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Studies in Asymmetric and Heterocyclic Synthesis:
I. Chiral Ketones
II. Quinolones
III. Trifluoromethylated Pyrones

Coláiste na hOllscoile Corcaigh, Éire
University College Cork, Ireland

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A thesis presented for the degree of
Doctor of Philosophy
to
THE NATIONAL UNIVERSITY OF IRELAND, CORK

Department of Chemistry
University College Cork

Supervisor: Dr. Gerard P. McGlacken
Head of Department: Prof. Martyn Pemble

August 2015

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Declaration

I hereby confirm that the body of work described within this thesis for the degree of Doctor of Philosophy, is my own research work, and has not been submitted for any other degree, either in University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism.

Sarah L. Clarke

Date 19th August 2015

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To Mum and Dad

Abstract

This thesis is split into three sections based on three different areas of research.

In the first section, investigations into the α-alkylation of ketones using a novel chiral auxiliary is reported. This chiral auxiliary was synthesised containing a pyrrolidine ring in the chiral arm and was applied in the preparation of α-alkylated ketones which were obtained in up to 92% ee and up to 63% yield over two steps. Both 3-pentanone and propiophenone based ketones were used in the investigation with a variety of both alkyl and benzyl based electrophiles. The novel chiral auxiliary was also successful when applied to Michael and aldol reactions. A diamine precursor en route to the chiral auxiliary was also applied as an organocatalyst in a Michael reaction, with the product obtained in excellent enantioselectivity, albeit in very poor yield. It was also employed as a chiral ligand along with sparteine in the α-alkylation of a carboxylic acid.

In the second section, investigations into potential anti-quorum sensing molecules are reported. The bacteria *Pseudomonas aeruginosa* is an antibiotic-resistant pathogen that demonstrates cooperative behaviours and communicates using small chemical molecules in a process termed quorum sensing. A variety of C-3 analogues of the quorum sensing molecules used by *P. aeruginosa*, including chloro, bromo, iodo, fluoro and methyl, as well as the quinazolinone analogue, were synthesised. Expanding upon previous research within the group, investigations were carried out into alternative protecting group strategies of 2-heptyl-4-(1*H*)-quinolone with the aim of improving the yields of products of cross-coupling reactions.

In the third section, investigations into fluorination and trifluoromethylation of 2-pyrones, pyridones and quinolones is reported. The incorporation of a fluorine atom or a trifluoromethyl group into a molecule is important in pharmaceutical drug discovery programmes as it can lead to increased lipophilicity and bioavailability, however late-stage incorporation is rarely reported. Both direct fluorination and trifluoromethylation were attempted. Eight trifluoromethylated 2-pyrones, five trifluoromethylated 2-pyridones and a trifluoromethylated 2-quinolone were obtained in a late-stage synthesis from their respective iodinated precursors using methyl fluorosulfonyldifluoroacetate as a trifluoromethylating reagent.

Abbreviations

3-oxo-C_6-HSL	3-oxohexanoyl-homoserine lactone
3-oxo-C_{12}-HSL	3-oxododecanoyl-homoserine lactone
α	stereochemical descriptor
$[\alpha]_D^T$	specific rotation
Å	Amstrong
Ac	acetyl
ACC	amino cyclic carbamate
AcOH	acetic acid
AHL	acyl homoserine lactone
AIDS	acquired immune deficiency syndrome
anal.	analysis
aq.	aqueous
β	stereochemical descriptor
Bn	Benzyl
Boc	tert-butyloxycarbonyl
bs	broad singlet
Bu	Butyl
BuLi	*n*-butyllithium
c	centi (10^{-2})
	concentration, for rotation
ºC	Celsius degrees
^{13}C NMR	carbon nuclear magnetic resonance
ca.	circa, about
calcd	calculated
Cbz	carboxybenzyl
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
CFU	colony forming unit
CIS-D	complex-induced *syn*-deprotonation
CM	complex mixture
COD	1,5-cyclooctadiene
COSY	correlation spectroscopy

C_4-HSL	*N*-butyryl-L-homoserine lactone
δ	NMR chemical shift
d	doublet
DABCO	1,4-diazabicyclo[2.2.2]octane
DAST	diethylaminesulfur trifluoride
DBH	1,3-dibromo-5,5-dimethylhydantoin
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	N,N'-dicyclohexylcarbodiimide
DCM	dichloromethane
dd	doublet of doublets
ddd	doublet of doublets of doublets
DEPT	distortionless enhancement by polarization transfer
DHPB	3,4-dihydro-2*H*-pyrimido[2,1,-b]benzothiazole
DIBAL-H	diisobutylaluminium hydride
DKR	dynamic kinetic resolution
DMA	dimethylacetamide
DMF	dimethylformamide
DMH	dimethylhydrazones
DMI	1,3-dimethyl-2-imidazolidinone
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dq	doublet of quartets
dr	diastereomeric ratio
E	Electrophile
E	entgegen configuration
e.g.	for example
ee	enantiomeric excess
equiv.	equivalent(s)
ESI	electrospray ionization
Et	Ethyl
et al.	and others
FT-IR	Fourier-transform infrared spectroscopy
g	gram(s)

GC	gas chromatography
h	hour(s)
^{1}H NMR	proton nuclear magnetic resonance
halex	halogen exchange
HCLA	homo chiral lithium amide
HHQ	2-heptyl-4-hydroxy-quinolone
HIV	human immunodeficiency virus
HMBC	heteronuclear multiple-bond correlation spectroscopy
HMPA	hexamethylphosphoramide
HMPT	hexamethylphosphormaide
HRMS	High resolution mass spectrometry
HSL	homoserine lactone
HSQC	heteronuclear single-quantum correlation spectroscopy
Hz	Hertz
i	iso
IOPY	3-iodo-4-phenoxypyridone
IR	infrared
J	coupling constant
k	rate constant
L	Litre
LC-MS	liquid chromatography-mass spectrometry
LDA	lithium diisopropylamide
LDH	lactate dehydrogenase
lit.	literature
LRMS	low resolution mass spectrometry
μ	micro (10^{-6})
m	metre
	mili (10^{-3})
	multiplet
	medium
M	molar
m/z	mass-to-charge ratio
max	maximum

m-CPBA	*m*-chloroperoxybenzoic acid
Me	methyl
MeCN	acetonitrile
MEM	2-methoxyethoxymethyl
MFSDA	methyl fluorosulfonyldifluoroacetate
mg	milligram
MHz	megahertz
min	minute
ml	millilitre
mol	mole
	molecular
MOM	methoxymethyl acetal
mmol	millimole
mol%	mol percent
MMPP	magnesium monoperoxyphthalate
m.p.	melting point
MS	mass spectrometry
MV	membrane vesicle
MvfR	multiple virulence factor regulator
ν_{max}	frequency of maximum absorption
n	nano (10^{-9})
n	normal
NaBARF	sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate
NBS	*N*-bromosuccinimide
NFSI	*N*-fluorobenzenesulfonimide
NIS	*N*-iodosuccinimide
Nk_1	neurokinin-1
NMP	*N*-methyl-2-pyrrolidone
NMR	nuclear magnetic resonance
NNRTI	non-nucleoside reverse transcriptase inhibitor
NSAID	nonsteroidal anti-inflammatory drug
o	ortho
o/n	overnight

π	type of orbital, electron
p	para
PA01	moderately virulent wild-type *P. aeruginosa* strain
PA14	highly virulent wild-type *P. aeruginosa* strain
PG	protecting group
Ph	phenyl
phen	1,10-phenanthroline
PMN	polymorphonuclear leucocyte
PPL	porcine pancreatic lipase
ppm	parts per million
PQS	*Pseudomonas* quinolone signal
Pr	propyl
p-TsOH	*p*-toluenesulfonic acid
q	quartet
QS	quorum sensing
QSI	quorum sensing inhibitor
R	rectus configuration
RAMBO	(2*R*,3a*R*,6a*R*)-2-(methoxymethyl)hexahydrocyclopenta[*b*]pyrrol-1(2*H*)-amine
RAMP	(*R*)-1-amino-2-methoxymethylpyrrolidine
RMP	(*R*)-2-(methoxymethyl)pyrrolidine
RT	room temperature
s	second
	singlet
	strong
S	sinister configuration
SADP	(*S*)-2-(2-methoxypropan-2-yl)pyrrolidin-1-amine
SADT	self-accelerating decomposition test
SAEP	(*S*)-2-(3-methoxypentan-3-yl)pyrrolidin-1-amine
SAM	*S*-adenosylmethionine
SAMP	(*S*)-1-amino-2-methoxymethylpyrrolidine
SAPP	(*S*)-2-(methoxydiphenylmethyl)pyrrolidin-1-amine

SAR	structure-activity relationship
SDS	sodium dodecyl sulfate
SET	single electron transfer
SM	starting material
SMP	(*S*)-2-(methoxymethyl)pyrrolidine
SPHOS	2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl
t	triplet
t, tert	tertiary
TBAF	tetrabutylammonium fluoride
TCA	trichloroacetic acid
TEDA	triethylenediamine
$TESCF_3$	(trifluoromethyl)triethylsilyane
TFA	trifluoroacetic acid
TfO, triflate	trifluoromethanesulfonate
THF	tetrahydrofuran
THP	tetrahydropyranyl
TLC	thin-layer chromatography
TMAF	tetramethylammonium fluoride
TMEDA	tetramethylethylenediamine
TMS	tetramethylsilane
	tetramethylsilyl
TOF LC-MS	time-of-flight liquid chromatography-mass spectrometry
t_R	retention time
Ts	4-toluenesulfonyl, tosyl
UV	ultraviolet
vol	volume
w	weak
wt	weight
Z	zusammen configuration

Note 1: Descriptors of stereoisomer composition and stereoselectivity used throughout are in accordance with the original papers.

Note 2: In biological terminology, 'LuxI' denotes a protein, '*luxI*' denotes a gene.

Studies in Asymmetric and Heterocyclic Synthesis

I

Chiral Ketones

Chapter 1

Investigation of a novel diamine based chiral auxiliary

Introduction

How would you like to live in Looking-glass House, Kitty? I wonder if they'd give you milk in there? Perhaps looking-glass milk isn't good to drink…

Lewis Carroll

Alice's Adventures in Wonderland; 1865

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1.1 General introduction

The study of stereochemistry originated in the early 1800s during investigations by the French physicist Jean-Baptiste Biot into the nature of plane-polarised light.[1-3] Biot noted that when a beam of plane-polarised light passed through a solution of certain organic molecules, including sucrose and camphor, the plane of polarisation rotated. The compounds were termed optically active. Little research was carried out in this area until Louis Pasteur began his work on the study of crystalline tartaric acid salts derived from wine in 1848. When Pasteur crystallised a concentrated solution of sodium ammonium tartrate below 28 °C, he made the observation that two distinct types of crystals, which were mirror images of each other, precipitated.[4] Pasteur was able to painstakingly separate the two types of crystals by working carefully with tweezers. Although the original mixture was optically inactive, solutions of each type of crystal were optically active and their specific rotations were equal in amount but opposite in sign. This discovery of chirality two centuries ago has necessitated that organic chemists develop methods of forming enantiomerically pure substances, rather than synthesising a racemate and separating the two enantiomers.

Chiral compounds are used in a wealth of applications, including pharmaceuticals[5] and agrochemicals.[6] Biological activity of these compounds arises through their interaction with an enzyme or receptor, which are constructed from chiral building blocks such as amino acids or carbohydrates, meaning these sites of action are themselves chiral. Receptor sites in the body can interact differently with the two different enantiomers of a chiral compound. Although there is only a difference in the spatial arrangements of the atoms at a chiral centre of a compound, this variance can lead to different effects *in vivo*.[7-9]

Synthetic organic chemistry reactions that allow formation of carbon-carbon bonds are an important class of transformations, demonstrated by the numerous reviews published in this area.[10,11] Numerous well-known reactions successfully accomplish this feat, including for example cross-coupling reactions,[12] the aldol condensation[13] and annulation reactions.[14] Methodologies that allow selective formation of these bonds while also introducing new stereocentres are of particular interest and recent publications have shown progress in this area.[15-18]

1.2 Methods of synthesising enantiomerically pure compounds

There are three main routes to form enantiomerically pure compounds based on the types of starting material used (**Figure 1.1**).[19]

The use of chiral pool methodology allows the formation of enantiomerically pure compounds by the use of cheap, naturally occurring, commercially available chiral starting materials. An example of this is the use of chiral amino acids to direct chirality further in a synthesis, with the chiral starting material becoming part of the final product through various addition steps. However, a drawback to this method is that it is only useful when the chiral starting material is available in stoichiometric quantities and is enantiomerically pure.

Alternatively, a racemic mixture can be synthesised and the desired enantiomer indirectly isolated by either crystallisation or chromatography. This procedure relies on derivatisation by reacting a mixture of enantiomers with enantiomerically pure compounds, resulting in diastereomeric compounds which have different physical properties and can be separated by the methods previously mentioned. Dynamic kinetic resolution (DKR) is an option when the reaction can be carried out in conditions where the enantiomers of a substrate can interconvert. In this case, theoretically all of the substrate can be converted to an enantiomerically pure product.[20] If DKR is not an option, then this route suffers from the major disadvantage that half of the mixture is the undesired enantiomer, which is usually discarded. On reaction scale up, this can be problematic both in terms of cost and logistics.

The treatment of achiral substrates with a chiral reagent to yield a chiral product is an attractive means of forming enantiomerically pure compounds compared with methods previously outlined. This can be performed using enzymes in the process of biocatalysis or using chiral compounds such as chiral auxiliaries or asymmetric catalysts. Asymmetric synthesis is of particular importance in the production of pharmaceuticals, and it has been reported that more than half of drug candidate molecules have at least one chiral centre.[21] The requirements for a useful asymmetric synthesis include high (usually greater than 90%) regio-, diastereo- and enantioselectivities.[7] In an asymmetric synthesis, an achiral substrate is converted to an unequal mixture of the two enantiomers of a chiral product, ideally achieving the highest possible proportion of the desired enantiomer.

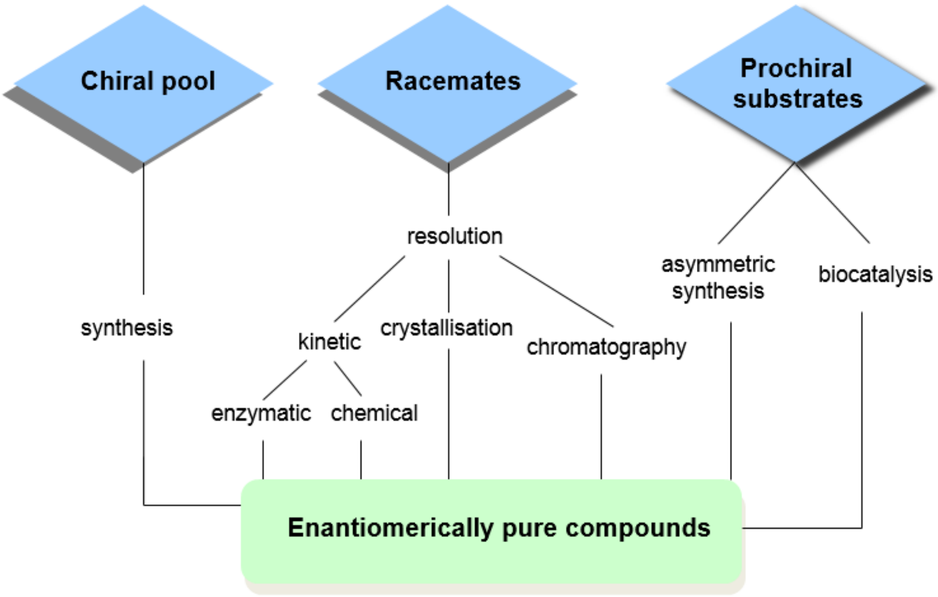
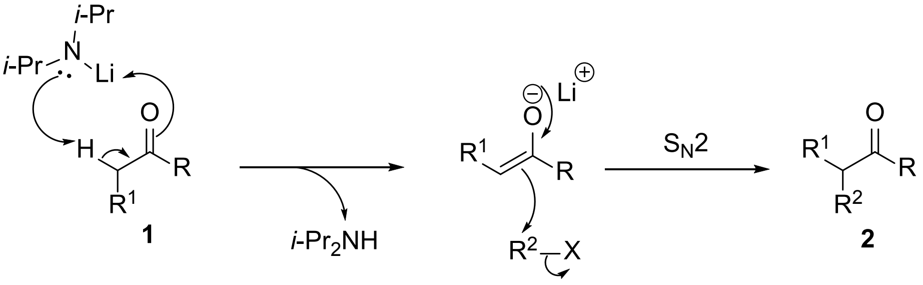

Chiral pool
Racemates
Prochiral substrates
synthesis
resolution
kinetic
enzymatic
chemical
crystallisation
chromatography
asymmetric synthesis
biocatalysis
Enantiomerically pure compounds

Figure 1.1 Routes to enantiomerically pure compounds.

1.3 α-Alkylation of aldehydes and ketones

Development of efficient and highly selective methods for carbon-carbon and carbon-heteroatom bond formation has been, and continues to be, a challenging yet exciting endeavour in organic chemistry, with new publications in the area constantly available.[16] The α-alkylation of aldehydes and ketones is considered one of the most fundamental reactions in synthetic organic chemistry.[22,23] These reactions consist of two steps. Firstly, the carbonyl compound is deprotonated at the α-position using a suitable base to provide a stabilised anion, also known as an enolate. Typically, a strong base such as LDA is used to ensure complete formation of the enolate. Deprotonation occurs in a cyclic mechanism.[24] The lone pair on the basic nitrogen atom of LDA abstracts an enolisable proton α- to the carbonyl moiety of **1** (**Scheme 1.1**). At the same time, the lithium coordinates to the oxygen to form the intermediate enolate. In the second step, an S_N2 substitution reaction takes place, with attack of the enolate on an electrophilic alkyl halide providing an α-alkylated product **2**. Both steps are usually undertaken at low temperatures, typically -78 °C. Choice of electrophile is important to ensure successful alkylation. Those that work best are methyl, allyl, primary alkyl and benzyl halides. Secondary alkyl halides can alkylate enolates, however the reaction tends to be slower than that which occurs when a more reactive alkyl halide is chosen. Tertiary halides on the other hand do not participate in alkylation reactions, instead undergoing unwanted E2 elimination reactions.[24]



Scheme 1.1 Depiction of two-step process in α-alkylation of carbonyl compounds.

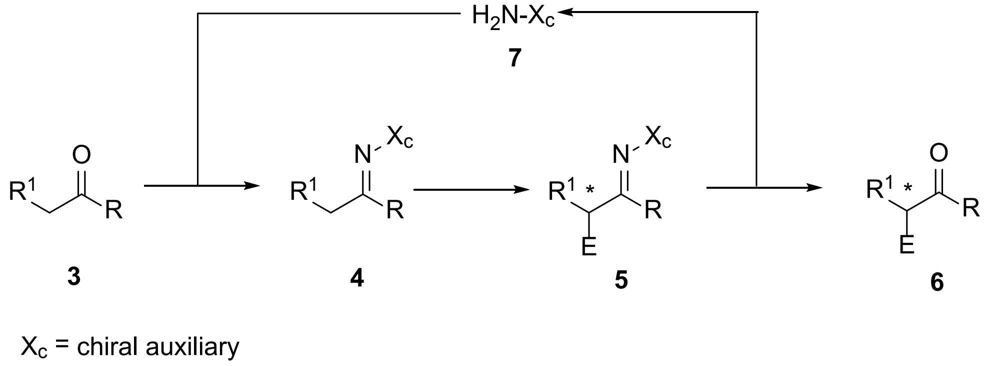
Due to the highly electrophilic nature of aldehydes, even with the reaction being carried out at the low temperatures required, the rate at which deprotonation takes place is not fast enough to outpace aldol self-condensation. Hence the reaction is more successful with ketones as substrates.[24]

Despite its usefulness and widespread application, classical carbonyl enolate chemistry is intrinsically complex and problematic. Most of the problems encountered in carbonyl enolate chemistry (aldol type self-condensation, di- and poly-alkylation, control of regiochemistry and lack of reactivity of corresponding enolates) have been solved in recent years by the use of nitrogen analogues of aldehydes and ketones, such as enamines, imines or hydrazones, which act as synthetic equivalents of the carbonyl compounds.[24-30]

The formation of α-alkylated aldehydes and ketones which also incorporate a new stereogenic centre are of utmost importance in organic chemistry. Fortunately, over the last four decades, techniques have become available to effectively allow this transformation, the most common approach of which is the use of chiral auxiliaries to induce the asymmetry in the product.[31]

1.4 Chiral auxiliaries

The use of chiral auxiliaries in asymmetric synthesis has been a major breakthrough towards easy access to enantiomerically pure compounds and it has become one of the preferred methods for carbon-carbon bond formation in the synthesis of chiral natural products.[8] In this approach, a prochiral substrate such as **3**, is attached to the enantiomerically pure chiral auxiliary such as **7** to yield **4**, before reaction with an achiral reagent (**Scheme 1.2**). The possible products **5** are thus diastereomeric and one is formed in excess due to the difference in activation energies. The chiral auxiliary is removed without racemisation to yield the final product **6** bearing a new stereocentre, with the formation of one enantiomer over the other favoured due to the chirality of the auxiliary.[7]



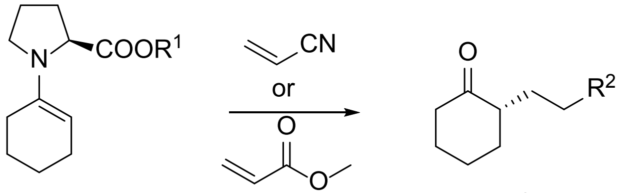
Scheme 1.2 Example of chiral auxiliary methodology.

For a chiral auxiliary to be considered practically useful it must be enantiomerically pure, cheap and easy to obtain in quantity. It must be both easy to attach to the substrate and easy to remove from the product without loss of stereochemical integrity and it should be separable and recoverable from the final product.[9]

There are four main factors which direct attack preferentially from one face. The main factor is steric hindrance of the bulk of the chiral auxiliary however the other factors that need to be considered include the chelation of metal ions, hydrogen bonding and electrostatic interactions.[7]

1.4.1 Development of chiral auxiliaries for asymmetric alkylation

In 1969, Yamada and co-workers reported the first asymmetric synthesis of α-alkylated cyclohexanones *via* enamine chemistry using a chiral auxiliary based on (*S*)-proline (**Scheme 1.3**).[32] Enamines (*S*)-**8a-c** were formed by reaction of cyclohexanone with their respective L-proline esters and reacted with either methyl acrylate or acrylonitrile to afford products (*S*)-**9a-c**. The authors found that the degree of enantioselectivity of **9** was enhanced with increasing steric bulk of the ester moiety. On optimisation of the reaction conditions, the authors noted that the best yield for the alkylation step (**9a** attained in 55% from **8b**) was obtained when the reaction was carried out in ethanol, so this was the solvent of choice for subsequent alkylations. The effect of reaction temperature on the asymmetric induction of **8c** with methyl acrylate was investigated. Results showed that increased reaction temperature resulted in increased yield (20 °C for 5 h provided **9b** in 17% yield, while reflux for 3 h provided **9b** in 33% yield), but a decrease in enantioselectivity (59% enantiomeric excess, ee, was observed when the reaction was carried out at 20 °C compared to 43% ee when the reaction was carried out at reflux).



(*S*)-**8a-c**

8a $R^1 = CH_3$
8b $R^1 = C_2H_5$
8c $R^1 =$ *t*-Bu

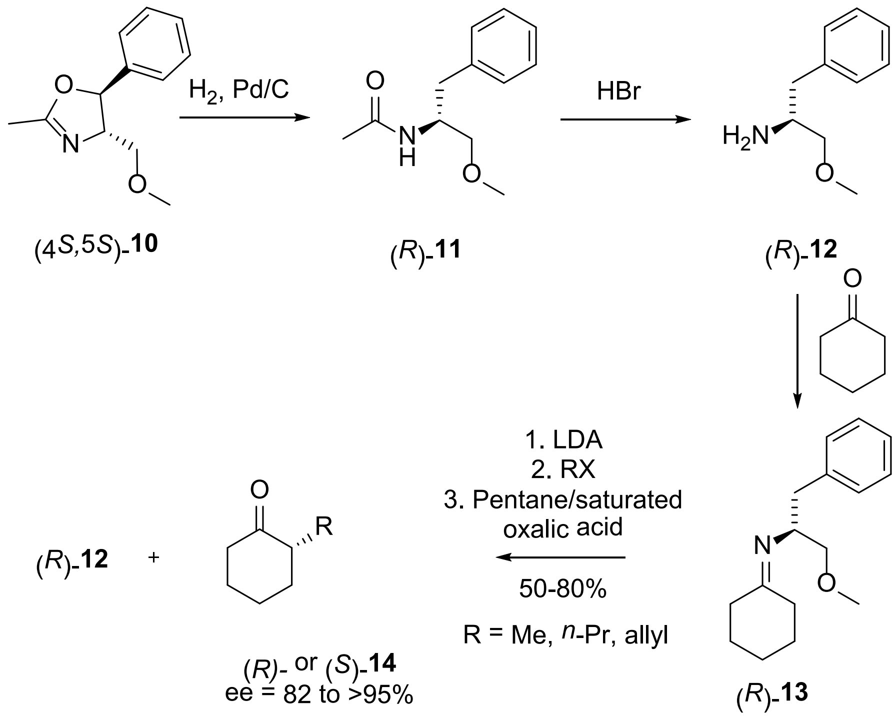
(*S*)-**9a-c**

9a $R^2 = CN$
9b $R^2 = COOCH_3$
9c $R^2 = COOH$

Scheme 1.3 Yamada's enamine based alkylation system.

Another example of the use of proline in asymmetric synthesis is the Eder-Sauer-Weichert-Hajos reaction which was more successful than Yamada's previous effort in terms of selectivity, with enantiomeric excess of up to 93% being achieved in cyclisation reactions used to form steroid building blocks.[33,34]

In 1976, Meyers reported results of >95% ee in alkylation reactions of cyclohexanone *via* metallated azaenolates using an acyclic amino-acid based auxiliary (**Scheme 1.4**).[35] Basing their studies on the results published by Yamada,[32] the authors proposed that a more rigid metalloenamine would be necessary to effect an efficient asymmetric alkylation. Hydrogenolysis of chiral oxazoline (4*S*,5*S*)-**10** to the amide (*R*)-**11** followed by acidic hydrolysis provided chiral amine (*R*)-**12** in 92% yield. (*R*)-**12** was reacted with cyclohexanone to provide imine (*R*)-**13** which was deprotonated, alkylated and hydrolysed to provide (*R*)- or (*S*)-**14** in excellent selectivity. Chiral auxiliary (*R*)-**12** could be recovered in good yield (73-85%) by neutralisation of the oxalic acid solution after hydrolysis.



Scheme 1.4 Meyer's synthesis of alkylated ketones using metallated azaenolates.

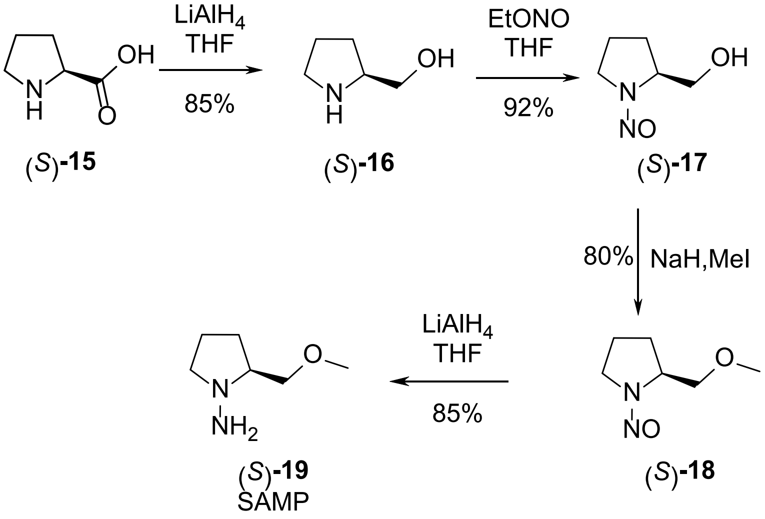
Around this time, the use of *N,N*-dimethylhydrazones (DMHs) as synthetic equivalents for azaenolates came to the fore.[36-40] The application of DMHs had major advantages over free carbonyl compounds as they are more reactive and higher regio- and stereoselectivity was observed.[25] The efficacy of these DMHs stems from the high reactivity of the hydrazone-derived organometallic species, in particular organolithium derivatives. The hydrazone moiety can be easily cleaved by a variety of methods, allowing regeneration of the carbonyl functionality.[25,41] The N-N bond is susceptible to reductive cleavage to yield primary amines.[25] Compared to the parent carbonyl compound, the acidity of the α-hydrogens of the hydrazone is *ca.* 10 orders of magnitude lower (the pK_a of the hydrazone is *ca.* 30 whereas the pK_a of the corresponding ketone is *ca.* 20). This is an advantage with hydrazones, as the lower acidity means their conjugate bases are more reactive towards electrophiles than the corresponding ketones. On addition of bases such as LDA,[36,39] *n*-BuLi[39] and *t*-BuLi[42] to solutions of hydrazones, stable metallated hydrazones are formed due to the coordination of the metal, in these cases lithium, to nitrogen atoms. The low acidity observed with α-hydrogens in hydrazones prevents racemisation of stereogenic centres at this positon by typical bases (such as carbonates, hydroxides and alkoxides), which is in direct contrast with the high racemisation rates observed with analogous ketones and aldehydes.[25] The use of hydrazones is also advantageous as regioselective deprotonation is usually high and predictable.[43,44] Unless there is an anion-stabilising group present at a competing site, deprotonation will occur at the least substituted carbon atom.[25] Alkylation of the metallated azaenolates provides regioselectively functionalised or branched hydrazones. Importantly, alkylation of hydrazones occurs selectively at the α-carbon unlike ketones or aldehydes, where alkylation of the oxygen often competes with alkylation at the α-carbon.[25]

1.4.2 SAMP/RAMP-methodology in asymmetric α-alkylation

The breakthrough in using chiral auxiliaries to synthesise α-substituted ketones in good yield and enantioselectivity occurred in 1976, when Enders and co-workers[45] pioneered the now frequently used SAMP/RAMP methodology. Employing (*S*)- or (*R*)-1-amino-2-methoxymethyl-pyrrolidine (SAMP or RAMP) as the chiral auxiliary, this approach has turned out to be very successful in the α-alkylation of both cyclic and acyclic ketones.

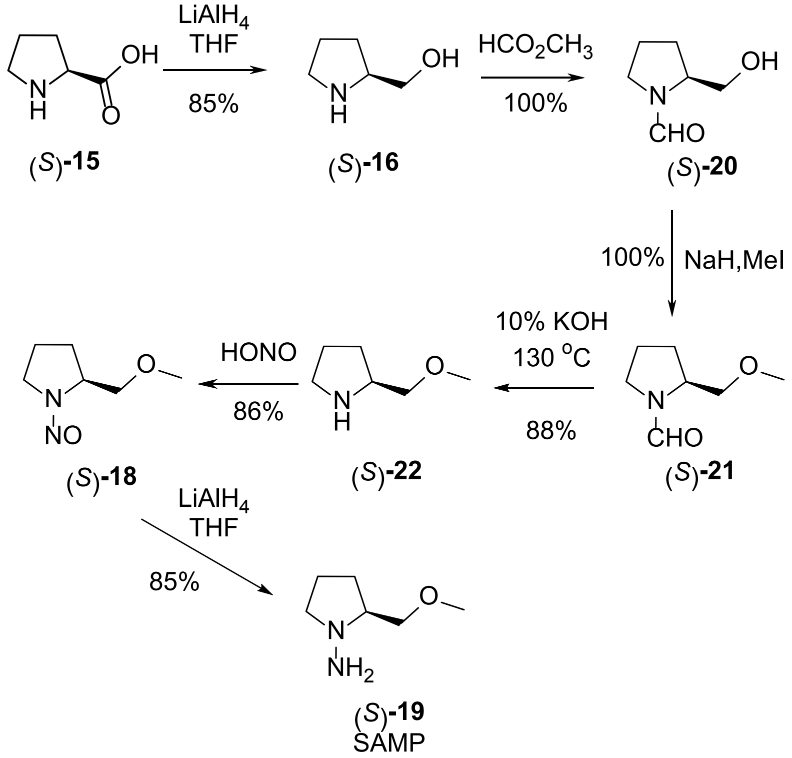
Starting from commercially available (*S*)-proline, the auxiliary SAMP is available in four steps in 58% yield, while RAMP, the (*R*)-enantiomer is obtainable in six steps starting from (*R*)-glutamic acid in 35% yield.[46,47]

The synthesis of SAMP (**Scheme 1.5**) begins with a lithium aluminium hydride reduction of (*S*)-proline (*S*)-**15** to alcohol (*S*)-**16**. Subsequent nitrosation using ethyl nitrite yields nitrosamine (*S*)-**17**, methylation is achieved using sodium hydride/methyl iodide to give (*S*)-**18** and a final lithium aluminium hydride reduction yields target compound SAMP (*S*)-**19**, with excellent yields being observed for each step.[45]


LiAlH4
THF
85%
EtONO
THF
92%
80%
NaH,MeI
LiAlH4
THF
85%
OH
O
N
H
NO
NH2
(S)-15
(S)-16
(S)-17
(S)-18
(S)-19
SAMP

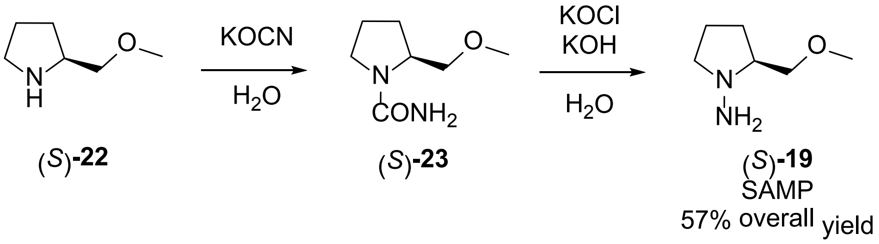
Scheme 1.5 Four step synthesis of SAMP.

SAMP (*S*)-**19** can also be obtained *via* a six step synthesis, again starting from (*S*)-proline (*S*)-**15** (**Scheme 1.6**). The first step is the same as for the previous synthesis shown. Alcohol (*S*)-**16** is treated with methyl formate to provide aldehyde-protected amine (*S*)-**20** in quantitative yield. Methylation yields (*S*)-**21** in excellent yield, whereupon treatment with base regenerates the free amine (*S*)-**22**. Nitrosation provides (*S*)-**18**, and a final lithium aluminium hydride reduction, as per **Scheme 1.5**, provides the desired product SAMP (*S*)-**19** in high yield.[45]


LiAlH4
THF
85%
HCO2CH3
100%
100%
NaH,MeI
10% KOH
130 °C
88%
HONO
86%
LiAlH4
THF
85%
(S)-15
(S)-16
(S)-20
(S)-21
(S)-22
(S)-18
(S)-19
SAMP

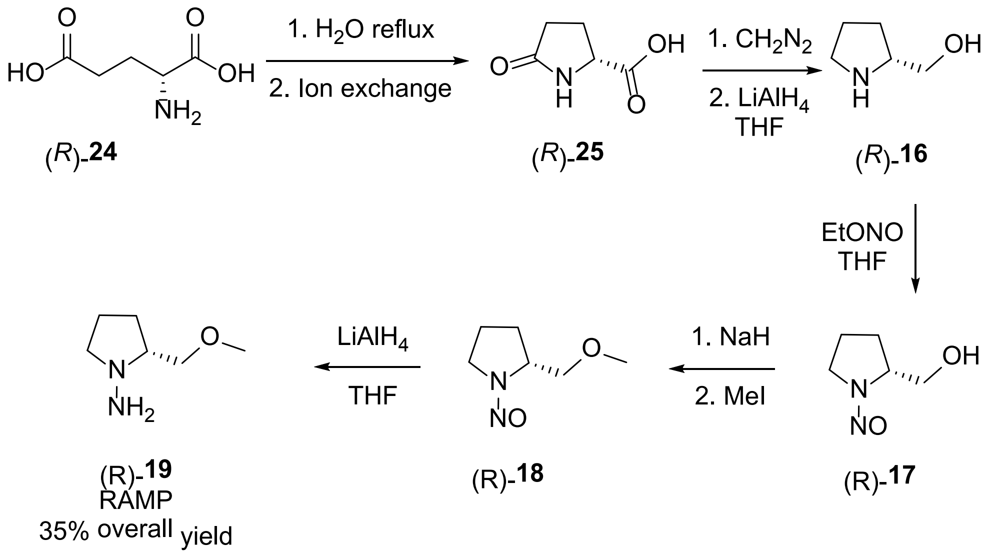
Scheme 1.6 Six step synthesis of SAMP.

An alternative route, in which the possibly toxic nitrosamine (*S*)-**18** intermediate in the reaction sequence is avoided, was developed by Enders *et al.* in 1987.[48] The nitrosamine is avoided by *N*-amination of (*S*)-**22** to yield (*S*)-**23** and subsequent Hofmann degradation to provide SAMP in 57% overall yield (**Scheme 1.7**).


KOCN
H2O
KOCl
KOH
H2O
(S)-22
(S)-23
(S)-19
SAMP
57% overall yield

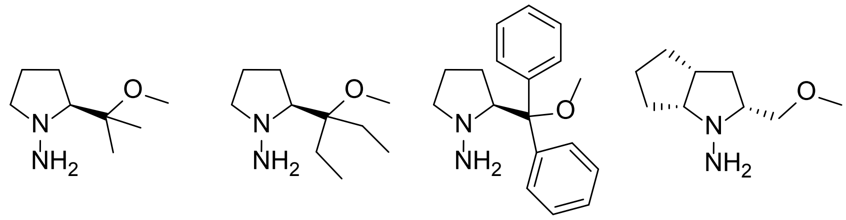
Scheme 1.7 Hofmann degradation route to SAMP.

(*S*)-Proline is the naturally occurring form of the amino acid and is available in abundance. However the (*R*)-enantiomer is not as readily available so RAMP is not synthesised in the same manner.[49] Instead, the synthesis begins with the more commonly available and relatively inexpensive (*R*)-glutamic acid (*R*)-**24**, which is refluxed in water and purified over an ion-exchange column to afford (*R*)-pyroglutamic acid (*R*)-**25**. Initial results indicated that it was possible to reduce (*R*)-**25** to (*R*)-**16** in 57% yield; however when the reaction was performed on larger scales it was noted that the yield dropped dramatically to *ca.* 15%. By transforming (*R*)-**25** into the methyl ester using diazomethane, the subsequent lithium aluminium hydride reduction of the lactam and ester moiety proceeds in one step in 76% yield. From this point, the synthesis is as per that of SAMP, whereby nitrosation with a suitable alkyl nitrite provides (*R*)-**17**. Subsequent methylation yields nitrosamine (*R*)-**18**. A final lithium aluminium hydride reduction affords RAMP (*R*)-**19** in 35% overall yield (**Scheme 1.8**).[49]



Scheme 1.8 Synthesis of RAMP.

Analogues of SAMP and RAMP chiral auxiliaries with increased steric bulk have also been developed. SADP (*S*)-**26**, SAEP (*S*)-**27** and SAPP (*S*)-**28** (**Figure 1.2**) can be prepared in a seven step sequence from (*S*)-**15**.[50] The even more sterically demanding RAMBO (*R*)-**29** can be obtained from the corresponding amino acid derivative[51,52] following the usual protocol for the preparation of these chiral auxiliaries.[53-55]

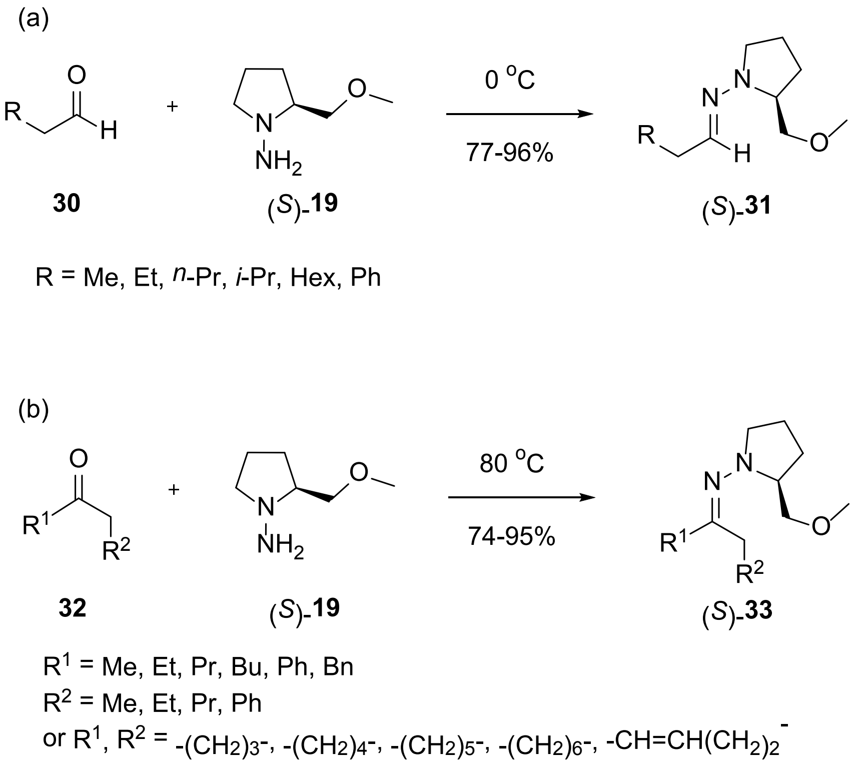


(*S*)-**26** SADP (*S*)-**27** SAEP (*S*)-**28** SAPP (*R*)-**29** RAMBO

Figure 1.2 Sterically demanding analogues of SAMP and RAMP.

1.4.2.1 Mechanism of α-alkylation of SAMP-hydrazones

Chiral hydrazones are formed in good to excellent yield (75-95%) by combining SAMP or RAMP with the desired ketone or aldehyde under water separation conditions (**Scheme 1.9**).[31] With ketones, the reaction is carried out at reflux with catalytic amounts of acid in benzene or cyclohexane. In the case of aromatic ketones, where carbonyl reactivity is lower, longer reaction times are necessary and water formed during the reaction must be removed azeotropically with a Dean-Stark trap. In the case of aldehydes, the reaction can be carried out neat at 0 °C.[47]

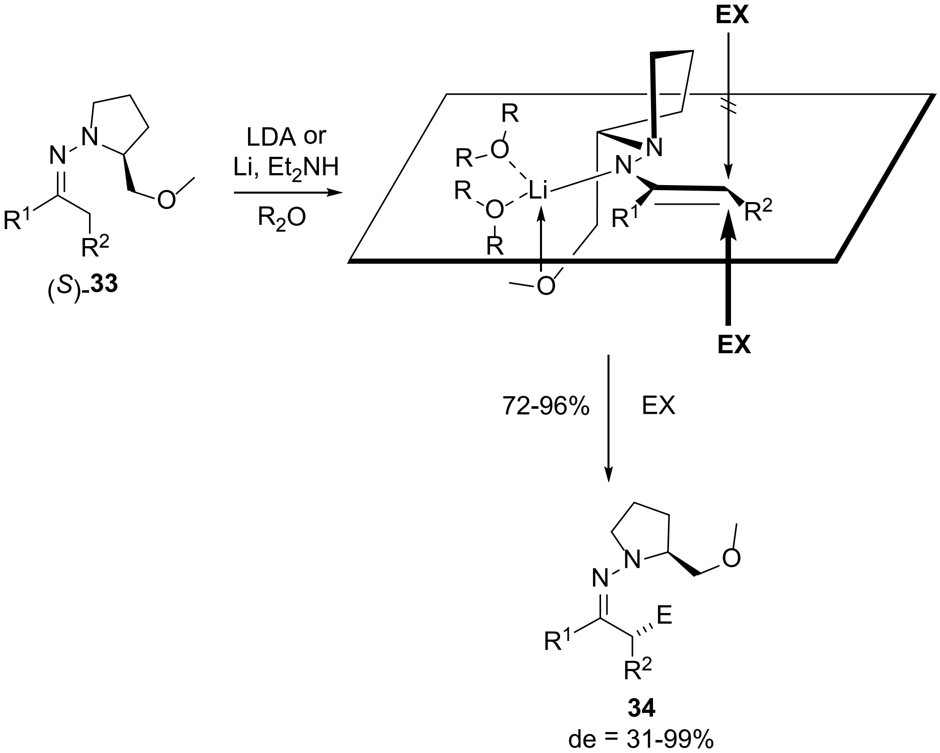


R = Me, Et, *n*-Pr, *i*-Pr, Hex, Ph

R^1 = Me, Et, Pr, Bu, Ph, Bn
R^2 = Me, Et, Pr, Ph
or R^1, R^2 = $-(CH_2)_3-$, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_6-$, $-CH{=}CH(CH_2)_2-$

Scheme 1.9 Synthesis of chiral SAMP-hydrazones from (a) aldehydes and (b) ketones.[31]

A lithium base, such as LDA, is typically used to deprotonate the hydrazone leading to an azaenolate intermediate. This can be trapped by an electrophile to obtain diastereomerically enriched products. In the deprotonation step, four geometrical isomers - $E_{\mathrm{CC}}E_{\mathrm{CN}}$, $E_{\mathrm{CC}}Z_{\mathrm{CN}}$, $Z_{\mathrm{CC}}E_{\mathrm{CN}}$ and $Z_{\mathrm{CC}}Z_{\mathrm{CN}}$ - can theoretically be generated. Investigations into the geometry of the deprotonation step showed that in the case of both cyclic ketones and the more flexible acyclic systems, only the $E_{\mathrm{CC}}Z_{\mathrm{CN}}$-species results (**Scheme 1.10**), as confirmed by trapping experiments,[45-47,56,57] spectroscopic investigations[58,59] and X-ray analysis.[60] Further determination of freezing point depression values of lithiated 2-acetylnapthalene-SAMP-hydrazones confirmed the monomeric structure of the enolate complexed by two solvent molecules.[61] In this structure, the lithium atom of the lithio enehydrazide is located *ca.* 20° below the CCNN-plane and is intramolecularly chelated by the methoxy group. This is a rigid intermediate and attack proceeds under high diastereofacial differentiation, yielding highly diastereomerically enriched hydrazones **34**.



(*S*)-**33**

34
de = 31-99%

R^1 = H, Me, Et, Pr; R_2 = Me, Et, Pr, *i*-Pr, Hex, Ph
and R^1, R^2 = $-(CH_2)_3-$, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_6-$, $-CH{=}CH(CH_2)_2-$
R = Et, $-(CH_2)_4-$
E = Me, Et, Pr, Bn, allyl
X = Br, I

Scheme 1.10 Origin of selectivity in SAMP-hydrazone alkylations.

Although much emphasis has been placed on the study of this and related newer approaches both experimentally[55,62-70] and theoretically,[71-78] the postulated S_E2'-front (metalloretentive) mechanism is based solely on the stereochemical outcome of the reaction. In this mechanism, the electrophile attacks from the front, which in this instance has to be the case as the back face is blocked by the steric bulk of the pyrrolidine ring. In 2011, Koch published findings on a computational study of the SAMP alkylation.[79] The results agreed with the previously postulated S_E2'-front mechanism. Detailed knowledge of the structure of the intermediate metallated SAMP hydrazone was required in order to understand the mechanism. The first step in the investigation by Koch was to find a reliable geometry of a model key intermediate **35**, which was formed from acetaldehyde and SAMP. Koch found that the resulting structures possess N-Li coordination as found in the X-ray structure, but with a varying number of additional contacts to the allylic carbon atoms and the second hydrazone nitrogen atom (**Figure 1.3**).[79] Compound **35b**, with the most coordinations, is predicted to be more stable in the gas phase (62 kJ mol^{-1} lower in energy compared to **35a**).

Due to the well-known fact that solvation plays a crucial role in lithium chemistry,[74,76,80-83] it was also considered during these investigations. Calculations on the model lithio azaenolate with either one or two THF molecules coordinated were performed. Two sets of optimised geometries were deemed to be possible: one in which the lithium cation sits "above" the NNCC moiety and one where it is displaced to the "back" of this NNCC semicircle (**Figure 1.3**). When one THF molecule was added, the energy difference between the two structures is reduced to less than 30 kJ mol^{-1}. When a second molecule of THF was added, the Li-O contacts become more important than those to the azaallylic part of the hydrazone, leading to almost equally stable structural motifs with three Li-O contacts within 8 kJ mol^{-1}. The two structures differ only slightly in the distances and number of azaallylic contacts (**Figure 1.3**). Based on this experimental evidence, Koch stated that the rate-determining step of the SAMP-alkylation proceeds *via* intermediate **35b**.

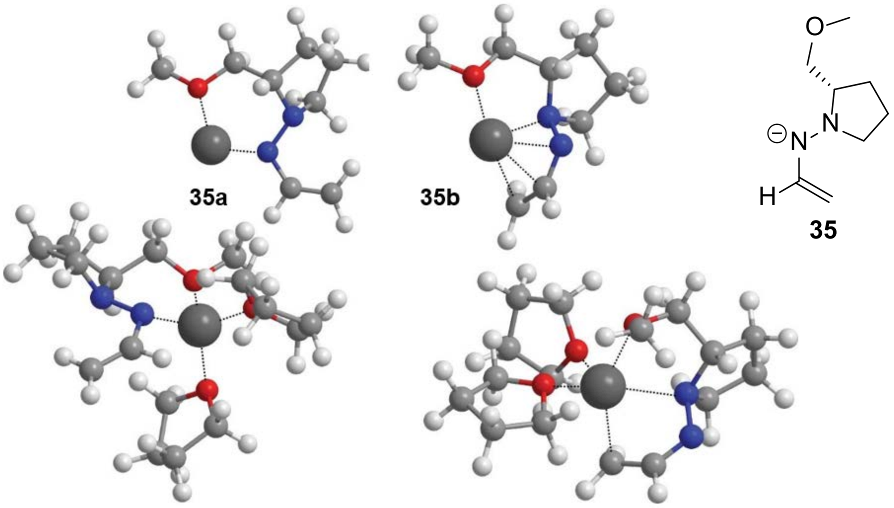


Figure 1.3 Postulated model lithio azaenolates as key intermediates in the SAMP alkylation. The disolvated (THF) structures are depicted in the lower half.[79]

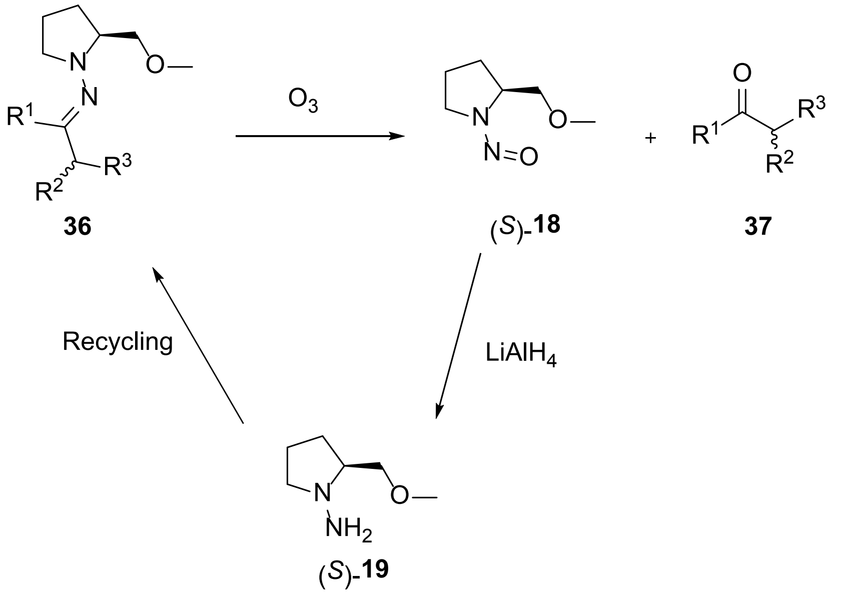
Added confirmation that the reaction proceeds by a metalloretentive S_E2'-front mechanism was attained when Koch calculated the transition states for several SAMP alkylations with known enantiomeric excesses, covering a wide range of enantiomeric purities.[79] Koch found that in all but one case, the metalloretentive *syn* attack of the electrophile from above onto the allylic moiety is preferential and that from all the data obtained, the reaction is likely to be an S_E2'-front or S_Ei-type mechanism, which are difficult to distinguish. In both cases a bimolecular mechanism is at play, whereby the new bond forms at the same time as the old bond breaks. Attack of the electrophile occurs from the front in both mechanisms, resulting in retention of configuration. The subtle difference is that in the S_Ei mechanism, the electrophile may assist in the removal of the leaving group by forming a bond with it at the same time as the new carbon-electrophile bond is being formed. In this case however, the relatively large distance between the electrophile and the leaving group suggested that the S_E2'-front was more likely. Koch also concluded that the selectivity of the reaction derives from the internal stabilisation of the transition state (where the electrophile attacks from above the lithiohydrazone plane) by electrophile-lithium interactions. It was stated that steric effects do not contribute to the selectivity.

1.4.3 Methods of cleavage of hydrazone to carbonyl

Over the last number of decades, many cleavage methods have been developed to allow transformation from the hydrazone back to the parent carbonyl functionality that are compatible with other functional groups.[41] In general, cleavage methods can be classified as either oxidative, hydrolytic or reductive.

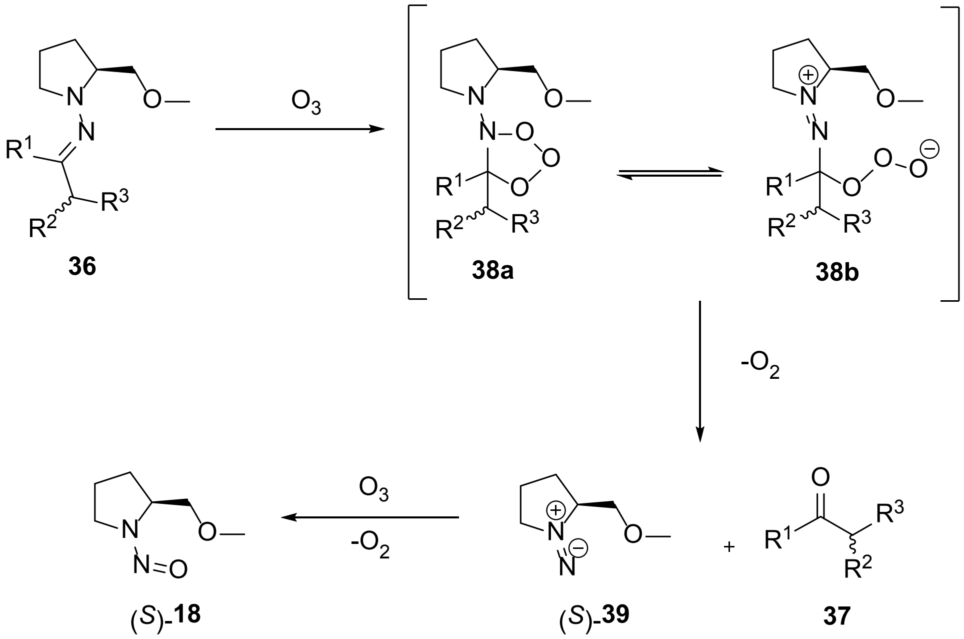
1.4.3.1 Oxidative methods

Oxidative methods include the use of ozone in an ozonolysis reaction, which has been extensively used in the case of DMHs and also SAMP/RAMP hydrazones (**Scheme 1.11**). In this methodology, a gentle stream of ozone is bubbled through a solution of the desired hydrazone **36** in dichloromethane at -78 °C. The colour of the solution turns green to blue (indicating excess O_3) when the reaction has gone to completion. Nitrogen or argon is then flushed through the solution as it is allowed to warm to room temperature.[46] This is a very clean reaction, yielding the desired carbonyl compound **37** and the nitrosamine (*S*)-**18** in quantitative yield, which allows for recycling of the chiral auxiliary (*S*)-**19** in up to 80% yield after separation by distillation or chromatography and subsequent reduction by lithium aluminium hydride. Advantages of this method of cleavage include the use of relatively mild reaction conditions, the requirement of neutral pH, short reaction times, the ease of detecting the end of the reaction, excellent yields and lack of racemisation of sensitive aldehydes and ketones. However this method cannot be applied when there are functional groups present which are incompatible with ozone under the reaction conditions. Due to the high reactivity of the C=N double bond towards ozone, the parent carbonyl functionality can be restored chemoselectively, even in the presence of functional groups that are sensitive to oxidation, such as thioethers,[84] protected α-hydrazino and aminoketones,[85] and borane-protected phosphines[86] by careful monitoring of the reaction by TLC or the addition of certain diazo dyes as indicators.



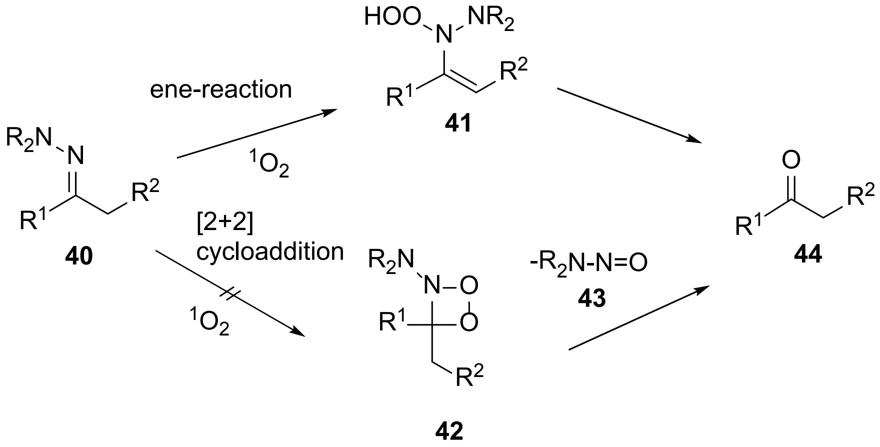
Scheme 1.11 Cleavage of hydrazone by ozonolysis.

Although the mechanism of ozonolysis is not exactly known, it is plausible that oxidative C=N bond cleavage starts with intermediates of type **38a** or **38b** (**Scheme 1.12**), which directly decompose to the desired carbonyl compound **37** and a diazene (*S*)-**39**, which is further oxidised under the reaction conditions to yield the nitrosamine (*S*)-**18**. Hence 2 equivalents of ozone are necessary for complete consumption of hydrazone.[87]



Scheme 1.12 Postulated mechanism for ozonolysis.

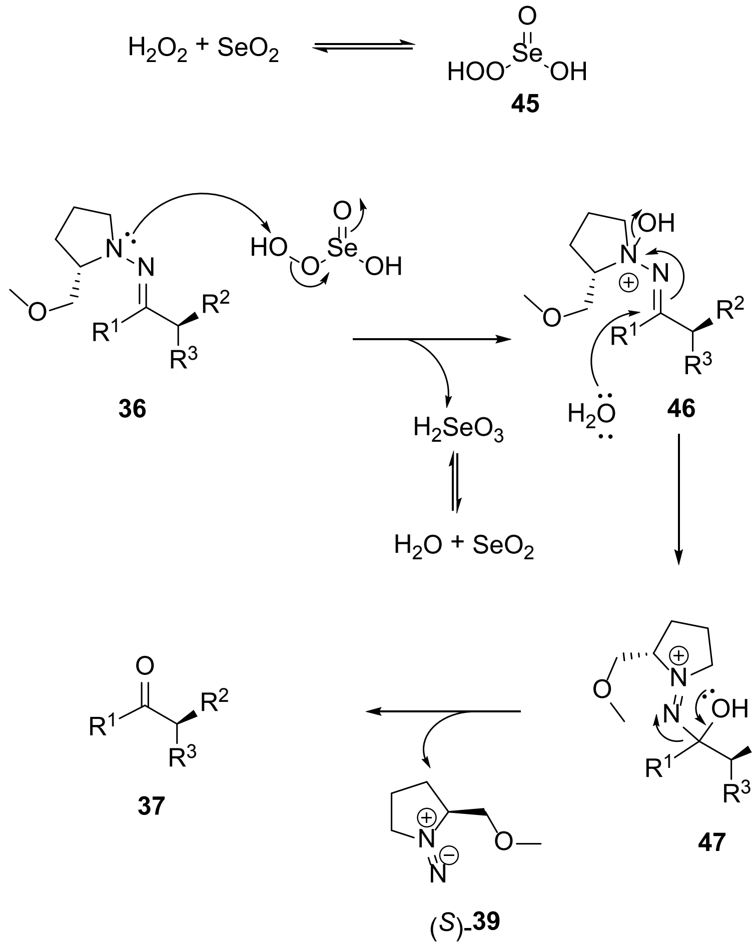
Another method of oxidative cleavage used to regenerate the parent carbonyl compound is the use of singlet oxygen, which has broad applicability for the regeneration of aldehydes, ketones and aldol adducts.[88] There are two plausible mechanistic pathways for the course of the oxidative cleavage from hydrazones **40**, either an ene-type reaction *via* a hydroperoxide **41**, or a cycloaddition reaction *via* the dioxazetidine **42** as an intermediate to provide carbonyl compounds **44** (**Scheme 1.13**). However, the cycloaddition route and autoxidation process can be ruled out as the dimethylnitrosamine **43** is not formed and the hydrazones **40** do not react in the absence of a sensitiser.



Scheme 1.13 Possible mechanistic routes for singlet oxygen hydrolysis of hydrazones.

Other less common oxidative cleavage methods include the use of sodium perborate ($NaBO_3$),[89,90] tetrabutylammonium peroxydisulfate ($(TBA)_2S_2O_8$),[91] [hydroxyl(tosyloxy)iodo]benzene (HTIB),[92] magnesium monoperoxyphthalate (MMPP),[93] meta-chloroperoxybenzoic acid (MCPBA),[94] peracetic acid[95] and hydrogen peroxide,[96] amongst others.[41]

A recent publication by Smith *et al.*[97] has shown that ketone SAMP hydrazones can be cleaved to the carbonyl compound by use of peroxyselenous acid, generated *in situ* from SeO_2 and 30% H_2O_2. The introduction of a pH 7 buffer was necessary to prevent epimerisation at the α-position of the ketone. The authors proposed a mechanism for the reaction (**Scheme 1.14**). The first step is the formation of peroxyselenous acid **45**. Oxidation of the pyrrolidine nitrogen of **36** takes place to generate **46**, which activates the hydrazone towards hydrolysis. Addition of water with abstraction of hydrogen leads to **47**, with the lone pair of electrons on the oxygen able to initiate fragmentation of the molecule, providing desired ketone **37** and diazene (*S*)-**39** as by-product.



R^1, R^2, R^3 = alkyl

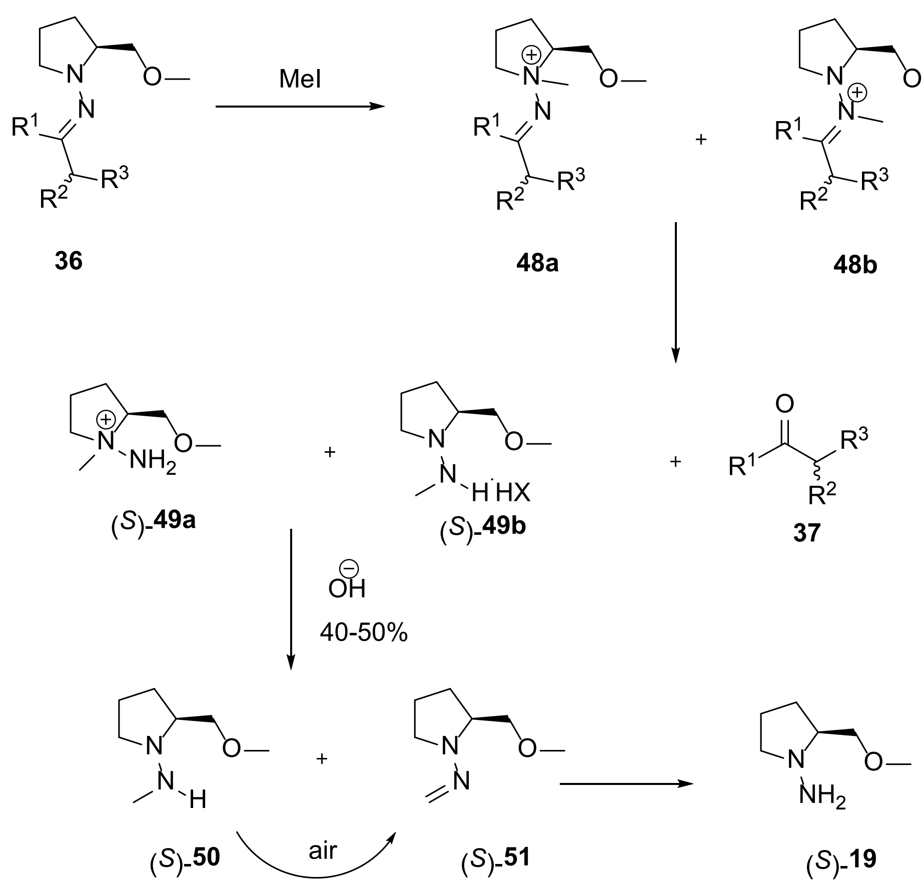
Scheme 1.14 Proposed mechanism for the oxidative hydrolysis of SAMP hydrazones with SeO_2 and H_2O_2 under buffered conditions.

1.4.3.2 Hydrolytic methods

A commonly used hydrolytic cleavage method used in SAMP/RAMP methodology is that of methyl iodide/HCl, also known as the salt method. According to Avaro *et al.*, much milder reaction conditions are required for the hydrolysis of dimethylhydrazones if they are first transformed to their corresponding trimethylhydrazonium iodides.[98] The conversion of SAMP/RAMP hydrazones involves a two-phase system.

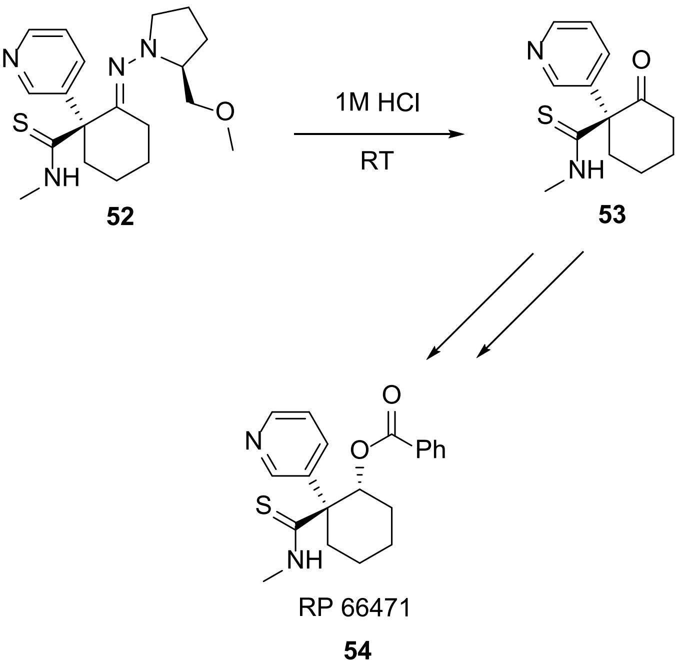
Treatment of **36** with excess methyl iodide at 60 °C leads to a quantitative mixture of methyliodides **48a** and **48b** (**Scheme 1.15**), which are hydrolysed without further purification in a biphasic system of 3-4M HCl and *n*-pentane to afford the parent carbonyl compounds without racemisation in good to excellent yields in reaction times of 15–60 min. The resultant carbonyl compounds are rapidly transferred with vigorous stirring into the pentane layer, which is free of acid. Chiral aldehydes and ketones which bear a stereocentre in a position α- to the carbonyl moiety are surprisingly resistant to racemisation in an acidic environment. However, great care must be taken with glassware as even traces of base lead to rapid and complete racemisation.[41]

The aqueous layer from this biphasic cleavage method contains the auxiliary in the form of salts (*S*)-**49a** and (*S*)-**49b**. When this is neutralised and extracted, the result is a mixture of SAMP hydrazone (*S*)-**50**, the hydrazine (*S*)-**51** and SAMP (*S*)-**19** in a ratio of 1:7:2 with a moderate yield of 40–50%. The ammonium salts (*S*)-**49a** remain in the aqueous phase whilst the trisubstituted hydrazine (*S*)-**50** is known to easily undergo oxidation in air to yield formaldehyde hydrazone (*S*)-**51**, which is hydrolysed to give (*S*)-**19**. In this way, it is possible to at least partially recycle the chiral auxiliary from this cleavage method (**Scheme 1.15**).[41]



Scheme 1.15 Cleavage of hydrazone *via* salt method.

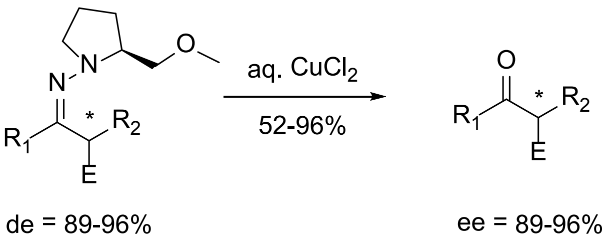
It is possible to carry out an efficient cleavage of the hydrazone to the parent carbonyl compound without first transforming the hydrazone to its corresponding methyliodide salt. The hydrazone is dissolved in a suitable solvent and stirred vigorously with 3-4M HCl in a biphasic system. This was the case in the synthesis of RP 66471 **54**, a potent potassium channel opener, where SAMP/RAMP methodology was used to form a quaternary centre α- to the carbonyl group.[99] Using acidic hydrolysis, the key intermediate **53** was formed from **52** without the need of first converting to salts (**Scheme 1.16**).



Scheme 1.16 HCl cleavage of hydrazone.

The use of acidic reagents for the cleavage of hydrazones to the parent carbonyl compounds is not ideal when acid-sensitive groups are present elsewhere on the compound. In these cases, alternative methods must be investigated. The use of silica gel for the cleavage of DMHs yields corresponding ketones with acid-sensitive groups such as THP groups, benzyl ether moieties and acetal groups remaining intact.[100] This method does appear to have limitations when attempting to cleave α-branched hydrazones.[101]

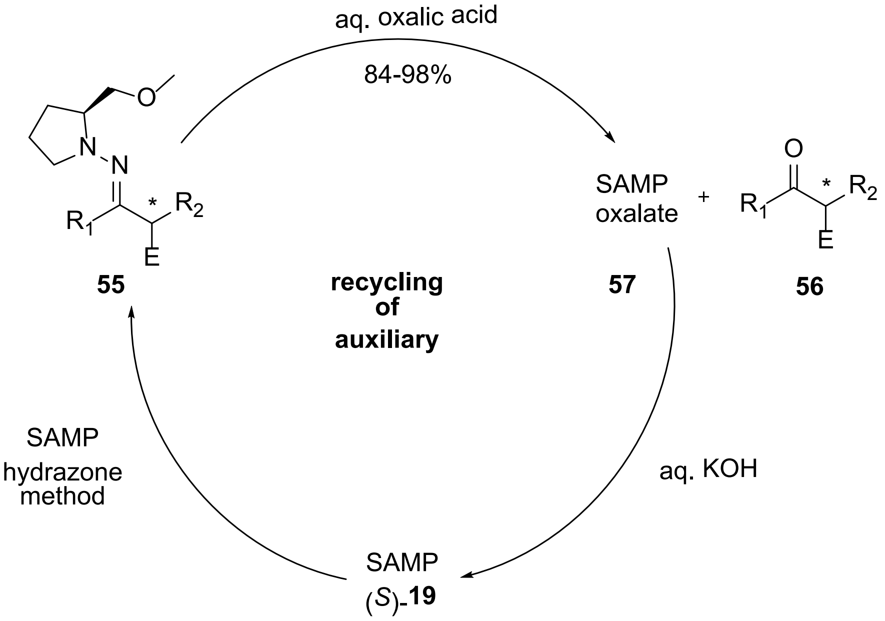
The hydrolysis of ketone and aldehyde DMHs can also be achieved using Cu(II) reagents,[102] in particular $CuCl_2$[103] and $Cu(OAc)_2$.[104] As the resulting hydrazine is oxidised to the diazene by Cu(II) the hydrolysis reaction is irreversible.[105] The Cu(II) hydrolysis method is ideal for compounds that contain functionalities that are sensitive to oxidation or strong acids (for example amines, alkenes, thioethers, acetals and silyl ethers) as these groups are unaffected under the conditions employed, and a higher yield is obtained than when subjected to other typical procedures such as ozonolysis or the salt method, due to associated side reactions. Enders *et al.* demonstrated the use of $CuCl_2$ in the cleavage of functionalised α-alkylated SAMP-hydrazones **55** in a 'one pot' alkylation/cleavage sequence to afford α-branched parent ketones **56** in good to high yield with high enantiopurity (**Scheme 1.17**).[103]



55 **56**

Scheme 1.17 $CuCl_2$ cleavage of hydrazone.

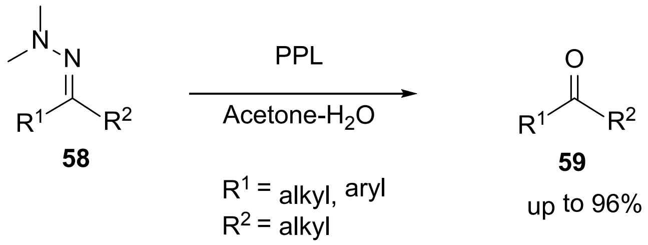
Cleavage of ketone SAMP-hydrazones **55** has been achieved with saturated aqueous oxalic acid solution in a mild, racemisation free biphasic method which also allows recycling of the chiral auxiliary.[106] The corresponding ketones **56** are available in excellent yields (84–98%) and high enantiomeric purity (90–99% ee). This procedure is particularly suited to compounds that contain functional groups that are sensitive to oxidative cleavage conditions (e.g. alkenes) or to strong acids (e.g. ketals). Recovery of the chiral auxiliary can be achieved by treating the corresponding oxalate salt **57** with sufficient base and extraction with a mixture of THF/diethyl ether (**Scheme 1.18**). To increase the recovery yield, the volume of water must be kept at a minimum due to the water solubility of the hydrazine. An added advantage of this method is that no potentially carcinogenic nitrosamine by-product is formed, as is the case with other cleavage methods, and there is no need to use potentially toxic methylating agents, as is required in the salt method. It must be noted that this method is not suitable for the hydrolysis of aldehyde SAMP hydrazones.[106]



Scheme 1.18 Oxalic acid method of cleavage of hydrazone.

Other less frequently used hydrolytic cleavage methods include the use of catalytic quantities of $BiCl_3$ in wet THF under microwave irradiation which allows hydrolytic cleavage of the C=N bond of DMHs to yield the corresponding ketones or aromatic aldehydes in good yields (75–98%) at atmospheric pressure within a few minutes.[107] Using a catalytic amount of $Pd(OAc)_2/SnCl_2$, ketone DMHs can be cleaved to parent ketones in moderate to good yields, without the use of acidic or oxidative reagents.[108] With this method, it is possible to hydrolyse α,β-unsaturated ketone DMHs without observing rearrangement. Halogens or nitro groups are also unaffected when using this methodology. The use of $BF_3{\cdot}OEt_2$ as a Lewis acid has proven to promote the hydrolysis of DMHs.[109] In comparison with ozonolytic cleavage, higher yields were obtained but reaction times were longer.[110] A method for the selective hydrolysis of ketone hydrazones in the presence of acetals has been published.[111] In this methodology, the hydrazone is dissolved in a biphasic mixture of THF and $NH_4H_2PO_4$ at pH 4.5 and proved successful for both cyclic and acyclic hydrazones.

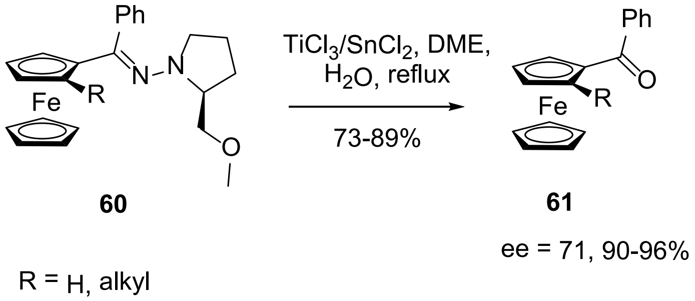
A wide scope of both ketone and aldehydes has been generated by biocatalytic conversion of phenyl- and *N,N*-DMHs in quantitative yield upon incubation with baker's yeast.[112] Another example of enzymatic cleavage is the use of a catalytic amount of porcine pancreatic lipase (PPL) in a biphasic acetone/water system at room temperature for the deprotection of ketone dimethylhydrazones **58** to ketones **59** with varying reaction times based on substrate structure (**Scheme 1.19**).[113] Both studies on the enzymatic hydrolysis were concerned only with racemic substrates.



Scheme 1.19 PPL hydrolysis of dimethylhydrazones.

1.4.3.3 Reductive methods

Although reductive cleavage procedures have been used to regenerate the carbonyl functionality from derivatives such as oximes and 2,4-dinitrophenylhydrazones, they have rarely been employed in the cleavage of dialkylhydrazones. Enders and co-workers synthesised ferrocenylketone SAMP hydrazones and required a reductive method to afford the corresponding ketones, due to the sensitivity of these compounds to oxidative and acidic reaction conditions.[114] The cleavage of the N-O bond of oximes and nitro compounds as well as the S-O bonds of sulfoxides can be achieved with the use of titanium(III). Due to the analogous nature of the N-N hydrazone bond, it was postulated that this method could be utilised en route to regenerate parent carbonyl functionality. Trivalent titanium has been employed in the cleavage of 2,4-dinitrophenylhydrazones,[115] with the resulting imines generated from the reduction being easily hydrolysed to their respective carbonyl compound. $TiCl_3$ and $SnCl_2$ have been used by Enders to successfully convert ferrocenylketone SAMP hydrazones **60** to their parent ketones **61** in good yields and enantioselectivities as an alternative to ozonolysis (**Scheme 1.20**).[114] During the cleavage, Sn(II) and Ti(III) are oxidised to Sn(IV) and Ti(IV) respectively, which are rapidly hydrolysed under the reaction conditions. It was noted that slight racemisation occurred with these cleavage methods, however *ortho*-functionalised ferrocenylketones were obtained with good to high enantiomeric purity.



Scheme 1.20 Cleavage of ferrocenylketone SAMP hydrazones.

1.4.4 α-Alkylation of *N*-amino cyclic carbamate chiral auxiliaries

For a number of years, the use of SAMP/RAMP chiral auxiliaries was the only method of forming asymmetric α-alkylated ketones in good yield and enantioselectivity. The methodology was successfully employed in a number of total syntheses of natural products.[31] Development of this methodology has been hindered due to some drawbacks. As the dialkylhydrazones used are only weakly acidic, longer exposure to LDA is required to ensure complete deprotonation. There is also the need for extremely low temperatures for alkylation (-110 to -78 °C). Both factors can be problematic and costly on larger scales. Finally, conditions for the removal of the chiral auxiliary to regenerate the parent ketone must be chosen carefully to avoid undesired reactions with other functional groups present on the molecule.[41]

In 2008, Lim and Coltart reported a major advance in the area with the development of chiral *N*-amino cyclic carbamates **62-65** (ACCs) (**Figure 1.4**), which were accessible by amination of the corresponding oxazolidinone.[116] Condensation of **62-65** with the desired carbonyl compound yields chiral hydrazones. It was noted that alkylation of these hydrazones could take place at -40 °C with less exposure time to LDA required for complete deprotonation, making this method more amenable to large scale reactions. Importantly, the use of these chiral auxiliaries allowed the formation of asymmetric α,α-bisalkylated ketones for the first time, due to their unique directing effect which overrides the inherent selectivity of LDA. Hydrazones **66-69** were synthesised by stirring a solution of 3-pentanone with each of the auxiliaries **62-65** and a catalytic amount of *p*-toluenesulfonic acid in DCM at reflux (**Figure 1.4**).

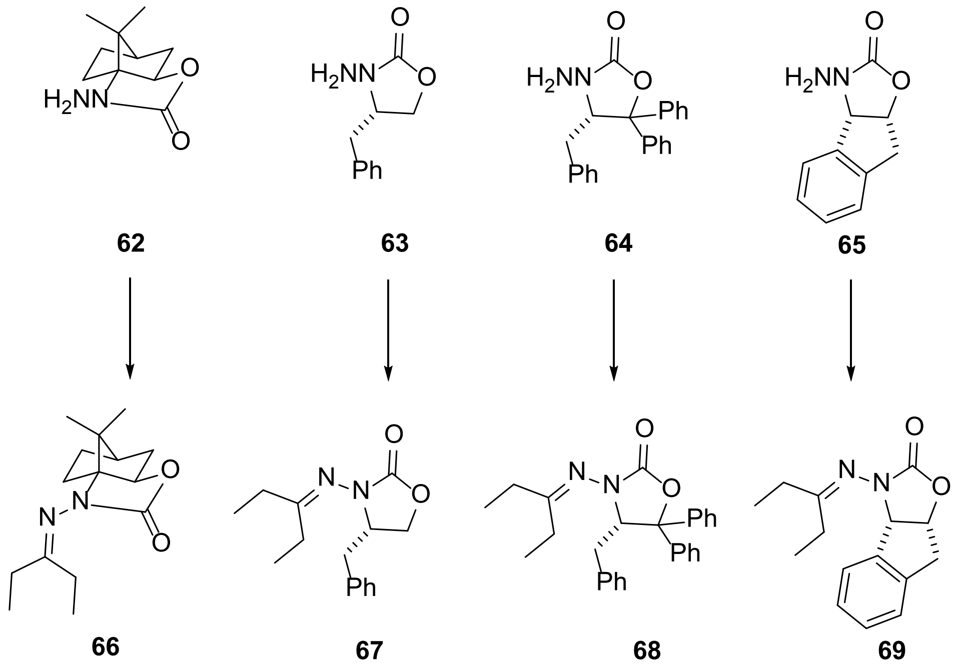
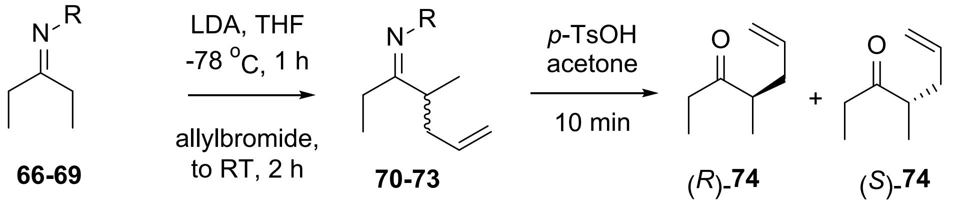


Figure 1.4 Chiral ACCs and respective hydrazones formed by reaction with 3-pentanone and *p*-toluenesulfonic acid in DCM, developed by Lim and Coltart.

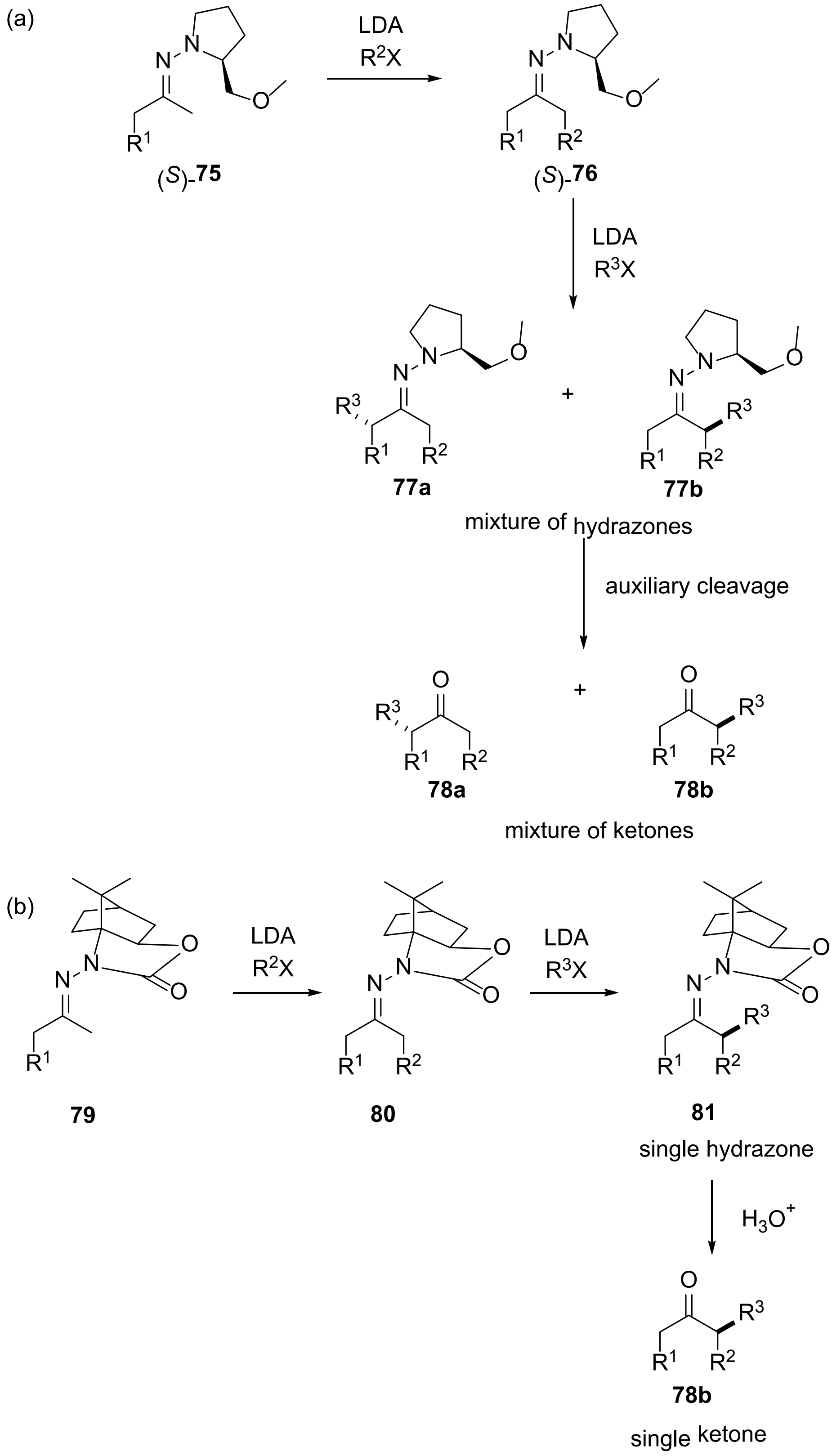
Initial studies on the asymmetric allylation of 3-pentanone ACC hydrazones **66-69** (**Scheme 1.21**) showed that using chiral hydrazone **67** resulted in the product ketone **74** being formed in excellent yield (90%) and good selectivity of (*R*)- and (*S*)-**74** in a 76:24 ratio. Repeating the reaction with hydrazone **69** resulted in better selectivity (86:14), however a slight decrease in yield was observed. From these results, it was postulated that if a chiral auxiliary was utilised that had greater steric bulk closer to the amino function it would result in greater enantioselectivity. Hydrazone **68** was subjected to allylation and cleavage to provide both (*R*)- and (*S*)-**74** in both excellent yield (93%) and enantioselectivity (91:9). The more conformationally rigid chiral auxiliary **62** was utilised to form hydrazone **66**, which contains a carbonyl group adjacent to the hydrazone moiety for enhanced α-proton acidity and tight chelation at the level of the azaenolate. Under the same reaction conditions using **66** a further improvement to both yield (96%) and enantioselectivity (96:4) was observed (**Scheme 1.21**).[116]



Scheme 1.21 Asymmetric allylation of ACC hydrazones.

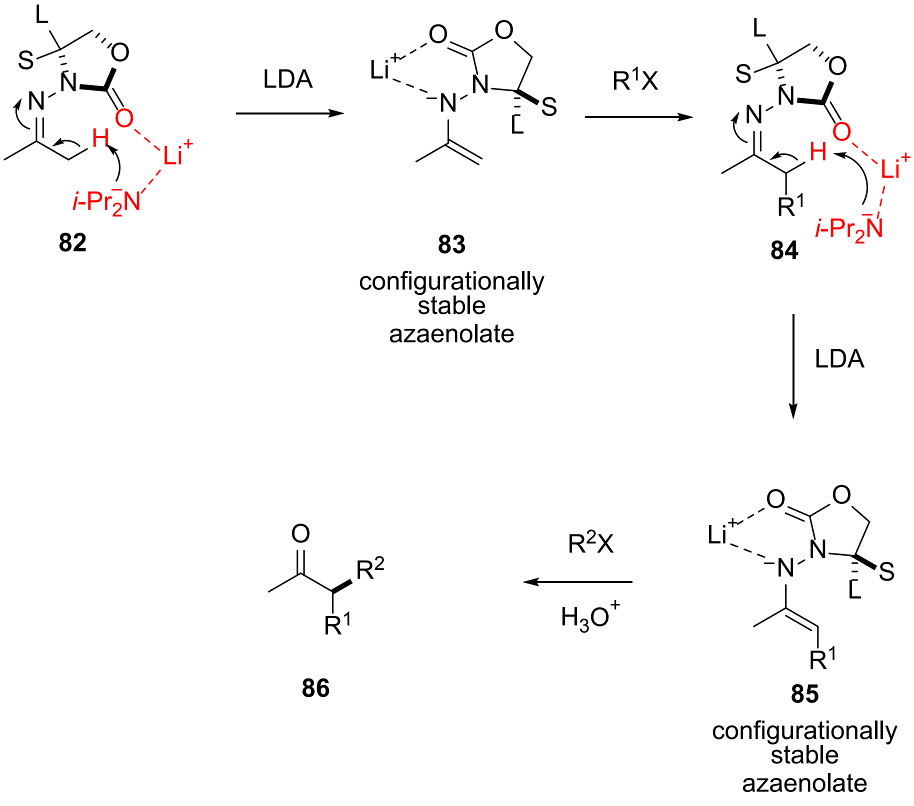
The authors have demonstrated that excellent enantioselectivites are available using a plethora of electrophiles and importantly, products are isolated in high yields. Best results were achieved using **66**, with excellent yields (up to 99%) and selectivities (up to 98:2) observed in alkylation reactions. Deprotonation occurs in 30 min at -40 °C and alkylation occurs in 2 h. Hydrazone cleavage is straightforward and efficient with no damage to or loss of the chiral auxiliary. These factors suggest that this method of α-alkylation of ketones is amenable to large-scale reactions.[116]

Addition of LDA to unsymmetrical ketone SAMP/RAMP-hydrazones **75** and **76** generally results in the removal of the most sterically accessible proton,[31,47,117] meaning that controlled asymmetric α,α-bisalkylation is not possible. This results in a mixture of alkylated hydrazones **77a** and **77b** and therefore ketones **78a** and **78b** (**Scheme 1.22, (a)**). In contrast, the use of ACC hydrazones in a process termed complex-induced *syn*-deprotonation (CIS-D) by Coltart and co-workers accounts for the α,α-bisalkylation of ketones observed.[117] The carbonyl group in the auxiliary was also intended to influence the regiochemistry of deprotonation to enable the α,α-bisalkylation of ketones which have both α- and α'-protons. Alkylation of **79** occurs to provide **80** with a second alkylation occurring at the same side to yield **81**. Cleavage of the hydrazone results in one single ketone **78b**. Interestingly, the regiochemical outcome is the opposite of that normally observed for LDA-mediated deprotonation of ketones and SAMP/RAMP-hydrazones (**Scheme 1.22, (b)**). An added advantage to this strategy is that access to either enantiomeric ketone is possible by using a single enantiomer of the auxiliary and by altering the alkylation sequence.


(a)
LDA
R²X
(S)-75
(S)-76
LDA
R³X
77a
77b
mixture of hydrazones
auxiliary cleavage
78a
78b
mixture of ketones
(b)
LDA
R²X
LDA
R³X
79
80
81
single hydrazone
H₃O⁺
78b
single ketone

Scheme 1.22 Asymmetric α,α-bisalkylation (a) *via* chiral imines and (b) *via* ACC hydrazones.

The postulated mechanism (**Scheme 1.23**) for the α,α-bisalkylation of ketones is based on directed deprotonation of the hydrazone, utilising the carbonyl lone pair on the auxiliary to coordinate with the base, thereby directing deprotonation to the same side of the C=N bond (**82**→**83**, **84**→**85**). Providing the resulting azaenolates **83** and **85** were configurationally stable and the monoalkylated product **84** did not isomerise when formed, the process should result in product ketone **86** *via* CIS-D.[116,118]


L
S
N
N
O
O
H
Li+
i-Pr2N
82
LDA
Li+
O
O
N
N
S
L
83
configurationally stable azaenolate
R1X
84
R1
LDA
85
configurationally stable azaenolate
R2X
H3O+
O
R2
R1
86

S = small substituent; L = large substituent

Scheme 1.23 Postulated mechanism for α,α-bisalkylation.

Coltart *et al*. carried out mechanistic studies into this reaction to prove the CIS-D route using 3-pentanone-derived ACC hydrazone and alkylating with *p*-bromobenzyl bromide.[117] A single monoalkylated product was formed and the alkylation took place on the same side of the C=N bond as the auxiliary, as confirmed by X-ray crystallography. Theoretical studies also support this method of alkylation.[118] Investigations into the scope of the monoalkylation reaction with a variety of alkyl halides showed that the reactions proceeded in high yield and selectively provided the α-regioisomer. These results provided further evidence of CIS-D occurring during formation of the azaenolate, as well as confirming the azaenolate intermediate was configurationally stable under the reaction conditions.[117] Although isomerisation of the hydrazone did not occur *in situ* after alkylation, the authors noted that if exposed to acidic conditions, the hydrazone was prone to isomerisation.[117]

The incorporation of a second alkyl group at the α-position requires an even more demanding application of CIS-D than that needed for the first alkylation. In this case, the ACC auxiliary must completely reverse the inherent preference of LDA for the removal of the most sterically accessible proton of the monoalkylated product and instead direct removal of the less accessible α-proton. Further mechanistic studies proved that a second alkylation occurred on the same side of the C=N bond as the auxiliary carbonyl, providing the α,α-bisalkylation compound as the major product, confirmed by X-ray crystallography.[117]

1.4.5 Chiral auxiliaries in drug synthesis

Although the use of chiral auxiliaries may seem outdated in light of the increasing scope of asymmetric catalysis, in many cases the chiral auxiliary approach is the only selective methodology available. Chiral auxiliaries also facilitate purification of products as single enantiomers by standard techniques including chromatography, recrystallisation and distillation.[119] As a result, chiral auxiliaries continue to play a key role in drug discovery and development in a variety of transformations.[120] With the current emphasis by various regulatory bodies towards single enantiomer drugs, straightforward access to enantiomerically pure pharmaceutical intermediates is essential to many drug discovery programmes, ensuring a continued importance for chiral auxiliaries in drug synthesis. Due to their high predictability in terms of stereochemical outcome, chiral auxiliaries continue to play an important role in the synthesis of many drugs, including atrasentan,[121] ontazolast,[122] and rupintrivir.[123]

Chiral auxiliaries have been used in diastereoselective aldol reactions, for example in the synthesis of (+)-methylphenidate hydrochloride (a mild nervous system stimulant that is primarily prescribed for the treatment of attention deficit hyperactivity disorder in children)[124] and in the synthesis of an optically pure trifluoromethyl alcohol which was required by researchers at Boehringer

Ingelheim Pharmaceuticals Inc. as part of a general drug discovery effort in the areas of inflammation, allergic and autoimmune disorders.[125]

Asymmetric hydrogenation reactions are quickly becoming one of the most powerful methods for installing chirality in both medicinal chemistry and process research.[126] This methodology was first reported by Knowles and Sabacky using a rhodium complex containing chiral tertiary phosphine ligands.[127] Although the corresponding chiral auxiliary route requires stoichiometric quantities of chiral reagent, the hydrogenation of chiral imines and enamines is an important method of synthesising optically pure amines required for medicinal chemistry and drug development.[128,129] This is demonstrated in the recent syntheses of sitagliptin, approved for the treatment of type II diabetes[130] and RWJ-53308, an orally active platelet fibrinogen receptor antagonist for the treatment of thrombotic disorders.[131]

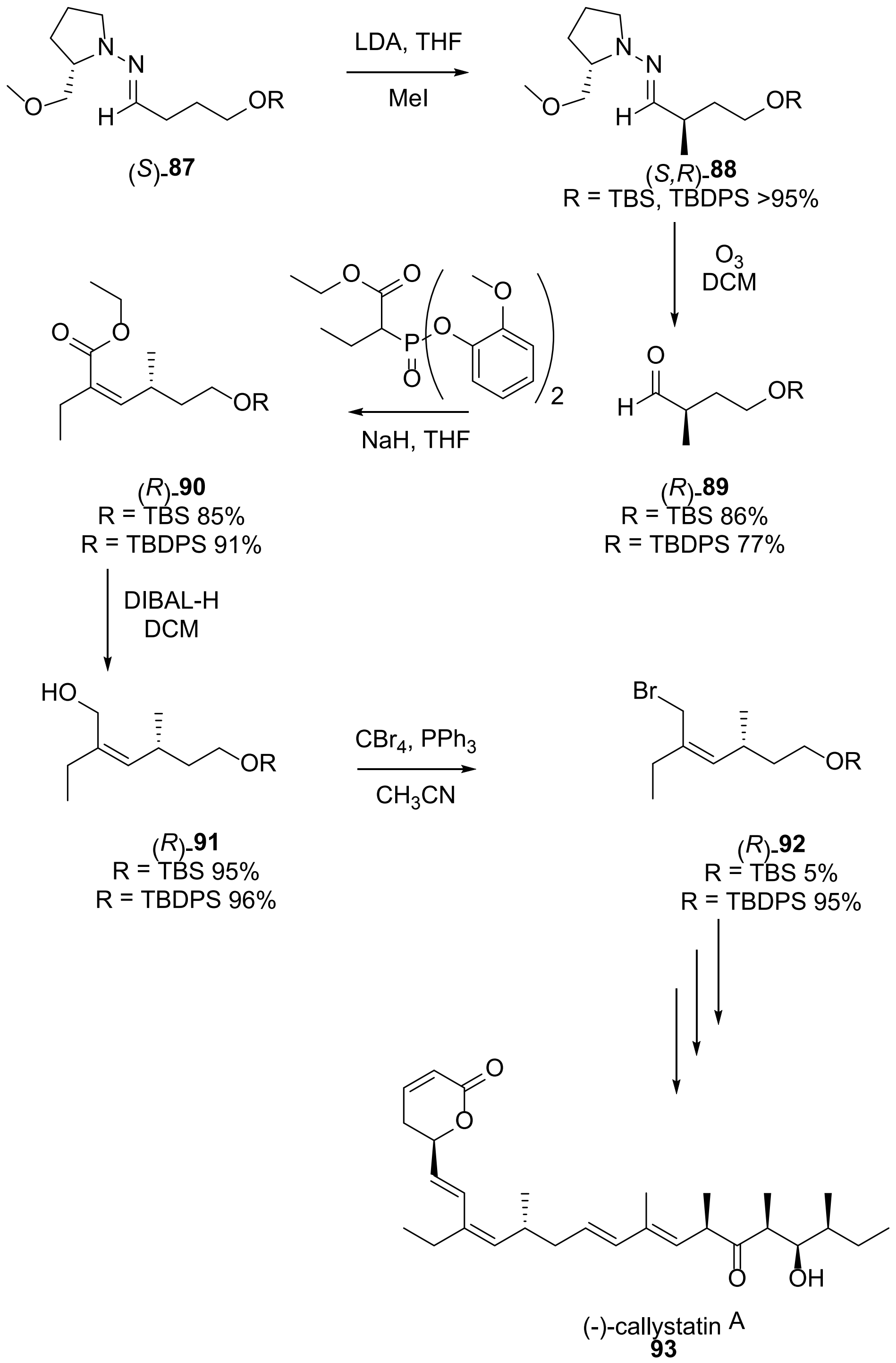
Chiral auxiliaries have also been employed in conjugate addition reactions in drug syntheses, for example in the synthesis of tipranavir,[132] (+)-(3*R*,4*R*)-3-(4-imidazolyl)-4-methylpyrrolidine dihydrochloride,[133] (3*R*)-*N*-methyl-2-oxo-[1,4'-bipiperidine]-3-acetamide[134] and in the synthesis of neurokinin-1 (NK_1) receptor antagonists.[135,136]

It has also been shown that chiral auxiliaries can be used in the synthesis of drugs in diastereoselective addition reactions to imines,[137] diastereoselective addition of trifluoromethyl anion[138] and diastereoselective chloromethylation.[139,140]

1.4.6 Chiral auxiliaries in natural product synthesis

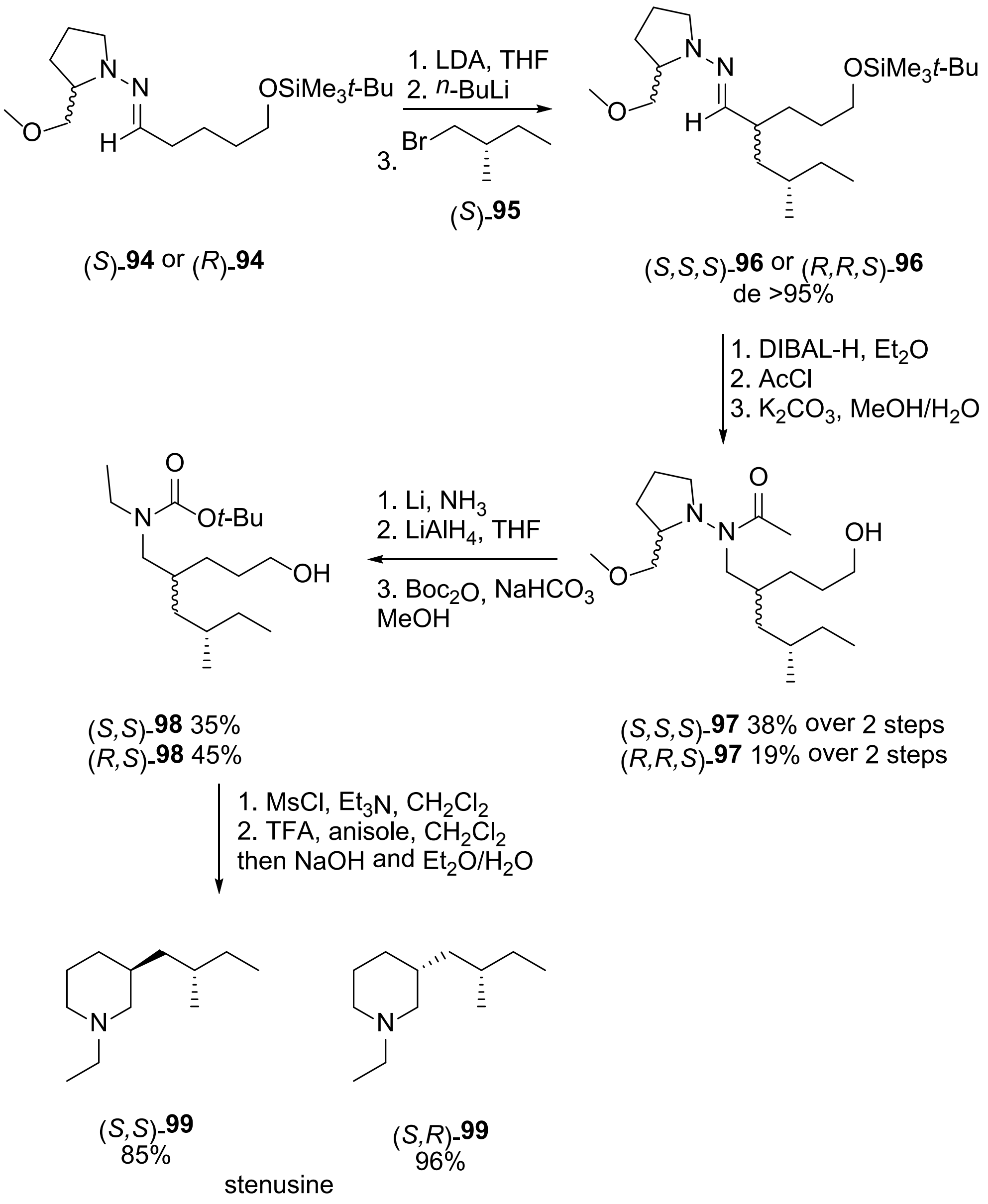
Chiral auxiliaries have found application in numerous total syntheses of natural products, a selection of which are outlined below.

SAMP (*S*)-**19** was used to synthesise an intermediate (*R*)-**92** (**Scheme 1.24**) en route to (-)-callystatin A **93**,[141] a potent cytotoxic polyketide isolated from the marine sponge *Callyspongia truncate* by Kobayashi *et al.* in 1997.[142] O-protected 4-hydroxybutanal hydrazones (*S*)-**87** were methylated by the usual SAMP alkylation procedure to provide (*S,R*)-**88**. Ozonolysis provided the parent aldehyde (*R*)-**89**, which was reacted with an aryl substituted phosphonate under modified Horner-Wadsworth-Emmons conditions to yield *Z*-α,β-unsaturated ester (*R*)-**90**. Subsequent reduction with DIBAL-H provided allylic alcohol (*R*)-**91** and bromination yielded the desired intermediate (*R*)-**92**, with the TBDPS group withstanding the reaction conditions. Further synthetic steps furnished **93**.


LDA, THF
MeI
(S)-87
(S,R)-88
R = TBS, TBDPS >95%
O3
DCM
(R)-89
R = TBS 86%
R = TBDPS 77%
NaH, THF
2
(R)-90
R = TBS 85%
R = TBDPS 91%
DIBAL-H
DCM
(R)-91
R = TBS 95%
R = TBDPS 96%
CBr4, PPh3
CH3CN
(R)-92
R = TBS 5%
R = TBDPS 95%
(-)-callystatin A
93

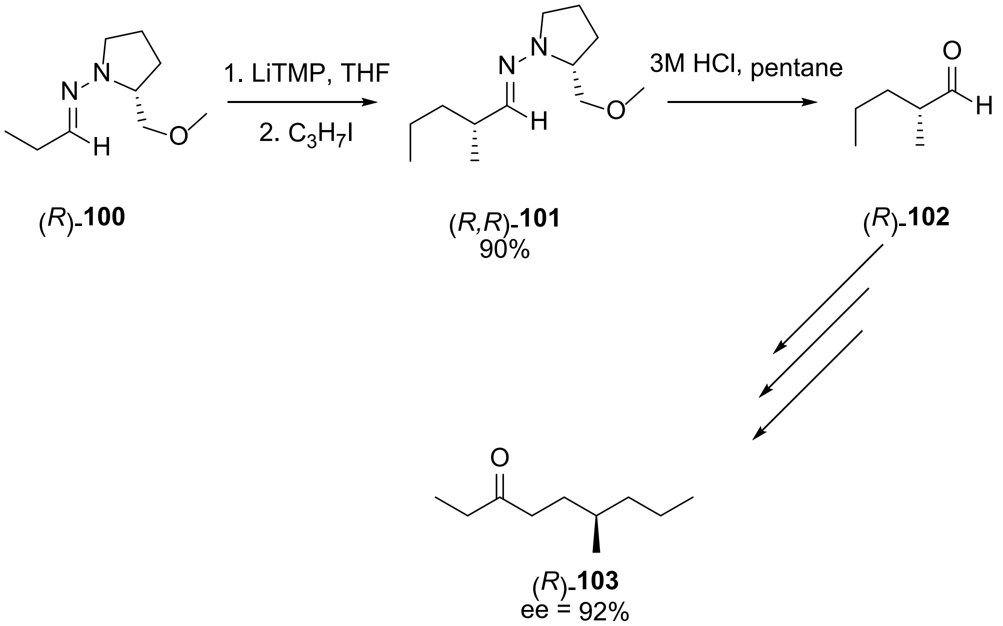
Scheme 1.24 Use of SAMP-hydrazone in the total synthesis of (-)-callystatin A **93**.

SAMP (*S*)-**19** and RAMP (*R*)-**19** have also been employed in the enantioselective synthesis of stenusine **99** (**Scheme 1.25**), a propulsion fluid expelled by the beetle *Stenus comma*.[143] It was envisaged that the stereogenic centre at the 3 position of the piperidine ring could be created by stereoselective alkylation of hydrazones derived from SAMP and RAMP. The electrophile could then be employed to introduce the side chain of the piperidine together with the second stereogenic centre. Lithiation of **94** was performed with LDA, followed by addition of 1 equiv. *n*-BuLi in order to deprotonate the formed diisopropylamine. The time required for deprotonation of these hydrazones was 14-16 h, longer than the usual time required for deprotonation of SAMP/RAMP-hydrazones.[47] The formed azaenolate was quenched by addition of bromide (*S*)-**95** to yield (*S,S,S*)-**96** when using SAMP and (*R,R,S*)-**96** when using RAMP. Both were produced in high diastereomeric purity (>95% de) and were carried through the next step without further purification due to their instability towards chromatography. Treatment with excess DIBAL-H resulted in simultaneous reduction of the C=N bond and removal of the silyl ether protecting group, and the ensuing formal aluminium hydrazide was quenched with acetyl chloride. Due to the presence of basic conditions, it was postulated that acylation could occur at either the hydrazine or the now unprotected hydroxyl group. However, as the reaction mixture was treated with potassium carbonate in aqueous methanol during the workup, hydroxyl hydrazides (*S,S,S*)-**97** and (*R,R,S*)-**97** were the products isolated. The acetyl group present in **97** was necessary to act as an activating group for the removal of the chiral auxiliary ((*S*)- or (*R*)-2-(methoxymethyl)pyrrolidine, SMP or RMP), which was achieved by reaction with lithium in liquid ammonia at -33 °C. The resulting crude acetamides were subjected to a lithium aluminium hydride reduction, with the acetyl group now acting as a precursor for the required *N*-ethyl substituent in the target molecules. Acylation with Boc_2O yielded the *N*-protected amino alcohols **98** with the same diastereomeric purity as was determined for the preceding hydrazones **96**. The final product was furnished by mesylation of the hydroxy functionality, cleavage of the carbamate group by treatment with trifluoroacetic acid and anisole which produced a syrupy ammonium salt, which cyclised spontaneously upon basic workup to provide stenusine (*S,S*)-**99** or (*S,R*)-**99**.


1. LDA, THF
2. n-BuLi
3.
Br
(S)-95
OSiMe3t-Bu
N
N
H
O
(S)-94 or (R)-94
(S,S,S)-96 or (R,R,S)-96
de >95%
1. DIBAL-H, Et2O
2. AcCl
3. K2CO3, MeOH/H2O
OH
(S,S,S)-97 38% over 2 steps
(R,R,S)-97 19% over 2 steps
1. Li, NH3
2. LiAlH4, THF
3. Boc2O, NaHCO3
MeOH
Ot-Bu
(S,S)-98 35%
(R,S)-98 45%
1. MsCl, Et3N, CH2Cl2
2. TFA, anisole, CH2Cl2
then NaOH and Et2O/H2O
(S,S)-99
85%
(S,R)-99
96%
stenusine

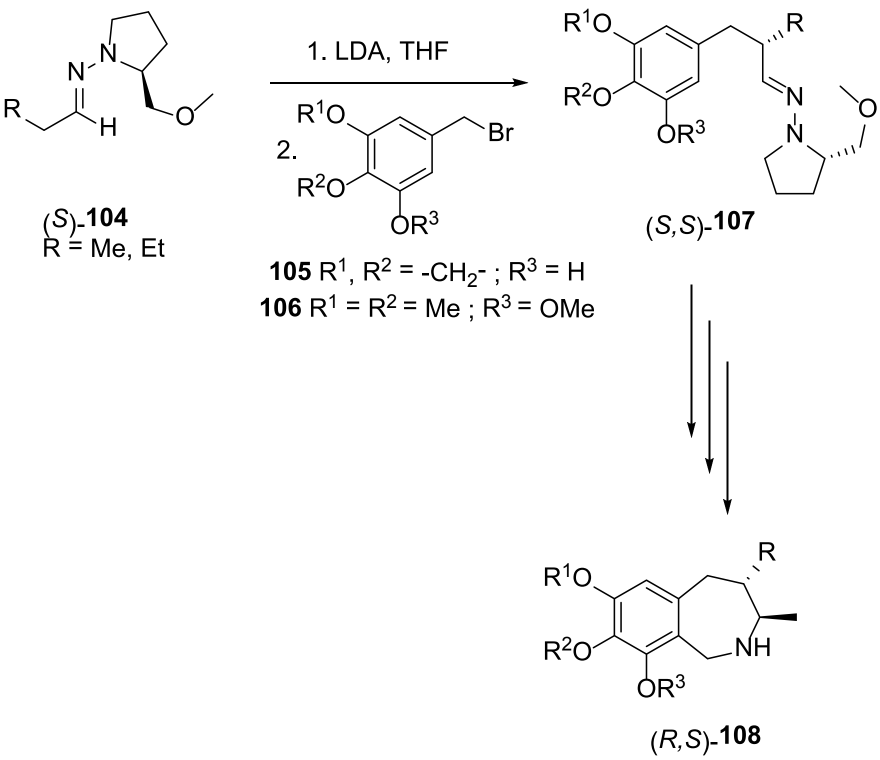
Scheme 1.25 Synthesis of stenusine using SAMP/RAMP hydrazones.

RAMP has also been utilised in the asymmetric synthesis of 6-methyl-3-nonanone **103**, the female-produced sex pheromone of the caddisfly *Hesperophylax occidentalis* (**Scheme 1.26**).[144] Both (*R*)- and (*S*)-enantiomers can be easily produced starting from RAMP or SAMP respectively, however (*R*)-**103** has been reported to be much more active than the (*S*)-**103**.[145] Hydrazone (*R*)-**100** was formed in virtually quantitative yield by reaction of propanal with RAMP (*R*)-**19**. Deprotonation of (*R*)-**100** was achieved by treatment with lithium tetramethylpiperidide (LiTMP) at 0 °C. Alkylation with iodopropane in the usual manner afforded the alkylated RAMP-hydrazone (*R*,*R*)-**101** in both excellent yield and selectivity (95% de).[47] Cleavage of the hydrazone was successfully achieved using a biphasic system to provide (*R*)-**102**. Due to the susceptibility of the liberated aldehyde to further oxidation, cleavage using the well-known ozone method could not be utilised on this occasion. Further transformations of (*R*)-**102** provided the desired product (*R*)-**103** in excellent yield and enantioselectivity. The same route was used in the synthesis of (+)-pectinatone using SAMP (*S*)-**19** as the chiral auxiliary.[146]



Scheme 1.26 Use of SAMP hydrazone in the synthesis of 6-methyl-3-nonanone.

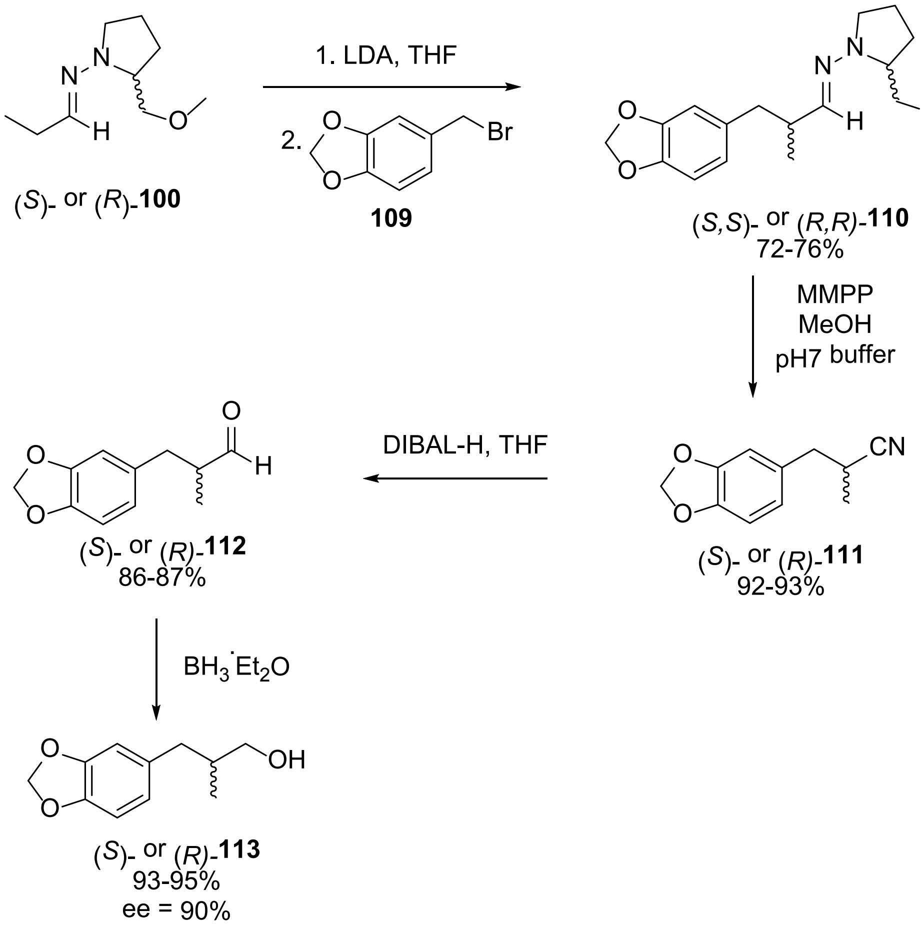
Compounds containing the benzazepine skeleton are of particular interest to medicinal chemists as this ring system lies at the heart of a wide range of constitutionally diverse models exhibiting profound chemotherapeutic properties.[147,148] A highly stereoselective route to 3,4-dialkylated-2,3,4,5-tetrahydro-2-benzazepines using aldehyde SAMP-hydrazones has been reported (**Scheme 1.27**).[149] SAMP-hydrazones (*S*)-**104** were prepared by combining the desired aldehydes with SAMP (*S*)-**19**. Exposure of (*S*)-**104** to LDA and subsequent alkylation with **105** or **106** led to the diastereomerically enriched arylated hydrazones (*S*)-**107** in moderate yield, observed as a single diastereomer by NMR. Further synthetic steps resulted in desired products (*R*,*S*)-**108** in moderate yield and high selectivity, with >96% de reported after chromatography.



Scheme 1.27 Use of SAMP-hydrazones in the synthesis of benzazepines.

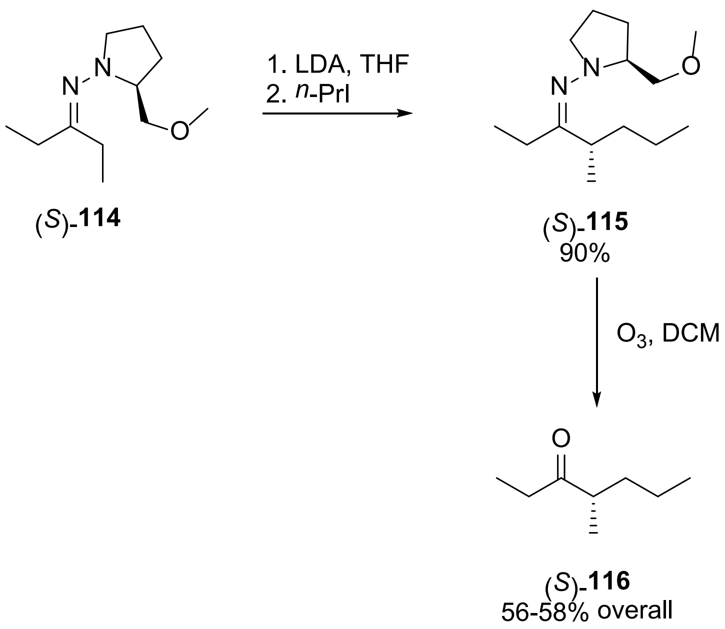
Many well-known odourants are chiral compounds and in many cases their enantiomers exhibit totally different scents. Tropional® is an α-branched aldehyde which is used in many perfumes to provide a fresh, marine scent.[150] In industry it is produced as a racemic mixture and as a result, nothing was known about the odour of each enantiomer of Tropional®. Enders and Backes reported the first asymmetric synthesis of Tropional® and differences in odour of the two enantiomers was described.[151] Each enantiomer of Tropional® could be successfully synthesised *via* an efficient four step synthesis using the SAMP/RAMP-hydrazone methodology (**Scheme 1.28**). (*S*)- or (*R*)-**100** was deprotonated using LDA and the resulting azaenolate trapped with 5-(bromomethyl)-1,3-benzodioxole **109**, resulting in alkylated hydrazone **110** in good yields and selectivity, with 90% de observed. Cleavage of the hydrazone was carried out *via* a two-step procedure. In the first step, the N-N bond was cleaved under oxidative conditions with magnesium monoperoxyphthalate (MMPP) to provide nitriles **111** in good yields. Reduction with DIBAL-H provided both enantiomers of Tropional® **112** in good yields and selectivity. The enantioselectivity was determined by reducing **112** to the corresponding alcohols

113. It was noted that the two enantiomers exhibited different odour characteristics, with the odour intensity of (*S*)-**112** about five times stronger than (*R*)-**112**. (*S*)-**112** exhibited represented the typical odour of the racemic mixture, whilst (*R*)-**112** showed a floral, citrus scent.


N
N
O
H
(S)- or (R)-100
1. LDA, THF
2.
O
O
Br
109
N
N
H
O
O
O
(S,S)- or (R,R)-110
72-76%
MMPP
MeOH
pH7 buffer
O
O
CN
(S)- or (R)-111
92-93%
DIBAL-H, THF
O
H
O
O
(S)- or (R)-112
86-87%
BH3·Et2O
O
O
OH
(S)- or (R)-113
93-95%
ee = 90%

Scheme 1.28 Use of SAMP/RAMP-methodology in the asymmetric synthesis of Tropional®.

The SAMP-hydrazone methodology has also been utilised in the synthesis of the principle alarm pheromone of the leaf-cutting ant *Atta texana,* (*S*)-(+)-4-methyl-3-heptanone (*S*)-**116**.[152] The simple, three step procedure (**Scheme 1.29**) begins with the formation of hydrazone (*S*)-**114** by reaction of 3-pentanone with SAMP (*S*)-**19**. Deprotonation with LDA leads to the azaenolate intermediate, which is trapped with iodopropane to provide (*S,S*)-**115** in 90% crude yield. Cleavage of the hydrazone moiety with ozone provided the desired product (*S*)-**116** in 56-58% overall yield.



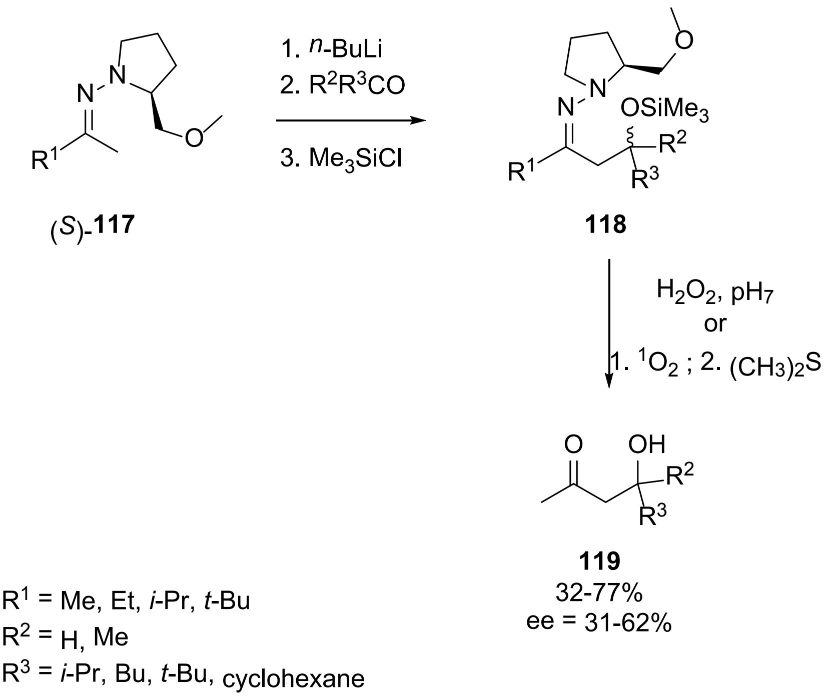
Scheme 1.29 Use of SAMP-hydrazone methodology in the synthesis of (*S*)-(+)-4-methyl-3-heptanone.

Other instances where SAMP-methodology has been used in the synthesis of natural products includes in the total synthesis of (+)-eremophilenolide[153] and (-)-methyl kolaventate.[154]

1.5 SAMP/RAMP-methodology in aldol reactions

The aldol reaction is an important carbon-carbon bond forming reaction in organic synthesis, highlighted by coverage in organic chemistry textbooks.[155,156] In the aldol reaction, aldehydes and ketones which possess an α-hydrogen atom are deprotonated with a suitable base, forming an enolate ion in what is the rate-determining step of the reaction. This enolate ion then reacts with another molecule of carbonyl compound to form the aldol product. Depending on the conditions, the elimination product may also form *via* an E1cB (unimolecular conjugate base elimination) mechanism. This mechanism is similar to E2 elimination, however in this case the leaving group can be a hydroxide, which is not the case in E2 elimination. Which product is formed partly depends on reaction conditions (use of stronger bases, higher temperatures and longer reaction times tend to promote formation of the elimination product) and partly on the structure of the reagents (some combinations are easy to stop at the aldol stage while some almost always give elimination product as well).[155]

It was not until 1978 that the first asymmetric intramolecular aldol reaction was reported, utilising SAMP-hydrazone methodology.[96] Hydrazones (*S*)-**117** were synthesised by combining the respective methyl ketone and SAMP (*S*)-**19** in the usual manner. Deprotonation with *n*-BuLi and subsequent treatment with carbonyl compounds provided β-hydroxyhydrazone intermediates. *In situ* trapping with chlorotrimethylsilane yielded protected hydroxyhydrazones **118** (**Scheme 1.30**). Two approaches were used to achieve cleavage of the hydrazone **118**: the reaction can be performed in one step with hydrogen peroxide at neutral pH to provide the desired aldol adduct **119** directly, or in two steps with singlet oxygen then dimethyl sulphide and hydrolysis to provide **119**. Aldol products **119** were achieved in moderate to good yields (32-77%) and enantioselectivities (31-62% ee).



(*S*)-**117**

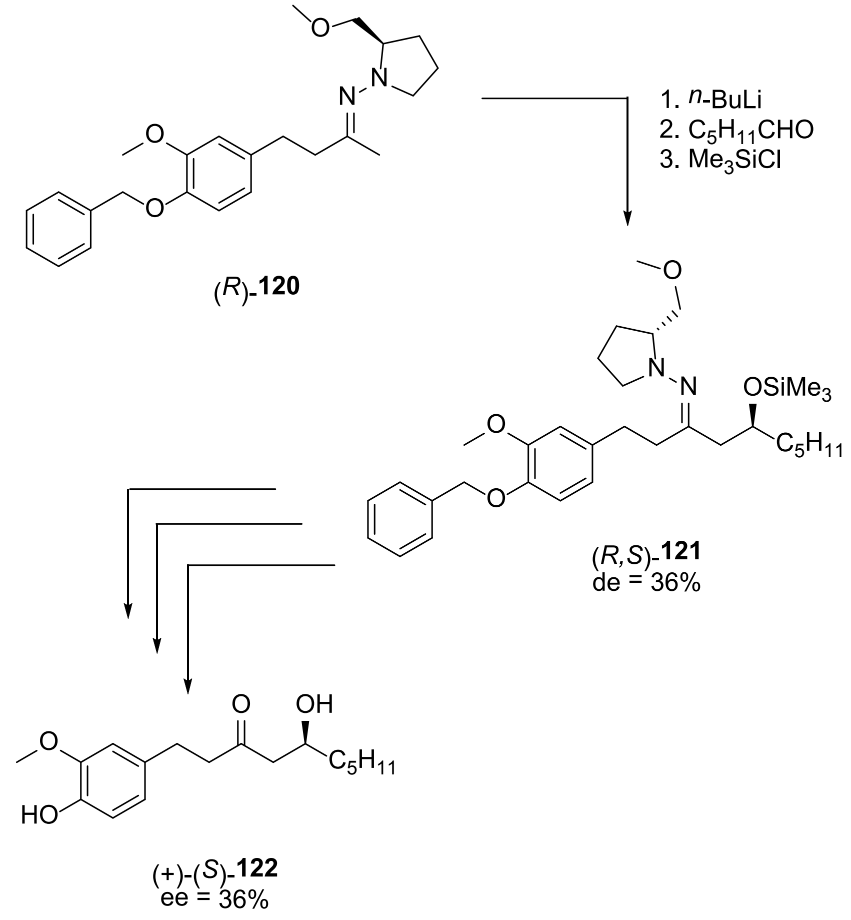
118

119
32-77%
ee = 31-62%

R^1 = Me, Et, *i*-Pr, *t*-Bu
R^2 = H, Me
R^3 = *i*-Pr, Bu, *t*-Bu, cyclohexane

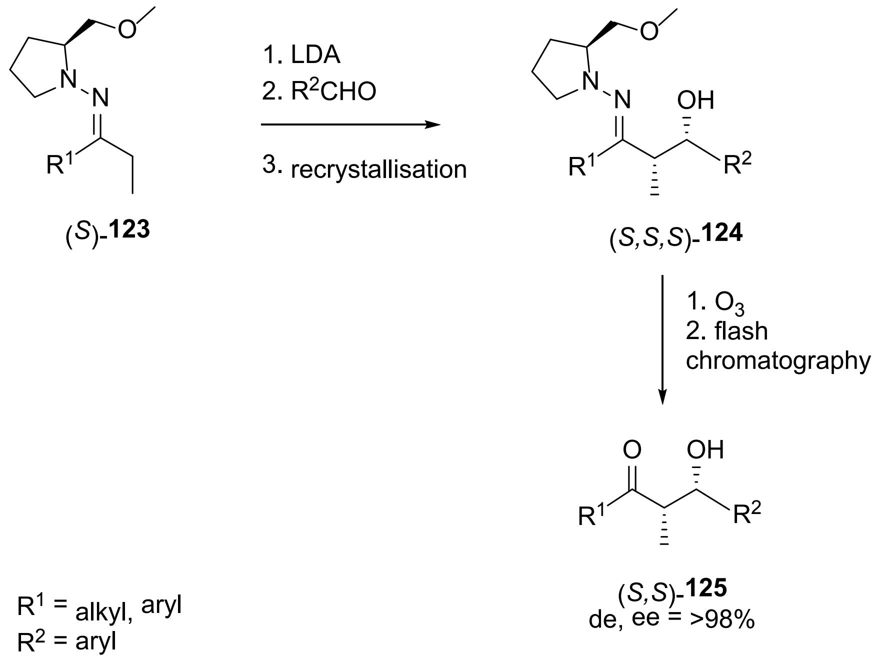
Scheme 1.30 Asymmetric aldol reactions using SAMP-hydrazone methodology.

This methodology has been applied to the synthesis of (+)- and (-)-[6]-gingerol, the principal odour compound of ginger (**Scheme 1.31**).[49] Deprotonation of (*R*)-**120** with *n*-BuLi, reaction with hexanal and subsequent trapping with chlorotrimethylsilane provided silyl-protected hydrazone (*R,S*)-**121** in 36% de. Further synthetic operations yielded (-)-[6]-gingerol (*S*)-**122** in 36% ee. When the reaction was performed with RAMP rather than SAMP as the chiral auxiliary, the opposite enantiomer was obtained.



Scheme 1.31 Synthesis of (+)-[6]-gingerol using RAMP-hydrazone methodology.

The asymmetric alkylation of SAMP-ethyl hydrazones (*S*)-**123** with aldehydes provided the *syn*-aldol adducts (*S,S,S*)-**124** with 51-80% de and 70-80% ee. When adducts (*S,S,S*)-**124** were recrystallised, pure stereoisomers of (*S,S,S*)-**124** were recovered and subsequent cleavage of the hydrazone moiety resulted in a variety of diastereomerically and enantiomerically pure *syn*-aldol products (*S,S*)-**125** for the first time (**Scheme 1.32**). The relative and absolute configurations were determined by X-ray structure analysis.[157]



Scheme 1.32 Synthesis of enantiomerically pure *syn*-aldol adducts.

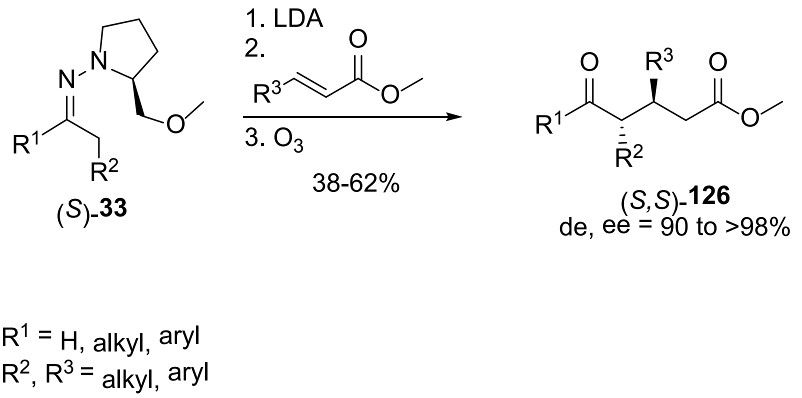
Other SAMP-analogues have been successfully utilised in aldol reactions. When SAEP (*S*)-**27** was used as the chiral auxiliary, the final aldol products were obtained in high enantiomeric purity; γ-hydroxy-α-ketoesters were obtained with >98% ee[158] and the biologically important isotetronic acid derivatives were provided in >96% ee.[159]

The use of titanated azaenolates in SAMP-hydrazone aldol reactions has provided excellent results. β-hydroxyhydrazones are formed as single *syn*-diastereomers, resulting in β-hydroxyketones in up to quantitative yields and with excellent de and ee values.[31,160]

1.6 SAMP/RAMP-methodology in Michael reactions

The Michael reaction is another important carbon-carbon bond forming reaction in organic chemistry.[161,162] This is a conjugate addition reaction between a nucleophilic enolate ion and an α,β-unsaturated carbonyl compound. The best Michael reactions are those that take place when a particularly stable enolate ion, such as that derived from a β-keto ester or other 1,3-dicarbonyl compound adds to an unhindered α,β-unsaturated ketone.[161] The asymmetric version of this reaction is of particular importance and various methods are available to carry out this reaction in an asymmetric fashion.[163,164]

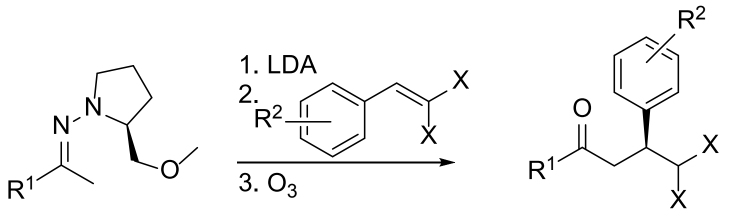
The asymmetric Michael reaction has been extensively investigated using SAMP/RAMP-methodology. The first reported reaction involved the addition of SAMP-hydrazones to α,β-unsaturated enoates (**Scheme 1.33**).[165,166] SAMP-hydrazone (*S*)-**33** is deprotonated with LDA, reacted with a suitable Michael acceptor to provide the corresponding 1,4-adducts, which are then cleaved using ozone to provide carbonyl compounds (*S,S*)-**126** in 38-62% overall yield with high diastereo- and enantiomeric excesses. This methodology has been successfully utilised in the synthesis of serine protease inhibitors by the formation of acyl enzyme complexes with α-chymotrypsin,[167,168] as well as in the enantioselective synthesis of pheromones of the small forest ant *Formica polyctena* and the red wood ant *F. rufa*.[169]



Scheme 1.33 Asymmetric Michael additions *via* SAMP-hydrazones.

A further study into this reaction proved that other Michael acceptors including 2-benzylidenemalonates and 2-benzylidenemalononitriles could be successfully utilised (**Scheme 1.34**).[170] SAMP-hydrazones (*S*)-**117** is transformed to the 2-substituted 4-oxo-diesters and dinitriles (*R*)-**127** after oxidative cleavage of the 1,4-adducts by ozonolysis in good overall yields of 50-82% and high enantioselectivities.

Numerous other Michael acceptors are tolerated under the reaction conditions.[171-173,31] The reaction has also been successful with cyclic SAMP/RAMP-hydrazones, with resultant ketones being obtained in high yields and selectivities.[174-176] A recent publication by Sammet *et al.*[177] provides an insight into the use of both acyclic and cyclic SAMP/RAMP-hydrazones as a traceless auxiliary in the asymmetric 1,4-addition of cuprates to enones.[177]



(*S*)-**117**

(*R*)-**127**
50-82%
ee = >95%

R^1 = Me, Et, Ph
R^2 = H, 3-OMe, 4-OBn, 4-OMe
X = CO_2CH_3, CN

Scheme 1.34 Asymmetric Michael additions using 2-benzylidenemalonates and 2-benzylidenemalononitriles as acceptors.

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Chapter 2

Investigation of a novel diamine based chiral auxiliary

Results and Discussion

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2.1 Background to project

The α-alkylation of ketones is a fundamental reaction in organic synthesis. However, there are few methodologies available to carry out this transformation in an asymmetric manner. The use of SAMP/RAMP-methodology almost exclusively accounts for these transformations. There remains significant scope for the exploration of new, easily prepared chiral auxiliaries for use in the synthesis of chiral α-alkylated ketones.

The aim of this project was to investigate if a nitrogen (as part of a pyrrolidine system) could ligate to lithium as effectively as in the SAMP/RAMP system (where a methoxy group is utilised) and whether alkylation with benzyl based alkylating reagents, which is rarely reported in this area, would provide the desired products in good selectivity.

In the following section, the chromatography-free synthesis of a novel chiral auxiliary incorporating a pyrrolidine ring is reported. Previous work within the group had established this novel chiral auxiliary as being a viable alternative to SAMP, however it was thought that improvements to the synthesis could be made, as well as attempting to improve both the low yields and very limited substrate scope previously investigated. The chiral hydrazine is available in five steps from *N*-protected proline or only two steps from commercially available (*S*)-(+)-1-(2-(pyrrolidinylmethyl)pyrrolidine). Subsequent reaction with symmetrical and unsymmetrical ketones followed by deprotonation, alkylation (using both alkyl and benzyl electrophiles) and hydrolysis gave valuable chiral ketones in very good enantiomeric excess and moderate yields.

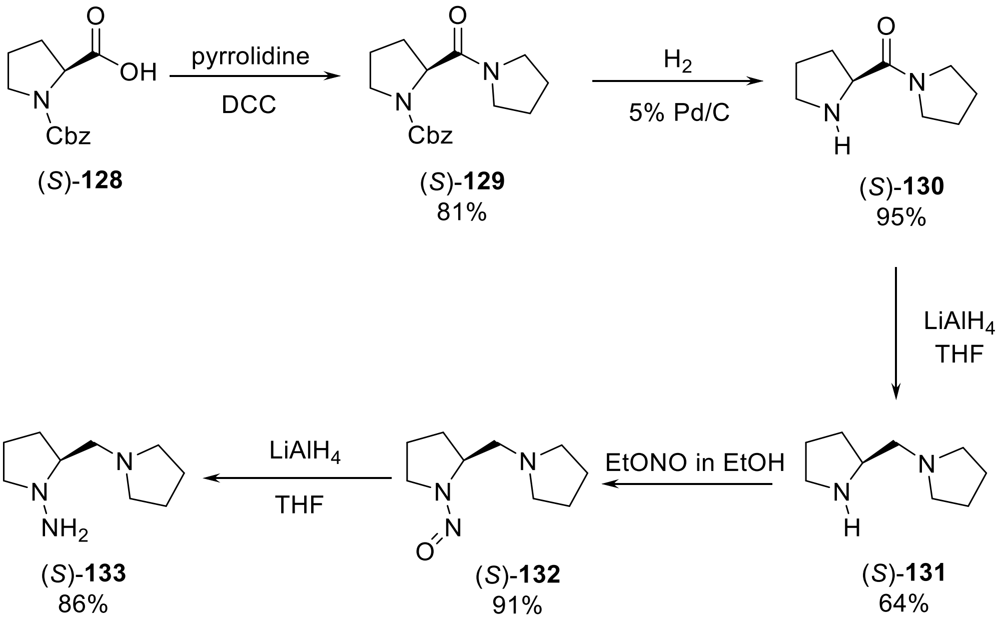
The chiral auxiliary also showed potential in both aldol and Michael reactions.

The diamine synthesised as an intermediate en route to the chiral auxiliary and chiral diamine sparteine were also investigated as chiral ligands in α-alkylations of a carboxylic acid.

2.2 Synthesis of chiral auxiliary

The novel chiral auxiliary had previously been synthesised within the group, however yields in many of the steps were moderate to low and problematic.[1] With this in mind, it was postulated that an improved synthetic methodology could be used to afford the desired chiral product.

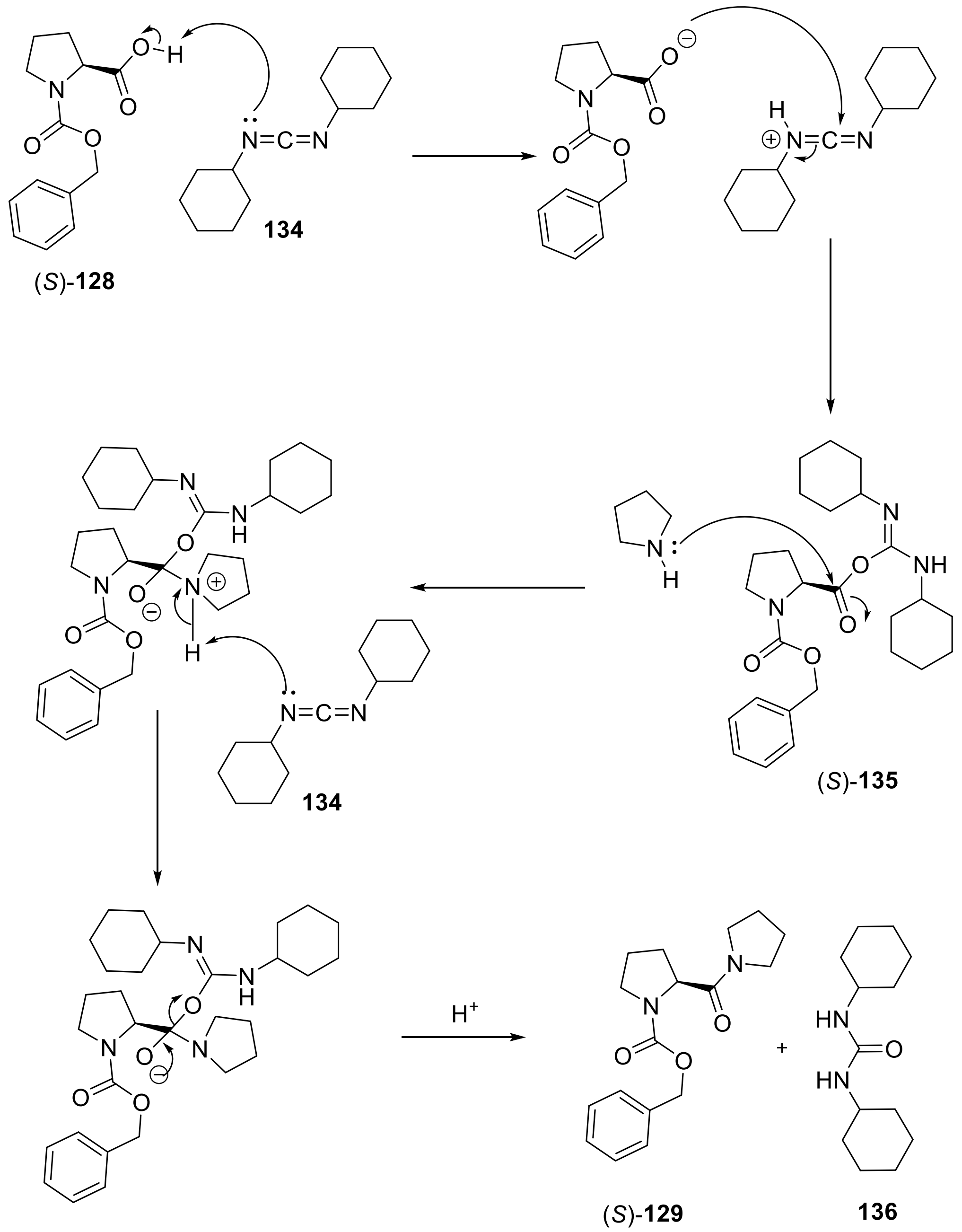
The novel chiral auxiliary is formed in a five step, chromatography-free sequence (**Scheme 2.1**) starting with a *N*,*N*'-dicyclohexylcarbodiimide (DCC) coupling reaction of commercially available (*S*)-*N*-(benzyloxycarbonyl)proline (*S*)-**128** with pyrrolidine to afford amide (*S*)-**129** in 81% yield.[2] Subsequent hydrogenolysis allows clean removal of the carboxybenzyl protecting group yielding the free amine (*S*)-**130** in 95% yield with no further purification required as the by-products (toluene and carbon dioxide) are removed *in vacuo*.[2] A lithium aluminium hydride reduction affords (*S*)-**131** in 64% yield, followed by nitrosation using ethyl nitrite to yield nitrosamine (*S*)-**132** in 91% yield.[3] A final lithium aluminium hydride reduction yields the novel chiral auxiliary, hydrazine (*S*)-**133** in 86% yield.



Scheme 2.1 Synthesis of novel chiral auxiliary.

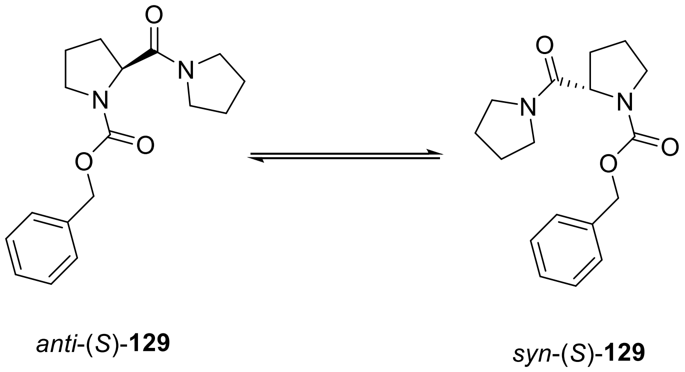
The mechanism for formation of (*S*)-**129** (**Scheme 2.2**) begins with the lone pair of electrons on one of the amine nitrogens in DCC **134** being used to deprotonate the carboxylic acid of (*S*)-**128** to yield the carboxylate. The protonated

DCC now acts as an electrophile and the negative charge on the oxygen of the carboxylate attacks the carbon of the central bond of the protonated DCC, yielding a good leaving group. The lone pair on the nitrogen of pyrrolidine can then attack the carbon of the carbonyl bond of (*S*)-**135**, forming a tetrahedral intermediate which collapses to yield the desired amide (*S*)-**129** and side product, *N*,*N*'-dicyclohexylurea **136** which is removed by filtration of the reaction mixture over Celite®. The filtrate is washed with 0.5M HCl, saturated aq. $NaHCO_3$, H_2O and brine to provide the crude product, which is purified by recrystallisation from ethyl acetate to provide (*S*)-**129** as a white solid.



Scheme 2.2 Mechanism of formation of (*S*)-**129**.

The Cbz-protected product (*S*)-**129** was shown to exist as a mixture of *syn*- and *anti*-rotamers by NMR spectroscopy (**Scheme 2.3**), due to the specific character of the carbamate N-C bond.[4-6] There is precedence in the literature for the free rotation of carbamate bonds of proline derivatives at physiological temperatures.[7] The lone pair of electrons on the nitrogen atoms are tied up in conjugation with the carbonyl group, meaning the carbamate N-C bond has partial single and double bond property, hence the observation of rotamers (two sets of signals) in NMR spectra, most notable in ^{13}C NMR spectrum (**Figure 2.1**). Mechanistic studies would be required to determine which rotamer is most stable.


O
N
N
O
O
anti-(S)-129
O
O
N
N
O
O
syn-(S)-129

Scheme 2.3 Depiction of *syn*- and *anti*-(*S*)-**129** rotamers.

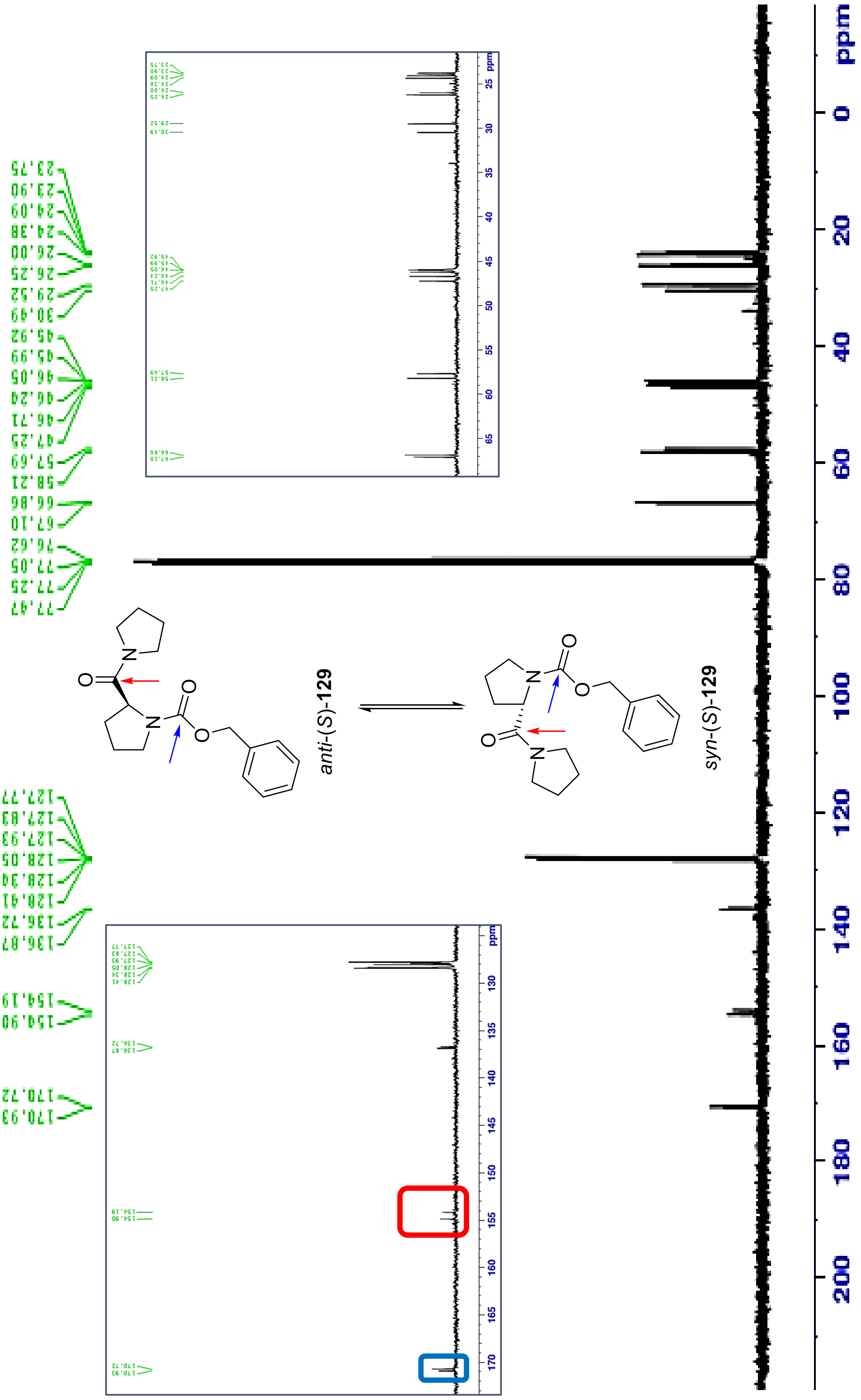
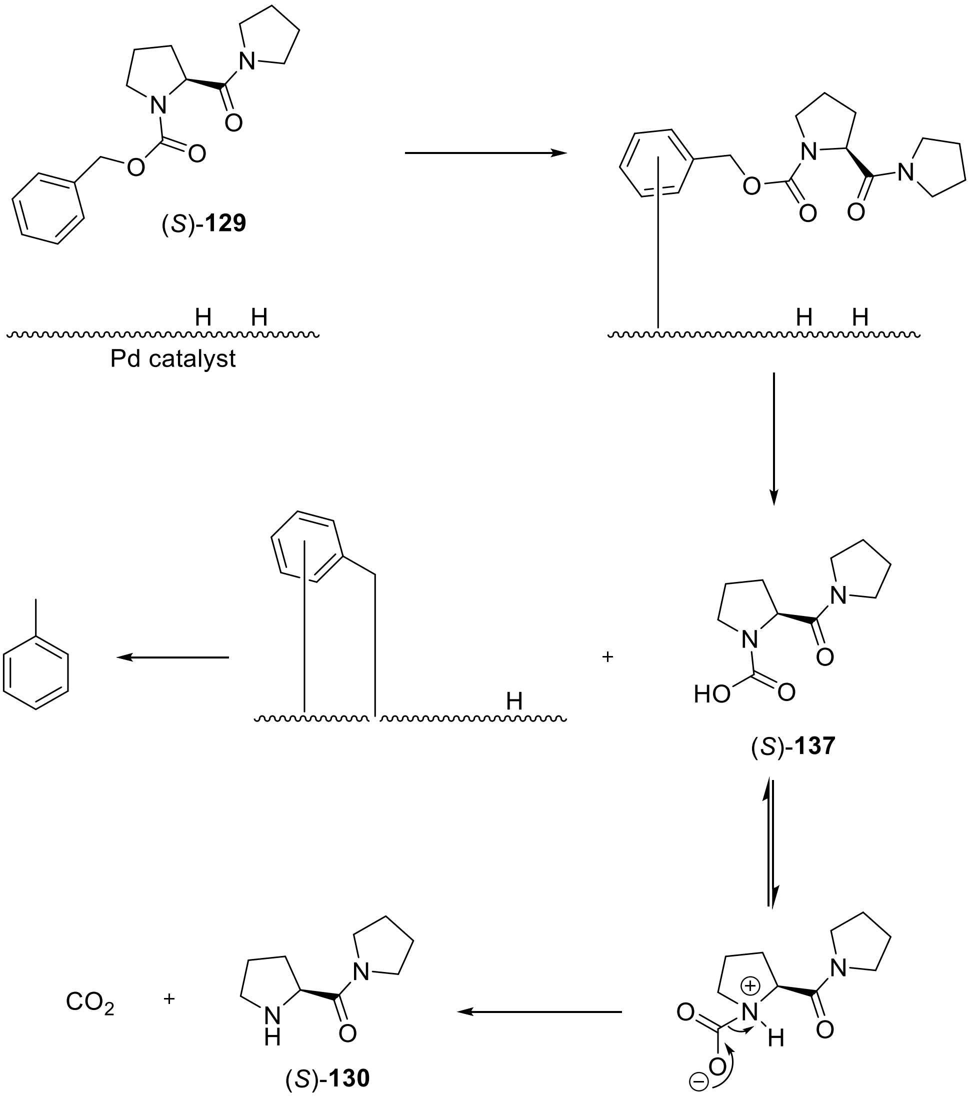


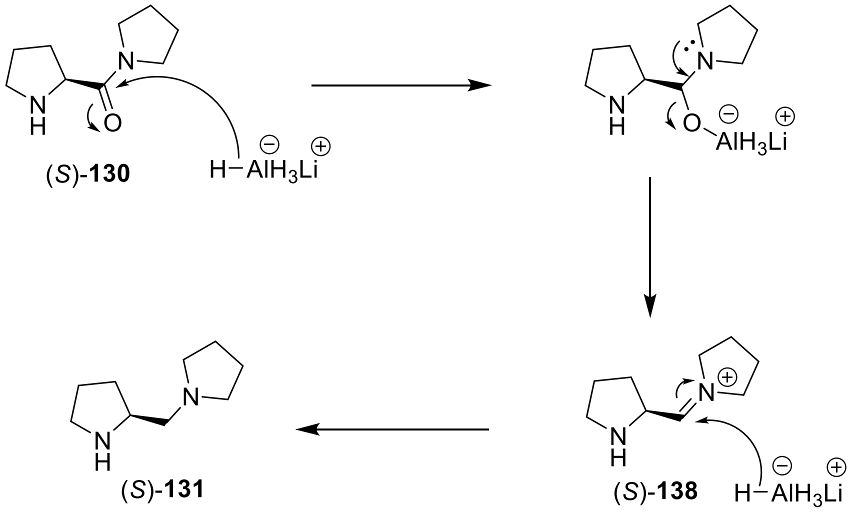
Figure 2.1 ^{13}C NMR spectrum of (*S*)-**129** with magnification of peaks and examples of peak doubling due to rotamers highlighted ($CDCl_3$, 75 MHz).

Removal of the carboxybenzyl group is achieved *via* hydrogenolysis which undergoes the following series of events (**Scheme 2.4**).[8] Cbz-protected amide (*S*)-**129** coordinates to the palladium catalyst *via* the electron-rich aromatic ring. This brings the benzylic C-O bond in close proximity to the palladium-bound hydrogen atoms and it is reduced, resulting in the carboxylic acid (*S*)-**137** and toluene. The acid is in equilibrium with its ionised form, which collapses to give (*S*)-**130** and carbon dioxide. Because of the need for initial coordination with the catalyst, only benzylic or allylic carbon-heteroatom bonds can be reduced. (*S*)-**130** was isolated as a yellow oil with no purification required.



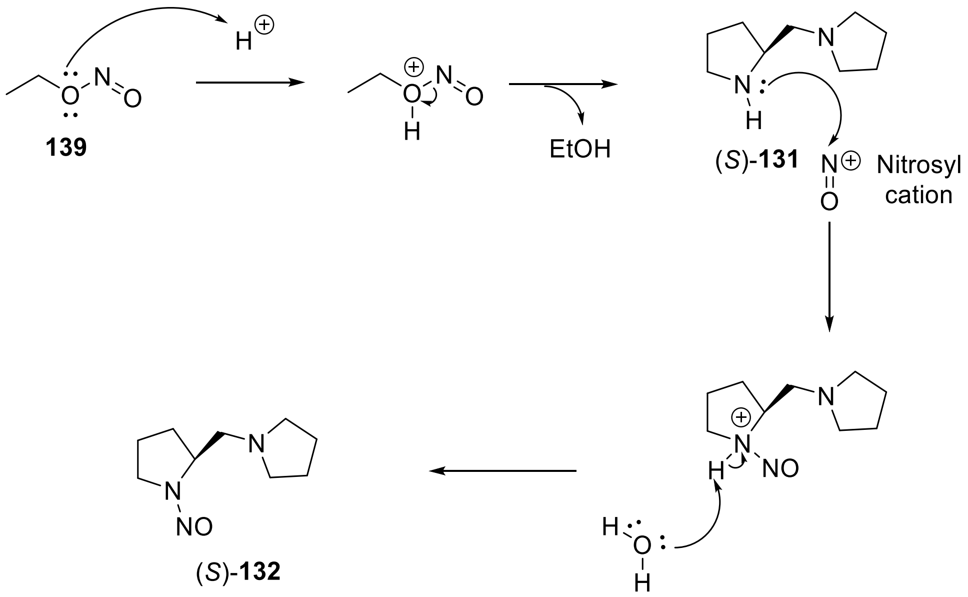
Scheme 2.4 Removal of protecting group by hydrogenolysis.

The third step in the synthesis of the chiral auxiliary is a standard lithium aluminium hydride reduction of an amide (*S*)-**130** to give amine (*S*)-**131** (**Scheme 2.5**). Lithium aluminium hydride acts as a source of "H^-", adding to the carbon of the carbonyl group in (*S*)-**130** to form a tetrahedral intermediate, which then collapses to form the iminium ion (*S*)-**138**. (*S*)-**138** is more electrophilic than (*S*)-**130**, so it reacts with a further equivalent of $LiAlH_4$ to be reduced to secondary amine (*S*)-**131**, which was purified by Kugelrohr distillation to yield the pure product as a colourless oil.



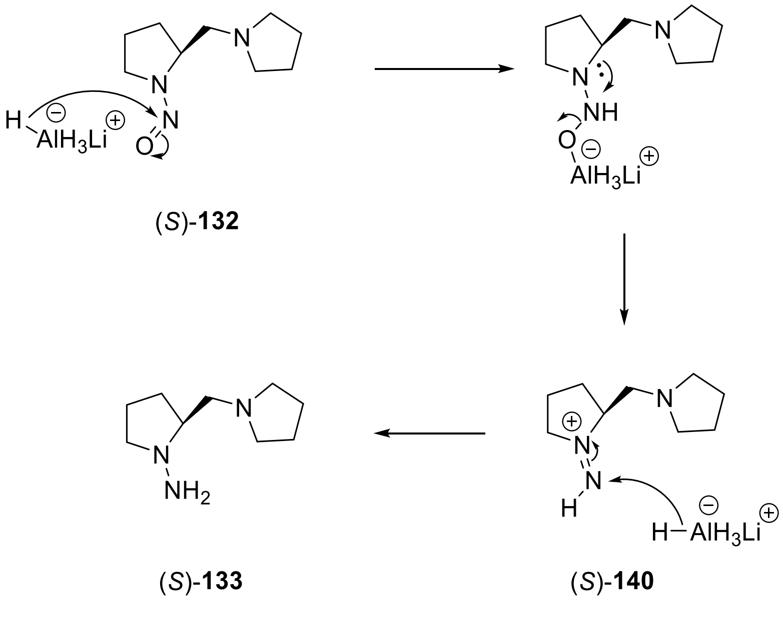
Scheme 2.5 $LiAlH_4$ reduction of amide (*S*)-**130**.

Amine (*S*)-**131** is then reacted with ethyl nitrite (supplied as a 10–20% solution in ethanol) to form nitrosamine (*S*)-**132** (**Scheme 2.6**). Ethyl nitrite **139** is converted to a nitrosyl cation, which is then able to pick up the lone pair of electrons on the secondary amine of (*S*)-**131**, forming a new N-N bond. Abstraction of the proton provided the target compound (*S*)-**132**. Caution was exercised with (*S*)-**132** due to the potential toxicity of the compound.[9-11] No purification was carried out and only ^1H and ^{13}C NMR data were obtained.


H⊕
139
EtOH
(*S*)-131
Nitrosyl cation
NO
(*S*)-132

Scheme 2.6 Nitrosation of amine (*S*)-**131**.

A lithium aluminium hydride reduction is carried out to convert nitrosamine (*S*)-**132** to the hydrazine chiral auxiliary (*S*)-**133** *via* a tetrahedral intermediate which collapses to give the azo compound (*S*)-**140** (**Scheme 2.7**).

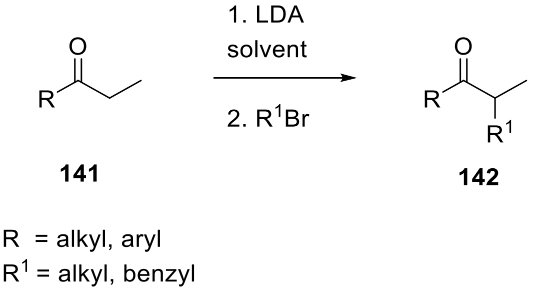

AlH3Li
(*S*)-132
NH
NH2
H−AlH3Li
(*S*)-133
(*S*)-140

Scheme 2.7 $LiAlH_4$ reduction of (*S*)-**132** to hydrazine.

2.3 Synthesis of racemic ketones

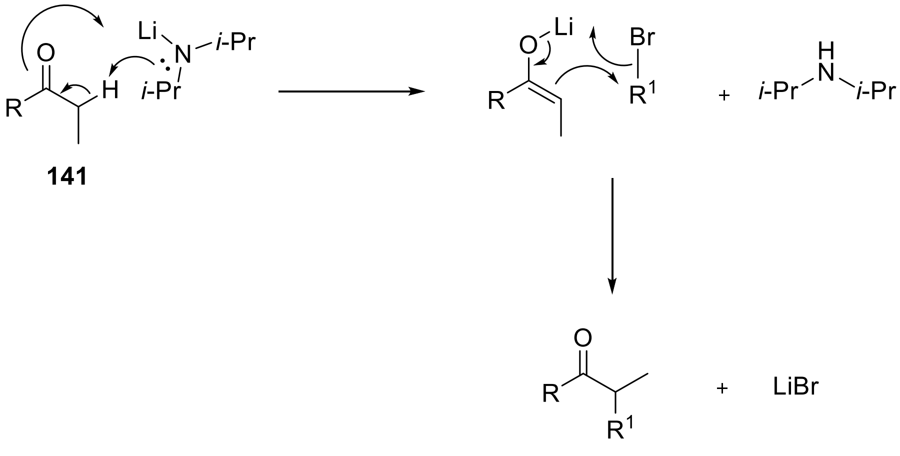
In order to be able to state enantiomeric excess of chiral compounds with certainty, it is necessary to have standards for comparison of gas chromatography (GC) traces. This can be achieved by synthesising racemic ketones and obtaining a GC trace prior to attaining the GC trace of the chiral ketone.

Racemic ketones **142** are formed by deprotonation of the desired ketone or aldehyde **141** using LDA in a suitable solvent, alkylation of the resulting enolate using the electrophile of choice and purification by silica gel column chromatography (**Scheme 2.8**). A sample of the pure ketone was then dissolved in distilled DCM to a concentration of 1 mg/mL and subjected to GC analysis using suitable conditions to allow 2 peaks of equal area to be identified.



Scheme 2.8 Formation of racemic α-alkylated ketones or aldehydes.

The reaction of lithium enolates with alkyl halides is undoubtedly one of the most important carbon-carbon bond forming reactions in organic chemistry.[12,13] The reaction consists of two steps. The first step involves formation of the enolate by deprotonation with base. A sufficiently strong base is required to ensure complete deprotonation of the enolisable proton at the α-position of ketones and aldehydes. Deprotonation occurs in a cyclic mechanism (**Scheme 2.9**), with the basic nitrogen atom of LDA removing the proton of **141** as the lithium is delivered to produce the enolate. In the subsequent step, the alkylating agent is added dropwise and adds to the enolate to provide **142** in an S_N2 fashion.[12] The reaction conditions employed in the synthesis of racemic ketones was that typical of enolate formation – deprotonation was carried out by addition of LDA dropwise at -78 °C in anhydrous THF. The alkylating agent is added dropwise, also at low temperature (-78 °C), as the lithium enolates may not be stable at higher temperatures. After allowing the reaction to stir for 30 min at this temperature, the reaction is allowed to warm to room temperature to increase the rate of reaction of the S_N2 alkylation. The low yields observed in this type of reaction may be due to self-condensation of the enolate, which may occur while the enolate is still forming.



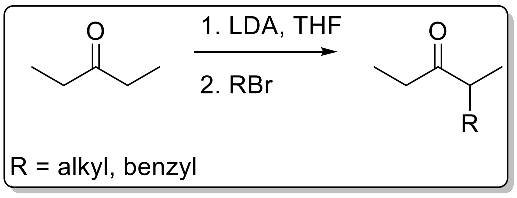
141

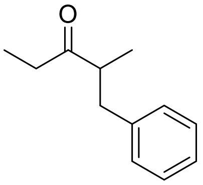
142

Scheme 2.9 Mechanism of deprotonation of ketones and aldehydes.

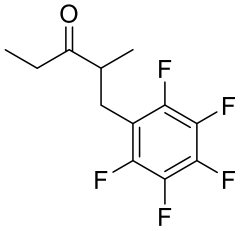
A number of racemic ketones were synthesised for this project based on either 3-pentanone (**Figure 2.2**) or propiophenone (**Figure 2.5**). A wide variety of electrophiles was selected to investigate if electronic effects had any effect on the observed yields of the reaction.

Yields for benzylation of 3-pentanone (**Figure 2.2**) varied from 12-55%, with no obvious correlation between the electrophile used and the yield of ketone obtained. Spectral characteristics of ketones **143**, **145**, **146** and **148** corresponded with that previously reported in the literature, with the structure of novel ketones **144** and **147** confirmed by spectral analysis. Elemental analysis of **147** provided additional structural confirmation. Allylation of 3-pentanone provided product ketones **149-151** in low to moderate yields, with no trend apparent between electrophile utilised and yield obtained (**Figure 2.2**). Spectral characteristics of **149** were consistent with that previously reported in the literature. The structure of novel allylated ketones **150** and **151** was confirmed by full experimental analysis.

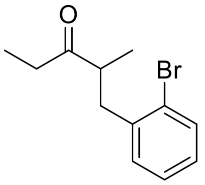

O
1. LDA, THF
2. RBr
O
R
R = alkyl, benzyl



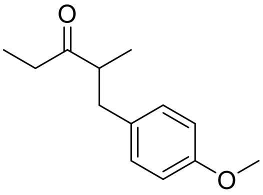
143
31%



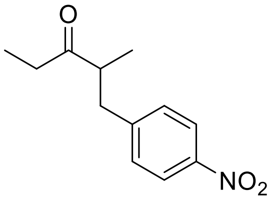
144
38%



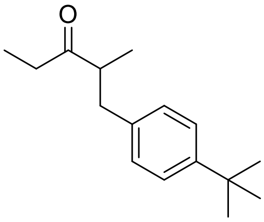
145
55%



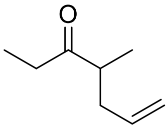
146
35%



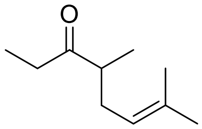
147
28%



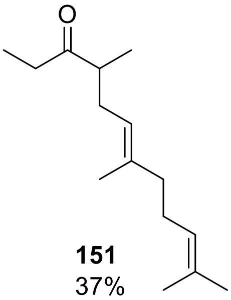
148
12%



149
38%



150
25%



151
37%

Figure 2.2 Racemic α-alkylated and α-benzylated 3-pentanone based ketones.

The ^{13}C NMR spectrum of **144** was particularly interesting due to the presence of five fluorine atoms on the benzyl ring (**Figure 2.3**).

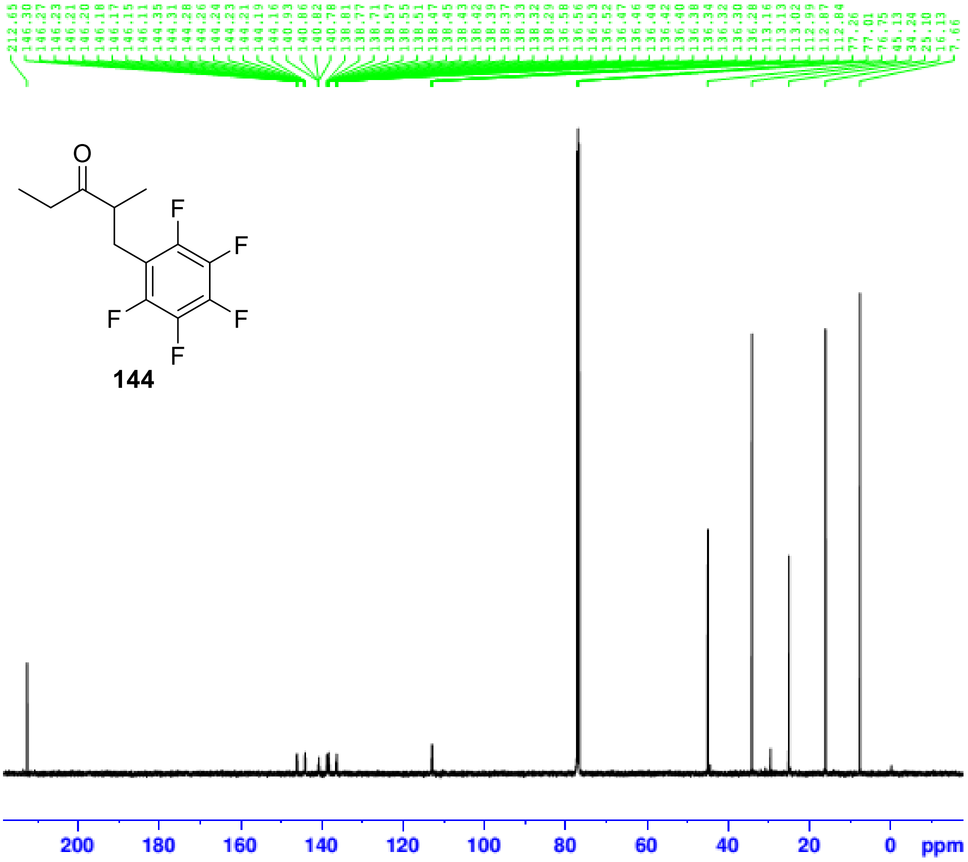


Figure 2.3 ^{13}C NMR spectrum of 2-methyl-1-(perfluorophenyl)pentan-3-one **144**.

On closer examination of the aromatic region (**Figure 2.4**), it was apparent that the quaternary carbon of the benzyl ring appeared as an apparent triplet of doublets at 113.0 ppm, with splitting caused by the neighbouring fluorine atoms. Similarly, a doublet of triplet of triplets is observed at 139.8 ppm, corresponding to the carbon atoms α- to the quaternary carbon of the benzyl ring, with the splitting due to the multiple neighbouring fluorine atoms. The other carbon atoms of the benzyl ring appeared as doublets of multiplets, with the coupling constants of the doublets around 250 Hz, typical of that observed for a carbon bonded to a fluorine atom. The observation of multiplets for these peaks is as expected as it is known that ^{19}F has the property of coupling across several bonds, even in saturated systems.[14] The ^{19}F NMR spectrum of **144** consisted of 3 peaks with the splitting patterns observed as expected.

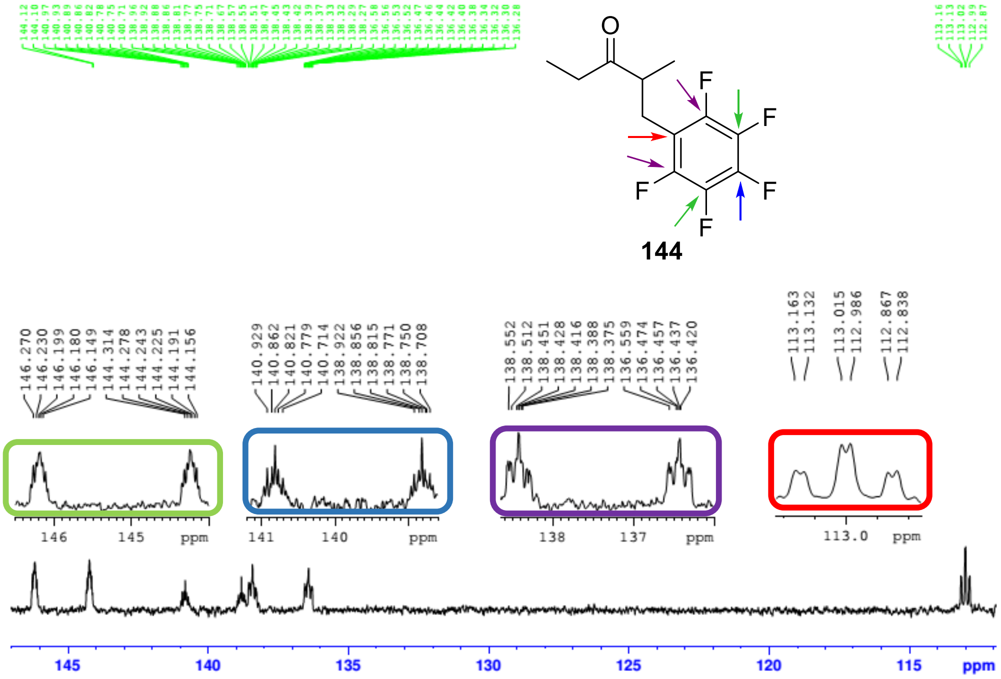
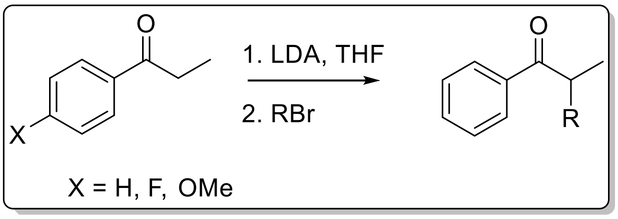
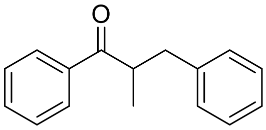


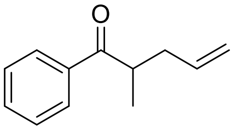
Figure 2.4 Magnified aromatic region of ^{13}C NMR spectrum of 2-methyl-1-(perfluorophenyl)pentan-3-one **144**.

Racemic α-alkylated and α-benzylated propiophenone-based ketones were synthesised as these were to be investigated using the chiral auxiliary method described in Chapter 1. Ketones **152-155** were synthesised in the same manner as the 3-pentanone based ketones, with deprotonation achieved with LDA in THF and alkylation with either benzyl bromide to provide **152** or allyl bromide to provide **153-155** (**Figure 2.5**). Spectral characteristics for **152-154** were consistent with previously reported data. The structure of novel alkylated ketone **155** was confirmed by full experimental analysis. The presence of a fluorine atom on the phenyl ring of **155** accounted for the doublets observed in the ^{13}C NMR spectrum. Yields of **152-155** are low, with the exception of **155** which was obtained in a moderate yield.

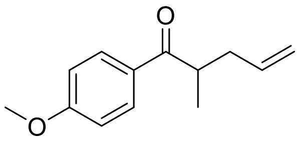




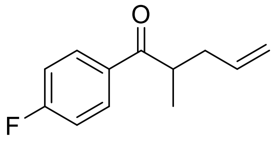
152
17%



153
22%



154
26%



155
52%

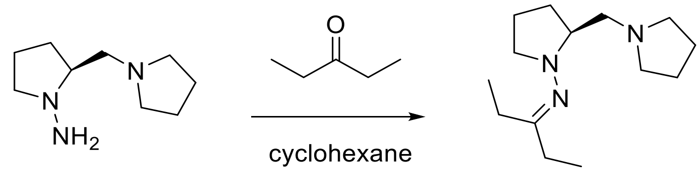
Figure 2.5 Racemic α-alkylated and α-benzylated propiophenone based ketones.

Racemic ketones were formed in low to moderate yields. In many cases, the product ketone has a small molecular weight and is volatile, so mass may have been lost whilst removing solvent *in vacuo* (a notable decrease in mass was observed that upon lengthy rotary evaporation).

2.4 Synthesis of chiral hydrazones

Chiral auxiliary (*S*)-**133** can be combined with either a ketone or an aldehyde to form the desired chiral hydrazone. Two ketones were used in this investigation. 3-Pentanone was chosen as an alkyl ketone for its symmetry to avoid the possible problems of regioselectivity and propiophenone (substituted and unsubstituted) as an aryl ketone with only one enolisable proton.

3-Pentanone hydrazone (*S*)-**156** is easily formed in 80% yield (46% yield after purification by Kugelrohr distillation) by reaction of 3-pentanone with chiral auxiliary (*S*)-**133** in cyclohexane at room temperature (**Scheme 2.10**), which is in contrast with formation of SAMP hydrazones which requires heating for the reaction to go to completion.[15]

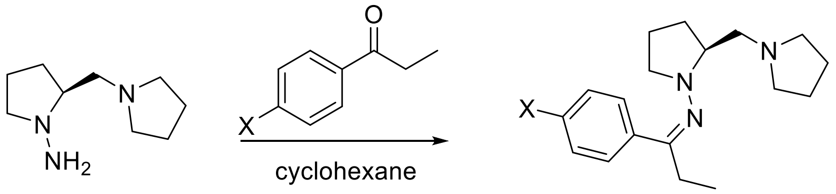


(*S*)-**133**

(*S*)-**156**
80%
(46% after purification by distillation)

Scheme 2.10 Synthesis of 3-pentanone hydrazone (*S*)-**156**.

In a similar manner, (*S*)-**133** is combined with propiophenone, *p*-methoxypropiophenone and *p*-fluoropropiophenone to afford hydrazones (*S*)-**157**, (*S*)-**158** and (*S*)-**159** in 52%, 54% and 48% yields respectively (**Scheme 2.11**). Purification of (*S*)-**157** was achieved by Kugelrohr distillation, whilst (*S*)-**158** and (*S*)-**159** were purified using silica gel column chromatography. It was postulated that the boiling point of (*S*)-**158** and (*S*)-**159** would be high, even under the vacuum conditions used in the Kugelrohr distillation. It was therefore decided to purify these compounds using silica column chromatography rather than expose them to high temperatures as a precaution against possible degradation.

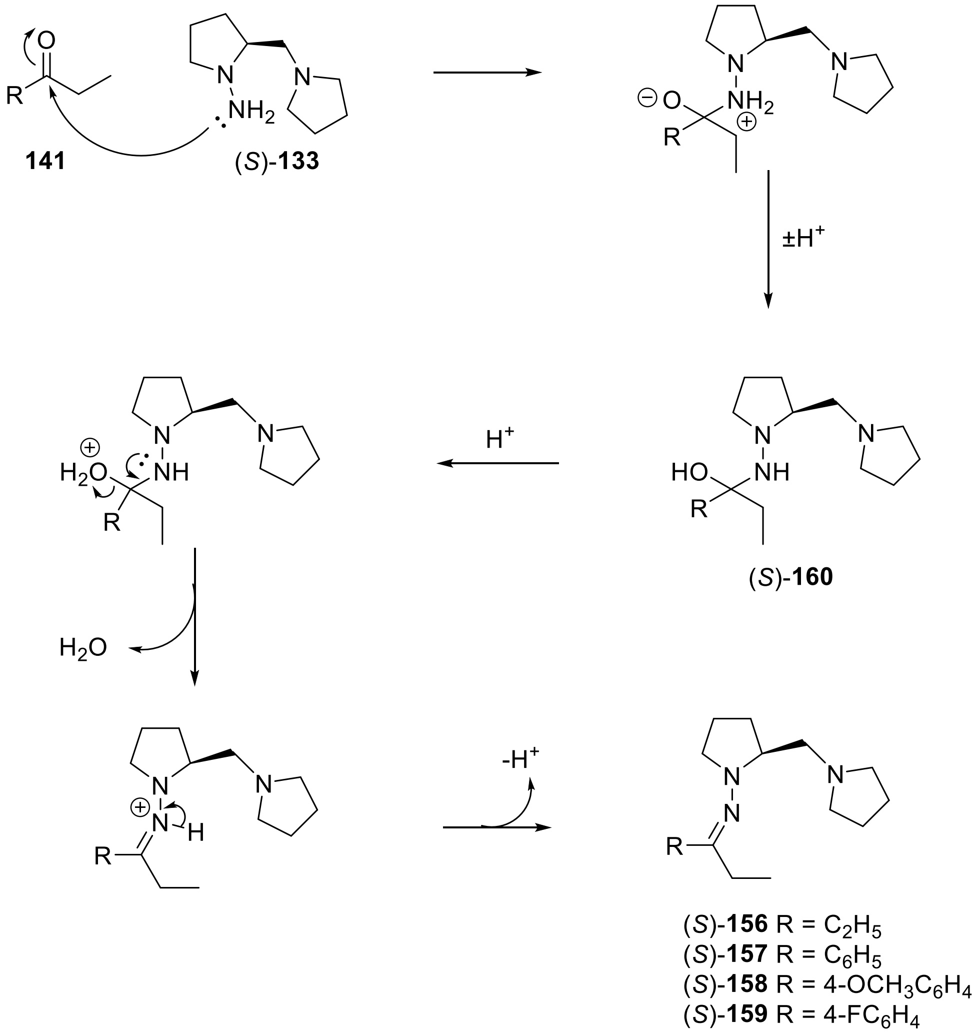


(*S*)-**133**

(*S*)-**157**
X = H, 62% (52% after purification)
(*S*)-**158**
X = OMe, 87% (54% after purification)
(*S*)-**159**
X = F, 80% (48% after purification)

Scheme 2.11 Synthesis of propiophenone-based hydrazones (*S*)-**157**-**159**.

The mechanism for hydrazone formation is analogous to that for imine formation. The lone pair on the nitrogen of the hydrazine chiral auxiliary (*S*)-**133** attacks the electrophilic carbon of the ketone or aldehyde **141**, pushing electrons from the double bond onto the oxygen. The now negatively charged oxygen picks up a proton from the positively charged nitrogen to provide tetrahedral intermediate (*S*)-**160**. The hydroxyl group picks up a proton to form water which acts a good leaving group, driving the formation of the carbon-nitrogen double bond. Loss of a proton neutralises the compound to provide (*S*)-**156**-**159** with concomitant loss of water (**Scheme 2.12**).



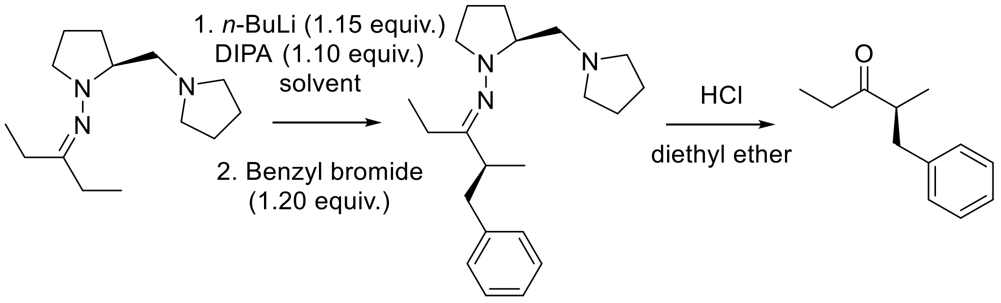
Scheme 2.12 Mechanism of formation of hydrazones.

2.5 Synthesis of chiral ketones

Chiral ketones are synthesised by deprotonation of the desired chiral hydrazone, alkylation with a suitable electrophile and cleavage of the hydrazone to the parent ketone using a suitable cleavage method.

The first step in this investigation was to perform a solvent screen to ascertain which solvent gave the best enantioselectivity for the alkylation of the chiral hydrazone. The alkylation of 3-pentanone hydrazone (*S*)-**156** was carried out using benzyl bromide as the electrophile in three different solvents (**Table 2.1**) to provide alkylated chiral hydrazone (*S*)-**161**. Cleavage was implemented using a biphasic HCl/diethyl ether system to provide desired chiral ketone (*S*)-**143**. The highest enantioselectivity of (*S*)-**143** was observed when diethyl ether was the solvent used in the reaction (**Table 2.1**, **entry 1**) and so deemed to be the solvent of choice for the rest of the investigation. This was as expected, as SAMP-hydrazone methodologies also usually involve diethyl ether as solvent.[15-17]

Table 2.1 Solvent Screen for alkylation of hydrazone.



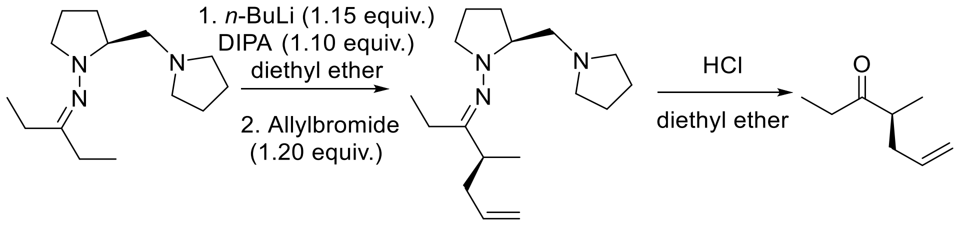
(*S*)-**156** (*S*)-**161** (*S*)-**143**

Entry	Solvent	Yield (*S*)-143 (%)[a]	ee (*S*)-143 (%)[b]
1	Diethyl ether	10	89
2	THF	12	66
3	Toluene	15	61

[a] Isolated yields quoted over 2 steps; [b] as determined by chiral GC analysis.

Next, it was necessary to establish how many equivalents of LDA would be required and at what temperature the deprotonation step was most successful. A range of equivalents of LDA and temperatures were chosen (**Table 2.2**) for the alkylation of 3-pentanone hydrazone (*S*)-**156** with allyl bromide.

Table 2.2 Investigation into deprotonation conditions required for alkylation.



(*S*)-**156** (*S*)-**162** (*S*)-**149**

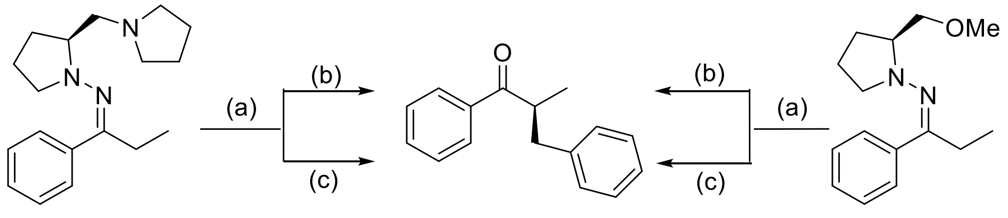
Entry	Deprotonation Conditions	LDA (equiv.)	Conversion[a] (*S*)-**156** → (*S*)-**162**
1	33 °C, 5 h	1.1	82%
2	RT, 5 h	2.1	100%
3	0 °C, 16 h	1.1	100%

[a]As determined by ^{1}H NMR.

Two of the conditions investigated achieved 100% conversion (**Table 2.2**, **Entries 2** and **3**). However, in the interest of keeping the amount of LDA used at a minimum, it was decided to proceed with deprotonation conditions using 1.1 equivalents of LDA at 0 °C for 16 h for the rest of the investigation.

Various methods for the cleavage of α-substituted hydrazones to the corresponding ketone are available.[18] Oxalic acid has been reported as a convenient, high yielding, racemisation-free method for the hydrolytic cleavage of SAMP hydrazones.[19] Two biphasic cleavage methods, oxalic acid/diethyl ether and the well-established HCl/diethyl ether, were investigated with both the novel chiral auxiliary and SAMP. Hydrazone (*S*)-**157** and the corresponding SAMP variant (*S*)-**163** were prepared and subjected to LDA and benzyl bromide (**Table 2.3**). Both alkylated hydrazones were hydrolysed using oxalic acid/diethyl ether and HCl/diethyl ether. Using the SAMP hydrazone (*S*)-**163**, benzylated propiophenone (*S*)-**152** was obtained in 92% ee and 88% ee using oxalic acid and HCl/diethyl ether cleavage methods, respectively (**Table 2.3**, **entries 3** and **4**). A larger variation in enantioselectivity was observed between the two cleavage methods when hydrazone (*S*)-**157** was employed in the reaction, with 51% and 78% ee observed when using oxalic acid and HCl/diethyl ether cleavage methods, respectively (**Table 2.3**, **entries 1** and **2**). The low yields of benzylated ketones observed using both the novel chiral auxiliary and SAMP reflect the challenges of this particular transformation.

Table 2.3 Racemisation studies of chiral hydrazones (*S*)-**157** and SAMP variant (*S*)-**163**.



(*S*)-**157** (*S*)-**152** (*S*)-**163**

Conditions: (a) 1. *n*-BuLi (1.15 equiv.)/DIPA (1.10 equiv.), diethyl ether; 2. Benzyl bromide (1.20 equiv.); (b) Oxalic acid, diethyl ether; (c) 4M HCl, diethyl ether.

Entry	**Hydrazone**	**Cleavage method**	**Yield (*S*)-152 (%)**[a]	**ee (*S*)-152 (%)**[b]
1	(*S*)-**157**	Oxalic acid, Et_2O	15	51
2	(*S*)-**157**	4M HCl, Et_2O	31	78
3	(*S*)-**163**	Oxalic acid, Et_2O	36	92
4	(*S*)-**163**	4M HCl, Et_2O	26	88

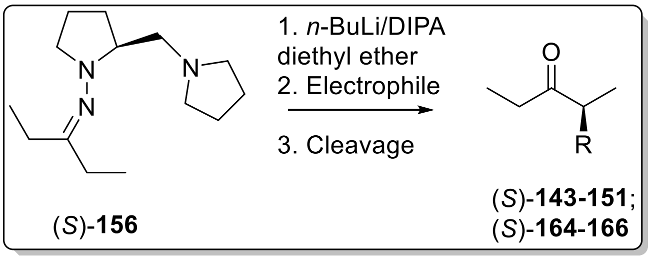
[a] Isolated yields quoted over 2 steps; [b] as determined by chiral GC analysis.

It is possible that the decrease in enantioselectivity was caused by either epimerisation of the chiral benzylated hydrazone or by racemisation of the chiral ketone itself. To investigate which of these was the case, (*S*)-**152** was exposed to the oxalic acid cleavage conditions for 28 h and subsequent enantiopurity determined (78% ee). As no change to the value of the enantiopurity (78% ee) was observed, this indicates that epimerisation of the chiral benzylated hydrazone occurs when oxalic acid is used in combination with our hydrazone. This may be due to protonation of the pyrrolidine in the chiral arm, resulting in increased solubility and exposure to the aqueous acidic layer. This result underlines the need for thorough investigation of cleavage methods in such cases.

With usable hydrolysis conditions in hand, a variety of electrophiles was reacted with the azaenolate derived from (*S*)-**156**. Cleavage of the hydrazone moiety resulted in chiral ketones with good to excellent enantioselectivity, albeit in low yield (**Figure 2.6**). In all cases, the alkylated hydrazone was not isolated.

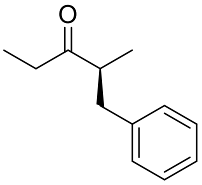
The use of benzyl bromides as electrophiles in hydrazone chiral auxiliary methodology has been very limited. In fact, no thorough investigation of benzyl based electrophiles has been reported using chiral hydrazone methodology. A plethora of electrophiles were used affording ketones (*S*)-**143**-**148** and (*S*)-**164**-**165**, all with moderate to high enantioselectivity. Substituted benzyl groups allowed investigation of the effect of electron donating and electron withdrawing groups present on the electrophile. From **Figure 2.6**, it is apparent that the presence of electron withdrawing groups on the benzyl moiety caused a decrease in enantioselectivity of the resultant ketone when compared to the unsubstituted benzyl bromide [(*S*)-**143**, 89% ee], which is most apparent with the use of perfluorobenzyl bromide [(*S*)-**144**, 48% ee] and *p*-nitrobenzyl bromide [(*S*)-**147**, 58% ee]. The presence of an electron donating group, for example the use of *p*-methoxybenzyl bromide [(*S*)-**146**, 84% ee] and *t*-butylbenzyl bromide [(*S*)-**148**, 87% ee], had little effect on the enantioselectivity observed. When 2-bromobenzyl bromide was used as the alkylating agent, the final ketone (*S*)-**145** was obtained in 86% ee. This is similar to the enantioselectivity of the ketone obtained when benzyl bromide is used as the electrophile [(*S*)-**143**], which suggests that the position of the substituent on the benzyl ring, as well as the nature of the substituent itself, is crucial to affecting the enantioselectivity. The cleavage of *p*-bromobenzylated and *p*-trifluoromethylbenzylated hydrazones was carried out using sat. aq. oxalic acid/diethyl ether prior to the discovery that this method resulted in racemisation. Thus the enantioselectivity of chiral ketones (*S*)-**164** and (*S*)-**165** obtained cannot be directly compared with that of (*S*)-**143**, however it can be speculated that the enantioselectivity obtained would be lower than that observed for (*S*)-**143** due to the presence of electron withdrawing groups at the *para*-position.

To the best of our knowledge, the enzymatic cleavage of chiral hydrazones has not been reported. Mino and co-workers[20] reported the use of porcine pancreatic lipase (PPL) as an effective method of deprotecting ketone dimethylhydrazones. The reaction was carried out on achiral substrates and achieved generally high yields. As a lipase-catalysed deprotection of chiral hydrazones had not been attempted to date, it was decided to investigate how successfully this methodology could be applied to our system. Results obtained from the cleavage of chiral benzylated 3-pentanone hydrazone gave the chiral product (*S*)-**143** in 83% ee, albeit in a low yield of *ca*. 10 % over 2 steps. Due to the increasing emphasis on green chemistry in both industry and academia, this result is promising and encouraging as a method for hydrolysis of chiral hydrazones as the reaction is carried out in a biphasic acetone/water mixture at ambient temperature.

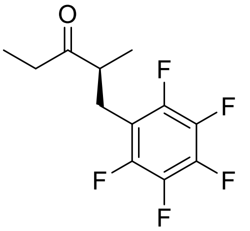

1. n-BuLi/DIPA
diethyl ether
2. Electrophile
3. Cleavage
N
N
N
O
R

(*S*)-**156**

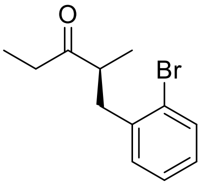
(*S*)-**143-151**;
(*S*)-**164-166**



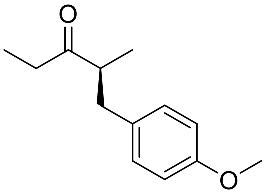
(*S*)-**143**
7%, ee = 89%^{a}
10%, ee = 83%^{b}



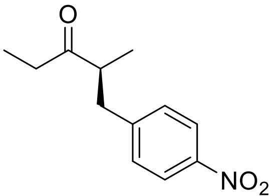
(*S*)-**144**
34%, ee = 48%^{a}



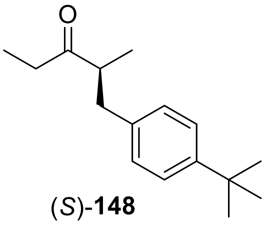
(*S*)-**145**
21%, ee = 86%^{a}



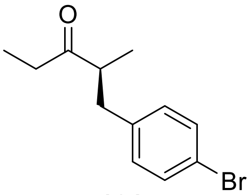
(*S*)-**146**
24%, ee = 84%^{a}



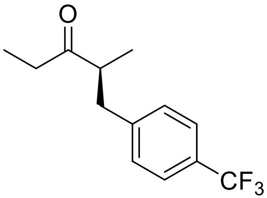
(*S*)-**147**
6%, ee = 58%^{a}



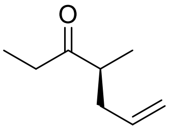
(*S*)-**148**
28%, ee = 87%^{a}



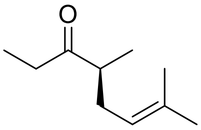
(*S*)-**164**
19%, ee = 62%^{c}



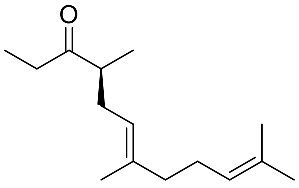
(*S*)-**165**
14%, ee = 73%^{c}



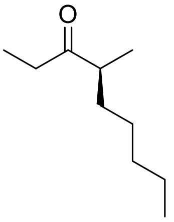
(*S*)-**149**
63%, ee = 55%^{c}
23%, ee = 90%^{a}



(*S*)-**150**
19%, ee = 89%^{a}



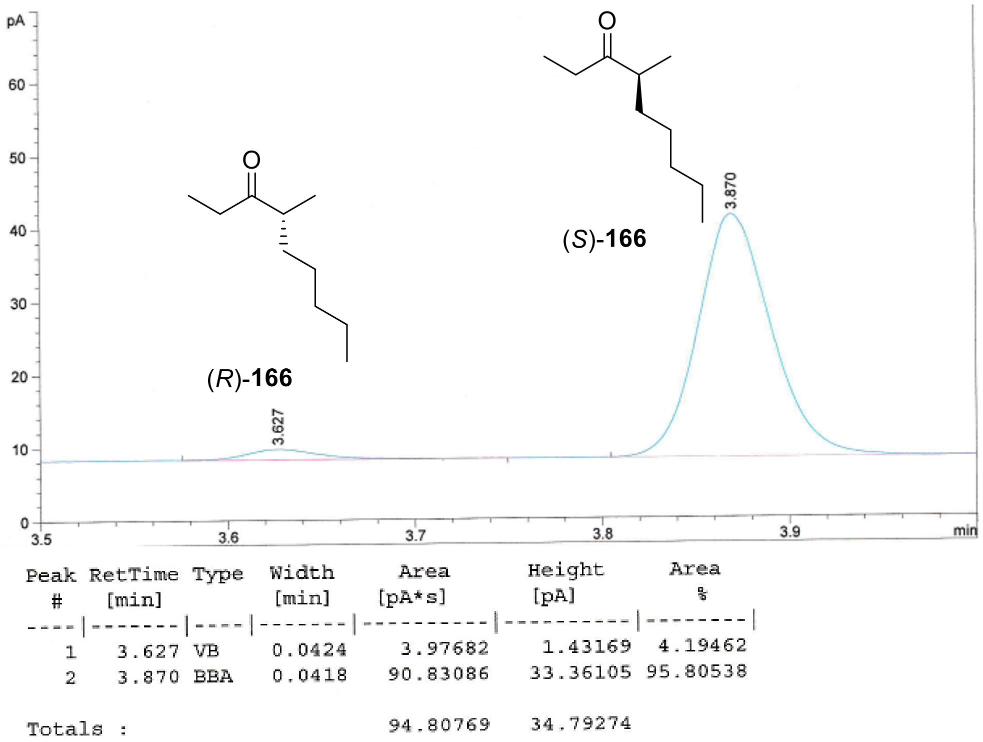
(*S*)-**151**
15%, ee = 86%^{a}



(*S*)-**166**
13%, ee = 92%^{a}
29%, ee = 82%^{a,d}

Figure 2.6 Chiral ketones synthesised using novel chiral hydrazone methodology. Yield is calculated over 2 steps – alkylated hydrazone not isolated. ^{a} HCl/Et$_2$O hydrolysis; ^{b} PPL hydrolysis; ^{c} sat. aq. oxalic acid hydrolysis; ^{d} *t*-BuLi used as base.

Reaction of (*S*)-**156** with allyl bromide, 3,3-dimethylallyl bromide and geranyl bromide provided (*S*)-**149-151** respectively in similar enantioselectivity (**Figure 2.6**), suggesting that an increase in alkyl chain length has no detrimental effect on the enantioselectivity of the product. The reaction of 3-pentanone hydrazone (*S*)-**156** with LDA and pentyliodide provided (*S*)-**166** in 92% ee (**Figure 2.7**), albeit in moderate yield. When *t*-BuLi was employed as the base instead of LDA, the selectivity dropped slightly to 82% ee, reaffirming that LDA is the optimum base for the reaction.



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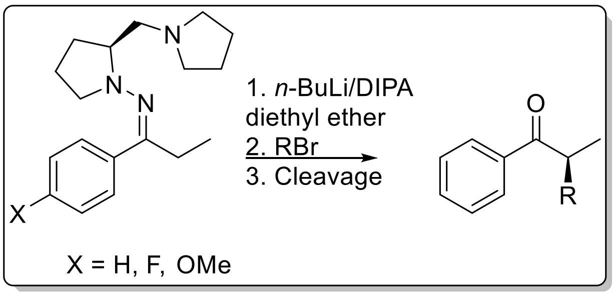
Peak RetTime Type  Width      Area       Height     Area
  #   [min]        [min]     [pA*s]       [pA]        %
----|-------|----|-------|----------|----------|--------|
   1   3.627 VB    0.0424    3.97682    1.43169  4.19462
   2   3.870 BBA   0.0418   90.83086   33.36105 95.80538

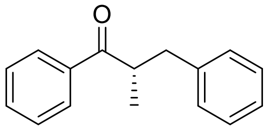
Totals :                    94.80769   34.79274
```

Figure 2.7 GC trace of product of reaction of (*S*)-**156** with *n*-pentyl iodide showing (*S*)-**166** obtained in 92% ee.

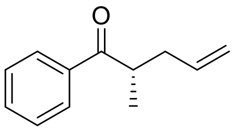
Further to these studies, it was decided to investigate the effect of electronic substituents present on the hydrazone moiety. Propiophenone, *p*-methoxypropiophenone and *p*-fluoropropiophenone hydrazones (*S*)-**157-159** were chosen as substrates and subjected to the standard conditions using allyl bromide as the electrophile. The resultant ketones (**Figure 2.8**) demonstrate that the presence of an electron donating substituent on the ring ((*S*)-**154**, 79% ee) results in a decrease in enantioselectivity when compared to the unsubstituted ketone ((*S*)-**153**, 89% ee). The presence of an electron withdrawing substituent ((*S*)-**155**, 90%

ee) had little effect on the enantioselectivity. It was also noted that an increase in enantioselectivity was observed when allylbromide was used as the electrophile ((*S*)-**153**, 89% ee) compared to when benzyl bromide was used as the electrophile ((*S*)-**152**, 78% ee), suggesting that the structure of the electrophile may have an influence on the enantioselectivity of the final product, at least when dealing with propiophenone-based hydrazones.

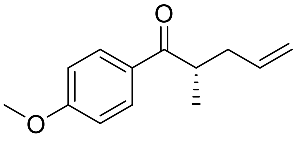




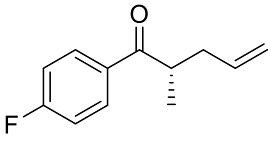
(*S*)-**152**
31%, ee = 78%[a]
15%, ee = 51%[b]



(*S*)-**153**
25%, ee = 89%[a]



(*S*)-**154**
29%, ee = 79%[a]



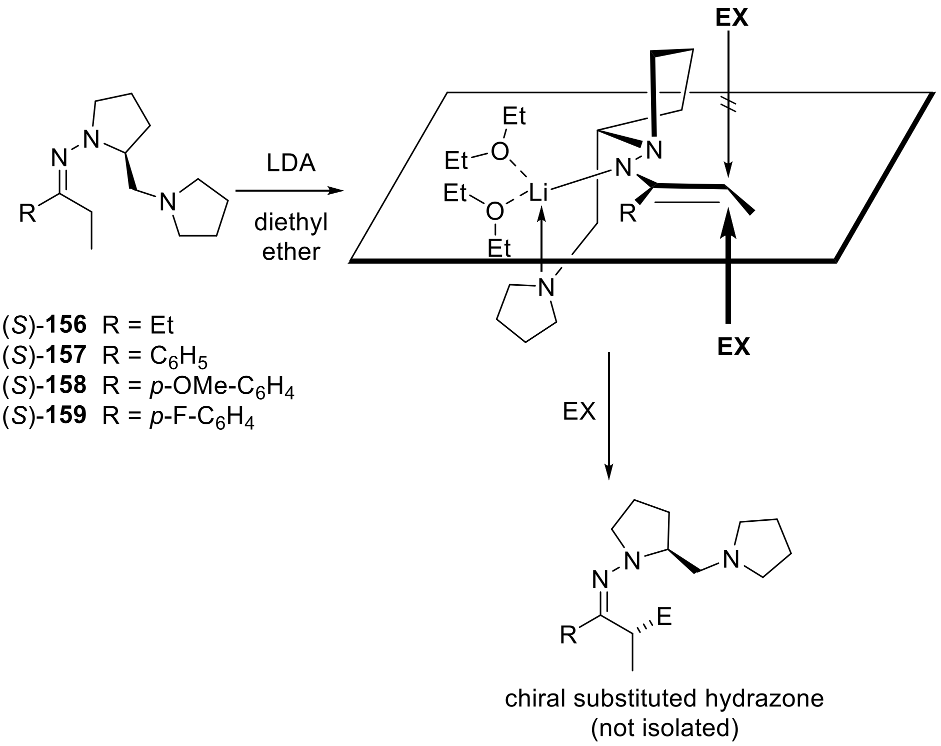
(*S*)-**155**
33%, ee = 90%[a]

Figure 2.8 Chiral ketones synthesised using novel chiral hydrazone methodology. Yield is calculated over 2 steps – alkylated hydrazone not isolated. [a] HCl/Et_2O hydrolysis; [b] sat. aq. oxalic acid hydrolysis.

The ketone products (*S*)-**143-155** and (*S*)-**164-166** have been assigned as (*S*) by comparison of the optical rotation value of chiral **153** with that reported in the literature[21] and others by analogy. All ee values were determined using chiral GC analysis and confirmed by comparison with independently prepared racemic ketones.

2.6 Origin of stereoselectivity in alkylation reactions with novel hydrazone

The selectivity of the chiral ketones obtained from the alkylation of novel hydrazones was observed to be the same as those obtained from the alkylation of SAMP hydrazones. This suggests that a similar azaenolate intermediate occurs, with an $E_{CC}Z_{CN}$ configuration resulting on deprotonation with LDA. This rigid intermediate allows attack of an electrophile to proceed under high diastereofacial differentiation, yielding highly diastereomerically enriched hydrazones and ultimately enantiomerically enriched α-substituted ketones upon cleavage of the chiral auxiliary. Attack of the electrophile from the top (*re*) face is sterically disfavoured, so preferential attack occurs from the bottom (*si*) face.



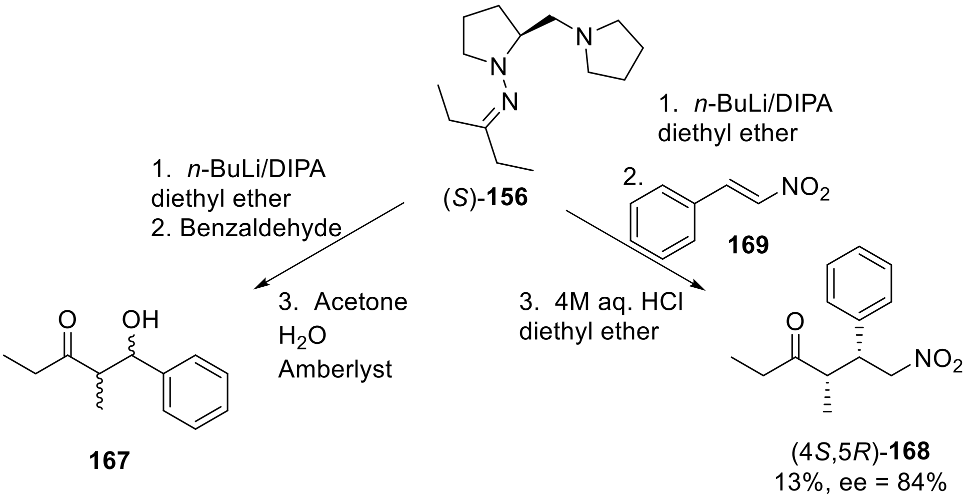
(*S*)-**156** R = Et
(*S*)-**157** R = C_6H_5
(*S*)-**158** R = *p*-OMe-C_6H_4
(*S*)-**159** R = *p*-F-C_6H_4

chiral substituted hydrazone
(not isolated)

Scheme 2.13 Origin of selectivity in novel hydrazone alkylations.

2.7 Application of chiral auxiliary to Michael and aldol reactions

Chiral auxiliaries, in particular SAMP and RAMP, have been successfully applied in both asymmetric aldol and Michael reactions.[22] With this in mind, it was postulated that the novel chiral auxiliary could also be applied to these reactions and provide similar results (**Scheme 2.14**).

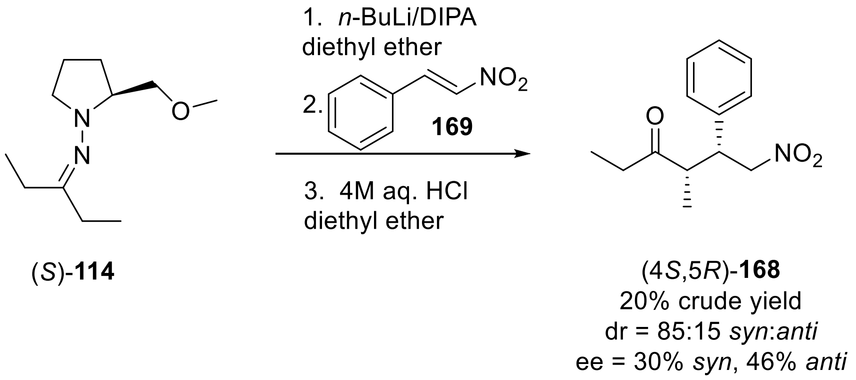


Scheme 2.14 Aldol and Michael reactions carried out using novel chiral auxiliary.

In both cases, (*S*)-**156** was treated with LDA in diethyl ether and allowed to stir at 0 °C for 16 h to ensure complete deprotonation. To furnish the aldol product **167**, a solution of benzaldehyde in diethyl ether was added dropwise to the reaction mixture. The resultant chiral hydrazone was converted to **167** by treatment with Amberlyst® 15 hydrogen form beads in a biphasic acetone/water mixture.[23,24] The crude product was subjected to chiral GC analysis and enantiomeric excesses of 63% and 15% were obtained for *anti*- and *syn*-**167** respectively. A diastereomeric ratio of 86:14 *anti*:*syn*, determined by chiral GC analysis, was identical to that observed by ^{1}H NMR spectroscopy. Purification was attempted using silica gel column chromatography, however, an inseparable mixture of diastereomers resulted. A point of note was that the relative stereochemistry observed (*anti*) was the opposite to that normally seen in aldol reactions using SAMP (*syn*).[22]

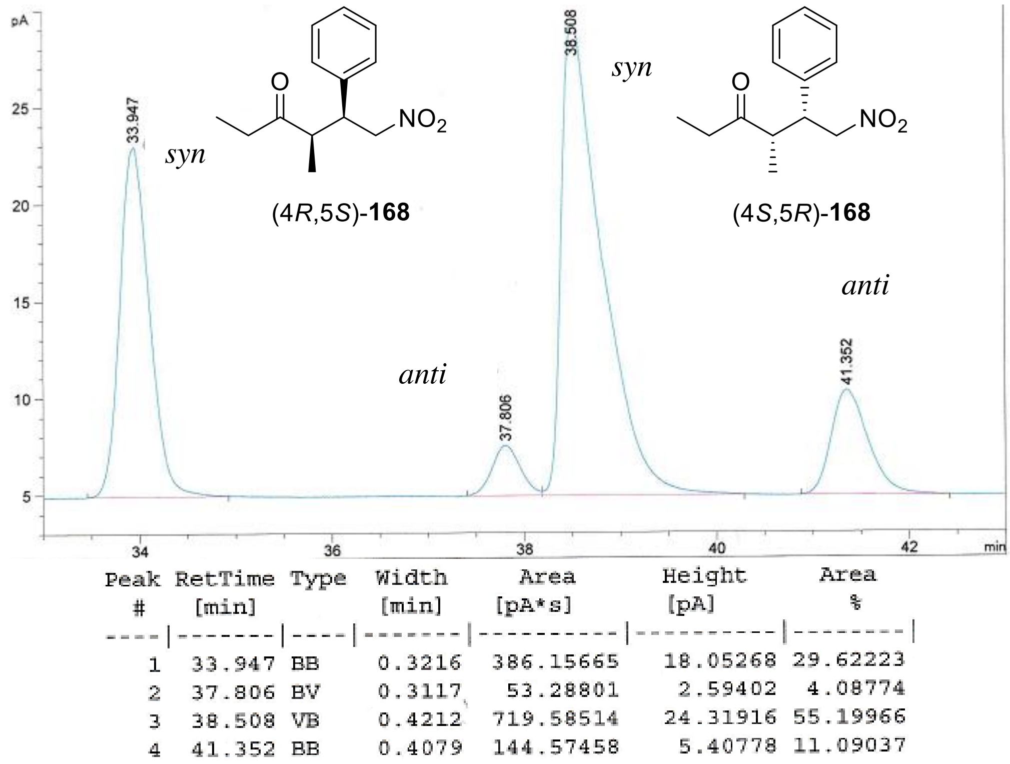
To furnish the Michael product, the deprotonated hydrazone was reacted with *trans*-β-nitrostyrene and subsequent hydrolysis afforded crude **168** which was subject to chiral GC analysis. Enantiomeric excesses of 84% and 47% were determined for *syn*- and *anti*-**168** respectively, with an excellent diastereomeric ratio of 94:6 *syn*:*anti* as determined by chiral GC analysis and ^{1}H NMR spectroscopy. Purification by silica column chromatography allowed isolation of *syn*-**168** in 84% ee and 13% yield over 2 steps. Again, the relative orientation was opposite to that usually found when using a SAMP chiral auxiliary in Michael reactions.[22]

There are no reports in the literature of either the aldol or Michael reaction using these specific conditions and reagents with SAMP as the chiral auxiliary. As relative orientation of the product in both cases was the opposite of what was expected, it was decided to carry out the Michael reaction using SAMP-based 3-pentanone hydrazone (*S*)-**114** (**Scheme 2.15**) using *trans*-β-nitrostyrene as the Michael acceptor as this has not been used in the literature with SAMP-based hydrazones.



Scheme 2.15 Michael reaction of SAMP-hydrazone (*S*)-**114** with trans-β-nitrostyrene.

Gratifyingly, crude **168** was determined to have a diastereomeric ratio of 85:15 *syn*:*anti* as determined by chiral GC analysis and ^{1}H NMR spectroscopy, with enantiomeric excesses of 30% and 46% observed for *syn*- and *anti*-**168** respectively (**Figure 2.9**). This confirmed that the *syn* diastereomer was the major product obtained, which is in keeping with the result observed when the novel chiral auxiliary was used for the Michael reaction. It follows that the relative stereochemistry observed in the aldol reaction using the novel chiral auxiliary (*anti*) should be the same as that which would be obtained if the reaction was carried out using SAMP. As these results are the opposite selectivity to that previously reported for these types of reaction in the literature, it suggests that the nature of the acceptor molecule (benzaldehyde and *trans*-β-nitrostyrene) and its interaction with the azaenolates intermediate causes the observed stereochemical outcome of the reaction.



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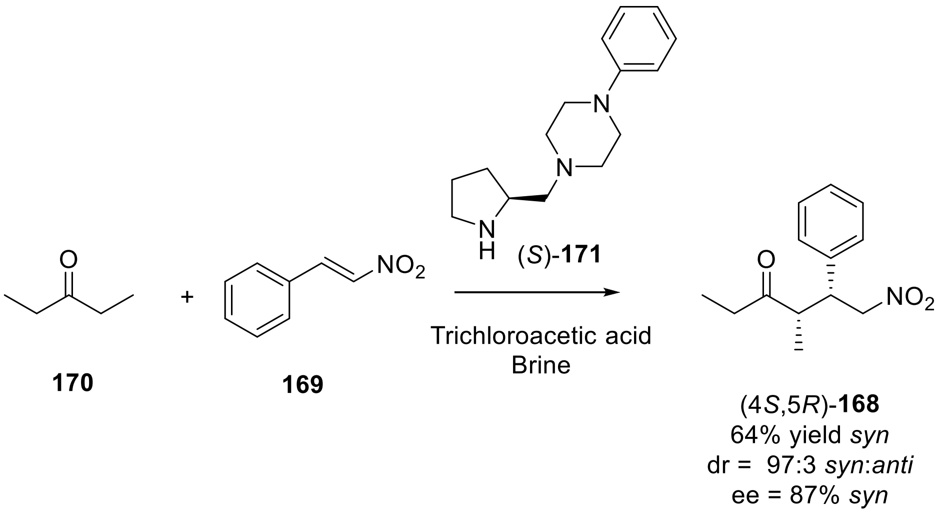
Peak RetTime Type  Width      Area      Height     Area
  #   [min]        [min]    [pA*s]      [pA]        %
----|-------|----|-------|----------|----------|--------|
  1   33.947 BB    0.3216  386.15665   18.05268 29.62223
  2   37.806 BV    0.3117   53.28801    2.59402  4.08774
  3   38.508 VB    0.4212  719.58514   24.31916 55.19966
  4   41.352 BB    0.4079  144.57458    5.40778 11.09037
```

Figure 2.9 GC trace of product of Michael reaction using SAMP.

With respect to the intermediate formed during the Michael reaction of SAMP-based hydrazones, little appears to be known about its nature. As the product of the Michael reaction using this particular acceptor (*trans*-β-nitrostyrene) provides the *syn* product as the major diastereomer, it would appear that the selectivity is based on the properties of the acceptor rather than the hydrazone donor. A plausible explanation for the observed *syn* selectivity of the final ketone product is as follows. Deprotonation of the hydrazone with LDA provides the azaenolate, which will react with the acceptor in the same way it would with an allylating or benzylating reagent, giving rise to the observed selectivity at the α-position. Due to the presence of the bulky pyrrolidine ring on one face of the hydrazone, the phenyl group will be orientated towards the opposite face as it would be more favoured sterically, resulting in the observed *syn* selectivity. Further mechanistic work would be required to determine the intermediate in this reaction sequence.

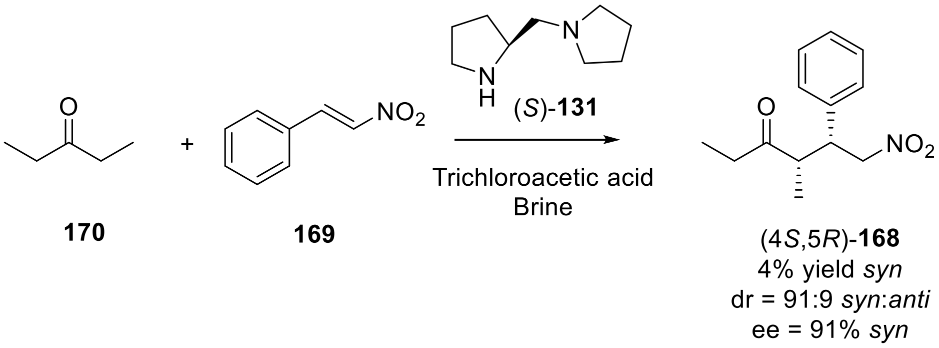
2.8 Organocatalytic Michael reaction using diamine

In a recent paper published by Singh and Chimni, various pyrrolidine based chiral di- and triamines have been successfully utilised as organocatalysts in the Michael reaction of various ketones with a variety of nitro-olefins in brine.[25] The authors chose to focus on pyrrolidine-based chiral amines as the organocatalysts based on the assumption that the pyrrolidine ring should act as the catalytic site by forming an enamine with the donor ketones, while the side chain would provide the chirality of the desired ketone product by appropriately orientating the nitro-olefins due to steric and hydrophobic interactions.[25] The aqueous solvent of choice was brine rather than water as it has been reported that nitrostyrenes can polymerise in the presence of amines, however if the reaction is carried out in an electrolyte solution, this polymerisation can be inhibited.[26] The authors found that on addition of trichloroacetic acid (TCA) as an acid co-catalyst, an improvement in both yield and enantioselectivity was observed. When their reaction was carried out with 3-pentanone **170** and *trans*-β-nitrostyrene **169** with catalyst (*S*)-**171** and TCA in brine, *syn*-**168** was obtained in 64% yield and 87% ee after stirring for 3 d at 25 °C (**Scheme 2.16**).



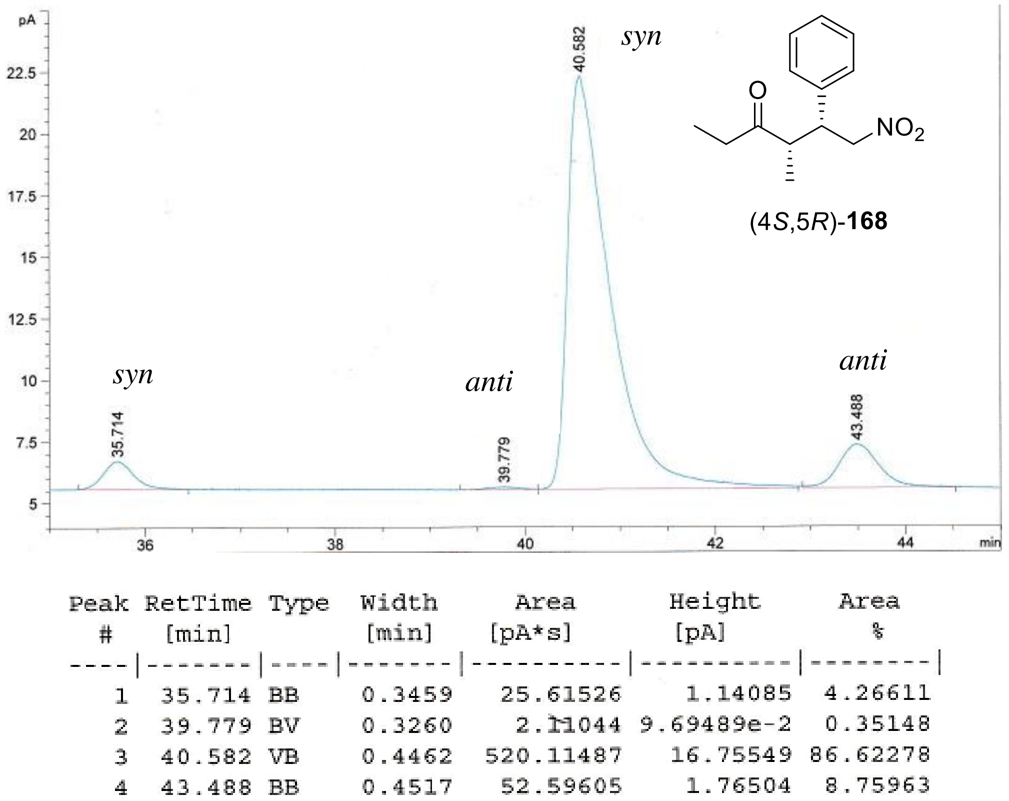
Scheme 2.16 Organocatalytic Michael reaction using (*S*)-**171** as catalyst.[25]

Considering these results, it was postulated that diamine (*S*)-**131** (formed as an intermediate in the synthesis of our novel chiral auxiliary) could be utilised in this reaction, as (*S*)-**131** has not been used by Singh and Chimni in their investigations. Thus 3-pentanone **170**, trans-β-nitrostyrene **169** and (*S*)-**131** were added to a solution of brine and TCA and allowed to stir at 25 °C (**Scheme 2.17**). The reaction was monitored by TLC analysis and was deemed to be complete after 36 h. Although **168** was obtained in a much lower yield (4%) than those observed by Singh and Chimni,[26] a gratifyingly high selectivity was attained, both in terms of diastereomeric ratio (91:9 *syn*:*anti*) and enantioselectivity (91% ee *syn*, 89% ee *anti*) (**Figure 2.10**).



Scheme 2.17 Organocatalytic Michael reaction using (*S*)-**131** as catalyst.

Reaction progress was monitored by TLC, with absence of **169** indicating completion of reaction. It is likely that due to the polar nature of **168**, product may have lost in aqueous washes during workup of the reaction, resulting in the observed low yield. The orientation of **168** was determined to be 4*S*, 5*R* by comparison of the optical rotation value with the literature value.[27]



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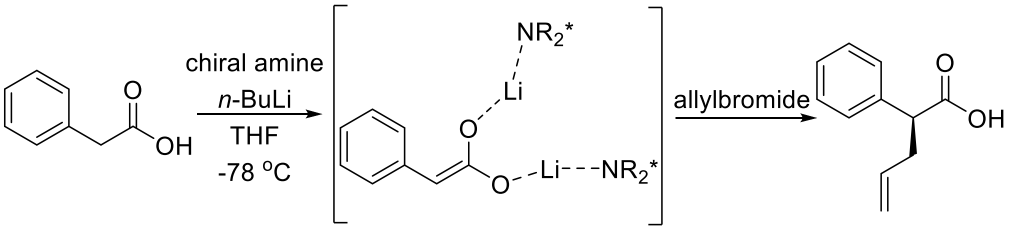
Peak RetTime Type  Width     Area       Height      Area
  #   [min]        [min]   [pA*s]       [pA]         %
----|-------|----|-------|----------|----------|--------|
   1  35.714 BB    0.3459   25.61526    1.14085  4.26611
   2  39.779 BV    0.3260    2.11044 9.69489e-2  0.35148
   3  40.582 VB    0.4462  520.11487   16.75549 86.62278
   4  43.488 BB    0.4517   52.59605    1.76504  8.75963
```

Figure 2.10 GC trace of product of organocatalytic Michael reaction.

2.9 α- and β-Allylations of carboxylic acids

Chiral lithium amides have been successfully utilised as traceless auxiliaries in the single-step α-allylation of arylacetic acids, with allylation of phenylacetic acid **172** providing (*S*)-**173** in excellent yield and enantioselectivity (**Scheme 2.18**).[28] High enantioselectivities were achieved with only a slight excess of chiral amine required (1.03 equiv.) which was easily recoverable from the aqueous layer of the workup.

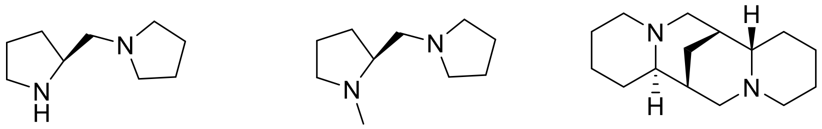


172

(*S*)-**173**
91% conversion
ee = 93%

Scheme 2.18 Allylation of phenylacetic acid using chiral lithium amides as traceless auxiliaries.[28]

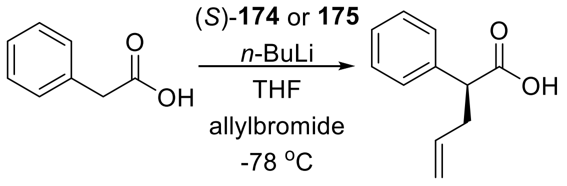
With these results in mind, it was postulated that similar results could be achieved using chiral diamine (*S*)-**131** and methylated variation (*S*)-**174** (**Figure 2.11**).



(*S*)-**131** (*S*)-**174** **175** (+)-sparteine

Figure 2.11 Diamines used as traceless auxiliaries in α-allylation of carboxylic acid.

(+)-Sparteine **175** (**Figure 2.11**) is a chiral diamine ligand which has previously been used in the research group[29] and as a supply was readily available, it was also used in the reaction. As the best published results had been achieved with phenylacetic acid and allylbromide, these reagents were used in the investigation. The reaction was first carried out using the conditions outlined by Stivala and Zakarian with (*S*)-**174** and **175** (**Scheme 2.19**), however yields were disappointingly low when compared with those obtained in the literature.[28]



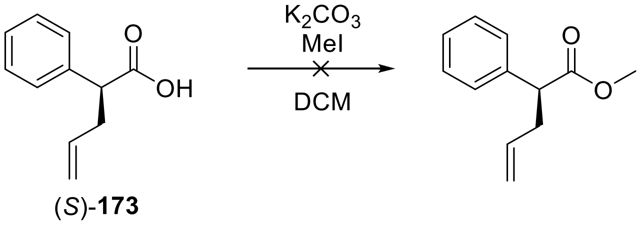
172 (*S*)-**173**

(*S*)-**174**: 22%
175: 10%

Scheme 2.19 α-Allylation of phenyl acetic acid using chiral diamine (*S*)-**174** and (+)-sparteine **175**.

Derivatisation of the alkylated phenyl acetic acid (*S*)-**173** was deemed necessary due to the nature of the chiral GC column available. Attempts were made to convert the carboxylic acid to a methyl ester using K_2CO_3 and methyliodide in DCM, however the reaction was unsuccessful (**Scheme 2.20**).

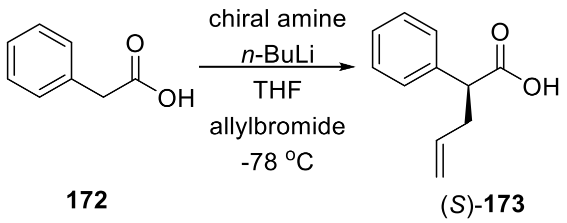
Due to the failure of the esterification and possible potential for racemisation of the chiral centre in basic conditions, it was decided to determine enantioselectivity by comparison of optical rotation of the synthesised α-alkylated phenyl acetic acids against racemic **173** and against literature data.

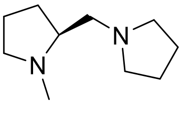
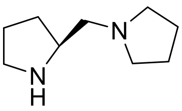
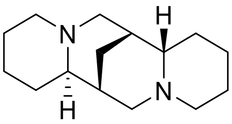
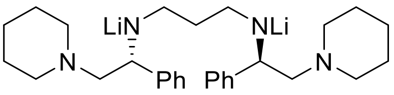


Scheme 2.20 Attempt at conversion of chiral carboxylic acid (*S*)-**173** to ester.

The allylation reaction was repeated using three chiral amines as ligands (**Table 2.4**). On comparison of optical rotation data of (*S*)-**173** obtained from reactions using chiral ligands (*S*)-**131**, (*S*)-**174** and **175** (**Table 2.4**, **entries 1-3**) with both racemic **173** and the published result from Stivala and Zakarian using Koga's amine **176** (**Table 2.4, entries 4 and 5**) our chiral ligands proved disappointing in terms of both yield and selectivity. It was also noted that in all three chiral reactions, a significant amount of starting material was present upon analysis of the crude mixture. This suggests that complete deprotonation has not occurred, perhaps due to the nature of the ligands involved.

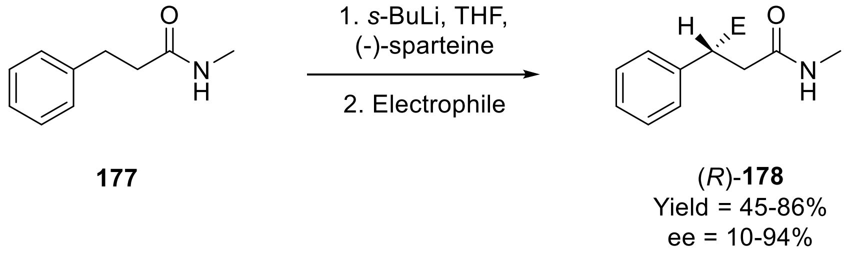
Table 2.4 α-Allylation of phenylacetic acid using chiral amines as ligands.



Entry	Ligand	*n*-BuLi (equiv.)	Yield (*S*)-173 (%)[a]	$[\alpha]^{23}_{D}$ (CHCl$_3$)
1	 (*S*)-**174**	2.1	22	-1.294 (c = 0.85)
2	 (*S*)-**131**	3.3	9	-1.667 (c = 0.9)
3	 **175**	2.2	5	+0.857 (c = 0.35)
4	None (racemic)	2.2	>98	+0.248 (c = 1.01)
5	 **176**	4.0	84	+77.2 (c = 1.01)

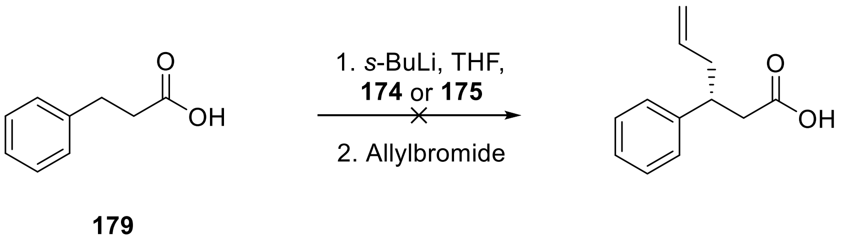
[a] Isolated yield.

Regioselective β-allylations of β-aryl secondary amides using 2.5 equiv. of (-)-sparteine has been reported (**Scheme 2.21**).[30] The authors found that the degree of enantioselectivity of the product was dependant on the electrophile used with a wide range of electrophiles tolerated under the reaction conditions.



Scheme 2.21 Enantioselective β-allylation of 3-phenylpropanamide **174** using (-)-sparteine as chiral ligand.

With these results in mind, the reaction was attempted using carboxylic acid **179** rather than the amide as the starting material. Supplies of (*S*)-**131** and (*S*)-**174** had been exhausted in attempts at α-allylation of phenylacetic acid, so the β-allylation of 3-phenylpropanoic acid was attempted with (+)-sparteine **175** and Koga's amine **176**, the chiral ligand which gave the best results in Stivala and Zakarian's α-allylation of phenylacetic acid.[28] Disappointingly in both cases, allylation occurred solely at the α-position with low enantioselectivity determined by optical rotation data: reaction with **175**: $[\alpha]_D^{25} = +0.58$ (c 1.12, $CHCl_3$); reaction with **176**: $[\alpha]_D^{25} = +3.66$ (c 0.56, $CHCl_3$); lit.[31] 99% ee: $[\alpha]_D^{25} = +0.58$ (c 1.12, $CHCl_3$), suggesting a nitrogen atom may be required to provide a coordination point for the chiral ligand to encourage allylation to take place at the β-position (**Scheme 2.22**).



Scheme 2.22 Attempts at β-allylation of 3-phenylpropanoic acid **179**.

2.10 Conclusions and future work

Novel chiral hydrazone methodology has been established involving 3-pentanone and propiophenone based ketones with the chiral auxiliary containing a pyrrolidine ring in the chiral arm. The chiral auxiliary has been formed in good yields in five steps from commercially available (*S*)-*N*-(benzyloxycarbonyl)proline (*S*)-**128** (or only two steps from commercially available (*S*)-(+)-1-(2-pyrrolidinylmethyl)pyrrolidine (*S*)-**131**) without the need for silica column chromatography purification. Enantiomeric excesses of up to 92% were achieved in the α-alkylated aliphatic ketones formed and up to 89% in less studied aromatic ketones. While the overall yields were moderate (in most cases due to product volatility), comparison studies with SAMP showed comparable yields. However, given the remarkably few methods available to access these compounds and the excellent enantioselectivities observed, the novel chiral auxiliary reported can be considered a viable route to these chiral synthons.

Initial unoptimised studies into the use of the novel chiral auxiliary in Michael reactions have proven to be successful and further research will be carried out in this area and in its potential in aldol reactions.

Although initial studies into the α- and β-allylation of carboxylic acids proved unsuccessful, future investigations in the area will involve alternative chiral amines including tertiary diamines and sparteine surrogates to act as ligands in these reactions.

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Chapter 3

Investigation of a novel diamine based chiral auxiliary

Experimental

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3.1 General experimental

Solvents and reagents were used as obtained from commercial sources and without purification with the following exceptions: THF and diethyl ether were freshly distilled from sodium/benzophenone under nitrogen. DCM and diisopropylamine were distilled from CaH_2 and stored under nitrogen.

Wet flash column chromatography was carried out using Kieselgel silica gel 60, 0.040–0.063 mm (Merck). TLC was carried out on pre-coated silica gel plates (Merck 60 PF254). Visualisation was achieved by UV and potassium permanganate staining.

Melting points were carried out on a uni-melt Thomas Hoover Capillary melting point apparatus.

IR spectra were recorded on Perkin-Elmer FT-IR Paragon 1000 spectrophotometer. Liquid samples were examined as thin films on NaCl plates. Solid samples were dispersed in KBr and recorded as pressed discs. The intensity of peaks were expressed as strong (s), medium (m) and weak (w).

NMR spectra were run in $CDCl_3$ using TMS as the internal standard at 20 °C unless otherwise specified. ^1H NMR (400 MHz) spectra and ^1H NMR (300 MHz) spectra were recorded on Bruker Avance 400 and Bruker Avance 300 NMR spectrometers respectively in proton coupled mode. ^{19}F NMR (282 MHz) spectra and ^{19}F NMR (470 MHz) were recorded on Bruker Avance 300 NMR and Bruker Avance 600 NMR spectrometers respectively in proton decoupled mode. ^{13}C NMR (150 MHz), ^{13}C NMR (125 MHz) and ^{13}C NMR (75.5 MHz) spectra were recorded on Bruker Avance 600 Bruker Avance 500 and Bruker Avance 300 NMR spectrometers respectively in proton decoupled mode. All spectra were recorded at University College Cork. Chemical shifts δ_H and δ_C are expressed as parts per million (ppm), positive shift being downfield from TMS; coupling constants (J) are expressed in hertz (Hz). Splitting patterns in ^1H NMR spectra are designated as s (singlet), bs (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), dq (doublet of quartets), and m (multiplet). For ^{13}C NMR spectra, the number of attached protons for each signal was determined using the DEPT pulse sequence run in the DEPT-90 and DEPT-135 modes. COSY, HSQC and HMBC experiments were routinely performed to aid the NMR assignment of novel chemical structures.

LRMS were recorded on a Waters Quattro Micro triple quadrupole instrument in ESI mode using 50% acetonitrile-water containing 0.1% formic acid as eluent; samples were made up in acetonitrile or methanol. HRMS were recorded on a Waters LCT Premier Tof LC-MS instrument in ESI mode using 50% acetonitrile-water containing 0.1% formic acid as eluent; samples were made up in acetonitrile or methanol.

Enantiopurity of the chiral compounds was determined by chiral gas chromatography performed on an Astec CHIRALDEX^{TM} G-TA, fused silica capillary column, 20 m × 0.25 mm × 0.12 μm film thickness. GC analysis was performed on an Agilent Technologies 7820 A GC system. All chiral columns were purchased from Sigma-Aldrich Supelco. Conditions for separation were determined using the following operating conditions as standard, flow rate: 1 mL/min, injection volume: 0.2 μL, split ratio: 10 : 1, front inlet temperature: 150 °C, detector temperature: 155 °C. Samples were prepared for GC analysis by dissolving in distilled DCM to 2 mg/ml and passing through silica gel.

Optical rotations were measure on a Perkin-Elmer Polarimeter 341 at 589 nm in a 10 cm cell; concentrations (*c*) are expressed in g/100 ml. $[\alpha]_D$ is the specific rotation of a compound and is expressed in units of 10^{-1} deg cm^2 g^{-1}. Specific rotations were employed to indicate the direction of enantioselection.

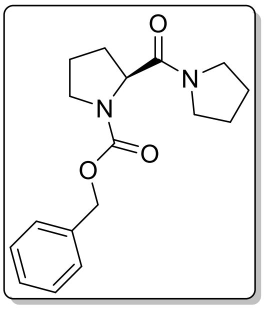
The Microanalysis Laboratory, National University of Ireland, Cork, performed elemental analysis using a Perkin-Elmer 240 and Exeter Analytical CE440 elemental analysers.

3.1.1 Analysis of known and novel compounds

^{1}H NMR spectra, ^{13}C NMR spectra, ^{19}F NMR (where applicable), LRMS and melting point (if solid) analyses were recorded for all previously prepared compounds. For novel compounds, in addition to the previously mentioned analysis, IR, HRMS and elemental analysis (if possible) were also obtained. Optical rotations were used to assign the absolute stereochemistry for known compounds.

3.2 Procedures for synthesis of hydrazones

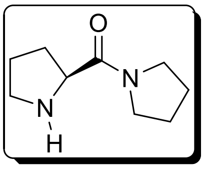
Benzyl (*S*)-2-(pyrrolidine-1-carbonyl)pyrrolidine-1-carboxylate, (*S*)-129



To a stirred solution of (*S*)-*N*-(benzyloxycarbonyl)proline (*S*)-**128** (74.57 g, 0.3 mol) in DCM (120 mL) was added dropwise a solution of DCC (61.69 g, 0.3 mol) in DCM (130 mL) at 0 °C under a N_2 atmosphere. After stirring for 30 min, a solution of pyrrolidine (24.7 mL, 0.3 mol) in DCM (120 mL) was added dropwise to the reaction mixture at 0 °C *via* an addition funnel. The reaction mixture was allowed slowly warm overnight. The precipitate was removed by filtration through a pad of Celite® and washed with DCM to elute the product. The filtrate was washed with 0.5 M HCl (2 × 150 mL), saturated aq. $NaHCO_3$ solution (150 mL), water (150 mL) and brine (150 mL). The organic layer was dried over $MgSO_4$, concentrated *in vacuo* and crude product (86.91 g, 96%) recrystallised from ethyl acetate to yield (*S*)-**129** as a white crystalline solid (73.52 g, 81%).

Spectral characteristics were consistent with previously reported data.[1] m.p. 129–131 °C [lit.[2] 130–133 °C]. $[\alpha]_D^{22}$= -13.3 (c 1.60, MeOH) [lit.[2] $[\alpha]_D^{22}$ = -14.1 (c 1.61, MeOH)]; ^{1}H NMR (300 MHz, $CDCl_3$): δ (mixture of rotamers) 1.56–2.20 (8H, m, 4 × C***H₂***), 3.25–3.75 (6H, m, 3 × NC***H₂***), 4.39–4.54 (1H, m, NC***H***), 4.97–5.22 (2H, m, OC***H₂***Ph), 7.28–7.37 (5H, m, 5 × C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ (mixture of rotamers) 23.8, 23.9; 24.1, 24.4; 26.0, 26.3 (3 × NCH_2***C***H_2), 29.5, 30.5 (NCH***C***H_2), 45.9, 46.0; 46.1, 46.3; 46.7, 47.3 (3 × N***C***H_2), 57.7, 58.2 (**C**H), 66.9, 67.1 (O***C***H_2), 127.8, 127.9; 128.0, 128.1; 128.4, 128.4 (5 × ***C***H arom.), 136.7, 136.8 ($COOCH_2$***C***), 154.2, 154.9 (CH***C***ON), 170.7, 171.0 (N***C***OO) ppm; MS (ESI) *m*/*z*: 303 [(M + H)$^+$, 100%].

(*S*)-Pyrrolidin-1-yl(pyrrolidin-1-yl)methanone, (*S*)-130

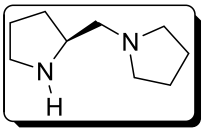


To a stirred solution of (*S*)-**129** (75.40 g, 250 mmol) in methanol (350 mL) was added palladium on carbon (5 wt. % loading, 4.78 g). The reaction mixture was stirred under hydrogen at atmospheric pressure for 22 h while monitoring the reaction progress by TLC analysis. The crude reaction mixture was filtered through a pad of Celite® and washed with methanol to elute the product. The filtrate was concentrated *in vacuo* to yield amide (*S*)-**130** as a yellow oil (39.84 g, 95%) with no purification required.

Spectral characteristics were consistent with previously reported data.[1]

$[\alpha]^{26}_{D}$ = -89.6 (c 1.7, EtOH) [lit.[3] $[\alpha]^{26}_{D}$= -112.2 (c 1.7, EtOH)]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.60–2.02 (7H, m, 3 × $\mathrm{NCH_2C}\boldsymbol{H_2}$, one of $\mathrm{NCHC}\boldsymbol{H_2}$), 2.05–2.14 (1H, m, one of $\mathrm{NCHC}\boldsymbol{H_2}$), 2.77–2.85 (1H, m, 1 of $\mathrm{NHC}\boldsymbol{H_2}$), 2.93 (1H, bs, N***H***), 3.15–3.22 (1H, m, 1 of $\mathrm{NHC}\boldsymbol{H_2}$), 3.36–3.57 (4H, m, 2 × $\mathrm{NC}\boldsymbol{H_2}\mathrm{CH_2}$), 3.73–3.77 (1H, dd, *J* = 6.5, 8.6 Hz, NHC***H***) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 24.0, 26.0, 26.5 (3 × $\mathrm{NCH_2}\boldsymbol{C}\mathrm{H_2}$), 30.4 ($\mathrm{NCH}\boldsymbol{C}\mathrm{H_2}$), 45.9, 46.0, 47.7 (3 × $\mathrm{N}\boldsymbol{C}\mathrm{H_2}$), 59.5 (N***C***H), 172.7 (***C***=O) ppm; MS (ESI) *m/z*: 169 [$(M + H)^+$, 100%].

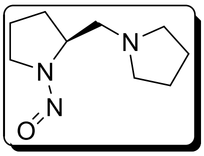
(*S*)-2-(1-Pyrrolidinylmethyl)-pyrrolidine, (*S*)-131



To a stirred suspension of $LiAlH_4$ (15 g, 396 mmol) in THF (140 mL) was added a solution of amide (*S*)-**130** (19.02 g, 113 mmol) in THF (80 mL) dropwise over 3 h at 0 °C under a N_2 atmosphere. The reaction mixture was slowly heated to reflux, then allowed to stir at this temperature for 4 h. The reaction was allowed to cool then quenched by dropwise addition of saturated aq. Na_2SO_4 solution (20 mL). The crude reaction mixture was then filtered through a pad of Celite® and washed with ethyl acetate to elute the product. The mother liquor was concentrated *in vacuo* to yield the crude product as a yellow oil (14.54 g, 83%), which was purified using Kugelrohr distillation to yield (*S*)-**131** as a colourless oil (11.22 g, 64%). Spectral characteristics were consistent with previously reported data.[1]

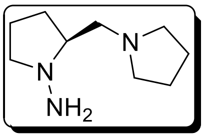
$[\alpha]^{20}_{D}$ = +8.2 (c 2.4, EtOH) [lit.[4] $[\alpha]^{20}_{D}$ = +8.9 (c 2.4, EtOH)]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22–1.37 (1H, m, one of $\mathrm{NCHC}\boldsymbol{H_2}$), 1.61–1.74 (6H, m, 3 × $\mathrm{NCH_2C}\boldsymbol{H_2}$), 1.76–1.91 (1H, m, one of $\mathrm{NCHC}\boldsymbol{H_2}$), 2.21 (1H, bs, N***H***), 2.28 (1H, dd, *J* = 5.2, 11.9 Hz, one of NHCHC***H₂***N), 2.38–2.54 (5H, m, 2 × NC***H₂***CH₂ pyrrolidine ring and one of NHCHC***H₂***N), 2.75–2.83 (1H, m, one of NHC***H₂***), 2.88–2.96 (1H, m, one of NHC***H₂***), 3.11–3.20 (1H, m, NHC***H***) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 23.4, 25.0 (3 × NCH₂***C***H₂), 30.1 (NHCH***C***H₂), 46.1 (NH***C***H₂), 54.6 (2 × N***C***H₂CH₂ pyrrolidine ring), 57.4 (NH***C***H), 62.1 (NHCH***C***H₂N) ppm; MS (ESI) *m/z*: 155 [$(M + H)^+$, 100%].

(*S*)-1-Nitroso-2-(pyrrolidin-1-ylmethyl)pyrrolidine, (*S*)-132



Ethyl nitrite in ethanol (10-20%, taken to be 15%) (5.45 mL, 8.63 mmol) was added to (*S*)-**131** (1.065 g, 6.90 mmol). The reaction vessel was covered in aluminium foil and allowed to stir at room temperature with progress monitored by ^{1}H NMR spectroscopy. After 45 h, ethanol was removed *in vacuo* to yield (*S*)-**132** as a yellow oil (1.15 g, 91%). *N.B. Due to the potential toxicity of this compound, only ^{1}H and ^{13}C spectra were obtained and the crude reaction mixture used without purification in the next step.* ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.76–1.81 (4H, m, 2 × NCH₂C***H₂*** pyrrolidine ring), 1.91–2.25 (4H, m, NCH₂C***H₂*** and NCHC***H₂***), 2.54–2.67 (4H, m, 2 × NC***H₂***CH₂ pyrrolidine ring), 2.80 (1H, dd, *J* = 8.8, 12.2 Hz, one of NCHC***H₂***N), 3.00 (1H, dd, *J* = 5.1, 12.2 Hz, one of NCHC***H₂***N), 3.52–3.62 (2H, m, NC***H₂***), 4.59–4.67 (1H, m, NC***H***) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.7 (NCH₂***C***H₂), 23.5 (2 × NCH₂***C***H₂ pyrrolidine ring), 28.7 (NCH***C***H₂), 45.6 (N***C***H₂), 54.7 (2 × N***C***H₂CH₂ pyrrolidine ring), 59.5 (NCH***C***H₂N), 60.3 (N***C***H) ppm.

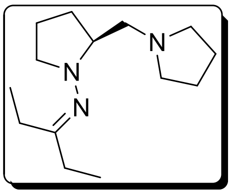
(*S*)-2-(Pyrrolidin-1-ylmethyl)pyrrolidin-1-amine, (*S*)-133



To a stirred suspension of $LiAlH_4$ (2.611 g, 68.8 mmol) in THF (120 mL) was added a solution of (*S*)-**132** (6.300 g, 34.4 mmol) in THF (60 mL) at 0 °C under a N_2 atmosphere. The reaction mixture was allowed stir at room temperature for 15 min before being heated to reflux for 4 h, then stirred at room temperature overnight. Reaction progress was monitored by ^{1}H NMR spectroscopy. The reaction was quenched by dropwise addition of saturated aq. Na_2SO_4 solution (20 mL). The crude reaction mixture was filtered through a pad of Celite® washing with diethyl ether to elute the product. The mother liquor was concentrated *in vacuo* to yield (*S*)-**133** as a yellow oil (4.98 g, 86%) with no purification required.

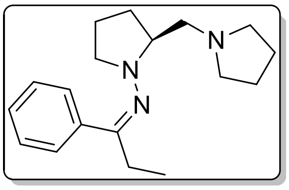
$[\alpha]_D^{20}$ = -49.4 (c 1.08, EtOH); IR (NaCl) ν_{max}: 3370 (N-H stretch, m), 2961, 2798 (C-H alkyl stretch, s), 1459, 1447 (C-H alkyl bend, m), 1139 (C-N stretch, m) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41–1.54 (1H, m, one of $NCHC\boldsymbol{H_2}$), 1.68–1.85 (6H, m, 2 × $NCH_2C\boldsymbol{H_2}$ pyrrolidine ring and $NCH_2C\boldsymbol{H_2}$), 1.93–2.07 (1H, m, one of $NCHC\boldsymbol{H_2}$), 2.26–2.41 (3H, m, one of $NCHC\boldsymbol{H_2}N$ and $NC\boldsymbol{H_2}$), 2.45–2.53 (2H, m, $N\boldsymbol{CH_2}CH_2$ pyrrolidine ring), 2.54–2.62 (2H, m, 2 × $N\boldsymbol{CH_2}CH_2$ pyrrolidine ring), 2.87 (1H, dd, *J* = 6.5, 11.8 Hz, one of $NCHC\boldsymbol{H_2}N$), 3.22–3.41 (3H, m, $NC\boldsymbol{H}$ and $N\boldsymbol{H_2}$) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.6 ($NCH_2\boldsymbol{C}H_2$), 23.5 (2 × $NCH_2\boldsymbol{C}H_2$ pyrrolidine ring), 28.7 ($NCH\boldsymbol{C}H_2$), 54.9 (2 × $N\boldsymbol{C}H_2CH_2$ pyrrolidine ring), 59.6 ($NCH\boldsymbol{C}H_2N$), 61.5 ($NH_2N\boldsymbol{C}H_2$), 67.8 ($NH_2N\boldsymbol{C}H$) ppm; HRMS (ESI) *m*/z calcd for $C_9H_{20}N_3$ [(M + H)$^+$]: 170.1657, found 170.1674.

(*S*)-*N*-(Pentan-3-ylidine)-2-(pyrrolidin-1-ylmethyl)pyrrolidin-1-amine, (*S*)-156



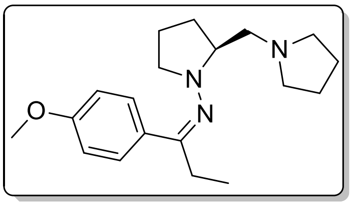
To a stirred solution of (*S*)-**133** (4.98 g, 29.4 mmol) in cyclohexane (10 mL) was added 3-pentanone (7.60 g, 88.2 mmol) dropwise under a N_2 atmosphere. The reaction mixture was allowed stir at room temperature overnight and reaction progress monitored by ^{1}H NMR spectroscopy. On completion, the reaction mixture was poured into a 6:1 DCM:water mixture, shaken and layers separated. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to give crude product (5.614 g, 80%) which was purified using Kugelrohr distillation to yield (*S*)-**156** as a pale yellow oil (4.52 g, 65%). $[\alpha]_D^{20}$ = +67.3 (c 1, EtOH); IR (NaCl) ν_{max}: 2874, 2794 (C-H alkyl stretch, s), 1658 (C=N stretch, s), 1460 (C-H alkyl bend, m), 1146 (C-N stretch, m) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.06 (3H, t, *J* = 7.5 Hz, $C\boldsymbol{H_3}$), 1.08 (3H, t, *J* = 7.5 Hz, $C\boldsymbol{H_3}$), 1.53–1.66 (1H, m, one of $NCHC\boldsymbol{H_2}$), 1.69–1.91 (6H, m, $NCH_2C\boldsymbol{H_2}$, 2 × $NCH_2C\boldsymbol{H_2}$ pyrrolidine ring), 2.02–2.14 (1H, m, one of $NCHC\boldsymbol{H_2}$), 2.17–2.29 (2H, m, $CH_3C\boldsymbol{H_2}$), 2.30–2.55 (9H, m, $CH_3C\boldsymbol{H_2}$, $NCHC\boldsymbol{H_2}N$, 2 × $NC\boldsymbol{H_2}CH_2$ pyrrolidine ring and one of $NC\boldsymbol{H_2}$), 2.97–3.10 (2H, m, one of $NC\boldsymbol{H_2}$ and $NC\boldsymbol{H}$) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 10.9 ($\boldsymbol{C}H_3$), 11.8 ($\boldsymbol{C}H_3$), 21.8 ($NCH_2\boldsymbol{C}H_2$), 23.4 ($CH_3\boldsymbol{C}H_2$), 23.5 (2 × $NCH_2\boldsymbol{C}H_2$ pyrrolidine ring), 28.6 ($CH_3\boldsymbol{C}H_2$), 28.7 ($NCH\boldsymbol{C}H_2$) 54.8 (2 × $N\boldsymbol{C}H_2CH_2$ pyrrolidine ring), 55.0 ($N\boldsymbol{C}H_2$), 61.4 ($NCH\boldsymbol{C}H_2N$), 66.1 ($N\boldsymbol{C}H$), 173.3 ($\boldsymbol{C}$=N) ppm; HRMS (ESI) *m*/z calcd for $C_{14}H_{28}N_3$ [$(M + H)^+$]: 238.2283, found 238.2288.

(*S*)-*N*-(1-Phenylpropylidene)-2-(pyrrolidin-1-ylmethyl)pyrrolidin-1-amine, (*S*)-157



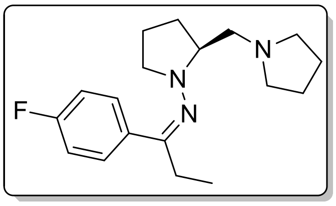
To a stirred solution of (*S*)-**133** (1.21 g, 7.14 mmol) in cyclohexane (4 mL) was added propiophenone (2.38 g, 17.7 mmol) dropwise under a N_2 atmosphere. The reaction mixture was allowed stir at room temperature overnight and the reaction progress monitored by ^1H NMR spectroscopy. On completion, the reaction mixture was poured into a 6:1 DCM:water mixture, shaken and layers separated. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to give crude product as a brown oil (1.27 g, 62%) which was purified by Kugelrohr distillation to yield (*S*)-**157** as a colourless oil (1.06 g, 52%). $[\alpha]_D^{20}$ = +418.5 (c 1.09, EtOH); IR (NaCl) ν_{max}: 2965, 2875, 2790 (alkyl C-H stretch, s), 1608 (C=N stretch, w), 1445, 1459 (aromatic C=C stretch, m), 1144 (C-N stretch, m), 696 (mono substituted benzene ring bend, s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ (mixture of *E*/*Z* isomers, 85:15 major:minor) 1.03 and 1.08 (3H, 2 × overlapping t, *J* = 7.6 Hz, $C\boldsymbol{H_3}CH_2$), 1.43–1.79 (6H, m , one of $NCHC\boldsymbol{H_2}$ and 2 × $NCH_2C\boldsymbol{H_2}$ pyrrolidine ring), 1.82–2.03 (1.9H, m, $NCH_2C\boldsymbol{H_2}$), 2.08–2.29 (1.4H, m, one of $NCHC\boldsymbol{H_2}$), 2.44–2.68 (7.7H, m, $NCHC\boldsymbol{H_2}N$, 2 × $NC\boldsymbol{H_2}CH_2$ pyrrolidine ring, one of $NC\boldsymbol{H_2}$), 2.70–2.94 (2H, m, $CH_3C\boldsymbol{H_2}$), 3.25–3.40 (2H, m, $NC\boldsymbol{H}$ and one of $NC\boldsymbol{H_2}$), 7.28–7.39 (3H, m, $C\boldsymbol{H}$ arom.), 7.67–7.71 (1.6H, m, $C\boldsymbol{H}$ arom.) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ (only major isomer reported) 11.7 ($\boldsymbol{C}H_3$), 22.47 ($NCH_2\boldsymbol{C}H_2$), 22.50 ($NCH\boldsymbol{C}H_2$), 23.5 (2 × $NCH_2\boldsymbol{C}H_2$ pyrrolidine ring), 28.7 ($CH_3\boldsymbol{C}H_2$), 55.0 ($N\boldsymbol{C}H_2$), 55.3 (2 × $N\boldsymbol{C}H_2CH_2$ pyrrolidine ring), 61.6 ($NCH\boldsymbol{C}H_2N$), 66.9 ($N\boldsymbol{C}H$), 126.8, 128.2, 128.6 (5 × $\boldsymbol{C}H$ arom.), 138.2 ($CH\boldsymbol{C}C$=N), 165.1 ($\boldsymbol{C}$=N); HRMS (ESI) *m*/z calcd for $C_{18}H_{28}N_3$ [$(M + H)^+$]: 286.2283, found 286.2288.

(*S*)-*N*-(1-(4-Methoxyphenyl)propylidene)-2-(pyrrolidin-1-ylmethyl)-pyrrolidin-1-amine, (*S*)-158



To a stirred solution of (*S*)-**133** (0.94 g, 5.57 mmol) in cyclohexane (9 mL) was added *p*-methoxypropiophenone (0.92 g, 5.57 mmol) dropwise under a N_2 atmosphere. The reaction mixture was allowed stir at reflux overnight and the reaction progress monitored by ^{1}H NMR spectroscopy. On completion, the reaction mixture was poured into a 6:1 DCM:water mixture, shaken and layers separated. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to give crude product as a dark orange oil (1.53 g, 87%) which was purified using silica column chromatography eluting with 90:10 hexane:ethyl acetate to yield (*S*)-**158** as an orange oil (0.95 g, 54%). $[\alpha]_D^{20}$ = +122.1 (c 1, Et_2O); IR (NaCl) ν_{max}: 2965 (C-H alkyl stretch, s), 1608 (C=N stretch, m), 1512 (C=C arom. stretch, s), 1250 (C-N stretch, s), 1176 (C-O stretch, m) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ (mixture of *E*/*Z* isomers, 96:4 major:minor) 1.08 (3.5H, 2 × overlapping t, *J* = 7.6 Hz, C$\boldsymbol{H_3}$CH$_2$ major and minor), 1.59–1.70 (1.2H, one of NCHC$\boldsymbol{H_2}$), 1.72–1.81 (4.5H, m, 2 × NCH$_2$C$\boldsymbol{H_2}$ pyrrolidine ring), 1.82–1.93 (2.3H, m, NCH$_2$C$\boldsymbol{H_2}$), 2.09–2.20 (1.4H, m, one of NCHC$\boldsymbol{H_2}$), 2.49–2.63 (8.1H, m, one of NC$\boldsymbol{H}$, NCHC$\boldsymbol{H_2}$N, 2 × NC$\boldsymbol{H_2}$CH$_2$ pyrrolidine ring), 2.71–2.89 (2H, m, CH$_3$CH$_2$), 2.96 (0.2H, q, CH$_3$C$\boldsymbol{H_2}$ minor), 3.20–3.35 (2.1H, m, NC$\boldsymbol{H}$ and one of NC$\boldsymbol{H_2}$), 3.83 (3H, s, OC$\boldsymbol{H_3}$), 3.87 (0.12H, s, OC$\boldsymbol{H_3}$ minor) 6.88 (2H, d, *J* = 8.9 Hz, 2 × C$\boldsymbol{H}$COCH$_3$), 7.37 (0.1H, d, *J* = 10.9 Hz, 2 × C$\boldsymbol{H}$COCH$_3$ minor), 7.65 (2H, d, *J* = 8.9 Hz, 2 × C$\boldsymbol{H}$CHCOCH$_3$), 7.96 (0.08H, d, *J* = 8.9 Hz, 2 × C$\boldsymbol{H}$CHCOCH$_3$ minor) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ (only major isomer reported) 12.0 ($\boldsymbol{C}$H$_3$), 22.2 (NCH$_2$$\boldsymbol{C}H_2$), 22.3 (NCH$\boldsymbol{C}H_2$), 23.5 (2 × NCH$_2$$\boldsymbol{C}H_2$ pyrrolidine ring), 28.7 (CH$_3$$\boldsymbol{C}H_2$), 55.1 (N$\boldsymbol{C}H_2$), 55.2 (2 × N$\boldsymbol{C}H_2CH_2$ pyrrolidine ring), 55.3 (O$\boldsymbol{C}$H$_3$), 61.6 (NCH$\boldsymbol{C}$H$_2$N), 66.7 (N$\boldsymbol{C}$H), 113.6 (2 × $\boldsymbol{C}$HCOCH$_3$), 128.2 (2 × $\boldsymbol{C}$HCHCOCH$_3$), 130.6 (CH$\boldsymbol{C}$C=N), 160.2 ($\boldsymbol{C}$OCH$_3$), 165.7 ($\boldsymbol{C}$=N) ppm; HRMS (ESI) *m*/z calcd for $C_{19}H_{30}N_3O$ [(M + H)$^+$]: 316.2389, found 316.2388.

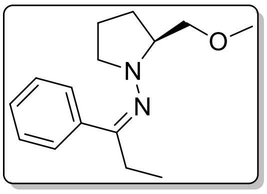
(S)-N-(1-(4-Fluorophenyl)propylidene)-2-(pyrrolidin-1-ylmethyl)pyrrolidin-1-amine, (S)-159



To a stirred solution of (S)-**133** (0.99 g, 5.85 mmol) in cyclohexane (9 mL) was added *p*-fluoropropiophenone (0.89 g, 5.85 mmol) dropwise under a N_2 atmosphere. The reaction mixture was allowed stir at reflux overnight and the reaction progress monitored by ^1H NMR spectroscopy. On completion, the reaction mixture was poured into a 6:1 DCM:water mixture, shaken and layers separated. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to give crude product as a brown oil (1.42 g, 80%) which was purified by silica column chromatography eluting with 90:10 hexane:ethyl acetate to yield (S)-**159** as an orange oil (0.86 g, 48%).

$[\alpha]_D^{20}$ = +289.4 (c 1, Et_2O); IR (NaCl) ν_{max}: 2969, 2934 (C-H alkyl stretch, s), 1600 (C=N stretch, s), 1508, 1460 (C=C arom. stretch, m), 1223(C-F stretch, m), 1157 (C-N stretch, m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ (mixture of major and minor *E*/*Z* isomers, 74:26 major:minor) 1.03 and 1.07 (3.3H, 2 × overlapping t, $C\boldsymbol{H_3}CH_2$), 1.61–1.71 (1.9H, m, one of $NCH\boldsymbol{C}H_2$), 1.73–1.81 (5.8H, m, 2 × $NCH_2C\boldsymbol{H_2}$ pyrrolidine ring), 2.09–2.20 (1.8H, m, one of $NCHC\boldsymbol{H_2}$), 2.46–2.66 (9.8H, m, one of $NC\boldsymbol{H_2}$, $NCHC\boldsymbol{H_2}N$, 2 × $NC\boldsymbol{H_2}CH_2$ pyrrolidine ring), 2.73–2.90 (2H, m, $CH_3C\boldsymbol{H_2}$ major), 2.94–3.01 (0.7H, q, *J* = 7.2 Hz, $CH_3C\boldsymbol{H_2}$ minor), 3.24–3.40 (3H, m, $NC\boldsymbol{H}$ and one of $NC\boldsymbol{H_2}$), 7.03 (2H, t, *J* = 8.8 Hz, 2 × $C\boldsymbol{H}CF$ major), 7.09–7.15 (0.6H, m, 2 × $C\boldsymbol{H}CF$ minor), 7.37 (0.7H, dd, *J* = 5.6, 8.8 Hz, 2 × $C\boldsymbol{H}CHCF$ minor), 7.67 (2H, dd, *J* = 5.5, 8.9 Hz, 2 × $C\boldsymbol{H}CHCF$ major) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ (only major isomer reported) 11.7 ($\boldsymbol{C}H_3CH_2$), 22.4 ($NCH_2\boldsymbol{C}H_2$), 22.5 ($NCH\boldsymbol{C}H_2$), 23.5 (2 × $NCH_2\boldsymbol{C}H_2$ pyrrolidine ring), 28.6 ($CH_3\boldsymbol{C}H_2C$=N), 55.0 ($N\boldsymbol{C}H_2$), 55.4 (2 × $N\boldsymbol{C}H_2CH_2$ pyrrolidine ring), 61.5 ($NCH\boldsymbol{C}H_2N$), 66.9 ($N\boldsymbol{C}H$), 115.1 (d, $^2J_{C\text{-}F}$ = 21.4 Hz, 2 × $\boldsymbol{C}HCF$), 128.5 (d, $^3J_{C\text{-}F}$ = 8.1 Hz, 2 × $\boldsymbol{C}HCHCF$), 134.2 (d, $^4J_{C\text{-}F}$ = 3.2 Hz $\boldsymbol{C}C$=N), 163.3 (d, $^1J_{C\text{-}F}$ = 248.7 Hz, $\boldsymbol{C}F$), 163.9 ($\boldsymbol{C}$=N) ppm; ^{19}F NMR (282 MHz, $CDCl_3$): -113.3 (major), -113.5 (minor) ppm; HRMS (ESI) *m*/*z* calcd for $C_{18}H_{27}N_3F$ [$(M + H)^+$]: 304.2189, found 304.2176.

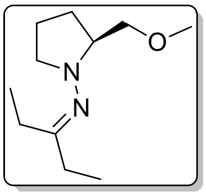
(*S*)-2-(Methoxymethyl)-*N*-(1-phenylpropylidene)pyrrolidin-1-amine, (*S*)-163



To a stirred solution of (*S*)-(-)-1-amino-2-(methoxymethyl)pyrrolidine (*S*)-**19** (0.300 g, 2.30 mmol) in cyclohexane (10 mL) was added propiophenone (0.309 g, 2.30 mmol) dropwise under a $\mathrm{N_2}$ atmosphere. The reaction mixture was allowed stir at 60 °C overnight and reaction progress monitored by TLC analysis. On completion, the reaction mixture was poured into a 6:1 DCM:water mixture and layers separated. The organic layer was dried over $\mathrm{MgSO_4}$ and concentrated *in vacuo* to give the crude product as an orange oil which was purified by silica column chromatography eluting with 90:10 hexane:ethyl acetate to yield (*S*)-**163** as a yellow oil (300 mg, 52%).

$[\alpha]^{20}_{\mathrm{D}}$ = +651.0 (c 1.3, $\mathrm{CHCl_3}$) [lit.[5] $[\alpha]^{20}_{\mathrm{D}}$ = +733.0 (c 1.3, $\mathrm{C_6H_6}$)]; IR (NaCl) ν_{max}: 2936, 2827 (C-H alkyl stretch, s), 1690 (C=N stretch, m), 1460, 1445 (C=C arom. stretch, s), 1101 (C-O stretch, s) $\mathrm{cm^{-1}}$; ^{1}H NMR (300 MHz, $\mathrm{CDCl_3}$): δ (mixture of *E*/Z isomers, 86:14 major:minor) 1.03 and 1.08 (3.4H, 2 × overlapping t, *J* = 7.6 Hz, $\mathrm{C\boldsymbol{H_3}CH_2}$ major and minor), 1.48–1.64 (0.4H, m, $\mathrm{NCHC\boldsymbol{H_2}}$ minor), 1.67–1.79 (1.5H, m, one of $\mathrm{NCHC\boldsymbol{H_2}}$ major and $\mathrm{NCH_2C\boldsymbol{H_2}}$ minor), 1.84–1.95 (2H, m, $\mathrm{NCH_2C\boldsymbol{H_2}}$ major), 2.01–2.12 (1H, m, one of $\mathrm{NCHC\boldsymbol{H_2}}$ major), 2.21–2.29 (0.15H, m, one of $\mathrm{NC\boldsymbol{H_2}}$ minor), 2.49–2.68 (1.4H, m, one of $\mathrm{NC\boldsymbol{H_2}}$ major and minor and one of $\mathrm{CH_3C\boldsymbol{H_2}}$ minor), 2.84 (2H, 2 × overlapping q, *J* = 7.7 Hz, $\mathrm{CH_3C\boldsymbol{H_2}}$ major), 3.00 (0.14H, q, *J* = 7.3 Hz, one of $\mathrm{CH_3C\boldsymbol{H_2}}$ minor), 3.27–3.43 (3.8H, m, one of $\mathrm{C\boldsymbol{H_2}OCH_3}$ major and minor, $\mathrm{NC\boldsymbol{H}}$ major and minor and one of $\mathrm{NC\boldsymbol{H_2}}$ and $\mathrm{OC\boldsymbol{H_3}}$ minor), 3.36 (3H, s, $\mathrm{OC\boldsymbol{H_3}}$ major), 3.51 (1H, dd, *J* = 3.7, 8.9 Hz, one of $\mathrm{C\boldsymbol{H_2}OCH_3}$ major), 3.61 (0.16H, dd, *J* = 3.7, 8.9 Hz, one of $\mathrm{C\boldsymbol{H_2}OCH_3}$ minor), 7.34–7.36 (3.49H, m, 3 × ***C***H arom. major and minor), 7.42–7.48 (0.13 H, m, C***H*** arom. minor), 7.66–7.69 (2H, m, 2 × ***C***H arom. major), 7.95–7.98 (0.13H, m, C***H*** arom. minor) ppm; ^{13}C NMR (75.5 MHz, $\mathrm{CDCl_3}$): δ (only major isomer reported) 11.8 ($\mathrm{\boldsymbol{C}H_3CH_2}$), 22.5 ($\mathrm{NCH_2\boldsymbol{C}H_2}$), 22.6 ($\mathrm{CH_3\boldsymbol{C}H_2}$), 26.7 ($\mathrm{NCH\boldsymbol{C}H_2}$), 55.6 ($\mathrm{N\boldsymbol{C}H_2}$), 59.2 ($\mathrm{O\boldsymbol{C}H_3}$), 66.8 ($\mathrm{N\boldsymbol{C}H}$), 75.6 ($\mathrm{\boldsymbol{C}H_2OCH_3}$), 126.8, 128.2, 128.7 (5 × ***C***H arom.), 138.1 ($\mathrm{CH_3CH_2C{=}N\boldsymbol{C}}$), 165.5 ($\mathrm{\boldsymbol{C}{=}N}$) ppm; HRMS (ESI) *m*/*z* calcd for $\mathrm{C_{15}H_{23}N_2O}$ [$\mathrm{(M + H)^+}$]: 247.1810, found 247.1800; Anal. calcd for $\mathrm{C_{15}H_{22}N_2O}$: C, 73.13; H, 9.00; N, 11.37%. Found: C, 73.53; H, 9.12; N, 11.49%.

(*S*)-2-(Methoxymethyl)-*N*-(pentan-3-ylidene)pyrrolidin-1-amine, (*S*)-114

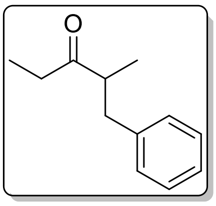


To a stirred solution of (*S*)-(-)-1-amino-2-(methoxymethyl)pyrrolidine (*S*)-**19** (0.500 g, 3.84 mmol) in cyclohexane (10 mL) was added 3-pentanone (0.331 g, 3.84 mmol) dropwise under a N_2 atmosphere. The reaction mixture was allowed stir at 60 °C overnight and reaction progress monitored by TLC analysis. On completion, the reaction mixture was poured into a 6:1 DCM:water mixture and layers separated. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to give (*S*)-**114** as an orange oil (0.578 g, 76%). $[\alpha]_D^{20}$ = +321.5 (c 1.1, $CHCl_3$) [lit.[5] $[\alpha]_D^{20}$ = +297.0 (c 1.0, C_6H_6)]; IR (NaCl) ν_{max}: 2970, 2877 (C-H alkyl stretch, s), 1636 (C=N stretch, m), 1100 (C-O stretch, s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.06 (3H, t, *J* = 7.5 Hz, C***H₃***), 1.08 (3H, t, *J* = 7.5 Hz, C***H₃***), 1.60–1.72 (1H, m, one of NC***H₂***), 1.77–1.87 (2H, m, $NCH_2C\boldsymbol{H_2}$), 1.95–2.07 (1H, m, one of NC***H₂***), 2.17–2.28 (2H, m, $CH_3C\boldsymbol{H_2}$), 2.32–2.54 (3H, m, $CH_3C\boldsymbol{H_2}$ and one of NC***H₂***), 3.01–3.16 (2H, m, NC***H*** and one of NCHC***H₂***), 3.21 (1H, dd, *J* = 9.2, 7.0 Hz, one of $CHC\boldsymbol{H_2}OCH_3$), 3.33 (3H, s, OC***H₃***), 3.39 (1H, dd, *J* = 9.2, 3.9 Hz, one of $CHC\boldsymbol{H_2}OCH_3$) ppm; δ_C ($CDCl_3$, 75.5 MHz) 10.9 (***C***H_3), 11.8 (***C***H_3), 21.9 ($NCH_2\boldsymbol{C}H_2$), 23.5 ($CH_3\boldsymbol{C}H_2$), 26.6 (N***C***H_2), 28.7 ($CH_3\boldsymbol{C}H_2$), 55.0 (N***C***H_2), 59.1 (O***C***H_3), 66.0 (N***C***H), 75.4 (***C***H_2OCH_3), 178.5 (***C***=N) ppm; HRMS (ESI) *m/z* calcd for $C_{11}H_{23}N_2O$ [(M + H)$^+$]: 199.1810, found 199.1812.

3.3 General procedure for synthesis of racemic α-alkylated ketones for use as GC standards

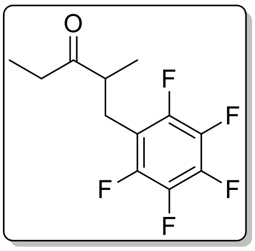
To a stirred solution of commercially available 1.6 M LDA (1.1 equiv.) in freshly distilled THF (5 mL) in a Schlenk tube under N_2 atmosphere was added ketone (1.0 equiv.) dropwise at -78 °C and the reaction was stirred at this temperature for 30 min. A solution of the electrophile (1.2 equiv.) in THF (3 mL) was added dropwise at -78 °C and reaction allowed to stir at this temperature for 1 h. The reaction was allowed warm to room temperature overnight. Saturated aq. NH_4Cl solution (10 mL) was added and the crude product extracted with ethyl acetate or diethyl ether (3 × 15 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product, which was purified by column chromatography on silica gel.

2-Methyl-1-phenylpentan-3-one, 143



3-Pentanone (91 mg, 1.06 mmol) was alkylated with benzyl bromide (200 mg, 1.17 mmol) as per general procedure **3.3** and purified by silica column chromatography 30:1 hexane:diethyl ether to yield **143** as a pale yellow oil (58 mg, 31%). Spectral characteristics were consistent with previously reported data.[6] ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.97 (3H, t, *J* = 7.3 Hz, $C\boldsymbol{H_3}CH_2$), 1.08 (3H, d, *J* = 6.9 Hz, $C\boldsymbol{H_3}CH$), 2.19–2.32 (1H, m, one of $CH_3C\boldsymbol{H_2}$), 2.37–2.50 (1H, m, one of $CH_3C\boldsymbol{H_2}$), 2.57 (1H, dd, *J* = 7.2, 13.2 Hz, one of $CH_3CHC\boldsymbol{H_2}$), 2.84 (1H, m, $CH_3C\boldsymbol{H}$), 2.97 (1H, dd, *J* = 7.2, 13.2 Hz, one of $CH_3CHC\boldsymbol{H_2}$), 7.12–7.16 (2H, m, 2 × C***H*** arom.), 7.18–7.21 (1H, m, C***H*** arom.), 7.24–7.30 (2H, m, 2 × C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 7.6 ($\boldsymbol{C}H_3CH_2$), 16.6 ($\boldsymbol{C}H_3CH$), 35.2 ($CH_3\boldsymbol{C}H_2$), 39.3 ($CH_3CH\boldsymbol{C}H_2$), 47.9 ($CH_3\boldsymbol{C}H$), 126.2 (***C***H arom.), 128.4 (2 × ***C***H arom.), 128.9 (2 × ***C***H arom.), 139.9 ($CH_3CHCH_2\boldsymbol{C}$), 214.8 (***C***=O) ppm; MS (ESI) *m/z*: 177 [$(M + H)^+$, 2%].

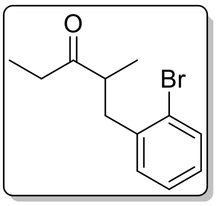
2-Methyl-1-(perfluorophenyl)pentan-3-one, 144



3-Pentanone (155 mg, 1.8 mmol) was alkylated with pentafluorobenzyl bromide (520 mg, 2.0 mmol) as per general procedure **3.3** and purified by silica column chromatography 90:10 hexane:diethyl ether to yield **144** as a yellow oil (136 mg, 28%). IR (NaCl) ν_{max}: 2980 (C-H alkyl stretch, m), 2941 (C-H alkyl stretch, m), 1717 (C=O stretch, s), 1503, 1522 (C=C arom. stretch, s), 1124 (C-F stretch, m) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.06 (3H, t, *J* = 7.3 Hz, $C\boldsymbol{H_3}CH_2$), 1.11 (3H, d, *J* = 6.9 Hz, $C\boldsymbol{H_3}CH$), 2.37–2.64 (2H, m $CH_3C\boldsymbol{H_2}$), 2.71–2.91 (2H, m, $CH_3CHC\boldsymbol{H_2}$), 2.97–3.03 (1H, m, $CH_3C\boldsymbol{H}$) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ 7.7 ($\boldsymbol{C}H_3CH_2$), 16.1 ($\boldsymbol{C}H_3CH$), 25.1 ($CH_3CH\boldsymbol{C}H_2$), 34.2 ($CH_3\boldsymbol{C}H_2$), 45.1 ($CH_3\boldsymbol{C}H$), 113.0 (apparent td, *J* = 3.8, 17.5 Hz, $CH_3CHCH_2\boldsymbol{C}$), 136.3–136.6, 138.3–138.7 (dm, *J* = 248.8 Hz, 2 × $CH_2C\boldsymbol{C}F$), 139.8 (dtt, *J* = 5.0, 13.8, 256.3 Hz, $CH_2CCFCF\boldsymbol{C}F$), 144.1–144.4, 146.1–146.3

(dm, *J* = 247.5 Hz, 2 × $CH_2CCF\boldsymbol{C}F$), 212.7 ($\boldsymbol{C}$=O) ppm; ^{19}F NMR (470 MHz, $CDCl_3$): δ -142.7 (dd, *J* = 4.7, 18.8 Hz, 2 × $CC\boldsymbol{F}CFCF$), -156.8 (t, *J* = 18.8 Hz, $CCFCFC\boldsymbol{F}$), -162.6 (m, 2 × $CCFC\boldsymbol{F}CF$) ppm; HRMS (ESI) *m/z* calcd for $C_{12}H_{12}F_5O$ [(M + H)$^+$]: 267.0808, found 267.0821.

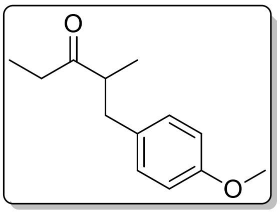
1-(2-Bromophenyl)-2-methylpentan-3-one, 145



3-Pentanone (860 mg, 10 mmol) was alkylated with 2-bromobenzyl bromide (2.750 g, 11 mmol) as per general procedure **3.3** and purified by silica column chromatography 40:1 hexane:ethyl acetate to yield **145** as a yellow oil (1.40 g, 55%). Spectral characteristics were consistent with previously reported data.[7]

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.98 (3H, t, *J* = 7.3 Hz, $C\boldsymbol{H_3}CH_2$), 1.09 (3H, d, *J* = 6.9 Hz, $C\boldsymbol{H_3}CH$), 2.28 (1H, dq, *J* = 7.3, 17.8 Hz, one of $CH_3C\boldsymbol{H_2}$), 2.47 (1H, dq, *J* = 7.3, 17.8 Hz, one of $CH_3C\boldsymbol{H_2}$), 2.68 (1H, dd, *J* = 6.9, 12.8 Hz, one of $CHC\boldsymbol{H_2}$), 2.95–3.13 (2H, m, $CH_3C\boldsymbol{H}$ and one of $CHC\boldsymbol{H_2}$), 7.04–7.09 (1H, m, $C\boldsymbol{H}$ arom.), 7.13–7.23 (2H, m, 2 × $\boldsymbol{C}H$ arom.), 7.53 (1H, dd, *J* = 1.2, 7.9 Hz, $C\boldsymbol{H}$ arom.) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 7.6 ($\boldsymbol{C}H_3CH_2$), 16.5 ($\boldsymbol{C}H_3CH$), 35.3 ($CH_3\boldsymbol{C}H_2$), 39.3 ($CH\boldsymbol{C}H_2$), 45.4 ($CH_3\boldsymbol{C}H$), 124.6 ($\boldsymbol{C}Br$), 127.3 ($\boldsymbol{C}H$ arom.), 128.0 ($\boldsymbol{C}H$ arom.), 131.6 ($\boldsymbol{C}H$ arom.), 132.9 ($\boldsymbol{C}H$ arom.), 139.2 ($CH_3CHCH_2\boldsymbol{C}$), 214.5 ($\boldsymbol{C}$=O) ppm; MS (ESI) *m/z*: 256 [(M + H)$^+$, 6%].

1-(4-Methoxyphenyl)-2-methylpentan-3-one, 146

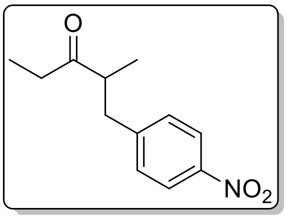


3-Pentanone (223 mg, 2.71 mmol) was alkylated with 4-methoxybenzyl bromide (600 mg, 2.98 mmol) as per general procedure **3.3** and purified by silica column chromatography 20:1 hexane:ethyl acetate to yield **146** as a pale yellow oil (194 mg, 35%). Spectral characteristics were consistent with previously reported data.[8]

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.97 (3H, t, *J* = 7.3 Hz, $C\boldsymbol{H_3}CH_2$), 1.07 (3H, d, *J* = 6.8 Hz, $C\boldsymbol{H_3}CH$), 2.24 (1H, dq, *J* = 7.3, 17.9 Hz, one of $CH_3C\boldsymbol{H_2}$), 2.42 (1H, dq, *J* = 7.3, 17.9 Hz, one of $CH_3C\boldsymbol{H_2}$), 2.51 (1H, dd, *J* = 6.9, 13.1 Hz, one of $CHC\boldsymbol{H_2}$), 2.80 (1H, m, $C\boldsymbol{H}$), 2.90 (1H, dd, *J* = 7.3, 13Hz, one of $CHC\boldsymbol{H_2}$), 3.78 (3H, s, $OC\boldsymbol{H_3}$), 6.81 (2H, d, *J* = 8.7 Hz, 2 × $C\boldsymbol{H}$ arom.), 7.05 (2H, d, *J* = 8.6 Hz, 2 × $C\boldsymbol{H}$

arom.) ppm; ^{13}C NMR (75.5 MHz, CDCl$_3$): δ 7.6 (***C***H$_3$CH$_2$), 16.6 (***C***H$_3$CH), 35.3 (CH$_3$***C***H$_2$), 38.5 (CH***C***H$_2$), 48.1 (CH$_3$***C***H), 55.2 (O***C***H$_3$), 113.8 (2 × ***C***H arom.), 129.9 (2 × ***C***H arom.), 131.9 (CH$_3$CHCH$_2$***C***), 158.0 (***C***OCH$_3$), 215.1 (***C***=O) ppm; MS (ESI) *m/z*: 207 [(M + H)$^+$, 10%].

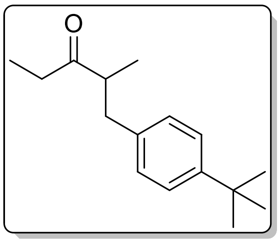
2-Methyl-1-(4-nitrophenyl)pentan-3-one, 147



3-Pentanone (172 mg, 2.0 mmol) was alkylated with 4-nitrobenzyl bromide (475 mg, 2.2 mmol) as per general procedure **3.3** and purified by silica column chromatography 90:10 hexane: diethyl ether to yield **147** as a colourless oil (124 mg, 28%).

IR (NaCl) ν_{max}: 2975 (C-H alkyl stretch, m), 2937 (C-H alkyl stretch, m), 1712 (C=O stretch, s), 1519 (NO$_2$ asymmetric stretch, s), 1346 (NO$_2$ symmetric stretch, s) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 0.99 (3H, t, *J* = 7.3 Hz, C***H$_3$***CH$_2$), 1.13 (3H, d, *J* = 7.0 Hz, C***H$_3$***CH), 2.26 (1H, dq, *J* = 7.3, 17.9 Hz, one of CH$_3$C***H$_2$***), 2.50 (1H, dq, *J* = 7.3, 17.9 Hz, one of CH$_3$C***H$_2$***), 2.68 (1H, dd, *J* = 6.8, 13.4 Hz, one of CHC***H$_2$***), 2.88 (1H, m, CH$_3$C***H***), 3.11 (1H, dd, *J* = 7.6, 13.4 Hz, one of CHC***H$_2$***), 7.31 (2H, d, *J* = 8.8 Hz, 2 × C***H*** arom.), 8.14 (2H, d, *J* = 8.8 Hz, 2 × C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, CDCl$_3$): δ 7.6 (***C***H$_3$CH$_2$), 17.0 (***C***H$_3$CH), 35.1 (CH$_3$***C***H$_2$), 38.6 (CH***C***H$_2$), 47.5 (CH$_3$***C***H), 123.7 (2 × ***C***HCNO$_2$, 129.8 (2 × ***C***HCHCNO$_2$) 146.6 (CH$_3$CHCH$_2$***C***), 147.9 (***C***N), 213.6 (***C***=O) ppm; HRMS (ESI) *m/z* calcd for $C_{12}H_{16}NO_3$ [(M + H)$^+$]: 222.1130, found 222.1122; Anal. calcd for $C_{12}H_{15}NO_3$: C, 65.14; H, 6.83; N, 6.33%. Found: C, 65.28; H, 6.77; N, 6.41%.

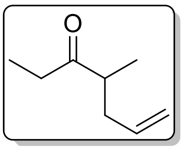
1-(4-(*tert*-Butyl)phenyl)-2-methylpentan-3-one, 148



3-Pentanone (200 mg, 2.32 mmol) was alkylated with 4-*t*-butylbenzyl bromide (631 mg, 2.78 mmol) as per general procedure **3.3** and purified by silica column chromatography 90:10 hexane:ethyl acetate to yield **148** as a pale yellow oil (63 mg, 12%). Spectral characteristics were consistent with previously reported data.[8] ^{1}H NMR (300 MHz, CDCl$_3$): δ 0.98 (3H, t, *J* = 7.3 Hz, C***H$_3$***CH$_2$), 1.08 (3H, d, *J* = 6.9 Hz, C***H$_3$***CH), 1.30 (9H, s, 3 × C***H$_3$***), 2.29 (1H, dq, *J* = 7.3, 17.8 Hz, one of

$\mathrm{CH_3C}\boldsymbol{H_2}$), 2.44 (1H, dq, *J* = 7.3, 17.8 Hz, one of $\mathrm{CH_3C}\boldsymbol{H_2}$), 2.53 (1H, dd, *J* = 7.4, 13.3 Hz, one of $\mathrm{CH_3CHC}\boldsymbol{H_2}$), 2.82 (1H, m, $\mathrm{CH_3C}\boldsymbol{H}$), 2.95 (1H, dd, *J* = 6.9, 13.3 Hz, one of $\mathrm{CH_3CHC}\boldsymbol{H_2}$), 7.06 (2H, d, *J* = 8.4Hz, 2 × $\mathrm{C}\boldsymbol{H}$ arom.), 7.29 (2H, d, *J* = 8.4 Hz, 2 × $\mathrm{C}\boldsymbol{H}$ arom.) ppm; ^{13}C NMR (75.5 MHz, $\mathrm{CDCl_3}$): δ 7.6 ($\boldsymbol{C}\mathrm{H_3CH_2}$), 16.6 ($\boldsymbol{C}\mathrm{H_3CH}$), 31.4 (3 × $\boldsymbol{C}\mathrm{H_3}$), 34.4 ($\boldsymbol{C}\mathrm{(CH_3)_3}$), 35.0 ($\mathrm{CH_3}\boldsymbol{C}\mathrm{H_2}$), 38.7 ($\mathrm{CH_3CH}\boldsymbol{C}\mathrm{H_2}$), 47.9 ($\mathrm{CH_3}\boldsymbol{C}\mathrm{H}$), 125.3 (2 × $\boldsymbol{C}\mathrm{H}$ arom.), 128.6 (2 × $\boldsymbol{C}\mathrm{H}$ arom.), 136.7 ($\mathrm{CH_3CHCH_2}\boldsymbol{C}$), 149.0 ($\boldsymbol{C}\mathrm{C(CH_3)_3}$), 215.0 ($\boldsymbol{C}\mathrm{=O}$) ppm; MS (ESI) *m/z*: 233 [$(\mathrm{M + H})^+$, 16%].

4-Methylhept-6-en-3-one, 149

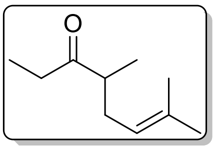


3-Pentanone (0.800 g, 9.29 mmol) was alkylated with allylbromide (1.349 g, 11.15 mmol) as per general procedure **3.3** and purified by silica column chromatography 90:10 hexane:diethyl ether to yield **149** as a yellow oil (0.441 g, 38%).

Spectral characteristics were consistent with previously reported data.[9, 10]

^{1}H NMR (300 MHz, $\mathrm{CDCl_3}$): δ 1.04 (3H, t, *J* = 7.3 Hz, $\mathrm{C}\boldsymbol{H_3}\mathrm{CH_2}$), 1.08 (3H, d, *J* = 7.0 Hz, $\mathrm{C}\boldsymbol{H_3}\mathrm{CH}$), 2.04–2.17 (1H, m, one of $\mathrm{CH_3CHC}\boldsymbol{H_2}$), 2.34–2.42 (1H, m, one of $\mathrm{CH_3CHC}\boldsymbol{H_2}$), 2.46 (2H, m, $\mathrm{CH_3C}\boldsymbol{H_2}$), 2.61 (1H, m, $\mathrm{CH_3C}\boldsymbol{H}$), 4.98–5.07 (2H, m, $\mathrm{C}\boldsymbol{H_2}$ alkene), 5.65–5.79 (1H, m, $\mathrm{C}\boldsymbol{H}$ alkene) ppm; ^{13}C NMR (75.5 MHz, $\mathrm{CDCl_3}$): δ 7.7 ($\boldsymbol{C}\mathrm{H_3CH_2}$), 16.2 ($\boldsymbol{C}\mathrm{H_3CH}$), 34.4 ($\mathrm{CH_3}\boldsymbol{C}\mathrm{H_2}$), 37.2 ($\mathrm{CH}\boldsymbol{C}\mathrm{H_2}$), 45.7 ($\mathrm{CH_3}\boldsymbol{C}\mathrm{H}$), 116.7 ($\boldsymbol{C}\mathrm{H_2}$ alkene), 135.8 ($\boldsymbol{C}\mathrm{H}$ alkene), 214.5 ($\boldsymbol{C}\mathrm{=O}$) ppm; MS (ESI) *m/z*: 127 [$(\mathrm{M + H})^+$, 20%].

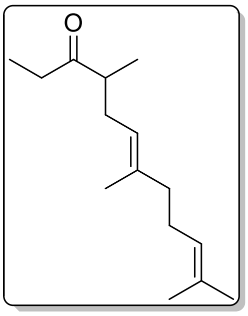
4,7-Dimethyloct-6-en-3-one, 150



3-Pentanone (0.600 g, 6.97 mmol) was alkylated with 3,3-dimethylallyl bromide (1.140 g, 7.67 mmol) as per general procedure **3.3** and purified by silica column chromatography 90:10 hexane:diethyl ether to yield **150** as a pale yellow oil (0.265 g, 25%). IR (NaCl) ν_{max}: 2972, 2935 (C-H alkyl stretch, s), 1714 (C=O stretch, s), 1458 (C=C alkene stretch, m) cm^{-1}; ^{1}H NMR (300 MHz, $\mathrm{CDCl_3}$): δ 1.03 (3H, t, *J* = 7.3 Hz, $\mathrm{C}\boldsymbol{H_3}\mathrm{CH_2}$), 1.06 (3H, d, *J* = 6.9 Hz, $\mathrm{C}\boldsymbol{H_3}\mathrm{CH}$), 1.60 (3H, s, $\mathrm{C}\boldsymbol{H_3}$ alkene), 1.68 (3H, s, $\mathrm{C}\boldsymbol{H_3}$ alkene), 2.00–2.10 (1H, m, one of $\mathrm{CH_3CHC}\boldsymbol{H_2}$), 2.24–2.34 (1H, m, one of $\mathrm{CH_3CHC}\boldsymbol{H_2}$), 2.45 (2H, q, *J* = 7.3 Hz, $\mathrm{CH_3C}\boldsymbol{H_2}$), 2.57 (1H, m, $\mathrm{C}\boldsymbol{H}$), 5.04

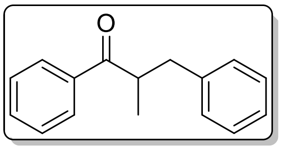
(1H, m, C***H*** alkene) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 7.7 (***C***H_3CH_2), 16.1 (***C***H_3CH), 17.7, 25.7 (2 × ***C***H_3 alkene), 31.6 (CH_3CH***C***H_2), 34.5 (CH_3***C***H_2), 46.3 (CH_3***C***H), 121.5 (***C***H alkene), 133.5 (C=**C**$(CH_3)_2$), 215.2 (***C***=O) ppm; HRMS (ESI) *m/z* calcd for $C_{10}H_{19}O$ $[(M + H)^+]$: 155.1436, found 155.1432.

4,7,11-Trimethyldodeca-6,10-dien-3-one, 151



3-Pentanone (0.860 g, 10 mmol) was alkylated with geranyl bromide (2.170 g, 10 mmol) as per general procedure **3.3** and purified by silica column chromatography 90:10 hexane:diethyl ether to yield **151** as a colourless oil (0.821 g, 37%). IR (NaCl) ν_{max}: 2970, 2932 (C-H alkyl stretch, s), 1715 (C=O stretch, s), 1671 (C=C stretch, m), 1456 (C-H alkyl bend, m) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.04 (3H, t, *J* = 7.3 Hz, C***H₃***CH_2), 1.06 (3H, d, *J* = 6.9 Hz, C***H₃***CH), 1.59 (6H, s, C***H₃***CC***H₃***), 1.67 (3H, s, C***H₃***C=$CHCH_2$), 1.93–2.11 (5H, m, $(CH_3)_2C$=CHC***H₂***C***H₂*** and one of CH_3CHC***H₂***), 2.25–2.35 (1H, m, one of CH_3CHC***H₂***), 2.44 (2H, q, *J* = 7.3 Hz, CH_3C***H₂***), 2.56 (1H, m, CH_3C***H***), 5.02–5.09 (2H, m, 2 × C***H*** alkene) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 7.8 (***C***H_3CH_2), 16.0 (***C***H_3CCH_3), 16.1 (***C***H_3CH), 17.7 (CH_3C***C***H_3), 25.7 (***C***H_3C=$CHCH_2$), 26.6 ($(CH_3)_2C$=CH***C***H_2), 31.6 (CH_3CH***C***H_2), 34.6 (CH_3***C***H_2), 39.8 ($(CH_3)_2C$=$CHCH_2$***C***H_2), 46.4 (CH_3***C***H), 121.5 (CH_3CHCH_2***C***H=C), 124.2 ($(CH_3)_2C$=***C***HCH_2), 131.4 (CH_3***C***CH_3), 137.1 (CH_3CHCH_2CH=***C***CH_3), 215.2 (***C***=O) ppm; HRMS (ESI) *m/z* calcd for $C_{15}H_{27}O$ $[(M + H)^+]$: 223.2062, found 223.2054; Anal. calcd. for $C_{15}H_{26}O$: C, 81.02; H, 11.79% Found: C, 80.70; H, 12.00%.

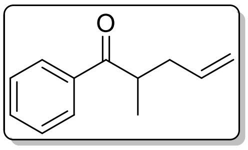
2-Methyl-1,3-diphenylpropan-1-one, 152



Propiophenone (0.500 g, 3.73 mmol) was alkylated with benzyl bromide (0.701 g, 4.10 mmol) as per general procedure **3.3** and purified by silica column chromatography 20:1 hexane:diethyl ether to yield **152** as a pale yellow oil (0.142 g, 17%). Spectral characteristics were consistent with previously reported data.[11] ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.20 (3H, d, *J* = 6.9 Hz, C***H₃***), 2.69 (1H, dd, *J* = 7.9, 13.7 Hz, one of CH_3CHC***H₂***), 3.17 (1H, dd, *J* = 6.3, 13.7 Hz, one of

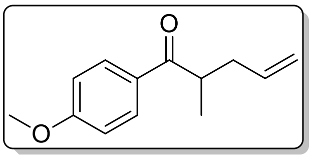
$CH_3CHC\boldsymbol{H_2}$), 3.75 (1H, m, $CH_3C\boldsymbol{H}$), 7.15–7.29 (5H, m, 5 × C***H*** arom.), 7.41–7.47 (2H, m, 2 × C***H*** arom.), 7.51–7.57 (1H, m, C***H*** arom.), 7.91–7.95 (2H, m, 2 × C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 17.4 (***C***H_3), 39.4 (***C***H_2), 42.8 (CH_3***C***HCH_2) 126.3 (CH_2CCHCH***C***H), 128.3 (2 × ***C***H arom.), 128.4 (2 × ***C***H arom.), 128.7 (2 × ***C***H arom.), 129.1 (2 × ***C***H arom.), 133.0 (C=OCCHCH***C***H), 136.4 (***C***C=OCH), 140.0 (***C***CH_2CH), 203.8 (***C***=O) ppm; MS (ESI) *m/z*: 225 [$(M + H)^+$, 10%].

2-Methyl-1-phenylpent-4-en-1-one, 153



Propiophenone (0.500 g, 3.73 mmol) was alkylated with allylbromide (0.496 g, 4.10 mmol) as per general procedure **3.3** and purified by silica column chromatography 90:10 hexane:diethyl ether to yield **153** as a pale yellow oil (0.141 g, 22%). Spectral characteristics were consistent with previously reported data.[12,13] ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21 (3H, d, *J* = 6.9 Hz, $C\boldsymbol{H_3}$), 2.15–2.25 (1H, m, one of $CH_3CHC\boldsymbol{H_2}$), 2.52–2.61 (1H, m, one of $CH_3CHC\boldsymbol{H_2}$), 3.54 (1H, m, $CH_3C\boldsymbol{H}$), 4.99–5.09 (2H, m, $C\boldsymbol{H_2}$ alkene), 5.72–5.86 (1H, m, C***H*** alkene), 7.47 (2H, t, *J* = 7.7 Hz, 2 × C***H***CHCCO), 7.56 (1H, t, *J* = 7.2 Hz, C***H***CHCHCCO), 7.96 (2H, d, *J* = 7.0 Hz, 2 × C***H***CCO) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 17.0 (***C***H_3), 37.6 (CH_3CH***C***H_2), 40.4 (***C***H), 116.8 (***C***H_2 alkene), 128.3 (2 × C***H***CHCCO), 128.7 (2 × C***H***CCO), 132.9 (***C***HCHCHCCO), 135.8 (***C***H alkene), 136.5 (CH_3CHCO***C***), 203.7 (***C***=O) ppm; MS (ESI) *m/z*: 175 [$(M + H)^+$, 100%].

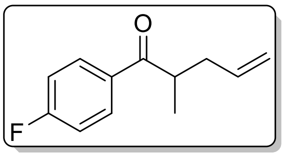
1-(4-Methoxyphenyl)-2-methylpent-4-en-1-one, 154



4-Methoxypropiophenone (0.600 g, 3.65 mmol) was alkylated with allylbromide (0.486 g, 4.02 mmol) as per general procedure **3.3** and purified by silica column chromatography 90:10 hexane:ethyl acetate to yield **154** as a yellow oil (0.194 g, 26%).

Spectral characteristics were consistent with previously reported data.[14] ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.20 (3H, d, *J* = 6.9 Hz, $C\boldsymbol{H_3}$), 2.13–2.24 (1H, m, one of $CH_3CHC\boldsymbol{H_2}$), 2.50–2.60 (1H, m, one of $CH_3CHC\boldsymbol{H_2}$), 3.50 (1H, m, $CH_3C\boldsymbol{H}$), 3.87 (3H, s, $OC\boldsymbol{H_3}$), 4.99–5.09 (2H, m, $C\boldsymbol{H_2}$ alkene), 5.72–5.85 (1H, m, C***H*** alkene), 6.95 (2H, d, *J* = 8.9 Hz, 2 × C***H***CHOCH$_3$), 7.95 (2H, d, *J* = 8.9 Hz, 2 × C***H***COCH$_3$) ppm; ^{13}C NMR (75.5 MHz, CDCl$_3$): δ 17.2 (***C***H$_3$), 37.8 (CH$_3$CH***C***H$_2$), 40.0 (CH$_3$***C***H), 55.5 (O***C***H$_3$), 113.8 (2 × ***C***HCOCH$_3$), 116.6 (***C***H$_2$ alkene), 129.4 (CH$_3$CHCO***C***), 130.6 (2 × ***C***HCHCOCH$_3$), 136.0 (***C***H alkene), 163.4 (***C***OCH$_3$), 202.2 (***C***=O) ppm; MS (ESI) *m/z*: 205.3 [(M + H)$^+$, 100%].

1-(4-Fluorophenyl)-2-methylpent-4-en-1-one, 155



4-Fluoropropiophenone (0.600 g, 3.94 mmol) was alkylated with allylbromide (0.524 g, 4.33 mmol) as per general procedure **3.3** and purified by silica column chromatography 90:10 hexane:ethyl acetate to yield **155** as a yellow oil (0.392 g, 52%).

IR (NaCl) ν_{max}: 2977, 2935 (C-H alkyl stretch, s), 1683 (C=O stretch, s), 1641 (C=C alkene stretch), 1598, 1506 (C=C arom. stretch, s), 1158 (C-F stretch, s) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 1.13 (3H, d, *J* = 6.9 Hz, C***H***$_3$), 2.08–2.18 (1H, m, one of CH$_3$CHC***H***$_2$), 2.43–2.53 (1H, m, one of CH$_3$CHC***H***$_2$), 3.42 (1H, m, CH$_3$C***H***), 4.92–5.01 (2H, m, C***H***$_2$ alkene), 5.63–5.77 (1H, m, C***H*** alkene), 7.07 (2H, t, *J* = 8.7 Hz, 2 × C***H***CF), 7.91 (2H, dd, *J* = 5.4, 8.9 Hz, 2 × C***H***CHCF) ppm; ^{13}C NMR (75.5 MHz, CDCl$_3$): δ 17.1 (***C***H$_3$), 37.6 (CH$_3$CH***C***H$_2$), 40.4 (CH$_3$***C***H), 115.7 (d, $^2J_{C-F}$ = 21.9 Hz, 2 × ***C***HCF), 116.9 (***C***H$_2$ alkene), 130.9 (d, $^3J_{C-F}$ = 9.3 Hz, 2 × ***C***HCHCF), 132.8 (d, $^4J_{C-F}$ = 2.9 Hz, CH$_3$CHCO***C***), 135.7 (***C***H alkene), 165.6 (d, J_{C-F} = 254.5 Hz, ***C***F), 202.0 (***C***=O) ppm; ^{19}F NMR (282 MHz, CDCl$_3$): δ -105.7 (s, ***F***) ppm; HRMS (ESI) *m/z* calcd for $C_{12}H_{14}OF$ [(M + H)$^+$]: 193.1029, found 193.1034.

3.4 General procedure for synthesis of chiral ketones

3.4.1 General procedure for alkylation of chiral hydrazone

To a stirred solution of freshly distilled diisopropylamine (1.1 equiv.) in freshly distilled diethyl ether (4 mL) in a N$_2$ backfilled Schlenk tube at -78 °C was added 1.6M *n*-BuLi (1.15 equiv.). The solution was allowed to stir at 0 °C for 30 min to generate a solution of LDA. Hydrazone (1.0 equiv.) was added dropwise at -78 °C and allowed to stir at 0 °C for 16 h. A solution of electrophile (1.2 equiv.) in dry diethyl ether (2-3 mL) in a separate Schlenk, which was previously evacuated and backfilled with N_2, was added dropwise to the solution of deprotonated hydrazone at -110 °C. The temperature of the reaction was held at -110 °C for 1 h, then at -70 °C for 5 h before being allowed to warm gradually to room temperature overnight. Saturated aq. NH_4Cl solution (10 mL) was added to quench the reaction, followed by extraction with diethyl ether (3 × 20 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo* to yield crude alkylated hydrazone which was hydrolysed without purification.

3.4.2 General procedures for hydrazone cleavage

Method A – HCl/diethyl ether hydrolysis

To a vigorously stirred solution of alkylated hydrazone in diethyl ether (5 mL/mmol alkylated hydrazone) was added 4 M HCl (3 mL/mmol alkylated hydrazone) and water (3 mL/mmol alkylated hydrazone). The reaction progress was monitored by TLC analysis every 10 min. On completion, water (10 mL) was added, followed by extraction with diethyl ether (3 × 25 mL). The organic layers were combined and washed with water (2 × 20 mL), dried over $MgSO_4$ and concentrated *in vacuo* to yield the desired ketone which was purified by silica column chromatography.

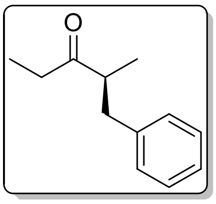
Method B – PPL hydrolysis

To a suspension of PPL (100 mg) in water (10 mL) was added a solution of alkylated hydrazone (1.05 mmol) in acetone (6 mL). The reaction was allowed to stir at room temperature for 23 h, diluted with diethyl ether (20 mL), washed with brine (3 × 15 mL), dried over $MgSO_4$ and concentrated *in vacuo* to yield the desired ketone which was purified by silica column chromatography.

Method C – Oxalic acid hydrolysis

To a vigorously stirred solution of alkylated hydrazone in diethyl ether (4 ml/mmol hydrazone) was added saturated aq. oxalic acid (1.5 ml/mmol hydrazone). The reaction progress was monitored by TLC analysis and on completion was added water (5 mL) and reaction mixture extracted with diethyl ether (3 × 20 mL). Combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo* to yield desired ketone which was purified by silica column chromatography.

(*S*)-2-Methyl-1-phenylpentan-3-one, (*S*)-143



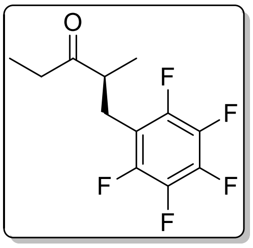
Hydrazone (*S*)-**156** (195 mg, 0.82 mmol (HCl/diethyl ether hydrolysis); 250 mg, 1.05 mmol (PPL hydrolysis)) was alkylated with benzyl bromide as per general procedure **3.4.1** and hydrolysed using either general procedure **3.4.2 Method A** to yield (*S*)-**143** as a pale yellow oil (10 mg, 7% over 2 steps, 89% ee), or **Method B** (19 mg, 10% over 2 steps, 83% ee).

Spectral characteristics were consistent with previously reported data[6] and with racemic **143**.

$[\alpha]_D^{23}$ = +94.4 (c 1.3, $CHCl_3$) [lit.[6] $[\alpha]_D^{23}$ = +70.9 (c 1.1, $CHCl_3$)].

Enantioselectivity was determined by GC analysis: **Method A**: 5.3 : 94.7 er, t_R = 25.6 (*R*-enantiomer) and 28.4 min (*S*-enantiomer) (90 °C hold for 33 min, ramp 10 °C/min to 140 °C, hold for 5 min). **Method C**: 8.4 : 91.6 er, , t_R = 7.5 (R-enantiomer) and 7.9 min (S-enantiomer) (120 °C hold for 10 min, ramp 5 °C/min to 140 °C, hold for 5 min). *Note difference in retention times and conditions due to use of older column for product of Method A and newer column for product of Method C*.

(*S*)-2-Methyl-1-(perfluorophenyl)pentan-3-one, (*S*)-144

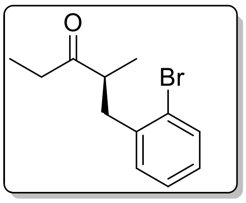


Hydrazone (*S*)-**156** (300 mg, 1.26 mmol) was alkylated with pentafluorobenzyl bromide as per general procedure **3.4.1** and hydrolysed using general procedure **3.4.2 Method A** to yield (*S*)-**144** as a yellow oil (115 mg, 34% over 2 steps, 49% ee). Spectral characteristics were identical to racemic **144**.

$[\alpha]_D^{20}$ = +4.7 (c 1, Et_2O).

Enantioselectivity was determined by GC analysis: 25.3 : 74.7 er, t_R = 5.9 (*R*-enantiomer) and 7.7 min (*S*-enantiomer) (120 °C hold for 10 min, ramp 5 °C/min to 140 °C, hold for 10 min).

(*S*)-1-(2-Bromophenyl)-2-methylpentan-3-one, (*S*)-145



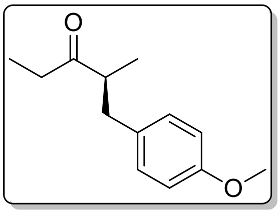
Hydrazone (*S*)-**156** (579 mg, 2.44 mmol) was alkylated with 2-bromobenzyl bromide as per general procedure **3.4.1** and hydrolysed using general procedure **3.4.2 Method A** to yield (*S*)-**145** as a yellow oil (131 mg, 21% over 2 steps, 86% ee).

Spectral characteristics were consistent with previously reported data[7] and with racemic **145**.

$[\alpha]_D^{20} = +48.6$ (c 1.5, Et_2O).

Enantioselectivity was determined by GC analysis: 7.0 : 93.0 er, $t_R = 18.6$ (*R*-enantiomer) and 19.7 min (*S*-enantiomer) (125 °C hold for 25 min, ramp 5 °C/min to 140 °C, hold for 5 min).

(*S*)-1-(4-Methoxyphenyl)-2-methylpentan-3-one, (*S*)-146



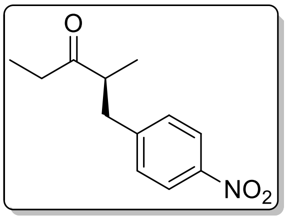
Hydrazone (*S*)-**156** (300 mg, 1.26 mmol) was alkylated with 4-methoxybenzyl bromide as per general procedure **3.4.1** and hydrolysed using general procedure **3.4.2 Method A** to yield (*S*)-**146** as a pale yellow oil (63 mg, 24% over 2 steps, 84% ee).

Spectral characteristics were consistent with previously reported data[8] and with racemic **146**.

$[\alpha]_D^{20} = +0.5$ (c 0.1, CH_2Cl_2).

Enantioselectivity was determined by GC analysis: 8.2 : 91.8 er, $t_R = 23.3$ (*R*-enantiomer) and 23.9 min (*S*-enantiomer) (120 °C hold for 15 min, ramp 5 °C/min to 140 °C, hold for 10 min).

(*S*)-2-Methyl-1-(4-nitrophenyl)pentan-3-one, (*S*)-147



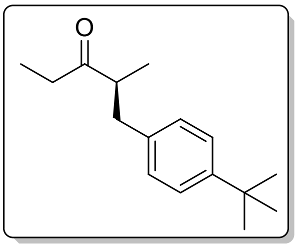
Hydrazone (*S*)-**156** (250 mg, 1.05 mmol) was alkylated with 4-nitrobenzyl bromide as per general procedure **3.4.1** and hydrolysed using general procedure **3.4.2 Method A** to yield (*S*)-**147** as a colourless oil (13 mg, 6% over 2 steps, 59% ee).

Spectral characteristics were consistent with racemic **147**.

$[\alpha]_D^{20}$ = +10.0 (c 0.3, Et_2O).

Enantioselectivity was determined by GC analysis: 20.5 : 79.5 er, t_R = 78.5 (*R*-enantiomer) and 91.8 min (*S*-enantiomer) (140 °C hold for 100 min).

(*S*)-1-(4-(*tert*-Butyl)phenyl)-2-methylpentan-3-one, (*S*)-148

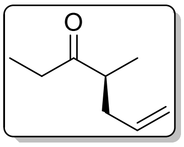


Hydrazone (*S*)-**156** (600 mg, 2.53 mmol) was alkylated with 4-*tert*-butylbenzyl bromide as per general procedure **3.4.1** and hydrolysed using general procedure **3.4.2** to yield (*S*)-**148** as a pale yellow oil (164 mg, 28% over 2 steps, 86% ee). Spectral characteristics were consistent with previously reported data[8] and with racemic **148**.

$[\alpha]_D^{20}$ = +59.4 (c 1.5, Et_2O).

Enantioselectivity was determined by GC analysis: 6.6 : 93.4 er, t_R = 12.8 (*R*-enantiomer) and 13.3 min (*S*-enantiomer) (140 °C hold for 25 min).

(*S*)-4-Methylhept-6-en-3-one, (*S*)-149



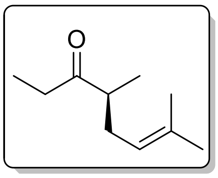
Hydrazone (*S*)-**156** was alkylated with either allyl iodide (213 mg, 0.90 mmol) or allyl bromide (295 mg, 1.24 mmol) as per general procedure **3.4.1** and hydrolysed using either general procedure **3.4.2 Method A** (allyl bromide) to yield (*S*)-**149** as a yellow oil (39 mg, 25% over 2 steps, 90% ee) or **Method B** (allyl iodide) (72 mg, 63% over 2 steps, 55% ee).

Spectral characteristics were consistent with previously reported data[9, 10] and with racemic **149**.

$[\alpha]_D^{20}$ = +2.8 (c 0.3, Et_2O).

Enantioselectivity was determined by GC analysis: **Method A**: 5.1 : 94.9 er, t_R = 2.9 (*R*-enantiomer) and 3.0 min (*S*-enantiomer); **Method B**: 22.5 : 77.5 er, t_R = 3.1 (*R*-enantiomer) and 3.2 min (*S*-enantiomer) (90 °C hold for 6 min, ramp 5 °C/min to 140 °C, hold for 10 min).

(*S*)-4,7-Dimethyloct-6-en-3-one, (*S*)-150



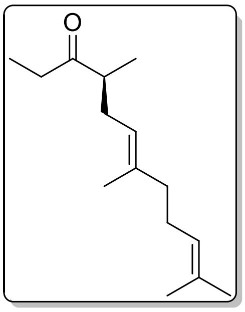
Hydrazone (*S*)-**156** (300 mg, 1.26 mmol) was alkylated with 3,3-dimethylallyl bromide as per general procedure **3.4.1** and hydrolysed using general procedure **3.4.2 Method A** to yield (*S*)-**150** as a pale yellow oil (36 mg, 19% over 2 steps, 90% ee).

Spectral characteristics were consistent with racemic **150**.

$[\alpha]^{20}_{D}$ = +2.5 (c 0.6, Et_2O).

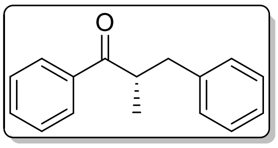
Enantioselectivity was determined by GC analysis: 4.9 : 95.1 er, t_R = 9.1 (*R*-enantiomer) and 9.4 min (*S*-enantiomer) (75 °C hold for 10 min, ramp 5 °C/min to 140 °C, hold for 10 min).

(*S*)-4,7,11-Trimethyldodeca-6,10-dien-3-one, (*S*)-151



Hydrazone (*S*)-**156** (250 mg, 1.05 mmol) was alkylated with geranyl bromide as per general procedure **3.4.1** and hydrolysed using general procedure **3.4.2 Method A** to yield (*S*)-**151** as a colourless oil (36 mg, 15% over 2 steps, 86% ee). Spectral characteristics were consistent with racemic **151**. $[\alpha]^{20}_{D}$ = +13.02 (c 1.94, Et_2O). Enantioselectivity was determined by GC analysis: 7.1 : 92.9 er, t_R = 45.7 (*R*-enantiomer) and 46.3 min (*S*-enantiomer) (100 °C hold for 40 min, ramp 5 °C/min to 140 °C, hold for 5 min).

(*S*)-2-Methyl-1,3-diphenylpropan-1-one, (*S*)-152



Novel hydrazone method: Hydrazone (*S*)-**157** (146 mg, 0.51 mmol) was alkylated with benzyl bromide as per general procedure **3.4.1** and hydrolysed using either general procedure **3.4.2 Method A** (180 mg alkylated hydrazone) or general procedure **3.4.2 Method C** (120 mg alkylated hydrazone) to yield (*S*)-**152** as a pale yellow oil (**3.4.2 Method A**–52 mg, 48% or **3.4.2 Method C**–27 mg, 38%). *SAMP hydrazone method:* Hydrazone (*S*)-**163** (187 mg, 0.76 mmol) was alkylated with benzyl bromide as per general procedure **3.4.1** (using SAMP hydrazone in place of novel diamine hydrazone) and hydrolysed using either general procedure

3.4.2 Method A (156 mg alkylated hydrazone) or general procedure **3.4.2 Method C** (227 mg alkylated hydrazone) to yield (*S*)-**152** as a pale yellow oil (**3.4.2. Method A** – 30 mg, 29%, or **3.4.2 Method C** – 48 mg, 32%).

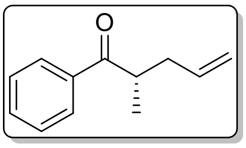
Spectral characteristics were consistent with previously reported data[11] and with racemic **152**.

$[\alpha]_D^{23}$ = +94.5 (c 2.5, $CHCl_3$) [lit.[6] $[\alpha]_D^{23}$ = + 88.9 (c 1.1, $CHCl_3$)]. Enantioselectivity was determined by GC analysis (**Table 3.1**): t_R = 48.7 (*R*-enantiomer) and 53.1 min (*S*-enantiomer) (140 °C hold for 30 min, ramp 5 °C/min to 110 °C, hold for 30 min).

Table 3.1 GC results for comparison of novel diamine auxiliary with SAMP and racemisation investigation.

Entry	Hydrazone	Hydrolysis Method	Enantiomeric ratio	ee (%)
1	(*S*)-**157**	HCl/diethyl ether	11.0 : 89.0	78
2	(*S*)-**157**	Oxalic acid	23.5 : 76.5	53
3	(*S*)-**163**	HCl/diethyl ether	4.8 : 95.2	91
4	(*S*)-**163**	Oxalic acid	4.6 : 95.4	91

(*S*)-2-Methyl-1-phenylpent-4-en-1-one, (*S*)-153

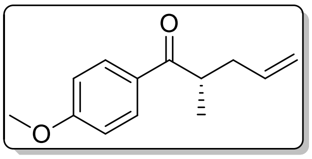


Hydrazone (*S*)-**157** (175 mg, 0.61 mmol) was alkylated with allyl bromide as per general procedure **3.4.1** and hydrolysed using general procedure **3.4.2 Method A** to yield (*S*)-**153** as a pale yellow oil (27 mg, 25% over 2 steps, 89% ee).

Spectral characteristics were consistent with previously reported data[12,13] and with racemic **153**.

$[\alpha]_D^{25}$ = +25.5 (c 1.25, CH_2Cl_2) [lit.[12] $[\alpha]_D^{25}$ = +39.7 (c 1.5, CH_2Cl_2)]. Enantioselectivity was determined by GC analysis: 5.4 : 94.6 er, t_R = 24.7 (*R*-enantiomer) and 25.7 min (*S*-enantiomer) (100 °C hold for 30 min, ramp 5 °C/min to 140 °C, hold for 10 min).

(*S*)-1-(4-Methoxyphenyl)-2-methylpent-4-en-1-one, (*S*)-154

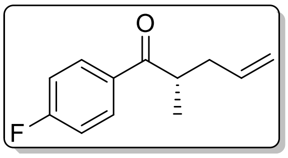


Hydrazone (*S*)-**158** (191 mg, 0.61 mmol) was alkylated with allyl bromide as per general procedure **3.4.1** and hydrolysed using general procedure **3.4.2 Method A** to yield (*S*)-**154** as a colourless oil (22 mg, 29% over 2 steps, 79% ee). Spectral characteristics were consistent with previously reported data[14] and with racemic **154**.

$[\alpha]_D^{20}$ = +3.8 (c 0.9, $CHCl_3$) [lit.[14] $[\alpha]_D^{20}$ = +31.77 (c 0.93, $CDCl_3$)].

Enantioselectivity was determined by GC analysis: 10.6 : 89.4 er, t_R = 78.0 (*R*-enantiomer) and 78.6 min (*S*-enantiomer) (110 °C hold for 60 min, ramp 2 °C/min to 140 °C, hold for 5 min).

(*S*)-1-(4-Fluorophenyl)-2-methylpenten-4-en-1-one, (*S*)-155



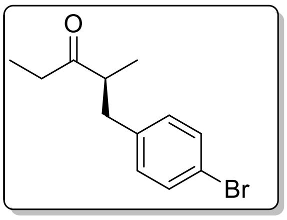
Hydrazone (*S*)-**159** (291 mg, 0.96 mmol) was alkylated with allyl bromide as per general procedure **3.4.1** and hydrolysed (276 mg, 0.80 mmol) using general procedure **3.4.2 Method A** to yield (*S*)-**155** as a pale yellow oil (51 mg, 33% over 2 steps, 90% ee).

Spectral characteristics were consistent with racemic **155**.

$[\alpha]_D^{20}$ = +27.2 (c 2.1, Et_2O).

Enantioselectivity was determined by GC analysis: 5.2 : 94.8 er, t_R = 42.0 (*R*-enantiomer) and 42.5 min (*S*-enantiomer) (90 °C hold for 40 min, ramp 5 °C/min to 140 °C, hold for 10 min).

(*S*)-1-(4-Bromophenyl)-2-methylpentan-3-one, (*S*)-164



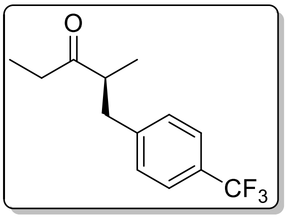
Hydrazone (*S*)-**156** (300 mg, 1.26 mmol) was alkylated with 4-bromobenzyl bromide as per general procedure **3.4.1** and hydrolysed using general procedure **3.4.2 Method C** to yield (*S*)-**164** as a colourless oil (59 mg, 23% over 2 steps, 62% ee). $[\alpha]_D^{20}$ = +22.2 (c 0.9, Et_2O).

IR (NaCl) ν_{max}: 2973, 2936 (C-H alkyl stretch, s), 1713 (C=O stretch, s), 1488, 1458 (C=C arom. stretch, s); ^1H NMR (300 MHz, $CDCl_3$): δ 0.97 (3H, t, *J* = 7.3 Hz, $C\boldsymbol{H_3}CH_2$), 1.07 (3H, d, *J* = 6.9 Hz, $C\boldsymbol{H_3}CH$), 2.25 (1H, dq, *J* = 7.3, 17.9 Hz, one of $CH_3C\boldsymbol{H_2}$), 2.45 (1H, dq, *J* = 7.3, 17.9 Hz, one of $CH_3C\boldsymbol{H_2}$), 2.51 (1H, dd, *J* = 6.9, 13.3 Hz, one of $CH_3CHC\boldsymbol{H_2}$), 2.80 (1H, m, $CH_3C\boldsymbol{H}$), 2.93 (1H, dd, *J* = 7.4, 13.3 Hz, one of $CH_3CHC\boldsymbol{H_2}$), 7.01 (2H, d, *J* = 8.5 Hz, 2 × C***H*** arom.), 7.38 (2H, dd, *J* = 8.4 Hz, 2 × C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 7.6 ($\boldsymbol{C}H_3CH_2$), 16.7 ($\boldsymbol{C}H_3CH$), 35.2 ($CH_3\boldsymbol{C}H_2$), 38.5 ($CH_3CH\boldsymbol{C}H_2$), 47.7 ($CH_3\boldsymbol{C}H$), 120.2 (***C***Br), 130.7 (2 × ***C***H arom.), 131.4 (2 × ***C***H arom.), 138.9 ($CH_3CHCH_2\boldsymbol{C}$), 214.2, (***C***=O) ppm; HRMS (ESI) *m/z* calcd for $C_{12}H_{16}BrO$ [$(M + H)^+$]: 255.0384, found 255.0385; Anal. calcd for $C_{12}H_{15}BrO$: C, 56.49; H, 5.93%. Found: C, 56.20; H, 5.96%.

Racemic **164** was previously synthesised within the group. Spectral characteristics were identical to chiral (*S*)-**164**.

Enantioselectivity was determined by GC analysis: 19.3 : 80.7 er, t_R = 16.3 (*R*-enantiomer) and 17.4 min (*S*-enantiomer) (140 °C hold for 20 min).

(*S*)-2-Methyl-1-(4-(trifluoromethyl)phenyl)pentan-3-one, (*S*)-165



Hydrazone (*S*)-**156** (300 mg, 1.26 mmol) was alkylated with 4-trifluoromethylbenzyl bromide as per general procedure **3.4.1** and hydrolysed using general procedure **3.4.2 Method C** to yield (*S*)-**165** as a colourless oil (44 mg, 14% over 2 steps, 72% ee).

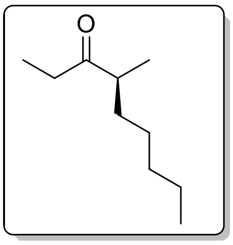
Spectral characteristics were consistent with previously reported data.[8]

$[\alpha]^{20}_{D}$ = +1.0 (c 1, Et_2O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.99 (3H, t, *J* = 7.3 Hz, $C\boldsymbol{H_3}CH_2$), 1.10 (3H, d, *J* = 6.9 Hz, $C\boldsymbol{H_3}CH$), 2.25 (1H, dq, *J* = 7.3, 17.7 Hz, one of $CH_3C\boldsymbol{H_2}$), 2.47 (1H, dq, *J* = 7.3, 17.8 Hz, one of $CH_3C\boldsymbol{H_2}$), 2.62 (1H, dd, *J* = 7.1, 13.4 Hz, one of $CH_3CHC\boldsymbol{H_2}$), 2.85 (1H, m, $CH_3C\boldsymbol{H}$), 3.05 (1H, dd, *J* = 7.3, 13.4 Hz, one of $CH_3CHC\boldsymbol{H_2}$), 7.25 (2H, d, *J* = 7.9 Hz, 2 × C***H*** arom.), 7.52 (2H, d, *J* = 8.0 Hz, 2 × C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 7.6 ($\boldsymbol{C}H_3CH_2$), 16.7 ($\boldsymbol{C}H_3CH$), 35.0 ($CH_3\boldsymbol{C}H_2$), 38.7 ($CH_3CH\boldsymbol{C}H_2$), 47.6 ($CH_3\boldsymbol{C}H$), 124.3 (q, $J_{C\text{-}F}$ = 271.7 Hz, ***C***F), 125.6 (q, $^3J_{C\text{-}F}$ = 3.8 Hz, 2 × ***C***H arom.), 128.6 (q, $^2J_{C\text{-}F}$ = 32.3 Hz, $\boldsymbol{C}CF_3$), 129.3 (2 × ***C***H arom.), 144.1 ($CH_3CHCH_2\boldsymbol{C}$), 213.0 (***C***=O) ppm; HRMS (ESI) *m/z* calcd for $C_{13}H_{16}OF_3$ [$(M + H)^+$]: 245.1153, found 245.1144.

Racemic **165** was previously synthesised within the group. Spectral characteristics were identical to chiral (*S*)-**165**.

Enantioselectivity was determined by GC analysis: 13.9 : 86.1 er, t_R = 10.8 (*R*-enantiomer) and 12.5 min (*S*-enantiomer) (120 °C hold for 10 min, ramp 5 °C/min to 140 °C, hold for 10 min).

(*S*)-4-Methylnonan-3-one, (*S*)-166



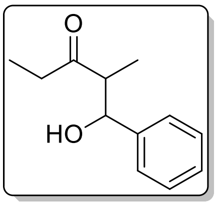
Hydrazone (*S*)-**156** (250 mg, 1.05 mmol) was alkylated with 1-iodopentane as per general procedure **3.4.1** and hydrolysed using general procedure **3.4.2 Method A** to yield (*S*)-**166** as a pale yellow oil (22 mg, 13% over 2 steps, 92% ee). $[\alpha]_D^{20}$ = +5.5 (c 0.2, Et_2O); IR (NaCl) ν_{max}: 2961, 2932 (C-H alkyl stretch, m), 1714 (C=O stretch, s), cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* = 6.8 Hz, $C\boldsymbol{H_3}CH_2CH_2$), 1.04 (3H, t, *J* = 7.3 Hz, $C\boldsymbol{H_3}CH_2CO$), 1.06 (3H, d, *J* = 6.9 Hz, $C\boldsymbol{H_3}CH$), 1.17–1.35 (8H, m, 4 × $C\boldsymbol{H_2}$), 2.46 (2H, dq, *J* = 1.5, 7.3 Hz, $CH_3C\boldsymbol{H_2}CO$), 2.48–2.58 (1H, m, $C\boldsymbol{H}$) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 7.8 ($\boldsymbol{C}H_3CH_2CO$), 14.1 ($\boldsymbol{C}H_3CH_2CH_2CH_2CH_2$), 16.5 ($\boldsymbol{C}H_3CH$), 22.5 ($CH_3\boldsymbol{C}H_2CH_2CH_2CH_2$), 27.0 ($CH_3CH_2CH_2\boldsymbol{C}H_2CH_2$), 31.9 ($CH_3CH_2\boldsymbol{C}H_2CH_2CH_2$), 33.1 ($CH_3CH_2CH_2CH_2\boldsymbol{C}H_2$), 34.2 ($CH_3C\boldsymbol{H_2}CO$), 46.1 ($\boldsymbol{C}H$), 215.7 ($\boldsymbol{C}$=O) ppm; HRMS (ESI) *m/z* calcd for $C_{10}H_{21}O$ [$(M + H)^+$]: 157.1592, found 157.1584.

Racemic **166** was previously synthesised within the group. Spectral characteristics were identical to chiral (*S*)-**166**.

The reaction was also carried out employing *t*-BuLi as base in place of LDA and hydrolysis using general procedure **3.4.2 Method A**, yielding (*S*)-**166** in 29% over 2 steps, 82% ee.

Enantioselectivity was determined by GC analysis: 4.2 : 95.8 er, t_R = 3.6 (*R*-enantiomer) and 3.9 min (*S*-enantiomer) (105 °C hold for 10 min, ramp °C/min to 140 °C, hold for 5 min).

1-Hydroxy-2-methyl-1-phenyl-3-pentanone, 167



To a stirred solution of dry diisopropylamine (0.2 mL, 1.39 mmol) in dry diethyl ether (4 mL) in a N_2 filled Schlenk tube at -78 °C was added 1.6M *n*-BuLi (0.91 mL, 1.45 mmol). The solution was allowed to stir at 0 °C for 30 min to generate a solution of LDA. Hydrazone (*S*)-**156** (299 mg, 1.26 mmol) was added dropwise at -78 °C allowed to stir at 0 °C for 16 h. A solution of benzaldehyde (245 mg, 1.64 mmol) in dry diethyl ether (3 mL) in a separate Schlenk tube, which was previously evacuated and filled with N_2, was added dropwise to the solution of deprotonated hydrazone at -110 °C. The temperature of the reaction was held at -110 °C for 1 h, then at -70 °C for 5 h before being allowed to warm gradually to room temperature overnight. Saturated aq. NH_4Cl solution (10 mL) was added to quench the reaction, followed by extraction with diethyl ether (3 × 20 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo* to yield crude alkylated hydrazone as a dark yellow solid. To a stirred solution of alkylated hydrazone in acetone (8 mL) and water (0.5 mL) was added Amberlyst® 15 hydrogen form beads[15,16] and reaction allowed stir at room temperature with progress monitored by TLC analysis. On completion, reaction mixture was filtered and acetone removed *in vacuo*. The residue was dissolved in diethyl ether (10 mL) and water (5 mL) and extracted with diethyl ether (3 × 15 mL). The organic layers were combined, dried over $MgSO_4$ and solvent removed *in vacuo* to yield the crude ketone as a mixture of *syn*- and *anti*-iosmers as a yellow oil (165 mg, 68% over 2 steps). Purification by silica column chromatography eluting with 90:10 hexane:diethyl ether afforded a mixture of inseparable diasteroisomers which was analysed by chiral GC (90 mg, 37% over 2 steps, 86:14 *syn*:*anti*, 15% ee *syn*-**167**, 63% ee *anti*-**167**).

Spectral characteristics were consistent with previously reported data.[17]

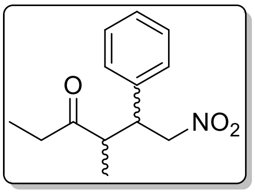
$[\alpha]_D^{20}$ mixture-**167**= -13.7 (c 0.9, Et_2O); ^1H NMR (300 MHz, $CDCl_3$): δ (mixture of *syn*- and *anti*-isomers) 0.94 (3H, d, J = 7.2 Hz, C***H*₃**CH), 1.04 (3H, t, J = 7.2 Hz, C***H*₃**CH_2), 2.36–2.62 (2H, m, CH_3C***H*₂**), 2.83–2.98 (2H, m, CH_3C***H***, O***H***), 4.76 (1H, d, J = 8.3 Hz, C***H***OH anti), 5.06 (0.16H, d, J = 4.0 Hz, C***H***OH syn), 7.29–7.36 (5H, m, C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 5.6 (***C***H_3CH_2), 12.6 (***C***H_3CH), 34.6 (CH_3***C***H_2), 50.8 (CH_3***C***H), 63.5 (***C***HOH), 124.7, 125.1, 126.7 (5 ×

C*H arom.), 139.1 (CH_2CHOHC), 214.3 (C***=O) ppm; MS (ESI) *m/z*: 191 [$(M - H)^-$, 6%].

Racemic **167** was previously synthesised within the group. Spectral characteristics were identical to chiral **167**.

Enantioselectivity was determined by GC analysis: t_R = 27.5 (*syn*), 29.1 (*anti*), 30.7 (*anti*) and 31.0 (*syn*) min (130 °C hold for 30 min, ramp 10 °C/min to 140 °C, hold for 5 min).

4-Methyl-6-nitro-5-phenylhexan-3-one, 168



Method A – alkylation of chiral hydrazone: To a stirred solution of dry diisopropylamine (0.2 mL, 1.39 mmol) in dry diethyl ether (4 mL) in a N_2 filled Schlenk tube at -78 °C was added 1.6M *n*-BuLi (0.91 mL, 1.45 mmol). The solution was allowed to stir at 0 °C for 30 min to generate a solution of LDA. Hydrazone (*S*)-**156** (299 mg, 1.26 mmol) was added slowly dropwise at -78 °C allowed to stir at 0 °C for 16 h. A solution of *trans*-β-nitrostyrene (245 mg, 1.64 mmol) in dry diethyl ether (3 mL) in a separate Schlenk tube, which was previously evacuated and filled with N_2, was added slowly dropwise *via* cannula to the solution of deprotonated hydrazone at -110 °C. The temperature of the reaction was held at -110 °C for 1 h, then at -70 °C for 5 h before being allowed to warm gradually to room temperature overnight. Saturated aq. NH_4Cl solution (10 mL) was added to quench the reaction, followed by extraction with diethyl ether (3 × 20 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo* to yield crude alkylated hydrazone as a dark yellow solid. The crude was hydrolysed using HCl/diethyl ether to yield the crude ketone as a pale brown oil (GC analysis of crude obtained) which was purified by silica column chromatography eluting with 90:10 hexane:diethyl ether afforded *syn*-**168** as a yellow oil (37 mg, 13% yield, 84% ee).

Method B – Organocatalytic reaction: To a stirred solution of diamine (*S*)-**131** (50 mg, 0.3 mmol) in brine (10 mL) was added trichloroacetic acid (50 mg, 0.3 mmol) at 25 °C. The solution was allowed to stir at 25 °C for 2 min. 3-Pentanone (741 mg, 8.6 mmol) and *trans*-β-nitrostyrene (426 mg, 2.9 mmol) were added and the reaction mixture allowed to stir at 25 °C for 36 h with reaction progress monitored by TLC analysis. On completion, the reaction mixture was extracted with ethyl acetate (3 × 15 mL). The organic layer was dried over $MgSO_4$ and solvent removed *in vacuo* to yield crude product (74 mg, 11%) as a yellow oil which was purified by silica column chromatography eluting with 80:20 hexane:diethyl ether to yield *syn*-**168** as a pale yellow oil (30 mg, 4%, 91% ee). Spectral characteristics were consistent with previously reported data for both *syn* and *anti*.[18, 19]

Syn: Pale yellow oil. $[\alpha]^{22}_{D}$ *syn*-**168**= +3.5 (c 0.2, $CHCl_3$) [lit.[20] $[\alpha]^{22}_{D}$ = +8.9 (c 0.2, $CHCl_3$).]; ^1H NMR (300 MHz, $CDCl_3$): δ 0.97 (3H, d, J = 7.1 Hz, $C\boldsymbol{H}_3CH$), 1.07 (3H, t, J = 7.3 Hz, $C\boldsymbol{H}_3CH_2$), 2.41 (1H, dq, J = 7.3, 18.0 Hz, $CH_3C\boldsymbol{H}_2$), 2.61 (1H, dq, J = 7.3, 18.0 Hz, $CH_3C\boldsymbol{H}_2$), 2.94–3.05 (1H, m, $CH_3C\boldsymbol{H}$), 3.66–3.73 (1H, m, $C\boldsymbol{H}C_6H_5$), 4.57–4.71 (2H, m, $C\boldsymbol{H}_2NO_2$), 7.14–7.17 (2H, m, 2 × C***H*** arom.), 7.29–7.33 (3H, m, 3 × C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 7.6 ($\boldsymbol{C}H_3CH_2$), 16.3 ($\boldsymbol{C}H_3CH$), 35.4 ($\boldsymbol{C}H_3CH_2$), 46.1 ($\boldsymbol{C}HC_6H_5$), 48.3 ($CH_3\boldsymbol{C}H$), 78.3 ($\boldsymbol{C}H_2NO_2$), 127.9, 129.0 (5 × CH arom.), 137.6 ($CH_3CHCH\boldsymbol{C}$), 213.6 ($\boldsymbol{C}$=O) ppm;

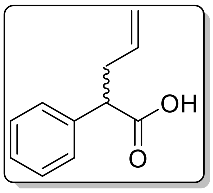
Anti: white solid. M.p. 60–62 °C [lit.[19] 61 °C]. ^1H NMR (300 MHz, $CDCl_3$): δ 0.83 (3H, t, J = 7.2 Hz, $C\boldsymbol{H}_3CH_2$), 1.19 (3H, d, J = 7.0 Hz, $C\boldsymbol{H}_3CH$), 1.98–2.12 (1H, m, one of $CH_3C\boldsymbol{H}_2$), 2.25–2.38 (1H, m, one of $CH_3C\boldsymbol{H}_2$), 2.93–3.03 (1H, m, $CH_3C\boldsymbol{H}$), 3.74–3.82 (1H, m, $C\boldsymbol{H}C_6H_5$), 4.71 (1H, dd, J = 9.7, 12.6 Hz, one of $C\boldsymbol{H}_2NO_2$), 4.80 (1H, dd, J = 5.3, 12.7 Hz, one of $C\boldsymbol{H}_2NO_2$), 7.16–7.19 (2H, m, 2 × C***H*** arom.), 7.24–7.33 (3H, m, 3 × C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 7.3 ($\boldsymbol{C}H_3CH_2$), 14.5 ($\boldsymbol{C}H_3CH$), 35.8 ($\boldsymbol{C}H_3CH_2$), 46.0 ($\boldsymbol{C}HC_6H_5$), 49.1 (2 × $CH_3\boldsymbol{C}H$), 77.6 ($\boldsymbol{C}H_2NO_2$), 127.85, 127.88, 128.9 (5 × CH arom.), 138.0 ($CH_3CHCH\boldsymbol{C}$), 212.5 ($\boldsymbol{C}$=O) ppm; MS (ESI) *m/z*: 236 [$(M + H)^+$, 20%].

Racemic **168** was synthesised in 52% yield as per general procedure **3.3**. Spectral characteristics were identical to chiral **168**.

Enantioselectivity was determined by GC analysis: t_R = 49.1 (*syn*), 54.9 (*anti*), 56.4 (*syn*) and 60.0 min (*anti*) (140 °C hold for 80 min).

3.5 Alkylation of carboxylic acid

2-Phenylpent-4-enoic acid, 173

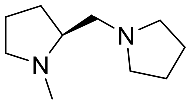
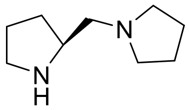
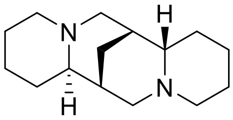
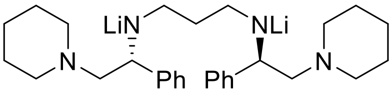


To a stirred solution of phenylacetic acid (1.0 equiv.) and ligand (1.03 equiv.) in THF (0.15 M) was added *n*-BuLi (2.1–3.3 equiv.) at 0 °C. The reaction mixture was allowed to stir at 0 °C for 15 min. The reaction mixture was cooled to -78 °C and stirred for an additional 5 min. Allyl bromide (4.0 equiv.) was added dropwise at -78 °C over 10 min. On completion of addition of the electrophile, the reaction was immediately quenched with a 3:1 THF:MeOH (8.0 equiv. MeOH) at -78 °C. 1M HCl (3 mL) was added after 4 min. The reaction mixture was diluted with ethyl acetate (8 mL) and water (8 mL). The aqueous layer was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with 1M aq. HCl (20 mL) and brine (20 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to yield the crude product which was purified by silica column chromatography eluting with 80:20 hexane:ethyl acetate to yield **173** as a pale yellow oil (**Table 3.2**).

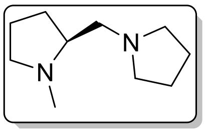
Spectral characteristics were consistent with previously reported data.[21]

^{1}H NMR (300 MHz, $CDCl_3$): δ 2.42–2.51 (1H, m, one of C***H*₂**CHCOOH), 2.72–2.82 (1H, m, one of C***H*₂**CHCOOH), 3.59 (1H, dd, *J* = 7.4, 7.5 Hz, C***H***COOH), 4.93 – 5.06 (2H, m, C***H*₂** alkene), 5.59 – 5.73 (1H, m, C***H*** alkene), 7.18 – 7.24 (5H, m, 5 × C***H*** arom.), 10.42 (1H, bs, O***H***) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 37.1 (***C***H_2CHCOOH), 51.4 (***C***HCOOH), 117.3 (***C***H_2 alkene), 127.6, 128.1, 128.8 (5 × ***C***H arom.), 134.9 (***C***H alkene), 137.8 (***C***CHCOOH), 179.8 (***C***=O) ppm. MS (ESI) *m/z*: 176 [(M + H)$^+$, 58%].

Table 3.2 Optical rotations of α-alkylated phenylacetic acid with different chiral ligands used in the reaction.

Entry	**Ligand**	***n*-BuLi (equiv.)**	**Yield (*S*)-173 (%)**[a]	**$[\alpha]^{23}_{D}$ (CHCl$_3$)**
1	 (*S*)-**174**	2.1	22	-1.294 (c = 0.85)
2	 (*S*)-**131**	3.3	9	-1.667 (c = 0.9)
3	 **175**	2.2	5	+0.857 (c = 0.35)
4	None (racemic)	2.2	>98	+0.248 (c = 1.01)
5	 **176**	4.0	84	+77.2 (c = 1.01)

(S)-1-methyl-2-(pyrrolidin-1-ylmethyl)pyrrolidine, (*S*)-174



To a stirred solution of (*S*)-**131** (0.885 g, 5.74 mmol) in water (7 mL) was added formic acid (4.7 mL, 126 mmol) and formalin (2.3 mL, 63.1 mmol) dropwise at room temperature. The reaction mixture was allowed stir at reflux for 24 h then cooled to room temperature, basified by addition of 10% NaOH to pH 11 and extracted with ethyl acetate (3 × 20 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo* to yield (*S*)-**174** as a pale yellow oil which did not require purification (0.472 g, 49%).

Spectral characteristics were consistent with previously reported data.[4]

$[\alpha]_D^{21}$ = -84.0 (c 0.55, EtOH) [lit[22] $[\alpha]_D^{21}$ = -84.5 (c 0.53, EtOH)]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.53–1.85 (7H, m, 3 × $NCH_2C\boldsymbol{H_2}$ and one of $NCHC\boldsymbol{H_2}$), 1.95–2.08 (1H, m, one of $NCHC\boldsymbol{H_2}$), 2.12–2.36 (3H, m, $CH_3NC\boldsymbol{H_2}$ and one of $CH_3NCHC\boldsymbol{H_2}N$), 2.39 (3H, s, $C\boldsymbol{H_3}$), 2.46–2.54 (4H, m, 2 × $NC\boldsymbol{H_2}CH_2$ pyrrolidine ring), 2.66 (1H, dd, *J* = 3.9, 11.5 Hz, one of $CH_3NCHC\boldsymbol{H_2}N$), 3.02–3.08 (1H, m, $CH_3NC\boldsymbol{H}$) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 22.6, 23.5 (3 × $NCH_2\boldsymbol{C}H_2$), 31.1 ($NCH\boldsymbol{C}H_2$), 41.4 ($\boldsymbol{C}H_3$), 55.0 (2 × $N\boldsymbol{C}H_2CH_2$ pyrrolidine ring), 57.6 ($CH_3N\boldsymbol{C}H_2$), 61.6 ($CH_3NCH\boldsymbol{C}H_2N$), 64.9 ($CH_3N\boldsymbol{C}H$) ppm. MS (ESI) *m/z*: 169 [$(M + H)^+$, 100%].

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Appendix I

Tetrahedron: *Asymmetry* 25 (2014) 356–361

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Investigation of a novel diamine based chiral auxiliary in the asymmetric alkylation of ketones

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ABSTRACT

A novel chiral auxiliary containing a pyrrolidine ring has been utilised in the preparation of various chiral ketones with good to excellent enantioselectivities (up to 92%). It has been successfully employed in aldol and Michael reactions giving moderate to high selectivity.

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1. Introduction

The α-alkylation of ketones is a fundamental reaction in organic synthesis. However there exists a very limited number of methods to carry out this transformation in an asymmetric manner. The use of SAMP/RAMP methodology almost exclusively accounts for these types of transformations.[1] SAMP/RAMP hydrazones have been widely employed as key steps in the synthesis of numerous natural products, for example, indanomycine,[2] (+)-eremophilenolide[3] and stigmatellin A.[4] Previous alteration of the basic SAMP/RAMP framework has included the use of more sterically hindered groups on the arm to give chiral auxiliaries such as SADP, SAEP, SAPP[5] and RAMBO.[6] Replacement of the terminal methoxy group with a trimethylsiloxy group showed comparable enantioselectivities to SAMP in asymmetric α-alkylation reactions and very good selectivities with aldol reactions.[7] More recently, Coltart has successfully used chiral *N*-amino cyclic carbamate hydrazones as an alternative to SAMP-type hydrazones, allowing the preparation of both α-alkylated and α,α-bisalkylated ketones in a convenient and scalable manner.[8]

With such a limited number of routes available to chiral α-alkylated ketones, there remains significant scope for the exploration of new, easily prepared chiral auxiliaries for use in their synthesis. We set out to investigate if a nitrogen (as part of a pyrrolidine system) could ligate to lithium as effectively as in the SAMP/RAMP system (where a –OMe group is utilised). We herein report the chromatography-free synthesis of a novel chiral auxiliary incorporating a pyrrolidine ring. The chiral hydrazine is available in four steps from N-protected proline **1** or only two steps from commercially available (*S*)-(+)-1-(2-pyrrolidinylmethyl)pyrrolidine **3**. Subsequent reaction with symmetrical and unsymmetrical ketones followed by deprotonation, alkylation (using both alkyl and the rarely reported benzyl electrophiles) and hydrolysis gave valuable chiral ketones in very good ee and moderate yields. The chiral auxiliary can be applied to both aldol and Michael reactions.

2. Results and discussion

Chiral auxiliary **5** was formed in a five step sequence from commercially available (*S*)-*N*-(benzyloxycarbonyl)proline **1** via DCC coupling to provide amide **2** in 81% yield. Two reduction steps afforded chiral diamine **3** in good yield. Nitrosation gave **4** and a final $LiAlH_4$ reduction furnished hydrazine **5**. Chiral auxiliary **5** was reacted with 3-pentanone to give chiral hydrazone **6** in 80% yield (46% yield after purification by distillation) (Scheme 1). In a similar manner, **5** was combined with propiophenone, *p*-methoxypropiophenone and *p*-fluoropropiophenone to afford hydrazones **7a**, **7b** and **7c** in 52%, 54% and 48% yields, respectively (Scheme 2).

Chiral hydrazone **6** was then subjected to LDA (5 h, room temperature) deprotonation and alkylated with benzyl bromide (addition at −110 °C, temperature held for 1 h at −110 °C then for 5 h at −70 °C) in either diethyl ether, toluene or tetrahydrofuran. The resultant alkylated hydrazone **8** was hydrolysed using a biphasic 4 M HCl/diethyl ether system and ketone **9** was analysed for enantioselectivity using chiral gas chromatography (Scheme 3). The use of diethyl ether as the solvent for the alkylation step afforded **9** with very good enantioselectivity (89% ee) in comparison to toluene and tetrahydrofuran (66% and 61% ee, respectively) albeit in moderate yields (20–30%).[9]

Improved yields were obtained on extension of the deprotonation time to 16 h and by decreasing the temperature to 0 °C. In these cases complete conversion to the alkylated hydrazone was observed. Yields remained moderate, most likely due to the high volatility of the resulting ketones.[10]

Various methods for the cleavage of α-substituted hydrazones to the corresponding ketones have been utilised.[11] Oxalic acid is reported as a convenient, high yielding, racemisation-free method for the hydrolytic cleavage of SAMP hydrazones.[12] However, when

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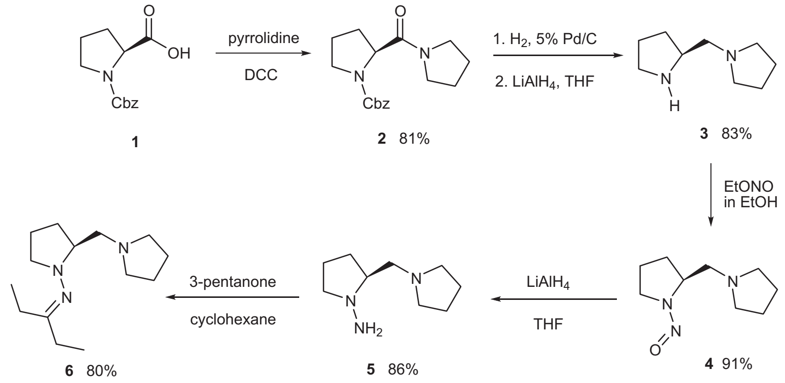
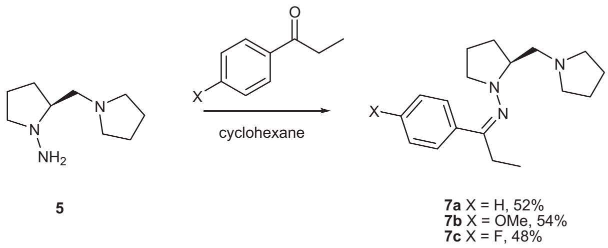

pyrrolidine
DCC
1. H2, 5% Pd/C
2. LiAlH4, THF
EtONO in EtOH
LiAlH4
THF
3-pentanone
cyclohexane
Cbz
OH
1
2 81%
3 83%
4 91%
5 86%
6 80%

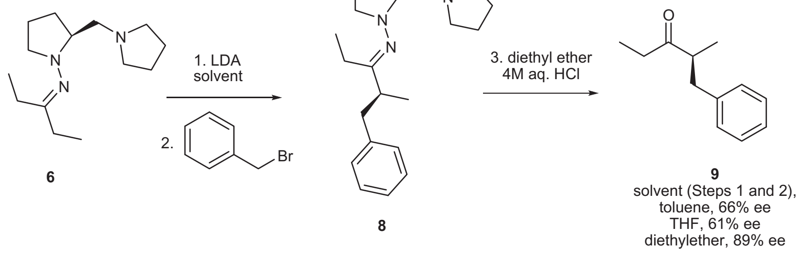
Scheme 1. Synthesis of the chiral auxiliary and the corresponding 3-pentanone hydrazone.


cyclohexane
5
7a X = H, 52%
7b X = OMe, 54%
7c X = F, 48%

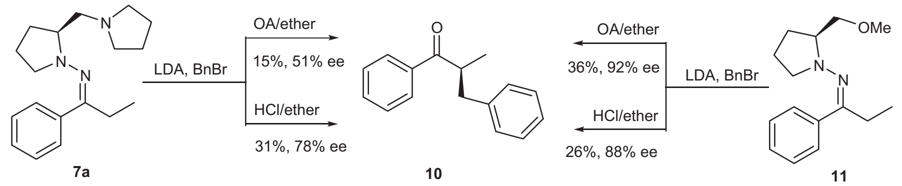
Scheme 2. Synthesis of propiophenone-based hydrazones.

we employed oxalic acid as a hydrazone cleavage method only moderate enantioselectivity was observed in the chiral ketones.[13] We suspected that racemisation was occurring, possibly due to some protonation of the pyrrolidine and increased solubility and exposure to the aqueous acidic layer. In order to investigate this possibility, both chiral hydrazone **7a** and the corresponding SAMP variant **11** were prepared and subjected to LDA and benzylbromide (Scheme 4). Both hydrazones were hydrolysed using oxalic acid and HCl/diethyl ether. Using the SAMP hydrazine, benzylated propiophenone **10** was obtained in 92% and 88% ee using oxalic acid and HCl/diethyl ether cleavage methods, respectively. A larger variation in the enantioselectivity was observed between the two cleavage methods when chiral auxiliary **7a** was employed in the reaction (51% and 78% ee). This clearly indicates that racemisation does occur when oxalic acid is used in combination with our chiral auxiliary and underlines the need for a thorough investigation of cleavage methods in such cases. To the best of our knowledge, the enzymatic cleavage of chiral hydrazones has not been reported. Porcine pancreatic lipase (PPL) was chosen as an appropriate enzyme because of its use in the cleavage of dimethylhydrazones.[14] Its use furnished ketone **9** in low (ca. 10%) yield (over two steps) albeit in 83% ee (Table 1, entry 8). Finally, a biphasic hydrolysis method (HCl/diethylether) was attempted. Clean conversion from alkylated hydrazones to ketones was observed with little or no racemisation occurring.

With usable hydrolysis conditions in hand, a variety of electrophiles were reacted with the azaenolate derived from **6**. The reaction of 3-pentanone hydrazone **6** with LDA and pentyliodide gave ketone **12** with 92% ee, albeit in moderate yield (Table 1, entry 1). When *t*-BuLi was employed as the base instead of LDA, the selectivity dropped to 82% ee (entry 2). Various other aliphatic electrophiles were employed to afford ketones **13**–**16** (entries 3–6) with very good enantioselectivities. We next turned our

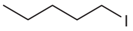
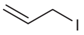
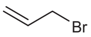
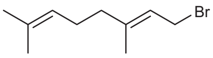
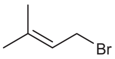
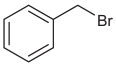
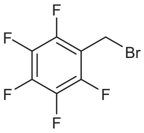
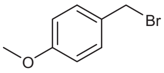
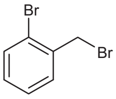
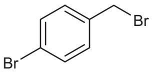
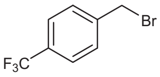
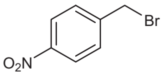
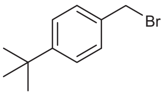
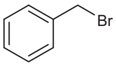
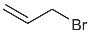

1. LDA
solvent
2.
Br
3. diethyl ether
4M aq. HCl
6
8
9
solvent (Steps 1 and 2),
toluene, 66% ee
THF, 61% ee
diethylether, 89% ee

Scheme 3. Solvent screen for the alkylation step of a chiral hydrazone.


LDA, BnBr
OA/ether
15%, 51% ee
HCl/ether
31%, 78% ee
OA/ether
36%, 92% ee
HCl/ether
26%, 88% ee
LDA, BnBr
OMe
7a
10
11

Scheme 4. Racemisation studies of chiral hydrazone **7a** and the SAMP variant **11** using oxalic acid (OA) or a biphasic 4 M HCl mediated cleavage. Isolated yields quoted over two steps.

Table 1
Results of alkylation reactions of hydrazones

Entry	Hydrazone	Electrophile	Product ketone	% Yield (over two steps)	% ee[e]
1	**6**		**12**	13	**92**[a]
2	**6**		**12**	29	82[a,b]
3	**6**		**13**	**63**	55[c]
4	**6**		**14**	23	90[a]
5	**6**		**15**	15	86[a]
6	**6**		**16**	19	89[a]
7	**6**		**9**	7	89[a]
8	**6**		**9**	10	83[d]
9	**6**		**17**	34	48[a]
10	**6**		**18**	24	84[a]
11	**6**		**19**	21	86[a]
12	**6**		**20**	19	62[c]
13	**6**		**21**	14	73[c]
14	**6**		**22**	6	58[a]
15	**6**		**23**	28	87[a]
16	**7a**		**10**	15	78[a]
17	**7a**		**24**	25	89[a]
18	**7b**		**25**	29	79[a]
19	**7c**		**26**	33	90[a]

Yield is calculated over two steps; alkylation of the parent hydrazone and hydrolysis of the alkylated hydrazone to the product ketone. Alkylated hydrazone is not isolated.

[a] HCl/diethyl ether hydrolysis.

[b] *t*-BuLi used as the base.

[c] Satd aq oxalic acid/diethyl ether hydrolysis.

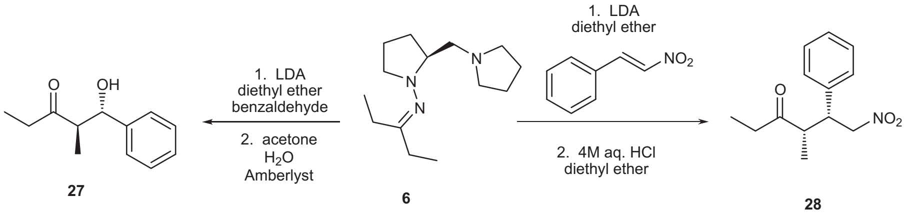
[d] PPL hydrolysis. The ketone products have been assigned as (*S*) by comparison of the specific rotation value of **24** with that reported in the literature and others by analogy.[15]

[e] All ee values were determined using chiral GC analysis and confirmed by comparison with independently prepared racemic ketones.

attention to the use of benzyl bromides as electrophiles. Their use in hydrazone chiral auxiliary methodology has been very limited. In fact, no thorough investigation of benzyl based electrophiles has been reported using chiral hydrazone methodology. A plethora of electrophiles were used affording ketones **9**, and **17**–**23**, all with good enantioselectivity. Substituted benzyl groups allowed us to probe the effect of electron withdrawing and donating groups present on the electrophiles. The presence of electron withdrawing groups on the benzyl moiety caused a decrease in the enantioselectivity of the resultant ketone when compared to the unsubstituted benzyl bromide (entry 7, 89%), which is most apparent with the use of perfluorobenzyl bromide (entry 9, 48%). The presence of an electron donating group, for example the use of *p*-methoxybenzyl bromide (entry 10, 84%), had little effect on the enantioselectivity observed.

Further to these studies it was decided to investigate the effect of the electronic substituents on the hydrazone moiety. Propiophenone, *p*-methyoxypropiophenone and *p*-fluoropropiophenone hydrazones **7a**–**c** were chosen as substrates and subjected to the standard conditions using allyl bromide as the electrophile. The resultant ketones **24**–**26** demonstrate that the presence of an electron donating substituent on the ring (entry 18, 79% ee) results in a decrease in the enantioselectivity when compared to the unsubstituted ketone (entry 17, 89% ee). The presence of an electron withdrawing substituent (entry 19, 90% ee), had little effect on the enantioselectivity.

We then applied our methodology to an aldol reaction (Scheme 5). Hydrazone **6** was deprotonated using LDA, reacted with benzaldehyde and hydrolysed using Amberlyst® to afford **27** in 39% yield over two steps. Enantiomeric excesses of 63% and



Scheme 5. Aldol and Michael reactions. Absolute stereochemistry unknown.

15% were obtained for *anti*- and *syn*-**27**, respectively. A diastereomeric ratio of 86:14 *anti*/*syn*, determined by GC, was identical to that observed by ^{1}H NMR.[16] The relative stereochemistry observed (*anti*) was opposite to that usually seen in aldol reactions using SAMP (*syn*).

Our novel chiral auxiliary was then applied to a Michael reaction (Scheme 5). Hydrazone **6** was treated with LDA and *trans*-β-nitrostyrene followed by subsequent hydrolysis to afford crude **28**, which was subjected to GC analysis. Enantiomeric excesses of 84% and 47% were determined for *syn*- and *anti*-**28**, respectively, with an excellent diastereomeric ratio of 94:6 *syn*/*anti* as determined by GC and NMR analysis. Again the relative orientation was opposite to that usually formed when using a SAMP chiral auxiliary in Michael reactions.[1c,17] Purification using column chromatography allowed isolation of *syn*-**28** in 84% ee and 13% yield over two steps.

3. Conclusion

A novel hydrazone-based chiral auxiliary has been established involving a pyrrolidine arm. The chiral auxiliary has been formed in good yields in five steps from commercially available (*S*)-*N*-(benzyloxycarbonyl)proline **1** (or only two steps from commercially available (*S*)-(+)-1-(2-pyrrolidinylmethyl)pyrrolidine **3**) without the need for silica column chromatography purification. Enantiomeric excesses of up to 92% were achieved in the α-alkylated aliphatic ketones formed and up to 89% in the less studied aromatic ketones. While the overall yields were moderate (in many cases due to product volatility), comparison studies with the SAMP chiral auxiliary showed comparable yields (Scheme 4). However, given the remarkably few methods available to access these compounds and the excellent enantioselectivities observed, we are pleased to report our novel chiral auxiliary as a viable route to these chiral synthons. Initial unoptimised studies into the use of our chiral auxiliary in Michael reactions have proven to be successful.

4. Experimental

4.1. Procedure for synthesis of the chiral auxiliary:

4.1.1. (*S*)-1-[*N*-(benzyloxycarbonyl)proly]-pyrrolidine 2[18]

To a CH_2Cl_2 solution (120 mL) of (*S*)-*N*-(benzyloxycarbonyl)proline (74.57 g, 0.3 mol) was added dropwise a CH_2Cl_2 solution (120 mL) of DCC (61.69 g, 0.3 mol) at 0 °C under a nitrogen atmosphere. After stirring for 30 min, a CH_2Cl_2 solution (120 mL) of pyrrolidine (24.7 mL, 0.3 mol) was slowly added dropwise to the reaction mixture at 0 °C via an addition funnel. The reaction mixture was allowed to warm to room temperature overnight. The precipitate was removed by filtration through a pad of Celite® and washed with CH_2Cl_2. The filtrate was washed with 0.5 M HCl (2 × 150 mL), satd aq $NaHCO_3$ solution (150 mL), H_2O (150 mL) and brine (150 mL). The organic layer was dried over $MgSO_4$, concentrated in vacuo and the crude product recrystallised from ethyl acetate to yield product **2** as a white, crystalline solid (73.52 g, 81% yield). $[\alpha]_D^{22} = -13.3$ (*c* 1.60, MeOH) {lit.[19] $[\alpha]_D^{22} = -14.1$ (*c* 1.61, MeOH)}. Mp 123–125 °C [lit.[19] 130–130 °C]. δ_H ($CDCl_3$, 300 MHz) (mixture of rotamers) 1.56–2.20 (8H, m, 4× CH_2), 3.25–3.75 (6H, m, 3× CH_2), 4.39–4.54 (1H, m, CH), 4.97–5.22 (2H, m, CH_2), 7.28–7.37 (5H, m, ArH). δ_C ($CDCl_3$, 75.5 MHz) (mixture of rotamers) 23.8, 23.9 (CH_2), 24.1, 24.4 (CH_2), 26.0, 26.3 (CH_2), 29.5, 30.5 (CH_2), 46.0, 46.0 (CH_2), 46.1, 46.3 (CH_2), 46.7, 47.3 (CH_2), 57.7, 58.2 (CH_2), 66.9, 67.1 (CH), 127.8, 127.9 (2× ArCH), 128.0, 128.1 (ArCH), 128.4, 128.4 (2× ArCH), 136.7, 136.8 (quaternary C), 154.2, 154.9 (C=O), 170.7, 171.0 (C=O). *m*/*z* (ES+) 303 [$(M+H)^+$, 100%].

4.1.2. (*S*)-2-(1-Pyrrolidinylmethyl)-pyrrolidine 3[20]

To a methanol (350 mL) solution of **2** (75.40 g, 250 mmol) was added Pd/C (5%, 4.78 g). The reaction mixture was then stirred under hydrogen at atmospheric pressure for 22 h while monitoring the reaction progress by TLC analysis. The crude reaction mixture was filtered through a pad of Celite® and washed with methanol to elute the product. The filtrate was concentrated in vacuo to yield the crude amide as a yellow oil (39.84 g, 95% yield). $[\alpha]_D^{26} = -89.6$ (*c* 1.7, EtOH) {lit.[21] $[\alpha]_D^{26} = -112.2$ (*c* 1.7, EtOH)}. δ_H ($CDCl_3$, 300 MHz) 1.60–2.02 (7H, m, 7× CH_2), 2.05–2.14 (1H, m, CH_2), 2.77–2.85 (1H, m, CH_2), 2.93 (1H, br s, NH), 3.15–3.22 (1H, m, CH_2), 3.36–3.57 (4H, m, 2× CH_2), 3.73–3.77 (1H, dd, *J* = 6.5, 8.6 Hz, CH). δ_C ($CDCl_3$, 75.5 MHz) 24.0, 26.0, 26.5, 30.4, 45.9, 46.0, 47.7 (7× CH_2), 59.5 (CH), 172.7 (C=O). *m*/*z* (ES+) 169 [$(M+H)^+$, 100%]. A solution of amide (19.02 g, 113 mmol) in dry THF (80 mL) was added dropwise over 3 h to $LiAlH_4$ (15.00 g, 396 mmol) in dry THF (140 mL) under a nitrogen atmosphere at 0 °C. The reaction mixture was allowed to stir at room temperature overnight, heated at reflux for 4 h, then allowed to stir at room temperature overnight. The reaction mixture was quenched by the dropwise addition of satd aq Na_2SO_4 solution (20 mL). The crude reaction mixture was filtered through a pad of Celite® and washed with ethyl acetate. The mother liquor was concentrated in vacuo to give the crude product as a yellow oil (14.54 g, 83% yield). Additional purification was achieved by Kugelrohr distillation yielding **3** as a colourless oil (11.22 g, 64% yield). $[\alpha]_D^{20} = +5.2$ (*c* 2.4, EtOH) [lit.[21] $[\alpha]_D^{20} = +8.9$ (*c* 2.4, EtOH)]. δ_H ($CDCl_3$, 300 MHz) 1.22–1.43 (1H, m, CH_2), 1.68–1.81 (6H, m, 3× CH_2), 1.82–1.95 (1H, m, CH_2), 2.31–2.37 (1H, dd, *J* = 5.2, 11.9 Hz, CH_2), 2.45–2.61 (6H, m, 3× CH_2, NH), 2.81–2.89 (1H, m, CH_2), 2.94–3.02 (1H, m, CH_2), 3.17–3.26 (1H, m, CH). δ_C ($CDCl_3$, 75.5 MHz) 23.4 (2× CH_2), 25.0, 30.1, 46.1 (3× CH_2), 54.6 (2× CH_2), 57.4 (CH), 62.1 (CH_2). *m*/*z* (ES+) 155 [$(M+H)^+$, 100%].

4.1.3. (*S*)-1-Nitroso-2-(pyrrolidin-1-ylmethyl)pyrrolidine 4

At first, 10–20% ethyl nitrite in ethanol (taken to be 15%) (5.45 mL, 8.63 mmol) was added to **3** (1.065 g, 6.90 mmol). The reaction vessel was covered in aluminium foil and allowed to stir at room temperature with progress monitored by ^{1}H NMR spectroscopy. After 45 h, ethanol was removed in vacuo to yield **4** as a yellow oil (1.15 g, 91% yield). δ_H ($CDCl_3$, 300 MHz) 1.76–

1.81 (4H, m, 2× CH_2), 1.91–2.25 (4H, m, 2× CH_2), 2.54–2.67 (4H, m, 2× CH_2), 2.80 (1H, dd, J = 8.8, 12.2 Hz, CH_2), 3.00 (1H, dd, J = 5.1, 12.2 Hz, CH_2), 3.52–3.75 (2H, m, CH_2), 4.59–4.67 (1H, m, CH). δ_C ($CDCl_3$, 75.5 MHz) 20.7 (CH_2), 23.5 (2× CH_2), 28.7, 45.6 (2× CH_2), 54.7 (2× CH_2), 59.5 (CH_2), 60.3 (CH). Since nitrosamines are potentially carcinogenic, no further data was obtained and the crude reaction mixture was used without purification in the next step.

4.1.4. (*S*)-2-(Pyrrolidin-1-ylmethyl)pyrrolidin-1-amine 5

To a solution of $LiAlH_4$ (2.61 g, 69 mmol) in dry THF (120 mL) was added dropwise a solution of **4** (6.30 g, 34 mmol) in dry THF (60 mL) under a nitrogen atmosphere at 0 °C. The reaction mixture was allowed to stir at 0 °C for 1 h, then at room temperature for 1 h before being heated at reflux for 4.5 h and stirred at room temperature overnight. The reaction progress was monitored by ^1H NMR spectroscopy. On completion, the reaction vessel was transferred to an ice bath and quenched by the dropwise addition of H_2O (2.6 mL), 3 M aq NaOH (2.6 mL) and H_2O (7.2 mL). The reaction mixture was filtered through a pad of Celite® using ether to elute the product. The mother liquor was concentrated in vacuo to yield **5** as a yellow oil (4.98 g, 86%). $[\alpha]_D^{20} = -11.4$ (c 1, EtOH). ν_{max}/cm^{-1} (KBr): 3306 (N–H stretch, m), 1591 (N–H bending, m), 1137 (C–N stretch, m). δ_H ($CDCl_3$, 300 MHz) 1.41–1.54 (1H, m, CH_2), 1.68–1.85 (6H, m, 3× CH_2), 1.93–2.07 (1H, m, CH_2), 2.26–2.41 (3H, m, 2× CH_2), 2.45–2.53 (2H, m, CH_2), 2.54–2.62 (2H, m, CH_2), 2.69–2.72 (3H, m/br s, CH_2/NH_2), 2.85–2.91 (1H, m, CH_2), 3.22–3.29 (1H, m, CH). δ_C ($CDCl_3$, 75.5 MHz) 20.6 (CH_2), 23.5 (2× CH_2), 28.7 (CH_2), 54.8 (2× CH_2), 59.6 (CH_2), 61.5 (CH_2), 67.8 (CH). Exact mass calcd for $C_8H_{11}IO_2$ [$(M+H)^+$], 170.1657. Found 170.1674.

4.1.5. (*S*)-*N*-(Pentan-3-ylidine)-2-(pyrrolidin-1-ylmethyl)pyrrolidin-1-amine 6

3-Pentanone (9.34 mL, 88 mmol) was added dropwise to a stirred solution of **5** (4.98 g, 29 mmol) in cyclohexane (8 mL) under an atmosphere of nitrogen. The reaction mixture was then allowed to stir at room temperature overnight and reaction progress monitored by ^1H NMR spectroscopy. On completion, the reaction mixture was poured into 6:1 DCM/H_2O and the organic layer extracted. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give the crude product as a yellow oil (5.61 g, 80% yield). Purification was achieved by Kugelrohr distillation to yield the product as a colourless oil (4.52 g, 65% yield). $[\alpha]_D^{20} = +114$ (c 1, EtOH). ν_{max}/cm^{-1} (NaCl): 1637 (C=N stretch, s), 1342, 1138 (C–N stretch, m). δ_H ($CDCl_3$, 300 MHz) 1.07 (6H, q, 2× CH_3), 1.53–1.66 (1H, m, CH_2), 1.69–1.91 (6H, m, 3× CH_2), 2.02–2.14 (1H, m, CH_2), 2.17–2.29 (2H, m, CH_2), 2.30–2.55 (9H, m, 4× CH_2, CH), 2.97–3.10 (2H, m, CH_2). δ_C ($CDCl_3$, 75.5 MHz) 10.9 (2× CH_3), 11.8, 21.8, 23.5, 23.5, 28.6, 28.7, 54.8, 55.0, 61.4 (10× CH_2), 66.1 (CH), 173.3 (CN). Exact mass calcd for $C_{14}H_{27}N_3$ [$(M+H)^+$], 238.2277. Found 238.2283.

4.2. General procedure for synthesis of racemic ketones

To THF (5 mL) was added commercially available LDA (1.1 equiv) at −78 °C. The reaction was stirred for 5 min and 3-pentanone was added dropwise. The reaction was stirred at −78 °C for 30 min and the electrophile (1.1 equiv) was added (in 3 mL THF if solid). The reaction was allowed to warm to room temperature overnight. Next, at. aq NH_4Cl solution (10 mL) was added and the crude product extracted with ethyl acetate or ether (3× 15 mL), dried over $MgSO_4$ and concentrated in vacuo to yield the crude product, which was purified by silica column chromatography.

4.3. General procedure for HCl/diethyl ether hydrolysis

At first, 4 M HCl (0.5 mL) and water (0.5 mL) were added to a vigorously stirred solution of alkylated hydrazone in diethyl ether (5 mL). The reaction progress was monitored by TLC analysis every 10 min. On completion, water (10 mL) was added, followed by extraction with diethyl ether (3× 25 mL). The organic layers were combined and washed with water (2× 10 mL), dried over $MgSO_4$ and concentrated in vacuo to yield the ketone, which was purified by silica column chromatography.

4.4. Procedure for PPL hydrolysis

To a solution of PPL (100 mg) in water (10 mL) was added a solution of alkylated hydrazone (1.05 mmol) in acetone (6 mL). The reaction was allowed to stir at room temperature for 23 h, diluted with diethyl ether (20 mL), washed with brine (3× 15 mL), dried over $MgSO_4$ and concentrated in vacuo. Purification was achieved using silica column chromatography to yield **9** as a yellow oil (19.3 mg, 10% yield over two steps).

4.5. General procedure for oxalic acid hydrolysis

At first, satd aq oxalic acid (1.5 vol with respect to mmol hydrazone) was added to a vigorously stirred solution of alkylated hydrazone in diethyl ether (4 vol with respect to mmol hydrazone). The reaction progress was monitored by TLC analysis and on completion were added water (5 mL) and diethyl ether (3× 20 mL). Organic extracts were combined, dried over $MgSO_4$ and concentrated in vacuo to yield the ketone which was purified by silica column chromatography.

4.6. Example procedure for the alkylation of chiral hydrazone

To a stirred solution of dry diisopropylamine (0.16 mL, 1.16 mmol) in dry diethyl ether (4 mL) in an N_2 filled Schlenk tube at −78 °C was added 1.6 M *n*-BuLi (0.86 mL, 1.21 mmol). The solution was allowed to stir at 0 °C for 30 min to generate a solution of LDA. Hydrazone **6** (250 mg, 1.05 mmol) was added slowly dropwise at −78 °C and allowed to stir at 0 °C for 16 h. A solution of *n*-pentyl iodide (250 mg, 1.26 mmol) in dry diethyl ether (2 mL) in a separate Schlenk, which was previously evacuated and filled with N_2 three times, was added dropwise to a solution of deprotonated hydrazone at −110 °C. The temperature of the reaction was kept at −110 °C for 1 h, then at −70 °C for 5 h before being allowed to warm gradually to room temperature overnight. Next, satd aq NH_4Cl solution (10 mL) was added to quench the reaction followed by extraction with diethyl ether (3 × 20 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated in vacuo to yield the crude alkylated hydrazone as a yellow oil, which was hydrolysed using HCl/diethyl ether to yield the crude product as a yellow oil. Purification was carried out using silica column chromatography eluting with 95:5 hexane/diethyl ether to afford **12** as a pale yellow oil (22 mg, 13% and 92% ee). $[\alpha]_D^{20} = +5.5$ (c 0.2, Et_2O). ν_{max}/cm^{-1} (film) 2961, 2932 (alkane CH stretches), 1714 (C=O). δ_H ($CDCl_3$, 300 MHz) 0.88 (3H, t, J = 6.8 Hz, CH_3), 1.04 (3H, t, J = 7.3 Hz, CH_3) 1.06 (3H, d, J = 6.9 Hz, CH_3), 1.17–1.35 (8H, m, 4× CH_2), 2.46 (2H, dq, J = 1.5, 7.3 Hz, CH_2), 2.48–2.58 (1H, m, CH). δ_C ($CDCl_3$, 125 MHz) 7.8, 14.1, 16.5 (3× CH_3), 22.5, 27.0, 31.9, 33.1, 34.2 (5× CH_2), 46.1 (CH), 215.7 (C=O). Exact mass calcd for $C_{10}H_{21}O$ [$(M+H)^+$], 157.1592. Found 157.1584. Sample for GC made up at 1 mg/mL in dry dichloromethane and run on Agilent Technologies 7820A GC System using G4513A Injector and Astec Chiraldex G-TA fused silica capillary column purchased from Sigma Aldrich Supelco using conditions 105 °C hold 10 min, ramp 10 °C/min to 140 °C hold 5 min, flow 1 mL/min, inj. vol. 0.2 μL, split ratio 10:1, front inlet 150 °C, detector 155 °C. Retention time: 3.63 min (minor), 3.87 min (major).

4.7. Example of the procedure for the Michael reaction

To a stirred solution of dry diisopropylamine (0.2 mL, 1.39 mmol) in dry diethyl ether (4 mL) in an N_2 filled Schlenk tube at −78 °C was added 1.6 M *n*-BuLi (0.91 mL, 1.45 mmol). The solution was then allowed to stir at 0 °C for 30 min to generate a solution of LDA. Hydrazone **6** (299 mg, 1.26 mmol) was slowly added dropwise at −78 °C and allowed to stir at 0 °C for 16 h. Next, *trans*-β-nitrostyrene (245 mg, 1.64 mmol) was dissolved in dry diethyl ether (3 mL), cooled to −78 °C and then slowly added dropwise to a solution of deprotonated hydrazone at −110 °C via a cannula. The temperature of the reaction was kept at −110 °C for 1 h, then at −70 °C for 5 h before being allowed to warm gradually to room temperature overnight. Next, satd aq NH_4Cl solution (10 mL) was added to quench the reaction followed by extraction with diethyl ether (3× 20 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated in vacuo to yield a product as a dark yellow solid, which was hydrolysed using HCl/diethyl ether to yield the crude product as a pale brown oil (GC analysis of crude obtained), which was purified using silica column chromatography eluting with 90:10 hexane/diethyl ether to afford *syn*-**28** as a yellow oil (37 mg, 13% and 84% ee). $[\alpha]_D^{22} = +3.5$ (c 0.2, $CHCl_3$). {lit.[22] $[\alpha]_D^{22} = +8.9$ (c 0.2, $CHCl_3$)}. δ_H ($CDCl_3$, 300 MHz) 0.97 (3H, d, $J = 7.1$ Hz, CH_3), 1.07 (3H, t, $J = 7.3$ Hz, CH_3), 2.41 (1H, dq, $J = 7.3$, 18.0 Hz, CH_3CH_2), 2.61 (1H, dq, $J = 7.3$, 18.0 Hz, CH_3CH_2), 2.94–3.05 (1H, m, CH_3CH), 3.66–3.73 (1H, m, CHAr), 4.57–4.71 (2H, m, CH_2NO_2), 7.14–7.17 (2H, m, ArH), 7.29–7.33 (3H, m, ArH). δ_C ($CDCl_3$, 75.5 MHz) 7.6, 16.3 (2× CH_3), 35.4 (CH_2), 46.1, 48.3 (2× CH), 78.3 (CH_2), 127.9, 129.0 (5× ArC), 137.6 (quaternary C), 213.6 (C=O). m/z (ES+) 235 $[(M+H)^+$, 78%]. Samples for GC made up at 1 mg/mL in dry dichloromethane and ran on Agilent Technologies 7820A GC System using G4513A Injector and Astec Chiraldex G-TA fused silica capillary column purchased from Sigma Aldrich Supelco using conditions 140 °C hold 70 min, flow 1 mL/min, inj. vol. 0.2 μL, split ratio 10:1, front inlet 150 °C, detector 155 °C. *anti*-**28** could not be isolated. Retention times: 44.95 min (*syn*), 51.05 min (*anti*), 52.40 min (*syn*), 55.53 min (*anti*).

Acknowledgements

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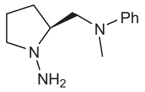

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Studies in Asymmetric and Heterocyclic Synthesis:

II

Quinolones

Chapter 4

Structure–function analysis of the C-3 position in analogues of microbial behavioural modulator, HHQ

Introduction

If you try to eradicate Nature, she will in time rise up silently and confound your foolish arrogance.

Horace

Epistles; 1st century BC

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4.1 General Introduction

One of the greatest technological achievements of the 20th century was the ability to control the growth of pathogenic and unwanted bacteria. Compounds that aim to either kill or inhibit the growth of microorganisms have been applied in many areas of everyday life. The most obvious applications are in food preservation, farming and in the treatment of bacterial infections. Today, it is impossible to imagine health care systems that are not able to cope with bacterial infections. The widespread use of antibiotics however, has led to a crisis in health care systems worldwide. The mode of action of antibiotics is to kill the bacteria they encounter but the result of this is that bacteria are under intense selective pressure to develop resistance, leading to strains of bacteria which are now multi-drug resistant. These are commonly known as superbugs.

In recent years, there has been a dramatic increase in the amount of multi-drug resistant bacteria, resulting in some strains which are now unharmed by nearly all the drugs designed to kill them and as a result, infections that were once easily treatable are almost untreatable.[1]

Between 1930 and 1962, more than twenty novel classes of antibiotics were identified, produced and marketed.[2] Since then, only two new classes of antibiotics, oxazolidinones[3] and cyclic lipopeptides,[4] have been marketed. The discovery of the newest antibiotic, teixobactin, was reported in early 2015.[5]

In the race against these superbugs we are already well behind. It is conceivable that in a number of years, routine medical procedures such as cancer chemotherapy and organ transplant surgery will become higher risk as the available antibiotics are rendered ineffective. Thus there is a need for the development of alternative strategies to tackle the problem of antibiotic resistance. An ideal approach is to target the control apparatus of the virulence and pathogenic traits of bacteria rather than killing the bacteria. In this way, there should be no natural selection of mutant strains and hence no resistance.

4.2 Bacteria

Between fifteen and twenty billion years ago, the universe arose as a cataclysmic eruption of hot, energy-rich, sub-atomic particles, resulting in the formation of the simplest elements, hydrogen and helium, within seconds.[6] As the universe expanded and cooled, stars were formed due to the condensing of material under the influence of gravity. Some stars became supernovae due to their incredibly large size, with their explosion releasing the energy required to fuse simple atomic nuclei into more complex chemical elements. In this manner, over billions of years, the Earth and all the chemical elements found on it today were produced. The introduction of life occurred around four billion years ago, and the first signs of life were that of simple microorganisms which had the ability to extract energy from organic compounds or from sunlight which they used to make a wealth of more complex biomolecules from the simple elements and compounds on the surface of the Earth – these were the early forms of bacteria.[6]

The first observation of bacteria was made in 1676 by Antoine van Leeuwenhoek. He described bacteria as “animalcules” and published his findings in a series of letters to the Royal Society.[7] It was more than 160 years later that the term bacterium would be introduced by Christian Gottfried Ehrenberg, in 1838.

The unity and diversity of organisms becomes apparent even at cellular level. Bacteria (prokaryotes) are single-celled, microscopic organisms, typically 1–2 μm in length, whereas larger organisms (for example animals and humans) are multicellular containing many different types of cells, which vary in shape, size and function. Animal and plant cells (eukaryotes) are typically 5–100 μm in diameter.[6]

All living organisms can be classified into one of three groups that define three branches of evolution from a common progenitor (**Figure 4.1**).[6] Prokaryotes can be split into two distinct groups based on their biochemical background – archaebacteria and eubacteria. Eubacteria can be found in soil and surface waters and in the tissues of other decaying organisms. Common eubacteria include the well-studied *Escherichia coli*.[8] Archaebacteria typically inhabit extreme environments such as salt lakes, hot springs, highly acidic bogs and ocean depths. It is thought that archaebacterial and eubacteria diverged early in evolution and so are seen as two separate domains, also known as Archae and Bacteria. All eukaryotic organisms evolved from the same branch that gave rise to the Archae, meaning archaebacterial are more closely related to eukaryotes than bacteria.

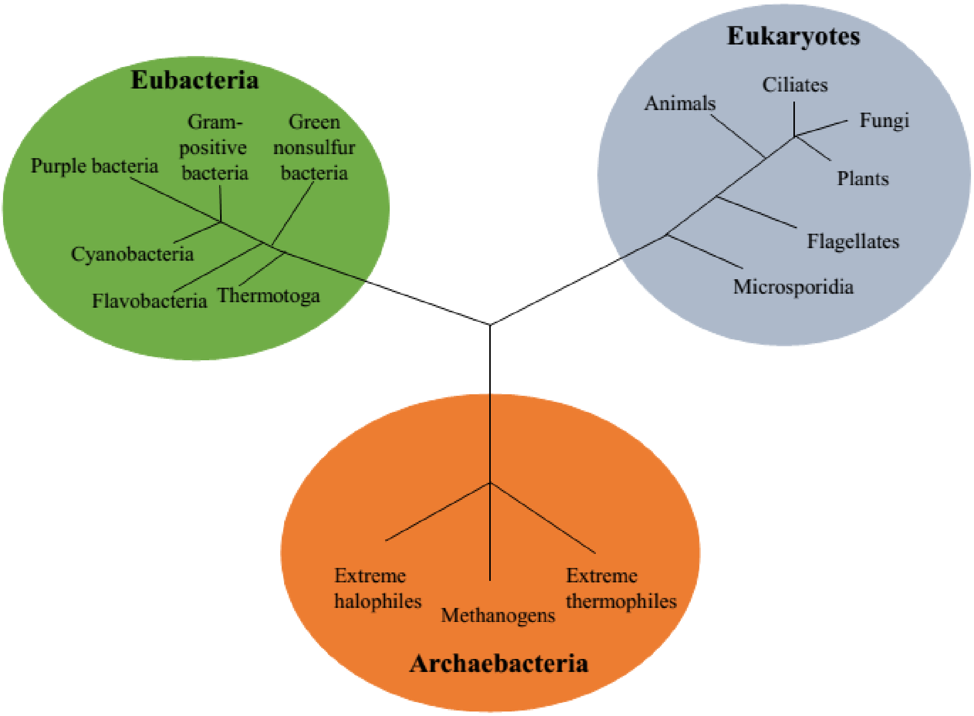
Eubacteria
Gram-positive bacteria
Green nonsulfur bacteria
Purple bacteria
Cyanobacteria
Flavobacteria
Thermotoga
Eukaryotes
Animals
Ciliates
Fungi
Plants
Flagellates
Microsporidia
Extreme halophiles
Methanogens
Extreme thermophiles
Archaebacteria

Figure 4.1 The three domains of life.

Bacteria can be divided into two main classes, Gram-positive and Gram-negative, based on their different abilities to retain an iodine-crystal violet stain when treated with organic solvents. Those that retain the stain are termed Gram-positive while those that do not are Gram-negative. Response to the staining technique depends primarily on the composition and morphology of the bacterial cell wall, the fundamental difference between the two types is the possession of an outer membrane within the cell wall of Gram-negative bacteria (**Figure 4.2**).[6]

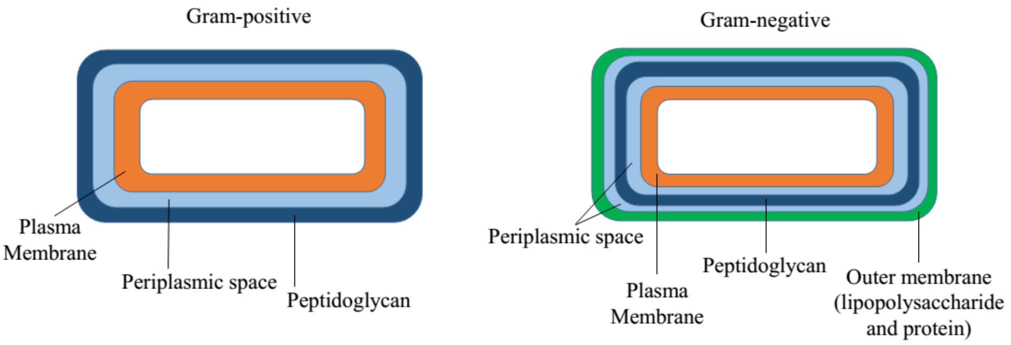
Gram-positive
Plasma Membrane
Periplasmic space
Peptidoglycan
Gram-negative
Periplasmic space
Plasma Membrane
Peptidoglycan
Outer membrane (lipopolysaccharide and protein)

Figure 4.2 Differences between Gram-positive and Gram-negative bacteria.

Bacterial cells all have certain common structural features (**Figure 4.3**),[6] but they also show group-specific specifications. *E. coli* is a well-known bacterial species which is usually a harmless inhabitant of the human intestinal tract. An *E. coli* cell is about 2 μm long and has a diameter of just less than 1 μm. The cytoplasm and nucleoid (containing all the genetic material of the bacterium) are enclosed by the inner plasma membrane and the protective outer layer with a layer of polymers called peptidoglycans in between the two layers, giving the cell its shape and rigidity. These layers form what is known as the cell envelope. The cytoplasm of bacteria will contain ribosomes, enzymes, numerous metabolites, co-factors and inorganic ions. The nucleoid contains a single, circular molecule of DNA, and the cytoplasm contains one or more smaller, circular segments of DNA, known as plasmids. In nature, some plasmids can confer resistance to toxins and antibiotics however they are also responsive to manipulation in the laboratory.[6]

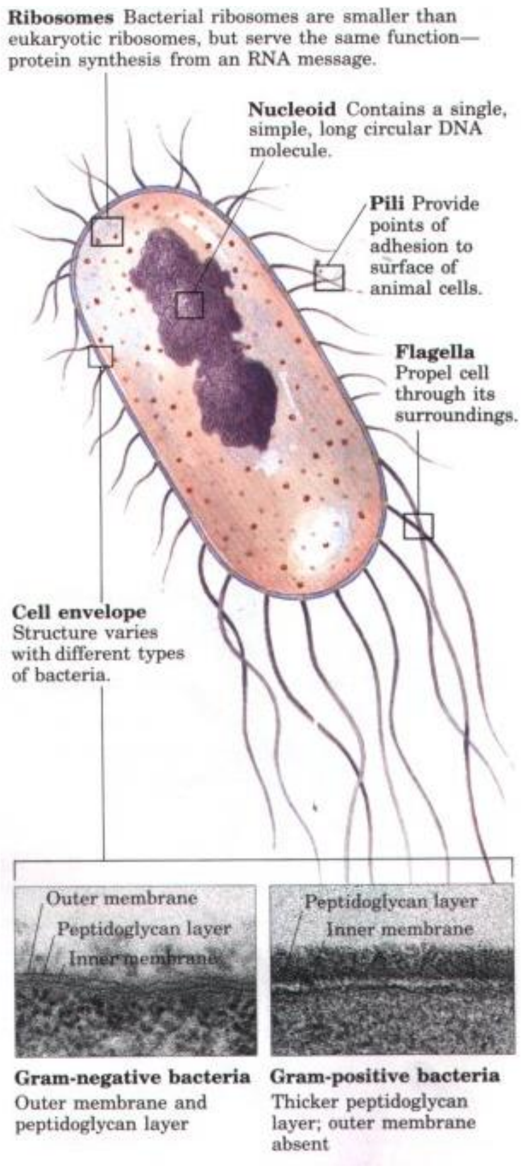
Ribosomes Bacterial ribosomes are smaller than eukaryotic ribosomes, but serve the same function—protein synthesis from an RNA message.
Nucleoid Contains a single, simple, long circular DNA molecule.
Pili Provide points of adhesion to surface of animal cells.
Flagella Propel cell through its surroundings.
Cell envelope Structure varies with different types of bacteria.
Outer membrane
Peptidoglycan layer
Inner membrane
Peptidoglycan layer
Inner membrane
Gram-negative bacteria Outer membrane and peptidoglycan layer
Gram-positive bacteria Thicker peptidoglycan layer; outer membrane absent

Figure 4.3 Common structural features of bacterial cells.[6]

Bacteria are ubiquitous on Earth, with typically 40 million bacterial cells in 1 g of soil and 1 million/mL of fresh water, for example. Overall, there are approximately 5 nonillion (5×10^{30}) bacteria on Earth, unsurprisingly forming much of the world's biomass.[9] Bacteria play a vital role in many important processes on Earth, such as putrefaction and in the fixation of nitrogen from the atmosphere. Interestingly though, most bacteria have not been characterised, and only about half of the phyla of bacteria have species that can be grown in the laboratory.[10]

Surprisingly, there are ten times more bacterial cells than human cells in the human body. Most of these bacterial cells are present on the skin and in the gut.[11] The majority of bacteria in the body are rendered harmless by the protective effects of the immune system. In fact, some are required as they have a crucial, beneficial effect. However, some species of bacteria have a detrimental effect on a human host as they are pathogenic and cause infectious diseases, for example the bacterium *Vibrio cholera* causes cholera and *Yersinia pestis* is the bacterium responsible for the bubonic plague, to name two. In developed countries, antibiotics are used for the treatment of bacterial infections, however their overuse has presented the problem of many strains of mutant bacteria which are now resistant to many, if not all, antibiotics currently available. Bacteria often attach to surfaces and form dense aggregations known as biofilms, which can range from a few micrometres up to half a metre in depth and contain multiple species of bacteria. Bacteria that live in biofilms display a complex arrangement of cells and extracellular components and form secondary structures such as microcolonies, which have the benefit of having networks of channels running through them to enable better diffusion of nutrients.[12,13] The majority of bacteria bound to surfaces in the natural environment, for example soil or the surfaces of plants, live in biofilms.[14] Bacteria are able to use biofilms as a method of protection against attack from antibiotics and are often present during chronic bacterial infections and in infections of implanted medical devices. Treatment of bacteria encapsulated by a biofilm is much more difficult than unprotected isolated bacteria.[15]

4.2.1 Antibiotics and the rise of antibiotic resistance

The remarkable success of antibiotics in the 1960s and 1970s led to the misconception that infectious diseases had been conquered. However, infectious diseases are still a major problem and in a report by the World Health Organisation in 2002, were cited as being the second-leading cause of death worldwide.[16]

Antibiotics are unique among pharmaceutical remedies in that they direct their action selectively towards foreign cells rather than host cells, meaning they can be prescribed less strictly than other pharmaceuticals. This selective action means that they must target physiological and biochemical differences between host cells and bacterial cells in order to exert their effect. Interestingly, it was noted that in the search for new antibiotics in moulds and other organisms, for example with *Penicillium*, many selective and useful antibiotics were found (including streptomycin and rifampicin), but others were also found which although had a good antibacterial effect, did not exhibit selectivity and so were deemed unusable for the clinical treatment of bacterial infections.[17]

In many cases, antibiotics are prescribed when signs of infection are present, without a strict bacterial diagnosis being made, which has contributed heavily to the unnecessarily large consumption of antibiotics worldwide.[17] In a 1983 World Health Organisation report, it was estimated that antibiotics are used by doctors irrationally or inappropriately on anything between two fifths and two thirds of all occasions.[18] Indeed, Tomasz reported in 1994 that every year in the USA, 60,000–70,000 patients die from hospital infections, half or more of which are caused by antibiotic-resistant superbugs and that hospital infections costs the US healthcare system a minimum of $4.5 billion per annum.[19]

Bacterial resistance to antibiotics has developed quickly over a short period of time. This phenomenon can be partially explained by the short generation span of bacteria, which allows them to undergo a Darwinian evolution in a much shorter time than is possible for animals – the smaller and simpler the species, the faster it can undergo evolution to respond to changes in its environment. Another aspect which explains the quick development of resistance to antibiotics is the fact that bacteria have the ability to manipulate their own genetic makeup, which leads to a faster adaption of the toxic effects of antibiotics and hence resistance. This can be seen as the natural genetic engineering of bacteria, including the uptake and incorporation of resistance-mediating genes from related organisms by adaption of evolutionary old genetic mechanisms to the new environmental situation involving the presence of antibiotics.[17]

Antibiotics function by either killing bacteria (bactericidal) or by preventing them from multiplying (bacteriostatic). Notably, a bacteriostatic drug can be bactericidal if it is used in high enough doses. Antibiotics can also be classed as either ‘narrow-spectrum’ or ‘broad-spectrum’ depending on the range of bacterial species they work against. The broad-spectrum antibiotics tend to do the most damage as the risk of developing antibiotic resistant bacteria is much higher. Penicillins, for example, sabotage the synthesis of the bacterial cell wall, rupturing the cell. However, the effectiveness of penicillins has decreased quickly due to the evolution of bacteria which produced β-lactamase or penicillase, which attacks the drug and cleaves the β-lactam ring.[20]

A great deal of the early prosperity of pharmaceutical companies was due to the development of antibacterial drugs, and as a consequence the market encompasses several of the oldest drug classes.[21] Despite the rise in the use of generic antibiotics, there is still growth in this area due to increasing sales volume, as well as the rise of premium-priced novel treatments for resistant bacteria (for example, Pfizer's Zyvox (linezolid) **180**, **Figure 4.4**).[21] The reason why this market is still expanding is due to the growing number of people with depressed immune systems, including the elderly, human immunodeficiency virus (HIV) patients and organ donors, for example.[21] Antibiotics are an unattractive target for the pharmaceutical industry for various reasons, including the fact that there is the current problem of antibiotic resistance, meaning drugs have relatively short life cycles and also that antibacterial therapy is acute rather than chronic. Indeed, 10 of the 15 largest pharmaceutical companies have either fully abandoned, or significantly diminished, their discovery efforts in the field of antibiotics since 1999.[22] This has left a niche for smaller companies to take over the drug development role, both by identifying innovative drugs or formulations, and/or by picking up clinical programmes aborted by the larger companies. However, the larger companies in the pharmaceutical industry often become involved in the latter stages of a promising drug as a strong salesforce is the key to success in the community antibacterial market.[21]

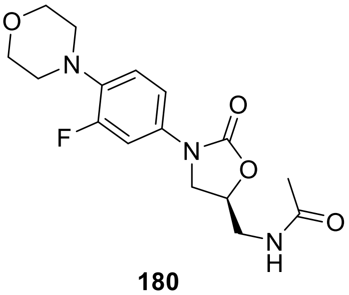

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Figure 4.4 Pfizer's Zyvox (linezolid).

The market for antibiotics can be split into two distinct groups. The larger community market (estimated at 62% of total antibacterial sales), with a lower average drug price and growth prospects and the smaller but more attractive hospital market, with a higher average drug price and growth prospects.[21]

The most widely used antibiotics are penicillins for Gram-positive bacteria and cephalosporins for both Gram-positive and Gram-negative bacteria. Fluoroquinolones are also widely used for Gram-negative pathogens.

Although there are good treatment options available for most common infections, there are several areas where choices are limited, for example there is a severe lack of oral drugs available to treat methicillin-resistant *Staphylococcus aureus* (MRSA), the prevalence of which has increased dramatically in recent years. Others include antibiotic resistant Gram-negative bacterial infections, in particular those from *Pseudomonas*. Few pharmaceutical companies are focusing on addressing the need for efficient treatment of these bacteria.[21]

During the last number of decades that antibiotics have been in ever-widening therapeutic use, the development of antibiotic resistance has followed. It is clear that whenever a new antibiotic, broader-spectrum forms of an existing antibiotic, or a new class of antibiotic is introduced into wide-spread use, clinically significant resistance appears. This can occur very quickly, even in a matter of months (**Table 4.1**). Vancomycin (**Table 4.1, entry 7**) is the exception to the rule as resistance came about 30 years after clinical introduction, most probably due to the limited use of vancomycin in the first 25 years. However, as it became more commonly used, resistance has developed.[23,24]

Due to the constant, intense exposure of bacteria to antibiotics in a hospital environment, bacteria become antibiotic-resistant much more rapidly than in the community. In these microenvironments, there is selective pressure for antibiotic-resistant bacteria to maintain those determinants, survive and even dominate the bacterial populations. Given a large population of bacteria which are exposed to a drug, there is a competition between the death of all the bacteria and the development of rare mutations that confer resistance. Given the short time required for bacterial division and replication along with a typical frequency of one error per 10^7 bases as their DNA polymerases copy DNA, then 100 million bacteria will contain about 10 mutants in the population.[25] If these mutations are randomly dispersed in the genome of a bacterium the size of *E. coli*, with 3000 genes, then 0.3% (10/3000) of the genes will have one mutation. If one of these gene mutations is a target for an antibiotic and the mutation confers some degree of resistance that means the bacteria is less sensitive to the antibiotic, it will have a selective survival advantage. As its neighbours without the mutation die, it will persist and have the advantage of having space to grow and dominate the culture. The resistant strain will continue to be selected for by the continuing presence of antibiotic in an environment such as a hospital ward.[25]

Table 4.1 Evolution of the resistance to antibiotics.[23]

Entry	Antibiotic	Year deployed	Resistance observed
1	Sulfonamides	1930s	1940s
2	Penicillin	1943	1946
3	Streptomycin	1943	1959
4	Chloramphenicol	1947	1959
5	Tetracycline	1948	1953
6	Erythromycin	1952	1988
7	Vancomycin	1956	1988
8	Methicillin	1960	1961
9	Ampicillin	1961	1973
10	Cephalosporins	1960s	Late 1960s

These findings show that bacterial resistance to antibiotics is an eventuality in every case, suggesting that there is a constant need for new cycles of antibiotic discovery and development. As soon as an antibiotic is introduced for widespread clinical use, selection for resistant strains will occur and hence a finite therapeutic lifetime will occur before the resistance becomes sufficiently widespread to lessen the efficacy of the drug.[25]

It is clear that alternative strategies must be investigated as another method for treatment of bacterial infection, whereby treatment would not involve killing the bacteria. Rather, there is a need to develop new drugs that circumvent the bacterial resistance mechanisms or that act on different bacterial targets.

4.3 *Vibrio Fischeri* and the discovery of quorum-sensing (QS)

Bacteria must constantly monitor their environment for changes that require an adaptive response. Bacteria are too small to exert their effect on a large host if they act as individuals. They must act in a different way in order to exert their pathogenicity. Bacteria have achieved this and have developed a method to communicate with one another using chemical molecules in a process termed quorum-sensing (QS).[26-29] QS bacteria produce, release, detect and respond to chemical signal molecules, termed autoinducers, whose external concentration increases in proportion to increasing cell-population density. Bacteria detect the accumulation of a minimal threshold stimulatory concentration of these autoinducers and alter gene expression, and ultimately behaviour, in response. The information supplied by these molecules is critical for synchronising the activities of large groups of bacteria and allowing them to alter their behaviour on a population-wide scale in response to changes in the number and/or species present in a community. Most processes that are controlled by QS are unproductive when undertaken by an individual bacterium acting alone, but when carried out simultaneously by a large number of cells it becomes beneficial. In a sense, QS blurs the barrier between prokaryotic and eukaryotic behaviour because it enables bacteria to act as multicellular organisms.[28]

The first described QS system is that of the bioluminescent marine bacterium *Vibrio fischeri*, which colonises a specially developed light organ in the Hawaiian bobtail squid (**Figure 4.5**).[30] In this light organ, *V. fischeri* grow to high cell density and induce the expression of genes required for bioluminescence. The squid and the *V. fisheri* have a symbiotic relationship. The squid is a nocturnal animal that inhabits shallow waters off the coast of Hawaii. On bright nights, the moonlight can penetrate the depth of the water that the squid lives in, meaning the squid itself will block the moonlight, cast a shadow and prey will see it coming. The squid can, however, use the bioluminsence of the *V. fischeri* to its advantage. The squid has a detector on its back that allows it to detect the amount of moonlight that is hitting it. It also has shutters over its light organ that it can open and close as desired. The squid can thus detect the amount of moonlight hitting its back and open the shutters on its light organ accordingly to allow the same amount of light to penetrate from its underside to the sea bed. This allows it to be able to mask its shadow and overall, this process acts as an anti-predation device.[31] The bacteria benefit from this symbiosis because the light organ is rich in nutrients and allows proliferation in numbers which would be unachievable in seawater,[28] so the QS system in *V. fischeri* has evolved to specifically enable the bacteria to produce light only under favourable conditions in which there is a positive selective advantage for light. This QS system is considered the paradigm for QS in most Gram-negative bacteria.

Figure 4.5 Hawaiian bobtail squid with large populations of *V. fischeri* colonising the light organ and showing bioluminescence due to QS.[32]

Studies carried out by Engebrecht and Silverman set the standard for all subsequent studies of QS in Gram-negative bacteria.[33] They identified, cloned and analysed the genes encoding the luciferase enzyme complex (the oxidative enzyme responsible for the bioluminescence) and the genes responsible for its density-dependent regulation from *V. fischeri*. The authors showed that bioluminescence in *V. fischeri* is controlled by two regulatory proteins, LuxI and LuxR. LuxI is the autoinducer synthase that is responsible for the synthesis of the acyl-homoserine lactone (AHL) 3-oxohexanoyl-homoserine lactone **181** (3-oxo-C_6-HSL) (**Figure 4.6**). LuxR is the autoinducer receptor/DNA-binding transcriptional activator, which is found in the cytoplasm.[34] Once the AHL molecules are produced, they can diffuse freely in and out of the cell. The amount of AHL present increases with increasing cell density.[35] Once the amount of AHL reaches a crucial threshold concentration, it is bound by LuxR and this complex activates transcription of the operon encoding luciferase.[36] This LuxR-AHL complex also induces expression of *luxI* as it is encoded in the luciferase operon. This operation is incredibly important as this regulatory configuration floods the environment with the signal, creating a positive feedback loop that causes the entire population to switch into QS mode and produce light (**Figure 4.6**).[28]

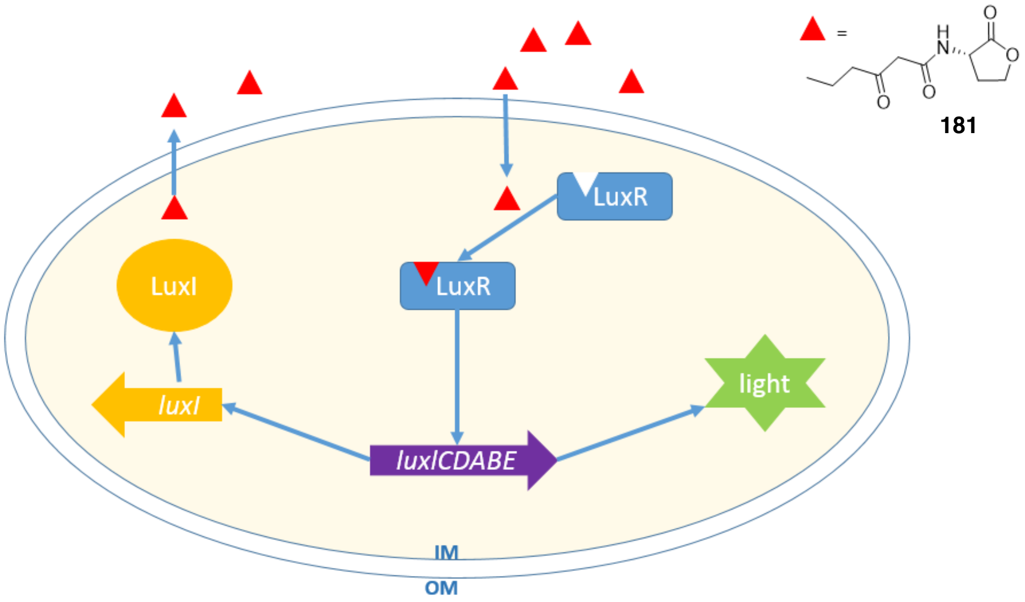


Figure 4.6 Quorum sensing regulation in *V. fischeri via* a LuxIR signalling circuit. Red triangles indicate the autoinducer, 3-oxo-C_6-HSL **181**, which is produced by LuxI. OM – outer membrane; IM – inner membrane.

Most other Gram-negative bacteria which possess LuxIR-type proteins communicate using AHL signalling molecules.[37] These systems are predominantly used for intraspecies communication as exquisite specificity exists between the LuxR proteins and their related AHL signals. These AHL signals are synthesised by LuxI-type proteins which link and lactonise the methionine moiety from *S*-adenosylmethionine (SAM) to specific acyl chains carried on acyl-acyl carrier proteins.[38,39] A diverse set of acyl side chains which vary in length, backbone saturation and side-chain substitution are found in AHL signalling molecules. These minor differences are crucial for signalling specificity.[40] AHLs **181** and **182**, for example, are signals used by bacteria which produce light, and **183** and **184** are some of the signalling molecules used by *Pseudomonas aeruginosa* (**Figure 4.7**).

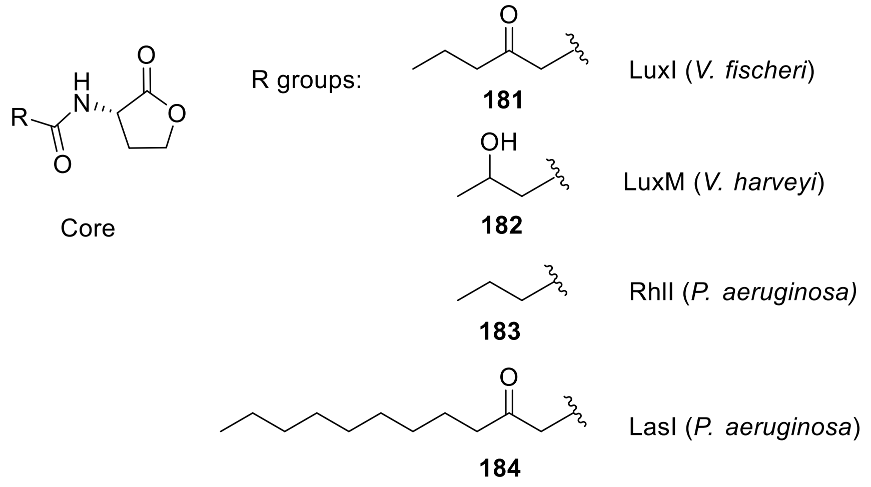


Figure 4.7 Examples of acyl-homoserine lactones used as signalling molecules in various species of bacteria.

Structural studies carried out in LuxI-type proteins indicate that each protein possesses a specific acyl-binding pocket that precisely fits a particular side chain moiety.[41,42] This structural feature configures specificity in signal production. This means that each LuxI protein can reliably produce the correct signal molecule. The structures of the LuxR proteins suggest that they too possess specific acyl-binding pockets that allow each LuxR to bind and be activated only by its specific signal.[43,44] Hence, in an environment where a number of different bacterial species are present, and therefore multiple AHL signal molecules, each species can easily distinguish, measure and respond only to the increase in concentration of its own signal. Another important factor is that bacteria rarely rely exclusively on one LuxIR QS system but instead use one or more LuxIR systems in collaboration with other types of QS circuits.[28]

As can be seen from **Figure 4.6**, the signal and detector can interact in the cytoplasm of the bacterial cell and so mechanisms must exist to prevent premature activation of LuxIR-type QS circuits. In 1999, Zhu and Winans demonstrated that one such mechanism is in play in the case of the LuxR homologue TraR in the plant pathogen *Agrobacterium tumefaciens*, where the stability of the LuxR-type proteins increases upon binding of autoinducer.[45] It was noted that in the absence of autoinducer, the half-life of TraR is only a few minutes. In contrast, when the AHL molecule is present, the half-life of TraR increase to over 30 minutes. A crystal structure of TraR has been obtained and it predicts that binding of the AHL is required for folding of the emerging polypeptide.[44] Radiolabelling studies demonstrated that radiolabelled TraR was only stabilised when its associated AHL was added prior to labelling of the protein.[46] This means that only when the concentration of AHL accumulates to a significant concentration, both outside and inside the cell, can TraR bind it, fold and then initiate the QS cascade. Another method that prevents premature activation of LuxIR systems is the active export of AHL molecules.[47] When a significant concentration of AHL has accumulated, which indicates the presence of a high number of bacterial cells, diffusion of AHL into the cell overwhelms export and thus engages the QS circuit. AHLs which contain long acyl side chains are thought to require active export in order to transverse the bacterial membrane.[47]

In contrast to Gram-negative bacteria, Gram-positive bacteria produce small linear or cyclic peptides for use as signalling molecules, which can be customised by chemical modifications that confer specificity. These cyclic peptides are perceived by a two-component regulatory system by means of a phosphorelay cascade to induce changes in gene expression.[48,49]

The use of QS as a means of cell density-dependent gene regulation is widely accepted, however two additional models have called into question its comprehensiveness and suggest alternative explanations as to why bacteria evolved diffusible signal molecules.[50,51] One of these models, known as diffusion sensing, proposes that signal molecules are inexpensive probes that allow a bacterium to assess the flux and mass transfer of an environment, which allows the cell the determine whether production of certain molecules, such as extracellular proteins and secondary metabolites, is viable.[51] The second method, termed efficiency sensing, combines the basic views of QS and diffusion sensing. In this method, flux, mass transfer, cell density and spatial distribution are important for signalling.[50]

4.4 *Pseudomonas aeruginosa*

At the end of the nineteenth century, after Pasteur's development of sterile culture medium, the first description of *Pseudomonas aeruginosa* as a distinct bacterial species was reported. The first scientific study on *P. aeruginosa* was published by Carle Gessard in 1882,[52] entitled "On the blue and green colouration of bandages." This characteristic pigmentation, which would later be attributed to pyocyanin (a phenazine derivative), is reflected in the old names *Bascillus pyocyaneus*, *Pseudomonas polycolor*, *Bakterium aeruginosa* and *Pseudomonas pyocyaneus*. As early as 1889, it was noted that *P. aeruginosa* was able to cause infections.[53] However, *P. aeruingosa* was not considered to be pathogenic[54] and in fact was seen as a source of potent antimicrobial substances.[55]

Prior to 1947, only 91 cases of septicaemia attributable to *P. aeruginosa* were reported in the literature.[56,57] It was not until the second half of the twentieth century that the importance of *P. aeruginosa* as a human pathogen, in particular in hospital patients, was realised.[58]

P. aeruginosa (**Figure 4.8**) is an antibiotic-resistant bacterium that is commonly found in soil and water, it can metabolise a wide range of carbon sources and is capable of growing in conditions of extremely low nutrient content.[59] With regards to water environments, *P. aeruginosa* is not found in seawater, most probably due to the high salt concentration restricting growth. In areas near to sewage outlets and other types of pollution it can be detected.[60-62] It has been identified in low concentrations in samples from private homes, schools, rivers and drinking water sources.[60,63] *P. aeruginosa* has been isolated from sink drains, toilets, showers and other bathroom fixtures, humidifiers and many medical devices and equipment that work with water.[63] In a study carried out in 1989, it was found that sinks in hospitals are generally more highly contaminated with *P. aeruginosa* than those in private homes.[64] In newer hospitals, sinks are mostly free of *P. aeruginosa* however they rapidly become contaminated.[65] This proves that humans that are contaminated or infected play an important, crucial role in transmission. The presence of *P. aeruginosa* has also been detected in warmer aquatic environments, such as on the recirculation system and filters in swimming pools and whirlpools, where higher temperatures are conducive to its growth.[60] Interestingly, it has been shown that *P. aeruginosa* can colonise many different types of surfaces, including stainless steel.[66,67] It appears to be almost impossible to purify water to the extent that prevents *P. aeruginosa* from growing. Even in distilled water with a purity of 71,000 ohms, multiplication of *P. aeruginosa* has, surprisingly, been observed.[68] *P. aeruginosa* has also been isolated from the soil, in particular from the rhizosphere.[69,70] It has also been found in a number of plants and vegetables.[70]

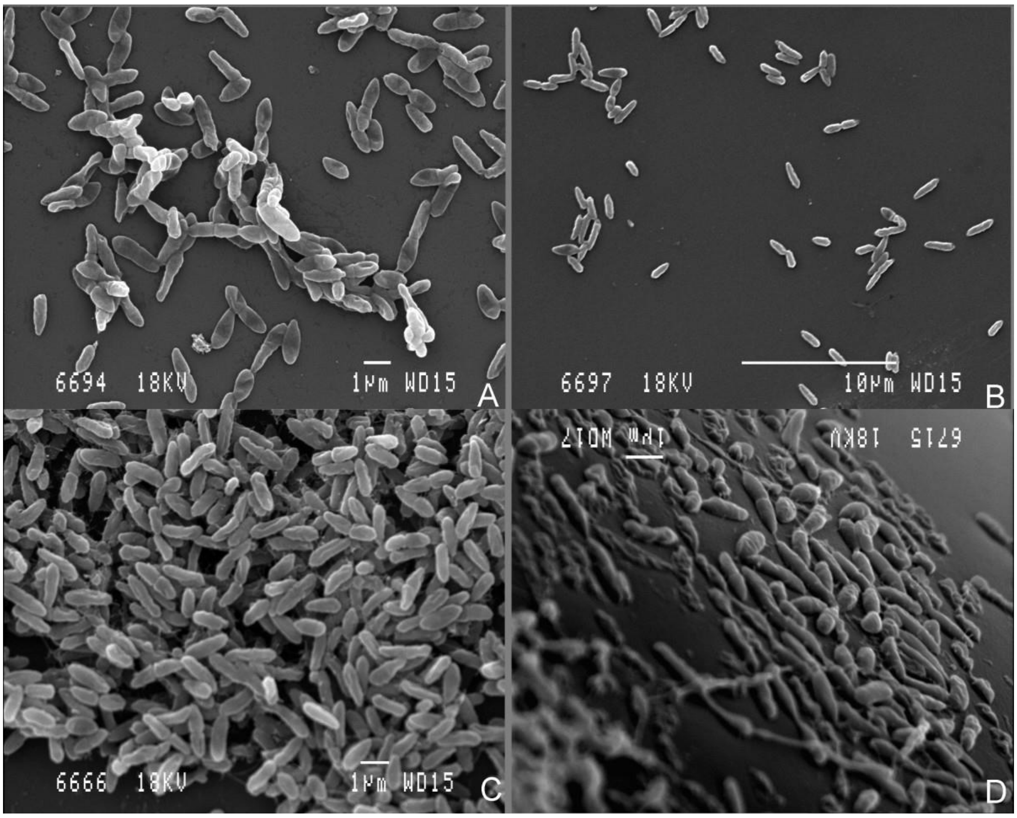


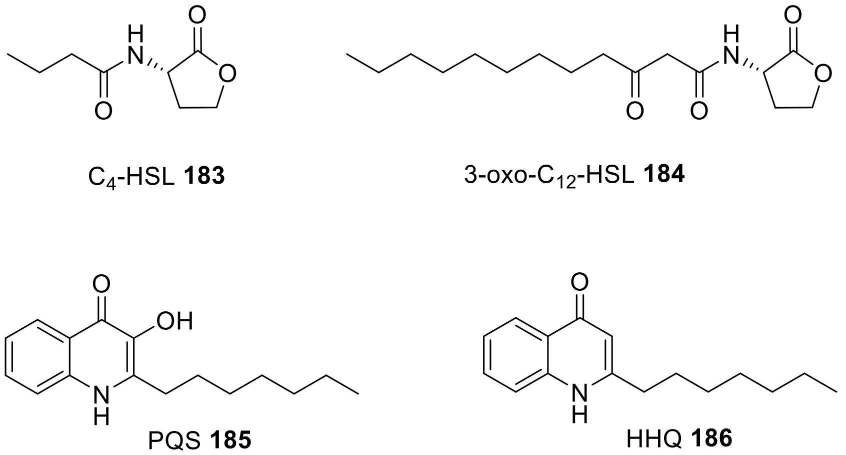
Figure 4.8 Scanning electron microscopy images of *P. aeruginosa* isolates attaching to glass surfaces. Weakly adherent *P. aeruginosa* isolated formed a monolayer (B and D), while the moderate and strongly adherent isolates formed clumps of cells (A and C) when biofilms were grown on glass cover slips. Microbial attachment first presented as clumps of cells (A and B, 7 and 14 h respectively after inoculation) and as the biofilm matured the spaces between the clumps were covered with a cell lawn (C and D, 20 and 40 h respectively).[71]

P. aeruginosa is classed as an opportunistic human pathogen as it can exploit a crack in a host defences to instigate an infection. In fact, it is the epitome of an opportunistic pathogen – the bacterium almost never infects uncompromised tissues but there is hardly any tissue it cannot infect if the defences are in some way compromised. Indeed, *P. aeruginosa* was only found in 1.2–2.3% of faeces from non-hospitalised, healthy individuals.[70,72-74] However, it is thought that in these cases, the source of the *P. aeruginosa* may be by ingestion of food contaminated with this organism.[75] Gastric acid and substances produced by anaerobic bacteria in the gut are thought to be involved in protection against colonisation by *P. aeruginosa*.[76] The formation of a biofilm, where the bacterial cells on a surface are embedded in a self-produced polymer matrix consisting of polysaccharide, protein and DNA, is necessary for persistent, chronic infection.[77] A bacterial biofilm causes a chronic infection because the bacteria show increased tolerance to antibiotics and they resist phagocytosis, as well as other components of both the innate and adaptive immune system.[77] *P. aeruginosa* causes a wide range of syndromes in humans that can vary from local to systemic, subacute to chronic, and superficial and self-limiting to life-threatening.[59] For example, it is the etiological agent for eye and ear infections, for super-infection of burn wounds and nosocomial pneumonia.[78] It primarily causes disease in predisposed or immunocompromised individuals and is one of the most common pathogens found in hospitals.[59] *P. aeruginosa* is the most common Gram-negative bacterium found in hospital-acquired and life-threatening infections of immunocompromised patients.[79] As it is naturally resistant to many commonly used antibiotics, it can persist in hospital disinfectants and sanitary facilities, more than likely contributing to its persistence in hospital environments. These facts are not surprising when it is considered that *P. aeruginosa* is one of the five most common pathogens isolated in hospital-wide surveillance and is the most common in intensive care units.[80]

In 1986, data from the US Centre for Disease Control in Atlanta publicised that *P. aeruginosa* was responsible for 11.4% of hospital-acquired infections of all sites, 12.7% of urinary tract infections, 16.9% of lower respiratory tract infections and 8.9% of surgical wound infections.[81] From 1984–1988 it was reported that *P. aeruginosa* went from being the fourth most frequent pathogen in hospital acquired septicaemia to being the most frequent.[82] The most probable reason for this trend is the increased resistance of *P. aeruginosa* to various antibiotics, including aminoglycosides, cephalosporins and quinolones.[83-85] Transmission of *P. aeruginosa* in a hospital setting can occur *via* a number of routes – either through patient to patient contact, contact between patients and hospital staff or contact between the patient and environmental sources. In particular, there are high contamination rates in hospital sinks and toilets. In a publication from 1991, it was found that when hand washing was performed in a sink contaminated with *P. aeruginosa* without the use of soap, 2400 colony forming units (CFU) of wild-type PAO1 strain were grown on the filter membrane after the hands were dried and placed in a sterile plastic bag containing 100 mL of physiological saline. In contrast, when soap was used, the result was 1200 PAO1 CFU per 100ml saline.[86] These results show that it is necessary to use appropriate disinfection while hand washing in sinks contaminated with *P. aeruginosa*. It follows that patients who are susceptible to infection are at a high risk of acquiring *P. aeruginosa*, or other bacterial infections, when they wash their hands or brush their teeth in contaminated sinks.

4.4.1 QS-signalling molecules in *P. aeruginosa*

P. aeruginosa uses QS in expressing virulence as well as in the production of biofilm.[87] There are two distinct but related QS circuits at play in *P. aeruginosa*. Both systems are genetically similar as they consist of genes encoding the transcriptional activator proteins, *lasR* and *rhlR*, as well as for the genes responsible for the production of AHLs, *lasI* and *rhlI*.[88-92] These systems are arranged hierarchically, with the *las* system on top of the signalling cascade, positively regulating expression of both *rhlR* and *rhlI*.[93] The QS systems of *P. aeruginosa* involve two distinct types of signalling molecules: two AHLs: *N*-butyryl-L-homoserine lactone (C_4-HSL) **183** and *N*-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C_{12}-HSL) **184** and the *Pseudomonas* quinolone signal (PQS) **185**, a 2-alkyl-4-quinolone (**Figure 4.9**).[94] 2-Heptyl-4(1*H*)-quinolone (HHQ) **186**, the biological precursor of PQS, has also shown to function as a signal molecule in *P. aeruginosa*.[95] In addition to HHQ and PQS, *P. aeruginosa* produces more than 50 other 2-alkyl-4-quinolones, most of which are functionally uncharacterised.[96,97] PQS was first isolated and chemically characterised in 1959 from *P. aeruginosa* culture supernatants, although its biological role was not known at this point.[98] AHL-dependent signalling is extensive among Gram-negative bacteria,[99] however 2-alkyl-4-quinolone signalling is more specific, and so far has only been detected in *P. aeruginosa* and certain *Burkholderia* and *Alteromonas* species.[100,101] Interestingly, the 2-alkyl-4-quinolones produced by *Burkholderia* species possess a methyl group at the C-3 position and it has been found that the presence of this methyl group is necessary for signalling.[101]



C_4-HSL **183**

3-oxo-C_{12}-HSL **184**

PQS **185**

HHQ **186**

Figure 4.9 Signalling molecules used by *P. aeruginosa*.

The intercellular signals for the *las* and *rhl* QS systems are C_4-HSL **183** and 3-oxo-C_{12}-HSL **184**, respectively.[102,103] These signals have been shown to control hundreds of genes, representative of 4–12% of the *P. aeruginosa* genome.[104-106] Studies have shown that PQS **185** induces the expression of the virulence factor elastase and *rhlI*, the gene which encodes C_4-HSL synthase,[94,107] suggesting that PQS acts as a connector signal between the *las* and *rhl* QS systems. In a study carried out by McGrath *et al*., it was found that transcription of *pqsA* and subsequent production of PQS was induced by the *las* QS system but repressed by the *rhl* QS system.[108] In the same study, it was found that production of PQS was dependent on the ratio of C_4-HSL **183** to 3-oxo-C_{12}-HSL **184**, providing more evidence of its role as a regulatory balance between QS systems.[108]

Unlike many QS signals, PQS is only slightly soluble in aqueous solutions, posing an interesting question as to how it is trafficked between cells. It has been shown that *in vitro*, PQS is solubilised by rhamnolipids, which are QS-regulated biosurfactants produced by *P. aeruginosa* that increase the solubility of molecules such as PQS which incorporate long alkyl chains.[109] It was later demonstrated that outer-membrane vesicles (MVs) are used to package and traffic PQS.[110]

It has been proven that the QS-signalling systems of *P. aeruginosa* are necessary for virulence and pathogenesis in multiple models of infection.[111] Several animal infection models have highlighted the involvement of QS-regulated virulence factors in the pathogenicity of *P. aeruginosa*. The simplest infection model is that in the nematode *Caenorhabditis elegans*, a worm which can feed on bacteria. In the case of an opportunistic pathogen such as *P. aeruginosa*, the worm is often killed within a short period of time after ingestion of the bacteria, due to the cyanide and phenazines secreted by the bacteria. However, when the worm feeds on bacteria which possess mutations in the QS regulatory systems, the worm remains alive.[112-114] It has been shown that QS plays a significant role in wound infections using the burned mouse model. The initial stages of chronic pulmonary infection can be modelled by casting *P. aeruginosa* into alginate beads which are then surgically inserted into the lungs of rats and mice.[115] When mice are infected with a strain of *P. aeruginosa* that have mutations in the QS systems, the mortality of the mice, horizontal spread of the infection, and dissemination of the bacteria throughout the body were all greatly reduced.[116] Additionally, the presence of a fully function QS system has been found to be important for both establishment and reduced clearance of a *P. aeruginosa* infection. Studies has proven that when animals are infected with QS mutants, the immune response is faster, the polymorphonuclear leucocytes (PMNs) respond by development of stronger oxidative bursts and antibodies accumulate faster in the infected lung.[117-120] Studies have found that AHL signalling can occur *in vivo* in the mouse lung, and AHL signal molecules are produced by *P. aeruginosa* growing *ex vivo* within sputum samples obtained from patients with Cystic Fibrosis (CF).[121,122]

The two different types of signalling (that using AHLs and that using PQS) are regulated differently. Whereas AHL signals are produced at a time of rapid population growth, PQS is produced maximally in the late stationary phase of growth.[107] These findings suggest that PQS signalling is important when *P. aeruginosa* cells are under stressful conditions, which would be typical of the conditions found during chronic infection in the lungs of a patient with CF.[123] PQS has been found in sputum samples of CF patients in estimated minimal concentration of *ca.* 2 μM, indicating that the presence of this molecule may be a crucial factor in the establishment of chronic infections, and thus may be a unique drug target for development of new therapies for treating *P. aeruginosa* infections.[123]

4.4.2 PQS and HHQ as modulators for interspecies and interkingdom behaviour

As stated previously, signalling molecules used by bacteria are often species-specific, however the ability of one species to be able to 'listen in' and decipher the messages of a competitive species, known as interspecies or interkingdom signalling, is a valuable asset in communities where a number of microbes coexist, such as those that exist during infection of the CF lung. This phenomenon is emerging as a key influence on the outcome of infectious diseases.

In 2006, Diggle *et al.*[100] examined genome databases and identified homologues of the *pqs* genes in other bacteria and found that *Burkholderia pseudomallei* and *B. thailandensis* contained the complete *pqsA-E* operon (termed *hhqA-E*), as in *P. aeruginosa*. When the authors introduced the *B. pseudomallei hhqA* and *hhqE* genes into *P. aeruginosa pqsA* and *pqsE* mutants, it was observed that virulence factor and PQS production was restored. It was also noted that *B. pseudomallei, B. thailandensis, B. cenocepacia* and *P. putida* all produce HHQ but not PQS. These findings divulge a role for alkyl-quinolone signalling in bacterial cell-to-cell communication beyond that seen in *P. aeruginosa*. A later study by Vial *et al.*[101] identified twenty nine different 4-hydroxy-2-alkylquinolines in three *Burkholderia* species, bearing a methyl group at the 3-position. While the function of HHQ analogues in these species has yet to be elucidated, strong structural similarities with the *P. aeruginosa* signalling molecule HHQ suggest the presence of a conserved interspecies signalling system.

In 2011, Reen *et al.*[124] investigated the possibility of HHQ and PQS playing a role in interspecies communication. In their study, the authors found that both HHQ and PQS were modulators of key phenotypes in Gram-positive and Gram-negative bacteria, as well as towards the eukaryotic yeast *Candida albicans*. Motility, which is associated with virulence in several bacterial species, was repressed in a broad range of bacteria, while biofilm formation in *Bacillus subtilis* and *Candida albicans* was repressed in the presence of HHQ. From their results, they were able to provide evidence for the structural requirements that define the interspecies role of these molecules. In particular, the presence of the alkyl chain at C-2 proved to be fundamental.

4.5 Cystic Fibrosis (CF)

CF is a genetically inherited disease which affects many organs in the body, but in particular the lungs.[125] Ireland has the highest incidence of CF in the world with approximately 1 in 19 people being carriers of the defective gene.[126] The main symptom of the disease is a build-up of thick, sticky mucus due to a mutation in both copies of the gene coding for CF transmembrane conductance regulator (CFTR) protein, which is expressed in many epithelial and blood cells. CFTR functions as a chloride ion transporter across the membrane of cells that produce mucus, sweat, saliva, tears and digestive enzymes, controlling the movement of water in tissues. A mutation in the CFTR gene disrupts the function of the chloride channel, preventing normal flow in and out of cells, resulting in the cells lining the passageways of the lungs, pancreas and other organs producing mucus that is abnormally think and sticky. This is problematic in the lungs in particular as it leads to bacterial infection and airway obstruction (**Figure 4.10**).[127]

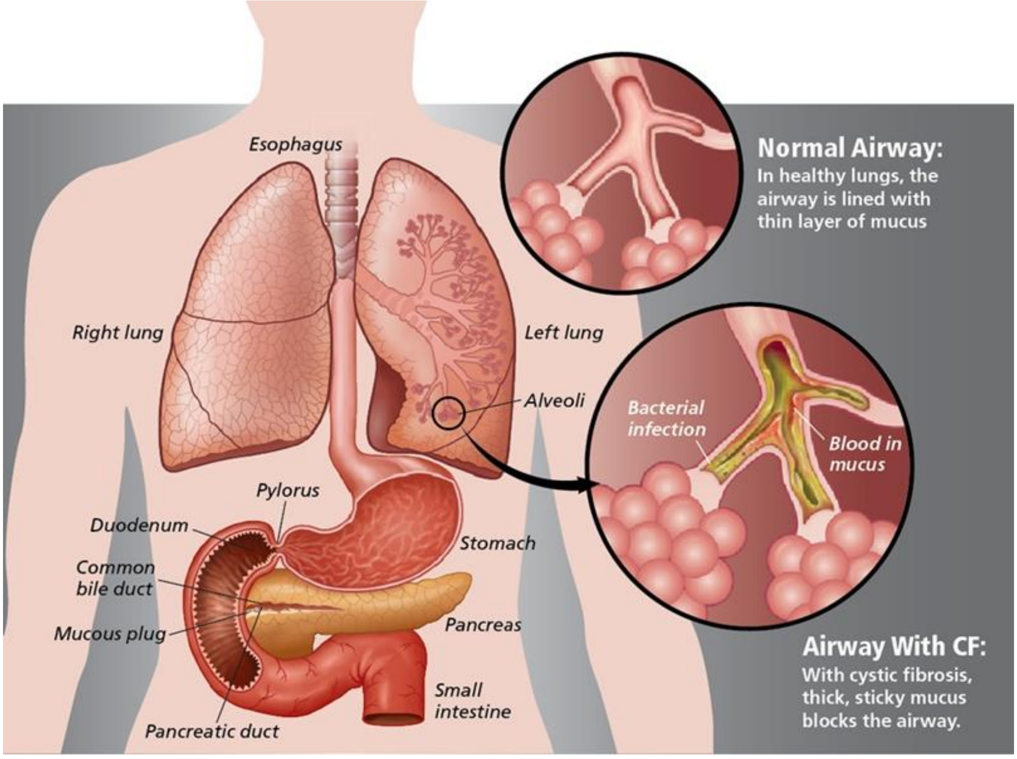

Esophagus
Normal Airway:
In healthy lungs, the airway is lined with thin layer of mucus
Right lung
Left lung
Alveoli
Bacterial infection
Blood in mucus
Pylorus
Duodenum
Stomach
Common bile duct
Mucous plug
Pancreas
Airway With CF:
With cystic fibrosis, thick, sticky mucus blocks the airway.
Small intestine
Pancreatic duct

Figure 4.10 Comparison of airway in healthy individual and individual with CF.[127]

Patients with CF are particularly disposed to infection from *P. aeruginosa* (**Figure 4.11**) and it is responsible for high rates of morbidity and mortality associated with the disease.[128] The chronic infection by *P. aeruginosa* is also problematic in that it has an important psychosocial impact on the quality of life of the sufferer as the regular doses of antibacterial chemotherapy required to control the infection are time-consuming to administer.[129,130] Airway infections with *P. aeruginosa* in CF patients are unique in that they chronically affect a host who is immunocompetent in terms of cellular and humoral responses but is immunocompromised by impaired airway clearance.[131] Most CF patients who have *P. aeruginosa* infection become colonised during school age or early adolescence. Once infection by *P. aeruginosa* has become established, the bacteria are notoriously resistant to eradication by chemotherapy.[131] The pseudomonads chronically colonise the bronchiolar lumen and virtually never breach the epithelial barrier.[132]

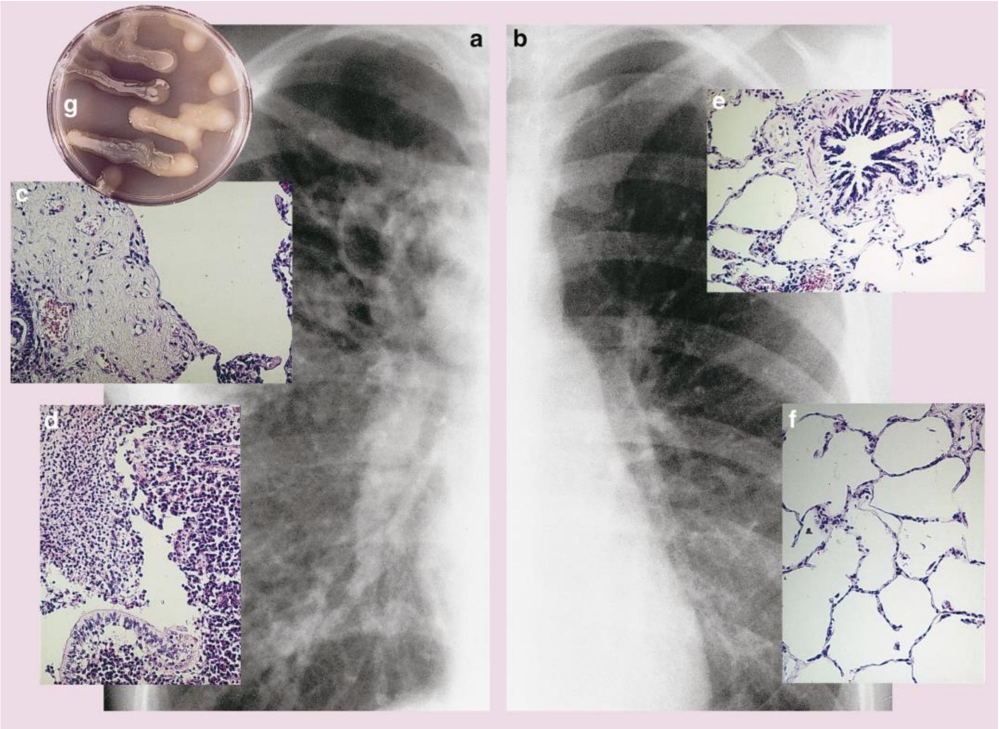


Figure 4.11 Comparison of (**a**) the radiograph of the right lung of an adolescent with CF who has been suffering from chronic *P. aeruginosa* infection for more than 10 years with (**b**) the left lung of a sex- and age-matched healthy non-CF control. The light micrographs show sections from (**c**) the bronchus and (**d**) the alveoli of a lung explant of another CF patient at the time of transplantation and normal (**e**) bronchus and (**f**) alveoli. Destructive emphysema, fibrosis (**c**) and massive immigration of neutrophils (**d**) are apparent in the remodelled CF tissue inhabited by *P. aeruginosa* for more than 20 years. The plate (**g**) shows typical colonies of *P. aeruginosa* found in mucus isolated from the sputum of a patient with CF and grown *in vitro* for 72 h at 37 °C.[80]

Genomic fingerprinting has shown that most CF patients become colonised with a single clone of *P. aeruginosa* that remains throughout their lifetime.[133] However, co-colonisation with more than one clone of *P. aeruginosa* occurs in 20-30% of patients in an either permanent or temporary manner.[80]

Biofilm growth associated with *P. aeruginosa* in the lungs of individuals with CF is particularly problematic. Due to the nature of the biofilm, chronic infection with the bacteria is quickly established and the result is an increased tolerance to antibiotics and the bacteria are also able to resist phagocytosis. A consequence of this is that a pronounced antibody response develops, leading to immune complex-mediated chronic inflammation, which is the major cause of lung tissue damage in individuals with CF. The growth of *P. aeruginosa* biofilm in the lungs of individuals with CF is associated with an increased frequency of mutations, slow growth and adaption of the bacteria to the conditions in the lungs and to antibiotic therapy. Low bacterial metabolic activity and increase of the doubling times of the bacterial cells in CF lungs are responsible for some of the tolerance to antibiotics. Biofilms can be prevented by early aggressive antibiotic prophylaxis or therapy, and they can be treated by chronic suppressive therapy.[77]

In a study by Yoon *et al*.[134] it was found that *P. aeruginosa* in the lungs of CF patients live in anaerobic biofilms and adopt a metabolic pattern and phenotype that differ significantly from those grown *in vitro* and from those that grow in aerobic biofilms. These results show not only that *P. aeruginosa* can grow in anaerobic conditions but also that this is the preferred mode of growth.[135]

4.5.1 Treatment of CF-associated *P. aeruginosa* airway infections

Antimicrobial chemotherapy is the basis of all attempts to control bacterial infections in CF.[136] If infection by *P. aeruginosa* is identified at an early stage while it is still in the initial phase of colonisation, long-term aerosol antibiotics are an efficient way of eradicating the infection.[137] If the infection by *P. aeruginosa* is at the chronic stage, treatment with antipseudomonal antibiotics in two to four-week courses per year is necessary[129,130] along with long-term administration of aerosolised antibiotics. *P. aeruginosa* co-exists with other species of bacteria in the airways of CF patients, including *Staphylococcus aureus*, which is treated with antistaphylococcal drugs and *Burkholderia cepacia*, the treatment of which is more difficult due to its intrinsic resistance to a broad range of antimicrobial agents. Usually treatment requires synergistic drug combinations in order to be effective. Other measures taken to treat chronic infection include following a high-calorie diet, the correction of nutritional deficits and the application of anti-inflammatory, mucolytic or antiobstructive drugs. Sports and physiotherapy sessions help with drainage and expectoration of the infected bronchial secretions.[80] The final stage therapeutic option for patients with end-stage lung disease is a lung transplant, however chronic rejection of the donor lung is a major issue. The average five-year survival after double lung transplantation of individuals with CF at the Hannover centre was 70% in 1998.[80]

It has been shown that when CF patients are isolated upon hospitalisation, there is a decrease in the number of *P. aeruginosa* pulmonary infections.[138,139] As it has been established that spread of both *P. aeruginosa* and *B. cepacia* has been demonstrated whenever adequate genotyping has been carried out,[130,131,133,140] grouping of patients according to bacteriological status has become a routine hygienic measure in many hospitals and CF centres. The natural habitat for both species of bacteria is an aquatic one, and filter devices reduce the contamination of water supplies, sinks, and basins with pseudomonads in CF clinics, but thermal and chemical disinfection are not useful. Care must also be taken when dealing with moist medical aids to ensure control of bacterial infection. Following these simple, but time-consuming and potentially expensive measures significantly reduce the incidence and prevalence of the major CF pathogens.[80] The ability of *P. aeruginosa* to rapidly develop antibiotic resistance, as well as the severe consequences it has on patients with CF and other diseases, has been a driving force in the search for novel therapeutic targets, such as the intercellular signals and their synthetic pathways.[131,141]

4.6 Quorum-sensing inhibitors as anti-pathogenic drugs

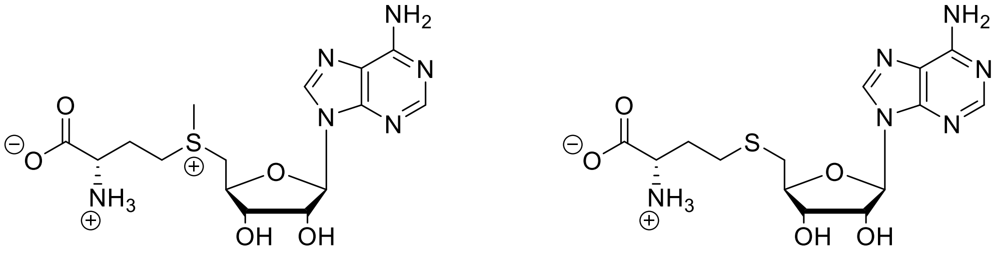
Treatment of a bacterial infection with traditional antibiotics has a major drawback – when growth of bacteria is blocked, the bacteria are under harsh pressure to develop resistance. A highly attractive target for the development of new alternatives to traditional antibiotics is to interrupt the QS-signalling systems of pathogens which use this method to regulate their pathogenicity, known as the anti-pathogenic drug principle.[142] By selectively blocking the control apparatus of virulence and pathogenic traits of bacteria, the infecting bacteria may fail to adapt to the host environment and be unable to establish an infection.[143] In interrupting the QS-signalling system, bacterial growth and survival are unaffected, eliminating the immediate risk of development of resistance. As bacterial growth is, therefore, normal, mutations affecting pathogenicity inhibition are not selected.

The bacterium does not know that it is exposed to an inhibitory agent and can continue to grow as normal but will not switch on the genes encoding for its pathogenicity.

There are three different targets in Gram-negative quorum sensing systems – the signal generator, the signal molecule itself and the signal receptor.[143] Screens can be carried out on compound libraries to investigate if signal molecules can be prevented from being synthesised by the relevant protein. If no signal molecule is produced then the bacteria will be unable to determine if and when a quorum has been achieved and QS-controlled genes will not be activated. The signal molecule itself can be targeted by either metabolic, chemical or enzymatic degradation, again preventing accumulation of the signal molecule. The most thoroughly studied target of the three is that of the signal receptor. If the signal molecule is prevented from binding to the receptor protein, it is stopped from being able to act as a transcriptional regulator.

4.6.1 Targeting the signal generator

In vitro tests have been performed by Parsek *et al.*[39] which have shown that analogues of SAM **187**, a precursor for the AHL signals produced by *P. aeruginosa*, are potent inhibitors of the *P. aeruginosa* AHL synthase, RhlI. Of the analogues tested, the most effective, *S*-adenosyl-L-homocysteine **188**, was found to lower the activity of RhlI by 97% (**Figure 4.12**).



S-Adenosyl methionine (SAM) **187**   *S*-Adenosyl-L-homocysteine **188**

Figure 4.12 Structures of *S*-adenosyl methionine, SAM, a precursor for the AHL signals produced by *P. aeruginosa* and the most potent analogue which proved to be an inhibitor of RhlI, *S*-adenosyl-L-homocysteine.

4.6.2 Targeting the signal molecule

Unsurprisingly, the homoserine lactone ring moiety present in AHLs (**Figure 4.9**) is unstable at pH levels above 7. At alkaline pH, the molecule undergoes lactonolysis and the biological activity is lost.[144] It has been shown that in stationary-phase cultures of *Erwinia carotovora*, *P. aeruginosa* and *Yersinia pseudotuberculosis* which are grown in unbuffered media have high pH levels and accordingly, only low levels of active AHL molecules are detected. AHLs which were introduced to the system exogenously also rapidly lose their ability to activate QS systems. It must be noted that pH dependency is not the only factor in play with regards to the ring opening. Temperature and the nature of the acyl side chain are factors which must also be considered. As would be expected, higher temperatures increase the rate of ring opening. In general, AHLs which possess longer acyl side chains take longer to undergo lactonolysis than those which have shorter acyl side chains. These findings indicate that in order for molecules to be active at physiological pH, the acyl side chain must be at least four carbons in length.[144,145] Interestingly, host organisms appear to take advantage of this property when they are attacked by bacteria which produce AHL signalling molecules. Byers *et al*. found that plants which are infected with the tissue-macerating pathogen *E. carotovora*, which uses QS to control expression of virulence factors, increase the pH level at the site of infection.[145] This alteration to an alkaline environment leads to inhibition of AHL dependent QS and reduced the extent of the degradative attack.

The marine alga *Laminaria digitata* has devised a different strategy to inactivate QS molecules, in this way avoiding biofouling.[146] *L. digitata* produces and secretes oxidised halogen compounds including hypochlorous and hypobromous acids, which have been used extensively for the eradication of microbes in industrial settings but they are also capable of reacting with oxidised AHL signal molecules. QS is known to be involved in the development of biofilms and their maintenance, and so secretion of compounds that destroy the AHL signal molecules, blocking QS, may be of benefit to the alga in its competition with biofouling organisms.[146]

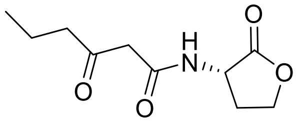
The identification of acyl-homoserine lactonase (AHL-lactonase) from a Gram-positive *Bacillus* species 240B1 brought to the fore another method of QS molecule inactivation.[147] This enzyme inactivates AHLs by hydrolysing the lactone bond of the molecules, and lowers the amount of bio-active AHL.[148,149] Indeed, Dong *et al*. demonstrated that complete inactivation of 20 μM 3-oxo-C_6-HSL can be achieved within two hours by a suspension culture.[148] The virulence of *E. caratovora* towards various plants and vegetables is greatly diminished when this enzyme is expressed from a plasmid transformed into the bacteria. Transgenic tobacco plants that express AHL-lactonase are much less prone to maceration by *E. caratovora* than their wild-type counterparts, suggesting that production of AHL-degrading enzymes would be a protective mechanism and an advantage for plants. Production of lactonases may also be a bacterial strategy in the competition with AHL-producing strains in their environment. AHL-lactonases hold potential commercial interest, however their application is more than likely limited to topical use as there are significant problems associated with the delivery of proteinaceous agents systematically. One must keep in mind the fact that the reaction is reversible, whether the lactonolysis occurs *via* chemical or enzymatic methods. In an acidic environment, a ring opened AHL will undergo ring formation producing the bio-active ring compound which is then able to activate QS-controlled virulence genes.[150]

Variovorax paradoxus and *P. aeruginosa* are able to grow on AHL molecules by using them as a source of carbon, energy and nitrogen. A correlation is observed between the length of the acyl side chain of the AHL and the molar growth yield, indicating that the side chain is used as a carbon source.[151] The bacteria achieve this by producing an enzyme that cleaves the amide bond of the signal molecule, an aminoacyclase, which yields a fatty acid and a homoserine lactone. The acid undergoes β-oxidation and it can be used as a source of both carbon and energy. The nitrogen is made available for the bacteria by the action of enzymes which release ammonia from the homoserine lactone ring moiety.[152] *P. aeruginosa* produces an AHL-inactivating enzyme encoded by *pvdQ*. When this gene is expressed in recombinant *E. coli*, the culture is able to rapidly inactivate exogenously supplied AHLs. When the gene is expressed in *P. aeruginosa*, the culture does not accumulate AHL molecules. When a strain is used which does not have the *pvdQ* gene present, *P. aeruginosa* is still able to utilise 3-oxo-C_{12}-HSL as the sole source of carbon, nitrogen and energy. This suggests that *P. aeruginosa* actually has several systems that it uses for degradation of AHL molecules.[152]

4.6.3 Targeting the signal receptor

The methodology of using small molecules to block the activation of LuxR homologues in order to inhibit QS is probably the most thoroughly studied of the three categories. Using a molecule that can be considered analogous to the native AHL molecules to block the receptor site is a classical pharmacological approach to receptor antagonism.

In a study carried out by Schaefer *et al.*,[153] analogues of 3-oxo-C_6-HSL **181** (**Figure 4.13**), the signalling molecule used by *V. fischeri*, involving substitution and other alterations to the acyl side chain, were identified which could displace ^{3}H-labelled 3-oxo-C_6-HSL from LuxR. It was found that the compounds that were able to bind to LuxR were able to displace the AHL and also activate the LuxR protein.



3-oxo-C_6-HSL **181**

Figure 4.13 Signalling molecule used by *V. fischeri*.

Another strategy to generate compounds to block the AHL receptor site has been to replace the HSL ring part of the AHL with an alternative ring structure. In the case of *P. aeruginosa*, alterations to two of the signal molecules used by the bacteria, 3-oxo-C_{12}-HSL **184** and C_4-HSL **183** (**Figure 4.14**) were made whereby an amino cycloalcohol or amino cycloketone containing either five or six carbon atoms in the ring were incorporated in place of the lactone ring (**189-196**).[154] The authors of this study identified new agonists of both HSL autoinducers used by *P. aeruginosa*, allowing important information about the unique R protein-autoinducer interaction in each cognate pair to be ascertained. It was found that **189** and **192** were activators of LasR, while **193** and **195** were activators of RhlR. Compound **190** was found to be a moderately active antagonist of **184**, and **191** a strong antagonist, inhibiting the QS cascade, which resulted in reduced expression of important virulence factors and biofilm.[154]

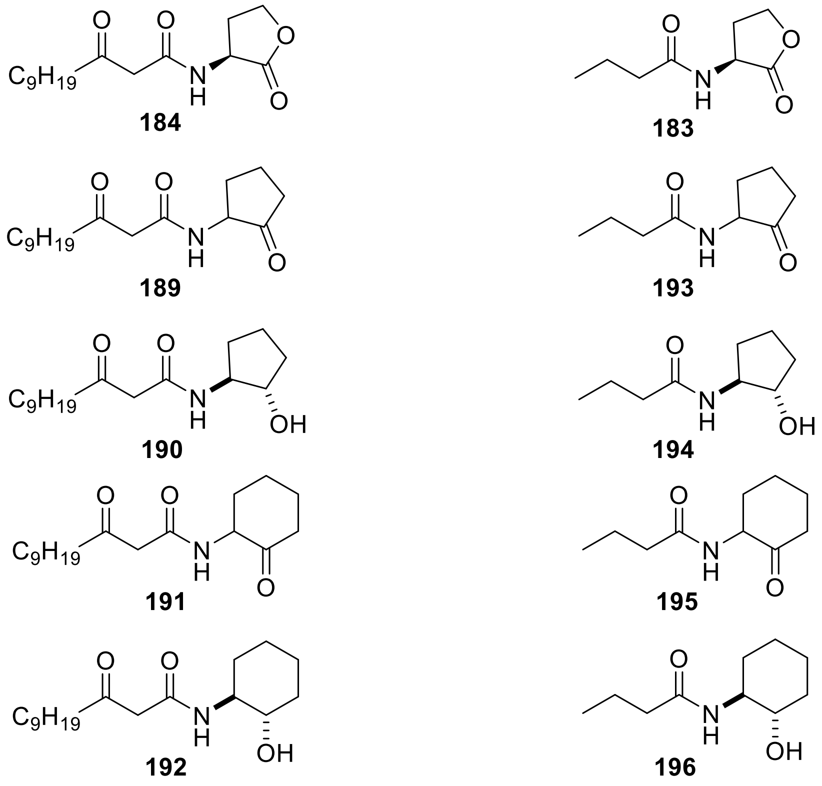


Figure 4.14 Structures of autoinducers and analogues.

Inihibitors of QS have been identified by random screening of pure compound libraries.[114] Using the QS inhibitor selector screen a number of compounds were identified that were able to block both LuxR- and LasR-based QS, with 4-nitropyridine-*N*-oxide **197** being the most effective inhibitor of this group of compounds (**Figure 4.15**). It was found that **197** significantly down regulated 37% of the QS regulated genes in *P. aeruginosa*. The genes targeted by **197** were regulated either by RhlR alone or else in conjunction with LasR, indicating that RhlR is the possible target for this compound. This screening system successfully identifies compounds able to interfere with LuxR-homologues protein but as the structure of these compounds is very different from the native AHLs, their mode of action is unknown.[114]

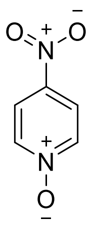


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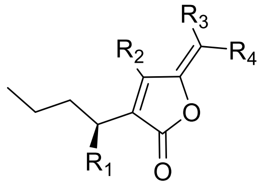
Figure 4.15 QS inhibitor 4-nitropyridine-*N*-oxide.

Halogenated furanones **198-202** (**Figure 4.16**) produced by the Australian macroalga *Delisea pulchra* have been shown to interfere with several AHL-regulated bacterial processes without having any effect on bacterial growth or general protein synthesis capability.[155-158] The current hypothesis is that furanones act as antagonists of AHL molecules as they compete for the binding site on the receptor protein. Manefield *et al.* reported that when halogenated furanones are present at concentrations similar to that produced by the alga, they are capable of displacing the native AHL molecules from the LuxR receptor protein.[157]



	R_1	R_2	R_3	R_4
198	H	Br	Br	Br
199	H	Br	H	Br
200	OAc	Br	H	Br
201	OH	Br	H	I
202	H	H	Br	Br

Figure 4.16 Halogenated furanones produced by *Delisea pulchra*.

With regards to *P. aeruginosa*, several studies that investigate both QS and inhibition of QS have shown that furanones can inhibit biofilm formation in *in vitro* experiments.[159,160] One such synthetic furanone is characterised by lack of a side chain at position 3 of the furanone ring, a bromine substituent at the methylene group and no bromine substituents on the furanone ring itself (**203**, **Figure 4.17**).[159] This furanone displays enhanced AHL-antagonistic properties, has little to no effect on the growth of *P. aeruginosa*, and is active against the *rhl* QS system.

Although early formation and attachment of the biofilm was not affected by **203**, when wild-type biofilm was grown in its presence, the biofilm failed to mature, suggesting **203** may inhibit expression of the gene responsible for biofilm maturation. These results posed an interesting question: Can these types of compounds be used to render a biofilm more susceptible to antimicrobial compounds? The answer is yes, demonstrated by Hentzer *et al.* when they reported that a *P. aeruginosa* biofilm, when treated with **203**, was easily killed by subsequent treatment with tobramycin, suggesting that a combination treatment of QS inhibitors and antibiotics may prove useful.[142] It was also noted that the furanone-treated biofilm was also more prone to dispersal by the detergent sodium dodecyl sulfate (SDS). Unfortunately, the halogenated furanones are unsuitable for clinical use due to their instability.[143]

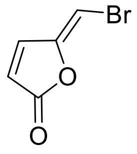


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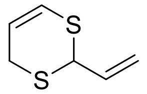
Figure 4.17 Halogenated furanone that inhibits maturation of biofilm in *P. aeruginosa*.

Due to the coexistence of prokaryotes and eukaryotes over billions of years, it is unsurprising to find that at least a few eukaryotes have developed bacterial defence systems based on inhibitors of QS systems. Many plants and vegetables, including carrots, garlic, habanero (chili) and water lily produce compounds that interfere with bacterial QS.[114] Garlic extracts contain two compounds **204** and **205** (**Figure 4.18**) which have shown to be inhibitors of bacterial QS, however they exist in very low concentration and are not as potent as other QS inhibitors, and use in a clinical setting would therefore be limited.[161]



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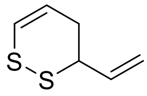

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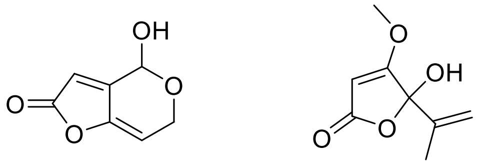
Figure 4.18 QS inhibitors found in garlic extracts.

Since Alexander Fleming's discovery of penicillin, several antibiotics and medically important compounds have been isolated from various fungi. Rasmussen *et al*.[162] carried out a screen on a selection of *Penicillium* species and were able to identify several strains which produced compounds that acted as inhibitors of QS. Two of these compounds were identified as patulin **206** and penicillic acid **207** (**Figure 4.19**), and were found to downregulate 45% and 60% of QS-regulated genes in *P. aeruginosa* respectively, indicating specificity for QS-regulated gene expression. Despite this result, **206** and **207** are known mycotoxins, which limits their pharmaceutical relevance.[162]



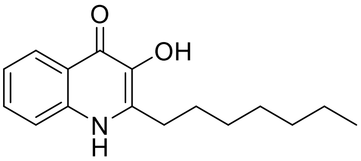
206 **207**

Figure 4.19 QS inhibitors isolated from *Penicillium* species.

4.6.3.1 Targeting the signal receptor in *P. aeruginosa* using analogues of PQS

PQS plays a particularly important role with regards to the virulence of *P. aeruginosa*. However there has only been one comprehensive structure-activity relationship (SAR) investigation carried out on a diverse range of PQS analogues (**208-223**), with variations on the alkyl chain as well as the anthranilate ring (**Figure 4.20**).[163]

Structure-activity studies prior to this focussed on the activity of analogues with variations in the alkyl chain, in particular with the effect of these analogues on membrane vesicle (MV) formation which are used to transport hydrophobic PQS out of the bacterial cells.[164] The authors found that both the hydroxyl group at the 3-position and the alkyl chain at the 2-position are necessary for stimulation of MV production.[164] In another study, the dependence of alkyl chain length on stimulating transcription of *pqsA* and *lecA* promoters was investigated with only slight variations in alkyl chain length being tolerated.[165]



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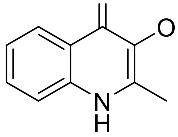

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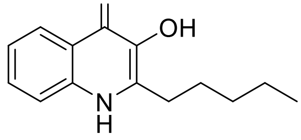

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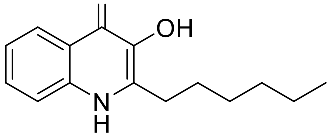

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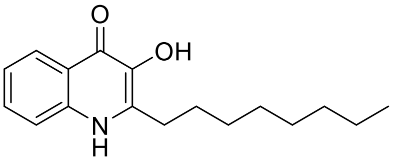

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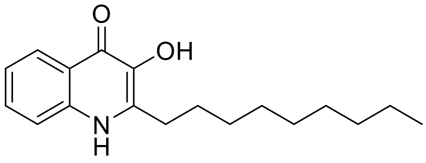

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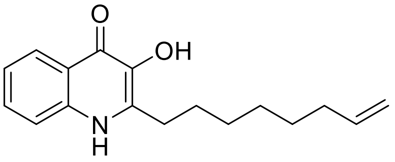

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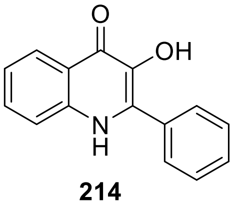

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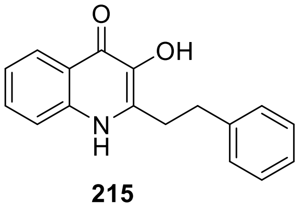

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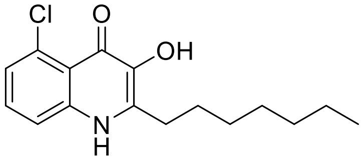

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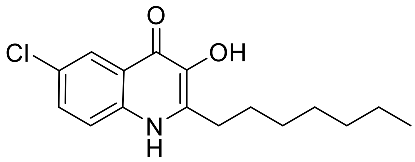

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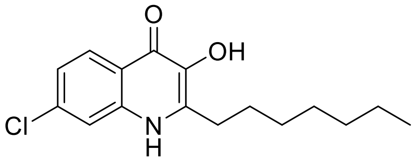

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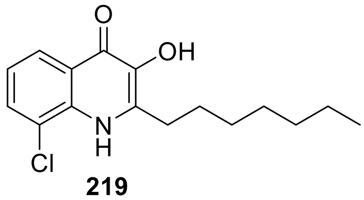

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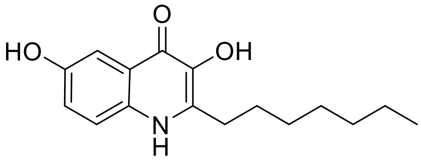

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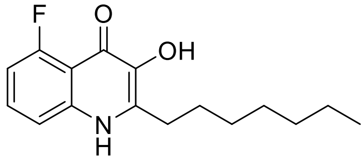

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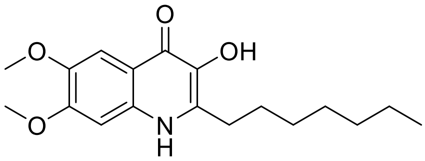

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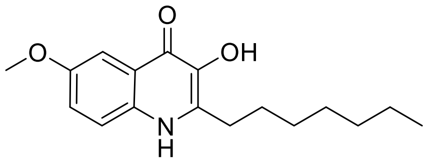

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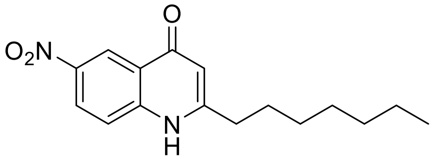
Figure 4.20 Structures of PQS analogues by Hodgkinson and co-workers.[163]

From the results of the comprehensive SAR study carried out by Hodgkinson *et al*., it was discovered that the length of the alkyl chain had a strong effect on the agonism of the compound towards PqsR.[163] PQS and HHQ are known to activate PqsR, also known as MvfR, the multiple virulence factor regulator in *P. aeruginosa* that drives the coordinated expression of nearly 200 genes and is necessary for full virulence.[166,95] Many of these genes are related to virulence factors, for example *phzA1-G1*, which are involved in the biosynthesis of pyocyanin; *hcnAB*, responsible for the production of hydrogen cyanide; *lasB*, which encodes elastase B; *rhlAB*, which is involved in the synthesis of rhamnolipids and *lecA*, which codes for Lectin A.[166,167] The production and formation of biofilm is also controlled by this system.[168] This signalling system is also used to control MV formation. These factors all contribute to making PqsR an attractive target for anti-QS molecules. Where a methyl group was in place at C-2 (**208**), it was noted that the compound was effectively inactive towards PqsR. As the length of the alkyl chain increased in length (**209** and **210**), so too did the agonism of the compounds. Interestingly, compounds which had alkyl chains of greater than 7 carbons (**211, 212** and **213**) retained substantial agonist activity, indicating the PQS receptor can accommodate these variants. These results are consistent with those obtained in an earlier SAR study by Fletcher and co-workers, who noted similar alkyl chain length dependency for agonism.[165] Compounds where phenyl groups were present at C-2 in place of the alkyl chain (**214** and **215**) were not tolerated by the binding pocket of the receptor as agonist activity was either dramatically reduced or eliminated. Substituents at various positions on the anthranilate ring were then investigated. An electron-withdrawing chloro substituent was placed at each of the four available positions on the anthranilate ring (**216-219**) however a decrease in agonist activity was observed in all cases. The introduction of an electronegative fluorine atom at position 5 however appeared to have no effect in comparison with the equivalent chloro analogue (**221** and **216** respectively). Conversely, the introduction of electron-donating hydroxyl or methoxy groups at position 6 (**220** and **223**) or position 7 (**222**) led to a significant decrease in activity, suggesting that the electron density of the aromatic ring may also have an influence.[163]

In 2012, the first antagonists of PqsR which acted as QS inhibitors were published.[169] The authors, following a ligand-based drug design approach, synthesised a set of HHQ and PQS analogues, with variations made on the alkyl chain and substitutions made at various positions on the anthranilate ring. As the protein structure for PqsR is yet to be determined and antagonists of the receptor have not been identified, the researchers used the natural ligands, which are agonists, for the design of possible antagonists (ligand-based approach) as it has previously been shown that structural modification of agonists can provide antagonists.[170] Although PQS is the more potent of the two natural ligands, analogues were based on the less potent HHQ.[95] This was chosen for a number of reasons. Firstly, it has been shown that the hydroxyl group at C-3 in PQS is responsible for interaction with the lipid A portion of lipopolysaccharides (the lipid A portion serves as the liquid anchor and is commonly composed of fatty acids, sugars and phosphate groups).[171] Synthesising analogues of HHQ, which do not possess this group at C-3, should therefore exhibit a lower tendency to membrane association. Another reason for this preference with regards to the synthesis of potential antagonist analogues is the fact that HHQ does not exhibit iron-chelating or pro-oxidant properties, and so modifications to HHQ should avoid these unwanted interactions.[172-174] From the analogues synthesised, those which possessed strong electron-withdrawing groups such as nitro, trifluoromethyl or nitrile (**224-226**, **Figure 4.21**), exhibited strong antagonistic properties by either completely or substantially inhibiting PqsR stimulation.



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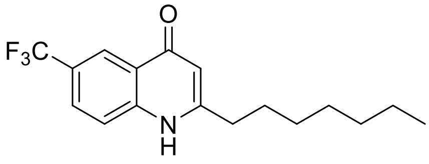

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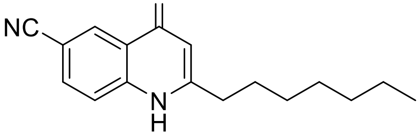

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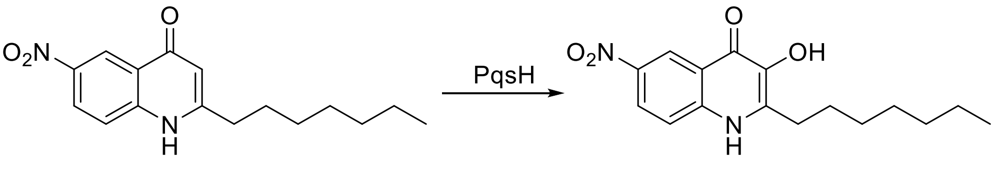
Figure 4.21 Analogues with electron-withdrawing groups present at C-6.

When the position of the electron-withdrawing group was varied to other positions on the anthranilate ring, the analogues lost their antagonistic ability. These results implied that the position of the electron-withdrawing group, as well as the electron-withdrawing effects, are of utmost importance in synthesising an antagonist. Compound **224** was deemed capable of reducing pyocyanin production in *P. aeruginosa* PA14 (a highly virulent strain of the bacteria) supernatants by 74% at a concentration of 3 μM.[169] An important finding was obtained regarding the growth kinetics of this strain in the presence of **225** and **226** – the growth of the bacteria remained unaffected despite the presence of the antagonists, proving that this methodology of selectively targeting bacterial QS-controlled virulence has no impact on bacterial viability and thus should not induce natural selection pressure.

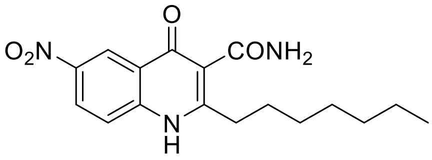
Although **224** was highly active towards PqsR, only a moderate reduction in pyocyanin production was observed. This prompted Lu *et al.* to further characterise the behaviour of the antagonist in *P. aeruginosa*.[175] Investigations suggested that a biotransformation of **224** to **227** may have occurred in *P. aeruginosa* by the action of the enzyme PqsH, which hydroxylates HHQ to form PQS (**Figure 4.22**). The study proved that this was indeed the case, and **227** restored PqsR stimulation.



224 **227**

Figure 4.22 Biotransformation of **224** by PqsH.

The authors then investigated blocking of the 3-position with an appropriate functional group which would prevent this biotransformation from occurring.[175] Of the 3-substituted compounds synthesised, carboxamide **228** proved to be a highly potent, antivirulence agent. This is a significant finding and suggests these compounds could overcome the shortcomings of traditional antibiotics, which are quickly rendered useless due to the problem of bacterial resistance.[169]


O
O2N
CONH2
N
H

228

Figure 4.23 Blocking the susceptible positon of **224**.

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Chapter 5

Structure–function analysis of the C-3 position in analogues of microbial behavioural modulator, HHQ

Results and Discussion

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5.1 Background to project

The aim of this project was to synthesise a number of analogues of communication molecules PQS and HHQ which could potentially act as QS inhibitors in *P. aeruginosa*. Work focused on introducing atoms at the C-3 position of the HHQ framework as this particular position has been understudied in the literature,[1-3] with only one recent publication beginning to probe the area.[4] In particular, atoms introduced at C-3 were chosen to have similar properties to those present in the quorum sensing molecules themselves (i.e. a hydroxyl group in PQS and a hydrogen in HHQ). These analogues would then be sent for biological testing in collaboration with Prof. Fergal O'Gara in the Department of Microbiology in University College Cork.

The first objective of the project was to synthesis HHQ, which would be achieved *via* a five-step synthesis.[5]

The second objective of the project was to synthesise various C-3 analogues of HHQ, including 3-bromo-HHQ, 3-chloro-HHQ, 3-iodo-HHQ and 3-fluoro-HHQ *via* electrophilic aromatic substitution using the appropriate reagents. Installation of a fluorine at the 3-position was of particular interest as the product would be sterically similar to the native molecule, HHQ. While previous work within the group has involved the synthesis of 3-bromo, 3-iodo and 3-chloro analogues, it was postulated that alternative methodologies of the 3-bromo and 3-iodo analogues could provide the desired products in increased yields. An analogue with a methyl group at the 3-position would also be synthesised and tested for biological activity as this particular compound is known to be produced by *Burkholderia* species.[6] The introduction of a nitrogen atom at the 3-position, rather than the usual carbon, was also achieved.

The next objective was to add protecting groups to HHQ and some halogenated analogues.[7-9] Two protecting groups were installed to HHQ and the3-bromo analogue and the products were isolated as *O*-protected quinolones. A Suzuki-Miyaura reaction was attempted on the Cbz protected 3-bromo analogue.

It was hoped that the analogues synthesised would block the binding site in PqsR and thus prevent the native QS molecules from binding (**Figure 5.1**).

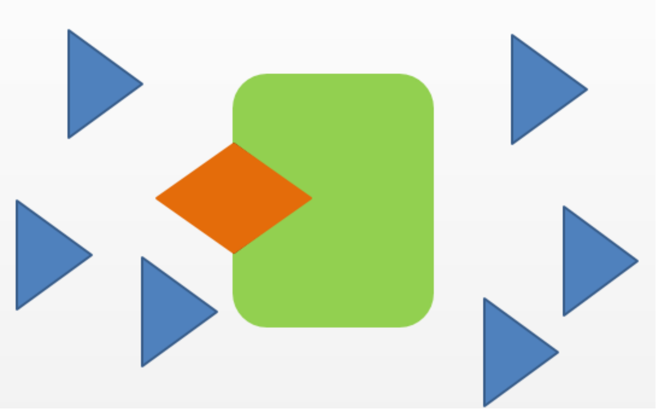
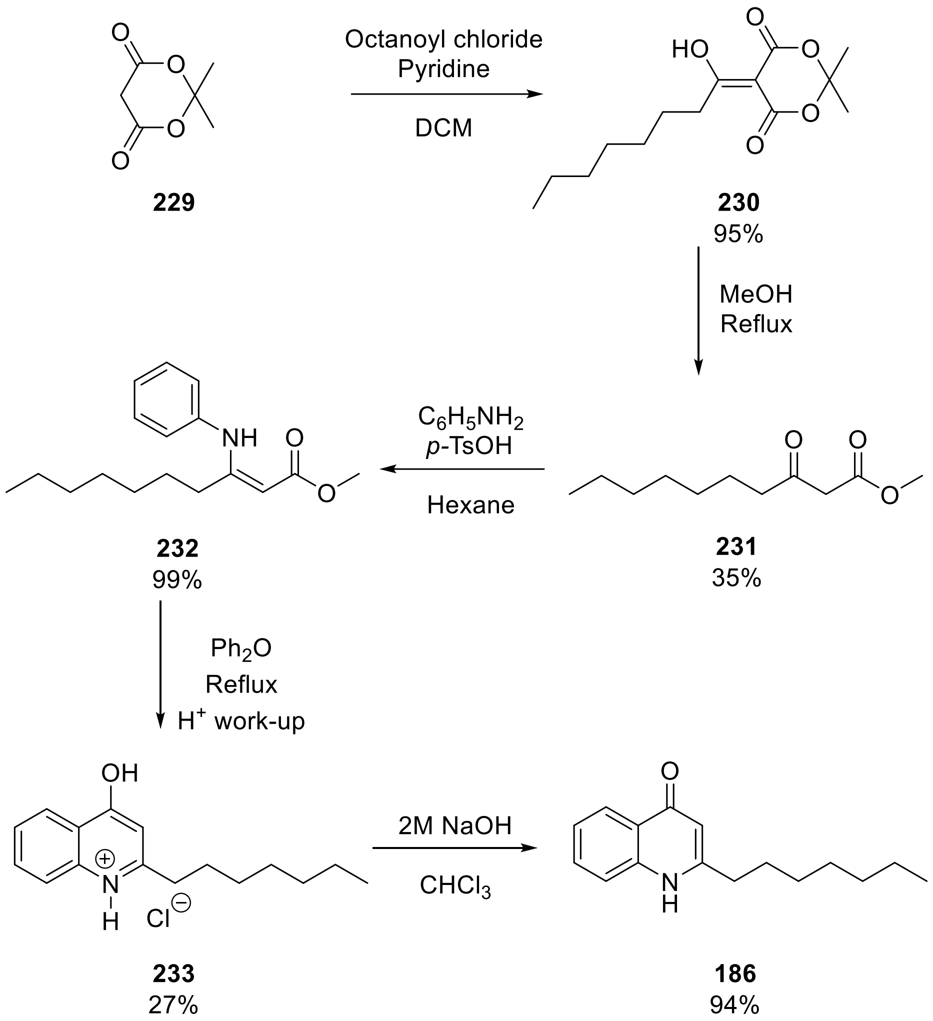


Figure 5.1 Simple depiction of native QS signalling molecules (blue triangles) blocked from the binding site in the receptor (green rectangle) as QS signalling analogue (orange triangle) is occupying that site.

5.2 Synthesis of HHQ

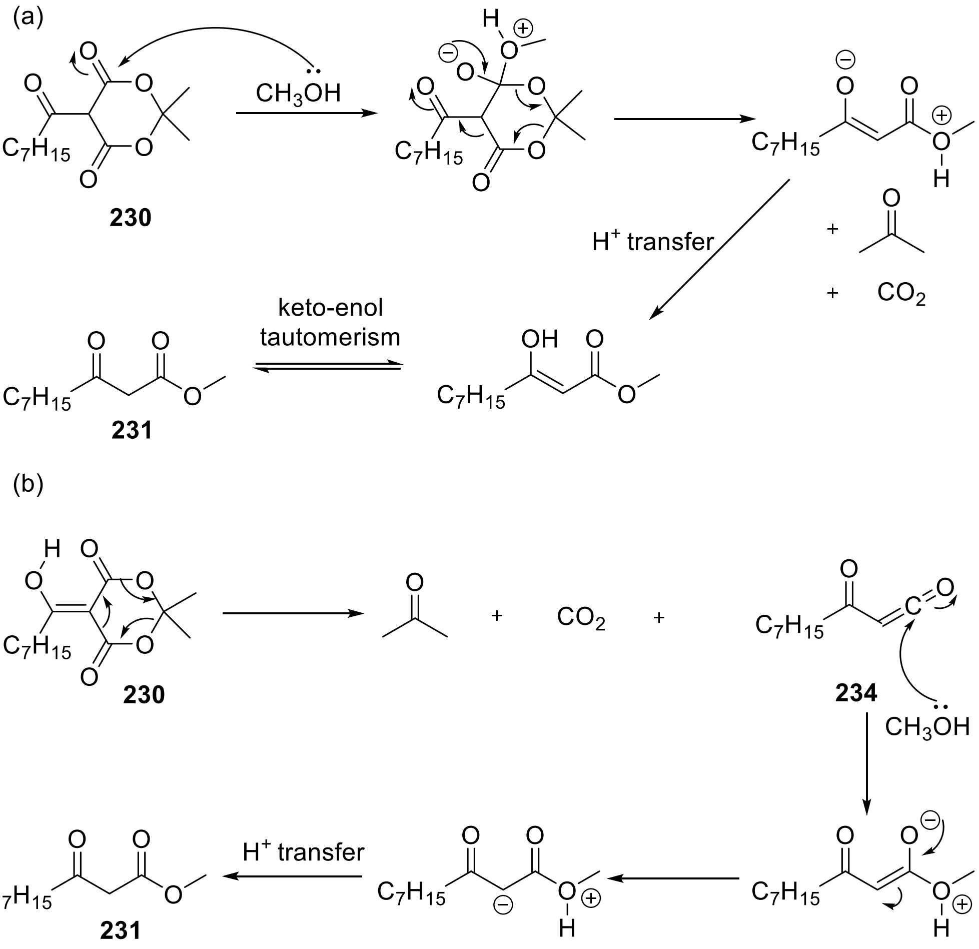
HHQ can be isolated as its HCl salt **233** *via* a four step synthesis, starting from commercially available 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) **229**. The HCl salt **233** can then be neutralised, as required, to afford free HHQ **186** in excellent yield (**Scheme 5.1**).


Octanoyl chloride
Pyridine
DCM
229
230
95%
MeOH
Reflux
C6H5NH2
p-TsOH
Hexane
232
99%
231
35%
Ph2O
Reflux
H+ work-up
2M NaOH
CHCl3
233
27%
186
94%

Scheme 5.1 Synthesis of HHQ **186**.

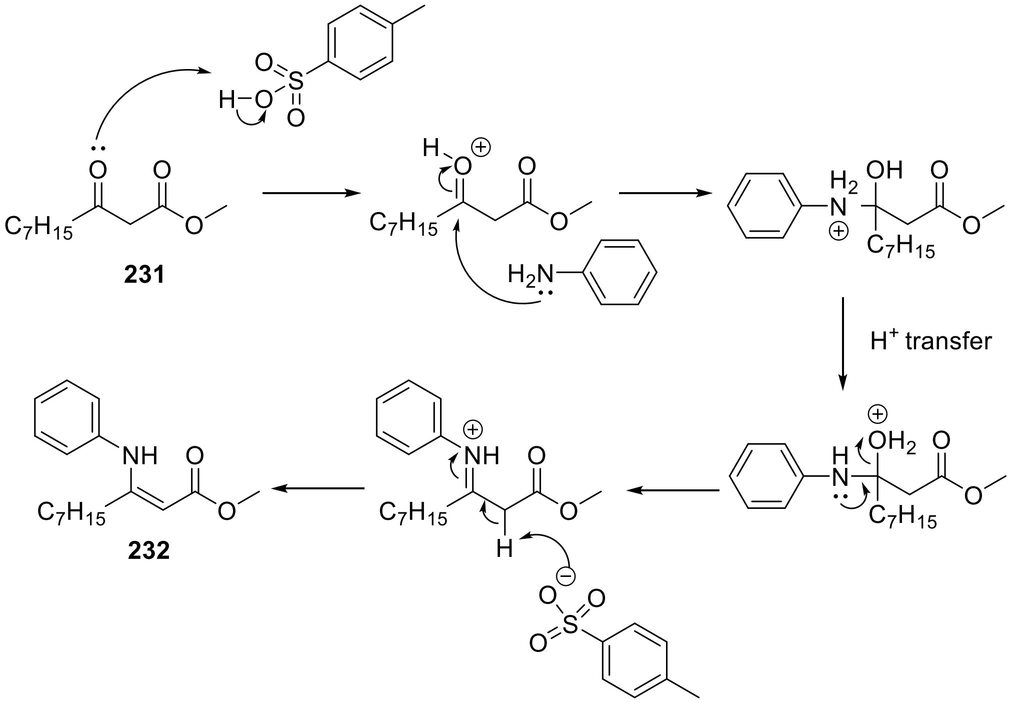
In the first step of the synthesis, a highly acidic proton (pK_a 4.97)[10] α- to the carbonyl group in Meldrum's acid **229** was deprotonated by pyridine and reacted with octanoyl chloride to form acylated Meldrum's acid **230** in almost quantitative yield, with the product existing predominantly in its enol form.[11]

β-Ketoester **231** was successfully prepared in 35% yield (after purification by vacuum distillation) by refluxing **230** in methanol.[12] It was originally thought that the mechanism proceeded via nucleophilic attack of one of the carbonyls of the acyl Meldrum's acid **230** at elevated temperature, with subsequent fragmentation expelling acetone and carbon dioxide, leaving the desired β-ketoester **231** (**Scheme 5.2** (**a**)).[11] In a more recent and detailed study by Xu *et al.*, a new mechanism was proposed that does not involve direct attack of the nucleophile and subsequent fragmentation.[13] Based on rate laws and monitoring of the reaction using IR spectroscopy, the authors have shown that heat initiates decomposition of the acyl Meldrum's acid **230** into an α-oxoketene species **234** which then accepts the nucleophile (**Scheme 5.2** (**b**)).



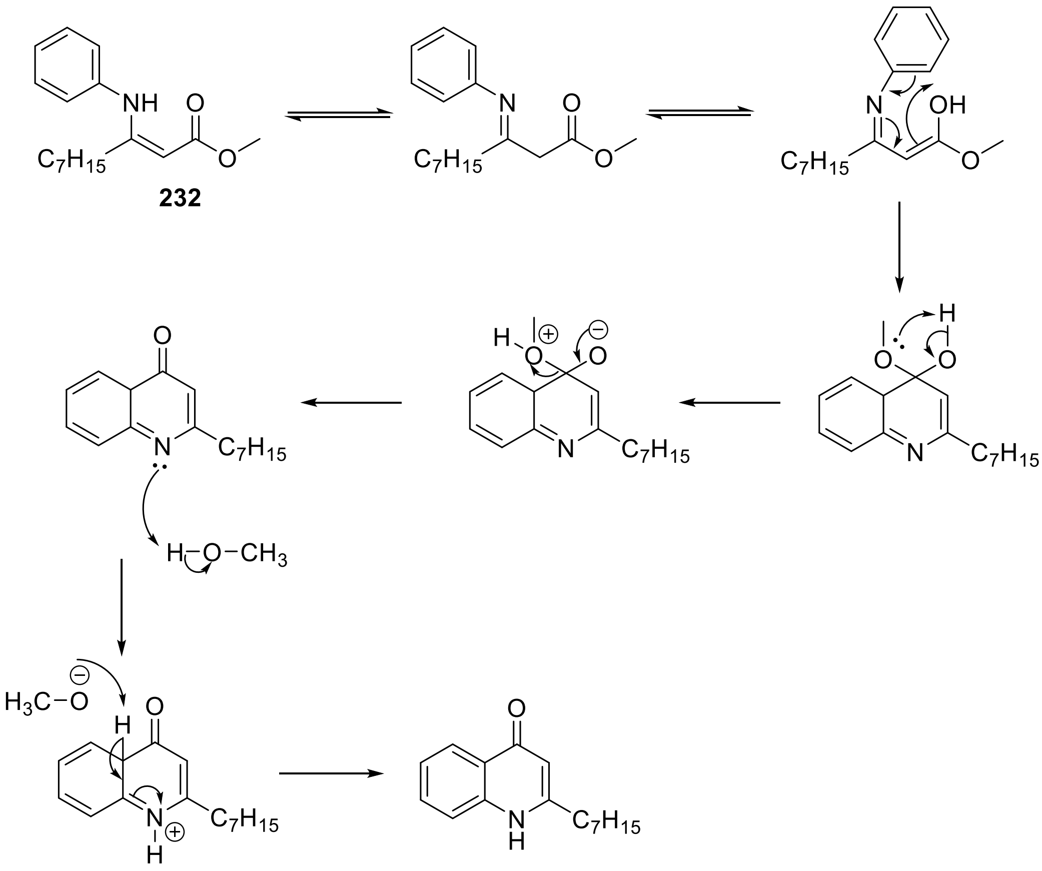
Scheme 5.2 (a) Initial mechanism proposed and (b) currently accepted mechanism for the fragmentation of acyl Meldrum's acid adduct to yield β-ketoester.

The next step of the synthesis involves the formation of enamine **232**. This was accomplished by refluxing the β-ketoester **231** with aniline and a catalytic amount of *p*-toluenesulfonic acid in hexane, affording the crude enamine **232** in almost quantitative yield, which was carried forward to the next step without purification.[14] The catalytic *p*-toluenesulfonic acid is necessary to protonate the ketone of β-ketoester **231** and encourage nucleophilic attack by aniline. Subsequent proton transfer allows for loss of water. An acidic proton is removed to allow the positive charge on the nitrogen to be neutralised to give enamine **232** (**Scheme 5.3**).



Scheme 5.3 Mechanism of formation of enamine from β-ketoester.

The final step involves formation of HHQ **186** from enamine **232**, *via* a Conrad-Limpach quinoline synthesis (**Scheme 5.4**).[15] A series of tautomerisations of the enamine **232** take place, firstly from the enamine to the imine and then keto-enol tautomerism of the carbonyl of the ester to the enol. This is now a high energy imine-enol tautomer and is the ultimate substrate for the rate-determining cyclisation step. A solvent with a very high boiling point must be employed for the cyclisation to the hemiketal as the aromaticity of the phenyl ring is being broken in this step.[16] The typical solvent of choice is diphenyl ether and the reaction was carried out at 270 °C. Once the cyclisation has occurred, aromaticity is restored by loss of methanol and a final deprotonation of the acidic hydrogen to provide quinolone product **186**. The quinolone was first isolated as its HCl salt **233** in low yield (27%) and subsequently converted to **186** by basification. Yields of the cyclisation step in high boiling point solvents are known to be low to moderate.[17]



Scheme 5.4 Mechanism of Conrad-Limpach cyclisation.

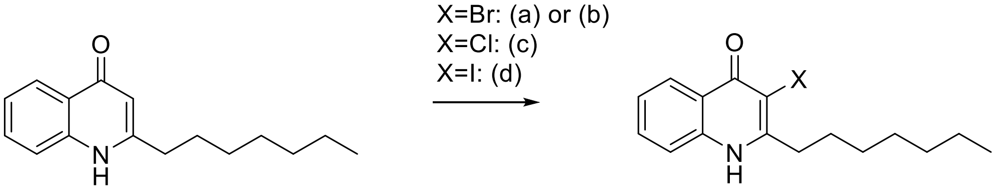
5.3 Synthesis of HHQ-halo analogues

The next step of the project involved the synthesis of analogues of PQS which contained small polar groups at the C-3 position. It was hoped that they would act as a mimic for the hydroxyl group present at C-3 in PQS, bind to PqsR and hence prevent binding of the native QS signalling molecules. It was decided to introduce various halogen atoms to this position in an effort to investigate the effect of electronegative atoms on the biological activity.

5.3.1 Synthesis of bromo-, chloro- and iodo- analogues

3-Bromo-2-heptylquinolin-4-one **235** was synthesised *via* two routes by reacting HHQ **186** with either *N*-bromosuccinimide to provide **235** in 37% yield, or bromine in glacial acetic acid to afford **235** in 76% yield, in a similar fashion to the published methodology for the halogenation of pyridones.[18] 3-Chloro-2-heptylquinolin-4-one **236** was synthesised in moderate yield by reacting HHQ **186** with sodium dichloroisocyanurate in methanol and water.[19] 3-Iodo-2-

heptylquinolin-4-one **237** was synthesised in moderate yield by reacting HHQ **186** with *N*-iodosuccinimide in glacial acetic acid, providing the product in moderate yield after purification by silica column chromatography (**Scheme 5.5**). The yields in all cases were moderate, perhaps due to the presence of a sterically demanding alkyl chain at C-2.

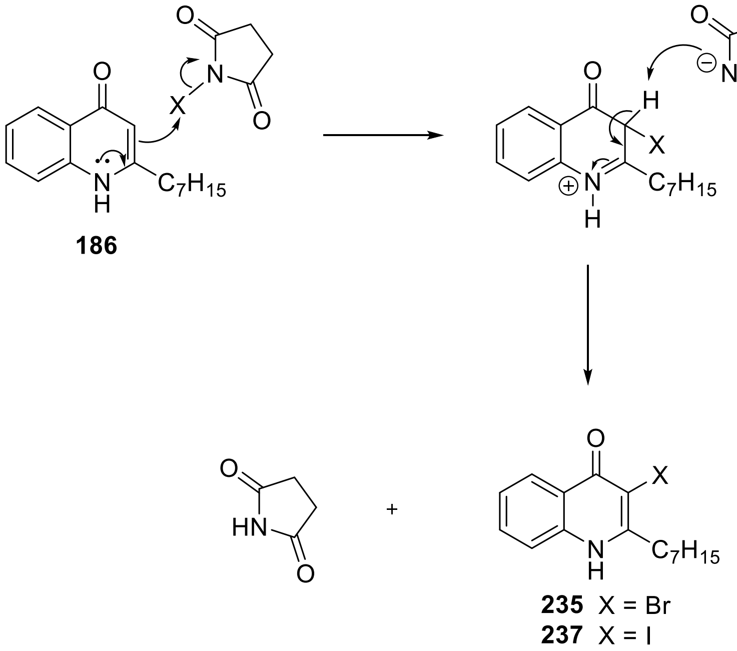


186

235 X = Br
236 X = Cl
237 X = I

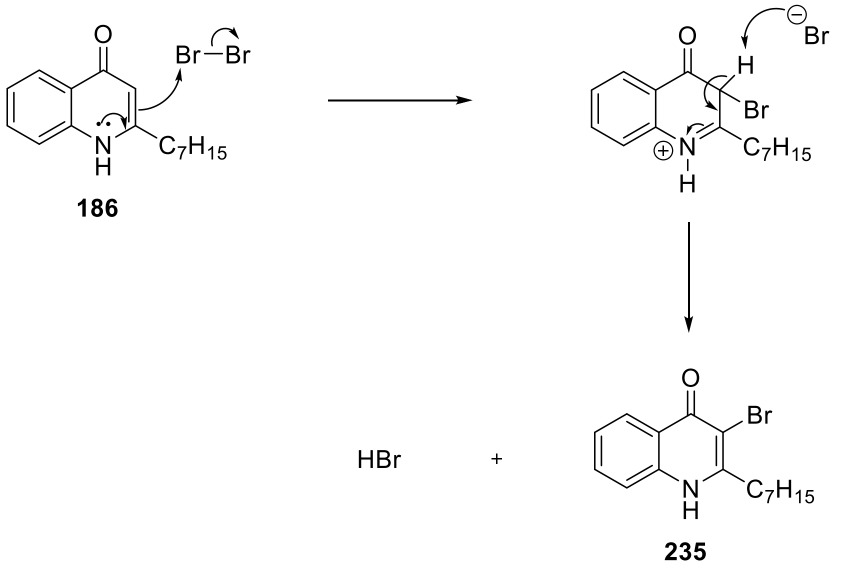
Scheme 5.5 (a) NBS (2.0 equiv.), MeOH, 37%; (b) Br_2 (1.1 equiv.), AcOH, 76%; (c) sodium dichloroisocyanurate (0.55 equiv.), MeOH/H_2O, 46%; (d) NIS (1.02 equiv.), AcOH, 48%.

The mechanism of halogenation using the *N*-halosuccinimides is likely to progress in similar fashion (**Scheme 5.6**). The available lone pair of electrons present on the nitrogen of the quinolone promotes attack of the double bond to the halogen atom on the *N*-halosuccinimide, with the new bond formed between the carbon at position 3 and the halogen atom. Aromaticity of the compound (while it could be suggested that the quinolone bear some aromaticity, certainly its tautomer, the quinoline, is aromatic) is restored by abstraction of a proton at C-3 and subsequent movement of electrons to neutralise the nitrogen of the quinolone. Succinimide is formed as a byproduct of the reaction and is removed in water washes upon workup.



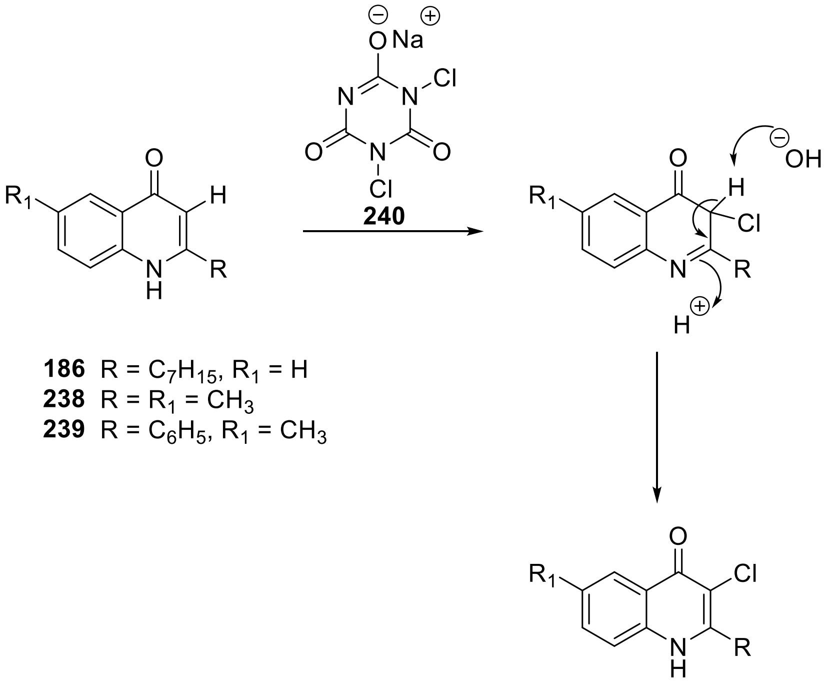
Scheme 5.6 Mechanism of halogenation using *N*-halosuccinimides.

Due to the electron-rich nature of the quinolone, it is possible to brominate using Br_2 in glacial acetic acid at C-3 without the presence of a Lewis acid catalyst. The mechanism is similar to that observed when using *N*-halosuccinimides as the halogen donor, however in this case HBr is the side product (**Scheme 5.7**). The use of glacial acetic acid as solvent is due to its polar, protic nature, allowing stabilisation of the intermediate with the added benefit that it can be easily removed in the workup of the reaction.



Scheme 5.7 Mechanism of bromination using bromine in glacial acetic acid.

Sodium dichloroisocyanurate (sodium 3,5-dichloro-2,4,6-trioxo-1,3,5-triazinan-1-ide) **240** is a solid *N*-halo imide, usually employed as a source of active chlorine for water treatment and as a disinfectant. Staskun demonstrated its use as a reliable reagent for the preparation of chlorinated quinolinones.[19] It was found that on treating 2,6-dimethyl-4(1*H*)-quinolinone **238** with 0.55 equiv. of sodium dichloroisocyanurate **240**, the 3-chloro derivative **241** was afforded in *ca*. 60% yield. Similarly, when the starting material possessed a phenyl group at C-2 (**239**), the 3-chloro product **242** was obtained in 71% yield. With these results in mind, it is proposed that chlorination of HHQ **186** takes place *via* the same pathway to provide 3-chloro-HHQ **236** in 46% yield (**Scheme 5.8**).



186 R = C_7H_{15}, R_1 = H
238 R = R_1 = CH_3
239 R = C_6H_5, R_1 = CH_3

236 R = C_7H_{15}, R_1 = H, 46%
241 R = R_1 = CH_3, *ca.* 60%
242 R = C_6H_5, R_1 = CH_3, 71%

Scheme 5.8 Route for synthesis of 3-chloro-quinolinone analogues.

Analogues **235-237** were found to be insoluble in many common organic solvents. NMR spectra of the final compounds were obtained in deuterated DMSO. In all cases the absence of a singlet peak corresponding to a proton at C-3 provided evidence that the substitution had successfully taken place. This is exemplified in comparison of the ^{1}H NMR spectra of **186** and **235** (**Figure 5.2**).

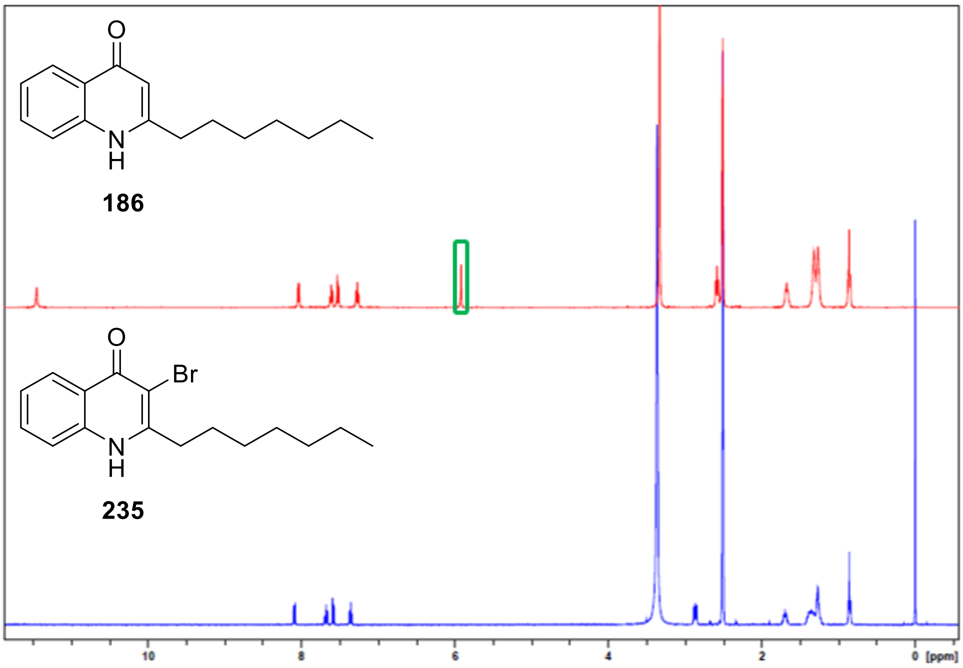


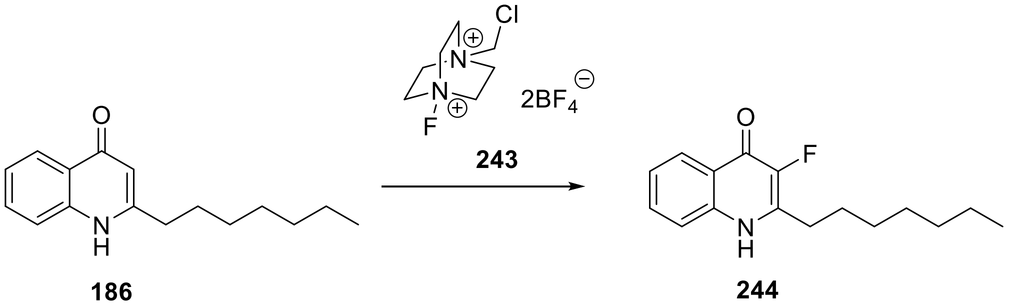
Figure 5.2 Comparison of ^{1}H NMR spectra of HHQ **186** and 3-bromo-HHQ **235**.

5.3.2 Fluorination attempts

The introduction of a fluorine atom at C-3 is of particular interest. Due to fluorine's steric and polar characteristics, the introduction of even a single fluorine substituent can have a remarkable effect on the physical and chemical properties of that molecule.[20] Replacement of a hydrogen or hydroxyl group in bioactive compounds with a fluorine atom is a common strategy in the field of medicinal chemistry. This substitution introduces minimal steric alterations, and it was hoped that interaction of fluorinated HHQ with the receptor site would be successful and thereby prevent the native signal molecules from binding.

5.3.2.1 Selectfluor®

Fluorination of HHQ **186** to provide HHQF **244** was initially attempted *via* electrophilic fluorination with the use of commercially available reagent Selectfluor® (**243**), an extremely stable, hazard-free, virtually non-hydroscopic crystalline solid (**Scheme 5.9**).[21]



Scheme 5.9 Synthesis of 3-fluoro-HHQ using Selectfluor®.

The reaction was carried out using a variety of conditions and solvents (**Table 5.1**) however overall results were disappointing. In some cases, the reaction conditions used were not suitable for the reagents. When DBU was added to a solution of **186** and **243** in acetonitrile, the reaction turned into a viscous black oil (**Table 5.1, entry 10**). Similarly when the reaction was attempted using DMSO as solvent, it was discovered that **243** is not compatible with this solvent and decomposed (**Table 5.1, entry 11**). Many of the reactions attempted resulted in complex mixtures of products as determined by ^{1}H NMR (**Table 5.1, entries 2, 8, 12** and **17**), whilst in others, only starting material was obtained (**Table 5.1, entries 3, 4, 6, 7, 9, 13** and **15**). In some cases, a trace amount of **244** was detected by mass spectrometry, however the product was not visible by ^{1}H NMR and purification of the crude reaction material was not attempted (**Table 5.1, entries 1** and **14**). When the reaction was carried out using 1.2 equiv. of **243** in a mixture of ethanol and acetonitrile at -10 °C for 3 days, the product was successfully isolated, albeit in a very low yield of 3% (**Table 5.1, entry 5**). When the reaction was carried out using higher equiv. of **243** in ethanol and the reaction allowed to stir at room temperature for 1 week, **244** was successfully isolated in 8% yield (**Table 5.1, entry 16**).

Table 5.1 Fluorination attempts of HHQ using Selectfluor®.

Entry	**Selectfluor® (equiv.)**	**Solvent**	**Additive (equiv.)**	**Conditions**	**Result (yield)**
1	1	EtOH	–	Reflux, o/n	**244** (trace)
2	1	MeCN	–	Reflux, o/n	CM
3	1	MeCN, 1.1 equiv. EtOH	–	Reflux, 2 d	SM
4	1	H_2O	Sodium tetraphenylborate	105 °C, 18 h	SM
5	1.2	MeCN, 10 equiv. EtOH	–	-10 °C, 3 d	**244** (3%)
6	1.2	EtOH	DBU (1.2)	Reflux, 5 d	SM
7	1.2	EtOH	–	µwave, 120 °C, 10 min	SM
8	1.2	5% H_2O in MeOH	–	Reflux, 3 d	CM
9	1.2	MeOH	H_2SO_4 (1)	RT, o/n, 50°C, o/n, 65 °C, 2 d	SM
10	1.2	MeCN	DBU (1.2)	–	–
11	1.2	DMSO	–	–	–
12	1.2	95:5 MeCN:H_2O	–	RT, o/n, reflux, 3 d	CM
13	6 × 0.2	MeCN, 1 equiv. EtOH	–	RT, 2 d	SM
14	1.3	MeCN	NaBARF (0.275)	RT, o/n, reflux, o/n	**244** (trace)
15	1.3	EtOH	NaBARF (0.275)	RT, o/n, reflux, o/n	SM
16	1.7	EtOH	-	RT, 1 week	**17** (8%)
17	2.5	EtOH		Reflux, 5 d	CM

SM = Starting material; CM = Complex mixture.

Evidence for the incorporation of fluorine was gleaned from the ^{1}H NMR spectrum of **244** (**Figure 5.3**), due to the lack of a singlet in the alkene region. An interesting point of note is that this analogue appeared to be soluble in deuterated chloroform, in contrast to previously synthesised 3-halo analogues. A second indication that the reaction had been successful is the fact that one peak was observed in ^{19}F NMR spectrum at -143.3 ppm which is typical of a fluorine bonded to an aromatic carbon (**Figure 5.4**). Splitting was also observed in the ^{13}C NMR spectrum, consistent with the presence of fluorine at C-3 (**Figure 5.5**). Further evidence for successful synthesis of **244** is obtained from the high resolution mass spectrum, with the mass found within 2.3 ppm of the calculated mass.

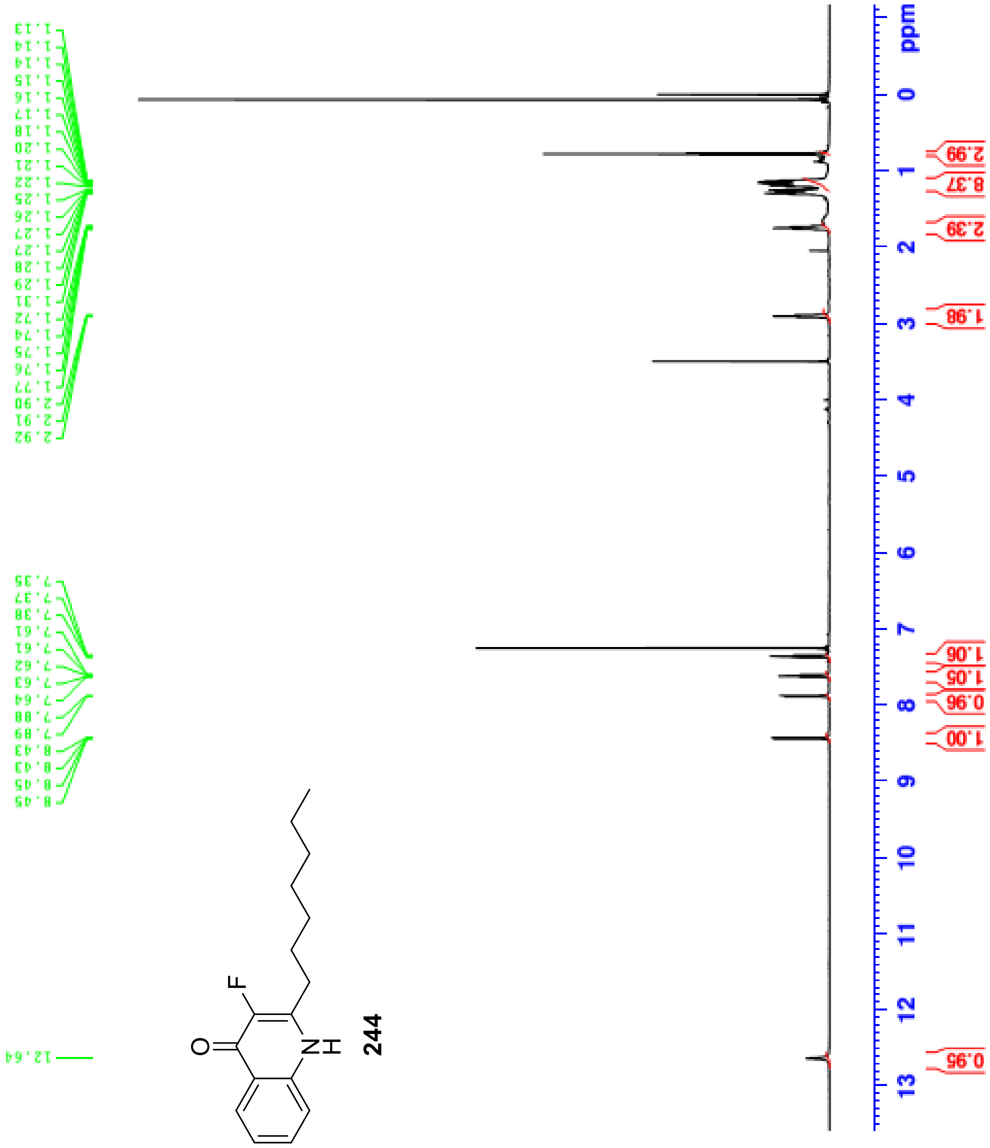


Figure 5.3 ^{1}H NMR spectrum of purified 3-fluoro-HHQ.

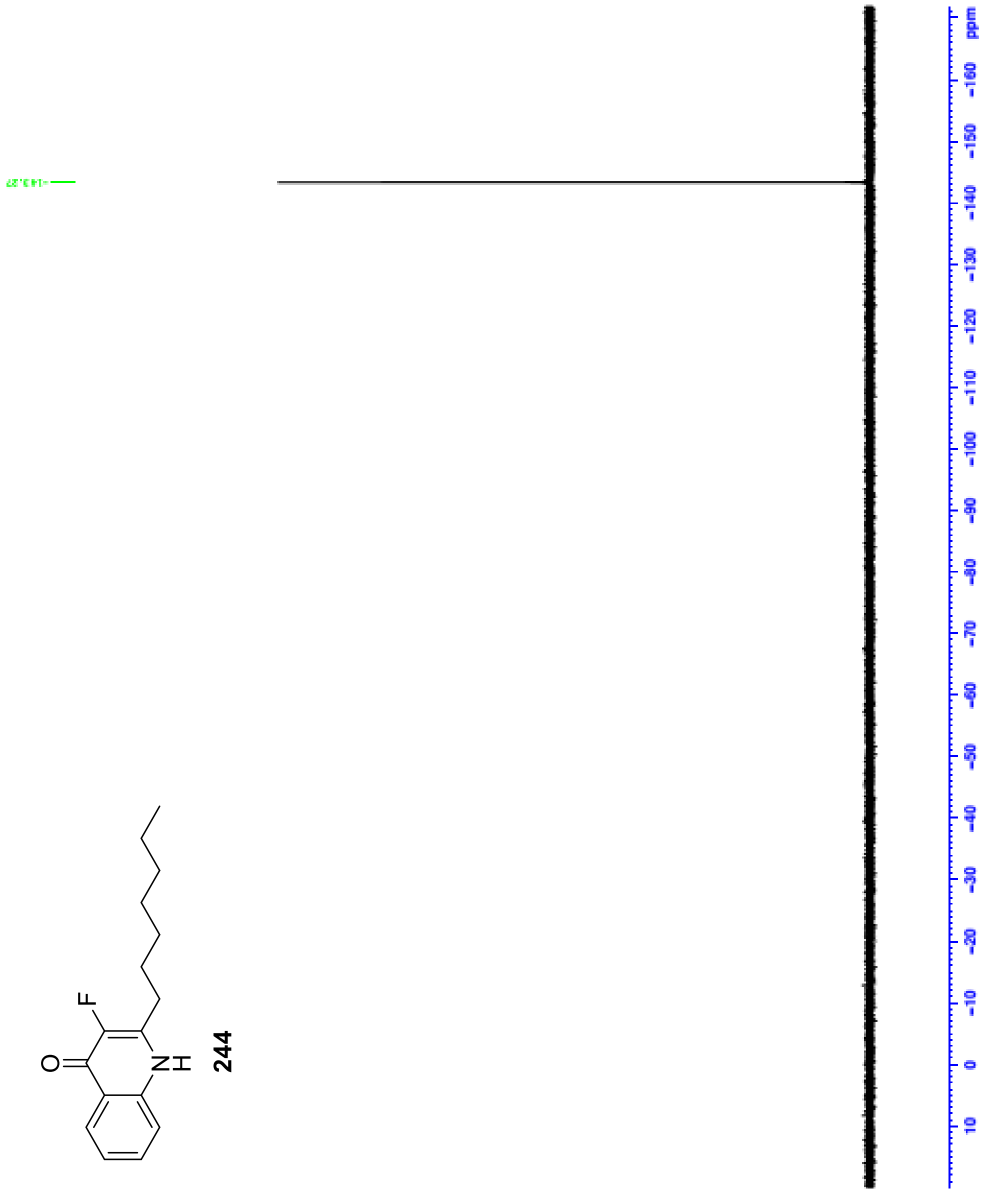
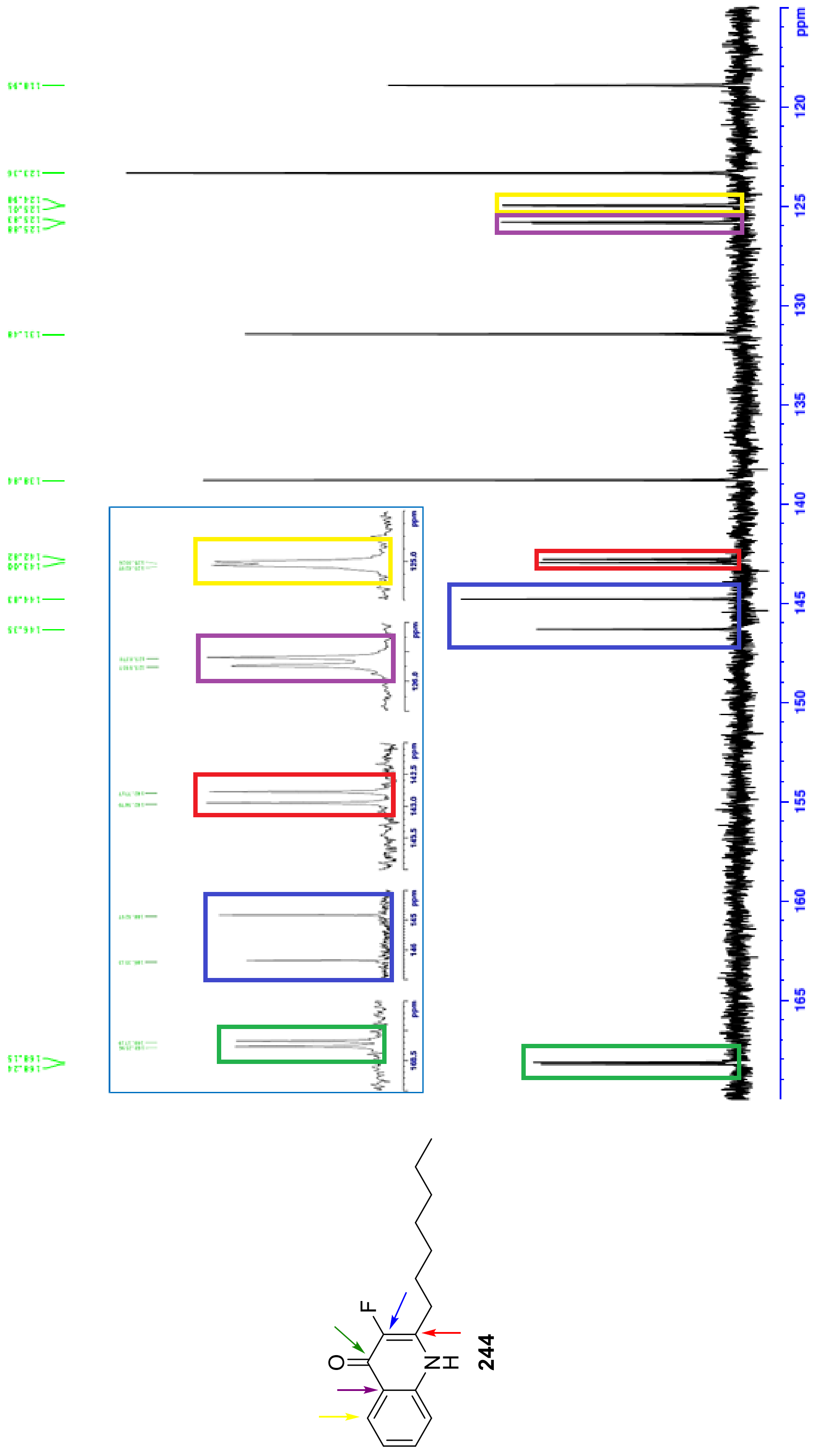


Figure 5.4 ^{19}F NMR spectrum of 3-fluoro-HHQ.



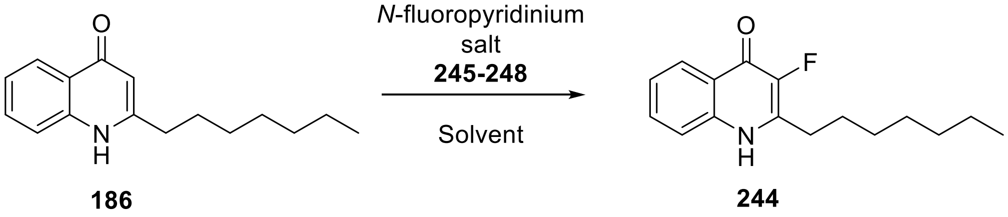
244

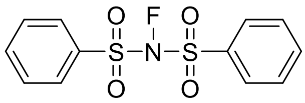
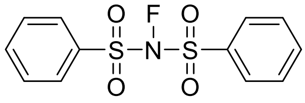
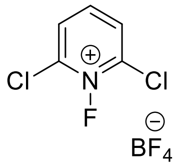
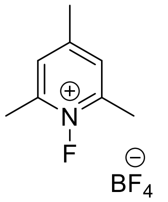
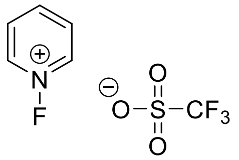
Figure 5.5 Magnified ^{13}C spectrum of 3-fluoro-HHQ showing doublets observed due to presence of fluorine at C-3.

5.3.2.2 Alternative electrophilic fluorinating agents

In an effort to synthesise 3-fluoro-HHQ **244** in better yield, it was necessary to investigate alternative electrophilic fluorination methods. A range of *N*-fluoropyridinium salts were chosen for this task (**Table 5.2**). *N*-fluorobenzenesulfonimide **245** was the first of these reagents to be investigated. Both DCM and THF were used as solvents (**Table 5.2, entries 1 and 2**), however results were disappointing, with either a complex mixture being observed when DCM was employed as solvent, or starting material being observed when THF was utilised. The use of other *N*-fluoropyridinium salts including 2,6-dichloro-1-fluoropyridinium tetrafluoroborate **246** (**Table 5.2, entry 3**) resulted in a complex mixture of products. Attempts using 1-fluoro-2,4,6-trimethylpyridinium tetrafluoroborate **247** (**Table 5.2, entry 4**) and 1-fluoropyridinium triflate **248** (**Table 5.2, entry 5**) were unsuccessful and only starting material was observed by ^{1}H NMR spectroscopy.

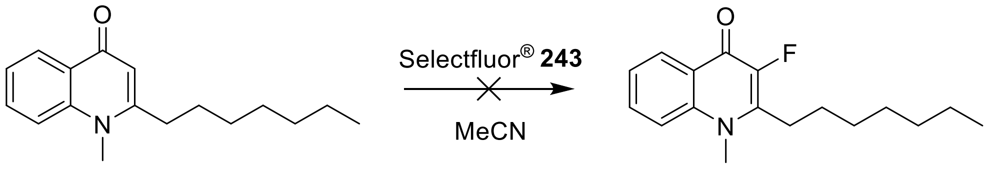
Table 5.2 Summary of reactions using *N*-fluoropyridinium salts.



Entry	Fluorinating agent	Solvent	Conditions	Result
1	 **245**	DCM	RT o/n, reflux o/n	CM
2	 **245**	THF	RT 48 h, reflux 48 h	SM
3	 **246**	DCM	RT, o/n	CM
4	 **247**	DCM	RT o/n, reflux 24 h	SM
5	 **248**	DCM	RT o/n, reflux 24 h	SM

5.3.2.3 Fluorination of *N*-methyl HHQ

Considering the limited success in the fluorination of HHQ **186**, it was thought that protecting the free –NH using a methyl group (previously prepared within the group) may give a better result. It was postulated that blocking of the –NH with a robust group would prevent the formation of N-F compounds. *N*-methyl HHQ **249** and Selectfluor® **243** were dissolved in acetonitrile and allowed to stir at room temperature. Samples were taken at regular intervals to monitor the progress of the reaction. After 5 days, although a trace of product was observed by mass spectrometry, only starting material was observed in the ^{1}H NMR spectrum of the crude material. (**Scheme 5.10**).



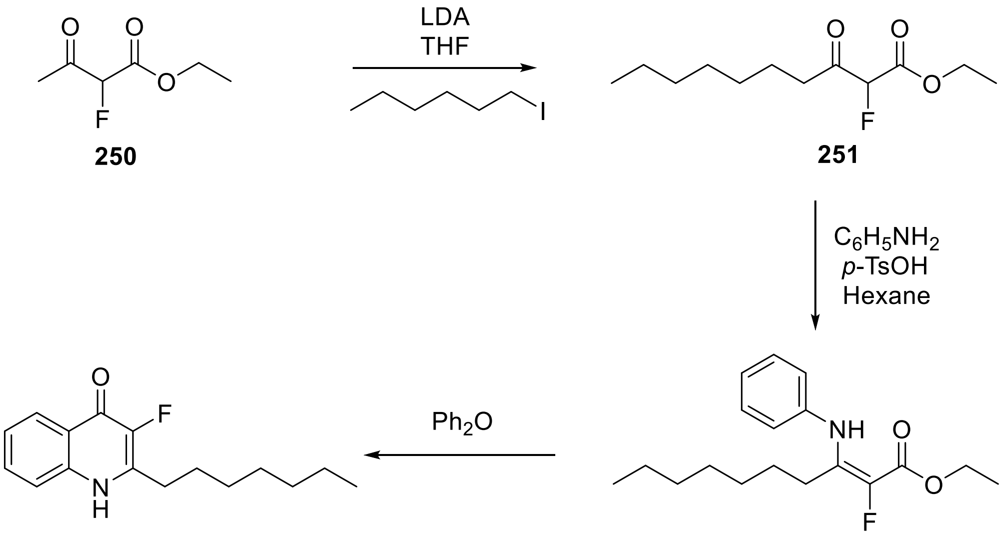
249

Scheme 5.10 Attempted fluorination of *N*-methyl HHQ.

Given that fluorination failed to occur with a methyl 'protecting group' on the nitrogen atom, no further easily-removable groups were tested.

5.3.2.4 Building block strategy

In an effort to improve the yield of the fluorinated product, our attention then turned to synthesis of the fluorinated analogue *via* a building block strategy. It was envisaged that HHQF **244** could be synthesised by firstly deprotonating ethyl 2-fluoroacetoacetate **250** using LDA and trapping the formed enolate with hexyliodide to yield fluorinated β-ketoester **251**.[22] Reaction with aniline and *p*-toluenesulfonic acid in hexane to give fluorinated enamine **252** and finally a Conrad-Limpach cyclisation of formed enamine **252** by refluxing in diphenyl ether would afford **244** (**Scheme 5.11**).



250

251

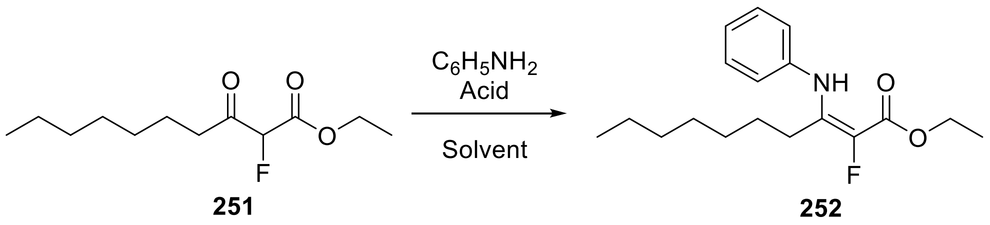
244

252

Scheme 5.11 Building block strategy for synthesis of 3-fluoro-HHQ.

Formation of fluorinated β-ketoester **251** was successfully achieved in 55% yield. The reaction required 2.1 equiv. of LDA as there are two sites for deprotonation. However, addition of hexyliodide under kinetic conditions allows selective addition at the least sterically hindered site. Formation of the fluorinated enamine **252** proved to be problematic. The enamine formation was attempted twice using the same conditions required as in the synthesis of HHQ. However on both occasions, only traces of fluorinated enamine were present by ^{1}H NMR (**Table 5.3, entries 1 and 2**). The reaction was repeated with the addition of 3Å molecular sieves in case water formation was preventing the reaction from going to completion. However by ^{1}H NMR, only starting material was observed (**Table 5.3, entry 3**). There is precedence in the literature for the formation of enamines from β-ketoesters in solvent-free conditions using ultrasound.[23] This methodology was attempted using fluorinated β-ketoester **251 (Table 5.3, entry 4)**. The reaction appeared to go to around 50% completion (as deduced by ^{1}H NMR); however on workup, this converted back to the fluorinated β-ketoester, suggesting the instability or reversibility of **252** may be why it is problematic to synthesise and isolate.

Table 5.3 Attempts at enamine formation.



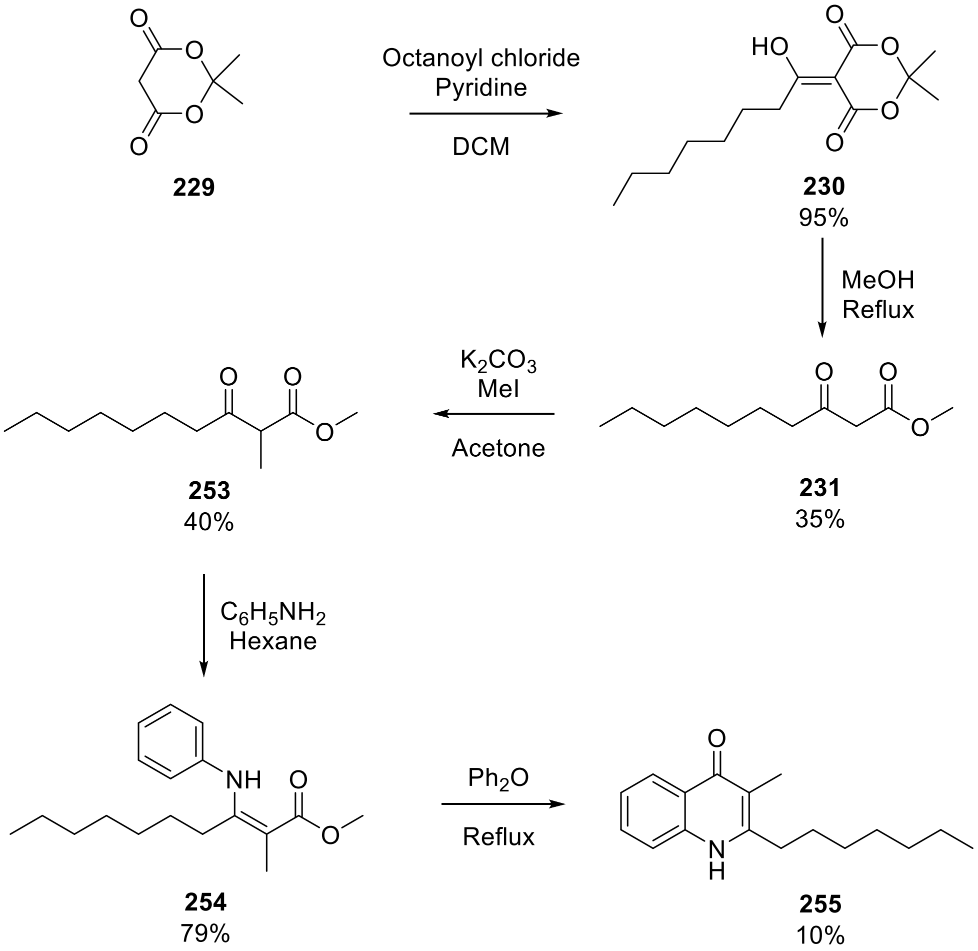
Entry	Conditions	Result
1	Aniline, *p*-TsOH, hexane	Trace of enamine
2	Aniline, *p*-TsOH, hexane	Trace of enamine
3	Aniline, *p*-TsOH, hexane, 3Å mol. sieves	SM
4	Aniline, AcOH Ultrasound	Trace of enamine

It was postulated that if the crude mixture from formation of fluorinated enamine **252** was carried through to the cyclisation step directly, the high temperature required may push the reaction to form the cyclised product **244**. Thus the reactions with traces of enamine obtained (**Table 5.3, entries 1, 2 and 4**) were added to refluxing diphenyl ether, however all reactions resulted in complex mixtures of products. A one-pot synthesis of HHQF **244** was attempted to convert the β-ketoester **251** directly to **244** however a complex mixture of products was again obtained.

Alternative cyclisation methods were explored in an effort to achieve **244** in good yield. Cyclisation of enamines to quinolones using polyphosphoric acid has previously been reported in the literature.[24] This method was attempted with our system and although a trace of product was identified by mass spectrometry, a complex mixture was revealed by ^{1}H NMR. Another cyclisation method that has been reported in the literature is that using diphenyl ether under microwave conditions.[25] Results using this methodology were also disappointing, with a complex mixture of products (deduced by ^{1}H NMR) resulting. It has been reported that the Conrad-Limpach cyclisation can be successfully carried out under solvent-free conditions by heating the desired β-ketoester and aniline at 100 °C with a catalytic amount of $InCl_3$, acting as a Lewis acid.[26] This method was attempted using both fluorinated β-ketoester **251** and β-ketoester **231**, however in both cases the reaction was unsuccessful and only starting material was observed.

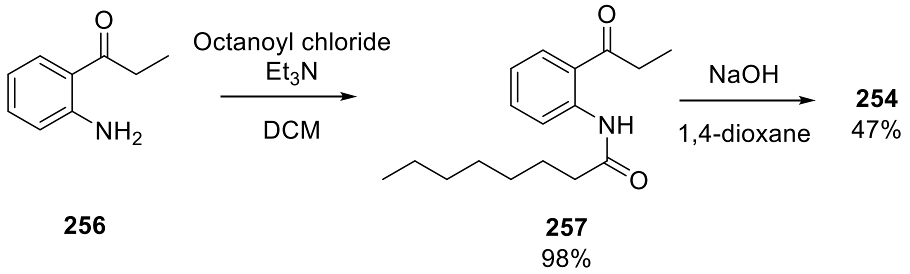
5.4 Synthesis of C-3 methyl analogue

The synthesis of 3-methyl HHQ proceeds in a similar fashion to the route used to afford HHQ. β-Ketoester **231** is synthesised as before, however an extra step is required at this point to afford methylated β-ketoester **253**.[27] The methylated enamine **254** and final methylated HHQ product **255** are synthesised *via* the same routes as per the synthesis of HHQ (**Scheme 5.12**).


229
Octanoyl chloride
Pyridine
DCM
230
95%
MeOH
Reflux
231
35%
K_2CO_3
MeI
Acetone
253
40%
$C_6H_5NH_2$
Hexane
254
79%
Ph_2O
Reflux
255
10%

Scheme 5.12 Synthesis of 3-methyl-HHQ.

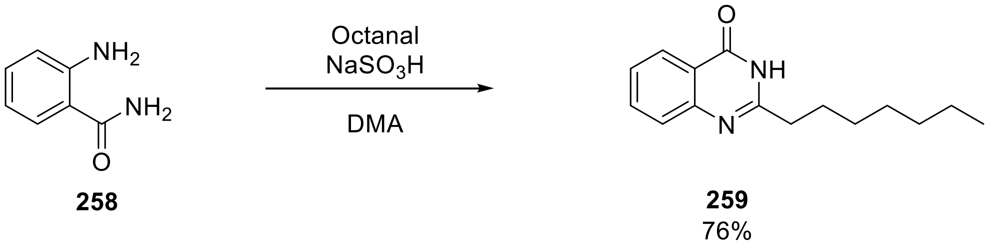
The desired product **255** was afforded in 10% yield after purification by recrystallisation from methanol, with analysis consistent with that previously reported.[28] However, a recent paper, published after this synthesis, affords **255** in 47% yield and in 2 steps (**Scheme 5.13**).[29]



Scheme 5.13 Alternative route to 3-methyl-HHQ.

5.5 Synthesis of N analogue

2-Heptylquinazolin-4(3*H*)-one **259** is synthesised in one step from anthranilamide **258** and octanal by refluxing in DMA with sodium bisulfite (**Scheme 5.14**), following the procedure published by Imai and co-workers.[30] In this analogue, a nitrogen is incorporated at the 3-position in place of carbon. This compound will show the effect a hydrogen-bonding group at the 3-position has on the phenotypes of *P. aeruginosa* and related species.



Scheme 5.14 Synthesis of 2-heptylquinazolin-4(3*H*)-one.

The reaction proceeds *via* a direct cyclodehydration-dehydrogenation of anthranilamide **258** with octanal in the presence of sodium bisulfite. The presence of sodium bisulfite is necessary to ensure the desired product is obtained in good yield. It is known that when aldehydes are reacted with diamines the major byproducts, and in many cases the major product, are disubstituted benzimidazoles, also known as aldehydines.[31] It is predicted that these disubstituted benzimidazoles arise from the diimines formed in the initial stages of the reaction. Under the reaction conditions utilised, the diimines undergo a rearrangement to produce the disubstituted benzimidazoles. With this knowledge, it is thought that the addition of bisulfite will produce the bisulfite adduct of the aldehyde. This is presumed to be less reactive than the free aldehyde, thereby allowing more time for ring closure and preventing formation of the disubstituted benzimidazoles.

On comparison of the ^{1}H NMR spectra of HHQ **186** and quinazolinone **259** (**Figure 5.6**), an obvious difference is the lack of a singlet around 6.2 ppm (highlighted by the green box in the ^{1}H NMR spectrum of **186**) in the ^{1}H NMR spectrum of **259**, proving there is no alkene proton present in the molecule.

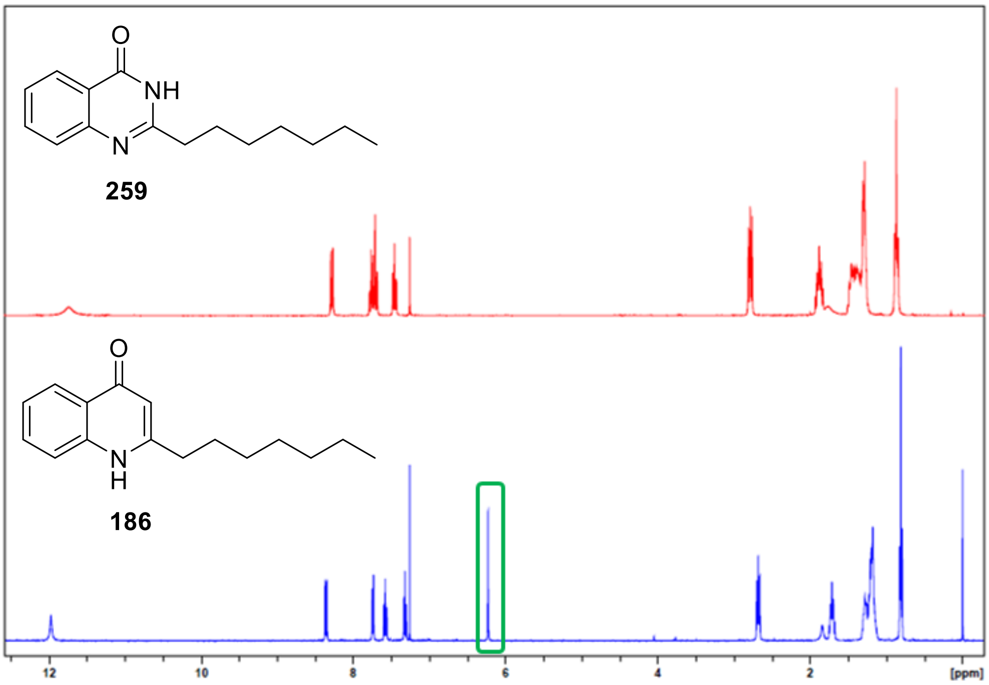
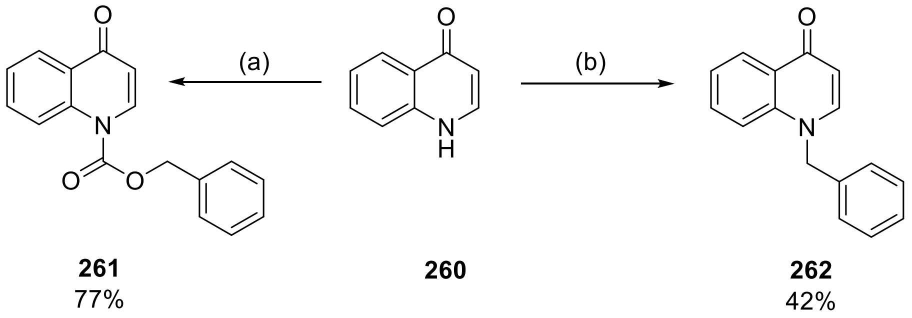


Figure 5.6 Comparison of ^{1}H NMR spectra of HHQ **186** and product **259**.

5.6 Synthesis of protected HHQ analogues

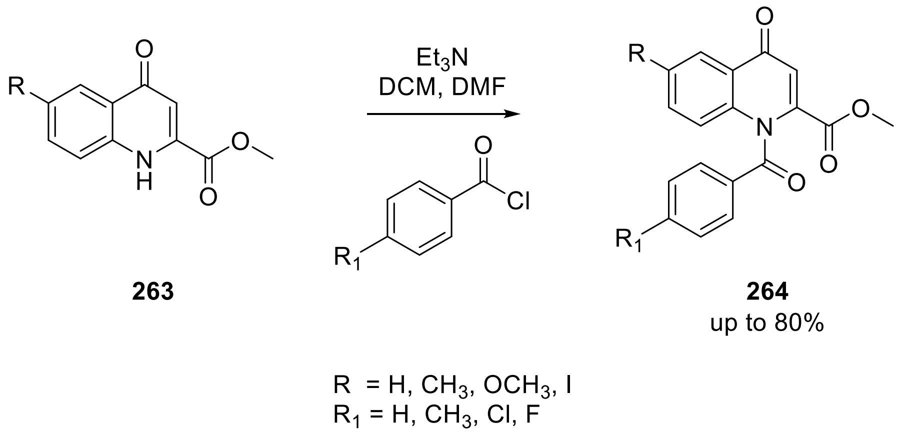
The next stage of the project was to attempt to add protecting groups (PG) to HHQ and various HHQ analogues to allow further functionalisation of the molecule (for example Pd mediated cross-coupling). Previous work in this area has shown that carbamate protection, in particular the use of a carboxybenzyl group (Cbz), can be achieved in good yield when starting with 4(1*H*)quinolone **260**, with the Cbz-product **261** formed in 77% yield (**Scheme 5.15**).[7] The first step of the reaction involved deprotonation of the amine hydrogen using sodium hydride followed by dropwise addition of benzyl chloroformate to yield **261**.

An additional protection was achieved by addition of benzylbromide to a methanolic solution of potassium hydroxide and 4(1*H*)quinolone **260** resulting in **262** in 42% yield.[8]



Scheme 5.15 Protection of 4(1*H*)quinolone. Conditions (a) 1. NaH, THF, 55 °C 15 min; 2. Benzylchloroformate, 21 h, RT. (b) KOH, MeOH, benzylbromide, RT.

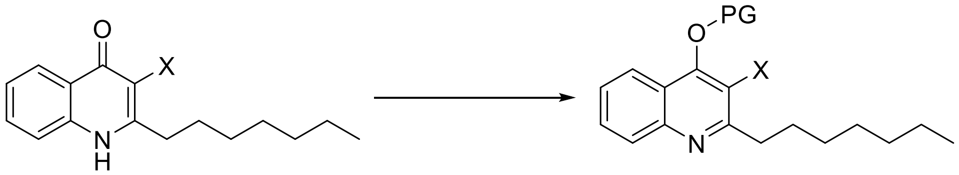
Benzoylation of a selection of 2-substituted, and thus sterically encumbered, quinolones **263** has been successful using a variety of *p*-substituted benzoyl chlorides in good to high yield. This was achieved by deprotonation using trimethylamine in a mixture of anhydrous DCM and DMF and refluxing with the desired benzoyl chloride for 3 h providing products **264** in excellent yield (**Scheme 5.16**).[9]



Scheme 5.16 Benzoylation of a variety of 2-carboxylate quinolones.

With these results in mind, it was postulated that protection of HHQ could be achieved in a similar manner. A variety of conditions and reagents were employed (**Table 5.4**). An interesting observation was that from spectral evidence, it was apparent that protection occurred at the oxygen of the quinolone in all cases, rather than the nitrogen, as was expected. When deprotonation occurs at the nitrogen, the resultant negative charge will be delocalised between the nitrogen and the oxygen due to the possible resonance forms of the compound. The fact that protection was only observed at the oxygen suggests that this is the most stable position for bulky groups to attach. This could be due to the presence of the sterically demanding alkyl chain present at C-2 which could make protection at the neighbouring nitrogen difficult. Initial results were promising, with Cbz-protected HHQ **265** synthesised in 20% yield using the conditions employed by Shintani and co-workers (**Table 5.4, entry 1**).[7] It was apparent that starting material was still present at the end of the reaction, as deduced by ^{1}H NMR spectroscopy, suggesting complete deprotonation or reaction with the acid chloride had not taken place. The deprotonation conditions were altered to allow for a longer deprotonation time (**Table 5.4, entry 2**), and indeed an increase to 52% yield of **265** was observed. In an effort to increase the scope of protecting groups being used, 2-methoxyethoxymethyl chloride (MEM-Cl) was then used in place of benzylchloroformate under the same deprotonation conditions (**Table 5.4, entry 3**). However results were disappointing and only starting material was observed by ^{1}H NMR spectroscopy. The next step in the investigation was to attempt to protect the halo-HHQ analogues using similar conditions. Gratifyingly, 3-bromo-HHQ **235** could be protected to give Cbz-protected analogue **265** in good yield using the shorter deprotonation time of 15 min (**Table 5.4, entry 4**). 3-Bromo-HHQ **235** can also be successfully benzylated using the conditions outlined by Li *et al*.[8] to provide **267**, albeit in a low yield (**Table 5.4, entry 5**). Protection of 3-iodo-HHQ **237** proved problematic, most probably due to the low solubility of the compound (**Table 5.4, entry 6**).

Table 5.4 Summary of protecting group strategies.



186 X = H
235 X = Br
237 X = I

265 X = H, PG = Cbz
266 X = Br, PG = Cbz
267 X = Br, PG = Bn

Entry	X	Deprotonation Conditions	Solvent	PG reagent	Result (yield)
1	H	NaH (4.7 equiv.), 55 °C,15 min	THF	Benzylchloroformate (1.5 equiv.) RT, 21 h	**265** (20%)
2	H	NaH (4.7 equiv.), 55 °C, 5 h	THF	Benzylchloroformate (1.5 equiv.) RT, 21 h	**265** (52%)
3	H	NaH (4.7 equiv.), 55 °C, 5 h	THF	MEM-Cl (1.5 equiv.) RT, 21 h	SM
4	Br	NaH (4.7 equiv.), 55 °C, 15 min	THF	Benzylchloroformate (1.5 equiv.) RT, 21 h	**266** (61%)
5	Br	KOH (1.5 equiv.)	MeOH	Benzylbromide (10.0 equiv.) RT, overnight	**267** (28%)
6	I	NaH (4.7 equiv.), 55 °C, 15 min	THF	Benzylchloroformate (1.5 equiv.) RT, 21 h	No reaction
7	I	KOH (1.5 equiv.)	MeOH	Benzylbromide (10.0 equiv.) RT, 21 h	(<10%)[a]

[a] Product could not be isolated without impurities.

When ^{1}H NMR spectra of HHQ **186** and Cbz-protected HHQ **265** are compared (**Figure 5.7**), noticeable shifts are observed for both the proton at C-3 (highlighted in green) and the CH_2 group of the alkyl chain bonded to C-2 (highlighted in purple). Further investigation using 2D-NMR methods provided more evidence that protection occurred at the oxygen of **186**. Using HMBC analysis, it was determined that the protons of the OCH_2 group were coupling to the quaternary carbon at 151.5 ppm, which was assigned as the carbon in position

4 of the ring attached to the oxygen. If protection had occurred at the nitrogen rather than the oxygen, this coupling would not have been observed. The IR spectrum of **265** showed only one carbonyl peak at 1#769 cm^{-1}, corresponding to the carbonyl group present in the Cbz moiety. In the IR spectrum of **186**, a carbonyl peak is present at 1640 cm^{-1}, which is absent in that of **265**, again providing proof that protection has occurred on the oxygen rather than the nitrogen.

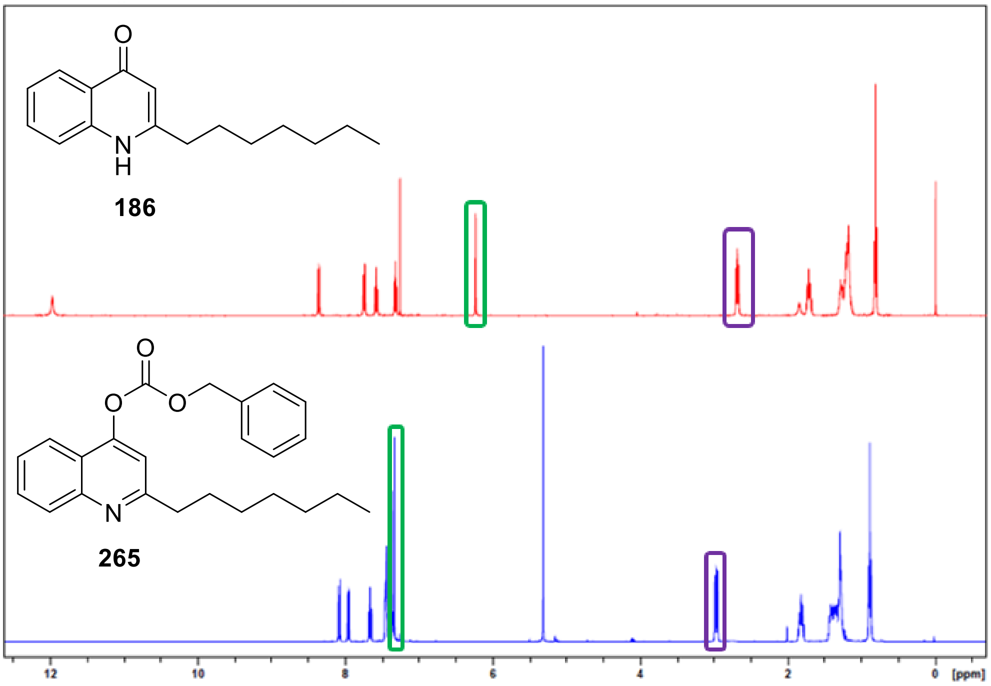


Figure 5.7 Comparison of ^{1}H NMR spectra of HHQ **186** and Cbz-protected HHQ **265**.

Similar conclusions can be drawn when the spectra of **266** and **267** are examined. In these cases, a direct comparison of ^{1}H NMR of products with 3-bromo-HHQ **235** cannot be made due to the difference in NMR solvents used. However a comparison can be made between ^{1}H NMR spectra of Cbz-protected HHQ **265** and 3-bromo Cbz-protected HHQ **266** (**Figure 5.8**). The spectra are similar, however for **266** a downfield shift is observed for the CH$_2$ of the alkyl chain bonded to C-2, most probably due to the influence of the bromine at C-3. There is a notable absence of a singlet alkene peak, as expected. Further evidence for protection occurring at the oxygen rather than the nitrogen is found when the IR spectrum of **266** is taken into account. Similar to **265**, there is only one carbonyl peak present at 1772 cm^{-1}, corresponding to the carbonyl group in the Cbz protecting group.

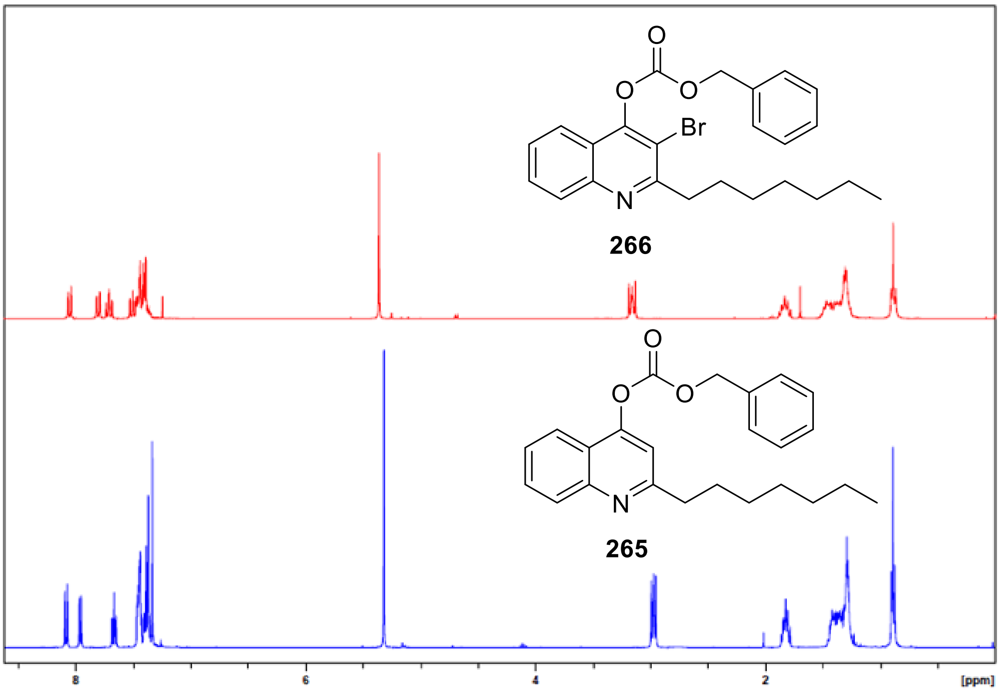


Figure 5.8 Comparison of ^{1}H NMR of **265** and **266**.

On changing the protecting group to a benzyl, there is little difference in the ^{1}H NMR spectra of **266** and **267** apart from in the aromatic region, which is as expected (**Figure 5.9**). 2D-NMR experiments again proved that benzylation occurs on the oxygen to provide **267**, with HMBC analysis showing coupling between the protons of the OCH_2 group and the quaternary carbon at 159.5 ppm, which was assigned as the carbon in position 4 of the ring attached to the oxygen. If protection had occurred at the nitrogen rather than the oxygen, this coupling would not have been observed. Confirmation of protection at the oxygen was obtained from the IR spectrum, where no carbonyl peaks are observed.

Overall, the addition of the benzoyl and benzyl groups at the oxygen was not deemed a problem. Subsequent to a cross-coupling, for example, reaction deprotection could be carried out and the quinolone structure would return *via* isomerisation.

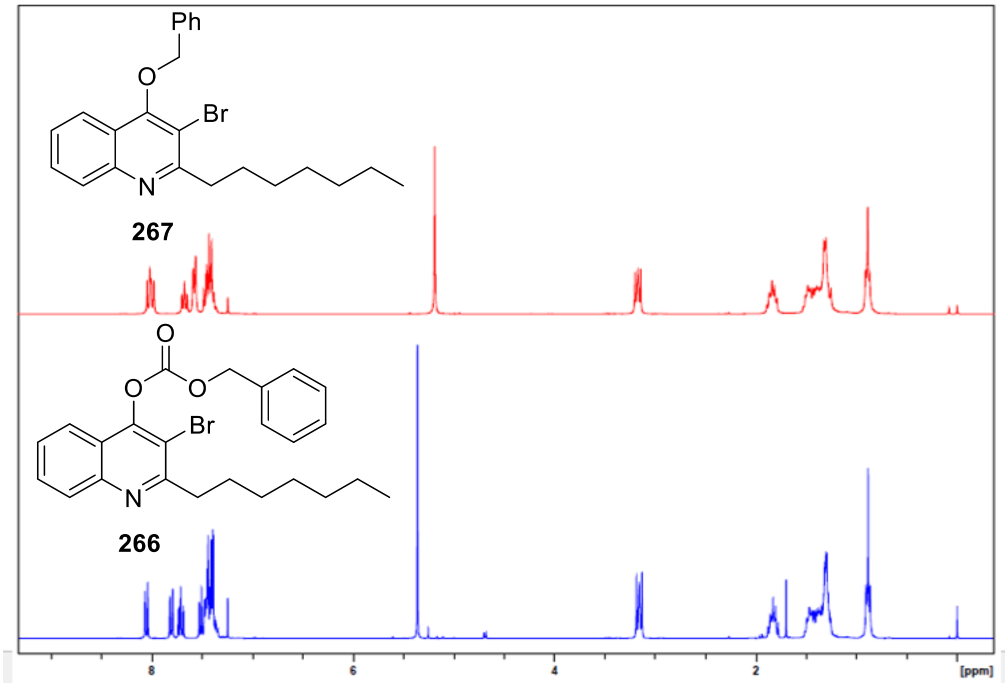
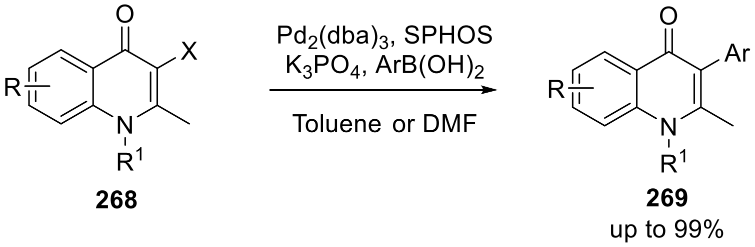


Figure 5.9 Comparison of ^{1}H NMR of **266** and **267**.

A point of note is that when bromination of protected HHQ using bromine in acetic acid was carried out after the protection step, it was found that the HBr produced in the reaction causes removal of the Cbz group, necessitating that the halogen group be in place prior to protection.

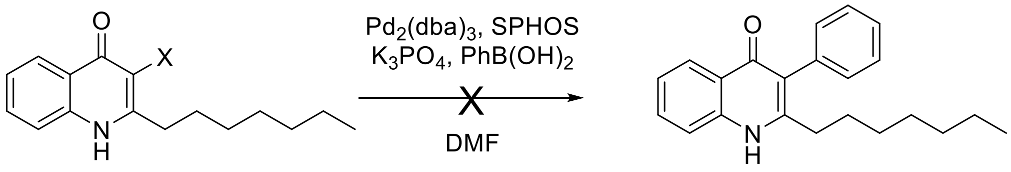
5.7 Suzuki reactions

In a recent paper by Cross and Manetsch,[32] palladium cross-coupling reactions were successfully carried out on 2-methyl-3-halo-quinolones **268** using a Pd/SPHOS catalyst/ligand system, with good to excellent yields of coupled product **269** being achieved (**Scheme 5.17**).



Scheme 5.17 Palladium cross-coupling reaction of 2-methyl-3-halo quinolones.

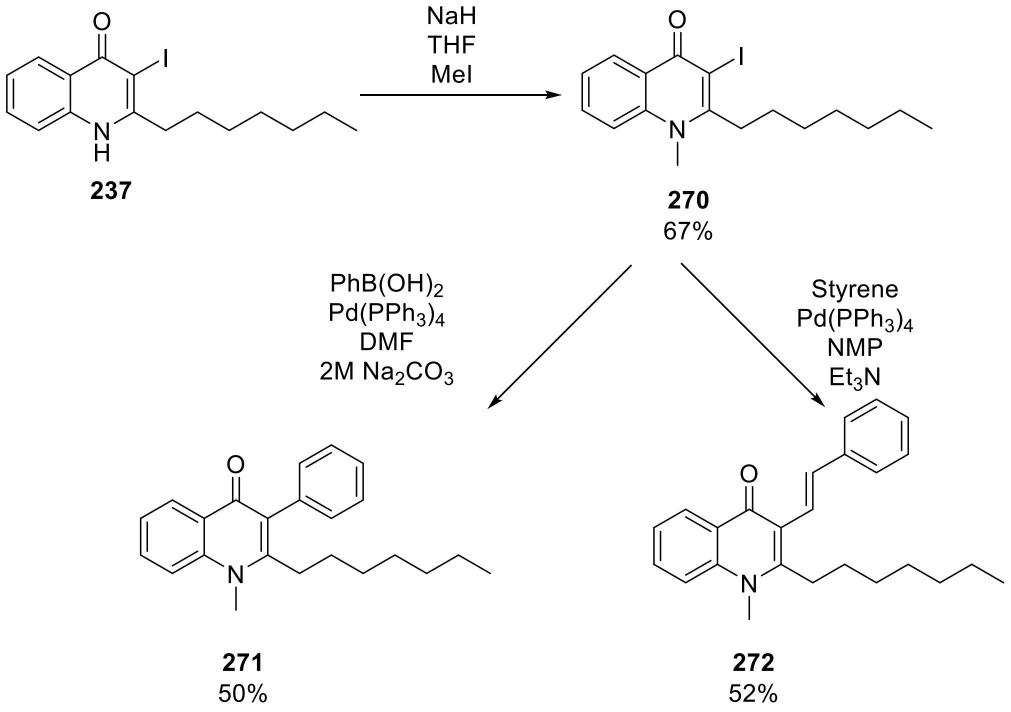
Compared to the use of the standard $Pd(PPh_3)_4$ catalyst, the reactions were complete in a shorter period of time and gave higher yields. Where *N*-methylated quinolones were employed, toluene was found to be the solvent of choice. In the case where both R and R[1] corresponded to H, the reaction was found to give a significantly higher yield of the product when carried out in DMF compared to when the reaction was carried out in toluene. With these results in mind, it was decided to test these reaction conditions with 3-iodo-HHQ **237** and 3-bromo-HHQ **235** (**Scheme 5.18**). The reactions were carried out as per the literature procedure,[32] however the resultant crude product in both cases was a complex mixture of products as determined by ^{1}H NMR spectroscopy.



235 X = Br
237 X = I

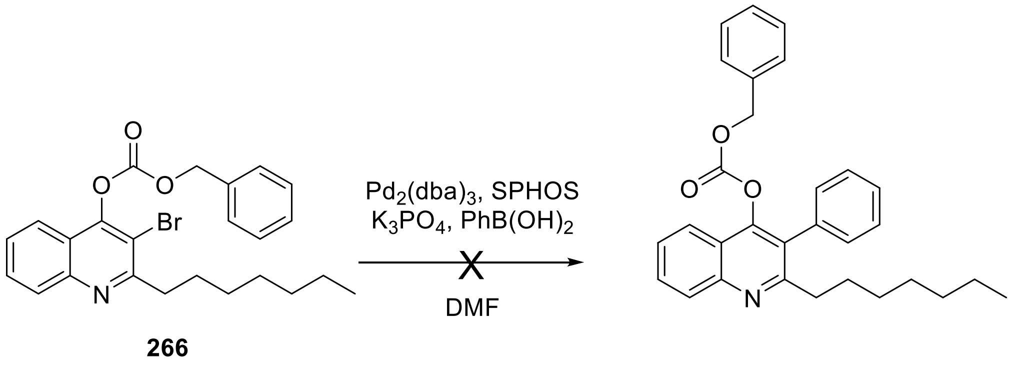
Scheme 5.18 Attempt at palladium cross-coupling reaction on HHQ using SPHOS.

Previous work within the group has shown that iodinated HHQ **237** can be easily methylated under standard conditions to provide **270**, which can then successfully undergo palladium catalysed cross-coupling reactions to afford **271** and **272** (**Scheme 5.19**).[5]


NaH
THF
MeI
237
270
67%
PhB(OH)2
Pd(PPh3)4
DMF
2M Na2CO3
Styrene
Pd(PPh3)4
NMP
Et3N
271
50%
272
52%

Scheme 5.19 Palladium cross-coupling reactions of *N*-methylated HHQ.

Although results for palladium cross-coupling with *N*-methylated 3-iodo-HHQ **270** were promising, there was scope for investigation into the reaction as it was envisaged that a better protecting group (which would be easier to remove after the coupling) could be utilised as well as using the conditions employed by Cross and Manetsch which gave high yields.[32] Cbz-protected 3-bromo HHQ **266** was subjected to these conditions (**Scheme 5.20**) however the reaction resulted in complex mixture of products as deduced by ^{1}H NMR spectroscopy. It could be that the steric size of the Cbz protecting group blocks the C-3 position and prevents the reaction from taking place.


Pd2(dba)3, SPHOS
K3PO4, PhB(OH)2
DMF
266

Scheme 5.20 Attempted cross-coupling reaction of Cbz-protected 3-bromo-HHQ.

5.8 Biological testing of analogues

In order to assess the importance of the C-3 position to the biological activity of HHQ and PQS, the capacity for analogues functionalised at this position (with the exception of 3-fluoro-HHQ **244**) to replace the native compounds in *P. aeruginosa* was investigated in collaboration with Professor Fergal O'Gara, Department of Microbiology, University College Cork.[28]

The PQS signalling system is known to control production of a range of virulence factors in *P. aeruginosa* including elastase, rhamnolipid and the phenazine redox compound pyocyanin.[33,34] It has been reported that phenazines, in µM concentrations, can support anaerobic survival of *P. aeruginosa*, typical of the conditions found in the lungs of CF patients, assisting in the formation of biofilms.[35] The first investigation on the synthesised analogues was using a *pqsA* mutant, which is a strain in which the biosynthetic steps for 2-alkyl-4-quinolones have been disrupted and these compounds are not produced. The analogues were assessed for restoration of phenazine production using this mutant. Whilst HHQ and PQS both restored phenazine production in the *pqsA* mutant, the analogues synthesised were much less effective in triggering production of the pigment, with **259** being the least effective (**Figure 5.10 (A)**). These results suggest that the C-3 position is crucial for control of phenazine production in *P. aeruginosa*. When the analogues were introduced in equimolar concentration to two wild-type strains which produce both HHQ and PQS (PAO1 and PA14) phenazine production was not interrupted (**Figure 5.10 (B)**).

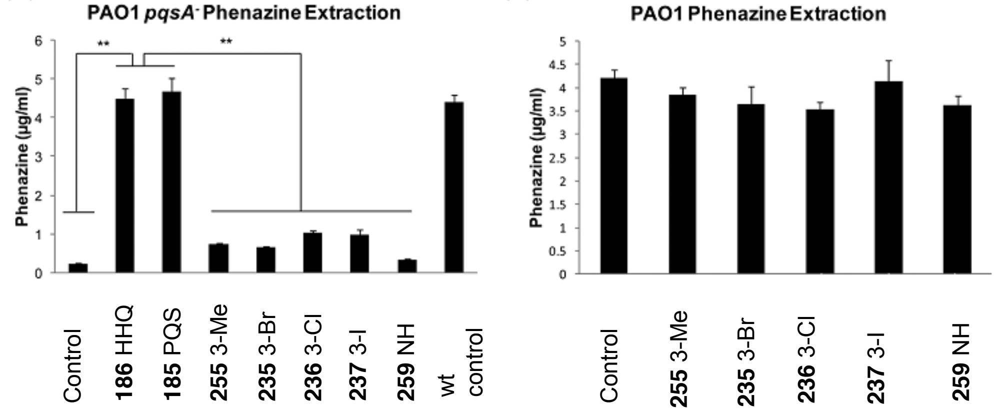


Figure 5.10 Influence of 3-substituted derivatives on PQS-dependent phenotypes in *P. aeruginosa*. **(A)** The ability of HHQ and PQS (10 µM) to restore phenazine

production in a *pqsA* mutant was lost to derivative compounds, indicating that the C-3 position is crucial in this regard. **(B)** Addition of 10 µM concentrations of derivative compounds did not interfere with phenazine production in the wild-type PAO1 strain. Data presented is representative of three independent experiments (Student's *t*-test, ***p*-value ≤ 0.005).

In *P. aeruginosa*, PQS plays a fundamental role in the formation of biofilms. Mutant strains which are deficient in PQS have been shown to produce thin, flat biofilms, which are markedly different to the mushroom shaped structures produced by the wild-type strain.[36] The addition of the analogues did not have an influence on biofilm formation in *P. aeruginosa* as seen in crystal violet multi-well assays (**Figure 5.11**).

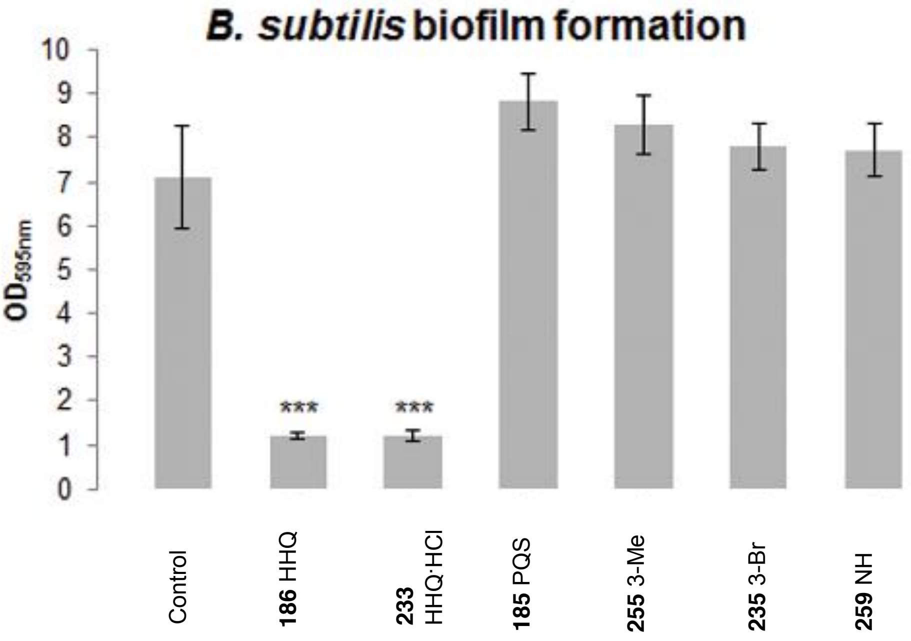


Figure 5.11 Crystal violet staining of 18 h cultures grown static in multi-well plates revealed HHQ interferes with *B. subtilis* biofilm formation irrespective of its tautomeric form. This anti-biofilm activity was abolished with C-3 substituted derivatives. All compounds were added at a final concentration of 10 µM and statistical significance was provided by paired Student's *t*-test (***, *p*-value ≤ 0.001).

Apart from their key role as signalling molecules in *P. aeruginosa*, both HHQ and PQS exert distinct influences on the behaviour of a range of microbial pathogens, modulating interspecies and interkingdom behaviour.[37] The fact that these two molecules differ only at the C-3 position yet display diverse biological functionalities suggests the C-3 position plays a key role in modulating interspecies microbial behaviour. Microbial swarming motility and biofilm formation require cooperative multicellular behaviour and provide a mechanism for bacterial cells to establish and persist during infection. In *S. aureus*, motility was shown to be altered in the presence of HHQ and PQS and in *C. albicans*, the formation of biofilm was found to be repressed in the presence of HHQ. Both phenotypes have been affected in *B. subtilis* (a bacterial species also found in soil) in the presence of HHQ, and so this organism was chosen as a model organism upon which to test the interspecies influence of the alkylquinolone analogues. Unlike HHQ and HHQ.HCl, analogues **235-237** and **259** did not exhibit anti-biofilm activity towards *B. subtilis*, thus highlighting the importance of the C-3 position in underpinning the biological role of these compounds. The influence of analogues **235-237**, **255** and **259** on microbial swarming motility was negligible in comparison to HHQ and PQS (**Figure 5.12**).

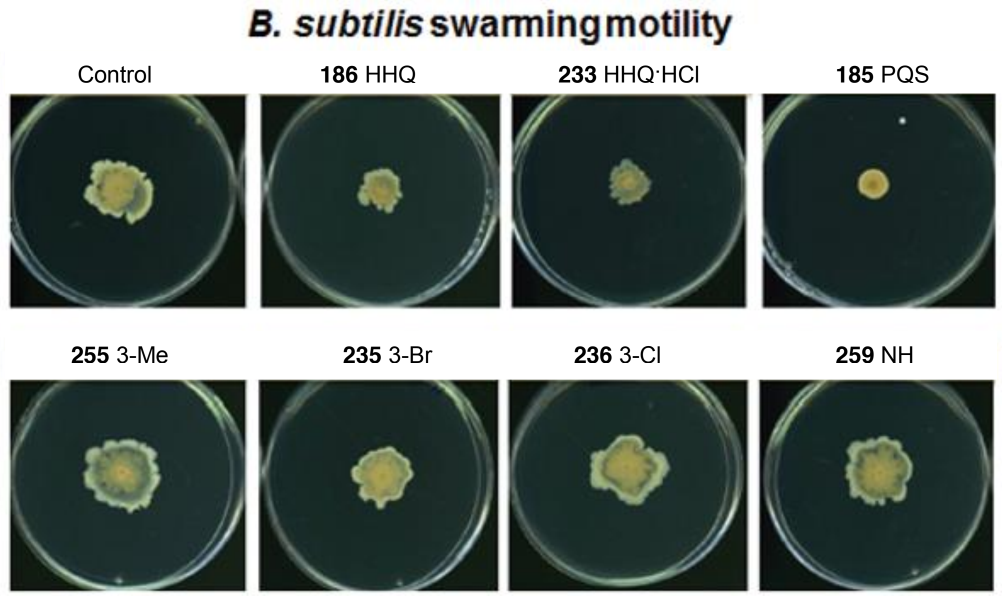


Figure 5.12 *B. Subtilis* swarming motility was assessed after 16 h on 0.3% (w/v) Trypticase Soy Agar (TSA) plates. C-3 substituted analogues (**235, 236, 255** and **259**) abolished the anti-swarming activity, highlighting the structural specificity underpinning the biological activity of these compounds.

The methyl and halogen substituted analogues **235-237** and **255** retained antimicrobial activity towards an *Algoriphagus* marine isolate, which had previously been shown to be susceptible towards HHQ, while PQS and quinazolinone **259** did not suppress growth of this species (**Figure 5.13**).

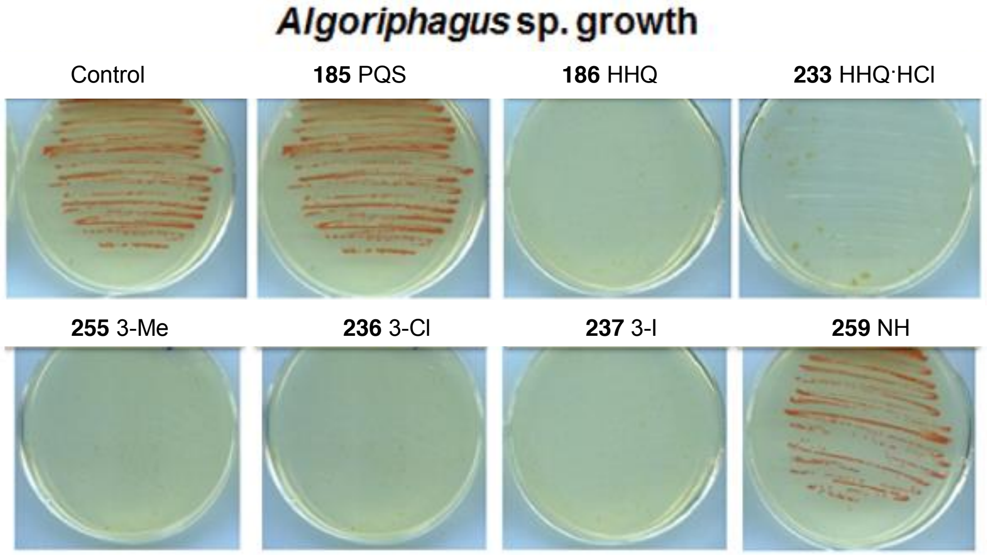


Figure 5.13 The ability of HHQ and C-3 substituted analogues to repress the growth of a marine isolate on Starch Yeast Peptone (SYP) marine agar.

Both HHQ and PQS have previously been shown to influence transcription in a mouse monocyte/macrophage cell line.[38] However, although PQS has been found in the sputum of CF patients,[39] the impact of potential cytotoxic effects of these compounds on airway epithelial cells has not been investigated. HHQ, PQS and analogues **235-237**, **255** and **259** were tested for cytotoxicity towards a human airway epithelial cell line (IB3-1 cells) for 16 h at concentrations ranging from 10 to 100 μM by quantification of the lactate dehydrogenase (LDH) release, in comparison with treatment by 0.1% Triton X-100, used as a positive control for cytotoxicity (**Figure 5.14**). HHQ was found to be cytotoxic towards IB3-1 cells however PQS did not exhibit any toxicity. Decreasing cytotoxicity of HHQ was observed with decreasing concentrations and was less than 10% at 10 μM. With the exception of quinazolinone **259** which exhibited a significant level of cytotoxicity, the C-3 substituted analogues **235-237** and **235** did not exhibit cytotoxicity towards IB3-1 cells, reinforcing the importance of the C-3 position in the functionality of the native signal molecules. When the concentration of HHQ was 100 μM, significant cellular damage of IB3-1 cells was observed by cellular morphological analysis. When analogue **259** was used, significant cellular change was noted by the same method. PQS and analogues **235-237** and **255** caused only moderate changes to cellular morphology and the plasma membrane remained intact, consistent with the lack of LDH release, in comparison with 0.1% Triton X-100.

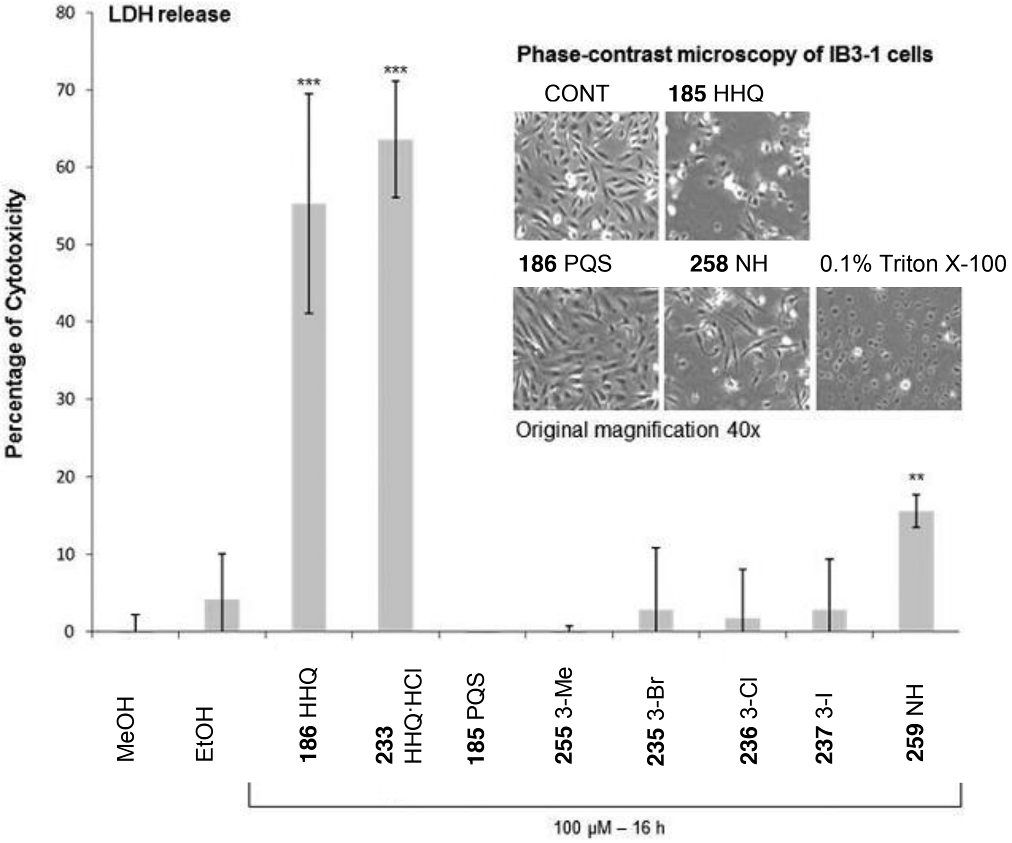


Figure 5.14 Substitution at C-3 abolishes cytotoxic activity of HHQ. Cytotoxicity is expressed as a percentage of the total amount of LDH released from cells treated with 0.1% Triton X-100. The release of LDH was measured in cell culture medium of IB3-1 cells treated with methanol or ethanol, or with 100 μM of HHQ, PQS or C-3 substituted analogues. Data (means ± SD) are representative of 3 independent biological experiments. Two-tailed unpaired student's *t*-test was performed by comparison of IB3-1 cells treated with HHQ analogue molecules with IB3-1 cells treated with methanol or ethanol (**, *p*-value ≤ 0.01; ***, *p*-value ≤ 0.001). Phase-contrast microscopy of IB3-1 cells untreated (CONT) or treated with HHQ, PQS

or **259** at a concentration of 100μM for 16 h. Triton X-100 (0.1%) was used as a control in these studies. Original magnification 40×.

Methylation at the C-3 position provided analogue **255** which is a known signal molecule produced by several *Burkholderia* species, which do not have the capacity to produce PQS. Interestingly, **255** appeared to have lost its ability to restore phenazine production or influence interspecies multicellular behaviour, although it did retain antibacterial activity against *Algoriphagus* species. Although **255** and PQS are structurally similar, and produced by important pathogens of the CF-lung, their interspecies profiles are distinct.

5.9 Conclusions and future work

Various C-3 analogues of HHQ have been successfully synthesised, including bromo **235**, chloro **236**, iodo **237**, fluoro **244**, methyl **255** and quinazolinone **259**.

The results from the biological testing of analogues **235-237**, **255** and **259** highlight, for the first time, the strict structural requirements at the C-3 position which underpin the biological activity of HHQ and PQS. The inability of any of the analogues to restore phenazine production in *P. aeruginosa pqsA* mutant suggests the C-3 position is crucial for control of phenazine production in this pathogen. When analogues were added to wild-type PAO1 and PA14 strains which produced both HHQ and PQS, there was no effect on phenazine production, suggesting they may not be effective inhibitors in *P. aeruginosa*. The analogues were also found not to interfere with the initial stages of biofilm formation in *P. aeruginosa*. The correlation between loss of function both within *P. aeruginosa* and towards other microbial species upon alteration of the C-3 position is remarkable.

If it was the case that a simple electron withdrawing group was required at the C-3 position, then halogenation at this point (analogues **235-237**) would be expected to produce molecules with similar biological activity. Alternatively, introduction of an NH group as in analogue **259** provides a molecule with hydrogen bonding properties at this position, potentially mimicking the hydrogen atom at this position in HHQ. However in both cases the capacity to modulate either *P. aeruginosa* or interkingdom behaviour was lost with these compounds. It is therefore clear that the hydrogen and hydroxyl groups present at C-3 in HHQ and PQS respectively, play a more complex role in these biological systems.

When a nitrogen was introduced in place of carbon at C-3 (**259**), a complete loss of biological activity in relation to HHQ was observed. Previous work within the research group has shown that when an aldehyde group was present at C-3, intermediate activity in relation to both compounds was observed.[37] From a chemical perspective, the bacterial biosynthesis of PQS *via* HHQ and the aldehyde analogue, would go some way to explaining this observation, however the evolutionary rationale supporting this has yet to be established.

Further investigation into manipulation of the C-3 position will be carried out, with a view towards attaining a deeper understanding of the complex roles of these molecules in bacterial and fungal species. Alternative methodologies for synthesis of 3-fluoro-HHQ will also be investigated in order to attempt to increase the yield of the reaction and allow subsequent biological testing of the molecule.

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Chapter 6

Structure–function analysis of the C-3 position in analogues of microbial behavioural modulator, HHQ

Experimental

Contents

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6.1 General experimental

Solvents and reagents were used as obtained from commercial sources and without purification with the following exceptions: THF was freshly distilled from sodium/benzophenone under nitrogen. DCM, hexane, acetone and diisopropylamine were distilled from CaH_2 under nitrogen.

Wet flash column chromatography was carried out using Kieselgel silica gel 60, 0.040–0.063 mm (Merck). TLC was carried out on pre-coated silica gel plates (Merck 60 PF254). Visualisation was achieved by UV light and potassium permanganate staining.

Melting points were carried out on a uni-melt Thomas Hoover Capillary melting point apparatus.

IR spectra were recorded on Perkin-Elmer FT-IR Paragon 1000 spectrophotometer. Liquid samples were examined as thin films interspersed on NaCl plates. Solid samples were dispersed in KBr and recorded as pressed discs. The intensity of peaks were expressed as strong (s), medium (m) and weak (w) and broad (b).

NMR spectra were run in $CDCl_3$ using TMS as the internal standard at 20 °C unless otherwise specified. ^1H NMR (600 MHz) spectra, ^1H NMR (400 MHz) spectra and ^1H NMR (300 MHz) spectra were recorded on Bruker Avance 600, Bruker Avance 400 and Bruker Avance 300 NMR spectrometers respectively in proton coupled mode. ^{19}F NMR (470 MHz) spectra and ^{19}F NMR (282 MHz) were recorded on Bruker Avance 600 NMR and Bruker Avance 300 NMR spectrometers respectively in proton decoupled mode. ^{13}C NMR (150 MHz) spectra and ^{13}C NMR (75 MHz) spectra were recorded on Bruker Avance 600 and Bruker Avance 300 NMR spectrometers respectively in proton decoupled mode. All spectra were recorded at University College Cork. Chemical shifts δ_H and δ_C are expressed as parts per million (ppm), positive shift being downfield from TMS; coupling constants (*J*) are expressed in hertz (Hz). Splitting patterns in ^1H NMR spectra are designated as s (singlet), bs (broad singlet), d (doublet), dd (doublet of doublets), dt (doublet of triplets), t (triplet), q (quartet) and m (multiplet). For ^{13}C NMR spectra, the number of attached protons for each signal was determined using the DEPT pulse sequence run in the DEPT-90 and DEPT-135 modes. COSY, HSQC and HMBC experiments were routinely performed to aid the NMR assignment of novel chemical structures.

LRMS were recorded on a Waters Quattro Micro triple quadrupole instrument in ESI mode using 50% acetonitrile-water containing 0.1% formic acid as eluent; samples were made up in acetonitrile or methanol. HRMS were recorded on a Waters LCT Premier Tof LC-MS instrument in ESI mode using 50% acetonitrile-water containing 0.1% formic acid as eluent; samples were made up in acetonitrile or methanol.

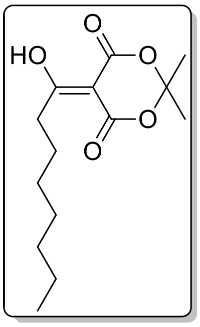
The Microanalysis Laboratory, National University of Ireland, Cork, performed elemental analysis using a Perkin-Elmer 240 and Exeter Analytical CE440 elemental analysers.

6.1.1 Analysis of known and novel compounds

^{1}H NMR spectra, ^{13}C NMR spectra, LRMS and melting point (if solid) analyses were recorded for all previously prepared compounds. For novel compounds, in addition to the previously mentioned analysis, ^{19}F NMR (where applicable), IR, HRMS and elemental analysis (if possible) were also obtained.

6.2 Synthesis of HHQ

5-(1-Hydroxyoctylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione, 230

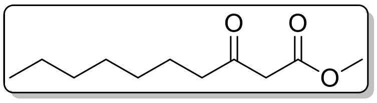


To a stirred solution of 2,2-dimethyl-1,3-dioxane-4,6-dione **229** (Meldrum's Acid) (40.32 g, 0.28 mol) in distilled DCM (350 mL) at 0 °C under a N_2 atmosphere was added pyridine (45.3 mL, 0.56 mol), followed by the dropwise addition of octanoyl chloride (54.6 mL, 0.32 mol) over 5 min. The resulting orange liquid was allowed stir at 0 °C for 1 h, then room-temperature for 1 h. The mixture was washed with 5% aq. HCl (3 × 110 mL) and water (110 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* to yield acyl Meldrum's acid **230** as a brown oil (75.16 g, 99%), which was used in the next step without further purification.

Spectral characteristics were consistent with previously reported data.[1]

^{1}H NMR (300 MHz, $CDCl_3$, only signals of the predominant enol tautomer are given): δ 0.88 (3H, t, *J* = 6.7 Hz, C***H***$_3$CH$_2$), 1.29–1.44 (8H, m, 4 × C***H***$_2$) 1.62–1.76 (2H, m, C***H***$_2$CH$_2$COH), 1.73 (6H, s, 2 × CC***H***$_3$), 3.07 (2H, t, *J* = 7.6 Hz, C***H***$_2$COH), 15.30 (1H, bs, O***H***) ppm; ^{13}C NMR (75 MHz, $CDCl_3$, only signals of the predominant enol tautomer are given): δ 14.1 (***C***H$_3$), 22.6, 26.1 (2 × ***C***H$_2$), 26.8 (2 × C***C***H$_3$), 28.9, 29.3, 31.6, 35.8 (4 × ***C***H$_2$), 91.2 (***C***COO), 104.8 (***C***(CH$_3$)$_2$), 160.2, 170.6 (2 × ***C***OO), 198.3 (***C***OH) ppm; MS (ESI) *m/z*: 269 [(M - H)$^-$, 100%].

Methyl 3-oxodecanoate, 231

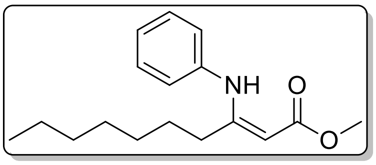


A stirred solution of acyl Meldrum's acid **230** (75.02 g, 0.28 mol) in methanol (350 mL) was heated at reflux for 3 h. The reaction mixture was allowed to cool to room-temperature and solvent removed *in vacuo* to yield an orange oil which was purified by fractional distillation to afford β-keto ester **231** as a colourless oil (45.02 g, 80%).

Spectral characteristics were consistent with previously reported data.[1]

^{1}H NMR (400 MHz, $CDCl_3$, only the signals of the predominant keto tautomer are given): δ 0.86 (3H, t, *J* = 6.9 Hz, C***H***$_3$CH$_2$), 1.22–1.27 (8H, m, 4 × C***H***$_2$), 1.53–1.59 (2H, m, C***H***$_2$CH$_2$CO), 2.50 (2H, t, *J* = 7.3 Hz, CH$_2$C***H***$_2$CO), 3.42 (2H, s, COC***H***$_2$CO), 3.71 (3H, s, OC***H***$_3$) ppm; ^{13}C NMR (75 MHz, CDCl$_3$, only the signals of the predominant keto tautomer are given): δ 14.0 (***C***H$_3$), 22.5, 23.4, 28.9, 29.0, 31.6, 43.0 (6 × ***C***H$_2$), 49.0 (CO***C***H$_2$CO), 52.2 (O***C***H$_3$), 167.7 (***C***=O ester), 202.8 (***C***=O ketone) ppm; HRMS (ESI) *m/z* calcd for $C_{11}H_{21}O_3$ [(M + H)$^+$]: 201.1491, found 201.1482.

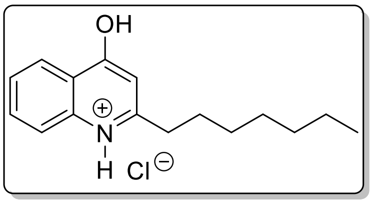
Methyl 3-(phenylamino)dec-2-enoate, 232



To a stirred solution of methyl-3-oxodecanoate **231** (44.97 g, 0.22 mol) in dry hexane (270 mL) was added aniline (21.9 mL, 0.24 mol) and *p*-toluene sulfonic acid (0.837 g, 4.4 mmol) and reaction vessel was fitted with Dean-Stark apparatus. The resulting reaction mixture was allowed to stir at reflux under a N$_2$ atmosphere for 16 h. The reaction mixture was allowed to cool and the solvent removed *in vacuo* to yield **232** as an orange oil (60.24 g, 99%) which was used in the next step without purification.

IR (NaCl) ν_{max}: 2928, 2857 (alkyl C-H stretch, s), 1736 (C=O stretch), 1623 (alkene C=C stretch, s), 1500, 1448 (aromatic C=C stretch, s), 1263 (C-N stretch, s), 1169 (ester C-O stretch, s) cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$): δ 0.84 (3H, t, *J* = 7.0 Hz, C***H***$_3$CH$_2$), 1.14–1.32 (8H, m, 4 × C***H***$_2$), 1.37–1.45 (2H, m, C***H***$_2$CH$_2$C), 2.26–2.30 (2H, m, C***H***$_2$C), 3.68 (3H, s, OC***H***$_3$), 4.73 (1H, s, C***H***), 7.09 (2H, d, *J* = 7.4 Hz, 2 × C***H*** arom.), 7.15–7.19 (1H, m, C***H*** arom.), 7.26–7.35 (2H, m, 2 × C***H*** arom.), 10.28 (1H, bs, N***H***) ppm; ^{13}C NMR (75 MHz, CDCl$_3$): δ 14.0 (***C***H$_3$), 22.6, 27.7, 28.7, 28.8, 29.4, 31.6 (6 × ***C***H$_2$), 50.7 (O***C***H$_3$), 84.5 (***C***HCOOCH$_3$), 125.1, 125.6, 129.1 (5 × ***C***H arom.), 139.2 (CHCH***C***NH) 163.8 (CH***C***NH), 171.8 (***C***=O) ppm; HRMS (ESI) *m/z* calcd for $C_{17}H_{26}NO_2$ [(M + H)$^+$]: 276.1964, found 276.1964.

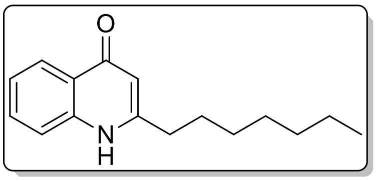
2-Heptyl-4-hydroxyquinolin-1-ium chloride, 233



To a refluxing solution of diphenyl ether (45 mL, 0.264 mol) was added enamine **232** (61.02 g, 0.22 mol) dropwise over 90 min, ensuring vigorous reflux was maintained. On completion of addition, the reaction mixture was allowed to reflux for 1 h. The reaction mixture was cooled to room temperature and the formed methanol removed *in vacuo*. To the isolated residue was added diethyl ether (120 mL) and 2M aq. HCl (120 mL) and the reaction mixture was allowed to stand at room temperature for 18 h, then the refrigerator for 2 h. The resulting precipitate was filtered and washed with diethyl ether to afford the crude product as a yellow solid which was purified by recrystallisation from ethyl acetate to yield product **233** as a cream solid (16.57 g, 27%).

m.p. 110–113 °C. IR (KBr) ν_{max}: 2930, 2738 (alkyl C-H stretch, s), 1639 (C=O stretch, s), 1594 (aromatic C=C stretch, s), 1488 (alkyl C-H bend, m) cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$): δ 0.77 (3H, t, *J* = 6.6 Hz, C***H***$_3$), 1.14–1.38 (8H, m, 4 × C***H***$_2$), 1.82–1.87 (2H, m, C***H***$_2$CH$_2$C), 3.17 (2H, t, *J* = 7.6 Hz, C***H***$_2$C), 7.61 (1H, t, *J* = 7.7 Hz, C***H*** arom.), 7.64 (1H, s, C***H***), 7.83 (1H, t, *J* = 7.5 Hz, C***H*** arom.), 8.31 (1H, d, *J* = 8.3 Hz, C***H*** arom.), 8.52 (1H, d, *J* = 8.4 Hz, C***H*** arom.) ppm; ^{13}C NMR (75 MHz, CDCl$_3$): δ 14.0 (***C***H$_3$), 22.5, 28.9, 29.3, 29.8, 31.6, 34.3 (6 × ***C***H$_2$), 105.5 (***C***H), 119.6 (CH***C***C=O), 119.9, 123.7, 127.2, 133.9 (4 × ***C***H arom.), 139.7 (CH***C***NH), 161.0 (CH$_2$***C***NH), 169.7 (***C***=O) ppm; HRMS (ESI) *m/z* calcd for $C_{16}H_{22}NO$ [(M + H)$^+$]: 244.1701, found 244.1696 (HHQ); Anal. calcd for $C_{16}H_{22}ClNO$: C, 68.68; H, 7.93; N, 5.01%. Found: C, 68.99; H, 7.91; N, 5.02%.

2-Heptylquinolin-4(1*H*)-one (HHQ), 186

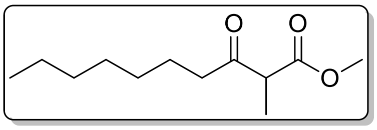


To a stirred solution of HHQ·HCl **233** (8.01 g, 28.6 mmol) in CHCl$_3$ (70 mL) was added 2M aq. NaOH to pH neutral. The layers were separated and the aqueous layer extracted with CHCl$_3$ (3 × 40 mL). Organic layers were combined, dried over MgSO$_4$ and concentrated *in vacuo* to yield **186** as an off-white solid (6.56 g, 94%).

Spectral characteristics were consistent with previously reported data.^{2} m.p. 143–146 °C [lit.^{3} 146–147 °C]. IR (KBr) ν_{max}: 2923, 2850 (alkyl C-H stretch, s), 1640 (C=O stretch, s), 1594 (aromatic C=C stretch, s), 1473 (alkyl C-H bend, m) cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$): δ 0.81 (3H, t, *J* = 6.9 Hz, C***H***$_3$), 1.14–1.30 (8H, m, 4 × C***H***$_2$), 1.68–1.75 (2H, m, C***H***$_2$CH$_2$C), 2.69 (2H, t, *J* = 7.7 Hz, C***H***$_2$C), 6.24 (1H, s, C***H***), 7.30–7.34 (1H, m, C***H*** arom.), 7.56–7.60 (1H, m, C***H*** arom.), 7.75 (1H, d, *J* = 8.3 Hz, C***H*** arom.), 8.36 (1H, dd, *J* = 1.3, 8.2 Hz, C**H** arom.), 11.97 (1H, bs, N**H**) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.0 (**C**H_3), 22.6, 29.0, 29.1, 29.2, 31.7, 34.4 (6 × **C**H_2), 108.2 (**C**H), 118.5, 123.6 (2 × **C**H arom.), 125.0 (CH**C**C=O), 125.3, 131.8 (2 × **C**H arom.), 140.6 (CH**C**NH), 155.1 (NH**C**CH_2), 178.9 (**C**=O) ppm; HRMS (ESI) *m/z* calcd for $C_{16}H_{22}NO$ [(M + H)$^+$]: 244.1701, found 244.1707; Anal. calcd for $C_{16}H_{21}NO$: C, 78.97; H, 8.70; N, 5.76%. Found: C, 79.19; H, 8.81; N, 5.83%.

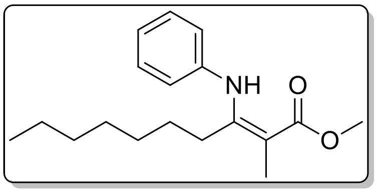
6.3 Synthesis of 3-methyl analogue

Methyl 2-methyl-3-oxodecanoate, 253



To a round bottomed flask containing dry potassium carbonate (2.46 g, 17.8 mmol) was added a solution of β-ketoester **231** (2.74 g, 13.7 mmol) in dry acetone (35 mL) under a N_2 atmosphere. The resulting mixture was allowed to stir for 20 min before the addition of methyliodide (1.02 mL, 16.4 mmol). The reaction mixture was allowed to stir at reflux for 6 h. The mixture was removed from the heat, allowed to cool and the solvent removed *in vacuo* to yield crude product which was purified by silica column chromatography eluting with 98:2 hexane:ethyl acetate to yield **253** as a pale yellow oil (1.17 g, 40%). IR (NaCl) ν_{max}: 2930, 2857 (alkyl C-H stretch, s), 1749 (ketone C=O stretch, s), 1717 (ester C=O stretch, s), 1204 (ester C-O stretch, m) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (3H, t, *J* = 6.7 Hz, C***H₃***CH_2), 1.22–1.28 (8H, m, 4 × C***H₂***), 1.32 (3H, d, *J* = 7.2 Hz, C***H₃***CH), 1.54–1.59 (2H, m, C***H₂***CH_2CO), 2.45–2.61 (2H, m, C***H₂***CO), 3.51 (1H, q, *J* = 7.1 Hz, CH_3C**H**), 3.71 (3H, s, OC***H₃***) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.8 (**C**H_3CH), 14.0 (**C**H_3), 22.6, 23.5, 28.98, 29.00, 31.6, 41.4 (6 × **C**H_2), 52.3 (CO**C**HCO), 52.7 (O**C**H_3), 171.1 (**C**=O ester), 205.9 (**C**=O ketone) ppm; HRMS (ESI) *m/z* calcd for $C_{12}H_{23}O_3$ [(M + H)$^+$]: 215.1647, found 215.1642; Anal. calcd for $C_{12}H_{22}O_3$: C, 67.26; H, 10.35%. Found: C, 67.15; H, 10.22%.

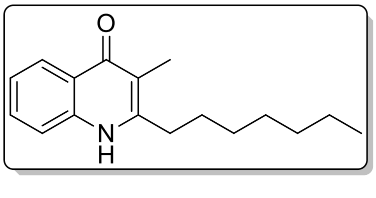
Methyl 2-methyl-3-(phenylamino)dec-2-enoate, 254



To a stirred solution of methyl 2-methyl-3-oxodecanoate **253** (1.27 g, 5.94 mmol) in dry hexane (30 mL) was added aniline (0.57 mL, 6.24 mmol) and *p*-toluene sulfonic acid (0.023 g, 0.12 mmol) and reaction vessel was fitted with Dean-Stark apparatus. The resulting reaction mixture was allowed to stir at reflux under a N_2 atmosphere for 16 h. The reaction mixture was allowed to cool and the solvent removed *in vacuo* to yield **254** as an orange oil (1.35 g, 79%) which was used in the next step without purification.

IR (NaCl) ν_{max}: 2952, 2928 (alkyl C-H stretch, s), 1744 (C=O stretch, s), 1657 (alkene C=C stretch, s), 1612, 1594 (aromatic C=C stretch, s), 1252 (C-N stretch, s), 1229 (C-O stretch, s) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, only signals of predominant enamine tautomer are given): δ 0.84 (3H, t, *J* = 7.0 Hz, C***H₃***CH₂), 1.17–1.29 (8H, m, 4 × C***H₂***), 1.37–1.46 (2H, m, C***H₂***CH₂C), 1.86 (3H, s, C***H₃***C), 2.34–2.38 (2H, m, C***H₂***C), 3.72 (3H, s, OC***H₃***), 7.06 (2H, d, *J* = 7.6 Hz, 2 × C***H*** arom.), 7.13–7.19 (1H, m, C***H*** arom.), 7.26–7.35 (2H, m, 2 × C***H*** arom.), 10.81 (1H, bs, N***H***) ppm; ^{13}C NMR (75 MHz, $CDCl_3$, only signals of predominant enamine tautomer are given): δ 12.5 (***C***H₃C), 14.0 (***C***H₃), 22.6, 27.7, 28.7, 28.8, 29.4, 31.6 (6 × ***C***H₂), 50.7 (O***C***H₃), 90.4 (CH₃***C***CO), 125.1, 125.6, 129.1 (5 × ***C***H arom.), 140.3 (CH***C***NH), 163.8 (CH***C***NH), 171.7 (***C***=O) ppm; HRMS (ESI) *m/z* calcd for $C_{18}H_{28}NO_2$ [(M + H)$^+$]: 290.2120, found 290.2116; Anal. calcd for $C_{18}H_{27}NO_2$: C, 74.70; H, 9.40; N, 4.84%. Found: C, 74.30; H, 9.20; N, 5.20%.

2-Heptyl-3-methylquinolin-4(1*H*)-one, 255



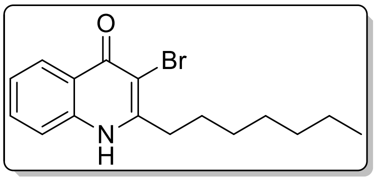
To a solution of refluxing diphenyl ether (45 mL) was added enamine **254** (1.35 g, 4.68 mmol) dropwise over 90 min. Reflux was maintained for 1 h before being allowed to cool to room temperature. The formed methanol was removed *in vacuo* to yield crude product as a brown oil. Purification was achieved by recrystallisation from methanol to yield **255** as a white solid (0.010 g, 10%).

Spectral characteristics were consistent with previously reported data.[4]

m.p. 228–230 °C [lit.[4] 227–228 °C]. ^{1}H NMR (400 MHz, CD$_3$OD): δ 0.88 (3H, t, *J* = 6.8 Hz, C***H***$_3$CH$_2$), 1.30–1.46 (8H, m, 4 × C***H***$_2$), 1.68–1.75 (2H, m, C***H***$_2$CH$_2$C), 2.15 (3H, s, CC***H***$_3$), 2.81 (2H, t, *J* = 7.9 Hz, C***H***$_2$C), 7.33 (1H, ddd, *J* = 1.1, 7.1, 8.1 Hz, C***H*** arom.), 7.53 (1H, d, *J* = 8.0 Hz, C***H*** arom.), 7.60–7.64 (1H, m, C***H*** arom.), 8.22 (1H, dd, *J* = 0.7, 8.4 Hz, C***H*** arom.) ppm; ^{13}C NMR (75 MHz, CD$_3$OD): δ 10.8 (C***C***H$_3$), 14.4 (***C***H$_3$), 23.7, 30.0, 30.2, 30.5, 32.9, 33.5 (6 × ***C***H$_2$), 116.2 (***C***CH$_3$), 118.7 (***C***H arom.), 124.4 (CH***C***C=O), 124.5, 126.2, 132.7 (3 × ***C***H arom.), 140.6 (CH***C***NH), 153.4 (CH$_2$***C***NH), 179.5 (C=O) ppm; HRMS (ESI) *m*/*z* calcd for $C_{17}H_{24}NO$ [(M + H)$^+$]: 258.1858, found 258.1849.

6.4 Synthesis of 3-halo analogues

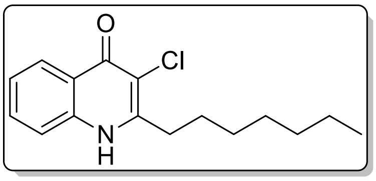
3-Bromo-2-heptylquinolin-4-ol, 235



Method A: To a stirred solution of HHQ **186** (2.094 g, 8.61 mmol) in acetic acid (10 mL) was added a solution of bromine (0.5 mL, 9.72 mmol) in acetic acid (5 mL) dropwise over 30 min. The reaction vessel was covered in aluminium foil and reaction allowed to stir at room temperature for 5 h. On completion, the reaction mixture was poured into 1% aq. sodium sulfite (100 mL). The precipitate was filtered and washed with water to yield the crude product as a pale yellow solid which was purified by recrystallisation from ethanol to yield **235** as a white solid (2.113 g, 76%). **Method B:** To a stirred solution of HHQ **186** (0.50 g, 2.05 mmol) in DCM (10 mL) and methanol (2.5 mL) was added *N*-bromosuccinimide (0.73 g, 4.10 mmol) portionwise and the resulting reaction mixture stirred at room temperature for 24 h with reaction progress monitored by TLC analysis. The solvent was removed *in vacuo* to yield the crude product as a yellow solid which was purified by recrystallisation from ethanol to yield **235** as a white solid (0.245 g, 37%). m.p. 245–248 °C. IR (KBr) ν_{max}: 3432 (NH stretch, w), 2926, 2855 (alkyl C-H stretch, s), 1631 (C=O stretch, m), 1582, 1559, 1550 (aromatic C=C stretch, s), 1475 (alkyl C-H bend, s), 1353 (C-N stretch, m) cm^{-1}; ^{1}H NMR (400 MHz, (CD$_3$)$_2$SO): δ 0.86 (3H, t, *J* = 6.9 Hz, C***H***$_3$CH$_2$), 1.23–1.41 (8H, m, 4 × C***H***$_2$), 1.66–1.74 (2H, m, C***H***$_2$CH$_2$C), 2.87 (2H, t, *J* = 7.9 Hz, C***H***$_2$C), 7.34–7.38 (1H, m, C***H*** arom.) 7.58 (1H, d, *J* = 8.2 Hz, C***H*** arom.), 7.66–7.70 (1H, m, C***H*** arom.)

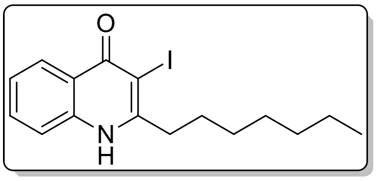
8.09 (1H, dd, *J* = 1.2, 8.2 Hz, C***H*** arom.), 12.01 (1H, bs, N***H***) ppm; ^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ 13.9 (***C***H$_3$), 22.0, 27.6, 28.3, 28.6, 31.1 34.5 (6 × ***C***H$_2$), 105.5 (***C***Br), 117.8 (***C***H arom.), 122.7 (CH***C***C=O), 123.6, 125.2, 131.9 (3 × ***C***H arom), 138.7 (CH***C***NH), 152.0 (CH$_2$***C***NH), 171.2 (***C*=**O) ppm; HRMS (ESI) *m/z* calcd for $C_{16}H_{21}BrNO$ [(M + H)$^+$]: 322.0807, found 322.0794; Anal. calcd for $C_{16}H_{20}BrNO$: C, 59.64; H, 6.26; N, 4.35%. Found: C, 59.55; H, 6.11; N, 4.28%.

3-Chloro-2-heptylquinolin-4(1*H*)-one, 236



To a stirred solution of HHQ·HCl **233** (0.839 g, 3.0 mmol) in methanol (50 mL), was added 2M aq. NaOH until neutral pH, followed by water (10 mL). Sodium dichloroisocyanurate (0.363 g, 1.65 mmol) was added and the reaction allowed to stir at room temperature overnight. The precipitate was filtered and washed with methanol. The filtrate was acidified to pH 4 and placed in the refrigerator overnight. The resulting precipitate was filtered to yield an off-white solid which was purified by recrystallisation from ethanol to yield **236** as a white solid (0.167 g, 46%). m.p. 269–272 °C. IR (KBr) ν_{max}: 3454 (NH stretch, w), 2927, 2857 (alkyl C-H stretch, s), 1634 (C=O stretch, m), 1562, 1504 (aromatic C=C stretch, s), 1477 (alkyl C-H bend, s), 1355 (C-N stretch, m) cm^{-1}; ^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ 0.85 (3H, t, *J* = 6.7 Hz, C***H₃***), 1.26–1.34 (8H, m, 4 × C***H₂***), 1.65–1.75 (2H, m, C***H₂***CH$_2$C), 2.84 (2H, t, *J* = 7.8 Hz, C***H₂***C), 7.32–7.37 (1H, m, C***H*** arom.), 7.58 (1H, d, *J* = 7.8 Hz, C***H*** arom.), 7.64–7.70 (1H, m, C***H*** arom.), 8.08–8.11 (1H, m, C***H*** arom.), 12.03 (1H, bs, N***H***) ppm; ^{13}C NMR (150 MHz, $(CD_3)_2SO$): δ 13.9 (***C***H$_3$), 22.0, 27.5, 28.4, 28.6, 31.1, 32.1 (6 × ***C***H$_2$), 113.3 (***C***Cl), 118.0 (***C***H arom.), 123.4 (CH***C***C=O), 123.5, 125.1, 131.8 (3 × ***C***H arom.) 138.6 (CH***C***N), 150.7 (CH$_2$***C***N), 170.9 (***C*=**O) ppm; HRMS (ESI) *m/z* calcd for $C_{16}H_{21}ClNO$ [(M + H)$^+$]: 278.1312, found 278.1317; Anal. calcd for $C_{16}H_{20}ClNO$: C, 69.18; H, 7.26; N, 5.04; Cl, 12.76%. Found: C, 68.67; H, 7.14; N, 5.09; Cl, 12.52%.

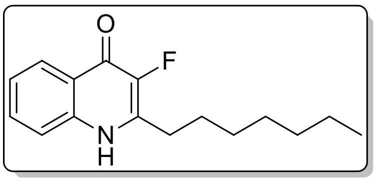
3-Iodo-2-heptylquinolin-4(1*H*)-one, 237



To a stirred solution of HHQ **186** (0.333 g, 1.37 mmol) in glacial acetic acid (10 mL) was added *N*-iodosuccinimide (0.315 g, 1.40 mmol) portionwise and the reaction mixture allowed to stir at room temperature for 2 h reaction. The precipitate was filtered and washed with acetic acid and acetonitrile. Purification was achieved using silica column chromatography eluting with 80:20 ethyl acetate:hexane to yield **237** as a white solid (0.22 g, 48%).

m.p. 221–225 °C. IR (KBr): ν_{max}: 3419 (N-H stretch, w), 2921 (alkyl C-H stretch, s), 1627 (C=O stretch, s), 1557 (aromatic C=C stretch, s), 1474 (alkyl C-H bend, s), 1351 (C-N stretch, m) cm^{-1}; ^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ 0.86 (3H, t, *J* = 6.9 Hz, C***H₃***CH₂), 1.27–1.36 (8H, m, 4 × C***H₂***), 1.63–1.70 (2H, m, CH₂C***H₂***C), 2.90 (2H, t, *J* = 7.9 Hz, C***H₂***C), 7.28–7.31 (1H, m, C***H*** arom.), 7.55–7.64 (2H, m, 2 × C***H*** arom.), 8.06 (1H, dd, *J* = 1.0, 8.2 Hz, C***H*** arom.), 12.08 (1H, bs, N**H**) ppm; ^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ 14.4 (***C***H₃), 22.5, 28.85, 28.94, 29.4, 31.7, 41.0 (6 × ***C***H₂), 86.7 (***C***I), 122.2, 122.6 (2 × ***C***H arom.), 123.0 (CH***C***C=O), 125.6, 130.4 (2 × ***C***H arom.), 144.0 (CH***C***N), 158.4 (CH₂***C***N), 172.4 (***C***=O) ppm; HRMS (ESI) *m/z* calcd for $C_{16}H_{21}INO$ [$(M + H)^+$]: 370.0668, found 370.0670.

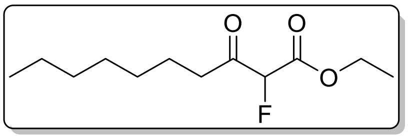
3-Fluoro-2-heptylquinolin-4(1*H*)-one, 244



Method A: To a stirred solution of Selectfluor® **242** (0.154 g, 0.436 mmol) in acetonitrile (4 mL) and ethanol (0.21 mL, 10 equiv.) was added HHQ **186** (0.088 g, 0.363 mmol). The reaction was allowed to stir at -10 °C for 3 days. The reaction mixture was filtered while cold and solvent removed *in vacuo* to yield the crude product as a bright yellow solid. Purification was achieved using silica column chromatography eluting with 90:10 ethyl acetate:hexane to yield **244** as a white solid (0.003 g, 3%). **Method B:** To a stirred solution of Selectfluor® **243** (0.509 g, 1.44 mmol) in ethanol (6 mL) was added HHQ **186** (0.206 g, 0.85 mmol). The reaction was allowed to stir at room temperature for 7 days. The reaction mixture was filtered and solvent removed *in vacuo* to yield the crude product as a bright yellow solid. Purification was achieved using silica column chromatography eluting with 90:10 ethyl acetate:hexane to yield **244** as a white solid (0.018 g, 8%).

m.p. 152–155 °C. IR (KBr) ν_{max}: 3369 (N-H stretch, m), 2957, 2928 (alkyl C-H stretch, s), 1694 (C=O stretch, s), 1615, 1508, 1483 (aromatic C=C stretch, s), 1108 (C-N stretch, s) cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.78 (3H t, *J* = 6.8 Hz, C***H₃***), 1.10–1.34 (8H, m, 4 × C***H₂***), 1.60–1.76 (2H, m, C***H₂***CH₂C), 2.89–2.93 (2H, m, C***H₂***C), 7.37 (1H, t, *J* = 7.5 Hz, C***H*** arom.), 7.62 (1H, t, *J* = 7.3 Hz, C***H*** arom.), 7.89 (1H, d, *J* = 7.2 Hz, C***H*** arom.), 8.44 (1H, d, *J* = 8.2 Hz, C***H*** arom.), 12.70 (1H, bs, N***H***) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ 14.0 (***C***H₃), 22.5, 28.4, 28.7, 28.9, 29.3, 31.6 (6 × ***C***H₂), 119.0, 123.4, 125.0 (d, $^4J_{C\text{-}F}$ = 4.5 Hz) (3 × ***C***H arom.), 125.8 (d, $^3J_{C\text{-}F}$ = 7.5 Hz, CH***C***C=O), 131.5 (***C***H arom.), 138.8 (CH***C***NH), 142.9 (d, $^2J_{C\text{-}F}$ = 27.2 Hz, CH₂***C***NH), 145.6 (d, $^1J_{C\text{-}F}$ = 229.5 Hz, ***C***F) 168.2 (d, $^2J_{C\text{-}F}$ = 13.6 Hz, ***C***=O) ppm; ^{19}F NMR (470 MHz, $CDCl_3$): δ -143.3 (C***F***) ppm; HRMS (ESI) *m/z* calcd for $C_{16}H_{21}FNO$ [(M + H)$^+$]: 262.1607, found 262.1601.

Ethyl 2-fluoro-3-oxodecanoate, 251



To a stirred solution of diisopropylamine (11.9 mL, 0.085 mol) in THF (210 mL) was added *n*-BuLi (60 mL, 0.085 mol) dropwise at -78 °C. The resulting reaction mixture was allowed to stir at 0 °C for 30 min to generate a solution of LDA. Ethyl-2-fluoroacetoacetate **250** (4.2 mL, 0.034 mol) was added dropwise at 0 °C and allowed to stir at this temperature for 1 h. Iodohexane (5.99 mL, 0.041 mol) was added dropwise at -78 °C. The resulting reaction mixture was allowed to warm to room temperature over 14 h with stirring. 10% aq. HCl (15 mL) was added to the reaction mixture and the layers separated. The aqueous layer was extracted with diethyl ether (3 × 25 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude product which was purified using silica column chromatography eluting with 90:10 hexane:ethyl acetate to yield **251** as a yellow oil (4.34 g, 55%).

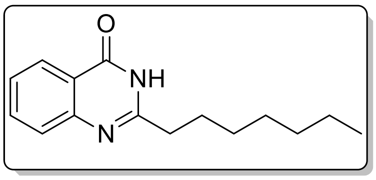
Spectral characteristics were consistent with previously reported data.[5]

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* = 6.8 Hz, C***H₃***CH₂), 1.26–1.34

(11H, m, 4 × C***H*₂** and OCH₂C***H*₃**), 1.58–1.65 (2H, m, C***H*₂**CH₂CO), 2.64–2.70 (2H, m, C***H*₂**CO), 4.31 (2H, q, *J* = 7.2 Hz, OC***H*₂**CH₃), 5.21 (1H, d, $^{1}J_{H\text{-}F}$ = 49.5 Hz, C***H***) ppm; ^{13}C NMR (75 MHz, (CDCl₃): δ 14.0 (2 × ***C***H₃), 22.5, 22.7 (d, $^{3}J_{C\text{-}F}$ = 1.5 Hz), 28.86, 28.89, 31.6, 38.4 (6 × ***C***H₂), 62.6 (O***C***H₂CH₃), 91.3 (d, $^{1}J_{C\text{-}F}$ = 197.8 Hz, ***C***H), 164.2 (d, $^{2}J_{C\text{-}F}$ = 23.9 Hz, ***C***=O ester), 201.3 (d, $^{2}J_{C\text{-}F}$ = 22.7 Hz, ***C***=O ketone) ppm; MS (ESI) *m*/*z*: 233 [(M + H)$^+$, 8%].

6.5 Synthesis of quinazolinone analogue

2-Heptylquinazolin-4(3*H*)-one, 259

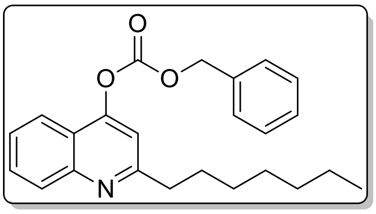


To a stirred solution of anthranilamide **258** (2.791 g, 20.5 mmol) in DMA (30 mL) was added *n*-octanal (3.2 mL, 20.5 mmol) and sodium bisulfite (3.2 g, 30.8 mmol). The resulting reaction mixture was allowed to stir at reflux for 2 h. The reaction mixture was poured into water (500 mL) and the precipitate filtered. The precipitate was recrystallised from ethanol to yield **259** as an off-white crystalline solid (3.82 g, 76%).

m.p. 124–127 °C. IR (KBr) ν_{max}: 2919, 2854 (alkyl C-H stretch, s), 1674 (amide C=O stretch, s), 1616 (C=N stretch, s), 1470 (alkyl C-H bend, s), 1341 (C-N stretch, m) cm^{-1}; ^{1}H NMR (300 MHz, CDCl₃): δ 0.88 (3H, t, *J* = 6.8 Hz, C***H*₃**), 1.25–1.51 (8H, m, 4 × C***H*₂**), 1.82–1.92 (2H, m, C***H*₂**CH₂C), 2.76 (2H, t, *J* = 7.7 Hz, C***H*₂**C), 7.43–7.49 (1H, m, C***H*** arom.) 7.70 (1H, d, *J* = 7.3 Hz, C***H*** arom.), 7.74–7.80 (1H, m, C***H*** arom.) 8.29 (1H, dd, *J* = 1.1, 8.0 Hz, C***H*** arom.), 11.74 (1H, bs, N***H***) ppm; ^{13}C NMR (75 MHz, CDCl₃): δ 14.0 (***C***H₃), 22.6, 27.6, 28.9, 29.2, 31.7, 36.0 (6 × ***C***H₂), 120.5 (CH***C***C=O), 126.2, 126.3, 127.2, 134.8 (4 × ***C***H arom.), 149.5 (CH***C***N), 156.9 (***C***=N), 164.2 (***C*=**O) ppm; HRMS (ESI) *m*/*z* calcd for $C_{15}H_{21}N_2O$ [(M + H)$^+$]: 245.1654, found 245.1654; Anal. calcd for $C_{15}H_{20}N_2O$: C, 73.74; H, 8.25; N, 11.47%. Found: C, 73.38; H, 8.20; N, 11.38%.

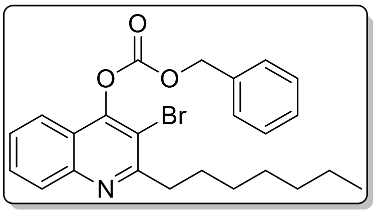
6.6 Synthesis of protected HHQ analogues

Benzyl (2-heptylquinolin-4-yl) carbonate, 265



To a stirred suspension of 60% NaH (0.605 g, 25.2 mmol) in dry THF (20 mL) was added a solution of HHQ **186** (1.315 g, 5.40 mmol) in dry THF (15 mL) and the resulting reaction mixture allowed to stir at 55 °C for 30 min. Benzyl chloroformate (1.16 mL, 8.11 mmol) was added dropwise, and the reaction mixture allowed to stir at room temperature for 21 h. The reaction mixture was quenched by addition of water (8 mL) and extracted with diethyl ether (3 × 15 mL). Organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product as a yellow oil which was purified using silica column chromatography eluting with 70:30 ethyl acetate:hexane to yield **265** as a pale yellow oil (0.385 g, 20%). IR (NaCl) ν_{max}: 2955, 2856 (alkyl C-H stretch, s), 1769 (C=O stretch, s), 1624 (C=N stretch, m), 1605, 1505 (aromatic C=C stretch, s), 1224 (ester C-O stretch, s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.87 (3H, t, *J* = 6.8 Hz, $C\boldsymbol{H}_3$), 1.26–1.44 (8H, m, 4 × $C\boldsymbol{H}_2$), 1.76–1.84 (2H, m, $C\boldsymbol{H}_2CH_2C$), 2.95 (2H, dd, *J* = 7.8, 8.0 Hz, $C\boldsymbol{H}_2C$), 5.30 (2H, s, $OC\boldsymbol{H}_2Ph$), 7.31 (1H, s, $C\boldsymbol{H}$), 7.33–7.45 (6H, m, 6 × $C\boldsymbol{H}$ arom.), 7.62–7.67 (1H, m, $C\boldsymbol{H}$ arom.), 7.94 (1H, dd, *J* = 0.8, 8.4 Hz, $C\boldsymbol{H}$ arom.), 8.06 (1H, d, *J* = 8.4 Hz, CH arom.) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1 ($\boldsymbol{C}H_3$), 22.7, 29.2, 29.5, 29.8, 31.8, 39.6 (6 × $\boldsymbol{C}H_2$), 70.9 ($O\boldsymbol{C}H_2Ph$), 111.8 ($\boldsymbol{C}H$), 120.4 ($CH\boldsymbol{C}CO$), 120.9, 126.1, 128.6, 128.8, 128.9, 129.0, 130.0 (9 × $\boldsymbol{C}H$ arom.), 134.5 ($OCH_2\boldsymbol{C}$), 149.6 ($CH\boldsymbol{C}N$), 152.4 ($\boldsymbol{C}=O$), 154.3 ($\boldsymbol{C}OCOOCH_2Ph$), 164.0 ($CH_2\boldsymbol{C}=N$) ppm; HRMS (ESI) *m/z* calcd for $C_{24}H_{28}NO_3$ [$(M + H)^+$]: 378.2069, found 378.2071; Anal. calcd for $C_{24}H_{27}NO_3$: C, 76.36; H, 7.21; N, 3.71%. Found: C, 76.37; H, 7.12; N, 3.47%.

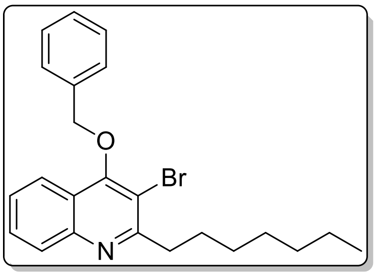
Benzyl (3-bromo-2-heptylquinolin-4-yl) carbonate, 266



To a stirred suspension of 60% NaH (0.097 g, 4.06 mmol) in dry THF (4 mL) was added a solution of 3-bromo-2-heptylquinolin-4(1*H*)-one **235** (0.280 g, 0.87 mmol) in dry THF (6 mL) and the resulting reaction mixture stirred at 55 °C for 30 min. Benzyl chloroformate (0.19 mL, 1.31 mmol) was added dropwise and reaction removed from oil bath and allowed to stir at room temperature for 21 h. The reaction mixture was quenched by addition of water (8 mL) and extracted with diethyl ether (3 × 15 mL). Organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product as a yellow oil which was purified using silica column chromatography eluting with 50:50 hexane:diethyl ether to yield **266** as a pale yellow oil (0.242 g, 61 %).

IR (NaCl) ν_{max}: 2927, 2856 (alkyl C-H stretch, s), 1772 (ester C=O stretch, s), 1589 (aromatic C=C stretch, m), 1224 (ester C-O stretch, s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.89 (3H, t, *J* = 6.7 Hz, $C\boldsymbol{H_3}$), 1.26–1.49 (8H, m, 4 × $C\boldsymbol{H_2}$), 1.78–1.88 (2H, m, $C\boldsymbol{H_2}CH_2C$), 3.16 (2H, dd, *J* = 6.1, 7.8 Hz, $C\boldsymbol{H_2}C$), 5.36 (2H, s, $OC\boldsymbol{H_2}Ph$), 7.36–7.53 (6H, m, 6 × C***H*** arom.), 7.68–7.75 (1H, m, C***H*** arom.), 7.80 (1H, dq *J* = 0.6, 8.3 Hz, C***H*** arom.), 8.05 (1H, d, *J* = 8.4 Hz, C***H*** arom.) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1 ($\boldsymbol{C}H_3$), 22.7, 28.5, 29.1, 29.6, 31.8, 38.7 (6 × $\boldsymbol{C}H_2$), 71.4 ($O\boldsymbol{C}H_2$), 112.2 (***C***Br), 120.8 (***C***H arom.), 122.0 (CH***C***CO), 127.1, 128.5, 128.8, 128.99, 129.04, 130.3 (8 × ***C***H arom.), 134.4 ($OCH_2\boldsymbol{C}$), 147.7 (CH***C***N), 151.4 (***C***=O), 151.5 ($\boldsymbol{C}OCOOCH_2Ph$), 162.0 ($CH_2\boldsymbol{C}N$) ppm; HRMS (ESI) *m/z* calcd for $C_{24}H_{27}BrNO_3$ [$(M + H)^+$]: 456.1174, found 456.1176; Anal. calcd for $C_{24}H_{26}BrNO_3$: C, 63.16; H, 5.74; N, 3.07%. Found: C, 63.46; H, 5.86; N, 2.96%.

4-(Benzyloxy)-3-bromo-2-heptylquinoline, 267



To a methanol solution of KOH (0.030 g, 0.53 mmol) and 3-bromo-2-heptylquinolin-4(1*H*)-one **235** (0.114 g, 0.35 mmol) in methanol (c = 1.0 M) was added benzyl bromide (0.42 mL, 3.54 mmol) in one aliquot and the resulting reaction mixture allowed to stir at room temperature overnight. The precipitate was removed by filtration, the solvent evaporated and the resulting residue purified by silica column chromatography eluting with 50:50 hexane:diethyl ether to yield **267** as a white solid (0.040 g, 28 %).

m.p. 62–64 °C. IR (KBr) ν_{max}: 2927, 2855 (alkyl C-H stretch, s), 1577 (C=N stretch, s), 1356 (ester C-O stretch, s) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 0.89 (3H, t, *J* = 6.7 Hz, C***H***$_3$), 1.26–1.53 (8H, m, 4 × C***H***$_2$), 1.79–1.89 (2H, m, C***H***$_2$CH$_2$C), 3.17 (2H, dd, *J* = 7.9. 8.1 Hz, CH$_2$C***H***$_2$C), 5.19 (2H, s, OC***H***$_2$), 7.36–7.48 (4H, m, 4 × C***H*** arom.), 7.56–7.59 (2H, m, 2 × C***H*** arom.), 7.64–7.70 (1H, m, C***H*** arom.), 7.99 (1H, dd, *J* = 1.0, 8.6 Hz, C***H*** arom.), 8.03 (1H, d, *J* = 8.5 Hz, C***H*** arom.) ppm; ^{13}C NMR (75 MHz, CDCl$_3$): δ 14.1 (***C***H$_3$), 22.7, 28.8, 29.2, 29.6, 31.8, 39.0 (6 × ***C***H$_2$), 76.0 (O***C***H$_2$), 112.1(***C***Br), 121.9 (***C***H arom.), 123.6 (CH***C***CO), 126.3, 128.3, 128.6, 128.7, 129.0, 129.9 (8 × ***C***H arom.), 136.2 (COCH$_2$***C***), 148.1 (CH***C***N=C), 159.5 (***C***OCH$_2$Ph), 162.6 (CH$_2$***C***=N) ppm; HRMS (ESI) *m/z* calcd for C$_{23}$H$_{27}$NOBr [(M + H)$^+$]: 412.1276, found 412.1270.

6.7 References

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Appendix II

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PAPER

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Structure–function analysis of the C-3 position in analogues of microbial behavioural modulators HHQ and PQS†

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2-Heptyl-3-hydroxy-4-quinolone (PQS) and its precursor 2-heptyl-4-quinolone (HHQ) are key signalling molecules of the important nosocomial pathogen *Pseudomonas aeruginosa*. We have recently reported an interkingdom dimension to these molecules, influencing key virulence traits in a broad spectrum of microbial species and in the human pathogenic yeast *Candida albicans*. For the first time, targeted chemical derivatisation of the C-3 position was undertaken to investigate the structural and molecular properties underpinning the biological activity of these compounds in *P. aeruginosa*, and using *Bacillus subtilis* as a suitable model system for investigating modulation of interspecies behaviour.

Microbial populations coordinate cellular behaviour through the mobilisation of diffusible signal molecules, which activate gene expression upon accumulation above a threshold or quorum.[1] This phenomenon (quorum sensing), is an essential communication system utilised by a broad spectrum of Gram-negative and Gram-positive bacteria, and is a central control mechanism for virulence and pathogenesis.[2] 2-Heptyl-3-hydroxy-4-quinolone, the Pseudomonas Quinolone Signal (PQS) is a key regulator of quorum sensing in *Pseudomonas aeruginosa*.[1,3,4] *P. aeruginosa* is best known as an antibiotic resistant human pathogen associated with hospital-acquired infections and is the primary cause of morbidity and mortality in people with cystic fibrosis (CF).[5] Controlling *P. aeruginosa* infection is thus of great clinical importance.[6–9] Research into PQS activity has revealed a vast and varied array of biological functions.[10–16] In addition to controlling expression of key components of the QS regulon, PQS also modulates biofilm formation, secondary metabolite production, pigment and virulence factor production, motility and membrane vesicle formation.[11–13,15] 2-Heptyl-4-quinolone (HHQ), the biological precursor of PQS, also possesses a plethora of roles including quorum sensing responsibilities.[13] PQS has been detected at 2 μM in CF samples from sputum, bronchoalveolar lavage fluid and mucopurulent fluid from distal airways of end-stage lungs removed at transplant.[17] Isolates obtained from infant CF-patients under 3 years of age overproduce PQS, suggesting that it may be instrumental in adaptation of *P. aeruginosa* to the airways of young CF-patients.[18] Transcriptomic and functional genomics studies have provided further evidence for the importance of PQS and its precursor HHQ during adaptation to the CF-lung[19,20] while Kim *et al.*[21,22] reported immunomodulation and inhibition of macrophage activation by HHQ and PQS. Diggle *et al.* reported that pathogenic bacteria other than *P. aeruginosa* synthesise 2-alkyl-4-quinolones (AQs). *Burkholderia pseudomallei*, for example, produces AQs and employs a structurally similar molecule to HHQ but does not produce PQS.[13,23] Intriguingly, it has recently been shown that both PQS and HHQ can also control the behaviour of other bacterial and fungal species.[24,25] We found that surface-associated phenotypes were repressed in a number of Gram-positive and Gram-negative bacteria as well as in pathogenic yeast in response to PQS and HHQ.[24] Motility was repressed in a broad range of bacteria, while biofilm formation in *Bacillus subtilis* and *Candida albicans* was repressed in the presence of HHQ, though initial adhesion was unaffected. Furthermore, HHQ exhibited potent bacteriostatic activity against several marine species of Gram-negative bacteria, including pathogenic *Vibrio vulnificus*.

To take advantage of signalling pathways in a clinical setting we take two routes: (1) the early detection of biomarkers such as HHQ and PQS[26–28] and (2) interference with bacterial signals by the synthesis of molecular analogues capable of interrupting key virulence traits such as biofilm formation and motility. To date, the limited structure–function analysis performed on HHQ and PQS has centred on the alkyl chain length[7,29] and substitution of the anthranilate ring.[7] The crucial C-3 position has not been investigated, notwithstanding the divergent biological activities

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† Electronic supplementary information (ESI) available: Full experimental procedures, biological data, ^{1}H spectra of novel compounds and additional crystallographical data are included. CCDC 885400. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2ob26823j

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identified for HHQ and PQS towards other bacterial and fungal pathogens. Therefore, the structure–function analysis detailed in this study was designed to provide key insights into the activity of the HHQ and PQS compounds both within *P. aeruginosa* and also towards non-pseudomonal bacterial and fungal species. These included several key cystic fibrosis pathogens such as *Staphylococcus aureus* and *C. albicans*, for which *B. subtilis* proved to be a suitable model for single species analysis of swarming motility and biofilm formation. Assigning the structural modules of the quinolone compounds to biological functions would provide significant insight into their underlying mechanism of action. This would form the basis for development of innovative therapies, for example where disruption of biofilm formation would expose microbial pathogens to normal antibacterial action. In this report we present our findings on the biological activity of eight molecules of interest, 2-heptyl-4-quinolone (**3**, **HHQ**), aldehyde **4**, 2-heptyl-3-hydroxyquinolin-4(1*H*)-one (**5**, **PQS**), 3-methyl analogue **8**, halogenated versions **9**, **10** and **11** and finally **12** which possesses a potential hydrogen donor/acceptor at the 3-position.

Chemical synthesis

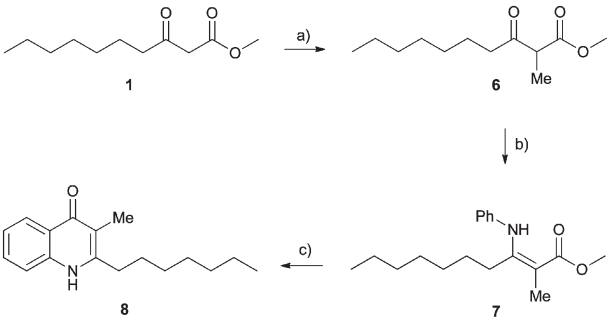
HHQ was prepared starting with Meldrum's acid (0.14 mol) which was reacted with octanoyl chloride followed by boiling in methanol (MeOH), reaction with aniline[30] and a Conrad–Umpach cyclisation (Scheme 1).†[31] An alternative cyclisation method reported by Woschek *et al.* failed to give any product in our hands.[32] As quantities of **4** and PQS were also required, their synthesis from HHQ was achieved using conditions described by *Pesci et al.*[33] although an excellent method for the direct synthesis of PQS has recently been reported.[34] The Duff formylation of HHQ proved problematic and 2 equivalents of hexamine (HMTA) was found to be crucial to obtaining decent yields.[35] Reaction of aldehyde **4** with MCPBA gave PQS in 29% yield.

The 3-methyl analogue **8** was synthesised in a similar fashion over five steps (Scheme 2).† Again Meldrum's acid was reacted with octanoyl chloride followed by β-ketoester formation. Methylation was carried out using K_2CO_3 and MeI giving **6**. Reaction with aniline gave enamine **7** and a final Conrad–Limpach cyclisation in diphenyl ether afforded analogue **8**.[31,35]

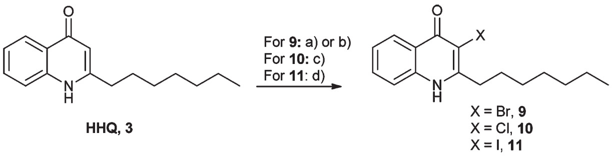
Bromo-analogue **9** was prepared using *N*-bromosuccinimide in 37% yield (after recrystallisation) or using Br_2 in 47% yield (Scheme 3). The 3-chloro-quinolone **10** was synthesised in one step using sodium dichloroisocyanurate (DCIC).[36] Recrystallisation afforded **10** in 46% yield. Iodo-analogue **11** was formed in the presence of *N*-iodosuccinimide in 48% yield (halogenation yields not optimised).

Novel 2-heptylquinazolin-4-one **12** was synthesised *via* a convenient one-step synthesis by reaction of anthranilamide with octanal, and following recrystallisation from ethanol, afforded the product in 76% yield (Scheme 4).

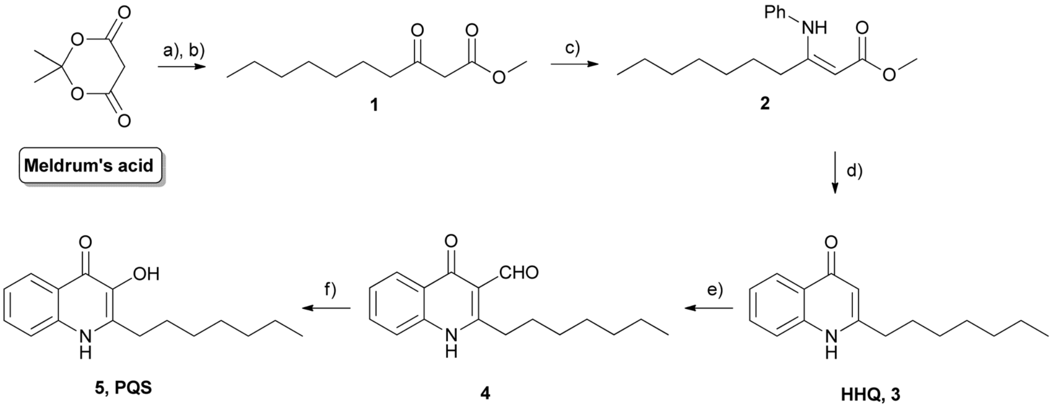
Given that both the quinolone and quinoline tautomeric structures (both structures have been arbitrarily depicted in the literature[37]) of HHQ were accessible, the latter as its hydrochloride salt (Fig. 1), we felt it would be valuable to confirm that both



Scheme 2 Synthesis of 3-Me analogue **8**. Conditions: (a) K_2CO_3, MeI, reflux, 40%. (b) $PhNH_2$, reflux, 79% (c) Ph_2O, reflux, 10%.



Scheme 3 Halogenation of HHQ. Conditions: (a) NBS, MeOH, 37% (b) Br_2, AcOH, 47% (c) DCIC, H_2O, 46% (d) NIS, AcOH, 48%.



Scheme 1 Synthesis of HHQ and PQS. Conditions: (a) octanoyl chloride, pyridine, DCM (b) MeOH, reflux, 34% over 2 steps (c) $PhNH_2$, reflux, 79% (d) Ph_2O, reflux, 33% (e) HMTA, TFA, reflux, 56% (f) H_2O_2, NaOH, 29%.

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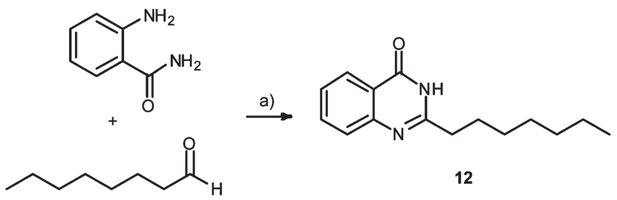
the free quinolone and quinoline hydrochloride exhibited identical biological activities. Both compounds **3** (HHQ) and **3·HCl** (HHQ·HCl) were found to be interchangeable when used in biological systems and exerted a common influence on inter-kingdom behaviour. Both motility and biofilm formation were similarly influenced in *B. subtilis* in the presence of **3** and **3·HCl** when tested under physiological pH (Fig. 3 and 4).

Biological studies

In order to assess the importance of the C-3 position to the biological activity of HHQ and PQS, the capacity for analogues functionalised at this position to replace the native compounds in *P. aeruginosa* was investigated. The PQS signalling system, a key component of QS in *P. aeruginosa*, is known to control production of a range of virulence factors, including elastase, rhamnolipid and the phenazine redox compound pyocyanin.[11,38] Therefore, the analogues were first assessed for restoration of phenazine production in a *pqsA* mutant, in which the biosynthetic steps required for AQ production have been disrupted. While both HHQ and PQS restored phenazine production in the *pqsA* mutant strain, the analogues were significantly less effective in triggering production of the pigment, with **12** being the least effective (Fig. 2A), suggesting that the C-3 position is crucial for control of phenazine production in *P. aeruginosa*. Interestingly, addition of equimolar concentrations of the analogues to the wild-type PAO1 and PA14 strains, which produce both HHQ and PQS, did not interfere with phenazine production (Fig. 2B). In *P. aeruginosa*, PQS also plays a fundamental role in the structural formation of biofilms and PQS-deficient mutants have been shown to produce thin flat biofilms, which are markedly different to the mushroom shaped structures produced by the wild-type strain.[39] However, neither mutation of *pqsA* nor addition of analogues markedly influenced the initial stages of biofilm formation in *P. aeruginosa* as seen in crystal violet multi-well assays (ESI†).



Scheme 4 Synthesis of PQS analogue. Conditions: (a) $NaSO_3H$, DMA, reflux, 76%.

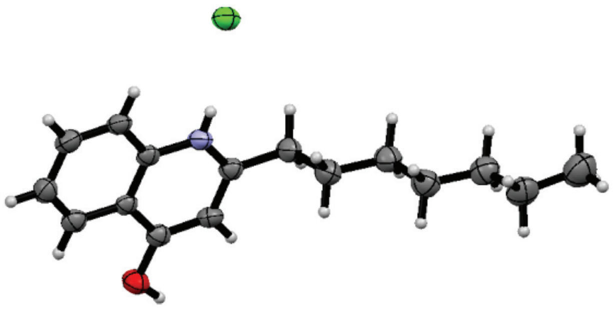


Fig. 1 Crystal structure of **3·HCl** (HHQ·HCl).†

Aside from their key role as signalling compounds in *P. aeruginosa*, both HHQ and PQS exert distinct influences on the behaviour of a range of microbial pathogens.[24] Differing only at the 3-position, yet displaying diverse biological functionalities suggests a key role for the C-3 position in modulating inter-species microbial behaviour. Microbial swarming motility and biofilm formation require cooperative multicellular behaviour and provide a mechanism for bacterial cells to establish and persist during infection. While motility was shown to be altered in *S. aureus* in the presence of HHQ and PQS, *C. albicans* biofilm formation was repressed in the presence of HHQ. As we have previously shown both phenotypes to be affected in *B. subtilis* in the presence of HHQ, this species was chosen as a model organism upon which to test the interspecies influence of the alkylquinolone compounds.

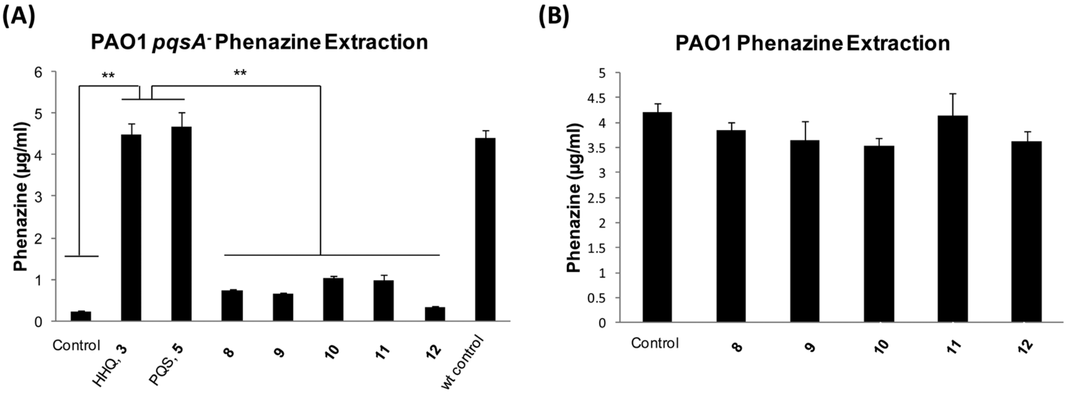


Fig. 2 Influence of functionalised derivatives on PQS-dependent phenotypes in *P. aeruginosa*. (**A**) The ability of HHQ and PQS (10 µM) to restore phenazine production in a *pqsA* mutant was lost to the derivative compounds indicating that the C-3 position is crucial in this regard. (**B**) Addition of 10 µM concentrations of derivative compounds did not interfere with phenazine production in the wild-type PAO1 strain. Data presented is representative of three independent experiments (Students *t*-test, ***p*-value ≤ 0.005).

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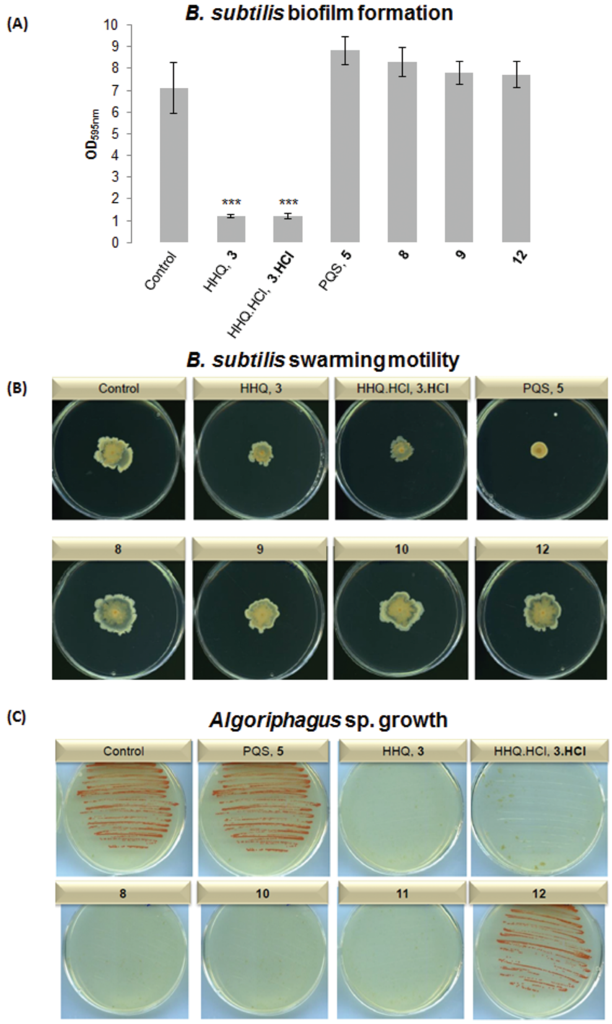

Fig. 3 Structural variation of the C-3 position interferes with the biological activity of *P. aeruginosa* AQ molecules. (**A**) Crystal violet staining of 18 h cultures grown static in multi-well plates revealed that HHQ interferes with *B. subtilis* biofilm formation irrespective of its tautomeric form (HHQone and HHQine). Furthermore, the anti-biofilm activity of the HHQ chloride salt was comparable to HHQ. However, this anti-biofilm activity was abolished upon substitution at the C-3 position, irrespective of the nature of that substitution (**8–12**). All compounds were added at a final concentration of 10 µM and statistical significance was provided by paired Student's *t*-test (***, p-value ≤ 0.001). (**B**) *B. subtilis* swarming motility was assessed after 16 h on 0.3% (w/v) TSA plates. Notably, substitution with methyl and halogen groups (**8–11**) which would be sterically consistent with PQS, was enough to abolish the anti-swarming activity of the parent compounds, again highlighting the structural specificity underpinning the biological activity of these compounds. All images are provided to scale. (**C**) The ability of HHQ to repress the growth of a marine isolate on SYP agar was maintained in both tautomeric forms, and upon substitution with methyl and halogen groups (**8–11**). However, **12** did not exhibit antibacterial activity towards the marine bacteria, similar to PQS. Data presented is representative of at least three independent biological replicates.

Unlike **3** and **3·HCl**, analogues **6–12** did not exhibit anti-biofilm activity towards *B. subtilis* (Fig. 3A), highlighting the importance of the C-3 position in underpinning the biological role of these compounds. Furthermore, the inability of **10** to affect biofilm formation in *C. albicans*, a human pathogenic yeast and an important CF pathogen (data not shown), underlines the importance of the C-3 position for biological functionality of the AQ compounds. The influence of **6–12** on microbial swarming motility was negligible compared to HHQ and PQS (Fig. 3B). Interestingly, the methyl and halogen substituted compounds **8–11** retained antimicrobial activity towards an *Algoriphagus* marine isolate, which was previously shown to be susceptible to HHQ, while PQS and quinazolinone **12** did not suppress growth of this species (Fig. 3C).

HHQ and PQS have previously been shown to influence transcription in a mouse monocyte/macrophage cell line.[40] However, although PQS has been found in CF sputum,[17] the impact and potential cytotoxic effects of these compounds on airway epithelial cells has not been investigated. Therefore, HHQ, PQS and compounds **8–12** were tested for cytotoxicity towards a human airway epithelial cell line (IB3-1 cells) for 16 h at concentrations ranging from 10 to 100 µM by quantification of the lactate dehydrogenase (LDH) release, in comparison with treatment by 0.1% Triton X-100, used as a positive control for cytotoxicity. Interestingly, HHQ was found to be cytotoxic towards IB3-1 cells while PQS did not exhibit any cytotoxic activity (Fig. 4). The cytotoxicity of HHQ decreased with decreasing concentrations and was less than 10% at 10 µM (data not shown). As above, the cytotoxicity of both the quinolone and quinoline compounds towards IB3-1 cells was comparable (~60%). With the exception of quinazolinone **12** which exhibited a significant level of cytotoxicity, the C-3 substituted analogues (**8–11**) did not exhibit cytotoxicity towards IB3-1 cells, reinforcing the importance of the C-3 position in the functionality of the HHQ and PQS molecules. Notably, IB3-1 cellular morphological analysis revealed massive cellular damage caused by HHQ at a concentration of 100 µM (Fig. 4) and significant cellular change for **12**. While PQS (Fig. 4) and to a lesser extent **8–11** (data not shown) caused moderate changes in cellular morphology, the plasma membrane remained intact, consistent with the lack of LDH release, compared to 0.1% Triton X-100.

Conclusions

The *P. aeruginosa* AQ signalling molecules are emerging as key components of the highly dynamic and bidirectional molecular dialogue that exists between pathogen and host and within the mixed microbial populations that are characteristic of infection. This is the first report highlighting the strict structural requirements at the C-3 position underpinning the biological activity of HHQ and PQS. The control of phenazine production in *P. aeruginosa* involves a complex interplay between PqsR, AQs and the last component of the PQS biosynthetic operon, PqsE.[38,41,42] The inability of any of the AQ analogues described in this study to restore phenazine production in a *P. aeruginosa pqsA* mutant suggests that the C-3 position is crucial for HHQ and PQS activity in this important nosocomial pathogen. Interestingly, addition of the analogues to wild-type cultures did not interfere with phenazine production, suggesting that they may not be effective inhibitors in *P. aeruginosa*. Multi-well biofilm assays suggest that the analogues do not interfere with the initial stages

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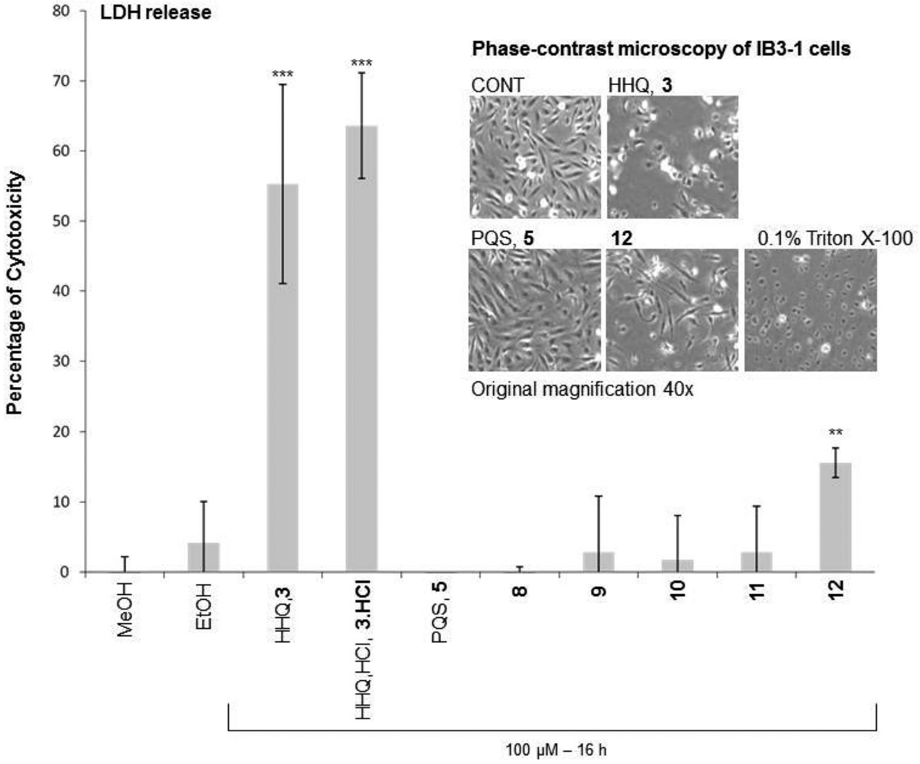

Fig. 4 C-3 substitution abrogates cytotoxic activity of HHQ. Cytotoxicity is expressed as a percentage of the total amount of LDH released from cells treated with 0.1% Triton X-100 (given the percentage of 100). The release of LDH was measured in cell culture medium of IB3-1 cells treated with methanol or ethanol, or with 100 µM of HHQ, the HHQ chloride salt, PQS, methylated HHQ analogue **8**, halogenated HHQ analogues **9–11** or analogue **12**. Data (means ± SD) are representative of three independent biological experiments. Two-tailed unpaired student's *t*-test was performed by comparison of IB3-1 cells treated with HHQ analogue molecules with IB3-1 cells treated with methanol or ethanol (**, *p*-value ≤ 0.01; ***, *p*-value ≤ 0.001). Phase-contrast microscopy of IB3-1 cells untreated (CONT) or treated with HHQ, PQS, or **12** at a concentration of 100 µM for 16 h. Triton X-100 (0.1%) was used as a control in these studies. Original magnification 40×.

of biofilm formation in *P. aeruginosa*, which is perhaps unsurprising as attachment and microcolony formation in *pqsA* mutants have previously been shown to be largely comparable to wild-type.[43,44] Flow-cell technology and confocal laser microscopy will be required to assess the full impact of these analogues on *P. aeruginosa* biofilm architecture and maturation. Notwithstanding this, the correlation between loss of function both within *P. aeruginosa* and towards other microbial species upon functionalisation of the C-3 position is striking. While the molecular mechanism underpinning the response to HHQ and PQS in other microbial species remains to be defined, it is interesting to speculate that some structural conservation may exist.

If a simple electron withdrawing group was required at C-3 then halogenation at this point (**9–11**) would be expected to produce molecules with similar biological activity. Alternatively introduction of an NH-group as in **12** (another tautomer can exist here also) could mimic the –OH group in PQS. However in both cases the capacity to modulate either *P. aeruginosa* or interkingdom behaviour was lost in these compounds. Therefore it is clear that the 3-H and 3-OH groups in HHQ and PQS respectively, play a more complex role in these biological systems. Methylation of the C-3 position (**8**) led to the generation of a known signal molecule produced by several *Burkholderia* species, which do not have the capacity to produce PQS. Interestingly, **8** appeared to have lost its ability to restore phenazine production or influence interspecies multicellular behaviour, although it did retain antibacterial activity against *Algoriphagus sp*. Therefore, although both compounds are structurally similar, and produced by important pathogens of the CF-lung, their interspecies activity profiles are distinct. Again introduction of a nitrogen at C-3 (**12**) led to complete loss of biological activity relative to HHQ, while we have previously shown that an aldehyde substituted analogue (**4**) had intermediate activity relative to both compounds.[24] Although from a chemical perspective, bacterial conversion of HHQ to PQS would go some way to explaining this observation, the evolutionary rationale underpinning this has yet to be established. Future structural studies will involve further manipulation of the 3-site with a view towards attaining a deeper understanding of the complex roles of these molecules in bacterial and fungal species.

Experimental (see ESI† for full details)

Preparation of 6

To 3-oxo-methyl-decanoate[45] (2.74 g, 13.7 mmol) was added dry acetone (35 mL). This solution was added to a flask containing dry potassium carbonate (1.76 g, 12.7 mmol) over a N_2

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atmosphere. The reaction mixture was allowed stir for 20 min before the addition of methyliodide (1.02 mL, 16.4 mmol). Stirring was continued at room temperature overnight before being heated at reflux for 6 h. The mixture was allowed to cool and the solvent was removed *in vacuo* to yield the crude product as a yellow oil. Purification was carried out using silica column chromatography to yield **6** as a pale yellow oil (1.17 g, 40%). (Found: C, 67.15; H, 10.2. $C_{12}H_{22}O_3$ requires C, 67.3; H, 10.35%.) ν_{max} (film)/cm^{-1} 2930 (CH stretch), 2857 (CH stretch), 1749 (C═O ketone), 1717 (C═O ester), 1456 (CH scissor, bending), 1204 (C–O ester). δ_H ($CDCl_3$, 300 MHz) 0.88 (3H, t, J = 6.7 Hz, CH_3), 1.23–1.27 (8H, m, 4 × CH_2), 1.33 (3H, d, J = 7.2 Hz, CO–CH(CH_3)–CO), 1.53–1.66 (2H, m, CH_2CH_2CO), 2.52 (2H, qt, J = 7.4, 17.2, 27.4, 44.6 Hz, CH_2CO), 3.53 (1H, q, J = 7.1 Hz, CO–CH(CH_3)–CO), 3.73 (3H, s, OCH_3). δ_C ($CDCl_3$, 75.5 MHz) 12.8, 14.0 (2 × CH_3), 22.6, 23.5, 28.98, 29.00, 31.6, 41.4 (6 × CH_2), 52.3 (CO–CH–CO), 52.7 (OCH_3), 171.1 (C═O ester), 205.9 (C═O ketone). Exact mass calculated for $C_{12}H_{23}O_3$ [(M + H)$^+$], 215.1647. Found 215.1642, *m*/*z* (ES$^+$) 215 [(M + H)$^+$, 30%].

Preparation of 7

To a solution of 2-methyl-3-oxo-methyl-decanoate (1.27 g, 5.94 mmol) in dry hexane (30 mL) was added aniline (0.57 mL, 6.24 mmol) and *p*-toluene sulfonic acid (0.023 g, 0.12 mmol). The reaction mixture was heated at reflux under a N_2 atmosphere for 16 h. The reaction was allowed to cool and the solvent was removed *in vacuo* yielding **7** as an orange oil (1.35 g, 79%). (Found: C, 74.3; H, 9.2; N, 5.2. $C_{18}H_{27}NO_2$ requires C, 74.7; H, 9.4; N, 4.8%.) ν_{max} (film)/cm^{-1} 3216 (NH stretch), 2952 (CH stretch), 2928 (CH stretch), 2856 (CH stretch), 1744 (C═O), 1657 (C═C), 1612, 1594 (NH bend), 1252 (C–O), 1229 (C–O), 1164 (C–O). δ_H ($CDCl_3$, 400 MHz) 0.84 (3H, t, J = 7.0 Hz, CH_3), 1.17–1.29 (8H, m, 4 × CH_2), 1.37–1.46 (2H, m, CH_2), 1.59 (1H, s, CH_3), 1.86 (1H, s, CH_3), 2.26–2.30 (1H, m, CH_2), 2.34–2.38 (1H, m, CH_2), 3.52–3.75 (4H, m, CH_3), 7.03–7.10 (2H, m, 2 × ArH), 7.13–7.19 (1H, m, ArH), 7.26–7.35 (2H, m, 2 × ArH), 10.81 (1H, bs, OH). δ_C ($CDCl_3$, 75.5 MHz) 12.5, 14.0 (2 × CH_3), 22.6, 27.7, 28.7, 28.8, 29.4, 31.6 (6 × CH_2), 50.7 (CH_3), 124.8 (quaternary C), 125.1, 125.6, 128.9, 129.1, 129.3 (5 × ArC), 160.8 (ArC–N), 163.8 (C–N), 171.7 (C═O). Exact mass calculated for $C_{18}H_{28}NO_2$ [(M + H)$^+$], 290.2120. Found 290.2116, *m*/*z* (ES$^+$) 290 [(M + H)$^+$, 56%].

Preparation of 8

Diphenyl ether (45 mL) was heated at reflux (270 °C) and the enamine (1.35 g, 4.68 mmol) was added dropwise over 90 min ensuring reflux was maintained and the mixture was heated for an additional 1 h. The mixture was then allowed cool to room temperature and the formed methanol was removed *in vacuo*. 4 M HCl (6 mL) was then added and the organic layer extracted with ethyl acetate (2 × 8 mL) dried over anhydrous $MgSO_4$, filtered and the solvent removed *in vacuo* to yield crude product as a brown oil. Purification was achieved using silica column chromatography to yield product as a dark brown solid followed by two recrystallisations from methanol to yield **8** as a white crystalline solid (10.6 mg, 10%). δ_H (CD_3OD, 400 MHz) 0.88 (3H, t, J = 6.8 Hz, CH_3), 1.30–1.46 (8H, m, 4 × CH_2), 1.68–1.75 (2H, m, CH_2), 2.15 (3H, s, CH_3), 2.81 (2H, t, J = 7.9 Hz, CH_2), 7.33 (1H, t, J = 8.1 Hz, ArH), 7.53–7.55 (1H, m, ArH), 7.60–7.64 (1H, m, ArH), 8.22 (1H, d, J = 7.5 Hz, ArH). δ_C (CD_3OD, 75.5 MHz) 10.8, 14.4 (2 × CH_3), 23.7, 30.0, 30.2, 30.5, 32.9, 33.5 (6 × CH_2), 116.2 (quaternary C), 118.7 (ArC), 124.4 (quaternary C), 124.5, 126.2, 132.7 (3 × ArC), 140.6, 153.4 (2 × quaternary C), 179.5 (C═O), *m*/*z* (ES$^+$) 258 [(M + H)$^+$, 100%].

Preparation of 3-bromo-2-heptylquinolin-4(1*H*)-one, 9

Method A. To a stirred solution of **3** (0.5 g, 2.05 mmol) in dichloromethane (10 mL) and methanol (2.5 mL) was added portionwise *N*-bromosuccinimide (0.73 g, 4.1 mmol) and the reaction was stirred at room temperature for 24 h. The solvent was removed *in vacuo* and the crude product was purified by recrystallisation in ethanol yielding **9** as a white solid (0.245 g, 37%).

Method B. To a stirred solution of **3** (0.389 g, 1.6 mmol) in acetic acid (4 mL) was added dropwise over 30 min, a solution of bromine (0.1 mL, 1.8 mmol) in acetic acid (1 mL). Reaction progress was monitored by TLC analysis. After 1 h, the reaction mixture was poured into 1% aqueous sodium sulfite (100 mL). The precipitate was filtered and washed with water yielding the product **9** as a white solid (0.245 g, 47%). Mp 245–248 °C (EtOH). ν_{max} (KBr)/cm^{-1} 3432 (OH stretch), 2926 (CH stretch), 2855 (CH stretch), 1631 (C═N), 1607 (aromatic), 1559 (C═N conjugated), 1475 (C═C stretch aromatic), 572 (C–Br). δ_H ($[CD_3]_2SO$, 300 MHz) 0.85 (3H, s, CH_3), 1.26–1.34 (8H, m, 4 × CH_2), 1.70 (2H, m, CH_2), 2.84–2.89 (2H, m, CH_2), 7.33–7.38 (1H, m, ArH), 7.57–7.70 (2H, m, 2 × ArH), 8.09 (1H, d, J = 7.9 Hz, ArH), 12.03 (1H, bs, OH). δ_C ($[CD_3]_2SO$, 75 MHz) 13.9 (CH_3), 22.0, 27.6, 28.3, 28.6, 31.1, 34.5 (6 × CH_2), 105.5 (C–Br), 117.8 (ArC), 122.7 (quaternary C), 123.6, 125.2 131.9 (3 × ArC), 138.7 (quaternary C), 152.0 (C═N), 171.24 (C–OH). Exact mass calculated for $C_{16}H_{21}NOBr$ [(M + H)$^+$], 322.0807. Found 322.0792, *m*/*z* ES$^+$ 322.3 [(M + H)$^+$, 100%].

Preparation of 3-chloro-2-heptylquinolin-4(1*H*)-one, 10

HHQ·HCl (**3·HCl**)† (0.839 g, 3.0 mmol) was dissolved in methanol (50 mL) before addition of 2 M NaOH until neutral followed by water (10 mL). Sodium dichloroisocyanurate (0.363 g, 1.65 mmol) was then added to the reaction mixture. The reaction was allowed stir at room temperature overnight. The precipitate was filtered and washed with methanol. The filtrate was then acidified to pH 4 and placed in the fridge overnight. The precipitate was filtered to give an off-white solid. Purification by recrystallisation in ethanol yielded **10** as a white crystalline solid (0.167 g, 46%). Mp 269–272 °C (EtOH). (Found: C, 68.7; H, 7.1; N, 5.1; Cl, 12.5. $C_{16}H_{20}ONCl$ requires C, 69.2; H, 7.3; N, 5.0; Cl, 12.8%.) ν_{max} (KBr)/cm^{-1} 3454 (OH stretch), 2927 (CH stretch), 2857 (CH stretch), 1634 (C═C stretch, conjugated), 1563 (C═N conjugated), 1504 (C–C stretch, in ring, aromatic), 1477 (C═C stretch, aromatic), 1356 (CN stretch), 584 (C–Cl). δ_H ($[CD_3]_2SO$, 300 MHz) 0.88 (3H, t, J = 6.7 Hz,

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CH_3), 1.26–1.34 (8H, m, 4 × CH_2), 1.65–1.75 (2H, m, CH_2), 2.84 (2H, t, J = 7.8 Hz, CH_2), 7.32–7.37 (1H, m, ArH), 7.57–7.70 (2H, m, 2 × ArH), 8.1 (1H, d, J = 8.1 Hz, ArH), 12.03 (1H, bs, OH). δ_C ($[CD_3]_2SO$, 150 MHz) 13.9 (CH_3), 22.0, 27.5, 28.4, 28.6, 31.1, 32.1 (6 × CH_2), 113.3 (C–Cl), 118.0 (Ar–CH), 123.4 (quaternary C), 123.5, 125.1, 131.8 (3 × Ar–CH), 138.6 (quaternary C), 150.7 (ArC), 170.9 (C═O). Exact mass calculated for $C_{16}H_{21}NOCl$ [(M + H)$^+$], 278.1312. Found 278.1317, m/z (ES$^+$) 278 [(M + H)$^+$, 100%].

Preparation of 3-iodo-2-heptylquinolin-4(1*H*)-one, 11

To a stirred solution of **3** (0.333 g, 1.37 mmol) in glacial acetic acid (10 mL) was added portionwise *N*-iodosuccinimide (0.315 g, 1.40 mmol). Reaction progress was monitored by TLC analysis and after 2 h, the precipitate was filtered, washed with acetic acid and acetonitrile. Purification was achieved by silica column chromatography (80/20 ethyl acetate/hexane, ramping to 100% ethyl acetate) to yield **11** as a white crystalline solid (0.22 g, 48%). Mp 221–225 °C (EtOAc). (Found: C, 52.4; H, 5.4; N, 3.9. $C_{16}H_{20}INO$ requires C, 52.0; H, 5.5; N, 3.8%.) ν_{max} (film)/cm^{-1} 3419 (OH stretch), 2921 (CH stretch), 1627 (C═N), 1557 (C═N conjugated), 1474 (C═C stretch aromatic), 1134 (C–O alcohol), 571 (C–I). δ_H ($[CD_3]_2SO$, 300 MHz) 0.84–0.88 (3H, m, CH_3). 1.27–1.37 (8H, m, 4 × CH_2), 1.63–1.71 (2H, m, CH_2), 2.88–2.93 (2H, m, CH_2), 7.30–7.35 (1H, m, ArH), 7.57–7.68 (2H, m, 2 × ArH), 8.05–8.08 (1H, d, J = 8.1 Hz, ArH). δ_C ($[CD_3]_2SO$, 75 MHz) 13.9 (CH_3), 22.0, 27.9, 28.3, 28.7, 31.1, 38.7 (6 × CH_2), 85.8 (C–I), 117.8 (ArC), 120.6 (quaternary C), 123.8, 125.4, 131.9 (3 × ArC), 139.0 (quaternary C), 154.5 (C═N), 173.1 (C–OH). Exact mass calculated for $C_{16}H_{21}NOI$ [(M + H)$^+$], 370.0668. Found 370.0664, m/z ES$^+$ 370.3 [(M + H)$^+$, 100%]. (Assigned as the quinoline tautomer.)

Preparation of 12

A mixture of anthranilamide (20.5 mmol, 2.791 g), *n*-octanal (20.5 mmol, 3.2 mL) and sodium bisulfite (30.75 mmol, 3.2 g) in dimethylacetamide (30 mL) was stirred at 150 °C for 2 h. Reaction progress was monitored by TLC analysis. The reaction mixture was poured into water (500 mL) and the precipitate filtered. The precipitate was recrystallised from ethanol to give product **12** as an off-white crystalline solid (3.82 g, 76%). Mp 124–127 °C (EtOH). (Found: C, 73.4; H, 8.2; N, 11.4. $C_{15}H_{20}N_2O$ requires C, 73.7; H, 8.25; N, 11.5%.) ν_{max} (KBr)/cm^{-1} 3448 (OH stretch), 3034 (C–H stretch aromatic), 2919 (CH stretch), 2855 (CH stretch) 1674 (C═C), 1616 (C═N), 1470 (CH_2 bend), 1341 (C–N stretch), 1149 (C–O alcohol). δ_H ($CDCl_3$, 300 MHz) 0.88 (3H, t, J = 6.8 Hz, CH_3), 1.25–1.51 (8H, m, 4 × CH_2), 1.82–1.92 (2H, m, CH_2), 2.74–2.79 (2H, m, CH_2), 7.44–7.49 (1H, m, ArH), 7.68–7.80 (2H, m, 2 × ArH), 8.29 (1H, dd, J = 1.1, 8.0 Hz, ArH), 11.10 (1H, bs, OH). δ_C ($CDCl_3$, 75 MHz) 14.1 (CH_3), 22.6, 27.6, 29.0, 29.2, 31.7, 36.1 (6 × CH_2), 120.5 (quaternary C), 126.3, 126.4, 127.2, 134.8 (4 × ArC), 149.4 (quaternary C), 156.8 (C═N), 164.0 (C–OH). Exact mass calculated for $C_{15}H_{20}N_2O$ [(M + H)$^+$], 245.1654. Found 245.1654, m/z ES$^+$ 245 [(M + H)$^+$, 80%].

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Analysis of pseudomonas quinolone signal and other bacterial signalling molecules using capillaries coated with highly charged polyelectrolyte monolayers and boron doped diamond electrode

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ABSTRACT

Coated capillary electrophoresis equipped with a boron doped diamond (BDD) electrode was developed for analysis of chemically synthesised 2-heptyl-3-hydroxy-4-quinolone (HHQ), 2-heptyl-3-hydroxy-4-quinolone (PQS), and 2-methyl analogues. Detection was then extended to biological samples. PQS and its biological precursor, HHQ, are two key regulators of bacterial cooperative behaviour known as quorum sensing in the nosocomial pathogen *Pseudomonas aeruginosa*. The fused silica capillary was coated with a thin layer of poly (diallyldimethylammonium) chloride to reverse the electroosmosis, allowing fast migration of PQS and HHQ with improved selectivity. The four model compounds were baseline resolved using a 50 mM H_3PO_4–Tris, pH 2.0 buffer with 20% (v/v) acetonitrile as buffer additive. With an injection time of 3 s, the detection limits of four analytes ranging from 60 to 100 nM (S/N = 3) were observed when the BDD electrode was poised at +1.5 V vs. 3 M Ag/AgCl. As expected, no PQS or HHQ was detected from the supernatant of the *P. aeruginosa* (*pqsA*) mutant. A concentration of HHQ of 247 μM was detected from the supernatant of the *pqsH* mutant, which catalyses the conversion of HHQ to PQS in the presence of molecular oxygen by monooxygenase. The separation and detection scheme was applicable to follow the conversion of HHQ to PQS in *P. aeruginosa* when entering the stationary phase of growth. The results obtained by coated capillary electrophoresis with BDD detection were validated and compared well with LC–MS data.

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1. Introduction

2-Heptyl-3-hydroxy-4-quinolone, the Pseudomonas Quinolone Signal (PQS) is a key regulator of bacterial cooperative behaviour known as quorum sensing in *Pseudomonas aeruginosa* [1–3]. *P. aeruginosa* is best known as an antibiotic resistant human pathogen associated with hospital-acquired infections and is the primary cause of morbidity and mortality in cystic fibrosis sufferers [4]. Controlling *P. aeruginosa* is thus of great clinical importance [5–8]. Research into PQS activity has revealed a vast and varied array of biological functions [9–15]. 2-Heptyl-4-quinolone (HHQ) is the biological precursor of PQS and also possesses a plethora of biological functions including signalling responsibilities [12]. It has recently been shown that HHQ can also control the behaviour of other bacteria species [16]. Diggle et al. reported that pathogenic bacteria other than *P. aeruginosa* synthesise 2-alkyl-4-quinolones (AHQs). *Burkholderia pseudomallei*, for example, produces AHQs and employs a structurally similar molecule to 2-heptyl-4-quinolone (HHQ) as a signalling molecule but does not produce PQS [12,17]. The chain-shortened analogue of PQS, quinolone 2 (Fig. 1) can act as an important iron entrapment molecule [12] and it is thought to be utilised by *Arthobacter nitroguajacolicus* [18]. Thus convenient, early stage detection of potential biomarkers PQS, HHQ and other 2-alkyl quinolones is of great clinical significance. To date, there have been very few efficient, simple and inexpensive methods for the detection of these chemically synthesised, or biologically produced, quinolones.

Analysis of 2-alkyl quinolones has been carried out by Ortori et al. using LC–MS/MS [19]. These, along with MS methods

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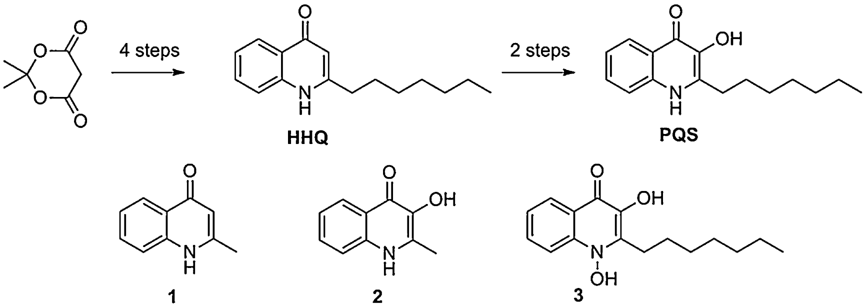

Fig. 1. Synthesis of quinolones.

developed by Lépine et al., require expensive instrumentation and the latter depends on the availability of the synthetic deuterated PQS standard [20]. Fluorometric methods lack selectivity and others such as chromatographic procedures require sample pretreatment, long analysis times and high costs, preventing them from being applied in routine analysis. Gas chromatography–mass spectrometry has been used to determine quinolone antibiotics in food and environmental samples [21]. High-performance liquid chromatography (HPLC) has also been applied for their separation and detection [22–25]. A biosensor based detection of PQS and HHQ has been reported by Williams and co-workers [26]. We recently showed that cyclic voltammetry and amperometry using a boron-doped diamond (BDD) thin-film electrode proved an excellent method for the sensitive detection of HHQ, PQS and other 3-alkyl quinolones [27]. Although the cyclic voltammetric system selectively detects PQS in the presence of its biological precursor HHQ, it is likely that PQS analogues (containing a 3-OH group) with shorter or longer C-2 alkyl chains are present in the supernatant, would not be suppressed, and may contribute to the response signal. Thus, accurate determination of individual components which bear very close similarity to PQS is difficult. Capillary electrophoresis (CE) is increasingly being applied to small molecule separation because of short analysis times and low consumption of reagents [28–33]. Improved separation can be achieved by judicious alteration of the capillary wall by electrostatic, hydrogen and hydrophobic interactions [34]. Cationic polymers such as poly (diallyldimethylammonium) chloride (PDDA) are usually more stable than neutral polymers [35,36] and make a suitable coating material for the capillary wall. PDDA coated capillaries performed very well for the analysis of peptides and proteins at acidic pH [37–39].

This work herein describes a new approach for the analysis of chemically synthesised PQS, HHQ and their 2-methyl analogues using a PDDA coated capillary coupled to a BDD electrode. The fused silica capillary is coated with a thin layer of PDDA to reverse the electroosmotic flow (EOF), allowing fast migration and excellent selectivity of chemically synthesised PQS and HHQ and other quinolones. Extension to biological systems is investigated for the detection of HHQ and PQS in several supernatant samples from *P. aeruginosa* wild-type and mutant strains.

2. Experimental

2.1. Chemical synthesis

Quinolones are synthesised as outlined vide infra.

2.2. Chemicals and materials

Poly (diallyldimethylammonium) chloride (PDDA, Mw = 200,000–350,000, 20 wt% in water), tris (hydroxymethyl) aminomethane (Tris), acetonitrile (ACN) and phosphoric acid (H_3PO_4) and all the standards were purchased from Sigma–Aldrich (Dublin, Ireland). The buffer solution for CE contained 50 mM H_3PO_4 solution adjusted to pH 2.0 with 0.5 M Tris solution, 20% (v/v) ACN. Stock solutions (5.0 mM) of PQS and HHQ were prepared in 50 mM $NaHPO_4$–Na_2HPO_4, pH 7, 50% (v/v) ACN. All solutions were prepared in Milli-Q ultrapure water and filtered through a 0.22 μm pore size membrane followed by sonication for 5 min prior to use.

2.3. Instrumentation

Amperometric measurement (I/t) and cyclic voltammetry (CV) were performed using a CHI 1040 electrochemical workstation (CH Instruments, Austin, TX) at room temperature. The three-electrode system consists of a boron doped diamond electrode (Windsor Scientific, Slough, Berkshire, UK), an Ag/AgCl (3 M NaCl) reference electrode (BAS, West Layette, IN) and a Pt wire counter electrode (Sigma, Dublin, Ireland).

2.4. Electrode preparation

A boron doped diamond (BDD) electrode, 3 mm diameter, 0.1% doped boron (Windsor Scientific, Slough, Berkshire, UK) was polished with polishing paper (grid 2000, Hand American Made Hardwood Products, South Plainfield, NJ) and subsequently with alumina (Buehler, UK) until a mirror finish was obtained. After thorough rinsing with deionised water, the electrode was sonicated in 2-propanol and deionised water for 5 and 10 min, respectively. The electrode was cyclised between −0.5 and +2.0 V versus Ag/AgCl (3 M NaCl, BAS, West Layette, IN) at 0.1 V s^{-1} in 50 mM phosphate buffer, pH 7 until a stable CV profile was obtained. Capillary electrophoresis with BDD electrode detection setup was as described previously [40] (see supplementary material).

2.5. Preparation of the coated capillary

A fused-silica capillary (50 μm ID and 365 μm OD) purchased from Polymicro Technologies (Composite Metal, Shipley, UK) was cut to 30 cm as the effective capillary length for analysis of the chemically synthesised quinolones. For the biological samples a longer capillary of 45 com length was needed to facilitate separation from other supernatant components. The fused-silica capillary was rinsed with 1.0 M NaOH and deionised water for 15 min each to expose the maximum number of silanol groups on the silica surface. The preconditioned capillary was then rinsed with the 0.2% (v/v) PDDA in H_2O solution followed by 15 min of incubation. The coated capillary was gently rinsed with deionised water for 3 min to flush out unsorbed coating materials. Before the first run, the coated capillary was equilibrated with the running buffer for 15 min but only 3 min between runs. All these procedures were performed at 25 °C. For overnight or prolonged storage, the capillary was rinsed

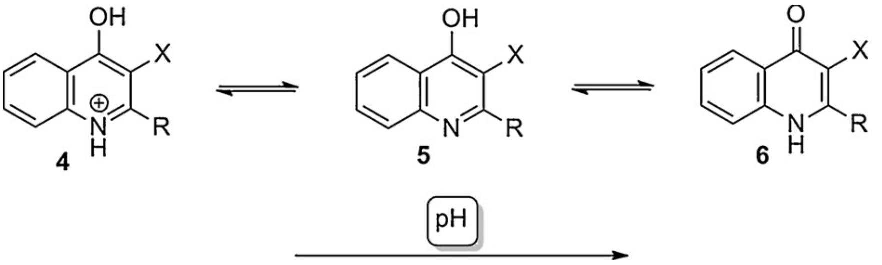

Fig. 2. Quinolone protonation and tautomerisation with pH variation.

with deionised water for 15 min and then stored with the capillary ends dipped in deionised water.

2.6. Preparation of the biological samples

Supernatant extracts for PQS analysis were obtained using a modified version of the Fletcher protocol [41]. Briefly, cultures of the *P. aeruginosa* mutant strain were incubated overnight in Luria Bertani broth at 37 °C (total 40 mL). Culture supernatants were obtained by centrifugation (5000 rpm for 10 min) and subsequently filter sterilised using Minisart (Sartorius) 0.2 μM filters into clean centrifuge tubes. An equal volume of acidified ethyl acetate (0.01% (v/v) glacial acetic acid) was added to the cell-free supernatant and vortexed for 30 s. After centrifugation (5000 rpm for 5 min), the top organic phase was removed and the process repeated a further two times to maximise the extraction (total 40 mL). The sample was then evaporated to dryness using a rotary evaporator. The running buffer (1 mL) was then added to the flask to dissolve the sample which was injected into the capillary.

3. Results and discussion

3.1. Chemical synthesis

HHQ was prepared starting with Meldrum's acid (0.14 mol) which was reacted with octanoyl chloride followed by boiling in MeOH, reaction with aniline [42] and a Conrad–Umpach cyclisation (Fig. 1) [43]. An alternative cyclisation method reported by Woschek et al. failed to give any product in our hands [44]. As quantities of PQS were also required, its synthesis from HHQ was achieved using conditions described by Pesci et al. [45]. The two-step procedure proved problematic and 2 equivalents of hexamine (HMTA) in the initial Duff formylation step was crucial to obtaining decent yields [46]. The chained-shortened compound 1 was prepared by refluxing ethyl acetoacetate and aniline followed by cyclisation in refluxing diphenylether [47]. Quinolone 2 was prepared as described by Hradil et al. [48]. The N-oxide 3 was prepared by protection of HHQ by esterification, followed by reaction with MCPBA and base hydrolysis (deprotection) [49]. While quinolones 1–3 may well be involved in bacterial cooperative behaviour, far more is known about HHQ and PQS. Thus, these two molecules are the main focus of this investigation.

3.2. Separation of PQS and HHQ on a bare capillary and a PDDA coated capillary

Capillary zone electrophoresis (CZE) performed at very high and very low pH values using high ionic-strength buffers is known to reduce the electrostatic interaction between the capillary wall and analytes and can aid separation [50]. Initially, basic conditions were tried for the CE separation of chemically synthesised quinolones HHQ and PQS. However baseline separation was not achieved. We next turned our attention to analysis at lower pH values. The pKa values and solubilities of the analytes were obtained using the ACD/Structure Designer software (Advanced Chemistry Development, Toronto, ON, Canada). 2-Alkyl-4-quinolones (of the form shown in Figs. 1 and 2, compounds 6) are tautomeric with 2-alkyl-4-hydroxyquinolines (Fig. 2, compounds 5) of which the predominance of one form is pH dependent [51–53]. It was reasoned by lowering the pH, the molecular structure would take the quinoline form depicted as 5, and may become protonated as depicted in form 4, Fig. 2. This would better facilitate separation based on differentiated pKa values. Based on the pKa calculations (Table 1) all the analytes are protonated at pH 2.

Table 1
Estimated pKa values using Marvin 6.8, ChemAxon software.

Analytes	pKa$_1$ (4)	pKa$_2$ (5/6)	pKa$_3$
PQS	3.43	9.89	13.86
HHQ	3.02	11.46	–
Qn1	3.57	9.96	13.89
Qn2	3.16	11.49	–

However, when the normal polarity of CZE was applied, poor peak shape and resolution was observed. At pH 2, the positively charged analytes may have adsorbed onto the untreated anode capillary wall, leading to decrease in peak efficiency and poor reproducibility [54] (Fig. 3A).

Coating the capillary wall minimises the electrostatic interaction between the capillary wall and analytes [55,56]. Thus the cationic polymer 0.2% poly (diallyldimethylammonium chloride) (PDDA) was used to coat the capillary inner wall of the capillary [57,58]. Importantly this leads to reversion of the surface charge of the capillary wall. In a previous report [59] we compared the electroosmotic mobility for bared fused silica and PDDA coated capillaries. The electroosmotic mobility of a PDDA-coated capillary (μ_{eof}), determined with mesityl oxide as the EOF marker, was estimated to be $-3.95 \times 10^{-4}\,\text{cm}^2\,\text{V}^{-1}\,\text{s}^{-1}$ (reversal of the flow) compared to $4.63 \times 10^{-4}\,\text{cm}^2\,\text{V}^{-1}\,\text{s}^{-1}$ for the bare fused silica counterpart. Such a result indicates that the PDDA-coated capillary become positively charged, owing to the abundance of the quaternary ammonium groups, effecting a reversal of the EOF. It was expected that at high PDDA concentrations, not all quaternary ammonium groups of the polymer were engaged in ionic interactions with SiOH of fused silica. As shown in Fig. 3B, all the analytes are well separated. PQS and HHQ are of higher molecular weight and are more hydrophobic than quinolones 1 and 2 and migrate faster in acetonitrile. At pH 2, PQS is more mobile in the EOF direction compared with HHQ. Interestingly N-oxide 3 shows no observable peak, perhaps indicative of the need for quinolinium cation formation for easy detection.

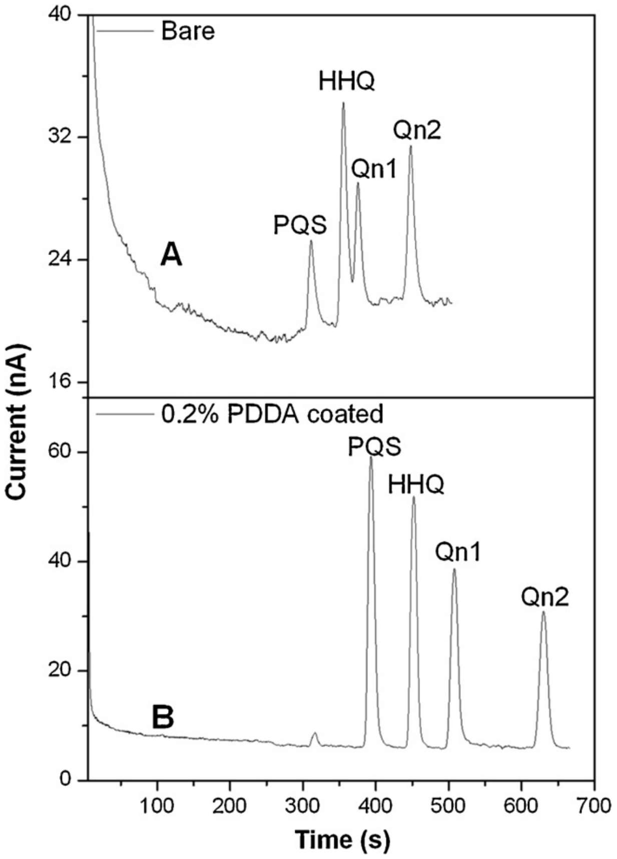

Fig. 3. Electropherograms obtained using (A) bare capillary, (B) 0.2% PDDA coated capillary (50 μm id and 40 cm effective length) for the separation of 100 μM (1) PQS, (2) HHQ, (3) Quinolone 1 (Qn1) and (4) Quinolone 2 (Qn2). The running buffer consisted of 50 mM H_3PO_4-Tris, pH 2, 20% (v/v) ACN. The separation voltage was applied at −10 kV with an injection time of 5 s at −10 kV. BDD poised at +1.5 V vs Ag/AgCl, 3 M NaCl.

3.3. Buffer condition optimisation

The separation of PQS and HHQ was investigated under different buffer conditions to examine the effect of pH. At pH 2, 25 mM (Fig. 4A(i)), good resolution of PQS, HHQ, quinolone 1 and 2 was achieved. In order to improve baseline separation for the PQS–HHQ pair and the HHQ–Qn1 pair, the buffer concentration was increased to 50 or 60 mM (Fig. 4A (ii) and (iii)). As ionic strength increases, EOF decreases since an increase in buffer electrolyte concentration has been known to affect the double layer thickness and the surface charge. The peak current was largely increased with longer migration time, particularly when the run was performed at 60 mM. A concentration of 50 mM was chosen as the optimal buffer concentration since all four peaks were baseline resolved within a migration time of ~330 s. Fine-tuning of the ideal pH for peak separation was then carried out using phosphate buffers. Well-separated peaks were obtained with good sensitivity at pH 1.8 and 2.0, respectively (Fig. 4B (i) and (ii)). The use of buffers with even slightly higher pH values resulted in poor peak resolution (Fig. 4B (iii)). Considering the hydrophobicity of PQS and HHQ, ACN was an essential component of the running buffer to improve the mobility and help avoid precipitation of the analytes in the capillary. 20% (v/v) ACN proved optimal (Fig. 4C (i–iv)). The final buffer conditions were thus optimised as 50 mM H_3PO_4–Tris, pH 2.0, with 20% (v/v) ACN as buffer additive.

To further improve peak shape, efficiency and resolution, separations were performed at different separation voltages (Fig. 5A) and injection times (Fig. 5B). Below 7.5 kV (Fig. 5A (ii)), migration times were quite long and peak broadening was observed. At 15 kV, the baseline noise proved a significant problem. The Ohm's law plot (Fig. 5A inset) was obtained using the 50 mM H_3PO_4–Tris, pH 2.0, with 20% (v/v) ACN as running buffer. Above 10 kV, Joule heating was observed. Therefore, 10 kV (Fig. 5A (iii)) was selected as the optimal voltage for further experiments. For the injection time, 7 s gave best results (Fig. 5B (iii)). The detection potential poised at +0.8 V (Fig. 5C (i)) failed to allow detection of quinolones 1 and 2. Although the detection focused on HHQ and PQS in biological samples, it would be advantageous if all four quinolones could be

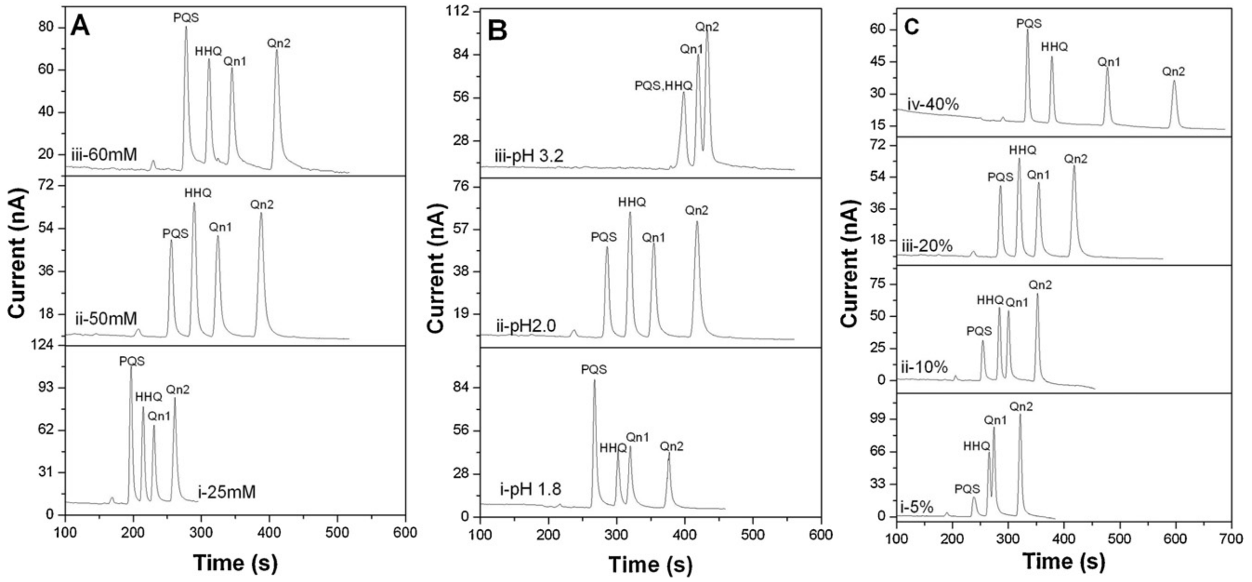

Fig. 4. Optimisation of (A) buffer concentration, (B) pH and (C) ACN content. Electropherograms obtained using PDDA coated capillary (50 μm id and 30 cm effective length) for the separation of 100 μM (1) PQS, (2) HHQ, (3) Quinolone 1 (Qn1) and (4) Quinolone 2 (Qn2). The separation voltage was applied at −10 kV with an injection time of 5 s at −10 kV. BDD poised at +1.5 V vs Ag/AgCl, 3 M NaCl. (A) Different buffer concentration: the running buffer consisted of (i) 25 mM, (ii) 50 mM, and (iii) 60 mM H_3PO_4–Tris, pH 2, 20% (v/v) ACN. (B) Different pH: the running buffer consisted of 50 mM H_3PO_4–Tris, pH (i) 1.8, (ii) 2.0 and (iii) 3.2, 20% (v/v) ACN. (C) Different percentage of ACN: the running buffer consisted of 60 mM H_3PO_4–Tris, pH 2.0, (i) 5%, (ii) 10%, (iii) 20%, and (iv) 40% (v/v) ACN.

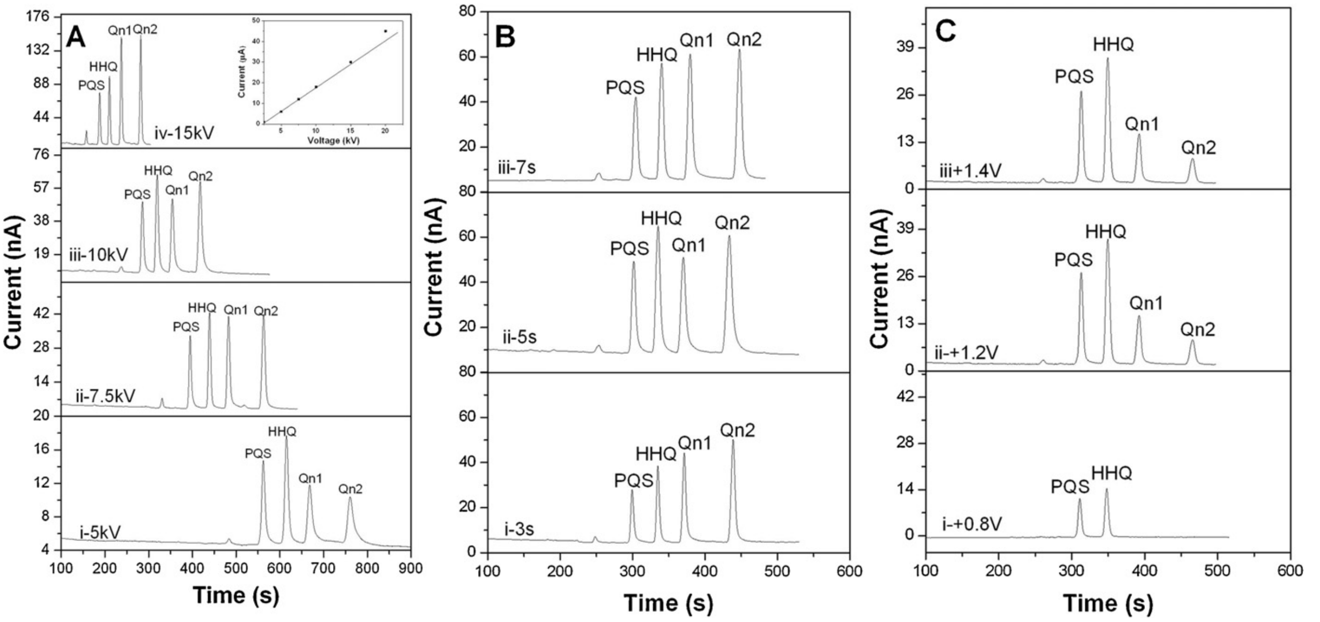

Fig. 5. Variation of (A) separation voltage, (B) injection time and (C) detection potential. Electropherograms obtained using PDDA coated capillary (50 μm id and 30 cm effective length) for the separation of 100 μM (1) PQS, (2) HHQ, (3) Quinolone 1(Qn1) and (4) Quinolone 2 (Qn2). The running buffer consisted of 60 mM H_3PO_4–Tris, pH 2, 20% (v/v) ACN. (A) The separation voltage was applied at (i) −5 kV, (ii) −7.5 kV, (iii) −10 kV, and (iv) −15 kV with an injection time of 5 s. Inlet: Ohm's law plot. (B) The separation voltage was applied at −10 kV with an injection time of (i) 3 s, (ii) 5 s and (iii) 7 s. BDD poised at +1.5 V vs Ag/AgCl, 3 M NaCl. (C) BDD electrode poised at (i) +0.8 V, (ii) +1.2 V and (iii) +1.5 V vs Ag/AgCl, 3 M NaCl.

detected and separated using a common optimised process. Finally, a detection potential of +1.5 V (Fig. 5C (iii)) vs. Ag/AgCl gave excellent amperometric detection of all four analytes using the BDD electrode.

3.4. Limit of detection

Under the optimal separation conditions, each analyte exhibited good linearity ($R^2 > 0.99$, $n = 5$) between peak height and the injection time (Table 2). Without loss of resolution, the LODs (signal-to-noise ratio of 3) of the four analytes range from 60 to 100 nM. The reproducibility values of migration time were less than 0.4%.

3.5. Determination of HHQ and PQS in supernatant samples of P. aeruginosa

3.5.1. PA14 pqsA mutant

Synthesis of HHQ proceeds through condensation of anthranilic acid and a β-keto acid, in a reaction catalysed by the PqsA enzyme[60]. PqsA is encoded as the first gene in a quintet operon *pqsA-E*, which encodes four enzymatic activities required for HHQ synthesis, as well as a fifth activity (PqsE) required for activation of the PQS molecule [61]. The *pqsA* mutant, therefore, does not produce HHQ or PQS, and typically lacks several virulence factors and toxins as a result [61]. A comparison of the supernatant sample with a spiked sample confirms that no HHQ or PQS was present (Fig. 6).

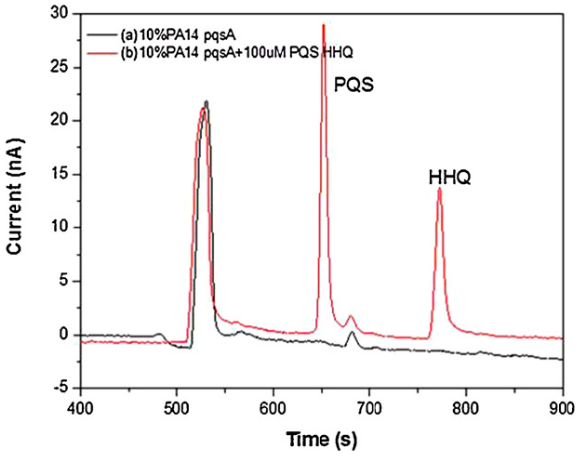

Fig. 6. Analysis of *P. aeruginosa pqsA* mutant strain using the PDDA coated capillary (50 μm id and 45 cm effective length). (a) *pqsA* mutant strains diluted 10-fold with the running buffer. (b) *pqsA* mutant strains diluted 10-fold with running buffer spiked with 100 μM standard (1) PQS and (2) HHQ. Running buffer, 50 mM H_3PO_4–Tris, pH 2, 20% (v/v) ACN; separation voltage, −10 kV; injection time, 5 s at −10 kV; BDD electrode poised at +1.5 V vs. Ag/AgCl.

3.5.2. PA14 pqsH mutant

Conversion of HHQ to PQS proceeds through the activity of monooxygenase (*PqsH*) encoded distant to the *pqsA-E* operon [61]. PqsH catalyses the conversion of HHQ to PQS in the presence of molecular oxygen [62], and is unique to *P. aeruginosa*. The *pqsH* mutant, therefore, produces HHQ but lacks the capacity to convert HHQ to PQS (Fig. 7). A concentration of HHQ of 247 μM was estimated from the electropherogram.

3.5.3. PA14 wild type

PA14 is a clinical isolate of *P. aeruginosa* originally obtained from a burn wound patient and has been shown to be highly pathogenic in a broad spectrum of animal models [63]. This wild-type clinical isolate converts HHQ to PQS upon entry into the stationary phase of growth (typically over a period of about 12 h). A sample taken after 8 h showed a conversion from HHQ to PQS of about 60% (Fig. 8).

Table 2
Calibration curve and limit of detection.

Compound	Linear range (μM)	Calibration equation	R^2	LOD (nM)	Migration time (s)
PQS	1–100	I (nA) = 0.549 C(μM) + 0.445	0.996	65	320
HHQ	1–100	I (nA) = 0.530 C(μM) − 0.4931	0.990	94	360
Quinolone 1	1–100	I (nA) = 0.411 C(μM) + 1.170	0.984	61	400
Quinolone 2	1–100	I (nA) = 0.405 C(μM) + 0.474	0.991	79	490

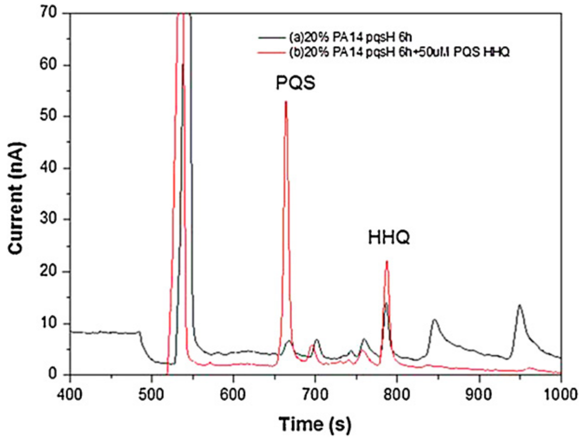

Fig. 7. Analysis of *P. aeruginosa pqsH* mutant strain using the PDDA coated capillary (50 μm id and 45 cm effective length). (a) *pqsH* mutant strains diluted 10-fold with the running buffer. (b) *pqsH* mutant strains diluted 10-fold with the running buffer spiked with 50 μM standard (1) PQS and (2) HHQ. Running buffer, 50 mM H_3PO_4–Tris, pH 2, 20% (v/v) ACN; separation voltage, −10 kV; injection time, 5 s at −10 kV; BDD electrode poised at +1.5 V vs. Ag/AgCl.

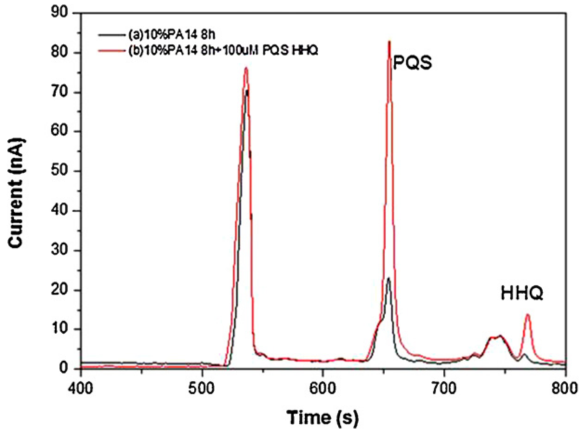

Fig. 8. Analysis of the *P. aeruginosa* PA14 wild type mutant strain using the PDDA coated capillary (50 μm id and 45 cm effective length). PA14 wild type cultured 8 h diluted 10-fold with the running buffer, (a) without spiked standard, (b) spiked with 100 μM standard (1) PQS, (2) HHQ. Running buffer, 50 mM H_3PO_4–Tris, pH 2, 20% (v/v) ACN; separation voltage, −10 kV; injection time, 5 s at −10 kV; BDD electrode poised at +1.5 V vs. Ag/AgCl.

3.5.4. PAO1 mutant

PAO1, a *P. aeruginosa* reference strain that was initially isolated from an infected burn wound patient [64]. The strain was the first *P. aeruginosa* isolate to have its genome sequenced [65] and has been the focus of the majority of research studies. This isolate, which is less virulent than PA14, nonetheless displayed the capacity to produce HHQ and PQS in a similar way to PA14, converting HHQ to PQS upon entry into the stationary phase of growth (Fig. 9). HHQ and PQS were also detected in this isolate and a conversion of 83% was calculated.

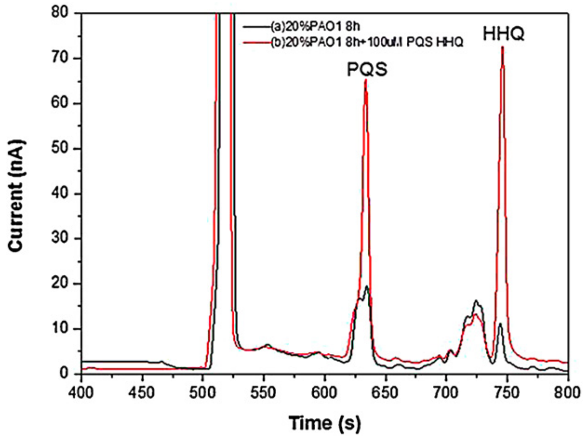

Fig. 9. Analysis of the *P. aeruginosa* PAO1 mutant strain using the PDDA coated capillary (50 μm id and 45 cm effective length). PAO1 cultured 8 h diluted 10-fold with running buffer, (a) without spiked standard, (b) spiked with 100 μM standard (1) PQS, (2) HHQ. Running buffer, 50 mM H_3PO_4–Tris, pH 2, 20% (v/v) ACN; separation voltage, −10 kV; injection time, 5 s at −10 kV; BDD electrode poised at +1.5 V vs. Ag/AgCl.

Table 3
Comparison of PQS and HHQ concentration detected from CE-ECD and LC–MS.[a]

Mutant	Concentration CE-ECD (μM)		Concentration LC–MS (μM)	
	PQS	HHQ	PQS	HHQ
PA14 *pqsA*	0	0	0	0
PA14 *pqsH*	0	248	0	219
PA14	399	282	323	287
PAO1	212	44	220	43

[a] Due to a shoulder peak eluted with PQS the area was calculated using OriginPro 8.5.1 software.

3.5.5. Comparison with LC–MS data

Selected samples were also analysed using LC–MS and were comparable with the values obtained using CE (Table 3 and supplementary data Fig. SM5). For PA14 *pqsA*, no PQS or HHQ was observed. For PA14 *pqsH*, a 248 μM HHQ concentration was determined by CE-ECD compared to 219 μM by LC–MS. PQS was detected at a concentration of 399 μM in PA14 by CE-ECD and 323 μM by LC–MS.

4. Conclusion

Our recently published report on the detection of quinolone biomarkers in *P. aeruginosa* utilised cyclic voltammetry and amperometric detection [27]. While this method proved efficient and cost effective for the positive and selective determination of HHQ and PQS, close analogues could contribute to the signal response and thus accurate determination of their concentrations is difficult. Herein we describe the use of capillary electrophoresis in the separation of key biomarkers for the antibiotic resistant bacterium *P. aeruginosa*. Thus interference from other quinolones can be greatly reduced. Capillary electrophoresis with amperometric detection using different electrode materials is a powerful analytical tool owing to its high sensitivity and tuneable applied potential for a variety of electroactive compounds. The experiments presented here illustrated that this technique is capable of satisfactorily separating a number of potential *P. aeruginosa* biomarkers. CE equipped with amperometric detection can be miniaturised and used together with solid-phase microextraction to improve detection sensitivity for clinical applications. A capillary-electrode holder could be easily constructed for positioning of the detecting electrode at the end of the capillary column without the aid of micropositioners or microscopes. The detection limit obtained was considerably below the normal physiological levels in, for example, sputum samples of Cystic Fibrosis patients [66]. The applicability of this technique to clinical isolates is a subject of future endeavour.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2012.06.064.

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Studies in Asymmetric and Heterocyclic Synthesis

III

Trifluoromethylated Pyrones

Chapter 7

Trifluoromethylation of 4-alkoxy-2-pyrones, pyridones and quinolones

Introduction

"The fury of the chemical world is the element fluorine. It exists peacefully in the company with calcium in fluorspar and also in a few other compounds; but when isolated, as it recently has been, it is a rabid gas that nothing can resist."

Scientific American

April 1888

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7.1 General introduction

Fluorine is the element of extremes.[1] It is the most electronegative of all the elements[2] and consequently, a carbon-fluorine bond is highly polar and is the strongest bond in organic chemistry.[3] Fluorine is the second smallest element in the periodic table,[4] with a van der Waals radius of 1.47Å, only 20% larger than that of hydrogen and significantly less than those of other halogens.[5] With regards to its steric impact, fluorine is the smallest substance that can replace a hydrogen in a molecule, other than an isotope of hydrogen.[6] Due to its similarity in size to hydrogen, it has been shown that microorganisms or enzymes often do not recognise the difference between a natural substrate and its analogue where a C-H group has been replaced by a C-F group.[4] Fluorine is the 13th most abundant element (and the most abundant halogen) in the Earth's crust, where it is trapped as fluorides in minerals such as fluorite and fluorspar.[7]

Despite its relatively high natural abundance, very few compounds containing fluorine, have been found in nature.[8] Surprisingly, fluorine has been identified as a component of only five secondary metabolites (**273-277**) produced in nature (**Figure 7.1**)[8,9] and around 30 minor lipid metabolites of **277** that are ω-fluorinated homologues of long chain fatty acids found as co-metabolites in the seeds of the same plant, *Dichapaetalum toxicarium*.[10] This is in contrast to the other halogens. Chlorine, bromine and even iodine feature in *ca*. 3000 secondary metabolites produced *via* biosynthetic pathways in plants, fungi and microorganisms.[11] The underlying reason for this scarcity of fluorinated natural products, compared with the relative abundance of other halogenated metabolites, is due to the unique chemical attributes of fluorine. Fluorine has very different properties to those of the other halogens and has even been described as a 'superhalogen'.[12]

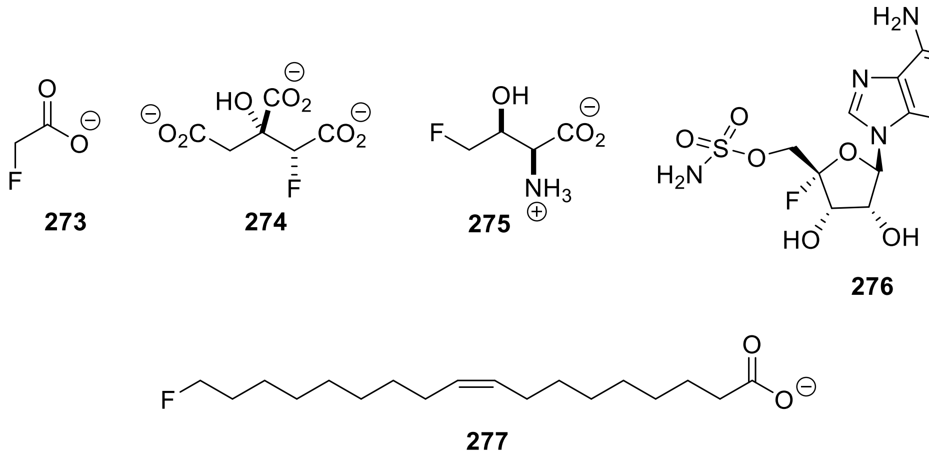


Figure 7.1 Naturally occurring fluorine-containing compounds.[8]

7.2 Development of organofluorine chemistry

The history of fluorine and its compounds is a relatively short one, most likely due to the hazardous nature of hydrofluoric acid and the difficulty in accessing elemental fluorine itself. The first synthesis of hydrofluoric acid from fluorspar and sulfuric acid was carried out in 1764 by Marggraf[13] and was repeated in 1771 by Scheele.[14] Surprisingly, it was over 100 years later that the real breakthrough in this area of organic chemistry came about, with the first synthesis of elemental fluorine in 1886 by Moissan.[15-17] The importance of this synthesis was highlighted by the Nobel Prize award in Chemistry to Moissan in 1906 "in recognition of the great services rendered by him in his investigation and isolation of the element fluorine, and for the adoption in the service of science of the electric furnace called after him."[18] The first examples of nucleophilic and electrophilic fluorinaton were reported in the late 19th century,[19-21] however the industrial application of organofluorine compounds did not start until the beginning of the 1930s with the introduction of chlorofluorocarbons (CFCs) as refrigerants.[22] The major turning point in the use of organofluorine compounds in industry was the Manhattan Project in 1941, which was the starting point for the development of nuclear weapons.[23,24] The Manhattan Project required highly resistant materials, lubricants, coolants and the improvement of technology for handling extremely corrosive inorganic fluorine-containing compounds. The main precursor for all of these materials was hydrofluoric acid and as a result, its consumption increased rapidly during the 1940s. There was further development in this area with the onset of the Cold War in 1945 and the subsequent arms race. After the Cold War, attention turned to the use of organofluorine compounds in civilian materials and pharmaceuticals.[25]

In 1974, a report published by Molina and Rowland predicted the devastating effects of CFCs on the ozone layer.[26] The Montreal Protocol, 13 years later was the beginning of phasing-out of the use of CFCs.[27] Some of the CFCs were replaced with other fluorine-containing chemicals, for example hydrofluorocarbons, HFCs,[28] but on the whole the fluorochemical industry had to turn their attention to other fields of application, for example fluoropolymers, fluorosurfactants and fluorinated intermediates for pharmaceuticals and agrochemicals.[25,29]

A rapidly growing market for organofluorine compounds involves the use of these chemicals as intermediates in the pharmaceutical, agrochemicals and electronics industries.[1] Indeed, in 2011, 3 of the 10 best-selling drugs and 7 of the 35 newly approved drugs contained fluorine atoms.[30] As a result, methods for the introduction of fluorine atoms into compounds at both early and late stages of a synthesis is an exciting aspect of modern synthetic organic chemistry.

7.3 Methods of fluorination

Despite being a relatively new area of organic chemistry, there are many methodologies available that allow the incorporation of fluorine into organic molecules. In recent years in particular, many new reagents and synthetic strategies have come to the fore which involve easier use and handling and extensive reviews on fluorination methodologies are available in the literature.[31-36] Fluorination procedures are conveniently divided into methods that involve electrophilic fluorine and those that involve nucleophilic fluorine.

7.3.1 Electrophilic fluorination

Organic chemists have developed F^+ equivalents which allow transfer of fluorine to an electron-rich centre. Various methodologies have been developed to allow this transformation, including the use of elemental fluorine, *O*-F reagents and *N*-F reagents.

Shortly after the isolation of elemental fluorine by Moissan in 1886, several reactions were carried out whereby organic substances were treated with this highly reactive gas.[15-17] Disappointingly, these reactions resulted in sometimes violent explosions, whether they were carried out at room temperature or extremely low temperatures and no major defined reaction products could be isolated. This is most probably due to the energy released by formation of the highly stable carbon-fluorine bonds (~116 kcal mol^{-1}) compared to the energy required for dissociation of carbon-carbon (~83 kcal mol^{-1}) or carbon-hydrogen bonds (~99 kcal mol^{-1}). Another contributory factor is the particularly low homolytic dissociation energy of elemental fluorine (37 kcal mol^{-1}), which permits swift initiation of uncontrollable radical chain reactions, even at low temperatures and in the absence of light.[1]

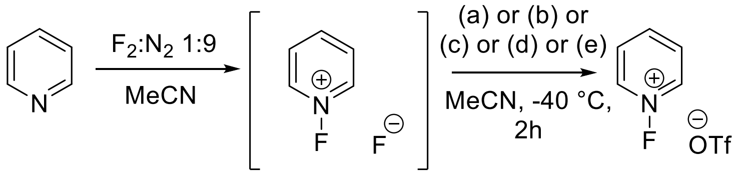
Direct fluorination of aliphatic compounds was achieved in liquid reaction media by Bockemüller in 1933.[37] By diluting the fluorine gas with either nitrogen or carbon dioxide and dissolving the organic substrate in a cooled inert solvent (such as CCl_4 or CF_2Cl_2), it was possible to control the reaction enthalpy and successfully achieve direct fluorination.[38]

Studies carried out by Fredenhagen and Cadenbach described fluorination of volatile organic substances in the gas phase on contact with a copper mesh.[39] This work was further developed by Fukuhara and Bigelow, who reported the successful preparation of polyfluorinated products from aliphatic hydrocarbons, benzene or acetone *via* vapour phase fluorination, however very specific apparatus was required to carry out these reactions.[40]

An improved methodology using a nickel reactor with different temperature zones and silver-doped copper filings as a catalyst was developed in the early 1970s by Lagow and Margrave, known as the LaMar direct fluorination process.[41,42] In this methodology, the concentration of fluorine to inert gas is slowly increased, allowing the energy to be liberated in a controlled manner.

Selective fluorination of pyridine, quinoline and quinoxaline derivatives have been successfully accomplished using fluorine–iodine mixtures.[43] In these cases, hydrogen atoms α- to the heteroatom were replaced by fluorine. Specialised equipment was required for this methodology.

Due to the special equipment and techniques required, as well as their potentially explosive, toxic, unstable and hygroscopic nature, alternative reagents for electrophilic fluorination were sought after. *N*-fluoropyridone[44,45] and *N*-alkylarenesulfonamides[46,47] were all successfully developed and although they proved much easier to handle than previous fluorination reagents, their reactivity was considerably lower. *N*-fluoropyridinium salts were first developed in the 1980s and have become an important source of electrophilic fluorine for fluorination reactions.[48] Studies were carried out by Umemoto *et al.* into various *N*-fluoropyridinium reagents where it was found that they functioned as useful fluorinating agents.[49,50] Previous work carried out by Meinert showed that when a dilute solution of F_2 in N_2 was bubbled into a solution of pyridine in $CFCl_3$ at -80 °C, a moisture-sensitive, unstable, white precipitate – a pyridine-F_2 adduct – was formed. This adduct was found to decompose violently at temperatures greater than -2 °C, leaving a red-brown oil containing 2-fluoropyridine. Although no physical or spectral data was reported for the adduct, an ionic structure was theorised.[51] Umemoto and Tomita suggested that the instability of the pyridine-F_2 adduct was due to some nucleophilicity of F^- in an ionic form **279**, and this could be improved by exchanging F^- for a non-nucleophilic anion. A variety of methods were used to transform pyridine **278** into *N*-fluoropyridinium triflate **280** (**Scheme 7.1**) in moderate to good yield.[48] The results of these reactions support the previous suggestion of Meinert that the pyridine-fluorine adduct exists in an ionic form.[51]



278 **279** **280**

Scheme 7.1 Methods for synthesis of *N*-fluoropyridinium triflate **280**. (a) Adduct **279** prepared in $CFCl_3$ then reacted with TfONa, 67%; (b) TfONa, 71%; (c) $TfOSiMe_3$, 45%; (d) TfOH, 44%; (e) TfONa present with F_2:N_2 mixture, 80%.

N-Fluoropyridinium salts possessing several different counter-anions **281-285** were prepared using these methodologies (**Figure 7.2**). A series of *N*-fluoropyridinium triflates containing electron-donating or electron-withdrawing substituents (**286-293**) was also synthesised (**Figure 7.2**). It was found that the electrophilic fluorinating power of these reagents increased with decreasing electron density of the N^+-F site, which can be controlled by altering substituents on the ring itself.[48] All of the *N*-fluoropyridinium salts were deemed to be highly stable under anhydrous conditions.[48] In 2003, a crystal structure of **280** was obtained by Banks *et al.*, with the N-F bond length established as being 1.357 Å, which suggests strong back donation of p-electrons of the fluorine atom to the nitrogen of the ring.[52]

Although specialised equipment is necessary for the initial fluorination step in the synthesis of these *N*-fluoropyridinium salts,[53] many are commercially available.[54]

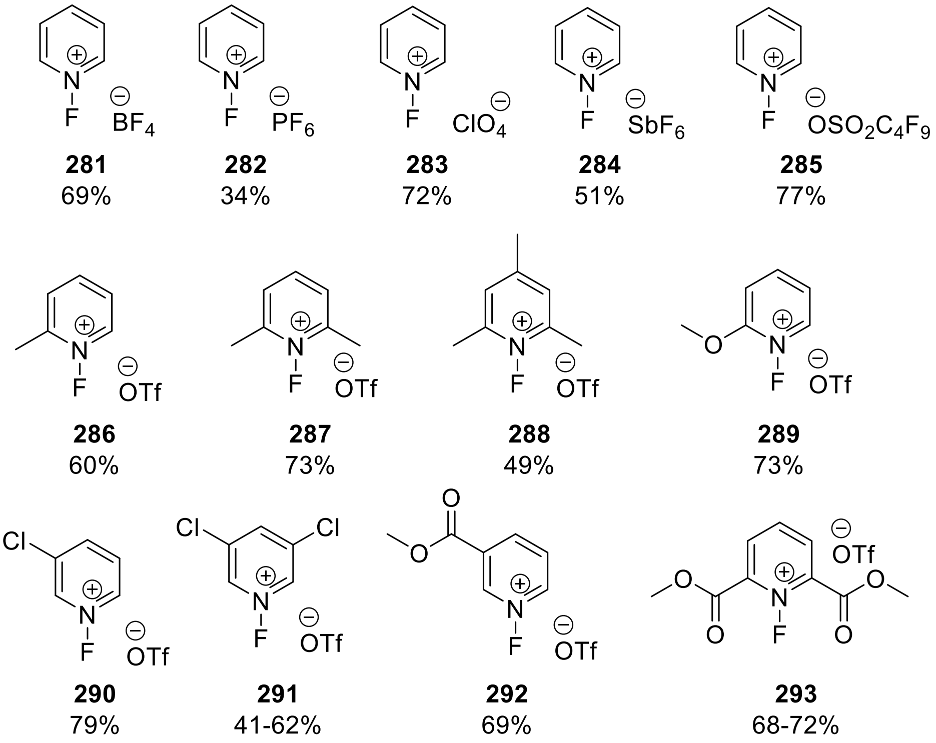


Figure 7.2 *N*-Fluoropyridinium salts with alternative counter-anions **281-285** and *N*-fluoropyridinium triflates with electron-withdrawing and electron-donating substituents **286-293**.

Fluorination using *N*-fluoropyridinium salts has been successfully applied to aromatics, carbanions, enol alkyl ethers, enol silyl ethers, vinyl esters, enamines and alkenes.[55]

These results led to further development of these classes of electrophilic fluorinating reagents. In 1984, a publication by Barnette highlighted the use of *N*-fluorosulfonamides **294** as a new class of broadly applicable fluorinating reagents that were easily prepared by the reaction of *N*-alkylsulfonamides with elemental fluorine.[46] This finding led the way for development of additional fluorinating agents of this type, including *N*-fluorobis[(trifluoromethyl)sulfonyl]imide[56] **295** or *N*-fluorobenzenesulfonimide (NFSI) **296** (**Figure 7.3**).[57]

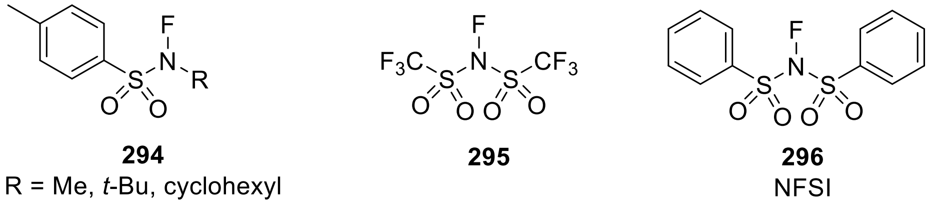

294
R = Me, *t*-Bu, cyclohexyl
295
296
NFSI

Figure 7.3 *N*-Fluoroalkylsulfonamides employed as fluorinating agents.

In recent years, structural alterations in *N*-fluoropyridinium salts have resulted in increased yield and specificity of fluorination as well as the elimination of side reactions.[55] Despite this, it is apparent that on the whole, *N*-fluoropyridinium salts are gradually being replaced by more effective and specific agents based on the diazabicyclooctane moiety, such as 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane (Selectfluor® or F-TEDA-BF_4) **243** (**Figure 7.4**).[58,59]

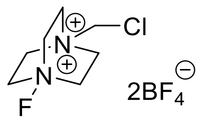
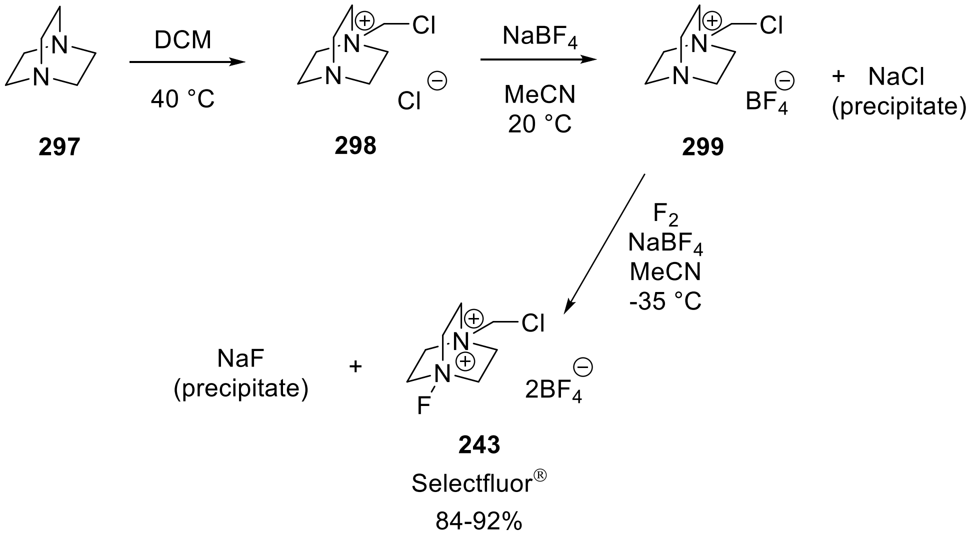

243
Selectfluor®

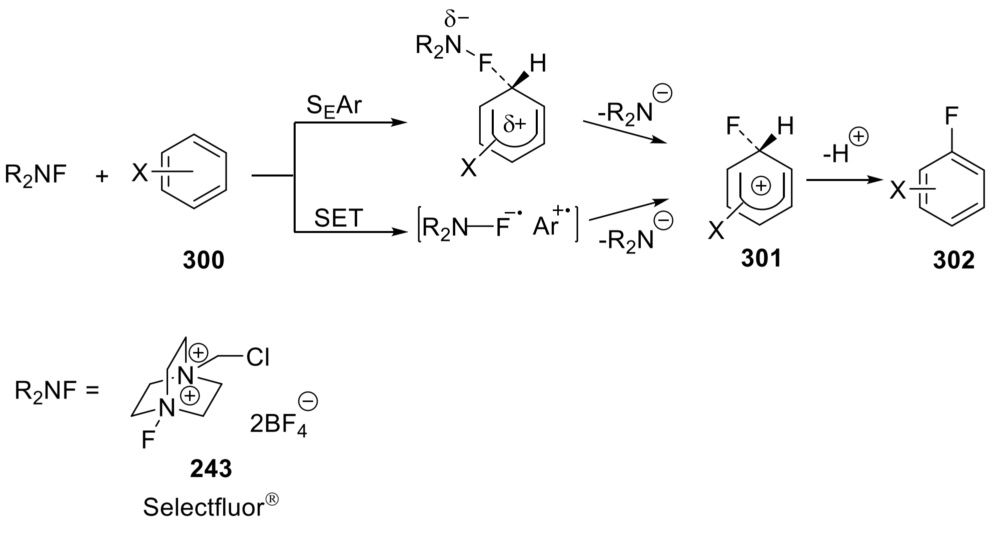
Figure 7.4 Commercially available 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane (Selectfluor®).

Selectfluor® is a stable, virtually non-hydroscopic crystalline solid that is particularly easy to handle and store. Indeed, in a self-accelerating decomposition test (SADT) carried out to demonstrate its stability, a 55-gallon drum was filled with Selectfluor® and heated to 56 °C for 7 days. During this time, the temperature remained constant within ±5 °C.[60] Selectfluor® has been found to be stable at temperatures up to 195 °C, however the inventors warn that when dealing with bulk samples, caution should be exerted when heating above 80 °C.[58] Selectfluor® is commercially available but can be easily prepared in the laboratory in three steps from 1,4-diazabicyclo[2.2.2]octane **297** (DABCO, also known as triethylenediamine (TEDA)) (**Scheme 7.2**). Alkylation of **297** using DCM takes place in the initial step. Counterion exchange with sodium tetrafluoroborate provides **298**, which is fluorinated to give **299** in good yield. Subsequent reaction of **299** with elemental fluorine and sodium tetrafluoroborate affords Selectfluor® **243** in high yield.[58]



Scheme 7.2 Synthesis of Selectfluor®.

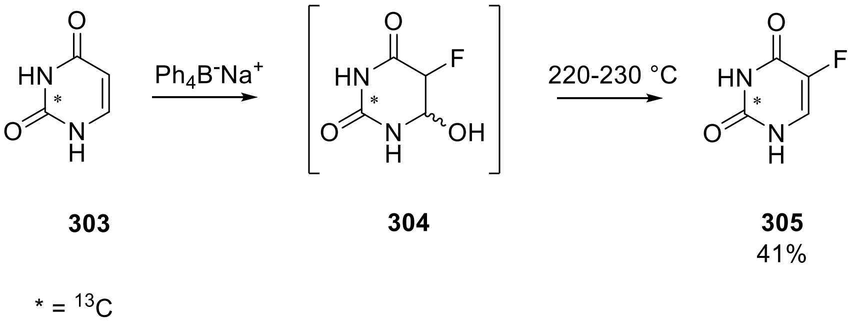
Since the introduction of Selectfluor® in the 1990s, it has demonstrated a broad scope of application, as demonstrated by a comprehensive review by Nyffeler *et al.*[61] Selectfluor® is soluble in only a few polar solvents, namely, acetonitrile, DMF and water.[58] Nitromethane has also been found to be a suitable solvent as it is inert and sufficiently polar to dissolve Selectfluor®.[62,63] In reactions where acidic conditions must be avoided, the use of nitromethane as solvent is ideal as it precludes the need for a base or proton sponge. Ionic liquids have also been successfully used in fluorination reactions involving Selectfluor®.[64,65] The mechanism of electrophilic fluorination of compounds using *N*-fluoropyrimidine salts has also been much debated and two possible pathways are suggested for the reaction: single-electron transfer (SET) or nucleophilic substitution at the fluorine atom (S_N2) (**Scheme 7.3**). Differding and coworkers have conducted several studies into the mechanism of the reactions, which support the S_N2 mechanism.[66,67] Vincent *et al.* reported on the electrophilic fluorination–nucleophilic addition reactions of Selectfluor® on glycals.[62] Using a hypersensitive radical probe, no product characteristic of a radical process was isolated, suggesting no single electron transfer occurs during the attack of the glycal on Selectfluor®. Borodkin *et al.* used kinetic isotope effects in studying the reaction of **243** and **230** to establish that fluorination reactions mediated by Selectfluor® proceed *via* a polar reaction mechanism to provide **302**.[68] Carbon-fluorine bond formation was found to be the rate-determining step in the reaction rather than the step involving formation of the Wheland complex **301**, based on the small values of k_H/k_D observed (0.86–1.00), meaning no primary kinetic isotope effect was found. In contrast, work by Zhang[69] and Kralj *et al.*[70] suggest that the reaction proceeds *via* a SET mechanism. There is precedence for electrophilic aromatic substitution occurring *via* SET[71-73] and a recent publication on the reaction of Selectfluor® and chloride provides more evidence for the reaction occurring *via* this mechanism.[74] In the most recent mechanistic work, Geng *et al.* reported on a systematic theoretical investigation of the fluorination of aromatic compounds using Selectfluor® using computational analysis.[75] The results indicating that the SET mechanism is preferred over the S_N2.



Scheme 7.3 Two possible mechanistic routes for fluorination of aromatic compounds using NF-reagents, illustrated with Selectfluor®.

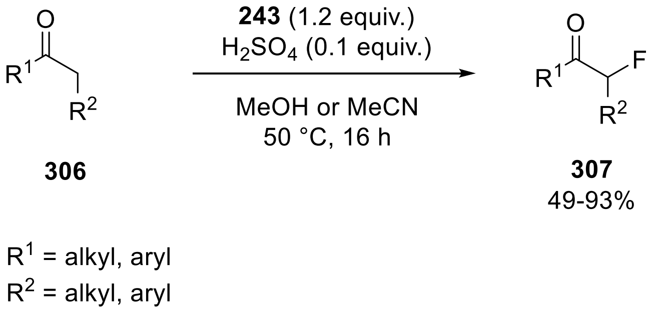
Selectfluor® has been used in the synthesis of α-fluorinated carbonyl compounds and thioethers,[76-81] glycosyl fluorides,[82] fluorinated aromatic compounds,[58,83,84] fluorinated alkenes[76,85-88,62,63,82,89] and indoles,[90,91] vinyl and alkyl fluorides[92-94] and fluorinated pyrimidines.[95] A recent publication by

Rangwala *et al.* demonstrated the use of Selectfluor® in the direct fluorination of uracil in the synthesis of radiolabelled 5-fluorouracil ([2-^{13}C]-5-fluorouracil), a potential diagnostic agent for measuring 5-fluorouracil induced toxicity in cancer patients.[96] The synthesis of radiolabelled 5-fluorouracil was achieved in three steps starting from reaction of [^{13}C]-urea and propiolic acid to provide [2-^{13}C]-pyrimidine-2,4(1*H*,3*H*)-ione **303**. Selectfluor® **243** was added to a solution of [2-^{13}C]-pyrimidine-2,4(1*H*,3*H*)-ione **303** in distilled water and stirred at 105 °C for 16 h. Sodium tetraphenylborate was added and the mixture stirred at 0 °C for 30 min to afford intermediate fluorohydrin **304**, which was converted to the desired product **305** *via* fractionated sublimation (**Scheme 7.4**). Addition of sodium tetraphenylborate was necessary to separate the spent reagent (consisting of a mixture of fluoride, bifluoride and tetrafluoroborate salts of 1-(chloromethyl)-4-protiotriethylenediamine) from the aqueous solution by conversion to the water insoluble salt 1-(chloromethyl)-4-protiotriethylenediamine bis(tetraphenylborate).[95]



Scheme 7.4 Use of Selectfluor® in the preparation of radiolabelled 5-fluorouracil.

Liu *et al.* reported that the direct fluorination of ketones, ketals and enamides was accomplished using Selectfluor® with sulphuric acid as an additive.[97] In order for direct α-fluorination of ketones to occur, initial formation of a nucleophilic enol intermediate must take place. The authors surmised that as acidic conditions promote enol formation, improved rate and yield of α-fluorinated ketones could be achieved. A variety of α-fluorinated products **307** were synthesised from **306** in this manner in good to high yield (**Scheme 7.5**).

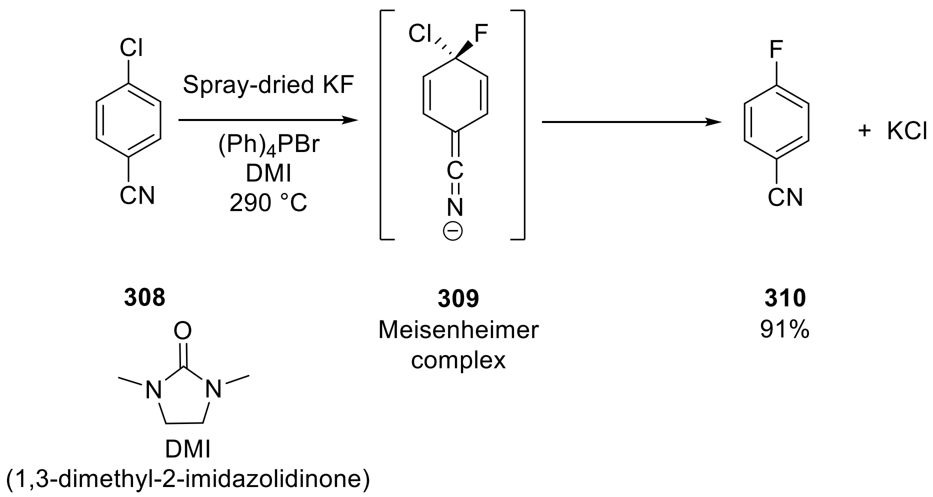


Scheme 7.5 Synthesis of α-fluorinated ketones using Selectfluor® and H_2SO_4 as an additive.

Fluorination reactions of heterocyclic nitro compounds have also been accomplished by irradiation with ultrasound in the presence of ammonium acetate as a base and Selectfluor®, providing products in low to good yield in 4–6 h.[98] Similarly, Selectfluor® performs well under microwave conditions, with 1,3-dicarbonyl compounds being successfully fluorinated in 10 min at 82 °C.[99]

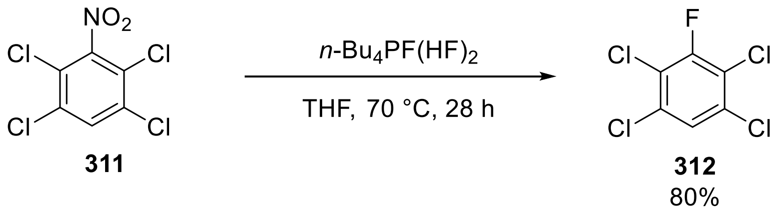
7.3.2 Nucleophilic fluorination

Fluorine can also be introduced into a molecule in a nucleophilic manner, in particular in electron-deficient arenes *via* nucleophilic aromatic substitution. The most common methodology for this transformation is a halogen exchange (halex) reaction, in which halogen atoms serve as leaving groups and inexpensive, inorganic fluoride sources are used as nucleophiles.[100] The most suitable substrates for halex reactions are arylchlorides due to the higher electronegativity of chlorine in comparison to bromine and iodine, however it is usually necessary to activate the chloro group by other moieties on the ring to ensure that substitution will take place. The rate determining step in this reaction is the addition of fluoride to **308** to form a Meisenheimer complex **309** as aromaticity is lost. Restoration of aromaticity is achieved by elimination of the leaving group to provide **310** (**Scheme 7.6**). The use of high reaction temperatures and phase-transfer catalysts can increase the efficiency of halex reactions due to the increased solubility of the fluoride.[100]


Cl
Spray-dried KF
(Ph)4PBr
DMI
290 °C
CN
Cl
F
C
N
F
CN
+ KCl
308
309
Meisenheimer complex
310
91%
O
N
N
DMI
(1,3-dimethyl-2-imidazolidinone)

Scheme 7.6 A typical halex reaction.[101]

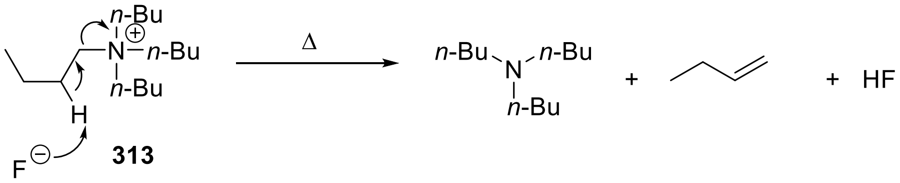
Due to the limitations of halex reactions, alternatives such as fluorodenitration have been developed. In fluorodenitration reactions, the nitro group functions as a leaving group however activation by other groups on the ring is still necessary to ensure a successful substitution reaction. This is demonstrated in the fluorodenitration of 2,3,5,6-tetrachloronitrobenzene **311** to provide **312** (**Scheme 7.7**).[102]


NO2
Cl
Cl
Cl
Cl
311
n-Bu4PF(HF)2
THF, 70 °C, 28 h
F
Cl
Cl
Cl
Cl
312
80%

Scheme 7.7 Illustration of fluorodenitration.

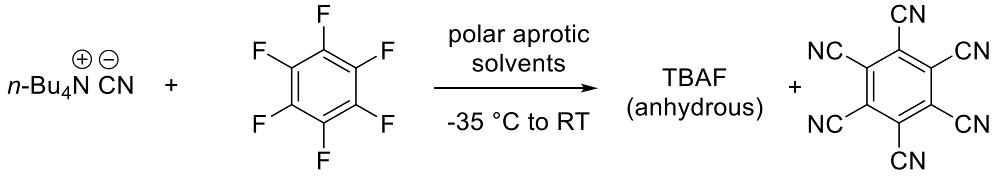
Other leaving groups that are suitable for displacement by fluoride in nucleophilic fluorination reactions are ammonium substituents. In particular, this methodology is useful for the introduction of radio labelled fluoride ($^{18}F^{-}$). Trimethylammonium groups tend to be more electron-withdrawing than nitro groups and undergo the transformation to fluorinated compounds more effectively than nitroarenes.[103]

Another commonly used nucleophilic fluorinating agent is commercially available tetrabutylammonium fluroride (TBAF) **313**, which is available as a trihydrate. The use of tetraalkylammonium as counterions for fluoride results in a reduction in the strength of the ionic bond as well as an increase in solubility in organic solvents.[104] In general, the presence of water in the trihydrate is problematic as it reduces the nucleophilicity of the fluoride by hydrogen bonding and side reactions are more likely to occur due to the presence of a source of hydroxide. Attempts to dry quaternary ammonium fluorides by heat usually results in competing E2-elimination (Hofmann elimination) as fluoride acts as a strong base under anhydrous conditions (**Scheme 7.8**).[105]



Scheme 7.8 Hofmann elimination observed when attempting to dry TBAF using heat.

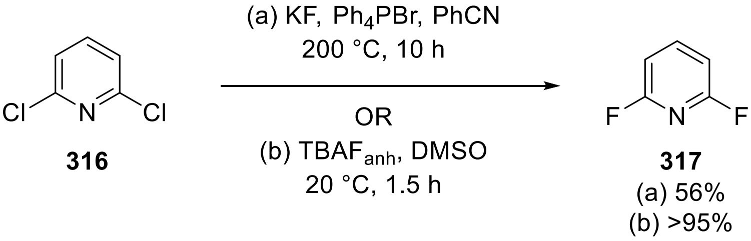
Due to the problems associated with generating anhydrous TBAF using heat, alternative methods were investigated. In 2005, Sun and DiMagno reported the successful synthesis of anhydrous TBAF directly from hexafluorobenzene **315** and tetrabutylammonium cyanide **314** in aprotic solvents (THF, acetonitrile or DMS) *via* nucleophilic aromatic substitution at low temperatures (**Scheme 7.9**).[106,107] The anhydrous TBAF was found to be stable under nitrogen at -35 °C for weeks, however it decomposed slowly in THF or in the solid state by Hofmann elimination if warmed above 0 °C. Conveniently, anhydrous TBAF can be prepared *in situ* in polar aprotic solvents at room temperature and used without isolation or purification.



314 **315** **313**
>95%
(70% isolated)

Scheme 7.9 Methodology for the successful preparation of anhydrous TBAF.

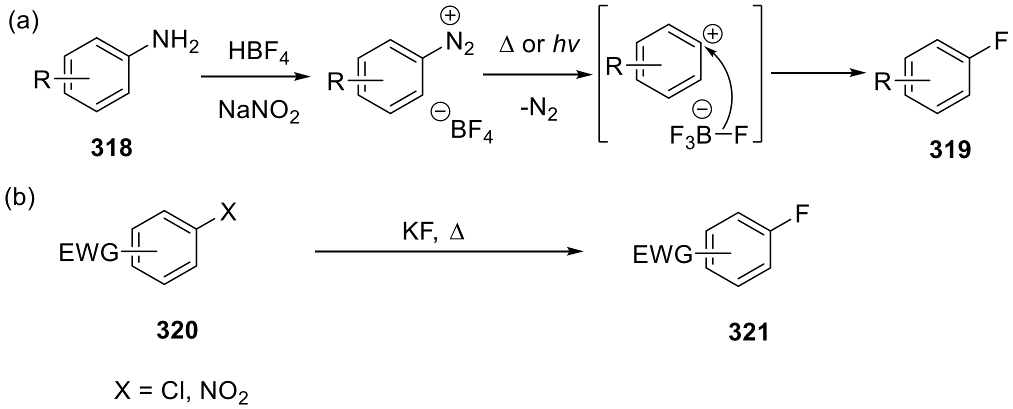
In general, fluorination reactions carried out using anhydrous TBAF have significant advantages over other nucleophilic fluorinating agents, with increased yield of fluorinated products obtained under less harsh conditions. An example of this is demonstrated in the synthesis of 2,6-difluoropyridine **317**. Under regular halex conditions, the fluorination of 2,6-dichloropyridine **316**, provided **317** in 56% yield after heating for 10 h at 200 °C (**Scheme 7.10 (a)**).[108] In contrast, when the same transformation was carried out using anhydrous TBAF the reaction proceeded much quicker and at a lower temperature, with **317** obtained in >95% yield after 1.5 h at 20 °C (**Scheme 7.10 (b)**).[107]



Scheme 7.10 Comparison of nucleophilic aromatic substitution under (a) regular halex conditions and (b) anhydrous TBAF.

Tetramethylammonium fluoride (TMAF) has been successfully used to provide arylfluroides and has an advantage over TBAF in that it lacks β-hydrogen atoms (meaning a Hofmann elimination pathway is avoided) and can be obtained as an anhydrous salt.[109]

Traditionally, aryl fluorides **319** are prepared from anilines **318** by the Balz-Schiemann reaction, a special class of nucleophilic aromatic fluorination reactions involving the pyrolysis of aromatic diazonium tetrafluoroborates ($ArN_2^+BF_4^-$) (**Scheme 7.11, (a)**).[110] Diazotisation of an aniline in the presence of hydrogen tetrafluoroborate (HBF_4) followed by either thermal or photochemical decomposition of the resulting diazonium tetrafluoroborate provides the desired aryl fluoride. However, due to the acidic conditions, the toxicity of the reagents and the potential for explosion, this reaction methodology is not ideal.[111] In an alternative methodology, aryl fluorides possessing electron-withdrawing groups **321** have been prepared by halex reactions, where electron-deficient aryl chlorides or nitroarenes **320** undergo nucleophilic aromatic substitution at high temperatures (**Scheme 7.11, (b)**).[100]



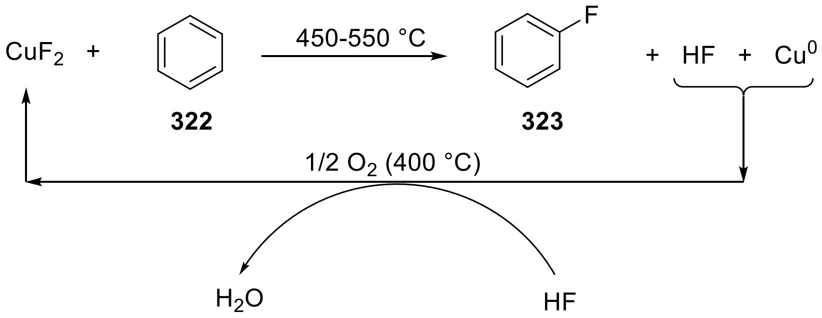
Scheme 7.11 (a) Balz-Schiemann preparation of aryl fluorides; (b) Halex process for the preparation of aryl fluorides.

7.3.3 Metal-catalysed fluorination

Reactions which are performed in the presence of a catalyst usually require much milder conditions as they selectively reduce the activation barriers from starting material to product. It is only recently that metal-catalysed cross-coupling fluorination reactions have been reported.[34] Conceptually, transition metal complexes have the potential to selectively reduce the barrier of activation for carbon-fluorine bond formation, making a thermodynamically favoured process more kinetically accessible. However due to the high strength of metal-fluorine bonds, design of appropriate catalysts to overcome the activation barrier to carbon-fluorine bond formation is challenging. It has been reported that the most challenging step in cross-coupling aryl fluorinations is the reductive elimination step, where both carbon and fluorine, initially bound to the metal, expel the catalyst and form a new carbon-fluorine bond.[112] In order for reductive elimination of two species to take place, there must be sufficient orbital overlap between both metal-ligand σ-bonds. Reductive elimination from arylpalladium alkyl complexes containing an electron withdrawing group occurs more slowly than complexes containing other functional groups.[36] Due to fluorine's high electronegativity and small size, metal-fluorine bonds are significantly polarised towards fluorine, meaning that electron density is lacking in the region where it is required for carbon-fluorine bond formation. The high polarisation of the metal-fluorine bond results in a significant ionic contribution to the bond, strengthening it and in turn increasing the energy barrier to carbon-fluorine reductive elimination. Methodologies which are used to form metal-fluorine bonds include ligand exchange with nucleophilic fluoride and oxidative addition with electrophilic fluorination reagents.[34]

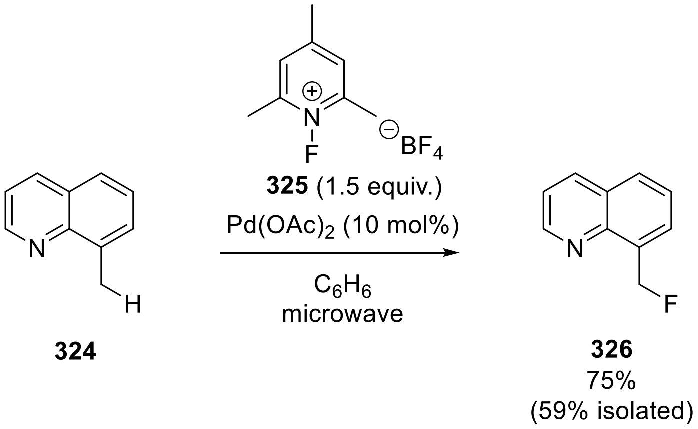
Choice of appropriate transition metals for carbon-fluorine bond formation can be directed by assessment of metal-fluorine bond strength: early transition metal fluorides generally have stronger metal-fluorine bonds compared to late transition metals. As a result, research into carbon-fluorine bond formation catalysis has largely focused on late transition metal complexes.[34]

In 2002, Subramanian and Manzer reported that the electrophilic reagent CuF_2 had been successfully used in the oxidation of benzene **322** to fluorobenzene **323**, albeit at high temperature (450–550 °C).[113] In this approach, the copper reagent can be regenerated after fluorination, however only structurally simple arenes appear to be amenable to this methodology. Also, low regioselectivity has been observed when substituents are present on the arene (**Scheme 7.12**).



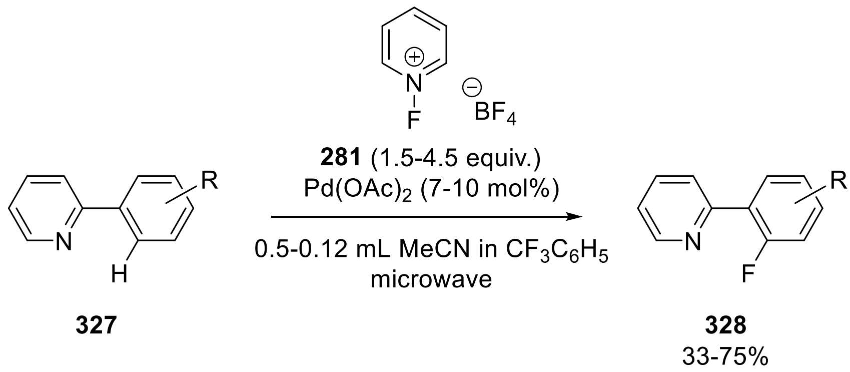
Scheme 7.12 Use of CuF_2 in the preparation of fluorobenzene.

By using directing groups, regioselective functionalisation of aryl carbon-hydrogen bonds can be achieved using milder conditions. When directing groups are attached to an aryl ring, they coordinate to a transition metal and preferentially lower the activation energy for carbon-hydrogen bond cleavage by positioning the transition metal in close proximity to specific carbon-hydrogen bonds.[114] The first report on the use of directing groups with transition metal catalysts to afford aryl fluorides was described in 2006 by Hull *et al.*[115] The authors described the first Pd-catalysed carbon-hydrogen activation/carbon-fluorine bond forming reaction, achieved under oxidising conditions using electrophilic (rather than nucleophilic) fluorinating reagents. Initial investigations were carried out on 8-methylquinoline as it undergoes facile quinoline-directed carbon-hydrogen activation at Pd^{II} to generate a σ-benzyl Pd species and therefore has proved to be an excellent substrate for related Pd-catalysed carbon-hydrogen activation/oxidative functionalisation reactions.[116-118] A screen was carried out using a variety of electrophilic fluorinating reagents and although initially yields were low to moderate, on carrying out the reaction under microwave irradiation, a vast improvement in yield was observed. *N*-Fluoro-2,4,6-trimethylpyridnium tetrafluoroborate **325** was deemed to be the most effective F^+ source of those tested, affording **326** from **324** in 75% yield (**Scheme 7.13**).



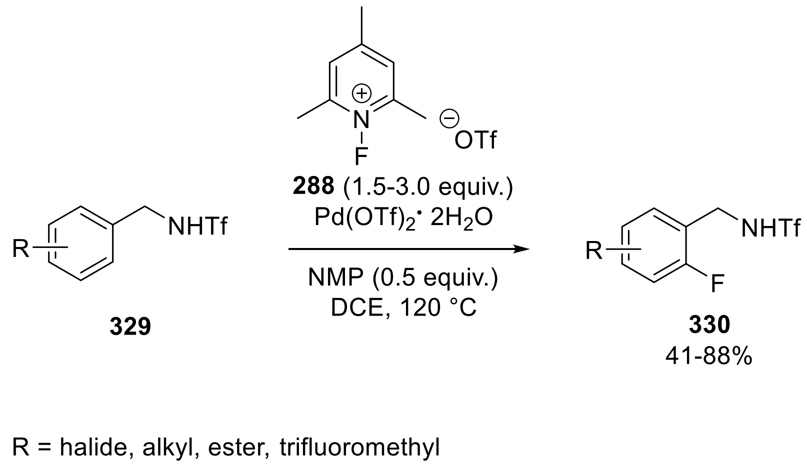
Scheme 7.13 Initial investigations into Pd-catalysed fluorination of carbon-hydrogen bonds.

The scope of the reaction was then investigated using quinoline/pyridine-directed benzylic and aromatic substrates **327**. A screen of fluorinating reagents determined *N*-fluoropyridinium tetrafluoroborate **281** to be the optimal source of F^+ in this case, and microwave irradiation under similar conditions to those used previously provided the *ortho*-fluorinated products **329** in moderate to good yield (**Scheme 7.14**). The methodology is tolerant of many common functional groups, including aryl halides, nonenolisable ketones and esters, trifluoromethyl substituents and methyl esters. The methodology is limited due to the harsh reaction conditions required as well as the necessity for *ortho*-directing groups and the need for blocking groups in the *ortho*'- or *meta*'-position to avoid difluorination.


281 (1.5-4.5 equiv.)
Pd(OAc)$_2$ (7-10 mol%)
0.5-0.12 mL MeCN in CF$_3$C$_6$H$_5$
microwave
327
328
33-75%

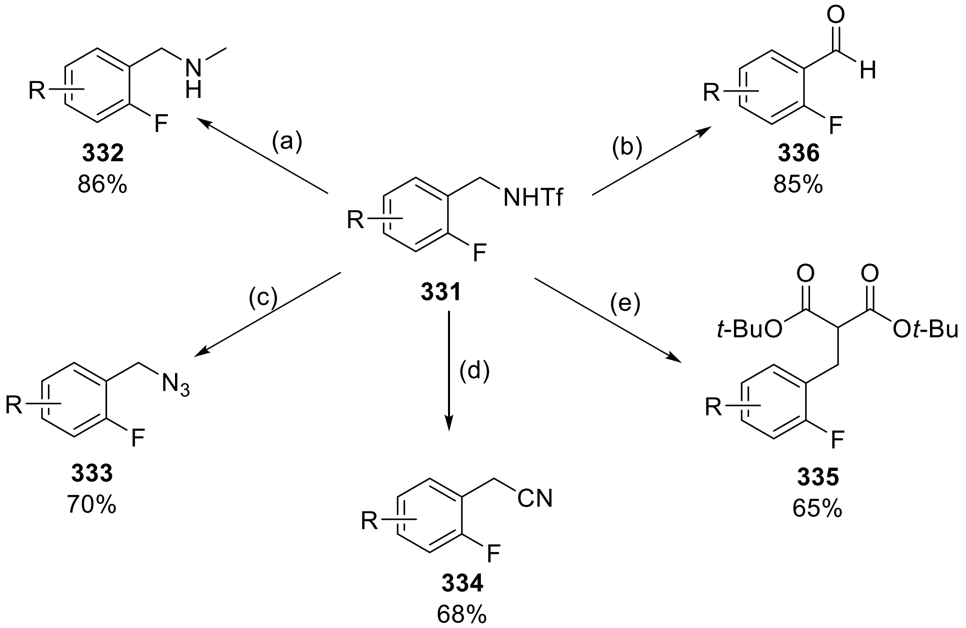
Scheme 7.14 Investigation of substrate scope in Pd-catalysed fluorination of carbon-hydrogen bonds.

In 2009, Wang *et al.* published their work on a similar palladium-catalysed directed electrophilic fluorination of aromatic carbon-hydrogen bonds of *N*-benzyltriflamide derivatives **329** to desired products **330** in moderate to good yield (**Scheme 7.15**).[119] In order for the reaction to proceed in synthetically useful yields, a new fluorinating reagent, *N*-fluoro-2,4,6-trimethylpyridinium triflate **288**, Pd(OTf)$_2$·2H$_2$O and 0.5 equivalents of NMP had to be employed. Although the reaction conditions required were not as harsh as those used by Hull *et al.*, the requirement of an *ortho*-directing group and *ortho*'- or *meta*'- blocking group was still necessary.


288 (1.5-3.0 equiv.)
Pd(OTf)$_2$· 2H$_2$O
NMP (0.5 equiv.)
DCE, 120 °C
329
330
41-88%
R = halide, alkyl, ester, trifluoromethyl

Scheme 7.15 Pd(OTf)$_2$·2H$_2$O-catalysed *ortho*-fluorination.

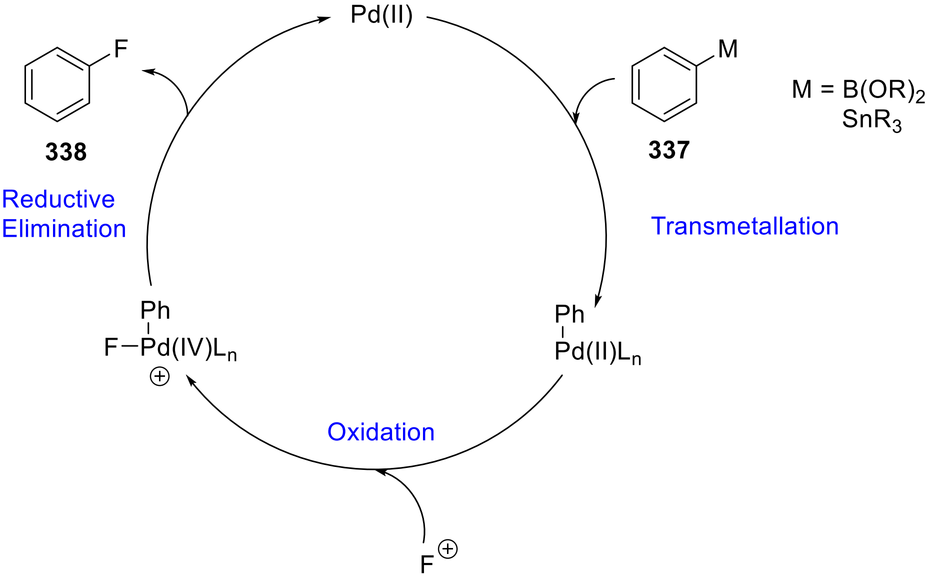
To improve the scope of aryl fluoride synthesis, the authors demonstrated that triflamide **331** can be readily transformed to a broad range of synthetically useful functional groups exploiting known reactivities.[120,121] These transformations provided access to five major classes of *ortho*-fluorinated synthons including benzylamine **332**, benzylazide **333**, phenylacetonitrile **334**, phenylpropanoate **335** and benzaldehyde **336** (**Scheme 7.16**).



Scheme 7.16 Transformation of triflamides: (a) (i) MeI (3.0 equiv.), K_2CO_3 (1.5 equiv.), acetone, reflux, 8 h; (ii) $LiAlH_4$ (2.0 equiv.), THF, reflux, 10 h; (b) (i) *ibid*; (ii) NaH (3.0 equiv.), DMF, 100 °C, 10 h; (iii) HCl (2N):THF (1:2), reflux, 2 h; (c-e) (i) NaH (1.0 equiv.), Tf_2O (1.0 equiv.), DCM, -78 °C–0 °C, 2 h; (ii) NaN_3, NaCN or NaCH(COO*t*-Bu)$_2$ (1.5 equiv.), HMPT, 24 °C, 8 h.

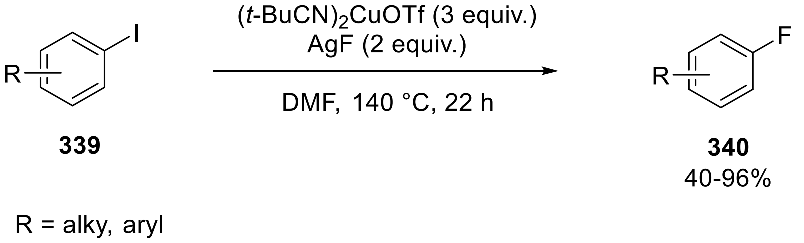
As yet, the mechanism for directed electrophilic fluorination has not been established. It is thought that after cyclopalladation, the crucial carbon-fluorine bond forming event could occur from a Pd(II) centre without change in the oxidation state of the metal (as in the electrophilic fluorination of an aryl Grignard reagent[122,123]) or from a higher oxidation state complex (such as Pd(III)[124] or Pd(IV)[125,126]) *via* carbon-fluorine reductive elimination. Preliminary mechanistic studies by Zhang *et al.* in 2015 on Pd(II)-catalysed fluorination of sp^3 C-H bonds suggest that the reaction mechanism proceeds *via* an inner sphere mechanism.[127] The results were consistent with direct C-F reductive elimination from a high-valent intermediate as previously predicted.

Palladium-mediated electrophilic fluorination without directing groups has also been reported, with the carbon-palladium bond introduced by transmetallation rather than cyclopalladation.[34] When no directing group is present on the substrate, the potential substrate scope is significantly larger than for directed fluorinations. However, this methodology does require the prefunctionalisation of substrates to allow the transmetallation step to occur. Oxidation of an arylpalladium(II) complex with an electrophilic fluorinating reagent can produce a high-valent arylpalladium(IV) complex which can then undergo reductive elimination to form a carbon-fluorine bond (**Scheme 7.17**). The reductive elimination from Pd(IV) species occurs much easier than from Pd(II) species.[128]



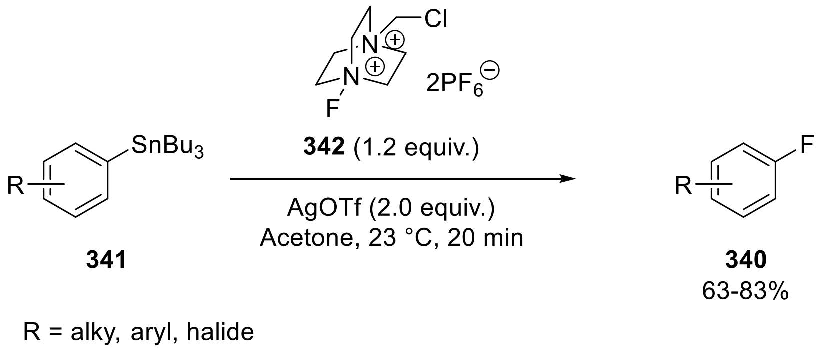
Scheme 7.17 General scheme of Pd-mediated electrophilic fluorination.

A 2012 publication by Fier and Hartwig reported on the fluorination of a functionally diverse set of aryl iodides with a simple copper reagent and a nucleophilic fluoride source.[129] The authors hypothesised that reductive elimination from an arylcopper(III) fluoride species would be enabled by a non-coordinating counterion and weakly donating ligands. Initial investigation by the team showed that when the reaction was carried out in the absence of copper, no reaction occurred, meaning a direct reaction between the aryl fluoride and aryl iodide did not take place. The authors next investigated the effect of nitrile ligands and counterions on the halex reaction, with the results showing that reactions conducted with *t*-BuCN-ligated CuOTf were more reproducible than those carried out with *t*-BuCN-ligated $CuSbF_6$. Also, in order for the reaction to occur in high yield, it was necessary to have an excess of AgF in the reaction. Investigation of substrate scope into the conversion of aryl iodides **339** into aryl fluorides **340** showed that both electron-rich and electron-poor aryl iodides are suitable for the reaction (**Scheme 7.18**). Reactions carried out with sterically hindered aryl iodides were particularly successful, with corresponding aryl fluorides produced in almost quantitative yield. Esters, amides, aldehydes, ketones and indole heterocycles were all well tolerated under the reaction conditions. The authors proposed that the reaction occurs by oxidative addition to form a Cu(III) intermediate and carbon-fluorine reductive elimination from an arylcopper(III)fluoride.[129]



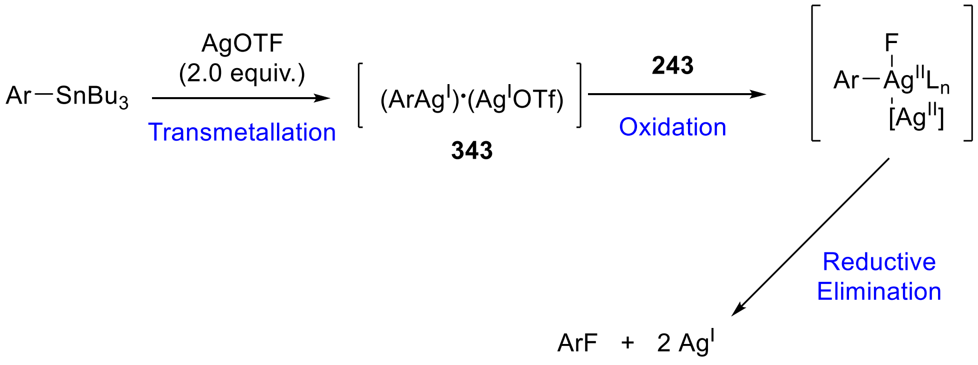
Scheme 7.18 Copper-mediated fluorination of aryl iodides.

In 2009, Furuya *et al*. reported their findings on silver-mediated fluorination of functionalised aryl stannanes,[130] based on literature reports of facile transmetallation from aryl stannanes to silver(I) nitrate.[131,132] Initial investigations using 2.0 equiv. of AgOTf as the Ag(I) source and 1.2 equiv. of Selectfluor® in acetone at 23 °C resulted in the aryl fluoride being isolated in 70% yield after 20 min. When the fluorinating agent F-TEDA-PF_6 **342** was used instead of Selectfluor®, the yield of fluorinated product increased to 83%. The authors suggested that the increase in yield observed may be due to arylation of the tetrafluoroborate anion of Selectfluor® by the aryl stannane to afford aryl borates.[133] A wide range of aryl stannane substrates **341** were subjected to the reaction conditions to provide arylfluorides **340** in good to high yield (**Scheme 7.19**). The silver-mediated fluorination tolerates electron-rich, electron-poor, *ortho,ortho*-disubstituted and heterocyclic aromatics, as well as protic functional groups. The authors then successfully applied the methodology to late-stage fluorination of biomedically active aromatics such as camptothecin and quinine.


Cl
N⊕
N⊕
F
2PF6⊖
SnBu3
R
342 (1.2 equiv.)
AgOTf (2.0 equiv.)
Acetone, 23 °C, 20 min
F
R
341
340
63-83%
R = alky, aryl, halide

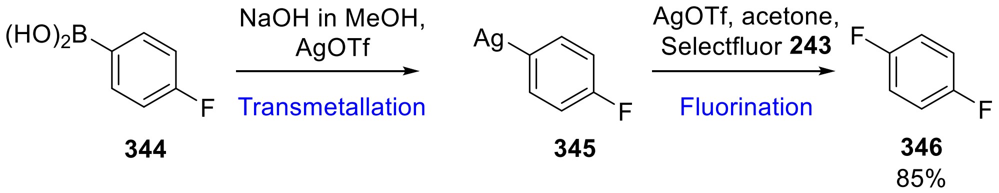
Scheme 7.19 Electrophilic fluorination of aryl stannanes.

Preliminary investigations into the mechanism of the reaction suggest that fluorination may occur from a redox active aryl silver species with the participation of more than one silver atom per carbon-fluorine bond formation event, in a bimetallic oxidation-reductive elimination process (**Scheme 7.20**). The postulated intermediate **343** was observed by ^{1}H and ^{19}F NMR but not isolated. The reductive elimination step could proceed in one of two ways: either in a two electron process or *via* one-electron redox participation of two silver atoms. Although the authors did not observe high-valent silver fluoride intermediates, the addition of various radical scavengers did not have an effect on the yield of fluorinated products obtained, suggesting that that formation of free radical intermediates is unlikely. A disadvantage of this method is the toxicity of the organotin compounds and consistent formation of 10-20% byproduct, resulting from photodestannylation, which can make purification problematic.


Ar—SnBu3
AgOTF
(2.0 equiv.)
Transmetallation
(ArAgI)·(AgIOTf)
343
243
Oxidation
F
Ar—AgIILn
[AgII]
Reductive
Elimination
ArF + 2 AgI

Scheme 7.20 Proposed bimetallic oxidation-reductive elimination.

This silver(I) triflate methodology was then further developed to allow the fluorination of boronic acids and esters using Selectfluor®.[134] The reaction uses commercially available reagents, does not require the addition of exogeneous ligands, displays high functional group tolerance and results in no detectable byproducts resulting from carbon-hydrogen bond formation. Upon addition of a methanol solution of sodium hydroxide to arylboronic acids such as **344**, arylsilver complexes such as **345** were formed *via* transmetallation. Subsequent evaporation of methanol and addition of acetone and Selectfluor® for the fluorination step provided arylfluorides such as **346** in a one pot synthesis (**Scheme 7.21**). Acetone was deemed to be the best solvent for the fluorination step, however this was not a suitable solvent for the transmetallation step. Methanol was necessary to ensure efficient transmetallation but cannot be used for the fluorination step due to the formation of aryl methyl ethers instead for arylfluorides. Finally, the authors found it necessary to include 3 Å molecular sieves as the presence of water resulted in undesired phenol formation. Electron-rich, electron-poor, protic, halogenated and *ortho,ortho*-disubstituted arenes and heterocycles were all tolerated under the reaction conditions. Unfortunately, the stoichiometric accumulation of the thermally unstable arylsilver complex proved to be increasingly problematic when more complex substrates were exposed to the reaction conditions, limiting the methodology somewhat. The authors suggested that the mechanism may proceed *via* a high-valent silver species from which carbon-fluorine reductive elimination can occur.



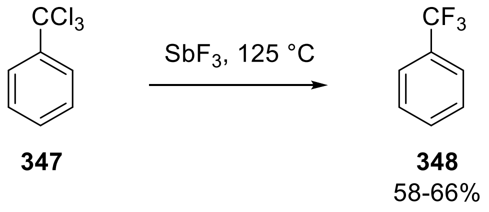
Scheme 7.21 One-pot fluorination of (4-fluorophenyl)boronic acid.

7.4 Methods of trifluoromethylation

The trifluoromethyl group is an important structural moiety which is present in many classes of bioactive organic molecules, most importantly in pharmaceuticals and agrochemicals.[135,136] The $-CF_3$ group is becoming increasingly popular in compounds synthesised for biological application due to its high lipophilicity, powerful electron-withdrawing properties and small size.[4,137,138] *In vivo* metabolism of drugs by cytochrome P450 oxidases can be problematic as it increases the rate of drug excretion from the body.[139] A common strategy to protect against *in vivo* metabolism is to incorporate the trifluoromethyl moiety at specific sites of drug candidate molecules.[140] When incorporated into small molecules, the trifluoromethyl group can often enhance drug efficacy by promoting electrostatic interactions with targets, improving cellular permeability and increasing robustness towards oxidative metabolism of the drug.[141-143] Trifluoromethylation has also found application in the dye industry, where trifluoromethylation of chromophores results in increased light fastness (resistance of colours to fading, changing shade or darkening on exposure to light) as well as a shift in colour in comparison to their non-fluorinated counterparts.[144,145] Polymers which contain the trifluoromethyl moiety have been reported to possess improved chemical and thermal stability, increased solubility and diverse mechanical and electrical properties.[146] Methodologies for the trifluoromethylation of various substrates have been extensively reviewed in the literature.[147,30,148,136,34]

The introduction of the trifluoromethyl moiety at a late stage in a synthesis is particularly challenging, as many of the synthetic methodologies to incorporate the $-CF_3$ group require harsh reaction conditions which can only be applied to fairly simple molecules. In many cases, if more complex trifluoromethylated molecules are required, it is necessary to start from a simple molecule containing the trifluoromethyl moiety and build the structure around it.[149]

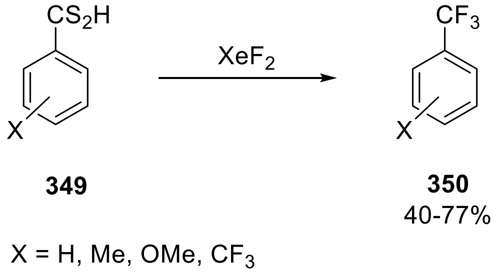
The first method of trifluoromethylation was a halex reaction developed by Swarts in 1898.[21] Here, antimony trifluoride was used to convert benzotrichloride **347** to benzotrifluoride **348** (**Scheme 7.22**).[21] Some years later, it was reported that anhydrous hydrogen fluoride could also be used to synthesise **348** from **347**.[150] A drawback of these methods is that they show low functional-group tolerance, the reagents used are hazardous and environmentally unfriendly and large volumes of chlorinated waste are formed.



Scheme 7.22 Early use of antimony trifluoride as a method of trifluoromethylation.

Attempts were made to develop alternative reaction conditions for this transformation and aluminium trichloride/fluorotrichloromethane[151-153] and silver tetrafluoroborate[154] proved to be successful substitutes for the more hazardous reagents.

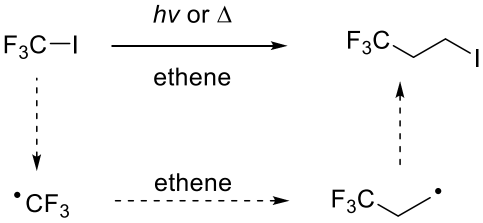
In a similar manner, aromatic orthothioesters can be used to synthesise aromatic trifluoromethyl compounds in two steps by the sequential addition of 1,3-dibromo-5,5-dimethylhydantoin (DBH) or NBS followed by HF in pyridine.[155] The reaction is carried out at a low temperature and yields range from moderate to good (34–67%) depending on the nature of the substrate. The preparation of aromatic trifluoromethyl compounds **350** has also been accomplished in moderate to good yield (40-77%) by reaction of xenon difluoride and aromatic dithiocarboxylic acids **349** (**Scheme 7.23**), however the high cost and toxicity of xenon difluoride diminishes the attractiveness of this methodology.[156]



Scheme 7.23 Trifluoromethylation of aromatic dithiocarboxylic acids with xenon difluoride.

Sulfur tetrafluoride has also been successfully utilised for trifluoromethylation reactions,[157] however application is rather limited due to the need for specialised equipment to minimise exposure to sulfur tetrafluoride and hydrogen fluoride.[158] Diethylaminosulfur trifluoride (DAST), a more easily handled derivative of sulfur tetrafluoride has been reported to trifluoromethylate benzoic acid to provide benzotrifluoride in 50% yield in the presence of sodium fluoride.[159]

In the late 1940s, Haszeldine published the first report on trifluoromethylation *via* a radical mechanism.[160] In his findings, he states that trifluoromethyl radicals are generated from iodotrifluoromethane through C-I bond homolysis upon irradiation or heating. In the presence of ethene, 3-iodo-1,1,1-trifluoropropane was observed as the major product from a radical addition/iodine transfer reaction (**Scheme 7.24**). The reaction proceeds by the addition of a trifluoromethyl radical to ethene to form the corresponding adduct radical, which then abstracts an iodine atom from the starting iodotrifluoromethane resulting in the observed product and the trifluoromethyl radical which propagates the chain.

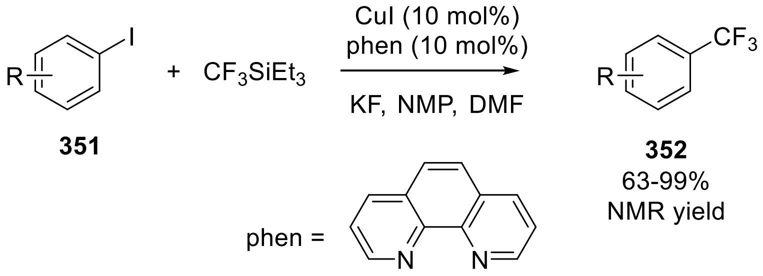


Scheme 7.24 Radical trifluoromethylation of ethene published by Haszeldine in 1949.

Due to the gaseous nature of iodotrifluoromethane, it is not a convenient reagent to work with. It is not easy to control the concentration, in particular when reactions are carried out at higher temperatures. This prompted the investigation of more convenient alternatives, including the electrochemical oxidation of trifluoroacetic acid to allow the clean generation of trifluoromethyl radicals[161-163] and the use of Barton thiohydroxamic trifluoromethyl ester as a precursor of trifluoromethyl radicals.[164]

7.4.1 Metal-catalysed trifluoromethylation

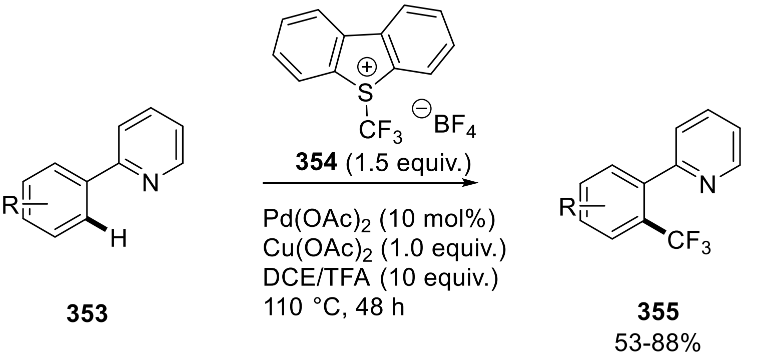
In 1969, the first cross-coupling reaction to form trifluoromethylated aromatic compounds was reported.[165] Upon heating iodobenzene and trifluoroiodomethane in DMF with activated copper at 150 °C, benzotrifluoride was obtained in 45% yield. This initial finding prompted more exploration in the area and modifications to reaction conditions and reagents have been reported.[166,167] The first copper-catalysed trifluoromethylation of aromatic compounds was accomplished in 2009, using a diamine ligand to form a copper(I)-diamine complex.[168] Electron-poor aryl iodides **351** were converted to benzotrifluorides **352** with a catalytic amount of CuI and 1,10-phenanthroline (**Scheme 7.25**). Although the mechanism of the reaction has not been investigated, it is proposed that it proceeds *via* generation of a trifluoromethylcopper ("$CuCF_3$") complex,[169-171] followed by oxidative addition to form an arylcopper(III) intermediate.[172-175]



Scheme 7.25 First reported copper-catalysed trifluoromethylation of aromatic compounds.

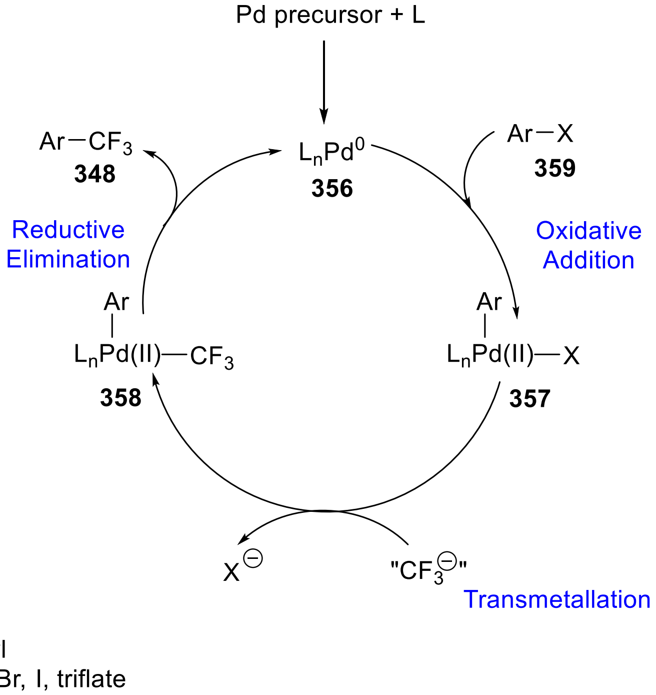
Copper-catalysed trifluoromethylation of aryl iodides has also been accomplished with potassium (trifluoromethyl)trimethoxyborate as a nucleophilic trifluoromethylation source. This reagent provides benzotrifluorides from aryl iodides in high yields in mild, base-free conditions in the presence of a catalytic amount of CuI and 1,10-phenanthroline.[176]

Wang *et al.* recently reported Pd(II)-catalysed arene trifluoromethylation *via* C-H activation using a directing group strategy (**Scheme 7.26**).[177] The authors found that the use of an electrophilic trifluoromethylating reagent *S*-(trifluoromethyl)dibenzothiophenium tetrafluoroborate **354** is more reactive with the ArPd(II) species than the corresponding triflate reagent. TFA was identified as a crucial additive for the success of the trifluoromethyl bond-forming protocol and $Cu(OAc)_2$ (the role of which remains to be elucidated) was found to be effective for enhancing the catalytic turnover. Electron-donating groups on substrates **353** were well-tolerated, as are moderately electron-withdrawing groups such as Cl. On the other hand, substrates which contained strong electron-withdrawing groups provided trifluoromethylated products in low yield. Various heterocycles such as pyridine, pyrimidine, imidazole and thiazole can be used as the directing group to provide products **355**.[177] Although this methodology obviates the need for pre-functionalisation, the requirement of a directing group as well as low functional group tolerance limits the general applicability of the reaction.



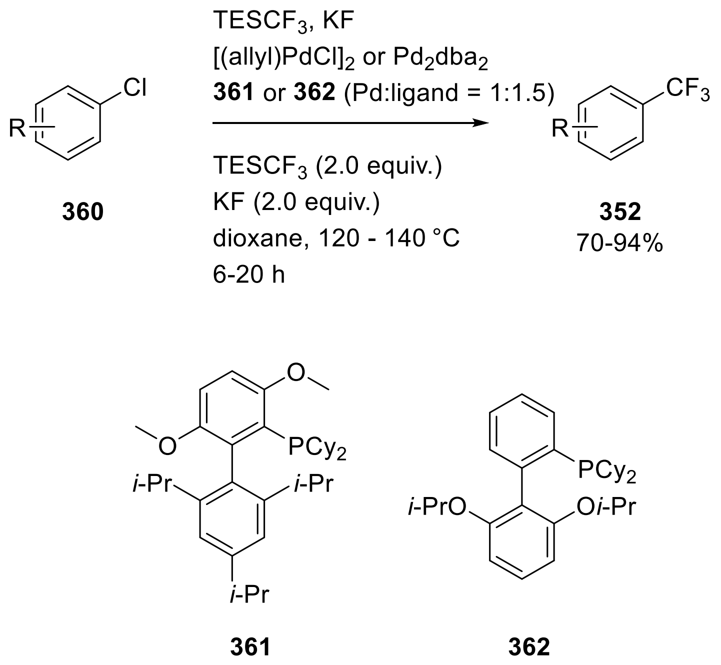
Scheme 7.26 Pd-catalysed directed electrophilic Ar-CF_3 bond forming reaction.

In 2010, Cho *et al.* reported the first palladium-catalysed trifluoromethylation of aryl halides using (trifluoromethyl)triethylsilyane ($TESCF_3$) and potassium fluoride (**Scheme 7.27**).[178] The authors proposed that this methodology would have the potential to overcome the limitations associated with previously reported copper-catalysed trifluoromethylations: Less harsh reaction conditions are required due to the use of a trifluoromethyl source as a transmetallating agent and a wide substrate scope for the reaction is possible. Previous attempts at this process have proven futile, with several complexes of type **358** bearing bidentate ligands providing either no[179,180] or only trace amounts[181] of benzotrifluoride products **348**. When chelating biphosphine ligands 1,2-bis(diphenylphosphino)ethane (dppe) and 1,3-bis(diphenylphosphino)propane (dppp) were employed the results were also disappointing, with benzotrifluoride **348** obtained in 10-60% yield after 64 h at 145 °C.[180] Benzotrifluoride could be obtained within 3 h *via* quantitative conversion of the complex XantphosPd(Ph)(CF_3), however replacement of the Xantphos ligand in **359** with trifluoromethyl ions competes with transmetallation to **358** and as a result, no catalytic system was reported.[182,183] Complexes **357** were treated with (trifluoromethyl)trimethylsilyane and a fluoride source such as CsF (necessary to activate the silyl group for transmetallation) to form complexes **358**, with the driving force of the reaction being the formation of a silicon-fluorine bond.[179-181] When using trifluoromethylsilanes in the presence of fluoride, fluoride-initiated self-decomposition of R_3SiCF_3 can occur, resulting in R_3SiF and difluorocarbene.[184] In catalytic reactions where high temperatures are necessary to promote reductive elimination from **358** to **348**, transmetallation must be significantly faster than this self-decomposition process in order for the reaction to proceed efficiently.


Pd precursor + L
L_nPd^0
356
Ar−X
359
Oxidative Addition
$L_nPd(II)—X$ with Ar
357
"$CF_3^\ominus$"
Transmetallation
$X^\ominus$
$L_nPd(II)—CF_3$ with Ar
358
Reductive Elimination
$Ar—CF_3$
348
Ar = aryl
X = Cl, Br, I, triflate

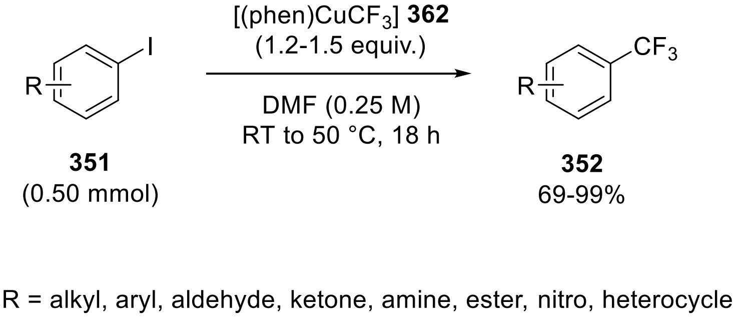
Scheme 7.27 Generalised catalytic cycle for Pd-catalysed aryl trifluoromethylation.

Previous work by Wang *et al.* has shown that trifluoromethylated arenes can be achieved from pyridine derivatives *via* C-H activation, oxidation of the Pd(II) intermediate with an electrophilic CF_3^+ source and a final reductive elimination, but the substrate scope was limited to compounds containing specific directing groups.[177] In contrast, the methodology developed by Cho *et al.* was suitable for a wide range of substrates, however those with protic functional groups did not undergo trifluoromethylation, likely due to the fact that such functional groups accelerate decomposition of $TESCF_3$ or aryl(trifluoromethyl)palladium(II) and arylpalladium(II) fluoride complexes.[178] As the nucleophilic trifluoromethyl group is slowly generated *in situ* in the reaction with $TESCF_3$ and KF, the potential for the occurrence of side reactions is diminished. In a typical experiment (**Scheme 7.28**), a solution of the palladium source and ligand **361** or **362** in dioxane was added to spray-dried KF and the aryl chloride. $TESCF_3$ was added and the reaction allowed to stir at 120–140 °C for 6–20 h. Due to the hygroscopic nature of KF, reactions were carried out in a nitrogen-filled glovebox to prevent the hydrolysis of $TESCF_3$ during the course of the reaction. A wide range of both electron-rich and electron-poor substrates **360** were exposed to the reaction conditions to provide trifluoromethylated products **352** in good to high yield. Various heteroaromatic substrates have also been successfully trifluoromethylated using these reaction conditions, such as indoles, carbazoles, quinolines and benzofuranes. It was found that substrates containing aldehyde or ketone moieties were not suitable for this transformation. Furthermore, substrates which contained unprotected OH or NH groups proved to be problematic under the reaction conditions, presumably due to protonation of the CF_3 anion to form fluoroform, reaction at the silicon centre of $TESCF_3$ and/or competing protonation at the palladium centre.[178]


Cl
R
360
TESCF3, KF
[(allyl)PdCl]2 or Pd2dba2
361 or 362 (Pd:ligand = 1:1.5)
TESCF3 (2.0 equiv.)
KF (2.0 equiv.)
dioxane, 120 - 140 °C
6-20 h
CF3
R
352
70-94%
O
O
PCy2
i-Pr
i-Pr
i-Pr
361
PCy2
i-PrO
Oi-Pr
362

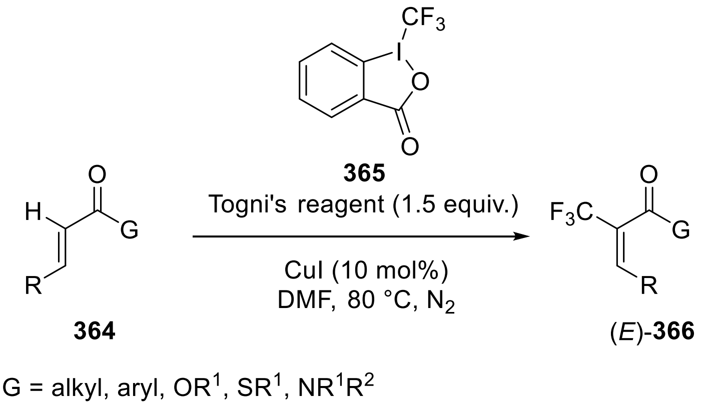
Scheme 7.28 Pd-catalysed trifluoromethylation of aryl and heteroaryl chlorides.

In 2011, a publication from Hartwig's group provided another methodology for the introduction of a trifluoromethyl group, in this case from a pre-functionalised iodinated starting material.[185] The authors isolated a trifluoromethylcopper(I) reagent ligated by 1,10-phenanthroline **363** that reacts with a range of aryl halides **351** in polar, aprotic solvents at mild temperatures (RT to 50 °C) to provide trifluoromethylated products **352** in good to excellent yield (**Scheme 7.29**). Compound **363** can be synthesised, isolated and stored under nitrogen or prepared *in situ* with similar yields of trifluoromethylated product obtained in each case. The reaction conditions are reported to tolerate a wide range of functional groups, basic heterocycles and substrates with steric bulk.



Scheme 7.29 Hartwig's trifluoromethylation using [(phen)CuCF$_3$].

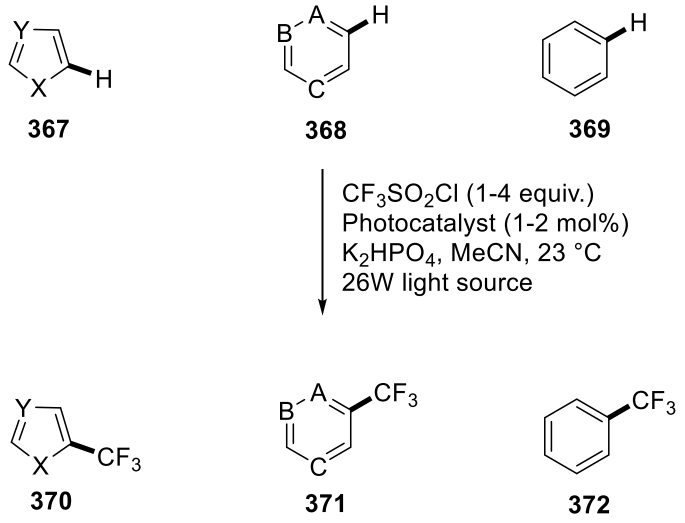
An exciting advance in the area of metal-catalysed trifluoromethylation was recently reported by Fang *et al.*, who developed a copper(I)-catalysed, regioselective α-trifluoromethylation of a diverse range of α,β-unsaturated carbonyl compounds **364** using Togni's reagent **365** to provide (*E*)-**366** in good to high yields (**Scheme 7.30**).[186] The authors carried out investigations into the mechanism of the reaction. A radical mechanism was proposed due to the significant inhibition of the reaction observed based on experiments using a radical scavenger.


CF3
I
O
O
365
Togni's reagent (1.5 equiv.)
CuI (10 mol%)
DMF, 80 °C, N2
H
O
G
R
364
F3C
O
G
R
(E)-366
G = alkyl, aryl, OR1, SR1, NR1R2

Scheme 7.30 Use of Togni's reagent to directly trifluoromethylate α,β-unsaturated carbonyl compounds.

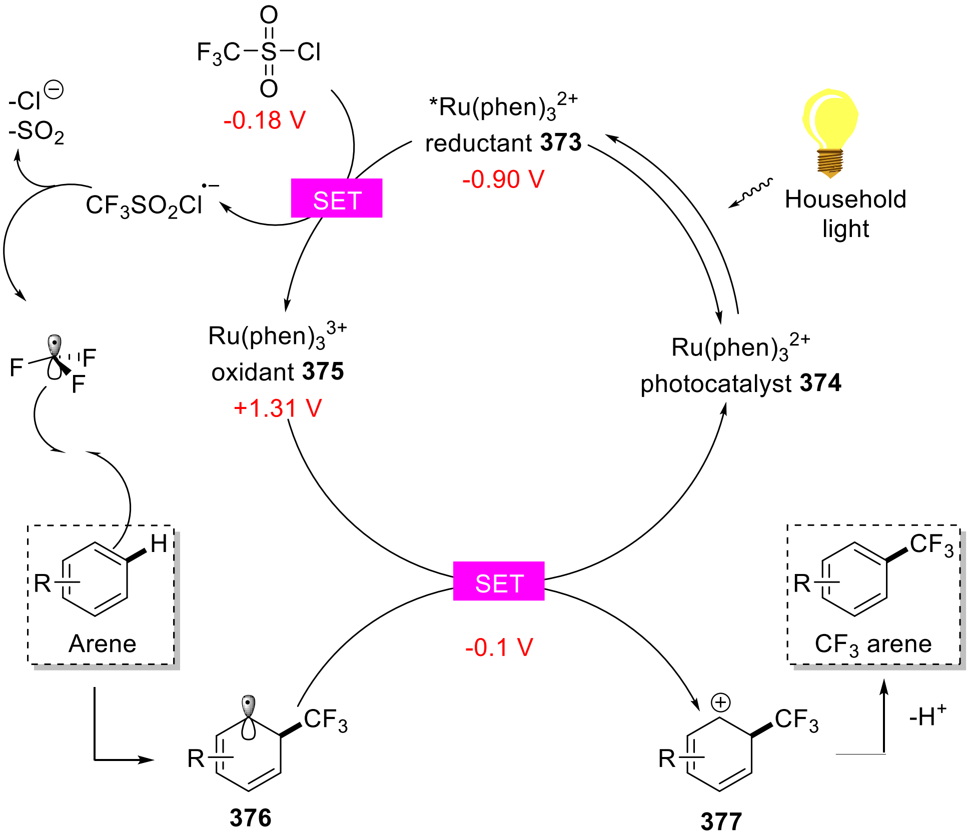
7.4.2 Trifluromethylation by means of photoredox catalysis

In 2011, Nagib and MacMillan reported a novel methodology for trifluoromethylation of unactivated arenes and heteroarenes through a radical-mediated mechanism using commercial photocatalysts and a household light bulb.[187] The authors sought to take advantage of photoredox catalysis, which provides a mild, efficient method for accessing electrophilic radicals, such as $^{\bullet}CF_3$, *via* photosynthesis-inspired redox chemistry.[188-190] This methodology employs polypyridyl organometallic complexes whose excitation at room temperature by a source of light (such as a common household light bulb) provides a strongly oxidising or reducing catalyst that can rapidly engage a variety of substrates to provide high energy, reactive species.[191] Two redox catalysts were used in the study, the choice of which depended on of the starting material they wished to functionalise. $Ru(phen)_3Cl_2$ was chosen as catalyst for reaction with five-atom electron rich heteroarenes **367** to provide trifluoromethylated products **370**. $Ir(Fppy)_3$, an iridium photocatalyst with a longer lived excitation state, was used with six-atom electron deficient heteroarenes **368** and unactivated arenes **369** to afford products **371** and **372** (**Scheme 7.31**). Triflyl chloride was the reagent of choice to introduce the trifluoromethyl moiety. The authors noted that the reaction tolerates several equivalents of water or alcohol with only a moderate decrease in efficiency. Trifluoromethylated products were obtained in excellent yields of up to 94% and the methodology has been successfully applied to the trifluoromethylation of biologically active molecules.[187] A disadvantage of this route is that the photoredox catalysts used are costly, although only 0.01 – 0.02 equivalents are required per reaction.



Scheme 7.31 Trifluoromethylation of unactivated arenes and heteroatoms by means of a photoredox catalyst.

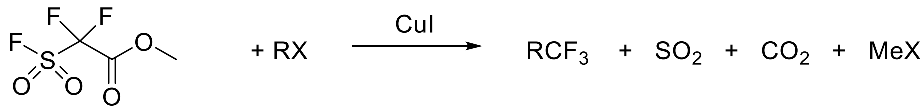
Nagib and MacMillan have proposed a mechanism for the reaction (**Scheme 7.32**). In the proposed photoredox catalytic cycle, initiation occurs *via* initiation of photocatalyst **374** to excited state **373** with a household light bulb. The authors assumed that SET reduction of triflyl chloride would be simultaneous with the oxidation of *Ru(phen)$_3^{2+}$ **374** to Ru(phen)$_3^{3+}$ **375**. This produced a CF_3SO_2Cl radical anion, which would be unstable and would quickly collapse to produce stabilised $^{\cdot}CF_3$, a process that should be entropically driven due to the release of SO_2 and chloride.[192] The resultant electron-deficient trifluoromethyl radical is an ideal candidate to add to the most electron-rich position of an arene or heteroarene in a selective manner. The resultant cyclohexadienyl radical **376** then undergoes a second SET with the now strongly oxidising Ru(phen)$_3^{3+}$ photocatalyst **375** which regenerates the ground-state photocatalyst **374**. Finally, deprotonation of **377** with a suitable base provides the desired trifluoromethylated arene. Overall, the reaction proceeds in a redox, catalytic fashion without the need for pre-functionalisation.[187]


F3C–SO2–Cl
-0.18 V
-Cl⊖
-SO2
CF3SO2Cl•−
SET
*Ru(phen)3 2+
reductant 373
-0.90 V
Household light
Ru(phen)3 3+
oxidant 375
+1.31 V
Ru(phen)3 2+
photocatalyst 374
Arene
CF3 arene
SET
-0.1 V
-H+
376
377

Scheme 7.32 Proposed mechanism for the direct trifluoromethylation of aryl C-H bonds *via* photoredox catalysis.

7.4.3 Methyl fluorosulfonyldifluoroacetate (MFSDA)

MFSDA was first reported as a suitable reagent for trifluoromethylation in 1989 by Chen and Wu.[193] It is an easy to handle liquid which is readily obtained from the corresponding acid fluoride, which is a starting material for producing commercial Nafion H® ion-exchange resins.[194] Gratifyingly, MFSDA is commercially available from a number of chemical suppliers at reasonable cost. However, if necessary, it may be prepared in the laboratory *via* a number of different methods. MFSDA can be prepared by reacting 3,3,4,4-tetrafluoro[1,2]oxathiethane-2,2-dioxide with sodium methoxide;[194] in two steps from difluoro(fluorosulfonyl)acetic acid;[195] or by the dropwise addition of methanol to trimethylsilyl fluorosulfonyldifluoroacetate.[196] Trifluoromethylated compounds **380** were obtained upon heating a solution of MFSDA **378**, aryl, alkenyl or alkyl halide **379** and a catalytic amount of CuI (12 mol%) in DMF at 60-80 °C for 2-6 h (**Scheme 7.33**).[193]



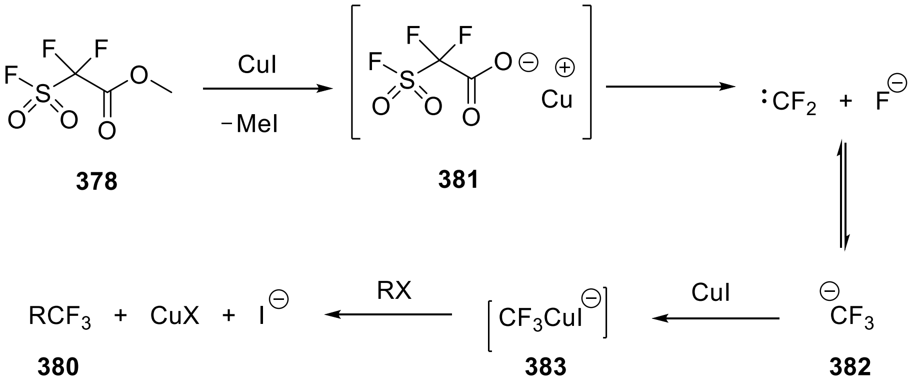
378 **379** **380**

R = aryl, alkenyl, alkyl
X = I, Br, Cl

Scheme 7.33 Reaction of MFSDA with halogenated compounds to form trifluoromethylated products.

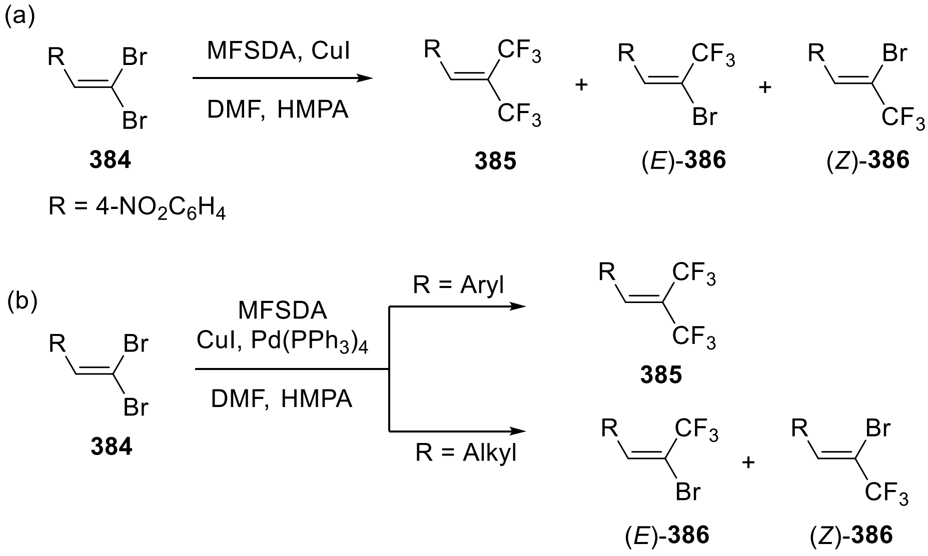
Chen and Wu deemed the order of reactivity of the halides to be RI > RBr > RCl, with the bromo derivatives being quite efficient and the chloro derivatives more sluggish. The presence of CuI is essential to the success of the reaction. When the reaction was carried out without a catalytic amount of CuI, no reaction occurred. The authors also tested KI as an alternative to CuI, however in this reaction the alkyl halide was recovered and fluoroform was the major product. DMF was used as solvent for the reaction, however the authors noted that DMSO could be used to provide products in comparable yields.[193]

The accepted mechanism for the reaction (**Scheme 7.34**) comprises an initial step involving the formation of a copper salt **381** from MFSDA **378** with the elimination of methyl halide. The salt then decomposes to release difluorocarbene and a fluoride ion, which are in equilibrium with a DMF stabilised trifluoromethyl anion **382**. In the presence of CuI, the equilibrium shifts to form $[CF_3CuI^-]$ **383**, which reacts with a halogenated starting material **379**, to provide trifluoromethylated product **380**, following release of CuX and I^-.[193] A radical mechanism has been ruled out on the basis of experimental and computational data.[197]



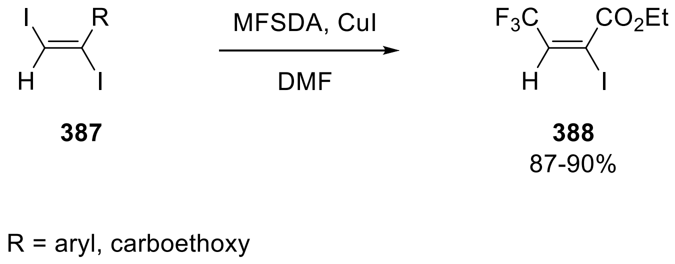
Scheme 7.34 Accepted mechanism for trifluoromethylation reactions using MFSDA.

MFSDA has been used to trifluoromethylate 1,1-dibromo-1-alkenes **384**, which resulted in a novel type of trifluoromethyl-containing building block.[198] When the reaction was carried out under the usual conditions required for trifluoromethylation using this reagent, a mixture of bistrifluoromethylated compound **385**, monotrifluoromethylated compounds (*E*)-**386** and (*Z*)-**386** along with unreacted **384** was isolated. These compounds were difficult to separate, with a ratio of 2:1:1 of **385**:(*E*)-**386**:(*Z*)-**386** (**Scheme 7.35, (a)**). Addition of a palladium catalyst ($Pd(PPh_3)_4$) in an attempt the provide a single product and improve the reaction efficiency greatly improved the bistrifluoromethylation, with **385** isolated in up to 90% (**Scheme 7.35, (b)**).[198]


(a)
MFSDA, CuI
DMF, HMPA
384
385
(E)-386
(Z)-386
R = 4-NO2C6H4
(b)
MFSDA
CuI, Pd(PPh3)4
DMF, HMPA
R = Aryl
R = Alkyl
384
385
(E)-386
(Z)-386

Scheme 7.35 Trifluoromethylation of 1,1-dibromo-1-alkenes (a) without palladium catalyst and (b) with palladium catalyst.

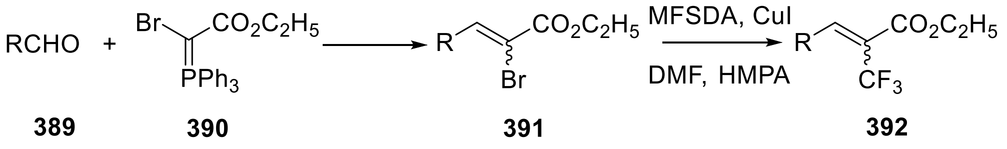
Stereospecific and highly regioselective trifluoromethylation of *trans*-1,2-diiodoalkenes was achieved in excellent yields using MFSDA.[199] In cases where the diiodoalkene is substituted by an aryl or a carboethoxy group **387**, the iodo substituent at the terminal position is replaced by a trifluoromethyl group to provide products **388** in high yield in a regio- and stereospecific manner (**Scheme 7.36**).


MFSDA, CuI
DMF
387
388
87-90%
R = aryl, carboethoxy

Scheme 7.36 Stereospecific and highly regioselective trifluoromethylation of *trans*-1,2-diiodoalkenes.

In 2000, Zhang *et al*. demonstrated that MFSDA could be used to provide trifluoromethylated α,β-unsaturated esters in very good yields.[200,201] In this route, aldehydes **389** were reacted with ylide **390** to provide α-bromo- α,β-unsaturated esters **391** with the (*Z*)-isomer as the major product. Trifluoromethylation was then accomplished using MFSDA to provide **392** in a mixture of (*E*)- and (*Z*)-

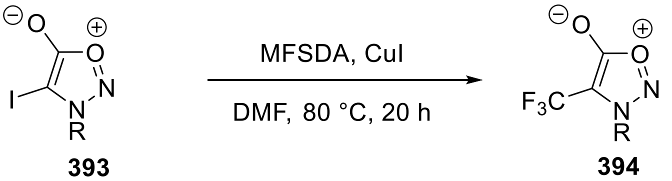
conformations (**Scheme 7.37**). This was later extended to a one-pot synthesis to provide a stereoselective synthesis of (*E*)-α-trifluoromethyl-α,β-unsaturated esters.[202]



R = alkyl, benzyl, 1,3-dioxolane

Scheme 7.37 Use of MFSDA to synthesis trifluoromethylated α,β-unsaturated esters.

MFSDA has also been used in the trifluoromethylation of 4-iodosydnones **393**, with products **394** being obtained in moderate to good yield (**Scheme 7.38**).[203] When the reaction was carried out with 4-iodo-*N*-phenylsydnone, the resultant trifluoromethylated product was obtained in 79% yield. The presence of an electron-donating *p*-methoxy phenyl group on the ring had little effect on the reaction and a similar yield was obtained (80%). When an electron-withdrawing *p*-nitro phenyl substituent was present, trifluoromethylation required a longer reaction time to provide the desired product in moderate yield (55%). Non-aromatic groups present on the nitrogen atom were also well tolerated under the reaction conditions.



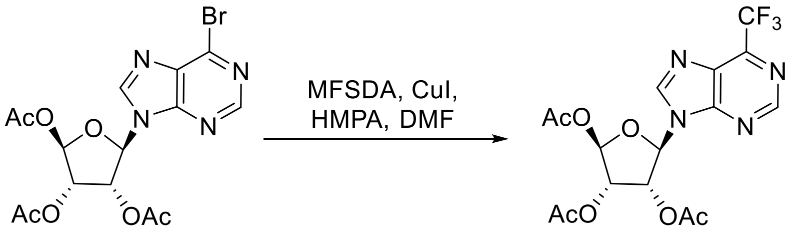
48-80%

R = Me, Bn, C_6H_5, 4-OMeC_6H_4, 4-$NO_2C_6H_4$

Scheme 7.38 Trifluoromethylation of 4-iodosydnones using MFSDA.

In 2001 the first report involving MFSDA with nucleosides was published.[204] In this paper, the authors reported the synthesis and characterisation of RNA containing an analogue of adenosine in which the C6 amine was replaced with a trifluoromethyl group to give 6-trifluoromethylpurine ribonucleoside **396**. Although **396** had previously been synthesised by the reaction of a sugar-protected

6-chloropurine ribonucleoside and trifluoromethyl copper,[205] the long reaction time (60 h) and low yield (29%) warranted investigation of alternative methods. Thus Véliz *et al*. attempted the trifluoromethylation with tri-*O*-acetyl-6-bromopurine ribonucleoside **395** *via* two methods.[204] Using MFSDA, compound **396** was prepared in 91% yield (**Scheme 7.39**), or in 96% using CF_3I/Zn/CuI/DMF. However, due to the ease of manipulation of the liquid MFSDA in comparison to the gaseous CF_3I, the former reaction is certainly more desirable.

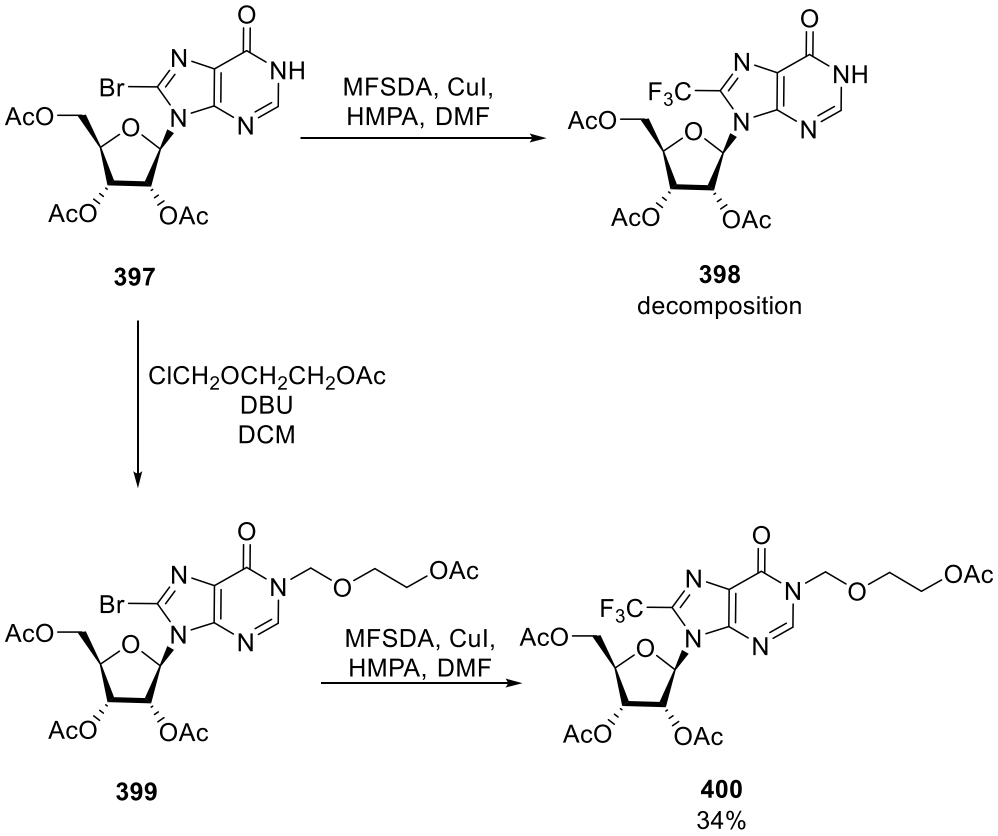


395

396
91%

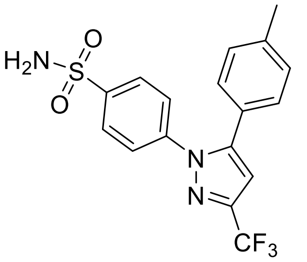
Scheme 7.39 The first reported use of MFSDA as a trifluoromethylating agent in nucleoside synthesis.

In 2010, Dong *et al*. reported the use of MFSDA to introduce the trifluoromethyl moiety at the 8-position of purine nucleosides.[206] When the trifluoromethylation of **397** was carried out using MFSDA, CuI and hexamethylphosphoric triamide (HMPA) in DMF, trifluoromethylated product **398** was obtained, however it was discovered that decomposition of **398** occurred after several hours in the reaction solution. If **397** was protected by reaction with 2-(chloromethoxy)ethyl acetate to provide **399** prior to trifluoromethylation, **400** could be obtained as a more stable compound (**Scheme 7.40**). This strategy was then successfully used to synthesise the first reported cyclic-ADP-ribose mimic.

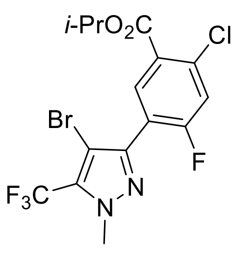

MFSDA, CuI,
HMPA, DMF
397
398
decomposition
$ClCH_2OCH_2CH_2OAc$
DBU
DCM
MFSDA, CuI,
HMPA, DMF
399
400
34%

Scheme 7.40 Model reaction for the trifluoromethylation at the 8-position of purine nucleoside.

Pyrazoles possessing fluorocarbon substituents are becoming increasingly prevalent as synthetic targets and building blocks within the fine chemical sector.[207,208] Some noteworthy examples of bioactive fluorinated pyrazoles include nonsteroidal anti-inflammatory drug (NSAID) celecoxib **401** (Celebrex®)[209] and the herbicide fluazolate **402** (**Figure 7.5**). Trifluoromethylated pyrazoles are classically synthesised using 1,1,1-trifluoromethyl-1,3-diketones, as this approach exploits the ready availability of trifluoroacetic acid derived precursors.[210-212] To some extent, regiocontrol can be achieved by careful choice of solvent,[213] however often mixtures of products result.



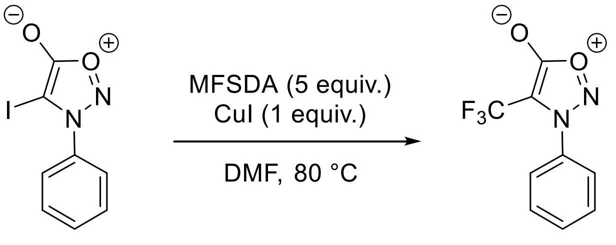
401



402

Figure 7.5 Examples of bioactive trifluoromethylated pyrazoles.

Foster *et al*. developed a more convenient strategy to allow late stage trifluoromethylation of pyrazoles using MFSDA.[214] Trifluoromethylation of 4-iodo-*N*-phenylsydnone **403** was carried out using typical conditions for MFSDA trifluoromethylations to provide product **404** in good yield (**Scheme 7.41**). The authors then successfully applied their methodology to the synthesis of the herbicide fluazolate5

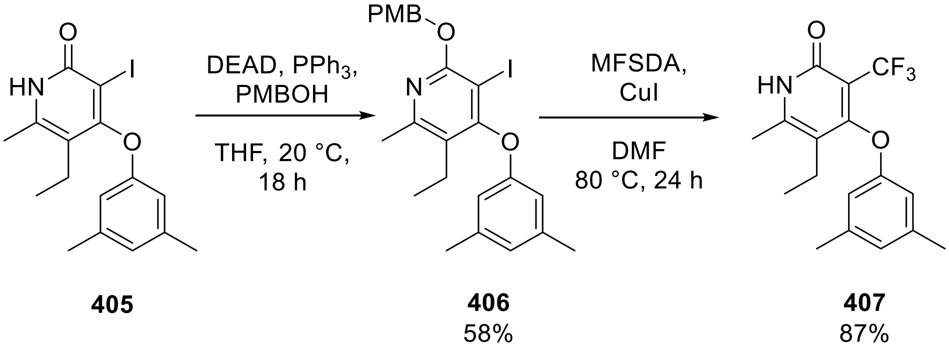


403

404
60-79%

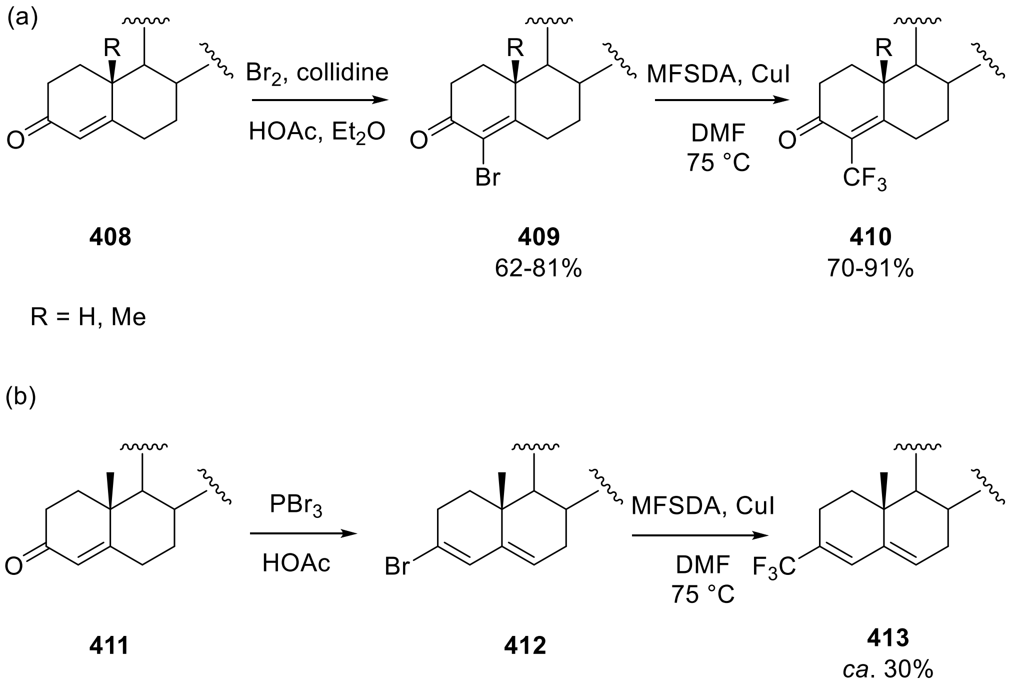
Scheme 7.41 Application of MFSDA in the synthesis of trifluoromethylated pyrazoles.

The 3-iodo-4-phenoxypyridinone (IOPY) is characteristic of a new family of pyridinone based non-nucleoside reverse transcriptase inhibitors (NNRTI's) which are highly active *in vitro* against a comprehensive panel of HIV-1 mutant strains found in AIDS patients.[215] It was conceived that the iodo substituent at the C-3 position could be substituted for a variety of different functional groups, providing a range of compounds which could be tested for anti-HIV activity. Benjahad *et al*. effectively utilised MFSDA to incorporate a trifluoromethyl group at the C-3 position.[216] Successful preparation of **407** involved first alkylating IOPY **405** under Mitsunobu conditions to provide 2-*O*-*para*-methoxybenzyl pyridine **406** in 58% yield. Subsequent reaction of **406** with MFSDA and CuI in DMF afforded the desired trifluoromethylated product **407** in 87% yield (**Scheme 7.42**). Compound **407** was then subjected to testing on wild-type HIV and showed anti-HIV activity similar to IOPY **405**. Further evaluation of **407** against HIV mutant strains, however, showed that it was less active than the parent compound **405**.[216]



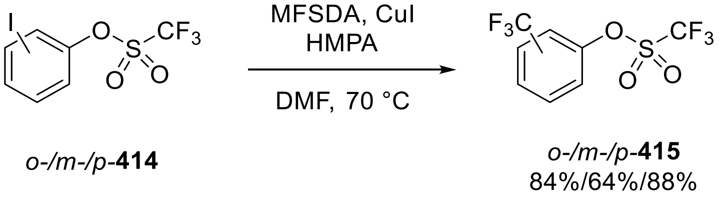
Scheme 7.42 Synthesis of 3-trifluoromethyl-4-phenoxypyridinone **407** using MFSDA.

Late-stage trifluoromethylation is of particular importance in the synthesis of steroids, where the use of a trifluoromethylated building block strategy can be extremely difficult. In 1998, Fei *et al*. published their findings on a new, convenient route for the trifluoromethylation of steroidal molecules.[217] Steroidal 4-en-3-ones **408** were first brominated with bromine in a mixture of acetic acid and diethyl ether in the presence of 2,4,6-trimethylpyridine (collidine), a proton acceptor. This suppressed allylic bromination and provided the monobromo derivative at C-4 **409** in 62-81% yield (**Scheme 7.43, (a)**). The authors found MFSDA to be the most suitable route for the trifluoromethylation of these steroidal compounds, with catalytic amounts of CuI required to provide the corresponding 4-trifluoromethylated products **410** in 70-91% yield.[218] Attempts were made to introduce the trifluoromethyl moiety at the C-3 position, firstly by brominating **411** to provide **412**[219] and subsequent reaction with MFSDA to yield **413**, however these products were obtained in disappointing yields (*ca*. 30%) (**Scheme 7.43, (b)**).[217]



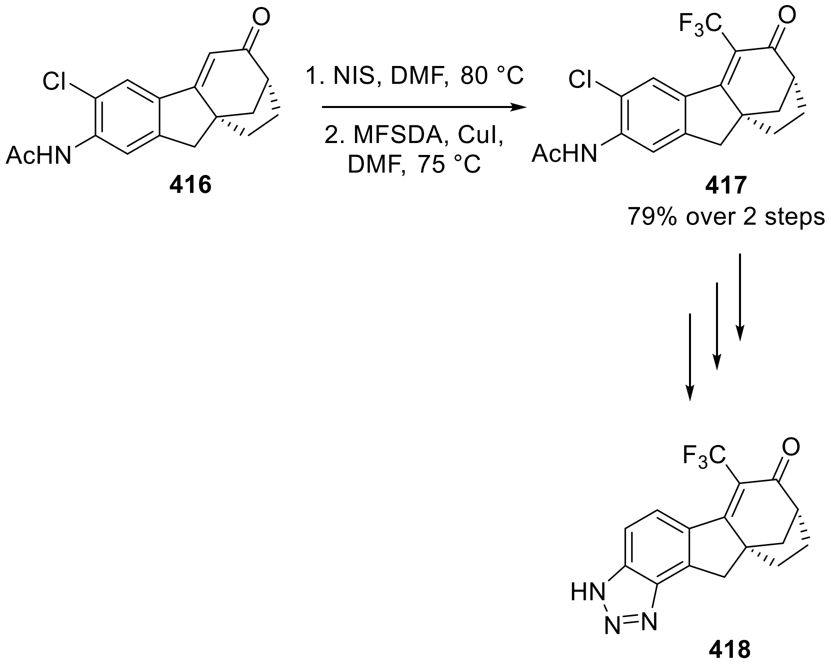
Scheme 7.43 Use of MFSDA in the trifluoromethylation of steroidal molecules at (a) C-4 and (b) C-3.

Aryl triflates are important synthetic molecules as they undergo regioselective cross-coupling reactions with different organometallic compounds.[220-223] It was postulated that trifluoromethylated aryl triflates would be ideal intermediates in the synthesis of bioactive trifluoromethylated aromatic compounds. In 1997, Qing *et al.* reported an efficient synthesis of *ortho*-trifluoromethylated aryl triflates using MFSDA.[224] In this methodology, the triflate group is introduced prior to the trifluoromethylation. Initial attempts at the MFSDA trifluoromethylation proved disappointing, with the reaction taking place slowly and only partial conversion to product taking place. When the reaction was carried out for longer times and higher reaction temperatures, it was found that decomposition occurred. This is most probably due to the concomitant formation of perfluoroethyl derivatives (which are difficult to remove during work up and purification) by the competing carbene insertion reaction to trifluoromethyl copper. Trifluoromethyl copper can be stabilised by addition of HMPA to the reaction mixture.[169] When Qing *et al.* carried out the MFSDA trifluoromethylation reaction on **414** with the addition of HMPA, *ortho*-trifluoromethylated aryl triflate **415** was obtained in 84% yield and no decomposition product was detected, indicating the importance of the stabilisation of the reactive trifluoromethylcopper species by HMPA (**Scheme 7.44**).[224] The methodology has also been successful for *meta*- and *para*-trifluoromethylation, as well as for more complex starting aryl iodides.



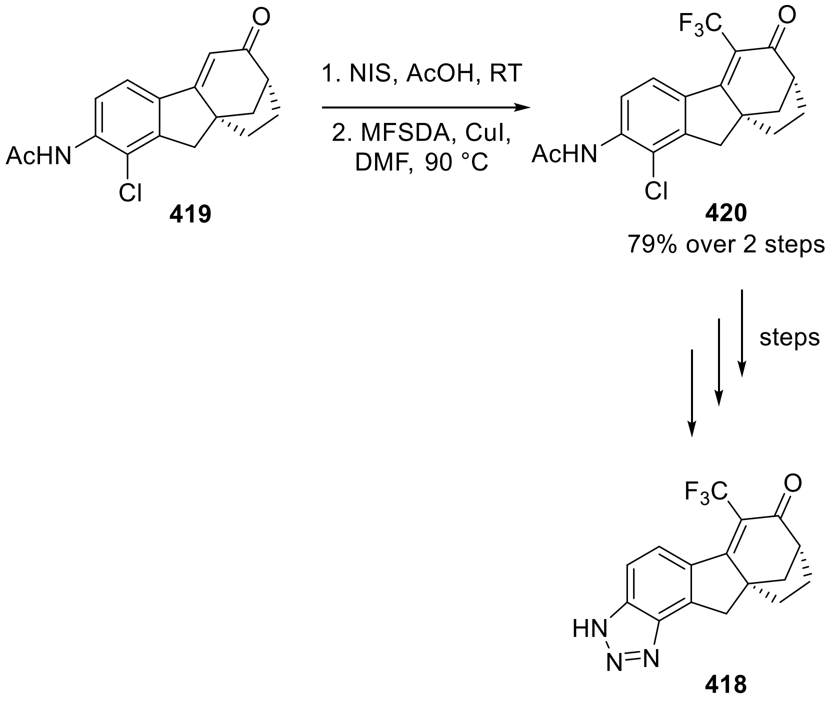
Scheme 7.44 Synthesis of trifluoromethylated aryl triflates using MFSDA with HMPA.

Tetrahydrofluoroene **418** has been identified as a potent agonist of oestrogen receptor subtype ERβ, a potential therapeutic agent for the treatment of symptoms associated with reduced oestrogen levels in postmenopausal women.[225,226] In the discovery chemistry of **418**, the trifluoromethyl moiety was successfully incorporated by iodination of **416** and subsequent reaction with MFSDA and CuI in DMF to provide trifluoromethylated product **417** in 79% yield. Further transformations provided the desired product **418** (**Scheme 7.45**).[227] However, due to the long linear synthesis required to produce **418**, this route was deemed not to be viable for scale-up.


Cl
AcHN
O
416
1. NIS, DMF, 80 °C
2. MFSDA, CuI, DMF, 75 °C
F_3C
417
79% over 2 steps
HN
N=N
418

Scheme 7.45 Use of MFSDA to incorporate a trifluoromethyl group in an intermediate en route to desired product **418**.

An alternative synthesis was used to provide **418** on scale-up.[227] Compound **419** was iodinated using NIS in acetic acid to provide iodinated product in 92% yield. Trifluoromethylation to afford **420** was accomplished using MFSDA and CuI in DMF, with the MFSDA added in a dropwise fashion to the preheated reaction mixture (90 °C) in order to control the heat of reaction on kilo-scale (**Scheme 7.46**).

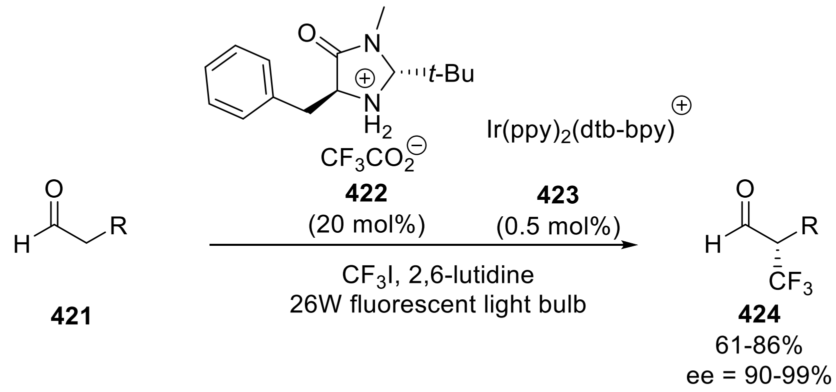


Scheme 7.46 Alternative synthesis of **418** with MFSDA used to incorporate the trifluoromethyl group.

MFSDA has also been used to introduce the trifluoromethyl moiety in the synthesis of various complex structures and bioactive molecules.[228-240]

7.4.4 Enantioselective trifluoromethylation

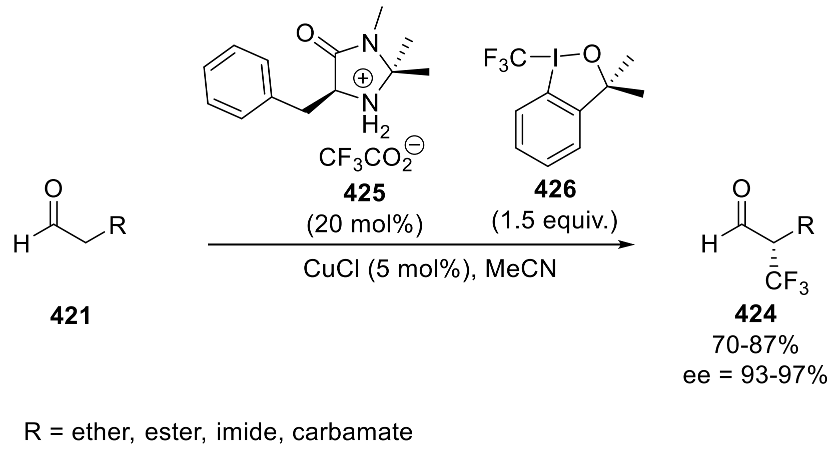
Enantioselective α-trifluoromethylation of carbonyls has also been successfully accomplished with appropriate electrophilic reagents. The enantioselective α-trifluoromethylation of aldehydes has been reported using two different methods. One methodology proceeds *via* photoredox catalysis *via* a radical mechanism. Using iodotrifluoromethane as trifluoromethylating agent, chiral organocatalyst **422**, iridium catalyst **423** and light from a fluorescent light bulb, aldehydes **421** were transformed into the corresponding α-trifluoromethyl aldehydes **424** in high yield (61–86%) and selectivity (90–99% ee) (**Scheme 7.47**).[241]



R = ether, ester, imide, carbamate

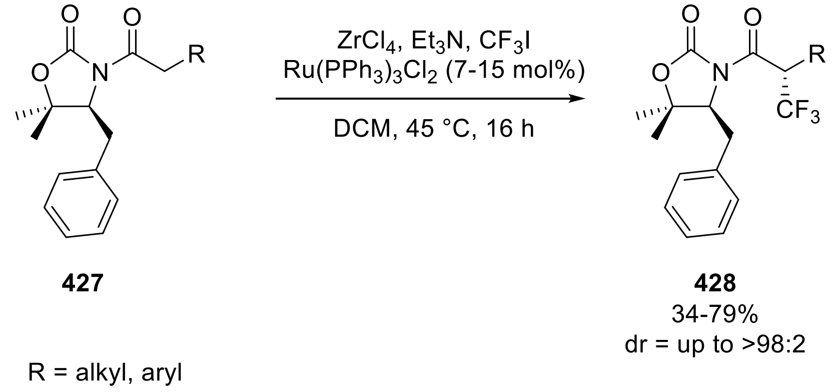
Scheme 7.47 Enantioselective trifluoromethylation of aldehydes using trifluoroiodomethane as the trifluoromethyl source, amine catalyst **422**, Ir catalyst **423** and light.

In a more recent publication from the MacMillan group, a novel methodology for the enantioselective synthesis of α-trifluoromethylated aldehydes has been developed *via* the merger of Lewis acid and organocatalysis with an electrophilic trifluoromethyl alkylating agent.[242] In this non-photolytic approach, the desired trifluoromethylated products can be obtained under mild reaction conditions using commercially available, bench-stable reagents and catalysts without the requirement of a light source. Products **424** were obtained in high yields (70–87%) and selectivities (93–97% ee) from simple aldehyde substrates **421** using Togni's reagent, a hypervalent iodine reagent **426** as the electrophilic trifluoromethylating reagent and a chiral imidazole catalyst **425** (**Scheme 7.48**).


425
(20 mol%)
426
(1.5 equiv.)
CuCl (5 mol%), MeCN
421
424
70-87%
ee = 93-97%
R = ether, ester, imide, carbamate

Scheme 7.48 Enantioselective trifluoromethylation of aldehydes using hypervalent iodine **426** as the trifluoromethyl source with amine catalyst **425**.

In 2012, a publication from Zakarian's group reported on a simple method for asymmetric trifluoromethylation of *N*-acyl oxazolidinones **427** *via* ruthenium-catalysed radical addition to zirconium enolates to provide trifluoromethylated products **428** in moderate to good yield (34-79%) and selectivities (up to >98:2 dr) (**Scheme 7.49**). The zirconium enolates generated *in situ* from $ZrCl_4$ and Et_3N act as radical acceptors in these transformations.[243]

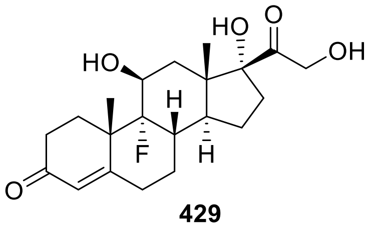

$ZrCl_4$, Et_3N, CF_3I
$Ru(PPh_3)_3Cl_2$ (7-15 mol%)
DCM, 45 °C, 16 h
427
428
34-79%
dr = up to >98:2
R = alkyl, aryl

Scheme 7.49 Ruthenium-catalysed diastereoselective radical trifluoromethylation of chiral *N*-acyloxazolidinones.

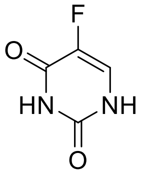
7.5 Importance of fluorine in pharmaceuticals and agrochemicals

Despite the scarcity of fluorine in natural products, a vast number of synthetic fluorine-containing compounds have been used in a multitude of applications due to the unique properties attributed to fluorine containing molecules. Due to the similarity in size between hydrogen and fluorine, it is often the case that enzymes or microorganisms cannot differentiate between a natural substrate and its fluorinated analogue – known as the "mimic effect" of fluorine for hydrogen. Also, the introduction of a single fluorine substituent can induce electronic effects on its neighbours by affecting the electron density of various functional groups, for example hydroxyl and amino groups. This results in a decrease in both the pK_a value and Lewis basicity of these functional groups and thus slows their oxidation.[4] The bioavailability of a drug molecule is highly dependent on its lipophilicity, hydrophobicity and ionisation. It is widely thought that incorporation of a fluorine atom or fluorinated group increases the lipophilicity of aromatic compounds, thereby increasing the bioavailability of the compound. In contrast, a decrease in lipophilicity is observed when fluorine is introduced into aliphatic compounds.[4] Due to the fact that fluorine is the most electronegative element, groups containing fluorine have unique inductive effects on the physicochemical properties of the molecules which possess them. Indeed, substantial changes in pK_a values of carboxylic acids, alcohols or protonated amines are noted when fluorine is incorporated into these molecules. Thus whenever fluorine atoms are incorporated into bioactive compounds, these substituents will exert strong effects on the binding affinity for the receptors or target enzymes, biological activities and pharmacokinetics.[4]

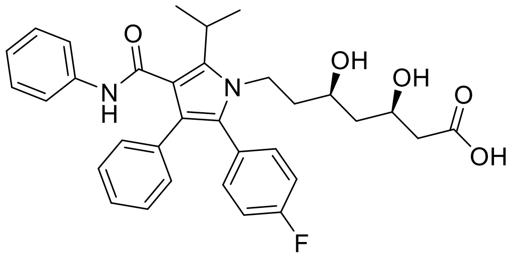
Two of the most early examples of pharmaceuticals which contain fluorine are 9α-fluorohydrocortisone **429**, an anti-inflammatory drug,[244] and 5-fluorouracil, an anticancer drug **430** (**Figure 7.6**).[245] Both **429** and **430** were developed in the 1950s, with the introduction of a single fluorine atom to the corresponding natural products bringing about remarkable pharmacological properties. Since then, the incorporation of fluorine into pharmaceutical and veterinary drugs to enhance their pharmacological properties has become almost standard practice.[4] Indeed, one of the best-selling drugs of all time, atorvastatin **431** (**Figure 7.6**) (marketed as Lipitor® by Pfizer), a member of the statin drug class used to lower blood cholesterol, contains a fluorine molecule in its structure. Over 14.5 years, from 1996 to 2011, Lipitor® generated over $125 billion in sales.[246]



429



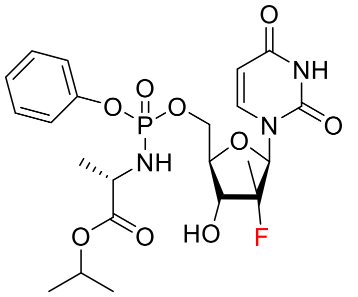
430



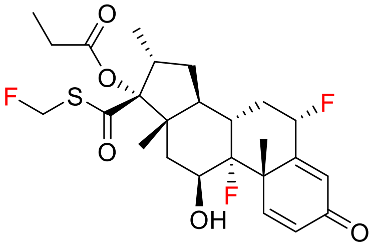
431

Figure 7.6 Structures of 9α-fluorohydrocortisone **429**, 5-fluorouracil **430** and atorvastatin **431**.

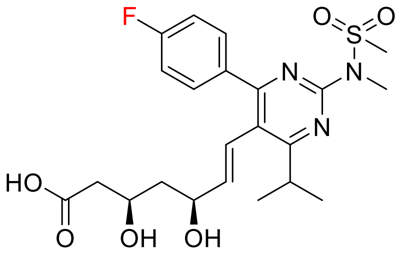
In a report in Genetic Engineering and Biotechnology News listing the top 25 best-selling drugs of 2014, 7 contained at least one fluorine atom.[247] In fact, one of the newest drugs to the market which contains a fluorine atom is sofosbuvir (Sovaldi®) **432**, a nucleotide analogue used in combination with other drugs for the treatment of Hepatitis C. Sofosbuvir reached sales of ~$10 billion in 2014, making it the second best-selling drug of 2014. Advair® (a combination of fluticasone **433** and salmeterol) and rosuvastatin (Crestor®) **434** also made it into the top 10. Sitagliptin (Januvia®) **435**, an antihyperglycemic drug for the treatment of diabetes mellitus type 2, placed 19th based on its annual sales, followed by HIV treatments Atripla® and Truvada® at 22nd and 23rd respectively, both of which contain fluorine containing components such as efavirenz **436** and emtricitabine **437** as part of a fixed-dose multi-drug combination. Based on sales of ~$2.7 billion in 2014, Celebrex® **401** which contains a trifluoromethyl group, was deemed to be the 25th best-selling drug of the year (**Figure 7.7**).



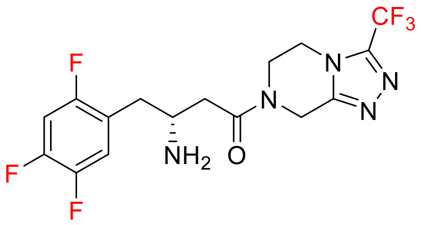
432
#2
$10.283 billion



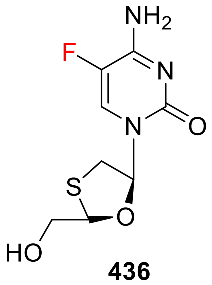
433
#9
$6.431 billion



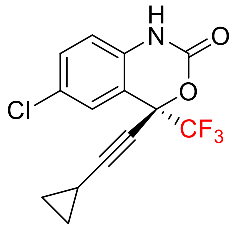
434
#10
$5.869 billion



435
#19
$3.931 billion



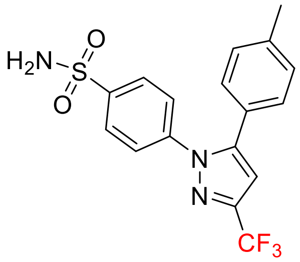
436



437

Atripla® (fixed-dose combination drug
containing **436**, **437**, and others)
#22, $3.470 billion

Truvada® (fixed-dose combination drug
containing **437** and others)
#23, $3.340 billion



401
#25
$2.699 billion

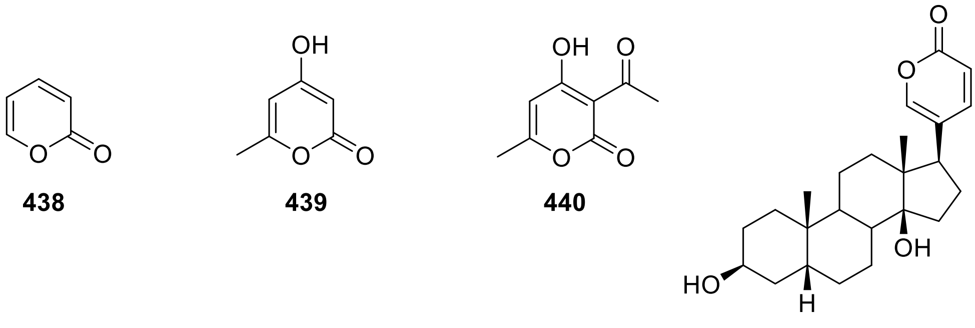
Figure 7.7 Top-selling drugs of 2014 which contain at least one fluorine atom.

The huge success observed with fluorine-containing drugs in recent years has inspired research into the incorporation of fluorine and trifluoromethyl groups (at both late-stage development and in early-stage building blocks) in many drug discovery programmes. Every new drug development programme, without exception, explores fluorine-containing drug candidates.[4] In many cases, a wide range of fluorine-containing compounds based either on known natural products or on novel skeletons have been synthesised and subject to biological evaluation. The interest in incorporation of fluorine into molecules has provided expansion and development into safe, efficient methodologies for these transformations. The limited availability of fluorochemicals for pharmaceutical and agrochemical applications is largely due to the exceptional properties and perilous nature of fluorine and fluorochemical sources. Also, in many cases, established synthetic methods for ordinary organic molecules do not work well for fluorochemicals owing to their distinctive reactivity.[4]

An additional role of fluorine is the use of radioactive ^{18}F–labelled organic compounds for positron emission tomography (PET) imaging in hospitals. Due to its optimal physical half-life of 110 min, ^{18}F is the most important positron emitting isotope as it allows for multistep radiosynthesis and longer *in vivo* investigation.[248,249]

7.6 Biological importance of 2-pyrones

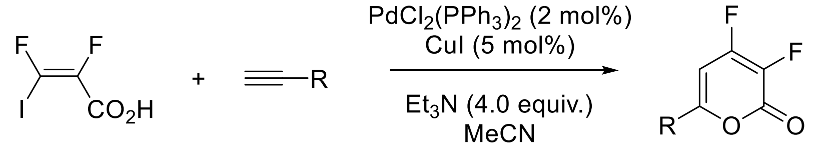
2-Pyrones are a privileged biological scaffold with broad-spectrum biological activity spanning cytotoxic, antibiotic and antifungal activity.[250-252] The 2-pyrone scaffold **438** is a six-membered cyclic unsaturated ester that shares chemical and physical properties reminiscent of alkene and aromatic compounds. This structural moiety is commonly found in bacteria, microbial, plant, insect and animal systems and partakes in a variety of biological processes, including defence against other organisms, as key biosynthetic intermediates and metabolites. Simple 2-pyrones such as triacetic acid lactone **439** and tetraacetic acid lactone **440** (**Figure 7.8**) are used as precursors in the synthesis of biologically important compounds including pheromones,[253] solanapyrones,[254] α-chymotrypsin,[255] elastase,[256] coumarins[257] and analogues.[258] Prominent examples of biologically active 2-pyrones include the bufadienolide class, an important group of steroidal molecules characterised by a 2-pyrone connected through a steroid nucleus, such as in bufalin **441**.[259] Bufadienolides have been shown to have diverse biological effects, including causing cardiac poisoning in animals and showing inhibitory activity towards leukaemia cell lines.[250]



438 439 440 441

Figure 7.8 Biologically important compounds containing the 2-pyrone moiety.

Numerous protocols have been designed for the synthesis and decoration of 2-pyrones.[260-265] More specifically, functionalisation of the 3-position of 4-alkoxy-2-pyrones has been accomplished under Suzuki–Miyaura[266-268] and Sonogashira[269] conditions. 3,4-Difluorinated pyrones **444** have been synthesised in moderate to good yield from (2*E*)-2,3-difluoro-3-iodoacrylic acid **442** with terminal acetylenes **443** under Sonogashira alkynylation conditions using $PdCl_2(PPh_3)_2$ in combination with CuI as a co-catalyst (**Scheme 7.50**).[270] Apart from palladium, the formation of 2-pyrones has also been successful with various other metal-based catalysts including gold,[271,272] rhodium,[273,274] ruthenium[275-277] and nickel.[278]



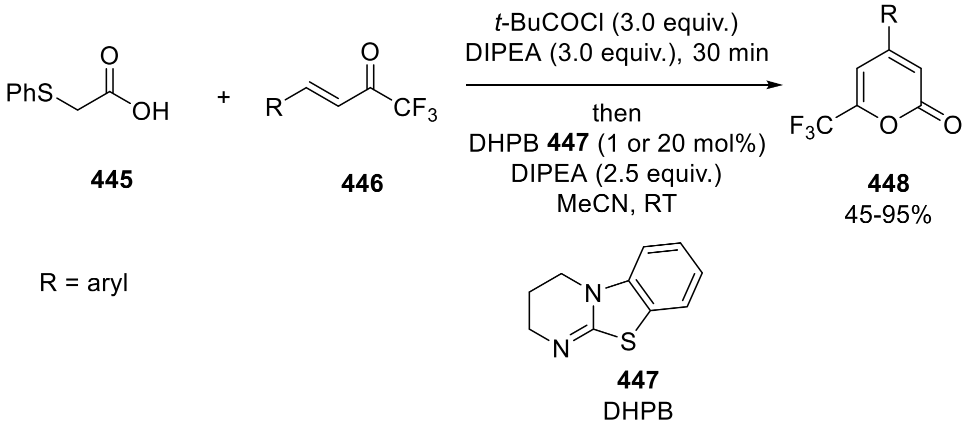
442 443 444

43-71%

R = alkyl, aryl

Scheme 7.50 Synthesis of difluorinated 2-pyrones involving Sonogashira-type alkynylation.

In a recent publication by Yeh *et al.* 6-trifluoromethyl-2-pyrones **448** have successfully been prepared in moderate to excellent yield in a one-pot isothiourea-mediated Michael addition/lacontisation/thiol elimination cascade sequence from (phenylthio)acetic acids **445** and α,β-unsaturated trifluoromethyl ketones **446** using an organocatalyst **447** (**Scheme 7.51**)[279]


PhS
OH
O
445
+
R
CF3
O
446
t-BuCOCl (3.0 equiv.)
DIPEA (3.0 equiv.), 30 min
then
DHPB 447 (1 or 20 mol%)
DIPEA (2.5 equiv.)
MeCN, RT
R
F3C
O
O
448
45-95%
R = aryl
N
N
S
447
DHPB

Scheme 7.51 Synthesis of 2-pyrones using an organocatalyst, DHPB (3,4-dihydro-2*H*-pyrimido[2,1-b]benzo-thiazole).

C-3 substituted 2-pyrones are present in bioactive polyketides such as nigerapyrone E **449** which possesses a methyl group at C-3 (**Figure 7.9**).[280] The trifluoromethyl moiety is present (incorporated prior to cyclisation and ring formation) at the C-3 position of the 2-pyrone moiety of fused bicyclic heterocycles **450** and **451** (**Figure 7.10**), which exhibit antifungal activity.[281]

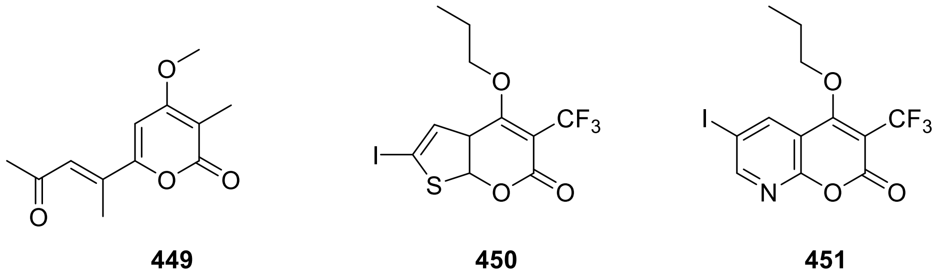

O
O
O
O
449
O
CF3
I
S
O
O
450
O
CF3
I
N
O
O
451

Figure 7.9 Biologically active structures containing the 3-substituted 2-pyrone moiety.

7.7 Biological importance of 2-pyridones

2-Pyridones are also an attractive target for synthetic organic chemists as a number of biologically active molecules contain this structural moiety.[282-284] The first pyridone alkaloid, a poisonous crystalline solid named ricinine **452**, was isolated from castor beans in the19th century by Tuson.[285] The 2-pyridone moiety is present in many naturally occurring compounds which possess both antibacterial and antifungal activity,[286] such as 2-pyridone alkaloids **453–455** (**Figure 7.10**) which have been isolated from a New Zealand marine-derived *Penicillium* species.[287] Some 2-pyridones are also reported to possess antitumour,[288] antibacterial[282] and other biological activities.[289,290] 2-Pyridones also play an important role as key intermediates in the synthesis of pyridine, piperidine, quinolizidine and indolizidine alkaloids.[291] In general, 2-pyridones are more highly active *in vitro* and *in vivo* and are more water soluble than their respective 4-quinolone analogues.[282]

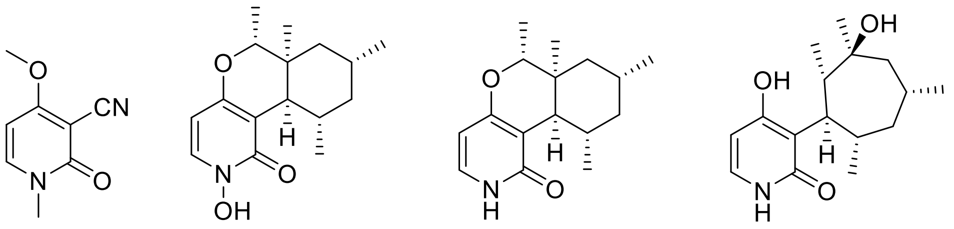
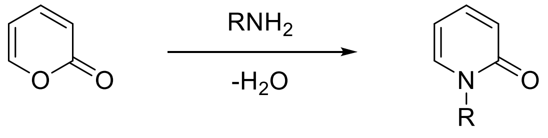

452 **453** **454** **455**

Figure 7.10 Structures of biologically active molecules containing the 2-pyridone moiety.

2-Pyridones can be synthesised either from other heterocyclic systems or by condensation of acyclic systems.[291] Conversion of 2-pyrones **456** to 2-pyridones **457** can be achieved by reaction with a desired amine (**Scheme 7.52**), however yields for these reactions are low to moderate.[292] Alternative syntheses include the oxidation of pyridines[293] and the Guareschi-Thorpe condensation of acyclic precursors such as cyanoacetamide with 1,3-diketones in the presence of ammonia.[294,295]

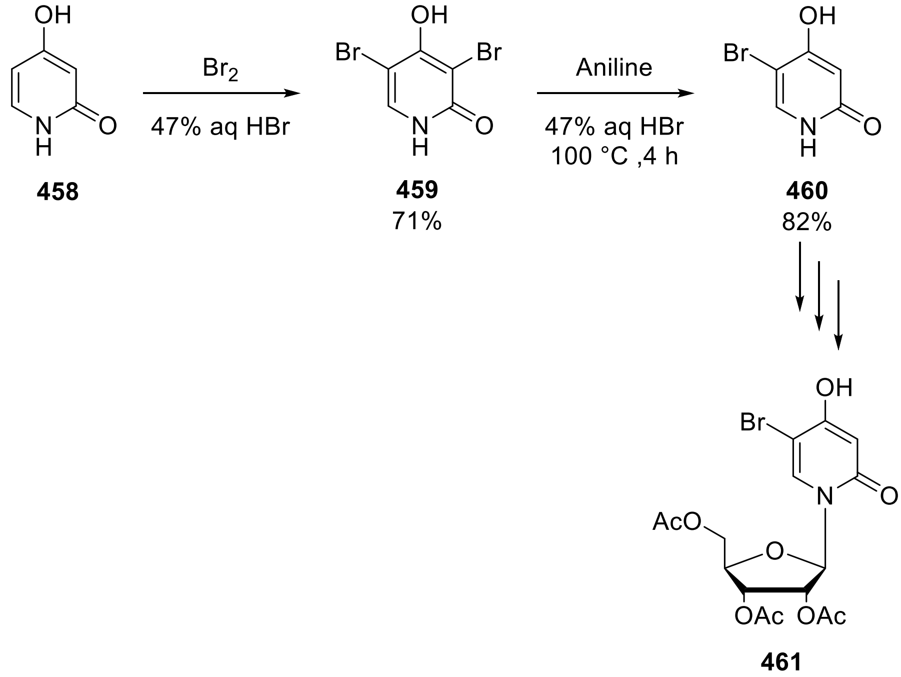


456 **457**

Scheme 7.52 Conversion of 2-pyrones to 2-pyridones by reaction with amines.

Direct functionalisation of the 3-position of 2-pyridones is limited and in general, the desired function groups are added to the acyclic precursor prior to cyclisation or are already present on the molecule before transformation to the 2-pyridone.[291] However there are some instances where direct functionalistion of 2-pyridones has been achieved, for example in the synthesis of nucleic acid related compounds such as 5-bromo-3-deazauridine **461**.[296] Bromination of 4-hydroxy-

2-pyridone **458** provided the dibrominated product **459** in good yield. Selective debromination at C-3 was then carried out to afford **460** in good yield, which was further transformed to the desired product **461** (**Scheme 7.53**).



Scheme 7.53 Direct functionalisation of 4-hydroxy-2-pyridone in the synthesis of 5-bromo-3-deazauridine **461**.

Another example of direct functionalisation includes iodination at the 3-position of 5-bromo-2-pyridone which was achieved in high yield by refluxing with NIS in acetonitrile.[297] Other successful functionalisation strategies include halogenation and nitration at the 3-position of 4-amino-2-pyridone or 4-hydroxy-2-pyridone.[298]

Many 2-pyridones with substitutions at the 3-position possess interesting biological activity. 2-Pyridone **462** (**Figure 7.11**) has been identified as a specific non-nucleoside reverse transcriptase inhibitor of human immunodeficiency virus-1 (HIV-1).[299,300] Other biologically active molecules containing substitutions at the 3-position include milrinone **463** and amrinone **464**[301] (**Figure 7.11**) and their analogues,[302-304] which are used for the treatment of cardiac failure. 2-Pyridone **407**, which contains a trifluoromethyl group at the C-3 position was synthesised using MFSDA and has potential as a HIV treatment.[216] However, a significant disadvantage in the synthesis of **407** was the requirement for carbonyl protection prior to trifluoromethylation.

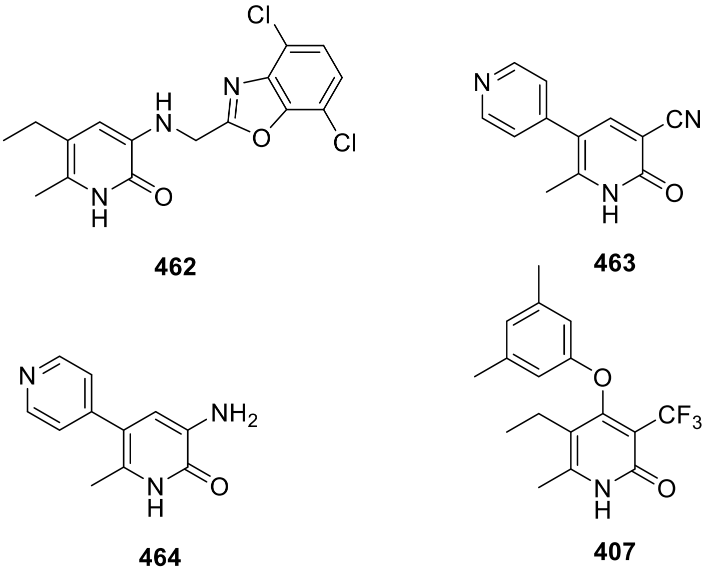
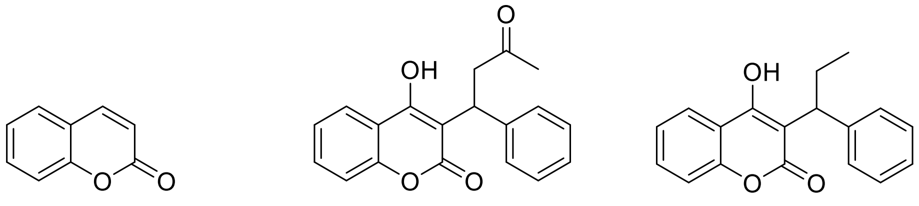


Figure 7.11 Structures of biologically active molecules containing the 2-pyridone moiety.

7.8 Biological importance of 2-coumarins

Coumarins are an important class of *O*-heterocyclic natural products. The parent substance of this benzo-α-pyrone group, coumarin **465** (**Figure 7.12**), was isolated in 1820 from tonka beans (*Dipteryx odorata*) by Vogel.[305] Naturally occurring coumarins are found in a variety of plant families and essential oils.[306-308] The coumarin moiety is found in a variety of compounds which display a wide range of biological activities such as anticoagulants (including warfarin **466** and phenprocoumon **467**),[309] antineurodegeneratives,[310] antioxidants,[311] anticancer[312] and antimicrobials.[313] Additionally, coumarins have attracted much attention as potential fluorescent probes recently due to their excellent fluorescent capabilities resulting from the electron-rich and good charge-transport properties of the π-π conjugated system.[314]

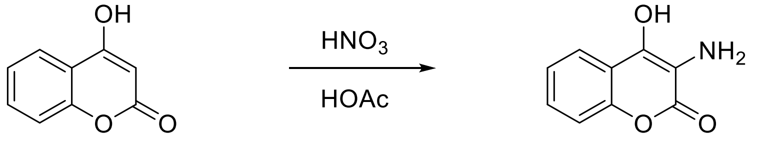


465 **466** **467**

Figure 7.12 Structure of some biologically active coumarins.

Due to the wide range of application of coumarin-containing compounds, methods for their synthesis is of particular interest to organic chemists and syntheses including the Pechmann condensation,[315,316] the Perkin reaction,[317] the Knoevenagel condensation,[318] the Wittig reaction[319-321] and the Baylis-Hillman reaction.[322,323]

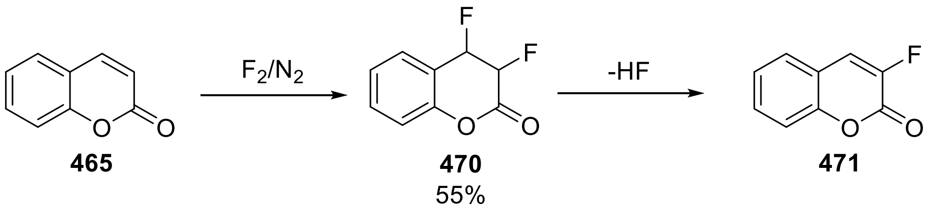
Functionalisation of coumarins at the 3-position is possible by either adding the desired groups prior to cyclisation to give the coumarin product or, less commonly, substitution at this position at a late stage in the synthesis. Typical late stage functionalization at the 3-position of coumarins involves simple substitution reactions such as amination and halogenation. 4-Hydroxycoumarin **468**, for example, can be aminated selectively at the 3-position to provide 3-amino-4-hydroxycoumarin **469** in moderate yield (**Scheme 7.54**).[324] The presence of an amino group at the 3-position allows the potential for further functional group transformations at this position.



468 **469** 52%

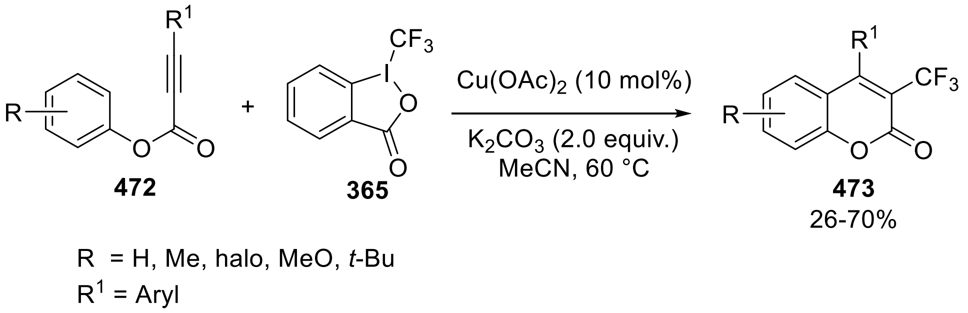
Scheme 7.54 Direct amination of the 3-position of 4-hydroxycoumarin.

Direct fluorination of coumarins was reported in 1986 by Rozen and Brand.[325] Coumarin **465** was treated with F_2/N_2 to provide difluoro adduct **470** in moderate yield. Subsequent elimination of HF (by adsorbtion of **470** on a silica gel column) provided dehydrofluorinated product **471** in quantitative yield (**Scheme 7.55**).


F
F
F
F_2/N_2
-HF
O
O
O
O
O
O
465
470
55%
471

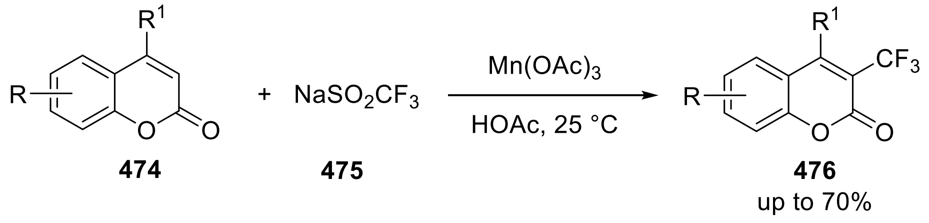
Scheme 7.55 Fluorination of coumarin using F_2/N_2.

The trifluoromethyl group has been successfully incorporated at the 3-position of coumarins. In 2014, Li *et al*. published a methodology that allowed the construction of trifluoromethylated coumarins **473** *via* copper-catalysed direct trifluoromethylation of propiolates **472** using Togni's reagent **365** (**Scheme 7.56**).[326] Preliminary mechanistic studies by the authors suggest that the reaction proceeds *via* a CF_3 radical addition to activated alkynes, followed by sequential oxidation cyclisation to provide the desired coumarin products.


R^1
R
O
O
+
CF_3
I
O
O
472
365
$Cu(OAc)_2$ (10 mol%)
K_2CO_3 (2.0 equiv.)
MeCN, 60 °C
R^1
CF_3
R
O
O
473
26-70%
R = H, Me, halo, MeO, *t*-Bu
R^1 = Aryl

Scheme 7.56 Synthesis of 3-trifluoromethylated coumarins using Togni's reagent.

Also in 2014, Cao *et al.* reported selective 3-trifluoromethylation of coumarins **474** with sodium trifluoromethylsulfinate **475** (Langlois reagent) in the presence of $Mn(OAc)_3$ *via* a radical mechanism.[327] The reaction proceeds under mild conditions in air to afford selective 3-trifluoromethyl coumarins **476** in moderate to good yields (**Scheme 7.57**).

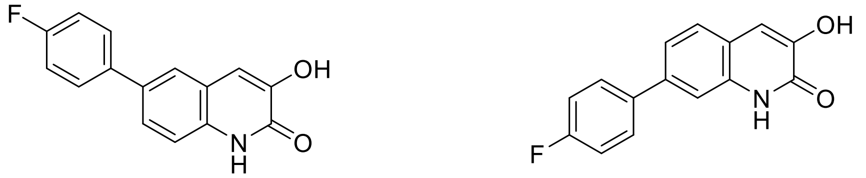


R = H, nitro, alkyl, halo, acetoxy
R^1 = H, Me, OMe

Scheme 7.57 Direct 3-trifluoromethylation of coumarins using Langlois reagent and $Mn(OAc)_3$.

7.9 Biological importance of 2-quinolones

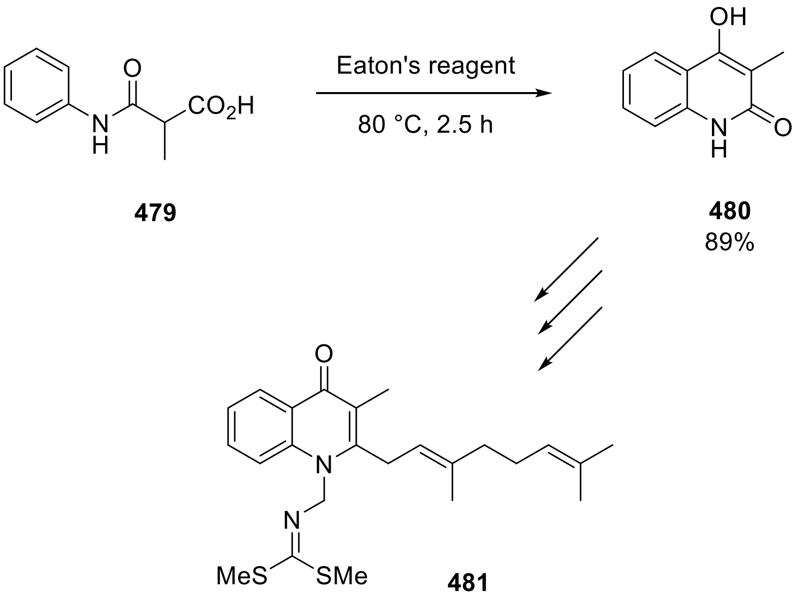
Many 2-quinolones have been discovered to possess biological activity.[328] In 2013, Sagong *et al.* synthesised a range of 3-hydroxyquinolin-2-(*1H*)-ones and tested their potential as inhibitors of the pandemic H1N1 influenza A endonuclease.[329] The authors reported that two of the compounds synthesised, 6- and 7-(4-fluorophenyl)-3-hydroxyquinoline-2-(1H)-one **477** and **478** (**Figure 7.13**) were potent inhibitors of H1N1 influenza A endonuclease, with the molecules found to chelate to two metal ions at the active site of the enzyme. Similar compounds have also been reported to possess selective inhibitory properties against HIV-1 reverse transcriptase associated ribonuclease H activity, however high cellular cytotoxicity limits their application as antiviral agents.[330]



477 **478**

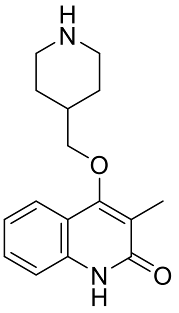
Figure 7.13 Biologically active 2-quinolones which were deemed to be potent inhibitors of H1N1 influenza A endonuclease.

In a recent publication by Abe *et al*., 4-hydroxy-3-methyl-2-quinolone **480** is synthesised in high yield from 2-methyl-3-oxo-3-(phenylamino)propanoic acid **479** using Eaton's reagent.[331] Subsequent steps provide intervenolin **481**, which inhibited tumour growth in model mice (**Scheme 7.58**).



Scheme 7.58 Synthesis of intervenolin, a natural quinolone.

The C-3 substituted quinolone moiety is found in potential therapeutic agents, including in **482** (**Figure 7.14**), which possesses a methyl group at C-3, a compound which has potential in the treatment of central nervous system disorders associated with phosphodiesterase 2 (PDE2).[332]



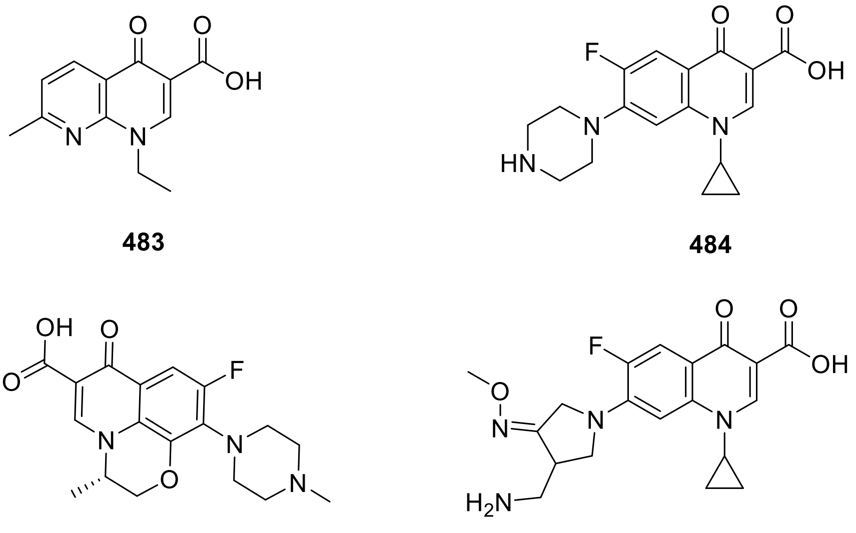
482

Figure 7.14 3-substituted-2-quinolone which is a potential treatment for CNS disorders.

7.10 Biological importance of 4-quinolones

Many 4-quinolones have also been discovered to possess biological activity. The 4-quinolone class of antimicrobial agents has generated considerable interest since the discovery of nalidixic acid **483** more than 50 years ago.[333-335] Development of this class led to the now well-known fluoroquinolones, which have been widely used as antimicrobial agents. The first marketed fluoroquinolone, ciprofloxacin **484**, came to the fore in the mid-1980s. Ciprofloxacin demonstrates excellent activity against both Gram-negative and Gram-positive bacteria and results in excellent clinical efficacy in a variety of infections.[336] Since then, third- and fourth-generation fluoroquinolones such as levofloxacin **485** and gemifloxacin **486** have been developed (**Figure 7.15**), which are active against *Streptococcus* (third-generation) and act against DNA gyrase and topoisomerase IV (fourth-generation) with this dual action slowing the development of resistance.[337,338,282,339]

In addition to possessing bactericidal properties, the quinolone scaffold is present in the structures of certain anti-cancer[340] and anti-viral[341] drugs and also in anti-oxidants.[342] The quinolone moiety is also present in compounds that display anti-malarial activity.[343]

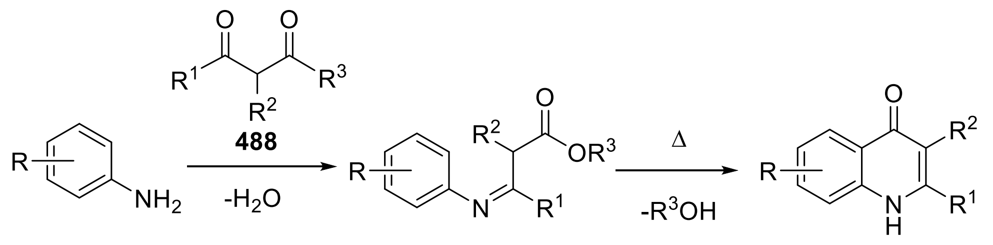


483 **484**

485 **486**

Figure 7.15 Biologically active molecules containing the quinolone moiety.

Numerous methodologies are available for the synthesis and functionalisation of quinolones.[344] A classical approach for the synthesis of 4-quinolones is the use of the Conrad-Limpach synthesis, in which the desired aniline **487** is condensed with a β-ketoester **488** to form a Schiff base **489** which is cyclised to form the desired quinolone **490** (**Scheme 7.59**).[345] High boiling point solvents are required for the final cyclisation step and yields for quinolones obtained from this methodology are known to be low.[346]

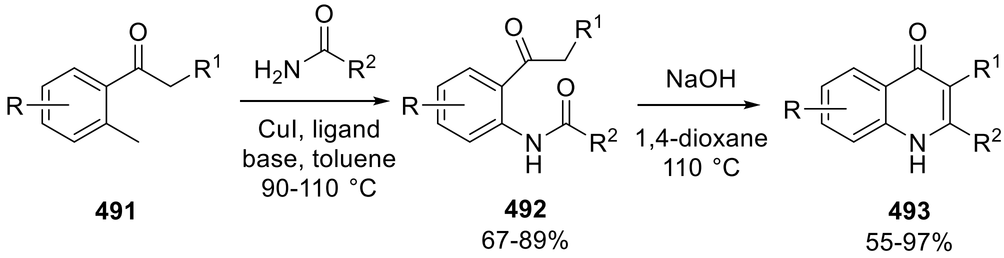


487 **489** **490**

Scheme 7.59 Conrad-Limpach quinolone synthesis.

Other classical approaches to form quinolones include the Niementowski reaction of anthranilic acids with ketones or aldehydes[347] and the Camps quinolone synthesis, in which an *o*-acylaminoacetphenone is reacted with a base to provide both 2- and 4-quinolones.[348]

Due to the significant biological activity associated with quinolones, interest in developing new methods for the preparation of these compounds has increased. These improved synthetic methodologies include the use of transition metal catalysis such as that reported by Jones *et al.* using copper to catalyse the amidation of *o*-halophenones **491** followed by a base-promoted Camps cyclisation of the resulting *N*-(2-ketoaryl)amides **492** to provide the desired quinolones **493** in up to 97% yield (**Scheme 7.60**).[349]

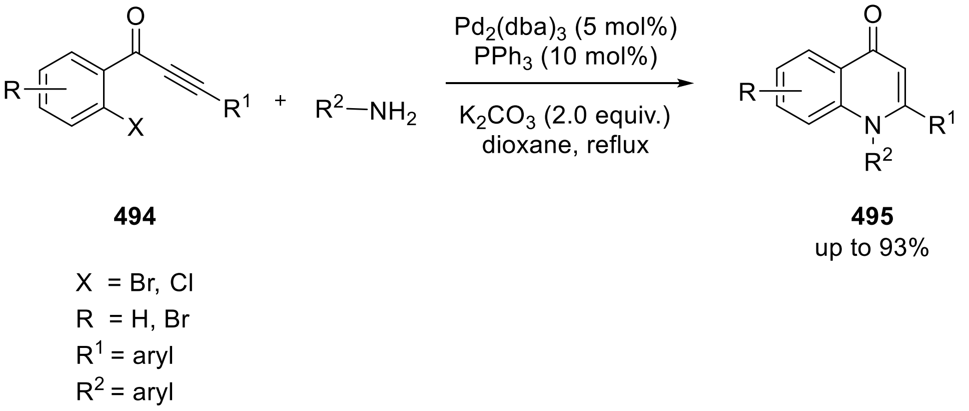


491 **492** 67-89% **493** 55-97%

R = H, F, OMe
R^1 = H, Me
R^2 = aryl

Scheme 7.60 Copper-catalysed formation of quinolones.

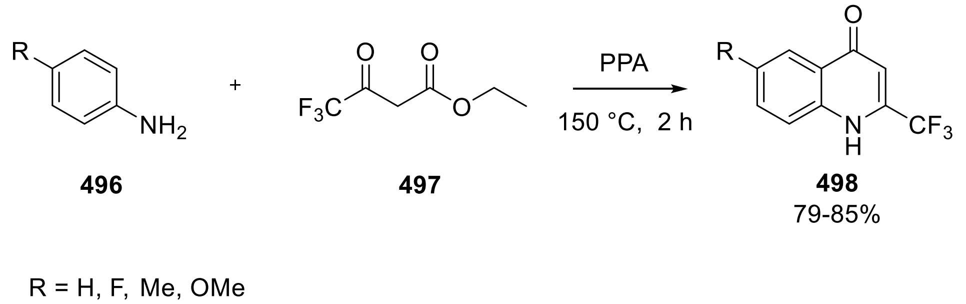
Palladium has also been successfully used to catalyse the formation of 4-quinolones. In 2009, Zhao and Xu reported an efficient one-step formation of functionalised 4-quinolones **494** *via* a palladium-catalysed tandem amination approach from easily accessible *o*-haloaryl acetylenic ketones **495** and primary amines with the desired product obtained in excellent yields (**Scheme 7.61**).[350] This methodology was then successfully extended to the use of alkyl amines and in these cases, palladium was not required to catalyse the reaction.[351]



Scheme 7.61 Palladium-catalysed formation of quinolones.

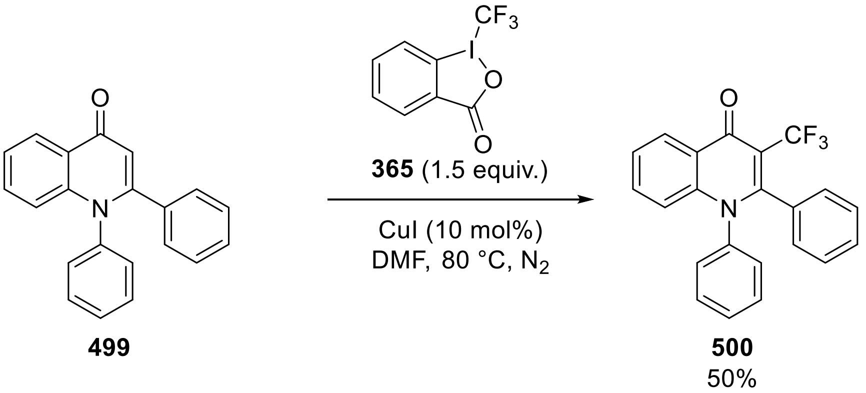
As can be seen from the above examples, functionalisation of quinolones is usually carried out prior to cyclisation, however there are some examples of late-stage functionalisation of these molecules, including a recent publication by Li *et al*. demonstrating the direct C-3 alkenylation of *N*-methylated quinolones *via* palladium-catalysed C-H functionalisation.[352] Direct halogenation of the 3-position of 4-quinolones has also be achieved using commercially available halogenating reagents.[353] Regioselective iodination at the 3-position of *N*-methylated 4-quinolone was recently achieved using a rhodium(III) catalyst.[354] Alternatively, specific groups can be incorporated prior to cyclisation which can be transformed to alternative functionalities.[344]

2-Trifluromethyl-4-quinolones **498** have found application as potential antimicrobial agents, with the library of compounds synthesised by Panda and Jain exhibiting good antibacterial activity towards Gram-positive bacteria and some showing moderate antifungal activity.[355] The authors chose to incorporate a trifluoromethyl group in the compounds for a number of reasons: To increase the stability of the compounds, reduce the toxicity to eukaryotic cells and improve the antibacterial activity. The library of 2-trifluoromethyl-4-quinolones were prepared in good yield by cyclocondensation of substituted anilines **496** with ethyl 4,4,4-trifluoro-3-oxobutanoate **497** in polyphosphoric acid at 150 °C for 2 h (**Scheme 7.62**). Again the trifluoromethyl group was in place before cyclisation.


R
NH2
+
F3C
O
O
O
PPA
150 °C, 2 h
R
O
N
H
CF3
496
497
498
79-85%
R = H, F, Me, OMe

Scheme 7.62 Formation of 2-trifluoromethyl-4-quinolones.

A recent publication by Fang *et al.* demonstrates late stage trifluoromethylation of *N*-phenyl-2-phenyl-4-quinolone **499** using Togni's reagent **365** with a catalytic amount of CuI. The reaction provides **500** in moderate yield in 25 h (**Scheme 7.63**).[186]


CF3
I
O
O
365 (1.5 equiv.)
CuI (10 mol%)
DMF, 80 °C, N2
O
N
499
O
CF3
N
500
50%

Scheme 7.63 Late stage trifluoromethylation of *N*-phenyl-2-phenyl-4-quinolone.

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Chapter 8

Trifluoromethylation of 4-alkoxy-2-pyrones, pyridones and quinolones

Results and Discussion

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8.1 Background to project

The aim of this project was to synthesise fluorinated and trifluoromethylated 2-pyrones, 2-pyridones and 2-quinolones using suitable methodologies. Work focused on introducing a fluorine or trifluoromethyl moiety at the C-3 position of these molecules as this was not yet accomplished in the literature. This was of particular interest as these molecules are privileged organic scaffolds which are found in numerous biologically active molecules.[1-6] Also, it is well-known that introduction of a fluoro or trifluoromethyl moiety can have a dramatic effect on biological activity by increasing the lipophilicity and bioavailability of the molecules.[7-9]

The first objective of the project was to fluorinate a set of privileged biological scaffolds, namely pyrones, using safe, easy-to-handle electrophilic fluorinating agents.

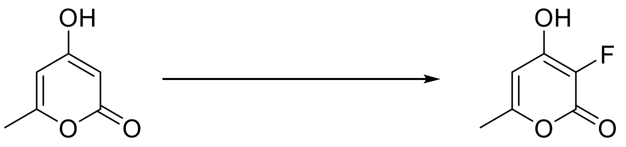
The second objective of the project was to trifluoromethylate pyrones with various substituents present elsewhere on the molecule.

The third objective of the project was to extend this methodology to isosteres such as coumarins, pyridones and quinolones, each of which have their own prominent biological profile.

8.2 Direct fluorination

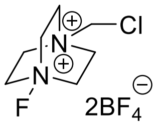
Initial work focused on the direct fluorination of cheap, commercially available 4-hydroxy-6-methyl-2-pyrone **439**. The preferred method would involve safe, easy-to-handle electrophilic fluorinating agents to accomplish this transformation. Methodologies that use fluorine gas were avoided due to the need for specialist equipment and the potentially explosive nature of the reactions. The reaction was first attempted using Selectfluor® **243** in water in a manner similar to that used for the synthesis of radiolabelled 5-fluorouracil.[10] Sodium tetraphenylborate was added to the reaction mixture to separate the spent reagent from the aqueous solution to a water insoluble salt. Unfortunately, this reaction resulted in a complex mixture of products as deduced by ^{1}H NMR spectroscopy (**Table 8.1, entry 1**). When the reaction of pyrone **439** with **243** was carried out in acetonitrile, no product was observed (**Table 8.1, entry 2**). The reaction was carried out using alternative solvents, additives and conditions, however the result in all cases was a complex mixture of products as determined ^{1}H NMR spectroscopy (**Table 8.1, entries 3 and 4**). The reaction was also attempted using NFSI **296** as the fluorinating agent, however no reaction occurred (**Table 8.1, entry 5**). Due to the lack of success thus far, attention turned to other commercially available *N*-fluoropyridinium salts **325**, **501** and **502**. The reactions were carried out in distilled DCM as this is the optimal medium for reactions involving these salts.[11] Unfortunately in all cases, only starting material was observed by ^{1}H NMR spectroscopy (**Table 8.1, entries 6-8**).

Table 8.1 Attempts at direct fluorination of 4-hydroxy-6-methyl-2-pyrone.



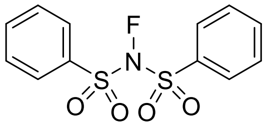
439

Fluorinating agents:



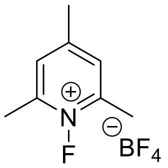
243

Selectfluor®

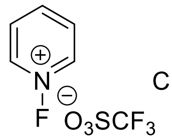


296

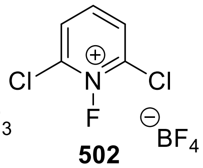
NFSI



325



501

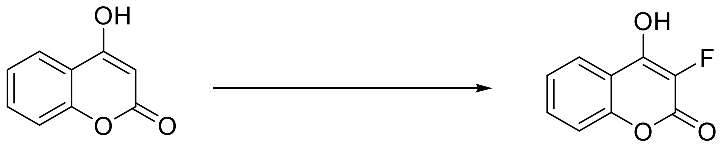


502

Entry	Fluorinating agent (equiv.)	Solvent	Additive	Conditions	Result
1	**243** (1.0)	H_2O	Sodium tetraphenyl borate (2.2 equiv.)	90 °C, 16 h	CM
2	**243** (1.2)	MeCN	None	RT, 5 d	SM
3	**243** (1.1)	$MeCN:H_2O$ 4:1	CF_3CO_2H (10% vol)	80 °C, 2 d	CM
4	**243** (1.2)	$MeNO_2:MeOH$ 5:1	None	RT, 2 d Reflux, 1 d	CM
5	**296** (1.1)	THF	None	Reflux, 4 d	SM
6	**325** (1.0)	DCM	None	RT, 3 d Reflux, 2 d	SM
7	**501** (1.0)	DCM	None	RT, 3 d Reflux, 2 d	SM
8	**502** (1.0)	DCM	None	RT, 3 d Reflux, 2 d	SM

Due to the disappointing results obtained with **439**, attention turned to the synthesis of fluorinated coumarins. 4-Hydroxycoumarin **468** was subjected to the same fluorinating conditions and reagents as used previously in attempt to synthesise fluorinated 4-hydroxy-6-methyl-2-pyrone (**Table 8.2**). However in all cases results were disappointing, with either a complex mixture of products or solely starting material present by ^{1}H NMR spectroscopy.

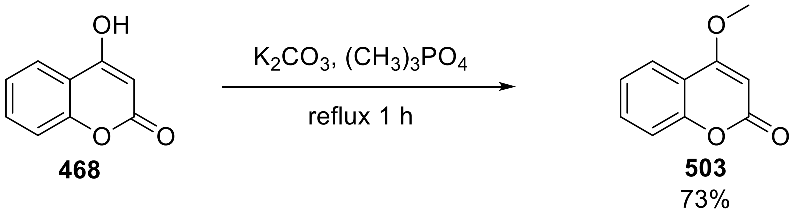
Table 8.2 Attempts at direct fluorination of 4-hydroxycoumarin.



468

Entry	Fluorinating agent (equiv.)	Solvent	Additive	Conditions	Result
1	**243** (1.0)	H_2O	Sodium tetraphenylborate (2.2 equiv.)	90 °C, 16 h	CM
2	**243** (1.2)	MeCN	None	RT, 5 d	SM
3	**243** (1.0)	MeCN:H_2O 4:1	CF_3CO_2H (10% vol)	80 °C, 2 d	CM
4	**243** (1.2)	$MeNO_2$:MeOH 5:1	None	RT, 2 d Reflux, 1 d	SM
5	**296** (1.1)	THF	None	Reflux, 4 d	SM

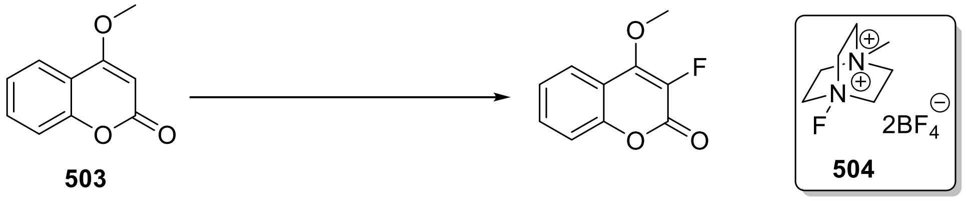
Given the lack of success with the fluorination of 4-hydroxy2-pyrone and 2-coumarin, it was surmised that if **468** was first methylated at the oxygen to provide 4-methoxycoumarin **503** (**Scheme 8.1**), the reaction may be more fruitful as the presence of a free nucleophilic hydroxyl group may be inhibiting the reaction. Additionally, many of the naturally-occurring and biologically active pyrones and coumarins possess an alkoxy group at the C-4 position.[12,13] Coumarin **503** was prepared in good yield as a white, fluffy solid by heating a stirred solution of **468**, potassium carbonate and trimethyl phosphate at 140 °C for 1 h.[14]



Scheme 8.1 Methylation of 4-hydroxycoumarin in solvent-free conditions.

Once methylated coumarin **503** was successfully synthesised, it was subjected to a variety of fluorinating agents and reaction conditions. Reactions of **503** with Selectfluor® **243** resulted in either a complex mixture of products or starting material being observed by ^{1}H NMR spectroscopy (**Table 8.3, entries 1-4, 8 and 11**). Selecfluor® II **504** was also employed in an attempt to achieve fluorination, however again these reactions resulted in either starting material or complex mixtures of products as observed by ^{1}H NMR spectroscopy (**Table 8.3, entries 6, 7 and 9**). Use of NFSI **296** and commercially available *N*-fluoropyrimidine salts **325**, **501** and **502** also proved disappointing (**Table 8.3, entries 5, 10, 12-14**).

Table 8.3 Attempts at the synthesis of 3-fluoro-4-methoxycoumarin.



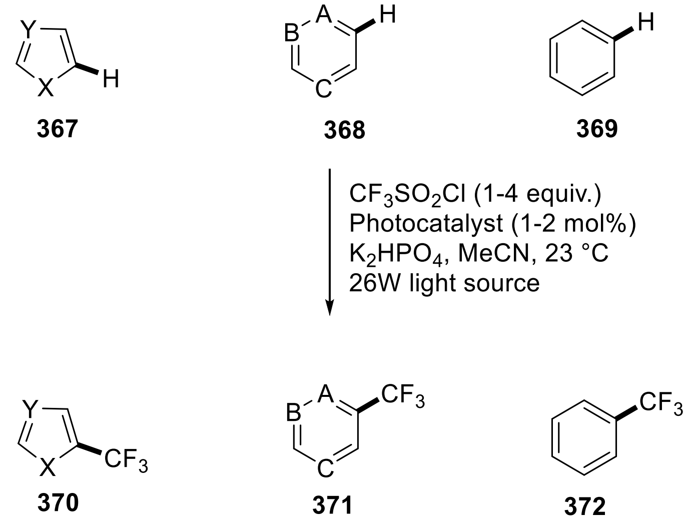
503

504

Entry	Fluorinating agent (equiv.)	Solvent	Additive	Conditions	Result
1	**243** (1.2)	EtOH	None	RT, 6 d Reflux, 2 d	CM
2	**243** (1.2)	MeOH	H_2SO_4 (0.1 equiv.)	Reflux, 6 d	SM
3	**243** (1.2)	MeCN	None	RT, 2 d 50 °C, 3 d	CM
4	**243** (1.2)	$MeNO_2$	None	RT, o/n Reflux, 3 d	CM
5	**296** (1.2)	MeOH	None	RT, o/n Reflux, 5 d	SM
6	**504** (1.2)	EtOH	None	RT, o/n Reflux, o/n	SM
7	**504** (1.2)	MeOH	H_2SO_4 (0.1 equiv.)	50 °C, 6 d	CM
8	**243** (1.2)	H_2O	None	Reflux, 6 d	SM
9	**504** (1.2)	MeCN	None	RT, 2 d Reflux, 3 d	SM
10	**296** (1.1)	THF	None	Reflux, 4 d	SM
11	**243** (1.2)	$MeNO_2$:MeOH 5:1	None	RT, 1 d Reflux, 2 d	CM
12	**325** (1.0)	DCM	None	RT, o/n Reflux, o/n	CM
13	**501** (1.0)	DCM	None	RT, o/n Reflux, o/n	SM
14	**502** (1.0)	DCM	None	RT, o/n Reflux, o/n	SM

8.3 Trifluoromethylation

Due to the lack of success with the fluorination of pyrones and coumarins, attention turned to methodologies to trifluoromethylate these important substrates. Initial attempts to gain access to trifluoromethylated products focused on the direct functionalisation of various starting materials using the published photoredox catalysts described by MacMillan.[15] In this methodology, polypyridyl organometallic complexes are employed, whose excitation at room temperature by a source of light provides a strongly oxidising or reducing catalyst that can react with a variety of substrates to provide high energy, reactive species.[16] Triflyl chloride was the trifluoromethylating agent of choice and the reaction could be carried out in acetonitrile at room temperature. The properties of the starting material determined which photocatalyst would be most appropriate for the reaction. Ru(phen)$_3$Cl$_2$ was employed with five-atom electron rich heteroarenes **367** to furnish **370**, whereas an iridium photocatalysis with a longer lived excitation state was used with six-atom electron deficient heteroarenes **368** and unactivated arenes **369** to provide **371** and **372** respectively (**Scheme 8.2**).

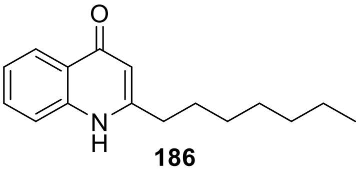
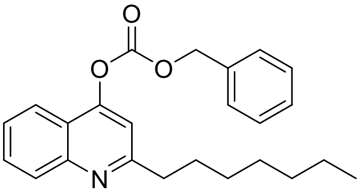
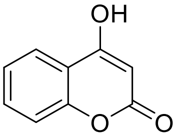
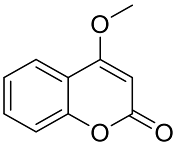
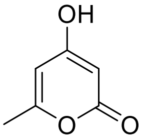
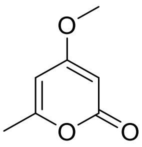


Scheme 8.2 Trifluoromethylation by means of a photoredox catalyst.

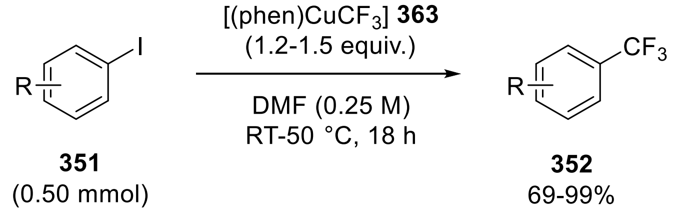
Attempts using both the iridium and ruthenium catalysts under the reported conditions were tried with a number of substrates (**Table 8.4**). Having previously synthesised HHQ **186** and its protected analogue **265**, these substrates were also subjected to the photocatalytic conditions. However in all cases, only starting material was observed by ^{1}H NMR spectroscopy (**Table 8.4, entries 1-4**). Both 4-hydroxycoumarin **468** and 4-methoxycoumarin **503** were also used as substrates, however the reactions were unsuccessful with only starting material (**Table 8.4, entries 5, 6 and 8**) or a trace amount of product being observed by ^{1}H NMR or mass spectrometry respectively. Attempts to trifluoromethylate 4-hydroxy-2-pyrone **439** or 4-methoxy-2-pyrone **505** also proved futile under the reaction conditions (**Table 8.4, entries 9-12**).

As trifluoromethylation reactions using MacMillan's conditions with a number of our substrates had proved unsuccessful, it was decided to investigate the reproducibility of the reaction using 4-(3*H*)-pyrimidinone. This substrate had been successfully used by MacMillan with the trifluoromethylated product obtained in 74% yield. When the same reaction was attempted, no product was obtained in our hands, suggesting this type of methodology can be difficult to replicate.

Table 8.4 Attempts at photocatalytic trifluoromethylation.

Entry	Starting Material	Catalyst (mol%)	CF_3SO_2Cl (equiv.)	Result
1	 **186**	Ir(dFppy)$_3$ (2)	4	SM
2		Ru(phen)$_3$Cl$_2$ (1)	2	SM
3	 **265**	Ir(dFppy)$_3$ (2)	4	SM
4		Ru(phen)$_3$Cl$_2$ (1)	2	SM
5	 **468**	Ir(dFppy)$_3$ (2)	4	SM
6		Ru(phen)$_3$Cl$_2$ (1)	2	SM
7	 **503**	Ir(dFppy)$_3$ (2)	4	Trace of product by MS
8		Ru(phen)$_3$Cl$_2$ (1)	2	SM
9	 **439**	Ir(dFppy)$_3$ (2)	4	Trace of product by MS
10		Ru(phen)$_3$Cl$_2$ (1)	2	SM
11	 **505**	Ir(dFppy)$_3$ (2)	4	SM
12		Ru(phen)$_3$Cl$_2$ (1)	2	SM

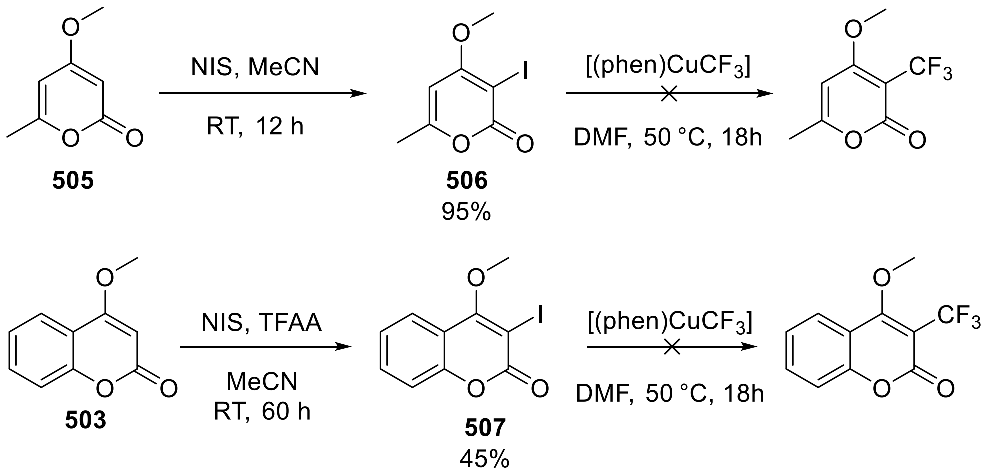
Attention then turned to an alternative strategy in order to generate the desired products. There have been numerous reports on the use of pre-functionalised starting materials to synthesise trifluoromethylated products.[17-19] One of the most successful methodologies to generate trifluoromethylated products **352** from a pre-functionalised substrate **351** is that reported by Hartwig's group in 2011 using a phenanthroline-ligated copper(I) complex [(phen)CuCF$_3$] **363** (**Scheme 8.3**).[20] A variety of iodinated substrates were successfully trifluoromethylated using this methodology in good to high yields.



R = alkyl, aryl, aldehyde, ketone, amine, ester, nitro, heterocycle

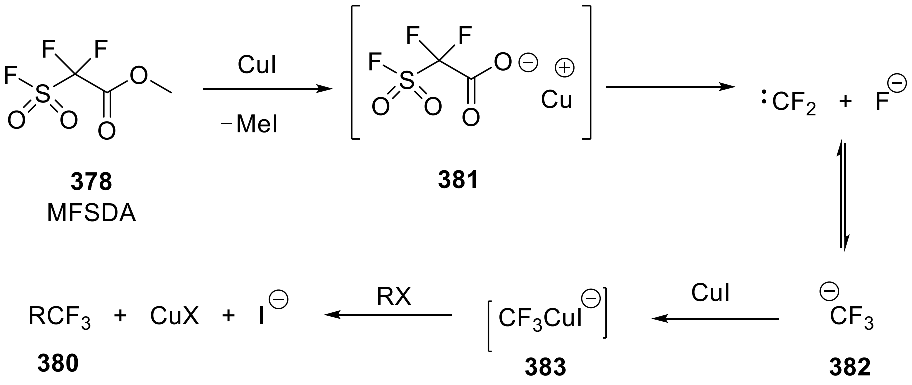
Scheme 8.3 Hartwig's trifluoromethylation using [(phen)CuCF$_3$].

With these results in mind, it was postulated that this methodology could be extrapolated to pyrones and related compounds. Pyrone **505** was iodinated in a regioselective manner at C-3, the more nucleophilic site, using NIS in acetonitrile to provide **506** in 95% yield. However, exposure to Hartwig's trifluoromethylation methodology yielded a complex mixture of products. The reaction was also attempted using a coumarin. In this case, iodination of **503** was achieved using NIS and trifluoroacetic acid in acetonitrile to provide **507** in 45% yield after purification by recrystallisation. When trifluoromethylation using Hartwig's conditions was attempted on **507**, a complex mixture of products again resulted (**Scheme 8.4**). Due to the high cost of the catalyst required for this reaction, an alternative strategy for trifluoromethylation was sought.



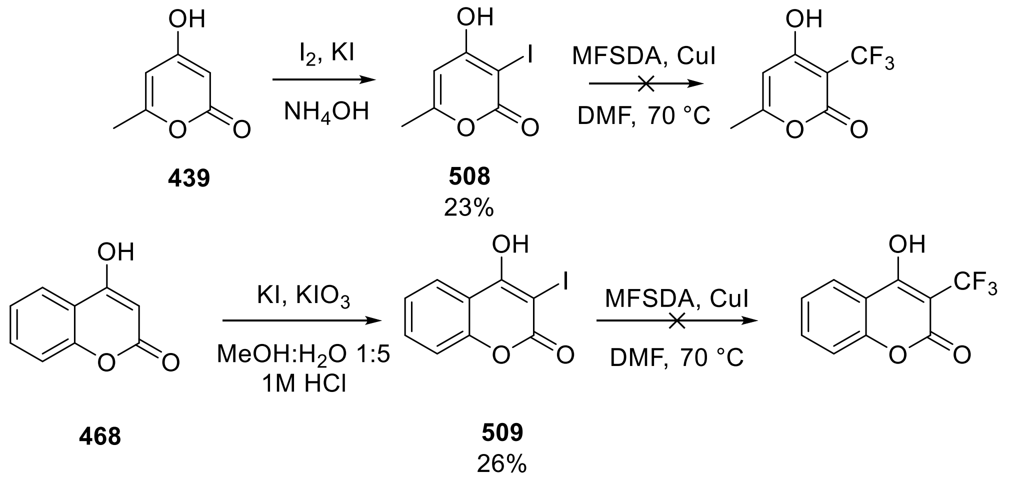
Scheme 8.4 Attempts at trifluoromethylation of pre-functionalised substrates using Hartwig's catalyst.

An alternative route to trifluoromethylated compounds involves reaction of pre-functionalised starting materials with methyl fluorosulfonyldifluoroacetate (MFSDA) **378** as a trifluoromethylating agent.[21] MFSDA came to the fore as a trifluoromethylating agent in the late 1980's. It was reported that trifluoromethylation of aryl, alkenyl and alkyl halides could be achieved in the presence of copper(I) iodide in DMF in good yields.[21] The accepted mechanism for the reaction (**Scheme 8.5**) comprises an initial step involving the formation of a copper salt **381** from MFSDA **378** with the elimination of methyl halide. The salt then decomposes to release difluorocarbene and a fluoride ion, which are in equilibrium with a DMF stabilised trifluoromethyl anion **382**. In the presence of CuI, the equilibrium shifts to form $[CF_3CuI^-]$ **383**, which reacts with a halogenated starting material to provide the trifluoromethylated product **380**, following release of CuX and I^-.[21] A radical mechanism has been ruled out on the basis of experimental and computational data.[22]



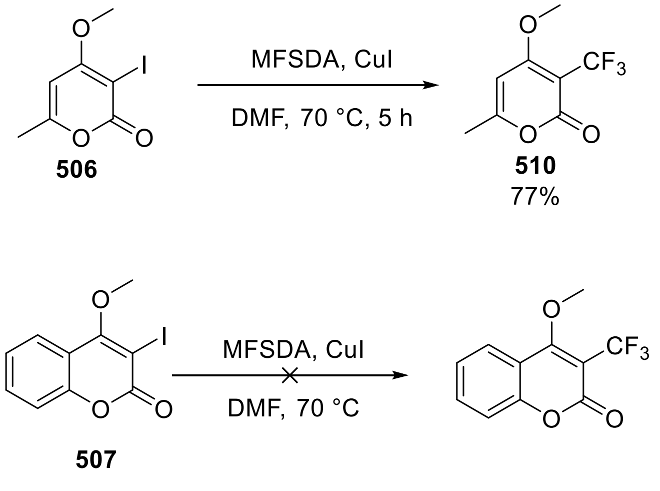
Scheme 8.5 Mechanism of trifluoromethylation using methyl fluorosulfonyldifluoroacetate (MFSDA).

Initial attempts at trifluoromethylation using this methodology were carried out on 4-hydroxy-6-methyl-2-pyrone **439** and 4-hydroxycoumarin **468**. Both **439** and **468** proved difficult to iodinate and low yields of both **508** and **509** were obtained. However pyrone **508** was obtained upon reaction of **439** with iodine in aqueous KI and the minimum amount of ammonium hydroxide. This methodology had previously been successfully used in the synthesis of 3-iodo-4-acetoxy-coumarins.[23] An alternative iodination strategy was used for the synthesis of coumarin **509**, whereby 1M HCl was added dropwise to a solution of **468**, potassium iodide and potassium iodide in a methanol/water mixture.[24] With the 4-hydroxy-3-iodo pyrone (**508**) and coumarin (**509**) in hand, the compounds were treated with 1.2 equiv. of MFSDA and 1.2 equiv. of CuI in DMF at 70 °C, however in both cases a complex mixture resulted and no trifluoromethylated product was obtained (**Scheme 8.6**).


OH
I₂, KI
NH₄OH
439
508
23%
MFSDA, CuI
DMF, 70 °C
CF₃
468
KI, KIO₃
MeOH:H₂O 1:5
1M HCl
509
26%

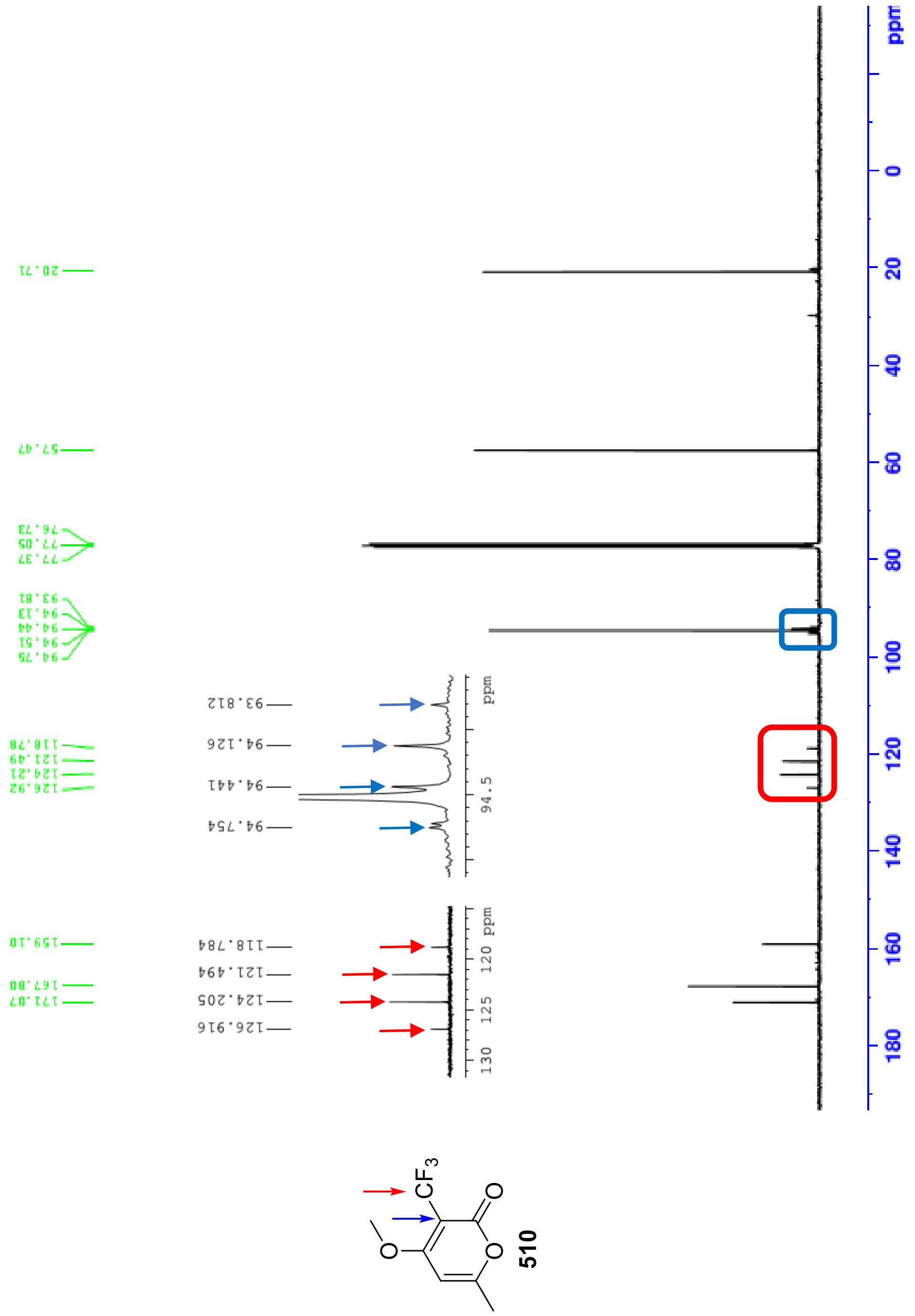
Scheme 8.6 Initial trifluoromethylation attempts using MFSDA.

Again we decided to utilise the 4-methoxy variants having previously methylated and iodinated the pyrone and coumarin starting materials (**Scheme 8.4**). Trifluoromethylation was attempted using 1.2 equiv. of MFSDA and 1.2 equiv. of CuI in DMF at 70 °C and although a complex mixture resulted on reaction with **507**, reaction with **506** allowed trifluoromethylated product **510** to be obtained in 77% yield after purification (**Scheme 8.7**).


506
MFSDA, CuI
DMF, 70 °C, 5 h
CF₃
510
77%
507
MFSDA, CuI
DMF, 70 °C

Scheme 8.7 Trifluoromethylation on methoxy-based starting materials.

Successful incorporation of the trifluoromethyl group into the pyrone was indicated by the presence of two quartets in the ^{13}C NMR spectrum of **510**. Due to one- and two-bond carbon-fluorine coupling, the three fluorine atoms present cause splitting of the carbon of the trifluoromethyl group and the quaternary carbon bonded to it (**Figure 8.1**). The noticeable disappearance of the signal corresponding to the proton at C-3 in the ^{13}C NMR spectrum also proves the trifluoromethyl group has been successfully incorporated.



510

Figure 8.1 ^{13}C NMR of trifluoromethylated pyrone **510**.

The presence of a singlet in the ^{19}F NMR spectrum of **510** also provides confirmation of successful incorporation of the trifluoromethyl group into the molecule (**Figure 8.2**). Further evidence of the formation of **510** was obtained from both the HRMS and elemental analysis.

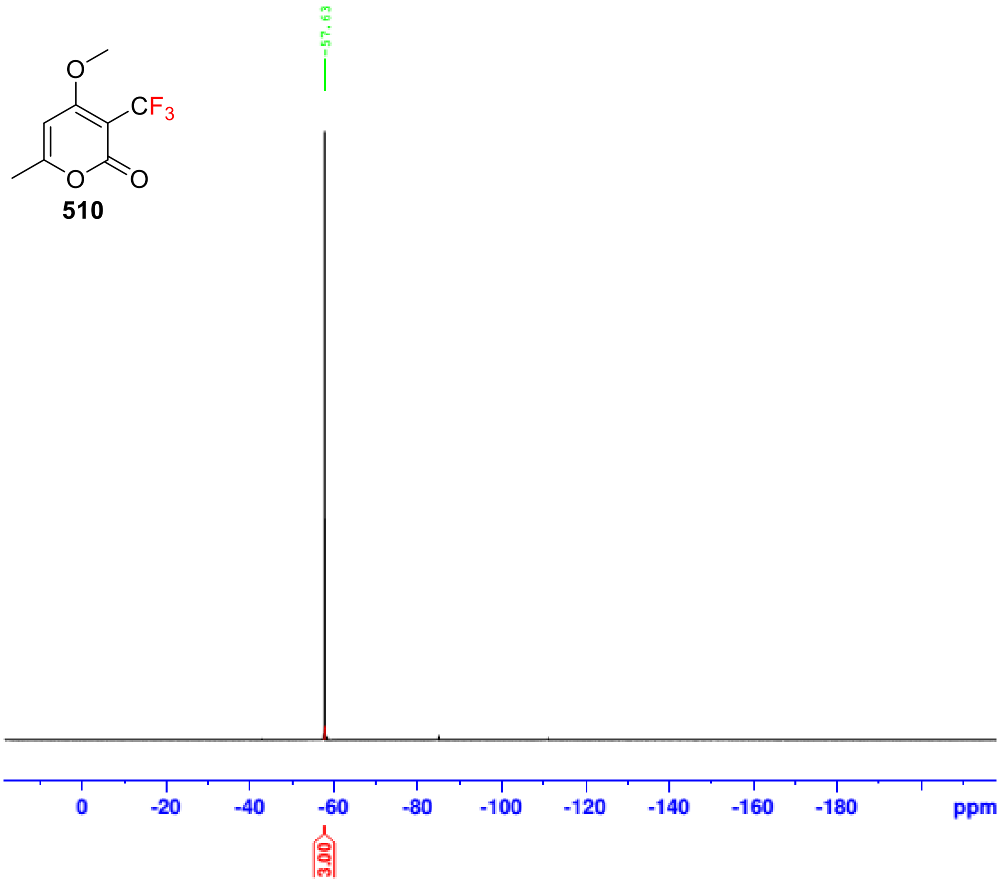
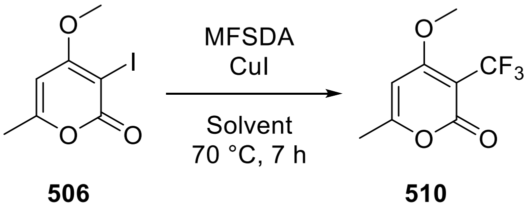


Figure 8.2 ^{19}F NMR spectrum of trifluoromethylated pyrone **510**.

With successful synthesis of trifluoromethylated pyrone **510** now achieved, it was necessary to carry out optimisation of the reaction to determine the ideal conditions. 3-Iodo-4-methoxy-2-pyrone **506** was treated with 1.2 equiv. of MFSDA in the presence of 1.2 equiv. of copper(I) iodide in a number of anhydrous solvents. When the reaction was carried out in THF and DMSO (**Table 8.5, entries 1 & 2**), no product was observed, however a modest yield (53%) of trifluoromethylated product **510** was achieved when NMP was used as solvent (**Table 8.5, entry 3**). Changing the solvent to DMF or DMA resulted in an increased yield of 77 and 79% respectively (**Table 8.5, entries 6 & 7**). Higher equivalents of MFSDA caused a slight decrease in yield (**Table 8.5, entry 5**).

Gratifyingly, the reaction can be successfully carried out in commercially available DMF which was used without purification, albeit with a slightly reduced yield of 63% (**Table 8.5, entry 4**). From these screening reactions, a balance of yield and cost was struck and it was decided to proceed using 1.2 equiv. of MFSDA in anhydrous DMF. The next step in the investigation involved determining the optimal amount of CuI required for the reaction. 3-Iodo-4-methoxy-2-pyrone **506** was treated with 1.2 equiv. of MFSDA in the presence of various equivalents of copper(I) iodide in anhydrous DMF. When the amount of CuI employed was equal to or less than 1.0 equiv., the yield of **510** was 61-65% (**Table 8.5, entries 8 & 9**). When 1.5 equiv. of CuI was used, the amount of **510** obtained increased (**Table 8.5, entry 10**), however the best yield was obtained when 1.2 equiv. of MFSDA were employed (**Table 8.5, entry 6**).

Table 8.5 Optimisation of trifluoromethylation reaction.

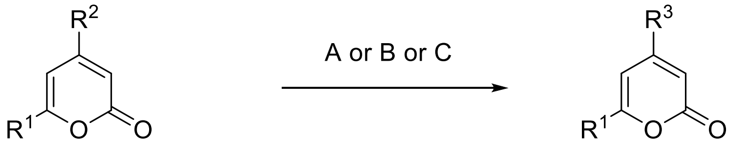


Entry	Solvent	CuI (equiv.)	MFSDA (equiv.)	Yield 510 (%)[a]
1	THF	1.2	1.2	No reaction
2	DMSO	1.2	1.2	No reaction
3	NMP	1.2	1.2	53
4	DMF[b]	1.2	1.2	63
5	DMF	1.2	5	66
6	DMF	1.2	1.2	77
7	DMA	1.2	1.2	79
8	DMF	0.5	1.2	61
9	DMF	1.0	1.2	65
10	DMF	1.2	1.5	71

[a] Isolated. [b] Commercially available DMF was employed as solvent without purification/drying.

With workable conditions in hand, the scope of the reaction was investigated. Different protecting groups were incorporated at the oxygen at C-4 to provide **511a-d** (**Table 8.6**). MOM-protected pyrone **511a** was obtained in high yield following deprotonation with triethylamine and subsequent reaction with methyl bromomethyl ether.[25] The same methodology was used to synthesise novel protected pyrones **511b** and **511c** however the yields achieved were more moderate. Pyrone **511d** was prepared in the same manner, however the yield obtained was much lower than that reported when potassium carbonate was used as the base.[26] The nature of the base used for deprotonation should not be the reason for the low yield, as previous reactions using triethylamine have provided alkylated products in high yield. This suggests that the problem may arise due to incomplete reaction with the benzylbromide. Compounds **511e** and **511f** were synthesised using the same methodology however yields were again low, likely due to the nature of the alkylating agents. Phenoxy pyrone **511g** was also obtained in high yield from 4-bromo-6-methyl-2-pyrone[27] **512**. Methoxy compounds **511i** and **511j** were synthesised from the corresponding 4-hydroxy-2-pyrones in low and high yield respectively.

Table 8.6 Synthesis of 4-substituted 2-pyrones.



439 R^1 = Me, R^2 = OH
512 R^1 = Me, R^2 = Br
513 R^1 = C_6H_4(*p*-Cl), R^2 = OH
514 R^1 = C_6H_4(*p*-CF_3), R^2 = OH

511a-j

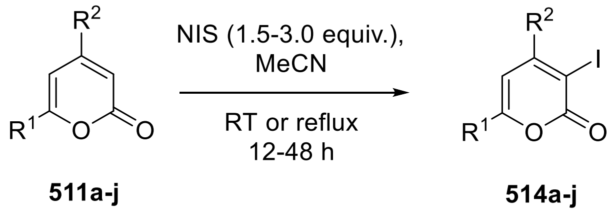
A: Et_3N (1.1 equiv.), DCM, 0°C, 10 min
B: K_2CO_3, acetone, phenol, reflux 24 h
C: K_2CO_3, trimethylphosphate, reflux o/n

Entry	SM	Reaction Conditions	Compound	R^1	R^2	Yield 510 (%)[a]
1	**439**	A	**a**	CH_3	OMOM	92
2	**439**	A	**b**	CH_3	OMEM	65
3	**439**	A	**c**	CH_3	OCbz	45
4	**439**	A	**d**	CH_3	OCH_2Ph	8
5	**439**	A	**e**	CH_3	OEt	12
6	**439**	A	**f**	CH_3	OPr	7
7	**512**	B	**g**	CH_3	OPh	91
8	**439**	N/A[b]	**h**	CH_3	Cl	18[b]
9	**513**	C	**i**	C_6H_4(*p*-Cl)	OCH_3	33[c]
10	**514**	C	**j**	C_6H_4(*p*-CF_3)	OCH_3	87[c]

[a] Isolated. [b] Previously synthesised within the group. [c] Starting materials **513** and **514** previously synthesised within the group.

Iodination of **511a-j** was smoothly accomplished using NIS in acetonitrile in all cases. Products were afforded in good to excellent yields (**Table 8.7**), with iodination occurring solely at the 3-position for all compounds synthesised.

Table 8.7 Synthesis of 3-iodo-2-pyrones.

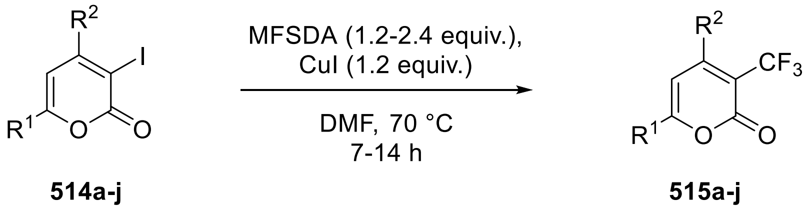


Entry	Compound	R^1	R^2	Yield 514 (%)[a]
1	**a**	CH_3	OMOM	74
2	**b**	CH_3	OMEM	42
3	**c**	CH_3	OCbz	81
4	**d**	CH_3	OCH_2Ph	84
5	**e**	CH_3	OEt	75
6	**f**	CH_3	OPr	96
7	**g**	CH_3	OPh	96
8	**h**	CH_3	Cl	75
9	**i**	C_6H_4(*p*-Cl)	OCH_3	77
10	**j**	C_6H_4(*p*-CF_3)	OCH_3	76

[a] Isolated.

Trifluoromethylation was generally successful, however pyrones containing commonly used protecting groups including MOM, MEM and Cbz did not tolerate the reaction conditions and resulted in a complex mixtures of products (**Table 8.8 entries 1-3**). When various other groups were present at the oxygen at C-4 (and a methyl group at C-6) (**Table 8.8 entries 4-8**), trifluoromethylated products were obtained in moderate yield and no trend was apparent. Variation of the substituent at C-6 was then investigated (**Table 8.8, entries 9 & 10**). A dramatic difference in yield of trifluoromethylated products was observed depending on the nature of the substituent on the phenyl ring at C-6.

Table 8.8 Synthesis of 3-trifluoromethyl-2-pyrones.



Entry	Compound	R^1	R^2	Yield 515 (%)[a]
1	**a**	CH_3	OMOM	CM
2	**b**	CH_3	OMEM	CM
3	**c**	CH_3	OCbz	CM
4	**d**	CH_3	OCH_2Ph	30
5	**e**	CH_3	OEt	34
6	**f**	CH_3	OPr	46
7	**g**	CH_3	OPh	43
8	**h**	CH_3	Cl	23
9	**i**	C_6H_4(*p*-Cl)	OCH_3	23
10	**j**	C_6H_4(*p*-CF_3)	OCH_3	72

[a] Isolated.

The ^{13}C NMR spectrum of **515j** is particularly interesting due to the two trifluoromethyl groups present on the molecule. In both cases the fluorine atoms cause splitting of adjacent carbon atoms into quartets (**Figure 8.3**). The presence of two trifluoromethyl groups is evident from the two quartets at 122.6 ppm and 123.4 ppm with coupling constants of 273.1 and 272.6 Hz respectively. The quartet at 96.3 ppm with a coupling constant of 31.9 Hz corresponds to the quaternary carbon bonded to a trifluoromethyl group. Similarly, the peak corresponding to the quaternary carbon of the phenyl ring which is directly bonded to the trifluoromethyl group appears as a quartet at 133.9 ppm with a coupling constant of 33.1 Hz. Three-bond carbon-fluorine coupling is also apparent in this spectrum, with the equivalent aromatic carbons appearing as a quartet at 126.2 ppm with a coupling constant of 3.7 Hz, typical of that for three-bond carbon-fluorine coupling. Structural assignment of **515j** was aided with 2D-NMR spectroscopy.

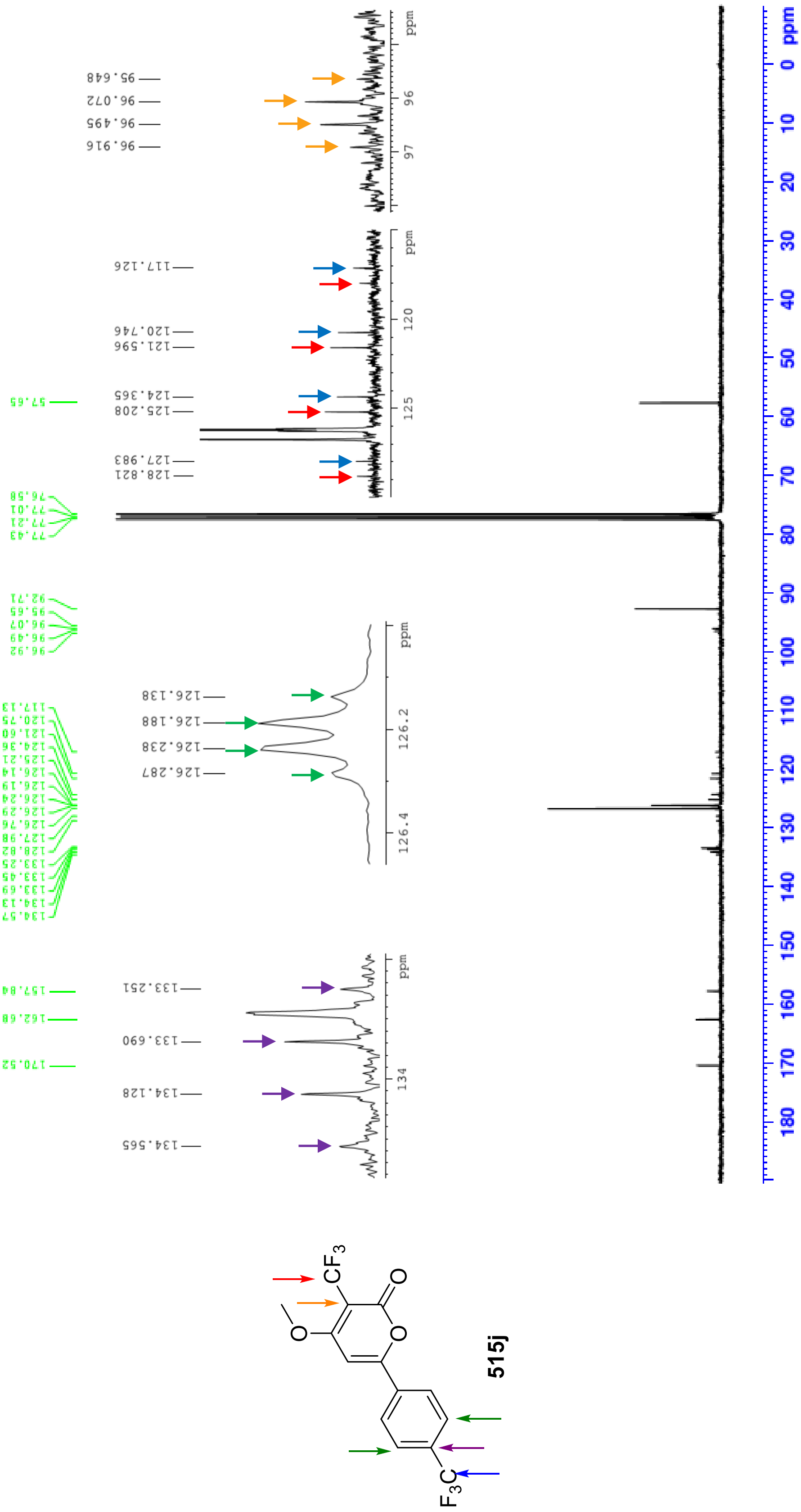
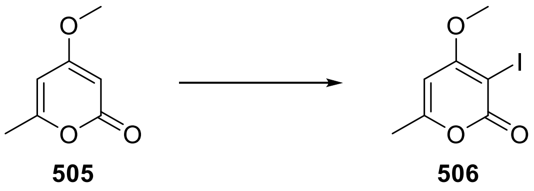


Figure 8.3 ^{13}C NMR spectrum of **515j** with magnification of quartets.

It was then postulated that it may be possible to achieve selective trifluoromethylation when more than one iodine atom is present on a molecule. In an effort to investigate this, iodination at both C-3 and C-5 of **505** was attempted. Initial efforts at diiodination were carried out on **505** (**Table 8.9**) however results were disappointing resulting in starting material, complex mixtures or monoiodination at C-3.

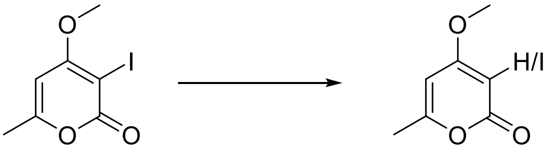
Table 8.9 Attempts at diiodination of 4-methoxy-6-methyl-2-pyrone.



Entry	Conditions	Result
1	NIS (4.0 equiv.), MeCN	Monoiodination at C-3 (**506**)
2	KI/KIO$_3$, MeOH:H$_2$O, 1M HCl	SM
3	30% aq. H$_2$O$_2$, I$_2$, H$_2$O	Monoiodination at C-3 (**506**)
4	NaIO$_4$/KI/NaCl, AcOH:H$_2$O 9:1, 25 °C	CM

The next logical step was to investigate if the monoiodinated product could be further iodinated to produce the desired diiodinated product. However results were again disappointing with either no reaction, a complex mixture resulting or even in some cases hydrodeiodination (**Table 8.10**) observed.

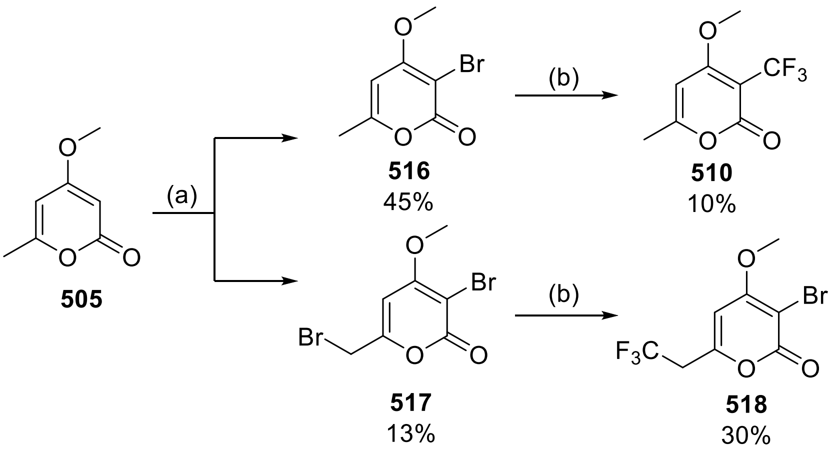
Table 8.10 Attempts at iodination of 3-iodo-4-methoxy-6-methyl-2-pyrone.



506 **505/506**

Entry	Conditions	Result
1	NIS (4.0 equiv.), MeCN, reflux	SM
2	TBAI, KOAc, Toluene	CM
3	30% aq. H_2O_2, I_2, H_2O	Hydrodeiodination (**505**)
4	KI/KIO_3, MeOH:H_2O, HCl (1M)	Hydrodeiodination (**505**)
5	NAIO_4/KI/NaCl, AcOH:H_2O 9:1, 25 °C	SM

The next step in the investigation was to test the optimised reaction conditions using a brominated analogue. Bromination of **505** in a regioselective manner was carried out using NBS to afford **516** in 45% yield. Trifluoromethylation was carried out as per the procedure used for the iodinated starting material, however **510** was obtained in only 10% yield. In comparison to trifluoromethylation of 3-iodo-4-methoxy-2-pyrone **506**, the yield was decreased dramatically. This could be rationalised based on the order of reactivity observed by Chen and Wu (RI > RBr > RCl).[21] In the formation of **516**, the dibrominated pyrone **517** was also observed and isolated in 13% yield. Dibrominated pyrone **517** was also subjected to the trifluoromethylation conditions to investigate the regioselectivity of the protocol. When 1.0 equiv. of MFSDA was employed, trifluoromethylation occurred at the sp^3 carbon affording **518** in 30% yield and no product resulting from trifluoromethylation at the sp^2 position was observed (**Scheme 8.8**).



Scheme 8.8 Trifluoromethylation of bromo compounds (a) NBS, MeCN; (b) MFSDA, CuI, DMF.

Confirmation that trifluoromethylation of **517** occurred at the sp^3 carbon rather than the sp^2 carbon was obtained from the ^{13}C NMR spectrum (**Figure 8.4**). On the spectrum, three quartets are evident, with coupling constants typical of those for one- two- and three-bond carbon-fluorine coupling (277.8 Hz, 32.0 Hz and 3.5 Hz respectively). The splitting of the CH_2 group into a quartet at 38.8 ppm is due to the presence of a neighbouring trifluoromethyl group, providing proof that trifluoromethylation occurred at the sp^3 carbon. Further evidence to corroborate this is that the quaternary carbon at C-6 is also split into a quartet due to the effect of the near-by fluorine atoms. If trifluoromethylation had instead occurred at the sp^2 carbon, only two quartets would have been observed, and most obviously, the peak corresponding to the CH_2 group would appear as a singlet. Further evidence for the successful incorporation of the trifluoromethyl group is obtained from the high resolution mass spectrum, with the found mass within 1.7 ppm of the calculated mass.

With regards to the mechanism of the reaction, a recent publication suggests that trifluoromethylation reactions of aryl halides involving a source of *in situ* trifluoromethyl copper do not procced *via* a radical mechanism. Rather, the authors state that the mechanism occurs *via* an oxidative addition-reductive elimination pathway.[22] In the case of the selective trifluoromethylation of **517** however, a radical mechanism for substitution at the sp^3 carbon should be considered. In this case, a benzylic-type radical would be formed which may promote this pathway. Further mechanistic studies are required in this area.

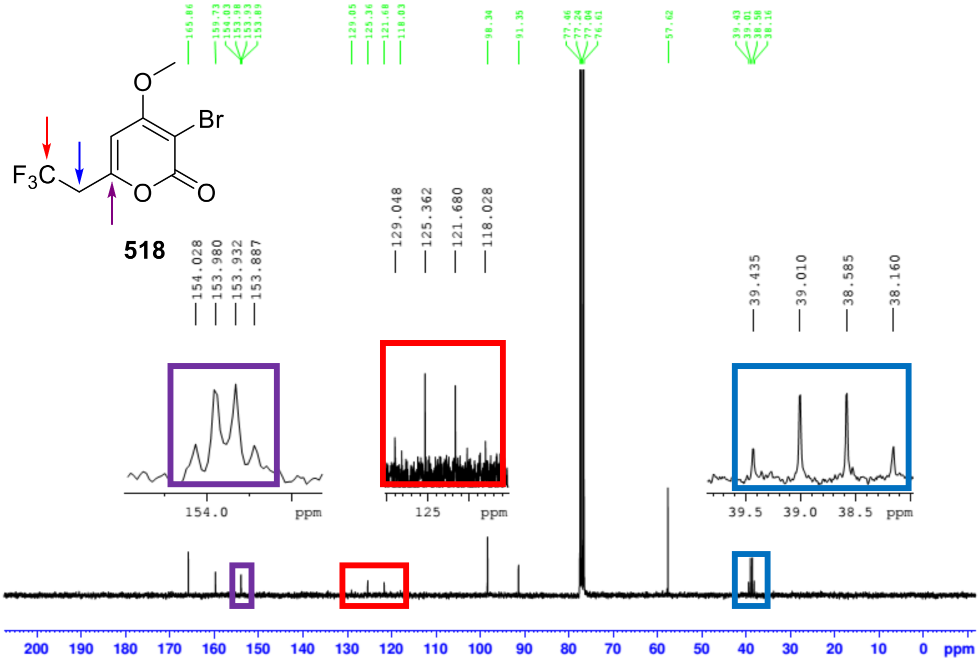
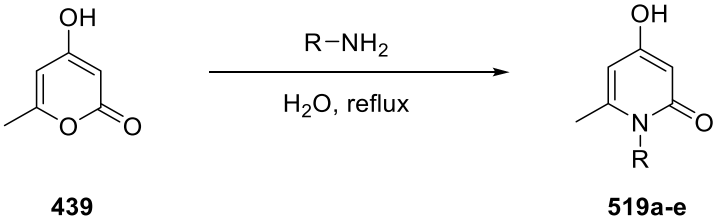


Figure 8.4 ^{13}C NMR spectrum of trifluoromethylated pyrone **518** showing that trifluoromethylation occurred at the sp^3 carbon.

Due to the number of biologically important compounds containing the pyridone moiety,[28-30] extension of the methodology to pyridones was then explored. Pyridones **519a-e** (**Table 8.11**) were synthesised following a literature procedure by heating the corresponding pyrone with the desired amine in water at reflux.[31] The crude product was purified by carrying out a trituration in hot ethanol and filtering the hot precipitate. Yields were low, however this is consistent with what has previously been found within the group.

Table 8.11 Synthesis of 2-pyridones from 2-pyrones.

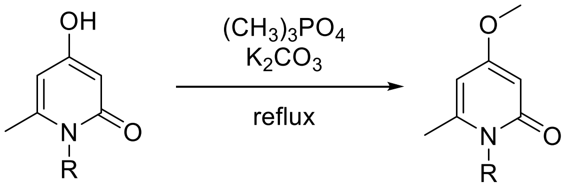


Entry	**Compound**	**R**	**Yield 519 (%)**[a]
1	**a**	CH_3	55
2	**b**	CH_2Ph	51[b]
3	**c**	Ph	14
4	**d**	$C_6H_4(p\text{-F})$	13
5	**e**	$C_6H_4(p\text{-OCH}_3)$	23

[a] Isolated. [b] Previously synthesised within the group.

Pyridones **519a-e** were then methylated at the oxygen using the same procedure that had been used for methylation of pyrone **439** and coumarin **468**. Methylated pyridones **520a-e** were obtained in variable yields with no obvious trend apparent between the nature of the substrate and the yield of product (**Table 8.12**).

Table 8.12 Methylation of 2-pyridones.



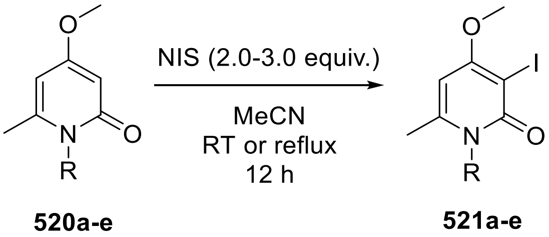
519a-e **520a-e**

Entry	**Compound**	**R**	**Yield 520 (%)**[a]
1	**a**	CH_3	36
2	**b**	CH_2Ph	57[b]
3	**c**	Ph	70
4	**d**	C_6H_4(*p*-F)	60
5	**e**	C_6H_4(*p*-OCH_3)	44

[a] Isolated.

Iodination was successfully carried out in all cases to afford **521a-e**. It was noted that in some cases, the yield of the iodination step was low (**Table 8.13, entries 1, 4 & 5**). This was discovered to be due to diiodination occurring as deduced by ^{1}H and ^{13}C NMR spectroscopy, however these products were not isolated from the reaction mixture. This finding means that iodination of **520a-e** must be carefully and frequently monitored to prevent this unwanted side reaction from occurring. Subsequent trifluoromethylation of **521a-e** provided the desired pyridones **522a-e** in moderate to good yield (**Table 8.14**).

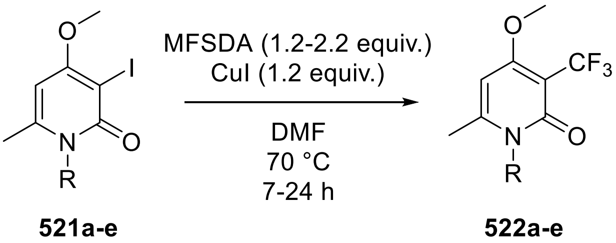
Table 8.13 Iodination of 4-alkoxy-2-pyrones.


NIS (2.0-3.0 equiv.)
MeCN
RT or reflux
12 h
520a-e
521a-e

Entry	**Compound**	**R**	**Yield 521 (%)**[a]
1	**a**	CH_3	36
2	**b**	CH_2Ph	70
3	**c**	Ph	84
4	**d**	C_6H_4(*p*-F)	31
5	**e**	C_6H_4(*p*-OCH_3)	13

[a] Isolated.

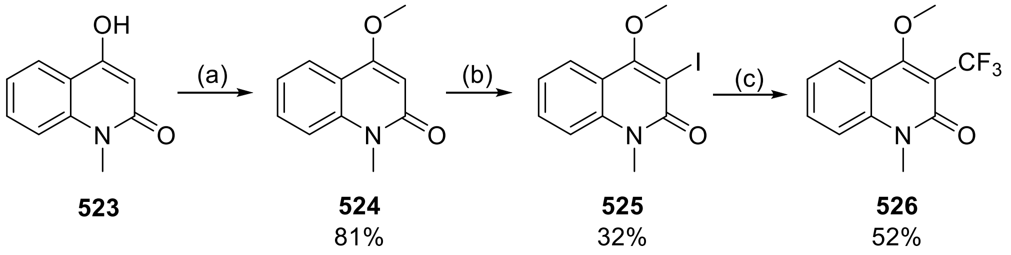
Table 8.14 Trifluoromethylation of 2-pyridones.


MFSDA (1.2-2.2 equiv.)
CuI (1.2 equiv.)
DMF
70 °C
7-24 h
521a-e
522a-e

Entry	**Compound**	**R**	**Yield 522 (%)**[a]
1	**a**	CH_3	61
2	**b**	CH_2Ph	62
3	**c**	Ph	29
4	**d**	C_6H_4(*p*-F)	52
5	**e**	C_6H_4(*p*-OCH_3)	46

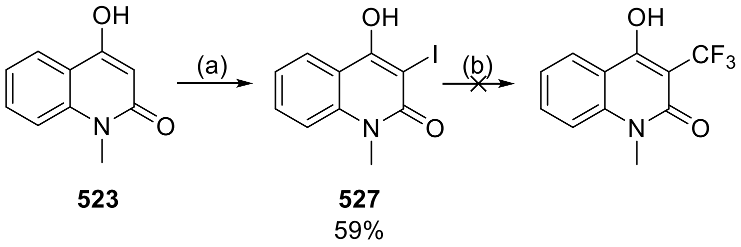
[a] Isolated.

The reaction conditions are also suitable for the trifluoromethylation of quinolones (**Scheme 8.9**), as demonstrated with commercially available **523**, which was methylated at the oxygen to yield **524**, iodinated to provide **525** and trifluoromethylated to provide **526** in good yield. Interestingly, in the ^{13}C NMR spectrum of **526** five-bond carbon-fluorine coupling is observed for the carbon of the methoxy group. However similar long-range coupling has previously been reported.[32]



Scheme 8.9 Formation of trifluoromethylated quinolone (a) $(CH_3)_3PO_4$, K_2CO_3; (b) NIS, MeCN, trifluoroacetic acid; (c) MFSDA, CuI, DMF.

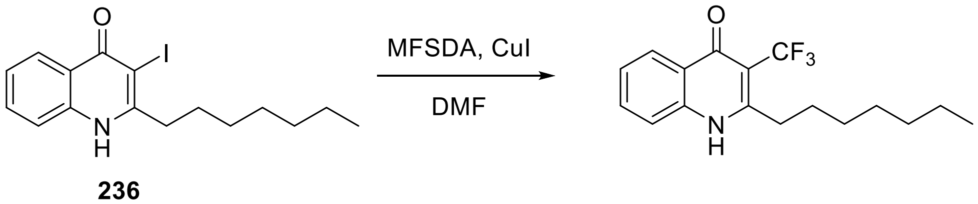
It was then decided to investigate if methylation of **523** was necessary to ensure successful trifluoromethylation. Thus **523** was successfully iodinated using potassium iodide/potassium iodate and isolated in a similar yield to that obtained when synthesised using a different methodology.[33] However, upon exposing **527** to our trifluoromethylation conditions, a complex mixture of products resulted (**Scheme 8.10**). This suggests that regardless of the substrate used in the trifluoromethylation reaction, hydroxyl groups must first be protected to ensure a successful reaction.



Scheme 8.10 Attempt at trifluoromethylation of non *O*-functionalised quinolone (a) KI/KIO_3, MeOH/H_2O, 1M HCl ; (b) MFSDA, CuI, DMF.

Attention then focussed on generating 2-heptyl-3-(trifluoromethyl)quinolin-4(1*H*)-one, the C-3 trifluoromethyl analogue of HHQ, a quorum sensing molecule used by *Pseudomonas aeruginosa*. Due to the ease of synthesising **526** in good yield and the similarity in structure to the 2- and 4-quinolone basic structure, trifluoromethylation was attempted using the same strategy. Thus, HHQI **237** (which had previously been synthesised for testing as a potential quorum sensing inhibitor (Chapter 5)) was exposed to 1.2 equiv. of MFSDA and 1.2 equiv. CuI in anhydrous DMF at 70 °C for 24 h. The result of this experiment was disappointing (**Table 8.15, entry 1**) and no reaction took place. A large scale repetition was also unsuccessful (**Table 8.15, entry 2**). The reaction was then repeated with a larger excess of MFSDA (**Table 8.15, entry 3**), however only a trace of product was observed by ^{1}H NMR spectroscopy. The lack of success with this reaction may be due to the presence of the long alkyl chain at C-2, which could be causing severe steric hindrance of the C-3 position, preventing the reaction from taking place.

Table 8.15 Attempts at synthesising trifluoromethylated HHQ.



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Entry	SM (mmol)	MFSDA (equiv.)	CuI (equiv.)	Solvent	Result
1	0.39	1.2	1.2	DMF	SM
2	1.42	1.2	1.2	DMF	SM
3	0.34	5.0	1.2	DMF	Trace

8.4 Conclusions and future work

A successful methodology for the convenient, late stage trifluoromethylation of pyrones, pyridones and quinolones has been developed, utilising the relatively cheap, commercially available trifluoromethylating agent, MFSDA. The remarkable biological activity of these compound classes, coupled with the growing significance attributed to the introduction of a trifluoromethyl group establishes the described protocol as a useful route to these compounds. Although substrates require pre-functionalisation, this route is more cost efficient than other methodologies that use iodinated starting materials and expensive catalysts. Despite typical protecting groups on the oxygen not tolerating the reaction conditions, a variety of alkyl and benzyl groups were deemed to be suitable.

Future work in the area involves expanding the substrate scope of the reaction, in particular quinolones as they are a particularly biologically active set of compounds. It is also hoped that the methodology can be applied to other heterocycles and in the synthesis of natural products. The diiodination of 2-pyridones **520a-e** will be optimised to drive the reaction to completion. The highly functionalised product will then be tested for selective trifluoromethylation. The remaining C-I site would then provide a handle for further synthetic manipulation (e.g. cross-coupling reactions). Finally, late stage trifluoromethylthiolation of these compounds will also be investigated due to the high prevalence of the SCF_3 group in biologically active compounds.

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Chapter 9

Trifluoromethylation of 4-alkoxy-2-pyrones, pyridones and quinolones

Experimental

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9.1 General experimental

Solvents and reagents were used as obtained from commercial sources and without purification with the exception of THF, which was freshly distilled from sodium/benzophenone under nitrogen.

Wet flash column chromatography was carried out using Kieselgel silica gel 60, 0.040–0.063 mm (Merck). TLC was carried out on pre-coated silica gel plates (Merck 60 PF254). Visualisation was achieved by UV light and potassium permanganate staining.

Melting points were carried out on a uni-melt Thomas Hoover Capillary melting point apparatus.

IR spectra were recorded on Perkin-Elmer FT-IR Paragon 1000 spectrophotometer. Liquid samples were examined as thin films interspersed on NaCl plates. Solid samples were dispersed in KBr and recorded as pressed discs. The intensity of peaks were expressed as strong (s), medium (m) and weak (w) and broad (b).

NMR spectra were run in $CDCl_3$ using TMS as the internal standard at 20 °C unless otherwise specified. ^{1}H NMR (600 MHz) spectra, ^{1}H NMR (400 MHz) spectra and ^{1}H NMR (300 MHz) spectra were recorded on Bruker Avance 600, Bruker Avance 400 and Bruker Avance 300 NMR spectrometers respectively in proton coupled mode. ^{19}F NMR (470 MHz) spectra and ^{19}F NMR (282 MHz) were recorded on Bruker Avance 600 NMR and Bruker Avance 300 NMR spectrometers respectively in proton decoupled mode. ^{13}C NMR (150 MHz) spectra and ^{13}C NMR (75 MHz) spectra were recorded on Bruker Avance 600 and Bruker Avance 300 NMR spectrometers respectively in proton decoupled mode. All spectra were recorded at University College Cork. Chemical shifts δ_H and δ_C are expressed as parts per million (ppm), positive shift being downfield from TMS; coupling constants (*J*) are expressed in hertz (Hz). Splitting patterns in ^{1}H NMR spectra are designated as s (singlet), bs (broad singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublet of doublets), t (triplet), q (quartet) and m (multiplet). For ^{13}C NMR spectra, the number of attached protons for each signal was determined using the DEPT pulse sequence run in the DEPT-90 and DEPT-

135 modes. COSY, HSQC and HMBC experiments were routinely performed to aid the NMR assignment of novel chemical structures.

LRMS were recorded on a Waters Quattro Micro triple quadrupole instrument in ESI mode using 50% acetonitrile-water containing 0.1% formic acid as eluent; samples were made up in acetonitrile or methanol. HRMS were recorded on a Waters LCT Premier Tof LC-MS instrument in ESI mode using 50% acetonitrile-water containing 0.1% formic acid as eluent; samples were made up in acetonitrile or methanol.

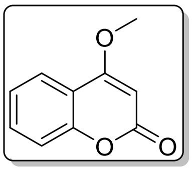
The Microanalysis Laboratory, National University of Ireland, Cork, performed elemental analysis using a Perkin-Elmer 240 and Exeter Analytical CE440 elemental analysers.

9.1.1 Analysis of known and novel compounds

^{1}H NMR spectra, ^{13}C NMR spectra, LRMS and melting point (if solid) analyses were recorded for all previously prepared compounds. For novel compounds, in addition to the previously mentioned analysis, ^{19}F NMR (where applicable), IR, HRMS and elemental analysis (if possible) were also obtained.

9.2 Synthesis of *O*-functionalised pyrones and coumarins

4-Methoxy-2*H*-chromen-2-one, 503

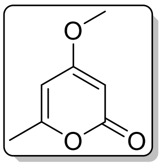


To a round bottomed flask containing 4-hydroxy-2*H*-chromen-2-one **468** (5.262 g, 32.5 mmol) and K_2CO_3 (5.376 g, 38.9 mmol) was added trimethyl phosphate (7.87 mL, 67.3 mmol) and the resulting reaction mixture stirred at reflux for 1 h. On completion, the reaction mixture was transferred to a separating funnel, washed with water (150 mL) and extracted with ethyl acetate (3 × 150 mL). The combined organic extract was dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product as an off-white solid which was purified by recrystallisation from ethanol to yield **503** as a fluffy, white solid (4.175 g, 73%). m.p. 123–125 °C [lit.[1] 122–124 °C].

Spectral characteristics were consistent with previously reported data.[1,2]

^{1}H NMR (300 MHz, $CDCl_3$): δ 4.00 (3H, s, C***H*3**), 5.70 (1H, s, C***H***), 7.25–7.34 (2H, m, 2 × C***H*** arom.), 7.52–7.58 (1H, m, ***CH*** arom.), 7.81 (1H, dd, *J* = 1.6, 7.7 Hz, C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 56.4 (O***C***H3), 90.1 (***C***HC=O), 115.6 (C=***C***COCH3), 116.7, 123.0, 123.9, 132.4 (4 × ***C***H arom.), 153.3 (***C***OC=O), 162.8 (***C***=O), 166.4 (***C***OCH3) ppm; MS (ESI) *m/z*: 177 [(M + H)$^+$, 90%].

4-Methoxy-6-methyl-2*H*-pyran-2-one, 505

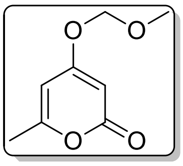


To a round bottomed flask containing 4-hydroxy-6-methyl-2*H*-pyran-2-one **439** (4.503 g, 35.7 mmol) and K_2CO_3 (5.915 g, 42.8 mmol) was added trimethylphosphate (8.65 mL, 73.9 mmol) and the resulting reaction mixture stirred at reflux for 1 h. On completion, the reaction mixture was transferred to a separating funnel, washed with water (150 mL) and extracted with ethyl acetate (3 × 150 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product as a yellow solid which was purified by recrystallisation from ethanol to yield **505** as a pale yellow, crystalline solid (2.303 g, 46%). m.p. 85–87 °C [lit.[3] 86–87.5 °C]. Spectral characteristics were consistent with previously reported data.[4]

^{1}H NMR (300 MHz, $CDCl_3$): δ 2.21 (3H, s, C***H*3**), 3.79 (3H, s, OC***H*3**), 5.41 (1H, C***H***C=O), 5.77–5.78 (1H, m, CH3CC***H***) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 19.8

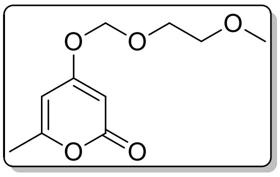
(***C***H$_3$), 55.8 (O***C***H$_3$), 87.4 (***C***HC=O), 100.3 (CH$_3$C***C***H), 162.0 (***C***=O), 164.9 (***C***CH$_3$), 171.3 (***C***OCH$_3$) ppm; MS (ESI) *m/z*: 141 [(M + H)$^+$, 100%].

4-(Methoxymethoxy)-6-methyl-2*H*-pyran-2-one, 511a



To a stirred suspension of 4-hydroxy-6-methyl-2-pyrone **439** (2.460 g, 19.5 mmol) in DCM (20 mL) was added triethylamine (3.00 mL, 21.5 mmol) dropwise and the mixture allowed stir at 0 °C for 10 min, followed by dropwise addition of methyl bromomethyl ether (1.76 mL, 21.5 mmol). The resulting mixture was stirred at room temperature for 12 h. Water (10 mL) was added to the reaction mixture, the organic layer separated and the aqueous layer extracted with DCM (3 × 20 mL). The combined organic extracts were dried over MgSO$_4$ and concentrated *in vacuo* to yield **511a** as a viscous pale white oil (3.059 g, 92%) which was subsequently used without purification due to sensitive nature of the protecting group. Spectral characteristics were consistent with previously reported data.[5] ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.20 (3H, s, C***H***$_3$), 3.45 (3H, s, OC***H***$_3$), 5.13 (2H, s, OC***H***$_2$O), 5.57 (1H, d, *J* = 1.9 Hz, C***H***C=O), 5.80–5.81 (1H, m, CH$_3$CC***H***) ppm; ^{13}C NMR (75.5 MHz, CDCl$_3$): δ 19.9 (***C***H$_3$), 57.0 (O***C***H$_3$), 90.3 (***C***HC=O), 94.2 (O***C***H$_2$O), 100.2 (CH$_3$C***C***H), 162.6 (CH$_3$***C***CH), 164.7 (***C***=O), 168.7 (***C***OCH$_2$OCH$_3$) ppm; HRMS (ESI) *m/z* calcd for $C_8H_{11}O_4$ [(M + H)$^+$]: 171.0657, found 171.0651.

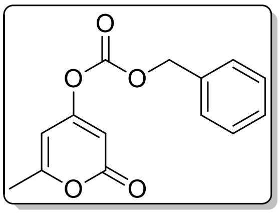
4-((2-Methoxyethoxy)methoxy)-6-methyl-2*H*-pyran-2-one, 511b



To a stirred suspension of 4-hydroxy-6-methyl-2-pyrone **439** (1.061 g, 8.41 mmol) in DCM (10 mL) was added triethylamine (1.29 mL, 9.26 mmol) dropwise and the mixture allowed stir at 0 °C for 10 min, followed by dropwise addition of 2-methoxy ethoxymethyl chloride (1.06 mL, 9.26 mmol). The resulting mixture was stirred at room temperature for 12 h. Water (10 mL) was added to the reaction mixture, the organic layer separated and the aqueous layer extracted with DCM (3 × 20 mL). The combined organic extracts were dried over MgSO$_4$ and concentrated *in vacuo* to yield crude product which was purified using silica column chromatography eluting with 70:30 hexane:ethyl acetate to yield **511b** as a white solid (1.167 g, 65%). m.p. 68–70 °C.

IR (KBr) ν_{max}: 2920, 2876 (C-H alkyl stretch, m), 1736 (C=O stretch, s), 1567 (aromatic C=C stretch, s), 1112 (ester C-O stretch, s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.21 (3H, s, $C\boldsymbol{H}_3$), 3.38 (3H, s, $OC\boldsymbol{H}_3$), 3.53–3.56 (2H, m, $C\boldsymbol{H}_2OCH_3$), 3.77–3.80 (2H, m, $C\boldsymbol{H}_2CH_2OCH_3$), 5.24 (2H, s, $OC\boldsymbol{H}_2O$), 5.61 (1H, d, J = 2.0 Hz, $CH_3CC\boldsymbol{H}$), 5.79 (1H, d, J = 0.9 Hz, $C\boldsymbol{H}C{=}O$) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.9 ($\boldsymbol{C}H_3$), 59.1 ($O\boldsymbol{C}H_3$), 68.9 ($\boldsymbol{C}H_2CH_2OCH_3$), 71.4 ($\boldsymbol{C}H_2OCH_3$), 90.3 ($\boldsymbol{C}HC{=}O$), 93.2 ($O\boldsymbol{C}H_2O$), 100.1 ($CH_3C\boldsymbol{C}H$), 162.6 ($CH_3\boldsymbol{C}CH$), 164.6 ($\boldsymbol{C}{=}O$), 168.8 ($CH\boldsymbol{C}OCH_2$) ppm; HRMS (ESI) *m/z* calcd for $C_{10}H_{15}O_5$ [$(M + H)^+$]: 215.0919, found 215.0910; Anal. calcd for $C_{10}H_{14}O_5$: C, 56.07; H, 6.59%. Found: C, 56.29; H, 6.23%.

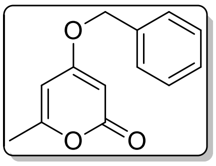
Benzyl (6-methyl-2-oxo-2*H*-pyran-4-yl) carbonate, 511c



To a stirred suspension of 4-hydroxy-6-methyl-2-pyrone **439** (2.049 g, 16.2 mmol) in DCM (20 mL) was added triethylamine (2.50 mL, 17.9 mmol) dropwise and the mixture allowed stir at 0 °C for 10 min, followed by dropwise addition of benzyl chloroformate (2.51 mL, 17.9 mmol). The resulting mixture was stirred at room temperature for 12 h. Water (20 mL) was added to the reaction mixture, the organic layer separated and the aqueous layer extracted with DCM (3 × 20 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product which was purified using silica column chromatography eluting with 80:20 hexane:ethyl acetate to yield **511c** as a white solid (1.896 g, 45%). m.p. 55–57 °C.

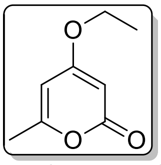
IR (KBr) ν_{max}: 2966 (C-H alkyl stretch, m), 1776, 1737 (C=O stretch, s), 1576 (aromatic C=C stretch, s), 1207 (ester C-O stretch, s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.24 (3H, s, $C\boldsymbol{H}_3$), 5.26 (2H, s, $OC\boldsymbol{H}_2Ph$), 6.00 (1H, dd, J = 0.9, 2.1 Hz, $CH_3CC\boldsymbol{H}$), 6.15 (1H, dd, J = 0.6, 2.1 Hz, $C\boldsymbol{H}C{=}O$), 7.36–7.42 (5H, m, 5 × $C\boldsymbol{H}$ arom.) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.0 ($\boldsymbol{C}H_3$), 71.1($O\boldsymbol{C}H_2Ph$), 99.7 ($\boldsymbol{C}HC{=}O$), 100.4 ($CH_3C\boldsymbol{C}H$), 128.6, 128.8, 129.1 (5 × $\boldsymbol{C}H$ arom.), 133.9 ($OCH_2\boldsymbol{C}$), 150.6 ($O\boldsymbol{C}{=}OO$), 163.0 ($CH\boldsymbol{C}{=}O$), 163.4 ($CH_3\boldsymbol{C}CH$), 163.6 ($CH\boldsymbol{C}OC{=}O$) ppm; HRMS (ESI) *m/z* calcd for $C_{14}H_{13}O_5$ [$(M + H)^+$]: 261.0763, found 261.0751; Anal. calcd for $C_{14}H_{12}O_5$: C, 64.61; H, 4.65%. Found: C, 64.75; H, 4.62%.

4-(Benzyloxy)-6-methyl-2*H*-pyran-2-one, 511d



To a stirred suspension of 4-hydroxy-6-methyl-2-pyrone **439** (1.557 g, 12.3 mmol) in DCM (15 mL) was added triethylamine (1.89 mL, 13.6 mmol) dropwise and the mixture allowed stir at 0 °C for 10 min, followed by dropwise addition of benzylbromide (1.62 mL, 13.6 mmol). The resulting mixture was stirred at room temperature for 12 h. Water (10 mL) was added to the reaction mixture, the organic layer separated and the aqueous layer extracted with DCM (3 × 20 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product which was purified using silica column chromatography eluting with 90:10 hexane:ethyl acetate to yield **511d** as a white solid (0.221 g, 8%). m.p. 90–92 °C [lit.[6] 92–94 °C]. Spectral characteristics were consistent with previously reported data.[7] ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.21 (3H, s, C***H*₃**), 5.00 (2H, s, OC***H*₂**Ph), 5.50 (1H, d, *J* = 2.1 Hz, C***H***C=O), 5.84 (1H, d, *J* = 1.1 Hz, CH_3CC***H***), 7.35–7.40 (5H, m, 5 × Ar***H***) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.8 (***C***H_3), 70.7 (***C***H_2), 88.5 (***C***HC=O), 100.5 (CH_3C***C***H), 127.8, 128.79, 128.83 (5 × ***C***H arom.), 134.4 (OCH_2***C***), 162.2 (CH_3***C***CH), 164.8 (***C***=O), 170.2 (***C***OCH_2Ph) ppm; HRMS (ESI) *m/z* calcd for $C_{13}H_{13}O_3$ [$(M + H)^+$]: 217.0865, found 217.0868.

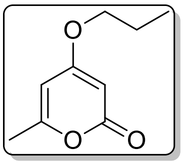
4-Ethoxy-6-methyl-2*H*-pyran-2-one, 511e



To a stirred suspension of 4-hydroxy-6-methyl-2-pyrone **439** (1.671 g, 13.3 mmol) in DCM (15 mL) was added triethylamine (2.03 mL, 14.6 mmol) dropwise and the mixture allowed stir at 0 °C for 10 min, followed by dropwise addition of bromoethane (1.09 mL, 14.6 mmol). The resulting mixture was stirred at room temperature for 12 h. Water (10 mL) was added to the reaction mixture, the organic layer separated and the aqueous layer extracted with DCM (3 × 20 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product which was purified using silica column chromatography eluting with 50:50 hexane:ethyl acetate to yield **511e** as a pale yellow solid (0.256 g, 12%). m.p. 63–64 °C [lit.[8] 61°C]. IR (KBr) ν_{max}: 2986 (alkyl C-H stretch, m), 1720 (C=O stretch, s), 1567 (aromatic C=C stretch, s), 1250 (ester C-O stretch, s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.37 (3H, t, *J* = 7.0 Hz, OCH_2C***H*₃**), 2.17 (3H, s, C***H*₃**), 3.98 (2H, q, *J* =

7.1 Hz, OC***H*2**CH3), 5.34 (1H, d, *J* = 2.0 Hz, C***H***C=O), 5.73 (1H, d, *J* = 1.0 Hz, CH3CC***H***) ppm; ^{13}C NMR (75.5 MHz, CDCl3): δ 14.0 (OCH2***C***H3), 19.8 (***C***H3), 64.5 (O***C***H2CH3), 87.6 (***C***HC=O), 100.6 (CH3C***C***H), 162.0 (CH3***C***CH), 165.1 (***C***=O), 170.5 (***C***OCH2CH3) ppm; HRMS (ESI) *m/z* calcd for $C_8H_{11}O_3$ [(M + H)$^+$]: 155.0708, found 155.0704.

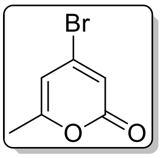
6-Methyl-4-propoxy-2*H*-pyran-2-one, 511f



To a stirred suspension of 4-hydroxy-6-methyl-2-pyrone **439** (1.338 g, 10.6 mmol) in DCM (15 mL) was added triethylamine (1.63 mL, 11.7 mmol) dropwise and the mixture allowed stir at 0 °C for 10 min, followed by dropwise addition of 1-iodopropane (1.14 mL, 11.7 mmol). The resulting mixture was stirred at room temperature for 12 h. Water (10 mL) was added to the reaction mixture, the organic layer separated and the aqueous layer extracted with DCM (3 × 20 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product which was purified using silica column chromatography eluting with 50:50 hexane:ethyl acetate to yield **511f** as a pale yellow viscous oil (0.122 g, 7%).

IR (KBr) ν_{max}: 2970 (alkyl C-H stretch, m), 1733 (C=O stretch, s), 1566 (aromatic C=C stretch, s), 1250 (ester C-O stretch) cm^{-1}; ^{1}H NMR (300 MHz, CDCl3): δ 0.93 (3H, t, *J* = 7.4 Hz, OCH2CH2C***H*3**), 1.71 (2H, m, OCH2C***H*2**CH3), 2.12 (3H, s, C***H*3**), 3.82 (2H, t, *J* = 6.5 Hz, OC***H*2**CH2CH3), 5.29 (1H, d, *J* = 1.1 Hz, C***H***C=O), 5.71 (1H, d, *J* = 2.1 Hz, CH3CC***H***) ppm; ^{13}C NMR (75.5 MHz, CDCl3): δ 10.2 (OCH2CH2***C***H3), 19.7 (***C***H3), 21.8 (OCH2***C***H2CH3), 70.2 (O***C***H2CH2CH3), 87.6 (***C***HC=O), 100.5 (CH3C***C***H), 161.9 (CH3***C***CH), 165.0 (***C***=O), 170.7 (***C***OCH2CH2CH3) ppm; HRMS (ESI) *m/z* calcd for $C_9H_{13}O_3$ [(M + H)$^+$]: 169.0865, found 169.0867.

4-Bromo-6-methyl-2*H*-pyran-2-one, 512

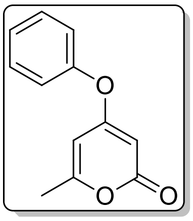


To a stirred solution of 4-hydroxy-6-methyl-2-pyrone **439** (4.173 g, 33.1 mmol) in toluene (150 mL) was added tetrabutylammoniumbromide (12.37 g, 38.4 mmol) and phosphorus pentoxide (22.55 g, 79.44 mmol). The reaction was allowed to stir at reflux for 3 h. The reaction mixture was filtered into a separating funnel and extracted with toluene (25 mL), washed with saturated aq. $NaHCO_3$ (40 mL) and brine (40 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product which was filtered through a pad of silica with 50:50 hexane:diethyl ether to provide **512** as an off-white solid (4.224 g, 68%). m.p. 85–87 °C [lit.[9] 87–89 °C].

Spectral characteristics were consistent with previously reported data.[9]

^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.25 (3H, s, C***H*₃**), 6.19 (1H, s, CH_3CC***H***), 6.46 (1H, s, C=OC***H***) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.7 (***C***H_3), 108.4 (CH_3C***C***H), 114.8 (C=OC***C***H*)*, 141.1 (***C***Br), 160.6 (***C***=O), 162.1 (CH_3***C***CH) ppm; HRMS (ESI) *m/z* calcd for $C_6H_6O_2Br$ [(M + H)$^+$]: 188.9551, found 188.9553.

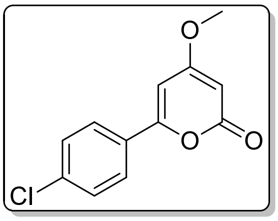
6-Methyl-4-phenoxy-2*H*-pyran-2-one, 511g



To a stirred solution of 4-bromo-6-methyl-2*H*-pyran-2-one **512** (2.225 g, 11.8 mmol) and K_2CO_3 (2.934 g, 21.2 mmol) in acetone (60 mL) was added phenol (1.666 g, 17.7 mmol) and the resulting reaction mixture stirred at reflux for 24 h. On completion was added H_2O (20 mL), the reaction mixture extracted with ethyl acetate (3 × 25 mL) and washed with 10 % aq. NaOH (2 × 25 mL). Organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield **511g** a beige solid (2.182 g, 91%) with no purification required. m.p. 86–88 °C [lit.[10] 89–91 °C].

IR (KBr) ν_{max}: 1722 (C=O stretch, s), 1564, 1447 (aromatic C=C stretch, s), 1290, 1232 (ester C-O stretch, s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.26 (3H, s, C***H₃***), 5.20 (1H, dd, *J* = 0.3, 2.2 Hz, C***H***C=O), 5.97 (1H, dd, *J* = 0.9, 2.2 Hz, CH_3CC***H***), 7.05–7.09 (2H, m, 2 × C***H*** arom.), 7.26–7.32 (1H, m, C***H*** arom.) 7.40–7.46 (2H, m, 2 × C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.0 (***C***H_3), 91.0 (***C***HC=O), 99.9 (CH_3C***C***H), 121.1, 126.5, 130.3 (5 × ***C***H arom.), 152.4 (CO***C*** arom.), 163.3(CH_3***C***CH), 164.6 (***C***=O), 170.8 (***C***OPh) ppm; HRMS (ESI) *m/z* calcd for $C_{12}H_{11}O_3$ [(M + H)$^+$]: 203.0708, found 203.0702; Anal. calcd for $C_{12}H_{10}O_3$: C, 71.28; H, 4.98%. Found: C, 71.20; H, 5.09%.

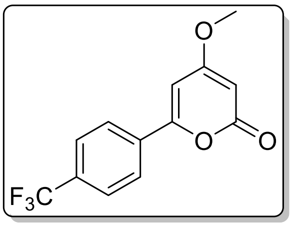
6-(4-Chlorophenyl)-4-methoxy-2*H*-pyran-2-one, 511i



To a round bottomed flask containing 6-(4-chlorophenyl)-4-hydroxy-2*H*-pyran-2-one (0.216 g, 0.97 mmol) and K_2CO_3 (0.161 g, 1.16 mmol) was added trimethylphosphate (2.36 mL, 20.1 mmol) and the resulting reaction mixture stirred at reflux for 1 h. On completion, the reaction mixture was washed with water (15 mL) and extracted with ethyl acetate (3 × 25 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product as a viscous, brown oil which was purified by silica column chromatography eluting with 50:50 hexane:ethyl acetate to yield **511i** as an off-white solid (0.077 g, 33%). m.p. 123–125 °C.

IR (KBr) ν_{max}: 1723 (C=O stretch, s), 1563 (aromatic C=C stretch, s), 1276 (ester C-O stretch, s), 730 (C-Cl stretch, w) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.86 (3H, s, OC***H*3**), 5.55 (1H, d, *J* = 2.1 Hz, C***H***C=O), 6.40 (1H, d, *J* = 2.1 Hz, C***H***), 7.42 (2H, d, *J* = 8.7 Hz, 2 × C***H*** arom.), 7.74 (2H, d, *J* = 8.7 Hz, 2 × C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 56.1 (O***C***H_3), 88.7 (***C***HC=O), 98.2 (***C***HCOCH_3), 127.0 (2 × ***C***H arom), 129.2 (2 × ***C***H arom), 129.6 (q***C*** arom.*)*, 137.1 (***C***Cl), 159.0 (***C***CHCOCH_3), 163.8 (***C***=O), 171.1 (***C***OCH_3) ppm; HRMS (ESI) *m/z* calcd for $C_{12}H_{10}O_3Cl$ [$(M + H)^+$]: 237.0318, found 237.0313.

4-Methoxy-6-(4-(trifluoromethyl)phenyl)-2*H*-pyran-2-one, 511j

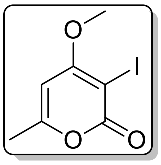


To a round bottomed flask containing 4-hydroxy-6-(4-(trifluoromethyl)phenyl)-2*H*-pyran-2-one (0.142 g, 0.55 mmol) and K_2CO_3 (0.091 g, 0.66 mmol) was added trimethylphosphate (2.36 mL, 20.1 mmol) and the resulting reaction mixture stirred at reflux overnight. On completion, the reaction mixture was washed with water (15 mL) and extracted with ethyl acetate (3 × 25 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product as an orange oil which was purified by silica column chromatography eluting with 50:50 hexane:ethyl acetate to yield **511j** a pale yellow solid (0.129 g, 87%). m.p. 154–155 °C. IR (KBr) ν_{max}: 1740 (C=O stretch, s), 1641 (C=C stretch, m), 1566 (aromatic C=C stretch, m), 1116 (ester C-O stretch, m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.87

(3H, s, OC**H_3**), 5.58 (1H, d, *J* = 2.1 Hz, C**H**C=O) 6.49 (1H, d, *J* = 2.1 Hz, C**H**COCH$_3$), 7.71 (2H, d, *J* = 8.3 Hz, 2 × C**H** arom.), 7.92 (2H, d, *J* = 8.3 Hz, 2 × C**H** arom.) ppm; ^{13}C NMR (75.5 MHz, CDCl$_3$): δ 56.2 (O**C**H$_3$), 89.4 (**C**HC=O), 99.5 (**C**HCOCH$_3$), 123.7 (q, $J_{\text{C-F}}$ = 272.4 Hz, **C**F$_3$), 125.8, 125.89, 125.94, 126.0 (4 × **C**H arom.), 132.6 (q, $^2J_{\text{C-F}}$ = 32.8 Hz, **C**CF$_3$), 134.4 (q**C** arom.), 158.4 (**C**CHCOCH$_3$), 163.6 (**C**=O), 170.9 (**C**OCH$_3$) ppm; ^{19}F NMR (282 MHz, CDCl$_3$): δ -63.2 (C**F_3**) ppm; HRMS (ESI) *m/z* calcd for $C_{13}H_{10}O_3F_3$ [$(M + H)^+$]: 271.0582, found 271.0583; Anal. calcd for $C_{13}H_9O_3F_3$: C, 57.79; H, 3.36%. Found: C, 57.69; H, 3.10%.

9.3 Iodination of *O*-functionalised pyrones and coumarins

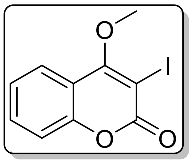
3-Iodo-4-methoxy-6-methyl-2*H*-pyran-2-one, 506



To a stirred solution of 4-methoxy-6-methyl-pyrone **505** (1.988 g, 14.2 mmol) in acetonitrile (40 mL) was added *N*-iodosuccinimide (4.790 g, 21.3 mmol). The reaction vessel was covered in aluminium foil and allowed stir at room temperature for 12 h. On completion, the solvent was concentrated *in vacuo*, the resulting orange solid dissolved in DCM (15 mL) and washed with 5% aq. sodium thiosulfate (3 × 20 mL). The organic layer was dried over MgSO$_4$ and concentrated *in vacuo* to yield **506** as a yellow solid (3.578 g, 95%) with no purification required. m.p. 144–146 °C [lit.[11] 144–146 °C].

Spectral characteristics were consistent with previously reported data.[11] ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.30 (3H, s, C**H_3**), 3.99 (3H, s, OC**H_3**), 6.00 (1H, s, C**H**) ppm; ^{13}C NMR (75.5 MHz, CDCl$_3$): δ 20.1 (**C**H$_3$), 57.5 (O**C**H$_3$), 62.3 (**C**I), 94.7 (**C**H), 161.7 (**C**=O), 164.2 (**C**CH$_3$), 170.5 (**C**OCH$_3$) ppm; MS (ESI) *m/z*: 267 [$(M + H)^+$, 100%].

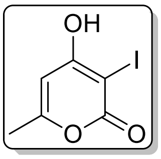
3-Iodo-4-methoxy-2*H*-chromen-2-one, 507



To a stirred solution of 4-methoxycoumarin **503** (2.56 g, 14.6 mmol) in acetonitrile (40 mL) was added *N*-iodosuccinimide (4.256 g, 18.9 mmol) and trifluoroacetic acid (1.12 mL, 14.6 mmol). The reaction vessel was covered in aluminium foil and allowed stir at room temperature for 60 h. On completion, the solvent was concentrated *in vacuo*, the resulting orange solid dissolved in DCM (15 mL) and washed with 5% aq. sodium thiosulfate (3 × 20 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product which was purified by recrystallisation from methanol to yield **507** as a cream solid (1.998 g, 45%). m.p. 88–90 °C [lit.[12] 88–89 °C].

Spectral characteristics were consistent with previously reported data.[12] ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.15 (3H, s, OC***H*₃**), 7.28–7.38 (2H, m, 2 × C***H*** arom.), 7.58–7.64 (1H, m, C***H*** arom.), 7.77 (1H, dd, *J* = 1.6, 7.9 Hz, C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 61.6 (O***C***H_3), 76.5 (***C***I), 116.7 (C=***C***$COCH_3$), 116.8 (***C***H arom.), 123.2 (***C***H arom.), 124.5 (***C***H arom.), 133.0 (***C***H arom.), 153.2 (***C***OC=O), 159.9 (***C***=O), 170.0 (***C***OCH_3) ppm; MS (ESI) *m/z*: 303 [$(M + H)^+$, 100%].

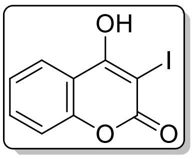
4-Hydroxy-3-iodo-6-methyl-2*H*-pyran-2-one, 508



To a stirred solution of 4-hydroxy-6-methyl-2*H*-pyran-2-one **439** (1.147 g, 9.10 mmol) in the minimum amount of ammonium hydroxide was added a solution of iodine (2.308 g, 9.10 mmol) in aq. KI (sufficient to dissolve iodine) dropwise at 0°C. The resulting reaction mixture was allowed stir at 0°C for 2 h before being placed in the refrigerator overnight. Reaction mixture was acidified to pH 4-5 and resulting precipitate was filtered and washed with water to yield crude product as a dark yellow solid which was purified by recrystallisation from ethyl acetate to yield **508** as a white solid (0.522 g, 23%). m.p. 184–186 °C.

IR (KBr) ν_{max}: 1728 (C=O stretch, s), 1643, 1545 (aromatic C=C stretch, s), 1398, 1347 (ester C-O stretch, s), 1216 (alcohol C-O stretch, m) cm^{-1}; ^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ 2.18 (3H, d, *J* = 0.7 Hz, C***H*₃**), 6.04 (1H, d, *J* = 0.8 Hz, C***H***) ppm; ^{13}C NMR (75.5 M89Hz, $(CD_3)_2SO$): δ 19.4 (***C***H_3), 60.2 (***C***I), 99.5 (***C***H), 162.0 (***C***=O), 163.0 (***C***CH_3), 170.7 (***C***OCH_3) ppm; HRMS (ESI) *m/z* calcd for $C_6H_6O_3I$ [$(M + H)^+$]: 252.9362, found 252.9353; Anal. calcd for $C_6H_5O_3I$: C, 28.60; H, 2.00%. Found: C, 29.16; H, 1.99%.

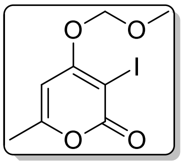
4-Hydroxy-3-iodo-2*H*-chromen-2-one, 509



To a stirred solution of 4-hydroxycoumarin **468** (2.227 g, 13.7 mmol), potassium iodide (1.528 g, 9.2 mmol) and potassium iodate (0.970 g, 4.5 mmol) in methanol (8 mL) and H_2O (40 mL) was added 1M HCl (14.1 mL) dropwise over 45 min. The resulting reaction mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with H_2O (50 mL) and extracted with DCM (3 × 25 mL). The combined organic extracts were washed with 5% aq. sodium thiosulfate (25 mL), water (25 mL) and brine (25 mL), dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product **509** as a brown solid (1.025 g, 26%) with no purification required. m.p. 155–157 °C [lit.[13] 152–153 °C].

IR (KBr) ν_{max}: 1725 (C=O stretch, s), 1548 (aromatic C=C stretch, s), 1156 (ester C-O stretch, m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.06 (1H, bs, O***H***), 7.31–7.40 (2H, m, 2 × C***H*** arom.), 7.61–7.66 (1H, m C***H*** arom.), 7.91 (1H, dd, *J* = 1.6, 8.0 Hz, C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 68.1 (***C***I), 113.4 (***C***COH), 116.6, 123.5, 124.6, 133.4 (4 × ***C***H arom.), 153.0 (***C***OC=O), 159.2 (***C***=O), 164.4 (***C***OH) ppm; HRMS (ESI) *m/z* calcd for $C_9H_6O_3I$ $[(M + H)^+]$: 288.9362, found 288.9357.

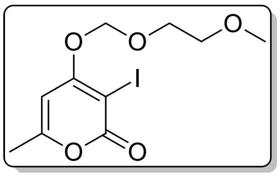
3-Iodo-4-(methoxymethoxy)-6-methyl-2*H*-pyran-2-one, 514a



To a stirred solution of 4-(methoxymethoxy)-6-methyl-2*H*-pyran-2-one **511a** (0.639 g, 3.76 mmol) in acetonitrile (20 mL) was added *N*-iodosuccinimide (2.542 g, 11.3 mmol). The reaction vessel was covered in aluminium foil and allowed stir at room temperature for 12 h. On completion, the solvent was concentrated *in vacuo*, the resulting orange solid dissolved in DCM (15 mL) and washed with 5% aq. sodium thiosulfate (3 × 20 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product **514a** as a yellow solid (0.829 g, 74%) which was subsequently used without purification due to sensitive nature of the protecting group. m.p. 109–111 °C.

IR (KBr) ν_{max}: 2925 (alkyl C-H stretch, m), 1716 (C=O stretch, s), 1523 (aromatic C=C stretch, s), 1158 (ester C-O stretch, m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.26 (3H, s, C***H*₃**), 3.50 (3H, s, OC***H*₃**), 5.27 (2H, s, OC***H*₂**O), 6.10 (1H, s, C***H***) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.0 (***C***H_3), 57.2 (O***C***H_3), 64.5 (***C***I), 94.7 (O***C***H_2O), 96.5 (***C***H), 161.9 (***C***=O), 163.5 (CH_3***C***CH), 168.9 (***C***OCH_2O) ppm; HRMS (ESI) calcd for $C_8H_{10}O_4I$ [(M + H)$^+$]: 296.9624, found 296.9618.

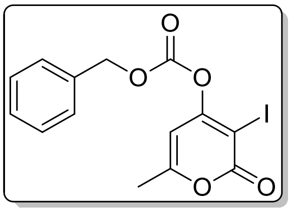
3-Iodo-4-((2-methoxyethoxy)methoxy)-6-methyl-2*H*-pyran-2-one, 514b



To a stirred solution of 4-((2-methoxyethoxy)methoxy)-6-methyl-2*H*-pyran-2-one **511b** (0.303 g, 1.41 mmol) in acetonitrile (20 mL) was added *N*-iodosuccinimide (0.955 g, 4.24 mmol). The reaction vessel was covered in aluminium foil and allowed stir at room temperature for 36 h. On completion, the solvent was concentrated *in vacuo*, the resulting orange solid dissolved in DCM (15 mL) and washed with 5% aq. sodium thiosulfate (3 × 20 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to give crude product which was purified using silica column chromatography eluting with 50:50 hexane:ethyl acetate to yield **514b** as a viscous yellow oil (0.200 g, 42%).

IR (NaCl) ν_{max}: 2925 (alkyl C-H stretch, w), 1718 (C=O stretch, s), 1525 (aromatic C=C stretch, s), 1110 (ester C-O stretch, s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.24 (3H, s, C***H***$_3$), 3.35 (3H, s, OC***H***$_3$), 3.52–3.55 (2H, m, OCH_2C***H***$_2$OCH_3), 3.81–3.84 (2H, m, OC***H***$_2$$CH_2OCH_3$), 5.35 (2H, s, OC***H***$_2$O), 6.13 (1H, s, C***H***) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.0 (***C***H_3), 59.0 (O***C***H_3), 64.5 (***C***I), 69.0 (O***C***$H_2$$CH_2OCH_3$), 71.2 (O$CH_2$***C***$H_2OCH_3$), 93.7 (O***C***$H_2$O), 96.5 (***C***H), 161.8 (***C***=O), 163.5 (CH_3***C***CH), 168.9 (***C***OCH_2O) ppm; HRMS (ESI) *m/z* calcd for $C_{10}H_{14}O_5I$ [(M + H)$^+$]: 340.9886, found 340.9880.

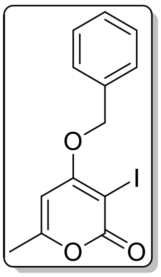
Benzyl (3-iodo-6-methyl-2-oxo-2*H*-pyran-4-yl) carbonate, 514c



To a stirred solution of benzyl (6-methyl-2-oxo-2*H*-pyran-4-yl) carbonate **511c** (0.559 g, 2.15 mmol) in acetonitrile (20 mL) was added *N*-iodosuccinimide (0.967 g, 4.30 mmol). The reaction vessel was covered in aluminium foil and allowed stir at reflux for 48 h. On completion, the solvent was concentrated *in vacuo*, the resulting orange solid dissolved in DCM (15 mL) and washed with 5% aq. sodium thiosulfate (3 × 20 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield product **514c** as a yellow solid

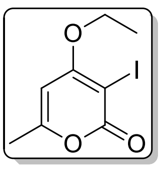
(0.670 g, 81%) with no purification required. m.p. 116–118 °C. IR (KBr) ν_{max}: 1764, 1729 (C=O stretch, s), 1549 (aromatic C=C stretch, m), 1266, 1241 (ester C-O stretch, m) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.26 (3H, s, C***H₃***), 5.32 (2H, s, OC***H₂***Ph), 6.11 (1H, s, C***H***), 7.39–7.45 (5H, m, 5 × C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, CDCl$_3$): δ 19.7 (***C***H$_3$), 71.7 (O***C***H$_2$Ph), 74.2 (***C***I), 101.4 (***C***H), 128.7, 128.8, 129.2 (5 × ***C***H arom.), 133.8 (OCH$_2$***C***), 150.1 (O***C***=OO), 161.2 (***C***=O), 163.1 (CH$_3$***C***CH), 164.5 (CH***C***OC=O) ppm; HRMS (ESI) *m/z* calcd for $C_{14}H_{12}O_5I$ [(M + H)$^+$]: 386.9730, found 386.9728.

4-(Benzyloxy)-3-iodo-6-methyl-2*H*-pyran-2-one, 514d



To a stirred solution of 4-(benzyloxy)-6-methyl-2*H*-pyran-2-one **511d** (0.204 g, 0.94 mmol) in acetonitrile (20 mL) was added *N*-iodosuccinimide (0.637 g, 2.83 mmol). The reaction vessel was covered in aluminium foil and allowed stir at room temperature for 48 h. On completion, the solvent was concentrated *in vacuo*, the resulting orange solid dissolved in DCM (15 mL) and washed with 5% aq. sodium thiosulfate (3 × 20 mL). The organic layer was dried over MgSO$_4$ and concentrated *in vacuo* to yield product **514d** as an off-white solid (0.272 g, 84%) with no purification required. m.p. 125–126 °C. IR (KBr) ν_{max}: 1705 (C=O stretch, s), 1524 (aromatic C=C stretch, s), 1318 (ester C-O stretch, m) cm^{-1}; ^{1}H NMR (CDCl$_3$, 300 MHz) : δ 2.22 (3H, s, C***H₃***), 5.35 (2H, s, OC***H₂***Ph), 5.97 (1H, s, C***H***), 7.32–7.40 (5H, m, 5 × C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, CDCl$_3$): δ 20.1 (***C***H$_3$), 63.3 (***C***I), 71.8 (O***C***H$_2$Ph), 95.8 (***C***H), 126.8, 128.7, 128.9 (5 × ***C***H arom.), 134.6 (OCH$_2$***C***), 161.8 (***C***=O), 164.0 (CH$_3$***C***CH), 169.8 (***C***OCH$_2$Ph) ppm; HRMS (ESI) *m/z* calcd for $C_{13}H_{12}O_3I$ [(M + H)$^+$]: 342.9831, found 342.9824.

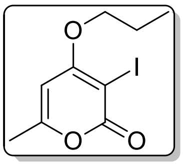
4-Ethoxy-3-iodo-6-methyl-2*H*-pyran-2-one, 514e



To a stirred solution of 4-ethoxy-6-methyl-2*H*-pyran-2-one **511e** (0.150 g, 0.97 mmol) in acetonitrile (20 mL) was added *N*-iodosuccinimide (0.657 g, 2.92 mmol). The reaction vessel was covered in aluminium foil and allowed stir at room temperature for 12 h. On completion, the solvent was concentrated *in vacuo*, the resulting orange solid dissolved in DCM (15 mL) and washed with 5% aq. sodium thiosulfate (3 × 20 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield product **514e** as a yellow solid (0.205 g, 75%) with no purification required. m.p. 130–131 °C.

IR (KBr) ν_{max}: 3102, 2981 (alkyl C-H stretch, m), 1699 (C=O stretch, s), 1521 (aromatic C=C stretch, s), 1314 (ester C-O stretch, m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.45 (3H, t, *J* = 7.0 Hz, $OCH_2C\boldsymbol{H_3}$), 2.25 (3H, s, $C\boldsymbol{H_3}$), 4.20 (2H, q, *J* = 7.1 Hz, $OC\boldsymbol{H_2}CH_3$), 5.92 (1H, s, $C\boldsymbol{H}$) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.7 ($OCH_2\boldsymbol{C}H_3$), 20.1 ($\boldsymbol{C}H_3$), 62.5 ($\boldsymbol{C}I$), 66.4 ($O\boldsymbol{C}H_2CH_3$), 95.3 ($\boldsymbol{C}H$), 161.9 ($\boldsymbol{C}$=O), 163.9 ($CH_3\boldsymbol{C}H$), 169.9 ($\boldsymbol{C}OCH_2CH_3$) ppm; HRMS (ESI) *m/z* calcd for $C_8H_{10}O_3I$ $[(M + H)^+]$: 280.967, found 280.9669; Anal. calcd for $C_8H_9O_3I$: C, 34.21; H, 3.24%. Found: C, 34.43; H, 3.00%.

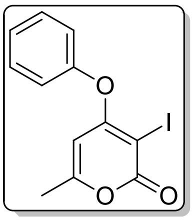
3-Iodo-6-methyl-4-propoxy-2*H*-pyran-2-one, 514f



To a stirred solution of 6-methyl-4-propoxy-2*H*-pyran-2-one **511f** (0.056 g, 0.33 mmol) in acetonitrile (15 mL) was added *N*-iodosuccinimide (0.225 g, 1.00 mmol). The reaction vessel was covered in aluminium foil and allowed stir at room temperature for 12 h. On completion, the solvent was concentrated *in vacuo*, the resulting orange solid dissolved in DCM (15 mL) and washed with 5% aq. sodium thiosulfate (3 × 20 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield product **514f** as a white solid (0.093 g, 96%) with no purification required. m.p. 113–115 °C.

IR (KBr) ν_{max}: 2969, 2920 (alkyl C-H stretch, s), 1709 (C=O stretch, s), 1522 (aromatic C=C stretch, s), 1317 (ester C-O stretch, s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.05 (3H, t, *J* = 7.4 Hz, $OCH_2CH_2C\boldsymbol{H_3}$), 1.83 (2H, m, $OCH_2C\boldsymbol{H_2}CH_3$), 2.25 (3H, s, $C\boldsymbol{H_3}$), 4.09 (2H, t, *J* = 6.4 Hz, $OC\boldsymbol{H_2}CH_2CH_3$), 5.93 (1H, s, $C\boldsymbol{H}$) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 10.4 ($OCH_2CH_2\boldsymbol{C}H_3$), 20.1 ($\boldsymbol{C}H_3$), 22.4 ($OCH_2\boldsymbol{C}H_2CH_3$), 62.5 ($\boldsymbol{C}I$), 72.0 ($O\boldsymbol{C}H_2CH_2CH_3$), 95.4 ($\boldsymbol{C}H$), 161.9 ($\boldsymbol{C}$=O), 163.9 ($CH_3\boldsymbol{C}CH$), 170.0 ($CH\boldsymbol{C}OCH_2CH_2CH_3$) ppm; HRMS (ESI) *m/z* calcd for $C_9H_{12}O_3I$ $[(M + H)^+]$: 294.9831, found 294.9820; Anal. calcd for $C_9H_{11}O_3I$: C, 36.76; H, 3.77%. Found: C, 36.93; H, 3.49%.

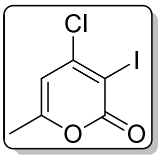
3-Iodo-6-methyl-4-phenoxy-2*H*-pyran-2-one, 514g



To a stirred solution of 4-phenoxy-6-methyl-2*H*-pyran-2-one **511g** (0.657 g, 3.25 mmol) in acetonitrile (20 mL) was added *N*-iodosuccinimide (1.096 g, 4.87 mmol). The reaction vessel was covered in aluminium foil and allowed stir at reflux for 36 h. On completion, the solvent was concentrated *in vacuo*, the resulting orange solid dissolved in DCM (15 mL) and washed with 5% aq. sodium thiosulfate (3 × 20 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield product **514g** as a yellow solid (1.022 g, 96%) with no purification required. m.p. 123–125 °C [lit.[10] 142 °C].

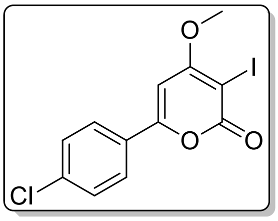
IR (KBr) ν_{max}: 1718 (C=O stretch, s), 1532, 1487 (aromatic C=C stretch, s), 1225 (ester C-O stretch, s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.16 (3H, s, C***H₃***), 5.54 (1H, s, C***H***), 7.07–7.09 (2H, m, 2 × C***H*** arom.), 7.29–7.34 (1H, m, C***H*** arom.), 7.42–7.47 (2H, m, 2 × C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.9 (***C***H_3), 64.9 (***C***I), 97.3 (***C***H), 120.9, 126.5, 130.4 (5 × ***C***H arom.), 153.0 (CHCO***C***), 161.8 (***C***=O), 163.6 (CH_3***C***CH), 169.4 (CH***C***OPh) ppm; HRMS (ESI) *m/z* calcd for $C_{12}H_{10}O_3I$ [$(M + H)^+$]: 328.9675, found 328.9667; Anal. calcd for $C_{12}H_9O_3I$: C, 43.93; H, 2.76%. Found: C, 43.96; H, 2.63%.

4-Chloro-3-iodo-6-methyl-2*H*-pyran-2-one, 514h



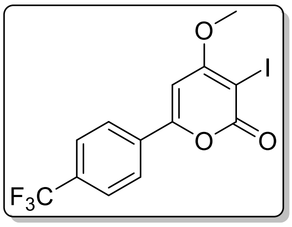
To a stirred solution of 4-chloro-6-methyl-2*H*-pyran-2-one **511h** (0.271 g, 1.87 mmol) in acetonitrile (20 mL) was added *N*-iodosuccinimide (1.265 g, 5.62 mmol). The reaction vessel was covered in aluminium foil and allowed stir at reflux for 18 h. On completion, the solvent was concentrated *in vacuo*, the resulting orange solid dissolved in DCM (15 mL) and washed with 5% aq. sodium thiosulfate (3 × 20 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield product **514h** as a yellow solid (0.381 g, 75%) with no purification required. m.p. 121–123 °C. IR (KBr) ν_{max}: 1719 (C=O stretch, s), 1509 (aromatic C=C stretch, s), 1267 (ester C-O stretch, m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.22 (3H, s, C***H₃***), 6.14 (1H, s, C***H***) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.4 (***C***H_3), 85.5 (***C***I), 106.4 (***C***H), 156.3 (***C***Cl), 159.5 (***C***=O), 161.4 (CH_3***C***CH) ppm; HRMS (ESI) *m/z* calcd for $C_6H_5O_2ICl$ [$(M + H)^+$]: 270.9023, found 270.9017.

6-(4-Chlorophenyl)-3-iodo-4-methoxy-2*H*-pyran-2-one, 514i



To a stirred solution of 6-(4-chlorophenyl)-4-methoxy-2*H*-pyran-2-one **511i** (0.070 g, 0.30 mmol) in acetonitrile (15 mL) was added *N*-iodosuccinimide (0.202 g, 0.90 mmol). The reaction vessel was covered in aluminium foil and allowed stir at room temperature for 18 h. On completion, the solvent was concentrated *in vacuo*, the resulting orange solid dissolved in DCM (15 mL) and washed with 5% aq. sodium thiosulfate (3 × 20 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield product **514i** as a pale yellow solid (0.084 g, 77%) with no purification required. m.p. 233–235 °C. IR (KBr) ν_{max}: 1687 (C=O stretch, s), 1508 (aromatic C=C stretch. s), 1380 (ester C-O stretch, s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 4.08 (3H, s, OC***H*3**), 6.52 (1H, s, C***H***), 7.45 (2H, d, *J* = 8.8 Hz, 2 × C***H*** arom.), 7.81 (2H, d, *J* = 8.8 Hz, 2 × C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 57.5 (O***C***H_3), 64.6 (***C***I), 91.8 (***C***HCOCH_3), 127.2 (2 × ***C***H arom.), 129.0 (q***C*** arom.*)*, 129.5 (2 × ***C***H arom.), 137.9 (***C***Cl), 160.72 (***C***=O), 160.74 (***C***CHCOCH_3), 170.2 (CH***C***OCH_3) ppm; HRMS (ESI) *m/z* calcd for $C_{12}H_9O_3ICl$ [$(M + H)^+$]: 362.9285 , found 362.9272.

3-Iodo-4-methoxy-6-(4-(trifluoromethyl)phenyl)-2*H*-pyran-2-one, 514j

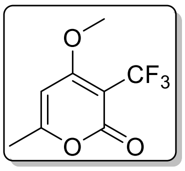


To a stirred solution of 4-methoxy-6-(4-(trifluoromethyl)phenyl)-2*H*-pyran-2-one **511j** (0.120 g, 0.44 mmol) in acetonitrile (15 mL) was added *N*-iodosuccinimide (0.200 g, 0.89 mmol). The reaction vessel was covered in aluminium foil and allowed stir at room temperature for 18 h. On completion, the solvent was concentrated *in vacuo*, the resulting orange solid dissolved in DCM (15 mL) and washed with 5 % aqueous sodium thiosulfate (3 × 20 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield product **514i** as a white solid (0.133 g, 76%) with no purification required. m.p. 207–208 °C.

IR (KBr) ν_{max}: 1699 (C=O stretch, s), 1521, 1508 (aromatic C=C stretch, s), 1111 (ether C-O stretch, s) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 4.11 (3H, s, OC***H₃***), 6.61 (1H, s, C***H***), 7.73 (2H, d, ***J*** = 8.3 Hz, 2 × C***H*** arom.), 7.99 (2H, d, ***J*** = 8.3 Hz, 2 × C***H*** arom.) ppm; ^{13}C NMR (75 MHz, CDCl$_3$): δ 57.6 (O***C***H$_3$), 65.8 (***C***I), 92.9 (***C***H), 123.5 (q, $^1J_{C\text{-}F}$ = 272.4 Hz, ***C***F$_3$), 126.1 (q, $^3J_{C\text{-}F}$ = 3.8 Hz, 2 × ***C***H arom.), 126.3 (2 × ***C***H arom.), 133.2 (q, $^2J_{C\text{-}F}$ = 32.9 Hz, CF$_3$***C***), 133.8 (q***C*** arom.), 160.0 (***C***CHCOCH$_3$), 160.6 (***C***=O), 170.0 (***C***OCH$_3$) ppm; ^{19}F NMR (282 MHz, CDCl$_3$): δ -63.1 (C***F₃***) ppm; HRMS (ESI) *m/z* calcd for $C_{13}H_9F_3O_3I$ [(M + H)$^+$]: 396.9549, found 396.9536.

9.4 Synthesis of trifluoromethylated *O*-functionalised pyrones

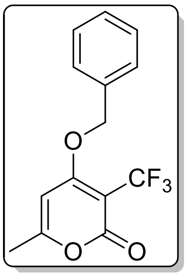
4-Methoxy-6-methyl-3-(trifluoromethyl)-2*H*-pyran-2-one, 510



To a stirred solution of 3-iodo-4-methoxy-6-methyl-2-pyrone **506** (0.101 g, 0.38 mmol) and copper (I) iodide (0.087 g, 0.46 mmol) in DMF (4 mL) was added methyl 2,2-difluoro-2-(fluorosulfonyl)acetate (0.06 mL, 0.46 mmol). The reaction mixture was allowed stir at 70 °C for 5 h. On completion, the reaction was cooled to room temperature, diluted with diethyl ether (20 mL) and filtered. The solution was poured into water (20 mL), extracted with diethyl ether (4 × 20 mL) and the combined organic extracts washed with water (3 × 5 mL). The organic layer was dried over MgSO$_4$ and concentrated *in vacuo* to yield crude product which was purified using silica column chromatography eluting with 50:50 hexane:ethyl acetate to yield **510** as a pale yellow solid (0.061 g, 77%). m.p. 120–122 °C.

IR (KBr) ν_{max}: 1747 (ester C=O stretch, s), 1560 (aromatic C=C stretch, s), 1266 (ether C-O stretch, s), 1100 (ester C-O stretch, s) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.33 (3H, s, C***H₃***), 4.00 (3H, s, OC***H₃***), 6.11 (1H, s, C***H***) ppm; ^{13}C NMR (100 MHz, CDCl$_3$): δ 20.7 (***C***H$_3$), 57.5 (O***C***H$_3$), 94.5 (***C***H), 94.3 (q, $^2J_{C\text{-}F}$ = 31.6 Hz, ***C***CF$_3$), 124.2 (q, $^1J_{C\text{-}F}$ = 272.7 Hz, ***C***F$_3$), 159.1 (***C***=O), 167.8 (***C***CH$_3$), 171.1 (***C***OCH$_3$) ppm; ^{19}F NMR (470 MHz, CDCl$_3$): δ -57.6 (C***F₃***) ppm; HRMS (ESI) *m/z* calcd for $C_8H_8F_3O_3$ [(M + H)$^+$]: 209.0426, found 209.0424; Anal. calcd for $C_8H_7F_3O_3$: C, 46.17; H, 3.39%. Found: C, 46.27; H, 3.49%.

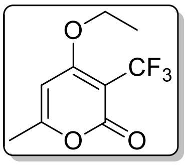
4-(Benzyloxy)-6-methyl-3-(trifluoromethyl)-2*H*-pyran-2-one, 515d



To a stirred solution of 3-iodo-4-benzyloxy-6-methyl-2-pyrone **514d** (0.259 g, 0.76 mmol) and copper (I) iodide (0.173 g, 0.91 mmol) in DMF (4 mL) was added methyl 2,2-difluoro-2-(fluorosulfonyl)acetate (0.12 mL, 0.91 mmol). The reaction mixture was allowed stir at 70 °C overnight. On completion, the reaction was cooled to room temperature, diluted with diethyl ether (20 mL) and filtered. The solution was poured into water (20 mL), extracted with diethyl ether (4 × 20 mL) and the combined organic extracts washed with water (3 × 5 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product which was purified using silica column chromatography eluting with 50:50 hexane:ethyl acetate to yield **515d** as a pale yellow solid (0.065 g, 30%). m.p. 149–151 °C.

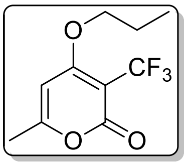
IR (KBr) ν_{max}: 1715 (C=O stretch, s), 1556, 1414 (aromatic C=C stretch, s), 1364 (ester C-O stretch, m) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.28 (3H, s, C***H*₃**), 5.27 (2H, s, OC***H*₂**Ph), 6.08 (1H, s, C***H***), 7.34–7.45 (5H, m, 5 × Ar***H***) ppm; ^{13}C NMR ($CDCl_3$, 150 MHz): δ 20.8 (***C***H_3), 71.9 (O***C***H_2Ph), 95.1 (q, $^{2}J_{C\text{-}F}$ = 29.4 Hz, ***C***CF_3), 95.3 (***C***H), 122.8 (q, $^{1}J_{C\text{-}F}$ = 271.5 Hz, ***C***F_3), 126.8, 128.9, 129.0 (5 × ***C***H arom.), 134.0 (OCH_2***C***), 159.0 (***C***=O), 167.5 (CH_3***C***CH), 170.2 (CH***C***OCH_2Ph); ^{19}F NMR (282 MHz, $CDCl_3$): δ -57.5 (C***F*₃**) ppm; HRMS (ESI) *m*/*z* calcd for $C_{14}H_{12}F_3O_3$ $[(M + H)^{+}]$: 285.0739, found 285.0728.

4-Ethoxy-6-methyl-3-(trifluoromethyl)-2*H*-pyran-2-one, 515e



To a stirred solution of 3-iodo-4-ethoxy-6-methyl-2-pyrone **514e** (0.190 g, 0.68 mmol) and copper (I) iodide (0.156 g, 0.82 mmol) in DMF (4 mL) was added methyl 2,2-difluoro-2-(fluorosulfonyl)acetate (0.21 mL, 1.63 mmol). The reaction mixture was allowed stir at 70 °C overnight. On completion, the reaction was cooled to room temperature, diluted with diethyl ether (20 mL) and filtered. The solution was poured into water (20 mL), extracted with diethyl ether (4 × 20 mL) and the combined organic extracts washed with water (3 × 5 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product which was purified using silica column chromatography eluting with 70:30 hexane:ethyl acetate to yield **515e** as a pale yellow solid (0.052 g, 34%). m.p. 129–131 °C. IR (KBr) ν_{max}: 1722 (C=O stretch, s), 1557 (aromatic C=C stretch, s), 1263 (ester C-O stretch, m) cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$): δ 1.44 (3H, t, *J* = 7.0 Hz, OCH$_2$C***H*₃**), 2.29 (3H, s, C***H*₃**), 4.23 (2H, q, *J* = 7.0 Hz, OC***H*₂**CH$_3$), 6.05 (1H, s, C***H***) ppm; ^{13}C NMR (100 MHz, CDCl$_3$): δ 14.4 (OCH$_2$***C***H$_3$), 20.7 (***C***H$_3$), 66.7 (O***C***H$_2$CH$_3$), 94.5 (q, $^{2}J_{C\text{-}F}$ = 31.5 Hz, ***C***CF$_3$), 95.0 (***C***H), 122.9 (q, $^{1}J_{C\text{-}F}$ = 272.8 Hz, ***C***F$_3$), 159.2 (***C***=O), 167.4 (CH$_3$***C***CH), 170.4 (***C***OCH$_2$CH$_3$) ppm; ^{19}F NMR (282 MHz, CDCl$_3$): δ -57.6 (C***F*₃**) ppm; HRMS (ESI) *m/z* calcd for $C_9H_{10}F_3O_3$ [(M + H)$^+$]: 223.0582, found 223.0574; Anal. calcd for $C_9H_9F_3O_3$: C, 48.66; H, 4.08%. Found: C, 48.48; H, 4.00%.

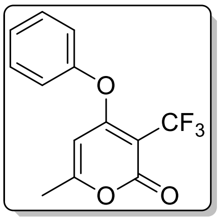
6-Methyl-4-propoxy-3-(trifluoromethyl)-2*H*-pyran-2-one, 515f



To a stirred solution of 3-iodo-4-propoxy-6-methyl pyrone **514f** (0.093 g, 0.32 mmol) and copper (I) iodide (0.073 g, 0.38 mmol) in DMF (4 mL) was added methyl 2,2-difluoro-2-(fluorosulfonyl)acetate (0.10 mL, 0.76 mmol). The reaction mixture was allowed stir at 70 °C for 12 h. On completion, the reaction was cooled to room temperature, diluted with diethyl ether (20 mL) and filtered. The solution was poured into water (20 mL), extracted with diethyl ether (4 × 20 mL) and the combined organic extracts washed with water (3 × 5 mL). The organic layer was dried over MgSO$_4$ and concentrated *in vacuo* to yield crude product which was purified using silica column chromatography eluting with 50:50 hexane:ethyl acetate to yield **515f** as a yellow solid (0.035 g, 46%). m.p. 107–109 °C.

IR (KBr) ν_{max}: 1719 (C=O stretch, s), 1552 (aromatic C=C stretch, s), 1113 (ester C-O stretch, m) cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$): δ 1.02 (3H, t, *J* = 7.4 Hz OCH$_2$CH$_2$C***H*₃**), 1.82 (2H, m, OCH$_2$C***H*₂**CH$_3$), 2.29 (3H, s, C***H*₃**), 4.11 (2H, t, *J* = 6.3 Hz, OC***H*₂**CH$_2$CH$_3$), 6.06 (1H, s, C***H***) ppm; ^{13}C NMR (100 MHz, CDCl$_3$): δ 8.2 (OCH$_2$CH$_2$***C***H$_3$), 18.8 (***C***H$_3$), 20.4 (OCH$_2$***C***H$_2$CH$_3$), 70.5 (O***C***H$_2$CH$_2$CH$_3$), 92.4 (q, $^{2}J_{C\text{-}F}$ = 31.6 Hz, ***C***CF$_3$), 93.3 (***C***H), 121.1(q, $^{1}J_{C\text{-}F}$ = 272.8 Hz, ***C***F$_3$), 157.4 (***C***=O), 165.6 (CH$_3$***C***CH), 168.9 (***C***OCH$_2$CH$_2$CH$_3$) ppm; ^{19} F NMR (282 MHz, CDCl$_3$): δ -57.6 (C***F*₃**) ppm; HRMS (ESI) *m/z* calcd for $C_{10}H_{12}O_3F_3$ [(M + H)$^+$]: 237.0739, found 237.0735.

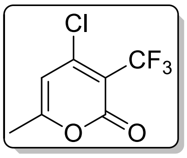
6-Methyl-4-phenoxy-3-(trifluoromethyl)-2*H*-pyran-2-one, 515g



To a stirred solution of 3-iodo-4-phenoxy-6-methyl-2-pyrone **514g** (0.289 g, 0.88 mmol) and copper (I) iodide (0.201 g, 1.06 mmol) in DMF (4 mL) was added methyl 2,2-difluoro-2-(fluorosulfonyl)acetate (0.27 mL, 2.12 mmol). The reaction mixture was allowed stir at 70 °C for 12 h. On completion, the reaction was cooled to room temperature, diluted with diethyl ether (20 mL) and filtered. The solution was poured into water (20 mL), extracted with diethyl ether (4 × 20 mL) and the combined organic extracts washed with water (3 × 5 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product which was purified using silica column chromatography eluting with 80:20 hexane:ethyl acetate to yield **515g** as a yellow solid (0.103 g, 43%). m.p. 128–129 °C.

IR (KBr) ν_{max}: 1730 (C=O stretch, s), 1563 (aromatic C=C stretch, s), 1261, 1123 (ester C-O stretch, s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.19 (3H, s, C***H₃***), 5.62 (1H, s, C***H***), 7.07–7.11 (2H, m, 2 × C***H*** arom.), 7.31–7.37 (1H, m, C***H*** arom.), 7.44–7.50 (2H, m, 2 × C***H*** arom.) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.5 (***C***H_3), 96.3 (q, $^2J_{C\text{-}F}$ = 32.1 Hz, ***C***CF_3), 96.9 (***C***H), 120.9 (2 × ***C***H arom.), 122.5 (q, $^1J_{C\text{-}F}$ = 273.1 Hz, ***C***F_3), 126.9 (***C***H arom.), 130.5 (2 × ***C***H arom.), 152.4 (CHCO***C***), 159.0 (***C***=O), 167.2 (CH_3***C***CH), 169.2 (CH***C***O) ppm; ^{19} F NMR (282 MHz, $CDCl_3$): δ -57.8 (C***F₃***) ppm; HRMS (ESI) *m/z* calcd for $C_{13}H_{10}F_3O_3$ $[(M + H)^+]$: 271.0582, found 271.0583; Anal. calcd for $C_{13}H_9F_3O_3$: C, 57.79; H, 3.36%. Found: C, 57.61; H, 3.40%.

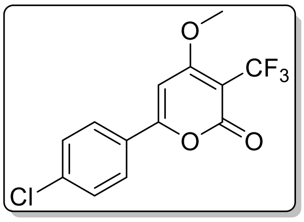
4-Chloro-6-methyl-3-(trifluoromethyl)-2*H*-pyran-2-one, 515h



To a stirred solution of 3-iodo-4-chloro-6-methyl-2-pyrone **514h** (0.363 g, 1.34 mmol) and copper (I) iodide (0.307 g, 1.61 mmol) in DMF (4 mL) was added methyl 2,2-difluoro-2-(fluorosulfonyl)acetate (0.41 mL, 3.22 mmol). The reaction mixture was allowed stir at 70 °C for 12 h. On completion, the reaction was cooled to room temperature, diluted with diethyl ether (20 mL) and filtered. The solution was poured into water (20 mL), extracted with diethyl ether (4 × 20 mL) and the combined organic extracts washed with water (3 × 5 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product which was purified using silica column chromatography eluting with 50:50 hexane:ethyl acetate to yield **515h** as a yellow solid (0.076 g, 23%). m.p. 50–52 °C.

IR (KBr) ν_{max}: 1732 (C=O stretch, s), 1548 (aromatic C=C stretch, m), 1138 (ester C-O stretch, s) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.31 (3H, s, C***H*$_3$**), 6.17 (1H, s, C***H***) ppm; ^{13}C NMR (100 MHz, CDCl$_3$): δ 19.9 (***C***H$_3$), 107.6 (***C***H), 111.4 (q, $^2J_{C\text{-}F}$ = 32.4 Hz, ***C***CF$_3$), 121.6 (q, $^1J_{C\text{-}F}$ = 274.5 Hz, ***C***F$_3$), 153.2 (***C***Cl), 156.3 (***C***=O), 164.8 (CH$_3$***C***CH) ppm; ^{19}F NMR (282 MHz, CDCl$_3$): δ -59.1 (C***F***$_3$) ppm; HRMS (ESI) *m/z* calcd for $C_7H_5O_2F_3Cl$ [(M + H)$^+$]: 212.9930, found 212.9938;

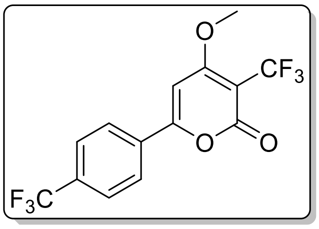
6-(4-Chlorophenyl)-4-methoxy-3-(trifluoromethyl)-2*H*-pyran-2-one, 515i



To a stirred solution of 6-(4-chlorophenyl)-3-iodo-4-methoxy-2*H*-pyran-2-one **514i** (0.060 g, 0.17 mmol) and copper (I) iodide (0.038 g, 0.20 mmol) in DMF (4 mL) was added methyl 2,2-difluoro-2-(fluorosulfonyl)acetate (0.03 mL, 0.20 mmol). The reaction mixture was allowed stir at 70 °C for 12 h. On completion, the reaction was cooled to room temperature, diluted with diethyl ether (20 mL) and filtered. The solution was poured into water (20 mL), extracted with diethyl ether (4 × 20 mL) and the combined organic extracts washed with water (3 × 5 mL). The organic layer was dried over MgSO$_4$ and concentrated *in vacuo* to yield crude product which was purified using silica column chromatography eluting with 50:50 hexane:ethyl acetate to yield **515i** as a white solid (0.012 g, 23%). m.p. 211–214 °C.

IR (KBr) ν_{max}: 1690 (C=O stretch, s), 1515 (aromatic C=C stretch. s), 1387 (ester C-O stretch, s) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 4.11 (3H, s, OC***H*$_3$**), 6.63 (1H, s, C***H***), 7.48 (2H, d, *J* = 8.8 Hz, 2 × C***H*** arom.), 7.82 (2H, d, *J* = 8.8 Hz, 2 × C***H*** arom.) ppm; ^{13}C NMR (100 MHz, CDCl$_3$): δ 57.5 (O***C***H$_3$), 91.4 (***C***H), 96.6 (q, $^2J_{C\text{-}F}$ = 31.9 Hz, ***C***CF$_3$), 122.7 (q, $^1J_{C\text{-}F}$ = 272.9 Hz, ***C***F$_3$), 127.2 (2 × ***C***H arom.), 128.7 (q***C*** arom.), 129.6 (2 × ***C***H arom.) 138.9 (***C***Cl), 158.1 (***C***=O), 163.4 (***C***OCH$_3$), 170.8 (CH$_3$***C***CH) ppm; ^{19}F NMR (282 MHz, CDCl$_3$): δ -57.6 (C***F***$_3$) ppm; HRMS (ESI) *m/z* calcd for $C_{13}H_9ClF_3O_3$ [(M + H)$^+$]: 305.0114, found 305.0119.

4-Methoxy-3-(trifluoromethyl)-6-(4-(trifluoromethyl)phenyl)-2*H*-pyran-2-one, 515j

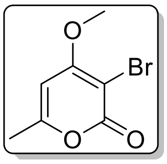


To a stirred solution of 3-iodo-4-methoxy-6-(4-(trifluoromethyl)phenyl)-2*H*-pyran-2-one **514j** (0.094 g, 0.24 mmol) and copper (I) iodide (0.054 g, 0.28 mmol) in DMF (4 mL) was added methyl 2,2-difluoro-2-(fluorosulfonyl)acetate (0.04 mL, 0.28 mmol). The reaction mixture was allowed stir at 70 °C for 7 h. On completion, the reaction was cooled to room temperature, diluted with diethyl ether (20 mL) and filtered. The solution was poured into water (20 mL), extracted with diethyl ether (4 × 20 mL) and the combined organic extracts washed with water (3 × 5 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product which was purified using silica column chromatography eluting with 70:30 hexane:ethyl acetate to yield **515j** as a white solid (0.058 g, 72%). m.p. 141–143 °C.

IR (KBr) ν_{max}: 1713 (C=O stretch, s), 1541 (aromatic C=C stretch, s), 1326 (ester C-O stretch, s), 1117 (ether C-O stretch, s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.13 (3H, s, OC***H*3**), 6.74 (1H, s, C***H***), 7.76 (2H, d, *J* = 8.3 Hz, 2 × C***H*** arom.), 7.99 (2H, d, *J* = 8.3 Hz, 2 × C***H*** arom.) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 57.7 (O***C***H3), 92.7 (***C***H), 96.3 (q, $^2J_{C-F}$ = 31.9 Hz, ***C***CF3), 122.6 (q, $^1J_{C-F}$ = 273.1 Hz, C***C***F3), 123.4 (q, $^1J_{C-F}$ = 272.6 Hz, ***C***F3 aryl), 126.2 (q, $^3J_{C-F}$ = 3.7 Hz, 2 × ***C***H arom.), 126.8 (2 × ***C***H arom.), 133.4 (q***C*** arom.), 133.9 (q, $^2J_{C-F}$ = 33.1 Hz, CF3***C***CH), 157.8 (***C***=O), 162.7 (***C***CHCOCH3), 170.5 (***C***OCH3) ppm; ^{19}F NMR (282 MHz, $CDCl_3$): δ -57.8 (C***F*3**), -63.2 (C***F*3** aryl) ppm; HRMS (ESI) *m/z* calcd for $C_{14}H_9F_6O_3$ [(M + H)$^+$]: 339.0456, found 339.0444.

9.5 Synthesis of 3-bromo-4-methoxy-2-pyrone and subsequent trifluoromethylation

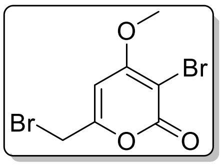
3-Bromo-4-methoxy-6-methyl-2*H*-pyran-2-one, 516



To a stirred solution of 4-methoxy-6-methyl-2*H*-pyran-2-one **505** (2.791 g, 19.9 mmol) in acetonitrile (30 mL) was added *N*-bromosuccinimide (7.089 g, 39.8 mmol). The reaction vessel was covered in aluminium foil and allowed stir at reflux overnight. On completion, the solvent was concentrated *in vacuo*, the resulting orange solid dissolved in DCM (20 mL) and washed with 5% aq. sodium thiosulfate (2 × 25 mL), water (25 mL) and brine (25 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product which was purified by silica column chromatography eluting with 50:50 hexane:ethyl acetate to yield **516** as a pale yellow solid (1.980 g, 45%). m.p. 149–151 °C. [lit.[14] 151–152 °C].

Spectral characteristics were consistent with previously reported data.[15] ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.29 (3H, s, C***H₃***), 3.98 (3H, s, OC***H₃***), 6.05 (1H, s, C***H***) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.3 (***C***H_3), 57.3 (O***C***H_3), 88.5 (***C***Br), 95.1 (***C***H), 160.8 (***C***=O), 162.8 (CH_3***C***CH), 166.8 (***C***OCH_3) ppm; HRMS (ESI) *m/z* calcd for $C_7H_8O_3Br$ [$(M + H)^+$]: 218.9657, found 218.9649.

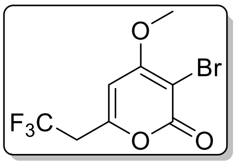
3-Bromo-6-(bromomethyl)-4-methoxy-2*H*-pyran-2-one, 517



3-Bromo-6-(bromomethyl)-4-methoxy-2*H*-pyran-2-one **517** was isolated as a side product from the bromination of 4-methoxy-6-methyl-2H-pyran-2-one **505** as an off white solid(0.794 g, 13%). m.p. 159–161 °C. [lit.[16] 162–164 °C]. Spectral characteristics were consistent with previously reported data.[16]

^{1}H NMR (300 MHz, $CDCl_3$): δ 4.03 (3H, s, OC***H₃***), 4.19 (2H, s, C***H₂***Br), 6.40 (1H, s, C***H***) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 26.3 (***C***H_2Br), 57.6 (O***C***H_3), 91.3 (***C***Br), 96.7 (***C***H), 159.1 (BrCH_2***C***CH), 159.7 (***C***=O), 166.0 (***C***OCH_3) ppm; HRMS (ESI) *m/z* calcd for $C_7H_7O_3Br_2$ [$(M + H)^+$]: 296.8762, found 296.8766.

3-Bromo-4-methoxy-6-(2,2,2-trifluoroethyl)-2*H*-pyran-2-one, 518

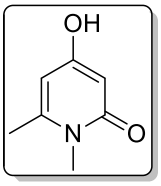


To a stirred solution of **517** (0.226 g, 0.76 mmol) and copper (I) iodide (0.173 g, 0.91 mmol) in DMF (4 mL) was added methyl 2,2-difluoro-2-(fluorosulfonyl)acetate (0.10 mL, 0.76 mmol). The reaction mixture was allowed stir at 70 °C for 5 h. On completion, the reaction was cooled to room temperature, diluted with diethyl ether (20 mL) and filtered. The solution was poured into water (20 mL), extracted with diethyl ether (4 × 20 mL) and the combined organic extracts washed with water (3 × 5 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product which was purified using silica column chromatography eluting with 50:50 hexane:ethyl acetate to yield **518** as a yellow solid (0.065 g, 30%). m.p. 146–148 °C.

IR (KBr) ν_{max}: 1702 (ester C=O stretch, s), 1537 (aromatic C=C stretch, s), 1327 (ester C-O stretch, s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.35 (2H, q, *J* = 9.7 Hz, $CF_3C\boldsymbol{H_2}$), 4.02 (3H, s, $OC\boldsymbol{H_3}$), 6.28 (1H, s, C***H***) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.8 (q, $^2J_{C\text{-}F}$ = 32.0 Hz, $CF_3\boldsymbol{C}H_2$), 57.6 ($O\boldsymbol{C}H_3$), 91.3 (***C***Br), 98.4 (***C***H), 123.5 (q, $^1J_{C\text{-}F}$ = 277.8 Hz, $\boldsymbol{C}F_3$), 154.0 (q, $^3J_{C\text{-}F}$ = 3.5 Hz, $\boldsymbol{C}CH_2CF_3$), 159.7 (***C***=O), 165.9 ($\boldsymbol{C}OCH_3$) ppm; ^{19}F NMR (282 MHz, $CDCl_3$): δ -64.2 ($C\boldsymbol{F_3}$) ppm; HRMS (ESI) *m/z* calcd for $C_8H_7O_3F_3Br$ [(M + H)$^+$]: 286.9531, found 286.9522.

9.6 Synthesis of 4-hydroxypyridones

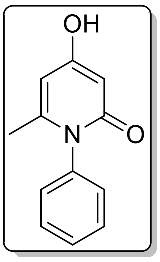
4-Hydroxy-1,6-dimethylpyridin-2(1*H*)-one, 519a



To a round bottomed flask containing a suspension of 4-hydroxy-6-methyl-2*H*-pyran-2-one **439** (3.661 g, 29.0 mmol) in water (20 mL) was added methylamine (2.6 mL, 58.0 mmol). The reaction vessel was placed in an oil bath set at 100 °C and reaction allowed to stir at reflux for 5 h. The reaction vessel was removed from oil bath and placed in an ice-water bath for 2 h. The resulting solid was filtered and triturated with hot ethanol to yield product **519a** as an off-white solid (2.221 g, 55%). m.p. 221–223 °C [lit.[17] 230 °C].

Spectral characteristics were consistent with previously reported data.[18] ^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ 2.26 (3H, s, $C\boldsymbol{H_3}$), 3.30 (3H, s, $NC\boldsymbol{H_3}$), 5.50 (1H, d, *J* = 2.4 Hz, C=OC***H***), 5.76 (1H, d, *J* = 2.1 Hz, $CH_3CC\boldsymbol{H}$) ppm; ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$): δ 20.1 ($\boldsymbol{C}H_3$), 30.2 ($N\boldsymbol{C}H_3$), 96.0 (***C***HC=O), 100.4 ($CH_3C\boldsymbol{C}H$), 148.3 ($CH_3\boldsymbol{C}CH$), 164.5 (***C***=O), 166.2 (***C***OH) ppm; HRMS (ESI) *m/z* calcd for $C_7H_{10}NO_2$ [(M + H)$^+$]: 140.0712, found 140.0711.

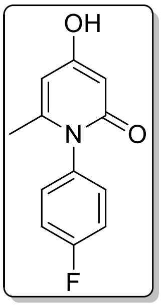
4-Hydroxy-6-methyl-1-phenylpyridin-2(1*H*)-one, 519c



To a round bottomed flask containing a suspension of 4-hydroxy-6-methyl-2*H*-pyran-2-one **439** (2.770 g, 22.0 mmol) in water (30 mL) was added aniline (2.02 mL, 22.2 mmol). The reaction vessel was placed in an oil bath set at 100 °C and reaction allowed to stir at reflux for 5 h. The reaction vessel was removed from oil bath and placed in an ice-water bath for 2 h. The resulting solid was filtered and triturated with hot ethanol to yield product **519c** as a white solid (0.639 g, 14%). m.p. > 250 °C. [lit.[19] 276 °C].

IR (KBr) ν_{max}: 2918 (alkyl CH stretch, m), 1639 (amide C=O stretch, s), 1483 (aromatic C=C stretch, s), 1252 (ether C-O stretch, m) cm^{-1}; ^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ 1.83 (3H, s, C***H₃***), 5.56 (1H, d, *J* = 2.3 Hz, C***H***C=O), 5.89 (1H, d, *J* = 1.9 Hz, CH_3CC***H***), 7.17 (2H, d, *J* = 7.0 Hz, 2 × C***H*** arom.), 7.40–7.52 (3H, m, 3 × C***H*** arom.), 10.59 (1H, bs, O***H***) ppm; ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$): δ 21.5 (***C***H_3), 96.6 (***C***HC=O), 100.5 (CH_3CC***H***), 128.6, 129.1, 129.7 (5 × ***C***H arom.), 139.3 (N***C***), 147.4 (CH_3***C***CH), 164.5 (***C***=O), 166.9 (***C***OH) ppm; HRMS (ESI) *m/z* calcd for $C_{12}H_{12}NO_2$ [(M + H)$^+$]: 202.0868, found 202.0858. Anal. calcd for $C_{12}H_{11}NO_2$: C, 71.63; H, 5.51; N, 6.96%. Found: C, 71.64; H, 5.71; N, 6.77%.

1-(4-Fluorophenyl)-4-hydroxy-6-methylpyridin-2(1*H*)-one, 519d

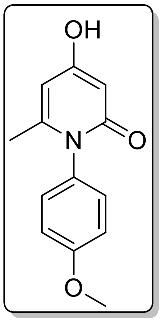


To a round bottomed flask containing a suspension of 4-hydroxy-6-methyl-2*H*-pyran-2-one **439** (2.641 g, 20.9 mmol) in water (30 mL) was added 4-fluoroaniline (2.00 mL, 21.2 mmol). The reaction vessel was placed in an oil bath set at 100 °C and reaction allowed to stir at reflux for 5 h. The reaction vessel was removed from oil bath and placed in an ice-water bath for 2 h. The resulting solid was filtered and triturated with hot ethanol to yield product **519d** as a white solid (0.597 g, 13%). m.p. > 250 °C.

IR (KBr) ν_{max}: 1619 (amide C=O stretch, s), 1492 (aromatic C=C stretch, s), 1213 (ether C-O stretch, s) cm^{-1}; ^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ 1.84 (3H, s, C***H₃***), 5.55 (1H, d, *J* = 2.1 Hz, C***H***C=O), 5.89 (1H, s, CH_3CC***H***), 7.23–7.34 (4H, m, 4 × C***H*** arom.), 10.62 (1H, bs, O***H***) ppm; ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$): δ 25.5

(***C***H$_3$), 96.5 (***C***HC=O), 100.6 (CH$_3$C***C***H), 116.5 (d, $^2J_{C\text{-}F}$ = 22.7 Hz, 2 × ***C***H arom.), 131.3 (d, $^3J_{C\text{-}F}$ = 8.8 Hz, 2 × ***C***H arom.), 135.4 (d, $^4J_{C\text{-}F}$ = 3.1 Hz, FCCHCH***C***N), 147.4 (CH$_3$***C***CH), 161.9 (d, $^1J_{C\text{-}F}$ = 244.7 Hz, F***C***CH), 164.6 (***C***=O), 167.0 (***C***OH) ppm; ^{19}F NMR (282 MHz, (CD$_3$)$_2$SO): δ -114.1 (C***F***) ppm; HRMS (ESI) *m/z* calcd for $C_{12}H_{11}NO_2F$ [(M + H)$^+$]: 220.0774, found 220.0781.

4-Hydroxy-1-(4-methoxyphenyl)-6-methylpyridin-2(1*H*)-one, 519e

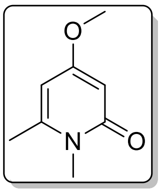


To a round bottomed flask containing a suspension of 4-hydroxy-6-methyl-2*H*-pyran-2-one **439** (2.784 g, 22.1 mmol) in water (30 mL) was added *p*-anisidine (2.72 mL, 22.3 mmol). The reaction vessel was placed in an oil bath set at 100 °C and reaction allowed to stir at reflux for 5 h. The reaction vessel was removed from oil bath and placed in an ice-water bath for 2 h. The resulting solid was filtered and triturated with hot ethanol to yield product **519e** as a white solid (1.185 g, 23%). m.p. > 250 °C.

IR (KBr) ν_{max}: 1619 (amide C=O stretch, s), 1531, 1507 (aromatic C=C stretch, s), 1246 (ether C-O stretch, s), 1110 (alcohol C-O stretch, m) cm^{-1}; ^{1}H NMR (300 MHz, (CD$_3$)$_2$SO): δ 1.83 (3H, s, C***H$_3$***), 3.80 (3H, s, OC***H$_3$***), 5.23 (1H, s, C***H***C=O), 5.86 (1H, s, CH$_3$CC***H***), 7.01 (2H, d, *J* = 8.5 Hz, 2 × C***H*** arom.), 7.09 (2H, d, *J* = 8.6 Hz, 2 × C***H*** arom.), 10.56 (1H, bs, O***H***) ppm; ^{13}C NMR (75.5 MHz, (CD$_3$)$_2$SO): δ 21.6 (***C***H$_3$), 55.8 (O***C***H$_3$), 96.5 (***C***HC=O), 100.3 (CH$_3$C***C***H), 114.8 (2 × ***C***H arom.), 130.1 (2 × ***C***H arom.), 131.8 (N***C***), 147.9 (CH$_3$***C***CH), 159.1 (***C***OCH$_3$), 164.7 (***C***=O), 166.8 (***C***OH) ppm; HRMS (ESI) *m/z* calcd for $C_{13}H_{14}NO_3$ [(M + H)$^+$]: 232.0974, found 232.0965.

9.7 Synthesis of 4-methoxypyridones

4-Methoxy-1,6-dimethylpyridin-2(1*H*)-one, 520a

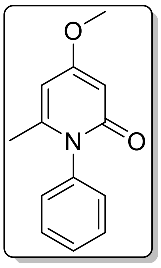


To a round bottomed flask containing 4-hydroxy-1,6-dimethylpyridin-2(1*H*)-one **519a** (0.959 g, 6.9 mmol) and K_2CO_3 (1.142 g, 14.3 mmol) was added trimethylphosphate (2.36 mL, 20.1 mmol) and the resulting reaction mixture stirred at reflux for 3 h. On completion, the reaction was washed with water (15 mL) and extracted with ethyl acetate (3 × 25 mL). The combined organic extracts were washed with brine

(50 mL), dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product as a pale yellow solid which was purified by silica column chromatography eluting with ethyl acetate to yield **520a** a pale yellow solid (0.375 g, 36%). m.p. 113–115 °C [lit.[20] 115–116 °C].

IR (KBr) ν_{max}: 1652 (amide C=O stretch, s), 1570 (aromatic C=C stretch, s), 1244, 1220 (ester C-O, s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.27 (3H, s, $C\boldsymbol{H_3}$), 3.43 (3H, s, $NC\boldsymbol{H_3}$), 3.71 (3H, s, $OC\boldsymbol{H_3}$), 5.57 (1H, dd, *J* = 0.8, 2.8 Hz, $CH_3CC\boldsymbol{H}$), 5.81 (1H, d, *J* = 2.8 Hz, C***H***C=O) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.8 ($\boldsymbol{C}H_3CCH$), 30.5 ($N\boldsymbol{C}H_3$), 55.2 ($O\boldsymbol{C}H_3$), 94.5 (***C***HC=O), 100.7 ($CH_3C\boldsymbol{C}H$), 146.0 ($CH_3\boldsymbol{C}CH$), 165.4 (***C***=O), 167.2 ($\boldsymbol{C}OCH_3$) ppm; HRMS (ESI) *m/z* calcd for $C_8H_{12}NO_2$ [$(M + H)^+$]: 154.0868, found 154.0867.

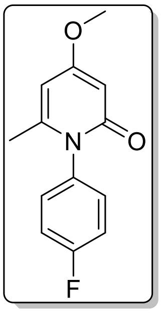
4-Methoxy-6-methyl-1-phenylpyridin-2(1*H*)-one, 520c



To a round bottomed flask containing 4-hydroxy-6-methyl-1-phenylpyridin-2(1*H*)-one **519c** (0.776 g, 3.86 mmol) and K_2CO_3 (0.640 g, 4.63 mmol) was added trimethylphosphate (0.93 mL, 8.01 mmol) and the resulting reaction mixture stirred at reflux overnight. On completion, the reaction was washed with water (15 mL) and extracted with ethyl acetate (3 × 25 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product as an orange oil which was purified by silica column chromatography eluting with ethyl acetate to yield **520c** a pale yellow solid (0.578 g, 70%). m.p. 162–163 °C.

IR (KBr) ν_{max}: 2968 (alkyl C-H stretch, m), 1650 (amide C=O stretch, s), 1597, 1562 (aromatic C=C stretch, s), 1243 (C-O stretch, m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.85 (3H, s, $C\boldsymbol{H_3}$), 3.73 (3H, s, $OC\boldsymbol{H_3}$), 5.81 (1H, s, $CH_3CC\boldsymbol{H}$), 5.85 (1H, d, *J* = 2.5 Hz, C***H***C=O), 7.14 (2H, d, *J* = 7.4 Hz, 2 × C***H*** arom.), 7.36–7.48 (3H, m, 3 × C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 21.5 ($\boldsymbol{C}H_3$), 55.4 ($O\boldsymbol{C}H_3$), 95.0 (***C***HC=O), 100.8 ($CH_3C\boldsymbol{C}H$), 128.3, 128.7, 129.7 (5 × ***C***H arom.), 138.7 (N***C***), 146.0 ($CH_3\boldsymbol{C}CH$), 165.5 (***C***=O), 167.9 ($\boldsymbol{C}OCH_3$) ppm; HRMS (ESI) *m/z* calcd for $C_{13}H_{14}NO_2$ [$(M + H)^+$]: 216.1025, found 216.1018; Anal. calcd for $C_{12}H_{11}NO_2$: C, 71.63; H, 5.51; N, 6.96%. Found: C, 71.64; H, 5.71; N, 6.77%.

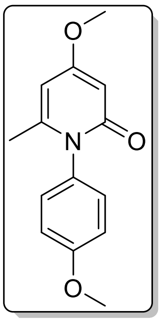
1-(4-Fluorophenyl)-4-methoxy-6-methylpyridin-2(1*H*)-one, 520d



To a round bottomed flask containing 1-(4-fluorophenyl)-4-hydroxy-6-methylpyridin-2(1*H*)-one **519d** (1.613 g, 7.36 mmol) and K_2CO_3 (1.221 g, 8.83 mmol) was added trimethylphosphate (1.79 mL, 15.3 mmol) and the resulting reaction mixture stirred at reflux for 2 h. On completion, the reaction was washed with water (15 mL) and extracted with ethyl acetate (3 × 25 mL). The combined organic extracts were washed with brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product as a pale yellow solid which was purified by silica column chromatography eluting with ethyl acetate to yield **520d** as a white solid (1.025 g, 60%). m.p. 188–190 °C.

IR (KBr) ν_{max}: 1651 (amide C=O stretch, s), 1558 (aromatic C=C stretch, s), 1245 (ether C=O stretch, s) cm^{-1}; ^{1}H NMR (300 MHz, ($CDCl_3$): δ 1.90 (3H, s, C***H₃***), 3.78 (3H, s, OC***H₃***), 5.84 (1H, d, *J* = Hz, C***H***C=O), 5.87 (1H, d, *J* = Hz, CH_3CC***H***), 7.16–7.18 (4H, m, 4 × C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, ($CDCl_3$): δ 21.5 (***C***H_3), 55.5 (O***C***H_3), 95.0 (***C***HC=O), 101.1 (CH_3C***C***H), 116.7 (d, $^2J_{C\text{-}F}$ = 22.8 Hz, 2 × C***H*** arom.), 130.1 (d, $^3J_{C\text{-}F}$ = 8.7 Hz, 2 × C***H*** arom.), 134.5 (d $^4J_{C\text{-}F}$ = 3.3 Hz, q***C*** arom.), 145.9 (CH_3***C***CH), 162.4 (d, $^1J_{C\text{-}F}$ = 248.3 Hz, F***C***), 165.6 (***C***=O), 168.0 (***C***OCH_3) ppm; ^{19}F NMR (282 MHz, ($CDCl_3$): δ -112.7 (C***F***) ppm; HRMS (ESI) *m/z* calcd for $C_{13}H_{13}NFO_2$ [(M + H)$^+$]: 234.0930, found 234.0920; Anal. calcd for $C_{13}H_{12}FNO_2$: C, 66.94; H, 5.19; N, 6.01%. Found: C, 66.92; H, 5.19; N, 6.01%.

4-Methoxy-1-(4-methoxyphenyl)-6-methylpyridin-2(1*H*)-one, 520e

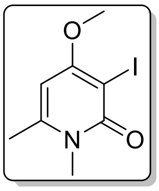


To a round bottomed flask containing 4-hydroxy-1-(4-methoxyphenyl)-6-methylpyridin-2(1*H*)-one **519e** (1.138 g, 4.92 mmol) and K_2CO_3 (0.816 g, 5.91 mmol) was added trimethylphosphate (2.40 mL, 20.4 mmol) and the resulting reaction mixture stirred at reflux for 2 h. On completion, the reaction was washed with water (15 mL) and extracted with ethyl acetate (3 × 25 mL). The combined organic extracts were washed with brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product as a pale yellow solid which was purified by column chromatography eluting with ethyl acetate to yield **520e** an off-white sold (0.528 g, 44%). m.p 171–173 °C.

IR (KBr) ν_{max}: 1652 (amide C=O stretch, s), 1560, 1511 (aromatic C=C stretch, s), 1244 (ether C-O stretch, s) cm^{-1}; ^{1}H NMR (300 MHz, (CDCl$_3$): δ 1.90 (3H, s, C***H₃***), 3.76 (3H, s, OC***H₃***), 3.82 (3H, s, OC***H₃*** phenyl ring), 5.81 (1H, s, CH$_3$CC***H***), 5.86 (1H, d, *J* = 2.6 Hz, C***H***C=O), 6.98 (2H, d, *J* = 8.9 Hz, 2 × C***H*** arom.), 7.08 (2H, d, *J* = 8.8 Hz, 2 × C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, (CDCl$_3$): δ 21.5 (***C***H$_3$), 55.3 (O***C***H$_3$), 55.5 (O***C***H$_3$ phenyl ring), 95.0 (***C***HC=O), 100.7 (CH$_3$C***C***H), 114.9 (2 × ***C***H arom.), 129.2 (2 × ***C***H arom.), 131.3 (q***C*** arom.), 146.5 (CH$_3$***C***CH), 159.5 (***C***OCH$_3$ phenyl ring), 165.8 (***C***=O), 167.8 (***C***OCH$_3$) ppm; HRMS (ESI) *m*/*z* calcd for $C_{14}H_{16}NO_3$ [(M + H)$^+$]: 246.1130, found 246.1126; Anal. calcd for $C_{14}H_{15}NO_3$: C, 68.56; H, 6.16; N, 5.71%. Found: C, 68.42; H, 6.15; N, 5.72%.

9.8 Iodination of 4-methoxypyridones

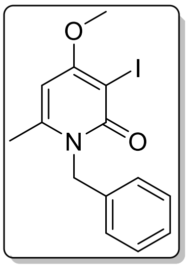
3-Iodo-4-methoxy-1,6-dimethylpyridin-2(1*H*)-one, 521a



To a stirred solution of 4-methoxy-1,6-dimethylpyridin-2(1*H*)-one **520a** (0.362 g, 2.36 mmol) in acetonitrile (15 mL) was added *N*-iodosuccinimide (1.603 g, 4.73 mmol). The reaction vessel was covered in aluminium foil and allowed stir at reflux for 12 h. On completion, the solvent was concentrated *in vacuo*, the resulting orange solid dissolved in DCM (20 mL) and washed with 5% aq. sodium thiosulfate (3 × 25 mL). The organic layer was dried over MgSO$_4$ and concentrated *in vacuo* to yield crude product which was purified by silica column chromatography eluting with 70:30 hexane:ethyl acetate to yield **521a** as a yellow solid (0.238 g, 36%). m.p. 191–193 °C.

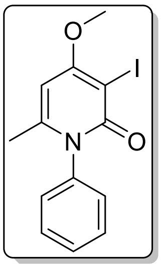
IR (KBr) ν_{max}: 1628 (amide C=O stretch, s), 1585 (aromatic C=C stretch, s), 1342 (ester C-O stretch, s) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.35 (3H, s, C***H₃***), 3.57 (3H, s, NC***H₃***), 3.88 (3H, s, OC***H₃***), 5.86 (1H, s, C***H***) ppm; ^{13}C NMR (75.5 MHz, CDCl$_3$): δ 21.4 (***C***H$_3$), 32.5 (N***C***H$_3$), 56.6 (O***C***H$_3$), 71.8 (***C***I), 94.7 (***C***H), 147.6 (CH$_3$***C***CH), 162.0 (***C***=O), 166.5 (***C***OCH$_3$), ppm; HRMS (ESI) *m*/*z* calcd for $C_8H_{11}NO_2I$ [(M + H)$^+$]: 279.9835, found 279.9827.

1-Benzyl-3-iodo-4-methoxy-6-methylpyridin-2(1*H*)-one, 521b



To a stirred solution of 1-benzyl-4-methoxy-6-methylpyridin-2(1*H*)-one **520b** (0.343 g, 1.50 mmol) in acetonitrile (20 mL) was added *N*-iodosuccinimide (1.009 g, 4.49 mmol). The reaction vessel was covered in aluminium foil and allowed stir at room temperature for 12 h. On completion, the solvent was concentrated *in vacuo*, the resulting orange solid dissolved in DCM (15 mL) and washed with 5% aq. sodium thiosulfate (3 × 20 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to give crude product which was purified using silica column chromatography eluting with 50:50 hexane:ethyl acetate to yield **521b** as a pale yellow solid (0.371 g, 70%). m.p. 146–148 °C. IR (KBr) ν_{max}: 2931, 2852 (alkyl C-H stretch, m), 1634 (C=O stretch, s), 1504 (aromatic C=C stretch, s), 1323 (ester C-O stretch, m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.30 (3H, s, C***H*₃**), 3.91 (3H, s, OC***H*₃**), 5.38 (2H, s, NC***H*₂**Ph), 5.88 (1H, s, C***H***), 7.14–7.16 (2H, m, 2 × C***H*** arom.), 7.23–7.32 (3H, m, 3 × C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 21.1 (***C***H_3), 48.7 (N***C***H_2Ph), 56.7 (O***C***H_3), 72.1 (***C***I), 95.3 (***C***H), 126.7, 127.5, 128.8 (5 × ***C***H arom.), 136.3 (NCH_2***C***), 147.9 (CH_3***C***CH), 162.2 (***C***=O), 166.7 (***C***OCH_3) ppm; HRMS (ESI) *m/z* calcd for $C_{14}H_{15}O_2NI$ [(M + H)$^+$]: 356.0148, found 356.0141.

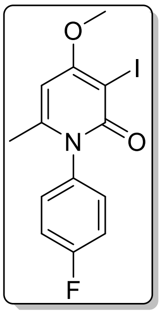
3-Iodo-4-methoxy-6-methyl-1-phenylpyridin-2(1*H*)-one, 521c



To a stirred solution of 4-methoxy-6-methyl-1-phenylpyridin-2(1*H*)-one **520c** (0.578 g, 2.69 mmol) in acetonitrile (25 mL) was added *N*-iodosuccinimide (1.208 g, 5.37 mmol). The reaction vessel was covered in aluminium foil and allowed stir at reflux for 12 h. On completion, the solvent was concentrated *in vacuo*, the resulting brown solid dissolved in DCM (20 mL) and washed with 5% aq. sodium thiosulfate (3 × 20 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to give crude product which was purified using silica column chromatography eluting with 50:50 hexane:ethyl acetate to yield **521c** as a pale brown solid (0.768 g, 84%). m.p. 222–224 °C.

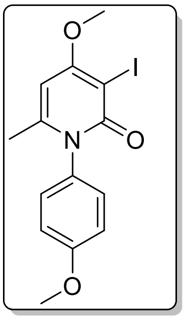
IR (KBr) ν_{max}: 1639 (amide C=O stretch, s), 1590, 1518 (aromatic C=C stretch, s), 1226 (ether C-O stretch, s) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.01 (3H, s, C***H*$_3$**), 3.97 (3H, s, OC***H*$_3$**), 5.99 (1H, s, C***H***), 7.16 (2H, d, *J* = 7.6 Hz, 2 × C***H*** arom.), 7.41 – 7.52 (3H, m, 3 × C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, CDCl$_3$): δ 22.0 (***C***H$_3$), 56.8 (O***C***H$_3$), 72.2 (***C***I), 94.7 (***C***H), 127.9, 128.9, 129.7 (5 × ***C***H arom.), 138.9 (N***C***), 147.6 (CH$_3$***C***CH), 162.2 (***C***=O), 167.2 (***C***OCH$_3$) ppm; HRMS (ESI) *m/z* calcd for $C_{13}H_{13}O_2NI$ [(M + H)$^+$]: 341.9991, found 341.9984; Anal. calcd for $C_{13}H_{12}O_2NI$: C, 45.77; H, 3.55; N, 4.11%. Found: C, 45.81; H, 3.57; N, 3.77%.

1-(4-Fluorophenyl)-3-iodo-4-methoxy-6-methylpyridin-2(1*H*)-one, 521d



To a stirred solution of 1-(4-fluorophenyl)-4-methoxy-6-methylpyridin-2(1*H*)-one **520d** (0.895 g, 3.84 mmol) in acetonitrile (25 mL) was added *N*-iodosuccinimide (1.727 g, 7.67 mmol). The reaction vessel was covered in aluminium foil and allowed stir at reflux for 12 h. On completion, the solvent was concentrated *in vacuo*, the resulting brown solid dissolved in DCM (20 mL) and washed with 5% aq. sodium thiosulfate (3 × 20 mL). The organic layer was dried over MgSO$_4$ and concentrated *in vacuo* to give crude product which was purified using silica column chromatography eluting with 50:50 hexane:ethyl acetate to yield **521d** as a pale brown solid (0.429 g, 31%). m.p. 196–198 °C. IR (KBr) ν_{max}: 1651 (amide C=O stretch, s), 1579, 1520 (aromatic C=C stretch, s), 1343 (ester C-O stretch, s), 1214 (ether C-O stretch, s) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.01 (3H, s, C***H*$_3$**), 3.97 (3H, s, OC***H*$_3$**), 6.00 (1H, s, C***H***), 7.15–7.19 (4H, m, 4 × C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, CDCl$_3$): δ 22.0 (***C***H$_3$), 56.8 (O***C***H$_3$), 72.1 (***C***I), 94.9 (***C***H), 116.7 (d, $^2J_{C\text{-}F}$ = 23.0 Hz, 2 × ***C***H arom.), 129.8 (d, $^3J_{C\text{-}F}$ = 8.7 Hz, 2 × ***C***H arom.), 134.7 (d, $^4J_{C\text{-}F}$ = 3.5 Hz, q***C*** arom.), 147.5 (CH$_3$***C***CH), 161.5 (d, $^1J_{C\text{-}F}$ = 248.8 Hz, ***C***F), 162.2 (***C***=O), 167.3 (***C***OCH$_3$) ppm; ^{19}F NMR (282 MHz, CDCl$_3$): δ -112.2 (C***F***) ppm; HRMS (ESI) *m/z* calcd for $C_{13}H_{12}O_2NFI$ [(M + H)$^+$]: 359.9897, found 359.9894.

1-(4-Methoxyphenyl)-3-iodo-4-methoxy-6-methylpyridin-2(1*H*)-one, 521e

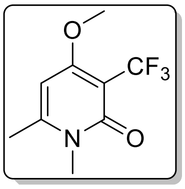


To a stirred solution of 4-methoxy-1-(4-methoxyphenyl)-6-methylpyridin-2(1*H*)-one **520e** (0.475 g, 1.94 mmol) in acetonitrile (25 mL) was added *N*-iodosuccinimide (0.872 g, 3.88 mmol). The reaction vessel was covered in aluminium foil and allowed stir at reflux for 12 h. On completion, the solvent was concentrated *in vacuo*, the resulting brown solid dissolved in DCM (20 mL) and washed with 5% aq. sodium thiosulfate (3 × 20 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to give crude product which was purified using silica column chromatography eluting with 50:50 hexane:ethyl acetate to yield **521e** as a yellow solid (0.090 g, 13%). m.p. 190–193 °C.

IR (KBr) ν_{max}: 1651 (amide C=O stretch, s), 1511 (aromatic C=C stretch, s), 1343 (ether C-O stretch, s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.02 (3H, s, C***H***$_3$), 3.84 (3H, s, OC***H***$_3$ phenyl ring), 3.96 (3H, s, OC***H***$_3$), 5.96 (1H, s, C***H***), 6.98 (2H, d, *J* = 8.6 Hz, 2 × C***H*** arom.), 7.07 (2H, d, *J* = 8.6 Hz, 2 × C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 22.0 (***C***H$_3$), 55.5 (O***C***H$_3$ phenyl ring), 56.7 (O***C***H$_3$), 72.2 (***C***I), 94.6 (***C***H), 114.9 (2 × ***C***H arom.), 128.9 (2 × ***C***H arom.), 131.5 (q***C***N arom.), 148.1 (CH$_3$***C***CH), 159.6 (***C***OCH$_3$ phenyl ring), 162.4 (***C***=O), 167.2 (***C***OCH$_3$); HRMS (ESI) *m/z* calcd for $C_{14}H_{15}O_3NI$ [(M + H)$^+$]: 372.0097, found 372.0099.

9.9 Trifluoromethylation of 3-iodo-4-methoxypyridones

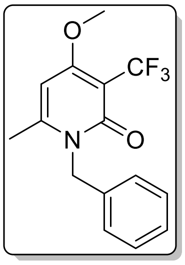
4-Methoxy-1,6-dimethyl-3-(trifluoromethyl)pyridin-2(1*H*)-one, 522a



To a stirred solution of 3-iodo4-methoxy-1,6-dimethylpyridin-2(1*H*)-one **521a** (0.235 g, 0.84 mmol) and copper (I) iodide (0.192 g, 1.01 mmol) in DMF (4 mL) was added methyl 2,2-difluoro-2-(fluorosulfonyl)acetate (0.13 mL, 1.01 mmol). The reaction mixture was allowed stir at 70 °C for 7 h. On completion, the reaction was cooled to room temperature, diluted with diethyl ether (20 mL) and filtered. The solution was poured into water (20 mL), extracted with diethyl ether (4 × 20 mL) and the combined organic extracts washed with water (3 × 5 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product which was purified using silica column chromatography eluting with 70:30 hexane:ethyl acetate to yield **522a** as a white solid (0.114 g, 61%). m.p. 136–138 °C.

IR (KBr) ν_{max}: 1651 (amide C=O stretch, s), 1598, 1563 (aromatic C=C stretch, s), 1130 (ester C-O stretch, s) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.40 (3H, s, C***H*$_3$**), 3.47 (3H, s, NC***H*$_3$**), 3.89 (3H, s, OC***H*$_3$**), 5.92 (1H, s, C***H***) ppm; ^{13}C NMR (75 MHz, CDCl$_3$): δ 21.8 (***C***H$_3$), 30.9 (N***C***H$_3$), 56.3 (O***C***H$_3$), 94.1 (***C***H), 99.9 (q, $^2J_{C\text{-}F}$ = 29.2 Hz, ***C***CF$_3$), 124.0 (q, $^1J_{C\text{-}F}$ = 272.8 Hz, ***C***F$_3$), 151.4 (CH$_3$***C***CH), 160.7 (***C***=O), 166.4 (***C***OCH$_3$) ppm; ^{19}F NMR (282 MHz, CDCl$_3$): δ -57.4 (C***F*$_3$**) ppm; HRMS (ESI) *m/z* calcd for $C_9H_{11}F_3NO_2$ [(M + H)$^+$]: 222.0742, found 222.0736; Anal. calcd for $C_9H_{10}F_3NO_2$: C, 48.87; H, 4.56; N, 6.33%. Found: C, 49.09; H, 4.43; N, 6.19%.

1-Benzyl-4-methoxy-6-methyl-3-(trifluoromethyl)pyridin-2(1*H*)-one, 522b

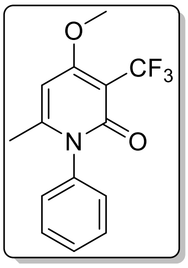


To a stirred solution of 1-benzyl-3-iodo-4-methoxy-6-methylpyridin-2(1*H*)-one **521b** (0.122 g, 0.34 mmol) and copper (I) iodide (0.079 g, 0.41 mmol) in DMF (4 mL) was added methyl 2,2-difluoro-2-(fluorosulfonyl)acetate (0.10 mL, 0.75 mmol). The reaction mixture was allowed stir at 70 °C for 24 h. On completion, the reaction was cooled to room temperature, diluted with diethyl ether (20 mL) and filtered. The solution was poured into water (20 mL), extracted with diethyl ether (4 × 20 mL) and the combined organic extracts washed with water (3 × 5 mL). The organic layer was dried over MgSO$_4$ and concentrated *in vacuo* to yield crude product as a white solid which was purified by silica column chromatography eluting with 50:50 hexane:ethyl acetate to yield **522b** as a pale yellow solid (0.064 g, 62%). m.p. 132–133 °C.

IR (KBr) ν_{max}: 1656 (amide C=O stretch, s), 1557, 1396 (aromatic C=C stretch, s), 1108 (ester C-O stretch, m) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.33 (3H, s, C***H*$_3$**), 3.91 (3H, s, OC***H*$_3$**), 5.30 (2H, s, NC***H*$_2$**Ph), 5.90 (1H, s, C***H***), 7.15–7.18 (2H, m, 2 × C***H*** arom.), 7.25–7.34 (3H, m, 3 × C***H*** arom.) ppm; ^{13}C NMR (100 MHz, CDCl$_3$): δ 21.5 (***C***H$_3$), 47.0 (N***C***H$_2$Ph), 56.3 (O***C***H$_3$), 94.5 (***C***H), 100.4 (q, $^2J_{C\text{-}F}$ = 29.4 Hz, ***C***CF$_3$), 123.9 (q, $^1J_{C\text{-}F}$ = 272.9 Hz, ***C***F$_3$), 126.6, 127.6, 128.9 (5 × ***C***H aromatic), 136.0 (NCH$_2$***C***), 151.6 (***C***CH$_3$), 160.7 (***C***=O), 166.6 (***C***OCH$_3$) ppm;

^{19}F NMR (282 MHz, CDCl$_3$): δ -57.2 (C***F*₃**) ppm; HRMS (ESI) *m/z* calcd for $C_{15}H_{15}NO_2F_3$ [(M + H)$^+$]: 298.1055, found 298.1047.

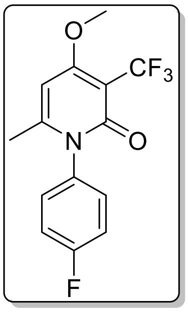
4-Methoxy-6-methyl-1-phenyl-3-(trifluoromethyl)pyridin-2(1*H*)-one, 522c



To a stirred solution of 3-iodo-4-methoxy-6-methyl-1-phenylpyridin-2(1*H*)-one **521c** (0.356 g, 1.04 mmol) and copper (I) iodide (0.239 g, 1.25 mmol) in DMF (4 mL) was added methyl 2,2-difluoro-2-(fluorosulfonyl)acetate (0.26 mL, 2.03 mmol). The reaction mixture was allowed stir at 70 °C for 24 h. On completion, the reaction was cooled to room temperature, diluted with diethyl ether (20 mL) and filtered. The solution was poured into water (20 mL), extracted with diethyl ether (4 × 20 mL) and the combined organic extracts washed with water (3 × 5 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product as a white solid which was purified by silica column chromatography eluting with 80:20 hexane:ethyl acetate to yield **522c** as a pale yellow solid (0.086 g, 29%). m.p. 205–207 °C.

IR (KBr) ν_{max}: 1658 (amide C=O stretch, s), 1551 (aromatic C=C stretch, s), 1392 (ether C-O stretch, s) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.03 (3H, s, C***H*₃**), 3.96 (3H, s, OC***H*₃**), 6.01 (1H, s, C***H***), 7.16–7.18 (2H, m, 2 × C***H*** arom.), 7.44–7.53 (3H, m, 3 × C***H*** arom.) ppm; ^{13}C NMR (75 MHz, CDCl$_3$): δ 22.4 (***C***H₃), 56.5 (O***C***H₃), 94.1 (***C***H), 100.5 (q, $^2J_{C\text{-}F}$ = 28.3 Hz, ***C***CF₃), 123.8 (q, $^1J_{C\text{-}F}$ = 273.0 Hz, ***C***F₃), 128.0, 129.1, 129.8 (5 × ***C***H arom.), 137.8 (N***C***), 151.5 (CH₃***C***CH), 160.9 (***C***=O), 167.3 (***C***OCH₃) ppm; ^{19}F NMR (282 MHz, CDCl$_3$): δ -57.2 (C***F*₃**) ppm; HRMS (ESI) *m/z* calcd for $C_{14}H_{13}NO_2F_3$ [(M + H)$^+$]: 284.0898, found 284.0893.

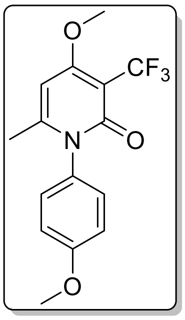
1-(4-Fluorophenyl)-4-methoxy-6-methyl-3-(trifluoromethyl)pyridin-2(1*H*)-one, 522d



To a stirred solution of 1-(4-fluorophenyl)-3-iodo-4-methoxy-6-methylpyridin-2(1*H*)-one **521d** (0.245 g, 0.68 mmol) and copper (I) iodide (0.156 g, 0.82 mmol) in DMF (4 mL) was added methyl 2,2-difluoro-2-(fluorosulfonyl)acetate (0.19 mL, 1.5 mmol). The reaction mixture was allowed stir at 70 °C for 24 h. On completion, the reaction was cooled to room temperature, diluted with diethyl ether (20 mL) and filtered. The solution was poured into water (20 mL), extracted with diethyl ether (4 × 20 mL) and the combined organic extracts washed with water (3 × 5 mL). The organic layer was dried over MgSO$_4$ and concentrated *in vacuo* to yield crude product as a pale yellow solid which was purified by silica column chromatography eluting with 80:20 hexane:ethyl acetate to yield **522d** as a white solid (0.107 g, 52%). m.p. 185–188 °C.

IR (KBr) ν_{max}: 1664 (amide C=O stretch, s), 1555, 1509, 1464 (aromatic C=C stretch, s), 1261 (ether C-O stretch, s) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.03 (3H, s, C***H***$_3$), 3.95 (3H, s, OC***H***$_3$), 6.03 (1H, s, C***H***), 7.12–7.21 (4H, m, 4 × C***H*** arom.) ppm; ^{13}C NMR (75 MHz, CDCl$_3$): δ 22.3 (***C***H$_3$), 56.6 (O***C***H$_3$), 94.4 (***C***H), 100.5 (q, $^2J_{\text{C-F}}$ = 29.5 Hz, ***C***CF$_3$), 116.8 (d, $^2J_{\text{C-F}}$ = 23.0 Hz, 2 × ***C***H arom.), 123.7 (q, $^1J_{\text{C-F}}$ = 273.1 Hz, ***C***F$_3$), 129.9 (d, $^3J_{\text{C-F}}$ = 8.8 Hz, 2 × ***C***H arom.), 133.6 (d, $^4J_{\text{C-F}}$ = 3.5 Hz, q***C*** arom.), 151.4 (CH$_3$***C***CH), 160.8 (***C***=O), 162.6 (d, $^1J_{\text{C-F}}$ = 248.8 Hz, F***C***CH), 167.4 (***C***OCH$_3$) ppm; ^{19}F NMR (282 MHz, CDCl$_3$): δ -112.0 (C***F***), -57.2 (C***F***$_3$) ppm; HRMS (ESI) *m/z* calcd for $C_{14}H_{12}NO_2F_4$ [(M + H)$^+$]: 302.0804, found 302.0796.

4-Methoxy-1-(4-methoxyphenyl)-6-methyl-3-(trifluoromethyl)pyridin-2(1*H*)-one, 522e

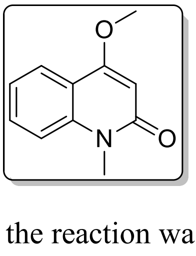


To a stirred solution of 1-(4-methoxyphenyl)-3-iodo-4-methoxy-6-methylpyridin-2(1*H*)-one **521e** (0.202 g, 0.54 mmol) and copper (I) iodide (0.124 g, 0.65 mmol) in DMF (4 mL) was added methyl 2,2-difluoro-2-(fluorosulfonyl)acetate (0.14 mL, 1.09 mmol). The reaction mixture was allowed stir at 70 °C for 24 h. On completion, the reaction was cooled to room temperature, diluted with diethyl ether (20 mL) and filtered. The solution was poured into water (20 mL), extracted with diethyl ether (4 × 20 mL) and the combined organic extracts washed with water (3 × 5 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product as a white solid which was purified by silica column chromatography eluting with 80:20 hexane:ethyl acetate to yield **522e** as a white solid (0.077 g, 46%). m.p. 153–156 °C.

IR (KBr) ν_{max}: 1662 (amide C=O stretch, s), 1552, 1514 (aromatic C=C stretch, s), 1251 (ether C-O stretch, s), 1108 (ester C-O stretch, s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.04 (3H, s, C***H₃***), 3.84 (3H, s, OC***H₃*** phenyl) 3.95 (3H, s, OC***H₃***), 5.99 (1H, s, C***H***), 6.98 (2H, d, *J* = 9.0 Hz, 2 × C***H*** arom.), 7.07 (2H, d, *J* = 9.0 Hz, 2 × C***H*** arom.) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.4 (***C***H₃), 55.5 (O***C***H₃ phenyl) 56.6 (O***C***H₃), 93.9 (***C***H), 100.6 (q, $^2J_{C\text{-}F}$ = 29.3 Hz, ***C***CF₃), 115.0 (2 × ***C***H arom.) 123.8 (q, $^1J_{C\text{-}F}$ = 273.1 Hz, ***C***F₃), 129.0 (2 × ***C***H arom.), 130.3 (q***C***N arom.), 151.9 (CH₃***C***CH), 159.8 (***C***OCH₃ phenyl), 161.0 (***C***=O), 167.2 (***C***OCH₃) ppm; ^{19}F NMR (282 MHz, $CDCl_3$): δ -57.3 (C***F₃***) ppm; HRMS (ESI) *m/z* calcd for $C_{15}H_{15}NO_3F_3$ [$(M + H)^+$]: 314.1004, found 314.0997.

9.10 Synthesis of trifluoromethylated quinolone

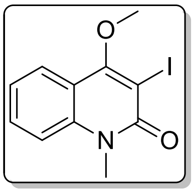
4-Methoxy-1-methylquinolin-2(1*H*)-one, 524



To a round bottomed flask containing 4-hydroxyquinolin-2(1*H*)-one **523** (1.085 g, 6.19 mmol) and K_2CO_3 (1.027 g, 7.43 mmol) was added trimethylphosphate (2.36 mL, 20.1 mmol) and the resulting reaction mixture stirred at reflux for 3 h. On completion, the reaction was washed with water (15 mL) and extracted with ethyl acetate (3 ×

25 mL). The combined organic extracts were washed with brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product as a pale yellow solid which was purified by recrystallisation from water to yield **524** an orange solid (0.953 g, 81%). m.p. 90–92 °C [lit.[20] 101–102 °C]. Spectral characteristics were consistent with previously reported data.[21] ^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ 3.56 (3H, s, NC***H₃***), 3.94 (3H, s, OC***H₃***), 6.04 (1H, s, C***H***), 7.26 (1H, t, *J* = 7.5 Hz, C***H*** arom.) 7.49–7.52 (1H, m, C***H*** arom.), 7.65 (1H, t, *J* = 7.8 Hz, C***H*** arom.), 7.88 (1H, dd, *J* = 1.5, 8.0 Hz, C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$): δ 29.1 (N***C***H_3), 56.6 (O***C***H_3), 96.8 (***C***H),s 115.1 (***C***H arom.), 116.0 (***C***$COCH_3$), 122.0 (***C***H arom.), 123.1 (***C***H arom.), 131.9 (***C***H arom.), 139.9 (***C***NCH_3), 162.3 (***C***OCH_3), 162.8 (***C***=O) ppm; HRMS (ESI) *m*/*z* calcd for $C_{11}H_{12}NO_2$ [$(M + H)^+$]: 190.0868, found 190.0864.

3-Iodo-4-methoxy-1-methylquinolin-2(1*H*)-one, 525

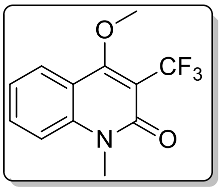


To a stirred solution of 4-methoxy-1-methylquinolin-2(1*H*)-one **524** (0.900 g, 4.76 mmol) in acetonitrile (15 mL) was added *N*-iodosuccinimide (1.605 g, 7.13 mmol) and trifluoroacetic acid (0.37 mL, 4.76 mmol). The reaction vessel was covered in aluminium foil and allowed stir at room temperature for 60 h. On completion, the solvent was concentrated *in vacuo*, the resulting orange solid dissolved in DCM (15 mL) and washed with 5% aq. sodium thiosulfate (3 × 20 mL) and 10% NaOH (3 × 20 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product which was purified by silica column chromatography eluting with 90:10 hexane:ethyl acetate to yield **525** as a yellow solid (0.485 g, 32%). m.p. 115–117 °C.

IR (KBr) ν_{max}: 1636 (amide C=O stretch, s), 1610, 1591 (aromatic C=C stretch, s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.81 (3H, s, NC***H₃***), 4.03 (3H, s, OC***H₃***), 7.25–7.31 (1H, m, C***H*** arom.), 7.41 (1H, d, *J* = 1.6 Hz, C***H*** arom.), 7.61–7.67 (1H, m, C***H*** arom.), 7.89 (1H, ddd, *J* = 8.0, 1.5, 0.4 Hz, C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 31.4 (N***C***H_3), 61.3 (O***C***H_3), 87.5 (***C***I), 114.5 (***C***H arom.), 117.5 (CH***C***$COCH_3$), 122.4 (***C***H arom.), 123.8 (***C***H arom.), 131.7 (***C***H arom.), 139.9 (CH***C***NCH_3), 160.8 (***C***=O), 166.3 (***C***OCH_3) ppm; HRMS (ESI) *m*/*z* calcd for

$C_{11}H_{11}NO_2I$ [(M + H)$^+$]: 315.9835, found 315.9828; Anal. calcd for $C_{11}H_{10}NO_2I$: C, 41.93; H, 3.20; N, 4.45%. Found: C, 41.61; H, 3.09; N, 4.08%.

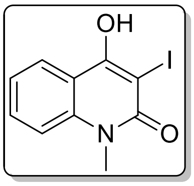
4-Methoxy-1-methyl-3-(trifluoromethyl)quinolin-2(1*H*)-one, 526



To a stirred solution of 3-Iodo-4-methoxy-1-methylquinolin-2(1*H*)-one **525** (0.283 g, 0.90 mmol) and copper (I) iodide (0.205 g, 1.08 mmol) in DMF (4 mL) was added methyl 2,2-difluoro-2-(fluorosulfonyl)acetate (0.14 mL, 1.08 mmol). The reaction mixture was allowed stir at 70 °C for 12 h. On completion, the reaction was cooled to room temperature, diluted with diethyl ether (20 mL) and filtered. The solution was poured into water (20 mL), extracted with diethyl ether (4 × 20 mL) and the combined organic extracts washed with water (3 × 5 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product which was purified using silica column chromatography eluting with 70:30 hexane:ethyl acetate to yield **526** as a white solid (0.120 g, 52%). m.p. 100–102 °C.

IR (KBr) ν_{max}: 1652 (amide C=O stretch, s), 1118 (ester C-O stretch, m) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.71 (3H, s, NC***H*₃**), 4.05 (3H, s, OC***H*₃**), 7.32 (1H, t, *J* = 7.6 Hz, C***H*** arom.), 7.40 (1H, d, *J* = 8.5 Hz, C***H*** arom.), 7.70 (1H, t, *J* = 7.9 Hz, C***H*** arom.), 7.96 (1H, dd, *J* = 1.4, 8.1 Hz, C***H*** arom.) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 29.6 (N***C***H₃), 64.3 (q, $^5J_{C\text{-}F}$ = 2.5 Hz, O***C***H₃), 111.0 (q, $^2J_{C\text{-}F}$ = 29.0 Hz, ***C***CF₃), 114.4 (***C***H arom.), 116.7 (***C***COCH₃), 122.6 (***C***H arom.), 122.7 (q, $^1J_{C\text{-}F}$ = 273.7 Hz, ***C***F₃), 125.2 (***C***H arom.), 133.5 (***C***H arom.), 140.8 (***C***NCH₃), 159.6 (***C***=O), 165.6 (q, $^3J_{C\text{-}F}$ = 1.3 Hz, ***C***OCH₃) ppm; ^{19}F NMR (282 MHz, $CDCl_3$): δ -59.7 (C***F*₃**) ppm; HRMS (ESI) *m/z* calcd for $C_{12}H_{11}F_3NO_2$ [(M + H)$^+$]: 258.0742, found 258.0731; Anal. calcd for $C_{12}H_{10}F_3NO_2$: C, 56.04; H, 3.92; N, 5.45%. Found: C, 55.82; H, 3.85; N, 5.12%.

4-Hydroxy-3-iodo-1-methylquinolin-2(1*H*)-one, 527



To a stirred solution of 4-hydroxy-1-methylquinolin-2(1*H*)-one **523** (0.621 g, 3.54 mmol), potassium iodide (0.395 g, 2.38 mmol) and potassium iodate (0.250 g, 1.17 mmol) in methanol (4 mL) and water (20 mL) was added 1M HCl (3.66 mL) dropwise over 45 min. The resulting reaction mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with water (50 mL) and extracted with DCM (3 × 25 mL). Combined organic extracts were washed with 5% aq. sodium thiosulfate (25 mL), water (25 mL) and brine (25 mL), dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product **527** as a yellow solid (0.633 g, 59%) with no purification required. m.p. 172–174 °C [lit.[22] 171–173 °C].

Spectral characteristics were consistent with previously reported data.[22]

^{1}H NMR (300 MHz, $CDCl_3$): δ 3.79 (3H, s, C***H₃***), 6.25 (1H, bs, O***H***), 7.24–7.29 (1H, m, C***H*** arom.), 7.38 (1H, d, *J* = 8.5 Hz, C***H*** arom.), 7.62–7.68 (1H, m, C***H*** arom.), 8.05 (1H, dd, *J* = 0.4, 8.0 Hz, C***H*** arom.) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 31.1 (***C***H_3), 77.8 (***C***I), 114.0 (CH***C***COH), 114.2, 122.2, 124.2, 132.1 (4 × ***C***H arom.), 139.3 (CH***C***NCH_3), 159.9 (***C***=O), 160.5 (***C***OH) ppm; MS (ESI) *m*/*z*: 302 [$(M + H)^+$, 100%].

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Appendix III

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Access to trifluoromethylated 4-alkoxy-2-pyrones, pyridones and quinolones

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ABSTRACT

2-Pyrones, pyridones and quinolones represent classes of molecules with remarkable and diverse biological activity. The introduction of a trifluoromethyl group to a molecule can have dramatic and beneficial effects in terms of lipophilicity and bioavailability. Herein we report a route to afford a library of novel trifluoromethylated 2-pyrones, 2-pyridones and a 2-quinolone in moderate to good yield using methyl fluorosulfonyldifluoroacetate as the trifluoromethylating agent.

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1. Introduction

2-Pyrones are a privileged biological scaffold with broad spectrum biological activity spanning cytotoxic, antibiotic and antifungal activity.[1–3] Prominent examples of biologically active 2-pyrones include the bufadienolide class, which have been shown to have diverse biological effects,[1] including causing cardiac poisoning in animals and showing inhibitory activity towards leukaemia cell lines. Numerous protocols have been designed for the synthesis and decoration of 2-pyrones.[4–9] More specifically, functionalisation of the 3-position of 4-alkoxy-2-pyrones has been accomplished under Suzuki–Miyaura[10–12] and Sonogashira[13] conditions. The 2-pyrone moiety is also present in bioactive polyketides such as Nigerapyrone E **1** (Fig. 1),[14] which possesses a methyl group at C-3. A methyl group is also present at C-3 in the 2-pyridone **2**, a potential therapeutic agent for treatment of central nervous system disorders associated with phosphodiesterase 2 (PDE2).[15] The trifluoromethyl (CF_3) moiety is present at the C-3 position of fused bicyclic heterocycles **3** and **4**, which exhibit fungicidal activity.[16] In the cases shown, the CF_3 groups were present prior to cyclisation and ring formation.

In vivo metabolism of drugs by cytochrome P450 oxidases can be problematic as it increases the rate of drug excretion from the body.[17] A common strategy to protect against in vivo metabolism is to incorporate the CF_3 moiety into drug candidate molecules.[18] When incorporated into small molecules, the CF_3 group can often enhance drug efficacy by promoting electrostatic interactions with targets, improving cellular permeability and increasing robustness towards oxidative metabolism of the drug.[19–21] Recent advances in catalysis have made the incorporation of −F and −CF_3 groups into complex organic molecules more manageable, but selective, general and practical fluorination and trifluoromethylation reactions remain elusive.[22] Herein we describe a protocol to access trifluoromethylated 4-alkoxy-2-pyrones, pyridones and quinolones.

2. Results and discussion

Initial attempts to gain access to trifluoromethylated 2-pyrones focused on the direct functionalisation of 4-methoxy-6-methyl-2-pyrone[23] **5** using a photoredox catalyst as described by MacMillan[24] (Scheme 1). However despite numerous attempts using both the iridium and ruthenium catalysts described, trifluoromethylation was unsuccessful in our hands. Attempts using a number of pyridone and quinolone substrates also proved fruitless. This led us to focus on the use of pre-functionalised starting materials. Thus pyrone **5** was iodinated in a regioselective manner using *N*-iodosuccinimide in acetonitrile yielding **6** in 95% yield. However, exposure to a trifluoromethylation catalyst, recently described by Hartwig[25] (Scheme 1) gave complex mixtures in all cases.

We then attempted the same trifluoromethylation reaction using methyl fluorosulfonyldifluoroacetate (MFSDA).[26] Since its

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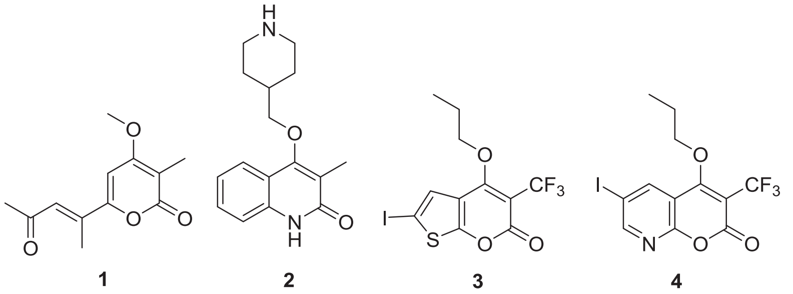
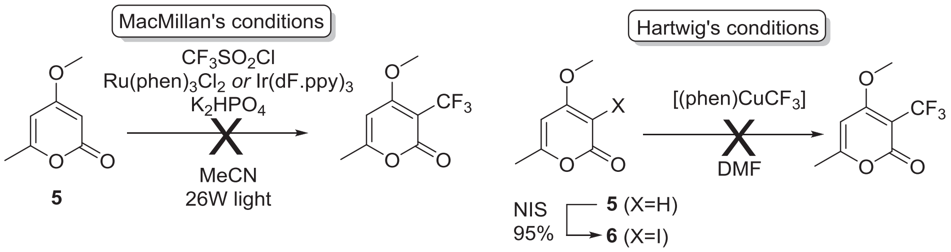


Fig. 1. Examples of biologically active compounds containing CH_3 and CF_3 functionalised, pyrone-derived moieties.



Scheme 1. Initial trifluoromethylation attempts.

introduction, MFSDA has been used as a convenient source of '$CuCF_3$' and has proved useful in the trifluoromethylation of subporphyrins,[27] pyrazoles,[28] purine nucleosides,[29,30] oxazolyl intermediates,[31] pyrazolopyridines,[32] and others.[33,34] The accepted mechanism comprises an initial step involving the formation of a copper salt with the elimination of methyl halide.[26] The salt then decomposes to release difluorocarbene and a fluoride ion, which are in equilibrium with a DMF stabilised trifluoromethyl anion. In the presence of CuI, the equilibrium shifts to form $[CF_3CuI^-]$, which reacts with a halogenated starting material, RX, to provide the trifluoromethylated product, RCF_3, following release of CuX and I^-. A radical mechanism has been ruled out on the basis of experimental and computation data.[35] Thus iodinated methoxypyrone **6** was treated with MFSDA in the presence of copper (I) iodide in a number of anhydrous solvents (Table 1). When the reaction was carried out in THF and DMSO (Table 1, entries 1 & 2), no product was observed. However a modest yield (53%) of trifluoromethylated product **7** was achieved using NMP as solvent (Table 1, entry 3). Changing the solvent to DMF or DMA resulted in an increased yield of 77 and 79% respectively (Table 1, entries 6 & 7). Higher equivalents of MFSDA caused a slight decrease in yield (Table 1, entry 5). Gratifyingly, the reaction can be successfully carried out in bulk DMF, albeit with a slightly reduced yield of 63% (Table 1, entry 4).

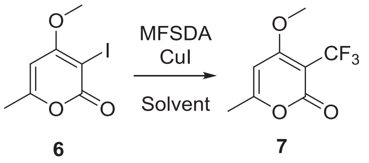
With workable conditions in hand, the scope of the reaction was investigated. Starting with 4-hydroxy-6-methyl-2-pyrone, a number of alkoxy groups were introduced using a literature procedure[11] to afford pyrones **8**–**10**, whilst **11** was prepare by heating 4-hydroxy-6-methyl-2-pyrone with phenol and K_2CO_3 in acetone. Pyrones **8**–**11** were then iodinated to give **12**–**15**. Finally the established protocol was applied and trifluoromethylated products **16**–**19** were obtained in moderate yield (Table 2, entries 1–4).

We then decided to test the optimised conditions using a bromo analogue. Bromination of **5** (again in a regioselective manner) was carried out using *N*-bromosuccinimide to afford **20** in 45% yield. Trifluoromethylation was carried out as per the procedure used for the iodo starting material, however **7** was obtained in only 10% yield. In the formation of **20**, the dibrominated compound **21** was also observed, and isolated in 13% yield. Dibromo **21** was subjected to our conditions to investigate the regioselectivity of the protocol. When 1 equiv MFSDA was employed, trifluoromethylation occurred at the sp^3 hybridised carbon affording **22** in 30% yield and no product resulting from trifluoromethylation at the 3-position of the pyrone was isolated (Scheme 2).

We next sought to extrapolate the methodology to 2-pyridones, which were prepared by heating the corresponding pyrone with the desired amine in water[36] to provide **23**–**25**. The resulting 2-pyridones were methylated to afford **26**–**28**, iodinated with *N*-iodosuccinimide in acetonitrile to afford **29**–**31** and finally trifluoromethylated to provide **32**–**34** in moderate yield (Table 3, entries 1–3). Both an electron withdrawing group (4-F–C_6H_4–) and an electron releasing group (4-MeO–C_6H_4–) on the nitrogen were tolerated.

The reaction conditions are also suitable for the trifluoromethylation of quinolones (Scheme 3). Commercially available **35**, was methylated to afford **36**, iodinated to provide **37** and trifluoromethylated, affording **38** in good yield.

Table 1
Optimisation studies

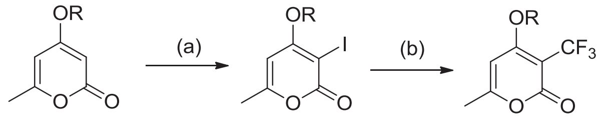


Entry	Solvent	MFSDA (equiv.)	Yield 7 (%)[a]
1	THF	1.2	No reaction
2	DMSO	1.2	No reaction
3	NMP	1.2	53
4	DMF[b]	1.2	63
5	DMF	5	66
6	DMF	1.2	77
7	DMA	1.2	79

[a] Isolated.

[b] Bulk DMF was employed as solvent.

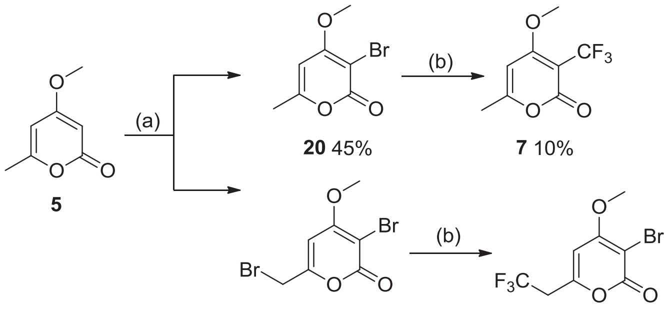
Table 2
Pyrone reaction scope. Conditions: (a) NIS, MeCN; (b) MFSDA, CuI, DMF



8 R = CH_2Ph **12** R = CH_2Ph **16** R = CH_2Ph
9 R = CH_2CH_3 **13** R = CH_2CH_3 **17** R = CH_2CH_3
10 R = $CH_2CH_2CH_3$ **14** R = $CH_2CH_2CH_3$ **18** R = $CH_2CH_2CH_3$
11 R = Ph **15** R = Ph **19** R = Ph

Entry	R	Yield (%)[a] Step (a)	Yield (%)[a] Step (b)
1	CH_2Ph	84	30
2	CH_2CH_3	75	34
3	$CH_2CH_2CH_3$	96	46
4	Ph	96	43

[a] Isolated.



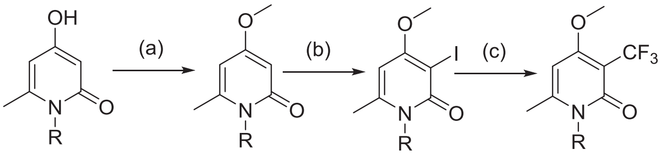
5

20 45% **7** 10%

21 13% **22** 30%

Scheme 2. Trifluoromethylation of bromo compounds (a) NBS, MeCN; (b) MFSDA, CuI, DMF.

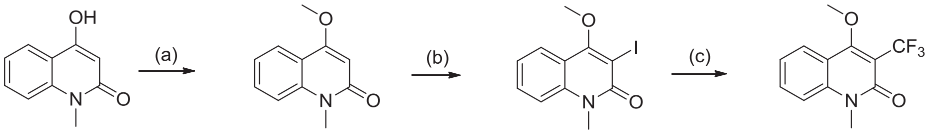
Table 3
Pyridone reaction scope. Conditions: (a) K_2CO_3, $(CH_3)_3PO_4$; (b) NIS, MeCN; (c) MFSDA, CuI, DMF



23 R = CH_2Ph **26** R = CH_2Ph **29** R = CH_2Ph **32** R = CH_2Ph
24 R = C_6H_4(*p*-F) **27** R = C_6H_4(*p*-F) **30** R = C_6H_4(*p*-F) **33** R = C_6H_4(*p*-F)
25 R = C_6H_4(*p*-OMe) **28** R = C_6H_4(*p*-OMe) **31** R = C_6H_4(*p*-OMe) **34** R = C_6H_4(*p*-OMe)

Entry	R	Yield (%)[a] Step (a)	Yield (%)[a] Step (b)	Yield (%)[a] Step (c)
1	CH_2Ph	57	70	62
2	C_6H_4(*p*-F)	60	31	52
3	C_6H_4(*p*-OMe)	44	13	46

[a] Isolated.



35 **36** 81% **37** 32% **38** 52%

Scheme 3. (a) $(CH_3)_3PO_4$, K_2CO_3; (b) NIS, MeCN, trifluoroacetic acid; (c) MFSDA, CuI, DMF.

3. Conclusion

A method for the formation of trifluoromethylated 2-pyrones, pyridones and quinolones has been established. The remarkable biological activity of these compound classes, coupled with the growing significance attributed to the introduction of the $-CF_3$ group establishes the described protocol as a useful route to these compounds. Efforts are currently underway to expand the substrate scope and to apply the methodology to natural product syntheses and in the formation of biologically active compounds such as those depicted in Fig. 1.

4. Experimental

4.1. General experimental

Solvents and reagents were used as obtained from commercial sources and without purification. Wet flash column chromatography was carried out using Kieselgel silica gel 60, 0.040–0.063 mm (Merck). Thin layer chromatography (TLC) was carried out on precoated silica gel plates (Merck 60 PF254). Visualisation was achieved by UV and potassium permanganate staining. Melting points were carried out on a uni-melt Thomas Hoover Capillary melting point apparatus. Infrared (IR) spectra were recorded on Perkin–Elmer FTIR Paragon 1000 spectrophotometer. Liquid samples were examined as thin films interspersed on sodium chloride plates. Solid samples were dispersed in potassium bromide (KBr) and recorded as pressed discs. NMR spectra were run in $CDCl_3$ using tetramethylsilane (TMS) as the internal standard at 20 °C unless otherwise specified. ^1H NMR (400 MHz) spectra and ^1H NMR (300 MHz) spectra were recorded on Bruker Avance 400 and Bruker Avance 300 NMR spectrometers respectively in proton coupled mode. ^{19}F NMR (282 MHz) spectra and ^{19}F NMR (470 MHz) were recorded on Bruker Avance 300 NMR and Bruker Avance 600 NMR spectrometers respectively in proton decoupled mode. ^{13}C NMR (125 MHz) spectra and ^{13}C NMR (75.5 MHz) spectra were recorded on Bruker Avance 500 and Bruker Avance 300 NMR spectrometers respectively in proton decoupled mode. All spectra were recorded at University College Cork. Chemical shifts δ_H and δ_C are expressed as parts per million (ppm), positive shift being downfield from TMS; coupling constants (*J*) are expressed in hertz (Hz). Low resolution mass spectra (LRMS) were recorded on a Waters Quattro Micro triple quadrupole instrument in electrospray ionisation (ESI) mode using 50% acetonitrile–water containing 0.1% formic acid as eluent; samples were made up in acetonitrile or methanol. High resolution precise mass spectra (HRMS) were recorded on a Waters LCT Premier Tof LC-MS instrument in electrospray ionisation (ESI) mode using 50% acetonitrile–water containing 0.1% formic acid as eluent; samples were made up in acetonitrile or methanol. The Microanalysis Laboratory, National University of Ireland, Cork, performed elemental analysis using a Perkin–Elmer 240 and Exeter Analytical CE440 elemental analysers.

4.2. General procedure for *O*-functionalisation

To a stirred suspension of 4-hydroxy-6-methyl-2-pyone (1.0 equiv) in CH_2Cl_2 (15 mL) was added triethylamine (1.1 equiv) dropwise. The mixture was allowed to stir at 0 °C for 10 min. Benzylbromide (1.1 equiv) was then added dropwise. The resulting mixture was stirred at room temperature for 12 h H_2O (10 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (3×20 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated in vacuo to yield the crude product, which was purified using silica column chromatography.

4.2.1. 4-(Benzyloxy)-6-methyl-2H-pyran-2-one, ***8***. White solid (0.221 g, 8% yield). Mp 90–92 °C [lit.[37] 92–94 °C]. Spectral characteristics were consistent with previously reported data.[38] ^1H NMR (300 MHz, $CDCl_3$): δ 2.21 (3H, s), 5.00 (2H, s), 5.50 (1H, d, *J*=2.1 Hz), 5.84 (1H, d, *J*=1.1 Hz), 7.35–7.40 (5H, m) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.8, 70.7, 88.5, 100.5, 127.8, 128.79, 128.83, 134.4, 162.2, 164.8, 170.2 ppm; HRMS (ESI) *m*/*z* calcd for $C_{13}H_{13}O_3$ $[(M+H)^+]$: 217.0865, found 217.0868.

4.2.2. 4-Ethoxy-6-methyl-2H-pyran-2-one, ***9***. Pale yellow solid (0.256 g, 12% yield). Mp 63–64 °C. IR (KBr) ν_{max}: 2986, 1720, 1567, 1250 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.37 (3H, t, *J*=7.0 Hz), 2.17 (3H, s), 3.98 (2H, q, *J*=7.1 Hz), 5.34 (1H, d, *J*=2.0 Hz), 5.73 (1H, d, *J*=1.0 Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.0, 19.8, 64.5, 87.6, 100.6, 162.0, 165.1, 170.5 ppm; HRMS (ESI) *m*/*z* calcd for $C_8H_{11}O_3$ $[(M+H)^+]$: 155.0708, found 155.0704.

4.2.3. 6-Methyl-4-propoxy-2H-pyran-2-one, ***10***. Pale yellow oil (0.122 g, 7% yield). IR (KBr) ν_{max}: 2970, 1733, 1566, 1250 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.93 (3H, t, *J*=7.4 Hz), 1.67–1.76 (2H, m), 2.12 (3H, s), 3.82 (2H, t, *J*=6.5 Hz), 5.29 (1H, d, *J*=1.1 Hz), 5.71 (1H, d, *J*=2.1 Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 10.2, 19.7, 21.8, 70.2, 87.6, 100.5, 161.9, 165.0, 170.7 ppm; HRMS (ESI) *m*/*z* calcd for $C_9H_{13}O_3$ $[(M+H)^+]$: 169.0865, found 169.0867.

4.2.4. 6-Methyl-4-phenoxy-2H-pyran-2-one, ***11***. To a stirred solution of 4-bromo-6-methyl-2*H*-pyran-2-one (2.225 g, 11.8 mmol) and K_2CO_3 (2.934 g, 21.2 mmol) in acetone (60 mL) was added phenol (1.666 g, 17.7 mmol) and the resulting mixture stirred at reflux for 24 h. H_2O (20 mL) was added and the aqueous layer extracted with ethyl acetate (3×25 mL) and washed with 10% aq NaOH (2×25 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated in vacuo to yield **11** as a beige solid (2.182 g, 91% yield) with no purification required. Mp 86–88 °C [lit.[39] 89–91 °C]. Spectral characteristics were consistent with previously reported data.[39] IR (KBr) ν_{max}: 1722, 1564, 1447, 1290, 1232 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.26 (3H, s), 5.20 (1H, dd, *J*=0.3, 2.2 Hz), 5.97 (1H, dd, *J*=0.9, 2.2 Hz), 7.05–7.09 (2H, m), 7.26–7.32 (1H, m) 7.40–7.46 (2H, m) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.0, 91.0, 99.9, 121.1, 126.5, 130.3, 152.4, 163.3, 164.6, 170.8 ppm; HRMS (ESI) *m*/*z* calcd for $C_{12}H_{11}O_3$ $[(M+H)^+]$: 203.0708, found 203.0702; Anal. Calcd for $C_{12}H_{10}O_3$: C, 71.28; H, 4.98%. Found: C, 71.20; H, 5.09%.

4.3. General procedure for formation of pyridones

To a suspension of 4-hydroxy-6-methyl-2*H*-pyran-2-one (1.0 equiv) in water (4 mL/mmol pyrone) was added amine (1.0 equiv). The reaction vessel was placed in an oil bath set at 100 °C and reaction was allowed to stir at reflux for 5 h. The reaction vessel was then placed in an ice–water bath for 2 h. The resulting solid was filtered and triturated with hot ethanol to yield product.

4.3.1. 1-Benzyl-4-hydroxy-6-methylpyridin-2(1H)-one, ***23***. Off-white solid (51% yield). Mp 201–207 °C [lit.[40] 205–208 °C]. Spectral characteristics were consistent with previously reported data.[40] ^1H NMR (300 MHz, $(CD_3)_2SO$): δ 2.17 (3H, s), 5.20 (2H, s), 5.61 (1H, d, *J*=2.6 Hz), 5.81 (1H, dd, *J*=2.7, 0.8 Hz), 7.02–7.14 (2H, m), 7.20–7.40 (3H, m), 10.51 (1H, br s); ^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ 20.4, 45.7, 96.3, 100.9, 127.3, 127.6, 129.0, 138.2, 148.1, 164.5, 166.4; MS (ESI) *m*/*z*: 214 $[(M-H)^-$, 42%].

4.3.2. 1-(4-Fluorophenyl)-4-hydroxy-6-methylpyridin-2(1H)-one, ***24***. White solid (0.597 g, 13% yield). Mp >250 °C. IR (KBr) ν_{max}: 1619, 1492, 1213 cm^{-1}; ^1H NMR (300 MHz, $(CD_3)_2SO$): δ 1.84 (3H, s), 5.55 (1H, d, *J*=2.1 Hz), 5.89 (1H, s), 7.23–7.34 (4H, m), 10.62 (1H, br s) ppm; ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$): δ 25.5, 96.5, 100.6, 116.5

(d, $^2J_{C-F}$=22.7 Hz), 131.3 (d, $^3J_{C-F}$=8.8 Hz), 135.4 (d, $^4J_{C-F}$=3.1 Hz), 147.4, 161.9 (d, $^1J_{C-F}$=244.7 Hz), 164.6, 167.0 ppm; ^{19}F NMR (282 MHz, $(CD_3)_2SO$): δ −114.1 ppm; HRMS (ESI) *m*/*z* calcd for $C_{12}H_{11}NO_2F$ [$(M+H)^+$]: 220.0774, found 220.0781.

4.3.3. 4-Hydroxy-1-(4-methoxyphenyl)-6-methylpyridin-2(1H)-one, ***25****.* White solid (1.185 g, 23% yield). Mp >250 °C. IR (KBr) ν_{max}: 1617, 1531, 1507, 1246 cm^{-1}; ^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ 1.83 (3H, s), 3.80 (3H, s), 5.23 (1H, s), 5.86 (1H, s), 7.01 (2H, d, *J*=8.5 Hz), 7.09 (2H, d, *J*=8.6 Hz), 10.56 (1H, br s) ppm; ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$): δ 21.6, 55.8, 96.5, 100.3, 114.8, 130.1, 131.8, 147.9, 159.1, 164.7, 166.8 ppm; HRMS (ESI) *m*/*z* calcd for $C_{13}H_{14}NO_3$ [$(M+H)^+$]: 232.0974, found 232.0965.

4.4. General procedure for methylation of pyrones, pyridones and quinolone

To 4-hydroxy starting material (1.0 equiv) and K_2CO_3 (1.2 equiv) was added trimethylphosphate (2.1 equiv) and the resulting mixture stirred at reflux with progress monitored by TLC analysis. On completion, H_2O (30 mL) was added and the aqueous layer extracted with ethyl acetate (3×30 mL). The organic layers were combined, washed with brine (50 mL), dried over $MgSO_4$ and concentrated in vacuo to yield crude product as a yellow solid, which was purified by recrystallisation from ethanol to yield product.

4.4.1. 4-Methoxy-6-methyl-2H-pyran-2-one, ***5****.* Pale yellow, crystalline solid (2.303 g, 46% yield). Mp 85–87 °C [lit.[41] 86–87.5 °C]. Spectral characteristics were consistent with previously reported data.[42] ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.21 (3H, s), 3.79 (3H, s), 5.41 (1H, d, *J*=2.2 Hz), 5.77–5.78 (1H, m) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 19.8, 55.8, 87.4, 100.3, 162.0, 164.9, 171.3 ppm; MS (ESI) *m*/*z*: 141 [$(M+H)^+$, 100%].

4.4.2. 1-Benzyl-4-methoxy-6-methylpyridin-2(1H)-one, ***26****.* Yellow solid (57% yield). Mp 115–116 °C. IR (KBr) ν_{max}: 1655 cm^{-1}; ^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ 2.19 (3H, s), 3.74 (3H, s), 5.22 (2H, s), 5.81 (1H, d, *J*=2.8 Hz), 5.91 (1H, dd, *J*=2.7, 0.7 Hz), 7.10 (2H, d, *J*=7.0 Hz), 7.35–7.24 (3H, m) ppm; ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$): δ 20.2, 45.9, 55.8, 94.4, 100.4, 126.5, 127.4, 129.0, 138.0, 147.6, 164.4, 167.5; HRMS (ESI) *m*/*z* calcd for $C_{14}H_{16}NO_2$ [$(M+H)^+$]: 230.1181, found 230.1172.

4.4.3. 1-(4-Fluorophenyl)-4-methoxy-6-methylpyridin-2(1H)-one, ***27****.* White solid (1.025 g, 60% yield). Mp 188–190 °C. IR (KBr) ν_{max}: 1651, 1558, 1510, 1246, 1213, 1165 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.90 (3H, s), 3.78 (3H, s), 5.84 (1H, d, *J*=1.8 Hz), 5.87 (1H, d, *J*=2.6 Hz), 7.16–7.18 (4H, m) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 21.5, 55.5, 95.0, 101.1, 116.7 (d, $^2J_{C-F}$=22.8 Hz), 130.1 (d, $^3J_{C-F}$=8.7 Hz), 134.5 (d $^4J_{C-F}$=3.3 Hz), 145.9, 162.4 (d, $^1J_{C-F}$=248.3 Hz), 165.6, 168.0 ppm; ^{19}F NMR (282 MHz, ($CDCl_3$)): δ −112.7 ppm; HRMS (ESI) *m*/*z* calcd for $C_{13}H_{13}NO_2F$ [$(M+H)^+$]: 234.0930, found 234.0920; Anal. Calcd for $C_{13}H_{12}O_2FN$: C, 66.94; H, 5.19; N, 6.01%. Found: C, 66.92; H, 5.19; N, 6.01%.

4.4.4. 4-Methoxy-1-(4-methoxyphenyl)-6-methylpyridin-2(1H)-one, ***28****.* Off-white solid (0.528 g, 44% yield). Mp 171–173 °C. IR (KBr) ν_{max}: 1652, 1560, 1511, 1244 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.90 (3H, s), 3.76 (3H, s), 3.82 (3H, s), 5.81 (1H, s), 5.86 (1H, d, *J*=2.6 Hz), 6.98 (2H, d, *J*=8.9 Hz), 7.08 (2H, d, *J*=8.8 Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 21.5, 55.3, 55.5, 95.0, 100.7, 114.9, 129.2, 131.3, 146.5, 159.5, 165.8, 167.8 ppm; HRMS (ESI) *m*/*z* calcd for $C_{14}H_{16}NO_3$ [$(M+H)^+$]: 246.1130, found 246.1126; Anal. Calcd for $C_{14}H_{15}O_3N$: C, 68.56; H, 6.16; N, 5.71%. Found: C, 68.42; H, 6.15; N, 5.72%.

4.4.5. 4-Methoxy-1-methylquinolin-2(1H)-one, ***36****.* Orange solid (0.953 g, 81% yield). Mp 90–92 °C. IR (KBr) ν_{max}: 1638, 1587, 1236 cm^{-1}; ^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ 3.56 (3H, s), 3.94 (3H, s), 6.04 (1H, s), 7.23–7.29 (1H, m) 7.49–7.52 (1H, m), 7.62–7.68 (1H, m), 7.87–7.89 (1H, m) ppm; ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$): δ 29.1, 56.6, 96.8, 115.1, 116.0, 122.0, 123.1, 131.9, 139.9, 162.3, 162.8 ppm; HRMS (ESI) *m*/*z* calcd for $C_{11}H_{12}NO_2$ [$(M+H)^+$]: 190.0868, found190.0864.

4.5. General procedure for iodination

To a stirred solution of 4-alkoxy starting material (1.0 equiv) in acetonitrile (30 mL) was added *N*-iodosuccinimide (1.5–3.0 equiv). The reaction vessel was covered in aluminium foil and allowed stir at room temperature with reaction progress monitored by TLC analysis. On completion, the solvent was concentrated in vacuo, the resulting solid dissolved in CH_2Cl_2 (15 mL) and washed with 5% aqueous sodium thiosulfate (3×20 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo to yield product, which was purified by silica column chromatography if required.

4.5.1. 3-Iodo-4-methoxy-6-methyl-2H-pyran-2-one, ***6****.* 1.5 equiv *N*-iodosuccinimide required. No purification required. Yellow solid (3.578 g, 95% yield). Mp 144–146 °C [lit.[11] 144–146 °C]. Spectral characteristics were consistent with previously reported data.[11] ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.30 (3H, s), 3.99 (3H, s), 6.00 (1H, s) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.1, 57.5, 62.3, 94.7, 161.7, 164.2, 170.5 ppm; MS (ESI) *m*/*z*: 267 [$(M+H)^+$, 100%].

4.5.2. 4-(Benzyloxy)-3-iodo-6-methyl-2H-pyran-2-one, ***12****.* 3.0 equiv *N*-iodosuccinimide required. No purification required. Off-white solid (0.272 g, 84% yield). Mp 125–126 °C. IR (KBr) ν_{max}: 1705, 1524, 1318 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.22 (3H, s), 5.35 (2H, s), 5.97 (1H, s), 7.32–7.40 (5H, m) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.1, 63.3, 71.8, 95.8, 126.8, 128.7, 128.9, 134.6, 161.8, 164.0, 169.8 ppm; HRMS (ESI) *m*/*z* calcd for $C_{13}H_{12}O_3I$ [$(M+H)^+$]: 342.9831, found 342.9824.

4.5.3. 4-Ethoxy-3-iodo-6-methyl-2H-pyran-2-one, ***13****.* 3.0 equiv *N*-iodosuccinimide required. No purification required. Yellow solid (0.205 g, 75% yield). Mp 130–131 °C. IR (KBr) ν_{max}: 3102, 2981, 1699, 1521, 1314 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (3H, t, *J*=7.0 Hz), 2.25 (3H, s), 4.20 (2H, q, *J*=7.1 Hz), 5.92 (1H, s) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.7, 20.1, 62.5, 66.4, 95.3, 161.9, 163.9, 169.9 ppm; HRMS (ESI) *m*/*z* calcd for $C_8H_{10}O_3I$ [$(M+H)^+$]: 280.967, found 280.9669; Anal. Calcd for $C_8H_9O_3I$: C, 34.21; H, 3.24%. Found: C, 34.43; H, 3.00%.

4.5.4. 3-Iodo-6-methyl-4-propoxy-2H-pyran-2-one, ***14****.* 3.0 equiv *N*-iodosuccinimide required. No purification required. White solid (0.093 g, 96% yield). Mp 113–115 °C. IR (KBr) ν_{max}: 2969, 2920, 1709, 1522, 1317 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.08 (3H, t, *J*=7.4 Hz), 1.80–1.91 (2H, m), 2.28 (3H, s), 4.10 (2H, q, *J*=6.4 Hz), 5.91 (1H, s) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 10.4, 20.1, 22.4, 62.5, 72.0, 95.4, 161.9, 163.9, 170.0 ppm; HRMS (ESI) *m*/*z* calcd for $C_9H_{12}O_3I$ [$(M+H)^+$]: 294.9831, found 294.9820; Anal. Calcd for $C_9H_{11}O_3I$: C, 36.76; H, 3.77%. Found: C, 36.93; H, 3.49%.

4.5.5. 3-Iodo-6-methyl-4-phenoxy-2H-pyran-2-one, ***15****.* 1.5 equiv *N*-iodosuccinimide required. No purification required. Yellow solid (1.022 g, 96% yield). Mp 123–125 °C. IR (KBr) ν_{max}: 1718, 1532, 1487, 1225 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.16 (3H, s), 5.54 (1H, s), 7.07–7.09 (2H, m), 7.29–7.34 (1H, m), 7.42–7.47 (2H, m) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.9, 64.9, 97.3, 120.9, 126.5, 130.4, 153.0, 161.8, 163.6, 169.4 ppm; HRMS (ESI) *m*/*z* calcd for $C_{12}H_{10}O_3I$

$[(M+H)^+]$: 328.9675, found 328.9667; Anal. Calcd for $C_{12}H_9O_3I$: C, 43.93; H, 2.76%. Found: C, 43.96; H, 2.63%.

4.5.6. 1-Benzyl-3-iodo-4-methoxy-6-methylpyridin-2(1H)-one, ***29***. 3.0 equiv *N*-iodosuccinimide required. Purification using silica column chromatography eluting with 50:50 hexane:ethyl acetate. Pale yellow solid (0.371 g, 70% yield). Mp 146–148 °C. IR (KBr) ν_{max}: 2931, 2852, 1634, 1504, 1323 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.30 (3H, s), 3.91 (3H, s), 5.38 (2H, s), 5.88 (1H, s), 7.14–7.16 (2H, m), 7.23–7.32 (3H, m) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 21.1, 48.7, 56.7, 72.1, 95.3, 126.7, 127.5, 128.8, 136.3, 147.9, 162.2, 166.7 ppm; HRMS (ESI) *m*/*z* calcd for $C_{14}H_{15}O_2NI$ $[(M+H)^+]$: 356.0148, found 356.0141.

4.5.7. 1-(4-Fluorophenyl)-3-iodo-4-methoxy-6-methylpyridin-2(1H)-one, ***30***. 2 equiv *N*-iodosuccinimide required. Purification using silica column chromatography eluting with 50:50 hexane:ethyl acetate. Pale brown solid (0.429 g, 31% yield). Mp 196–198 °C. IR (KBr) ν_{max}: 1651, 1579, 1520, 1343, 1214 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.01 (3H, s), 3.97 (3H, s), 6.00 (1H, s), 7.15–7.19 (4H, m) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 22.0, 56.8, 72.1, 94.9, 116.7 (d, $^2J_{C-F}$=23.0 Hz), 129.8 (d, $^3J_{C-F}$=8.7 Hz), 134.7 (d, $^4J_{C-F}$=3.5 Hz), 147.5, 161.5 (d, $^1J_{C-F}$=248.8 Hz), 162.2, 167.3 ppm; ^{19}F (282 MHz, $CDCl_3$): δ −112.2 ppm HRMS (ESI) *m*/*z* calcd for $C_{13}H_{12}O_2NFI$ $[(M+H)^+]$: 359.9897, found 359.9894.

4.5.8. 1-(4-Methoxyphenyl)-3-iodo-4-methoxy-6-methylpyridin-2(1H)-one, ***31***. 2.0 equiv *N*-iodosuccinimide required. Purification using silica column chromatography eluting with 50:50 hexane:ethyl acetate. Yellow solid (0.090 g, 13% yield). Mp 190–193 °C. IR (KBr) ν_{max}: 1651, 1511, 1343 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.02 (3H, s), 3.84 (3H, s), 3.96 (3H, s), 5.96 (1H, s), 6.98 (2H, d, *J*=8.6 Hz), 7.07 (2H, d, *J*=8.6 Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 22.0, 55.5, 56.7, 72.2, 94.6, 114.9, 128.9, 131.5, 148.1, 159.6, 162.4, 167.2; HRMS (ESI) *m*/*z* calcd for $C_{14}H_{15}O_3NI$ $[(M+H)^+]$: 372.0097, found 372.0099.

4.5.9. 3-Iodo-4-methoxy-1-methylquinolin-2(1H)-one, ***37***. 1.5 equiv *N*-iodosuccinimide required. Purification using silica column chromatography eluting with 80:20 hexane:ethyl acetate. Yellow solid (0.485 g, 32% yield). Mp 115–117 °C. IR (KBr) ν_{max}: 1636, 1610, 1591 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.81 (3H, s), 4.03 (3H, s), 7.25–7.31 (1H, m), 7.41 (1H, d, *J*=1.6 Hz), 7.61–7.67 (1H, m), 7.88–7.91 (1H, m) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 31.4, 61.3, 87.5, 114.5, 117.5, 122.4, 123.8, 131.7, 139.9, 160.8, 166.3 ppm; HRMS (ESI) *m*/*z* calcd for $C_{11}H_{11}NO_2I$ $[(M+H)^+]$: 315.9835, found 315.9828; Anal. Calcd for $C_{11}H_{10}NO_2I$: C, 41.93; H, 3.20; N, 4.45%. Found: C, 41.61; H, 3.09; N, 4.08%.

4.6. Bromination of 4-methoxy-6-methyl-2*H*-pyran-2-one

4.6.1. 3-Bromo-4-methoxy-6-methyl-2H-pyran-2-one, ***20***. To a stirred solution of 4-methoxy-6-methyl-2*H*-pyran-2-one **5** (2.791 g, 19.9 mmol) in acetonitrile (30 mL) was added *N*-bromosuccinimide (7.089 g, 39.8 mmol). The reaction vessel was covered in aluminium foil and resulting mixture allowed to stir at reflux overnight. The solvent was concentrated in vacuo, the resulting orange solid dissolved in CH_2Cl_2 (20 mL) and washed with 5% aqueous sodium thiosulfate (2×25 mL), water (25 mL) and brine (25 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo to yield crude product, which was purified by silica column chromatography to yield **20** as a pale yellow solid (1.980 g, 45% yield). Mp 149–151 °C [lit.[43] 151–152 °C]. Spectral characteristics were consistent with previously reported data.[44] ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.29 (3H, s), 3.98 (3H, s), 6.05 (1H, s) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.3, 57.3, 88.5, 95.1, 160.8, 162.8, 166.8 ppm; HRMS (ESI) *m*/*z* calcd for $C_7H_8O_3Br$ $[(M+H)^+]$: 218.9657, found 218.9649.

4.6.2. 3-Bromo-6-(bromomethyl)-4-methoxy-2H-pyran-2-one, ***21***. 3-Bromo-6-(bromomethyl)-4-methoxy-2*H*-pyran-2-one **21** was isolated as a side product from the bromination of 4-methoxy-6-methyl-2*H*-pyran-2-one as an off white solid (0.794 g, 13% yield). Mp 159–161 °C [lit.[45] 162–164 °C]. Spectral characteristics were consistent with previously reported data.[45] ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.03 (3H, s), 4.19 (2H, s), 6.40 (1H, s) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 26.3, 57.6, 91.3, 96.7, 159.1, 159.7, 166.0 ppm; HRMS (ESI) *m*/*z* calcd for $C_7H_7O_3Br_2$ $[(M+H)^+]$: 296.8762, found 296.8766.

4.7. General procedure for trifluoromethylation

To a stirred solution of iodo-substrate (1.0 equiv) and copper (I) iodide (1.2 equiv) in DMF (4 mL) was added methyl 2,2-difluoro-2-(fluorosulfonyl)acetate (1.2 equiv). The reaction mixture was allowed stir at 70 °C overnight. On completion, reaction was cooled to room temperature, diluted with diethyl ether (20 mL) and filtered. H_2O (20 mL) was added and the aqueous layer extracted with diethyl ether (4×20 mL). The combined organic extracts washed with water (3×5 mL), dried over $MgSO_4$ and concentrated in vacuo to yield crude product, which was purified using silica column chromatography.

4.7.1. 4-Methoxy-6-methyl-3-(trifluoromethyl)-2H-pyran-2-one, ***7***. Pale yellow solid (0.061 g, 77% yield). Mp 120–122 °C. IR (KBr) ν_{max}: 1747, 1560, 1266, 1100 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.33 (3H, s), 4.00 (3H, s), 6.11 (1H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.7, 57.5, 94.5, 94.3 (q, $^2J_{C-F}$=31.6 Hz), 124.2 (q, $^1J_{C-F}$=272.7 Hz), 159.1, 167.8, 171.1 ppm; ^{19}F NMR (470 MHz, $CDCl_3$): δ −57.6 ppm; HRMS (ESI) *m*/*z* calcd for $C_8H_8O_3F_3$ $[(M+H)^+]$: 209.0426, found 209.0424; Anal. Calcd for $C_8H_7F_3O_3$: C, 46.17; H, 3.39%. Found: C, 46.27; H, 3.49%.

4.7.2. 4-(Benzyloxy)-6-methyl-3-(trifluoromethyl)-2H-pyran-2-one, ***16***. Pale yellow solid (0.065 g, 30% yield). Mp 149–151 °C. IR (KBr) ν_{max}: 1715, 1556, 1414, 1364 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.28 (3H, s), 5.27 (2H, s), 6.08 (1H, s), 7.34–7.45 (5H, m) ppm; ^{13}C NMR ($CDCl_3$, 150 MHz): δ 20.8, 71.9, 95.1 (q, $^2J_{C-F}$=29.4 Hz), 95.3, 122.8 (q, $^1J_{C-F}$=271.5 Hz), 126.8, 128.9, 129.0, 134.0, 159.0, 167.5, 170.2; HRMS (ESI) *m*/*z* calcd for $C_{14}H_{12}O_3F_3$ $[(M+H)^+]$: 285.0739, found 285.0728.

4.7.3. 4-Ethoxy-6-methyl-3-(trifluoromethyl)-2H-pyran-2-one, ***17***. Pale yellow solid (0.052 g, 34% yield). Mp 129–131 °C. IR (KBr) ν_{max}: 1722, 1557, 1263, 1229, 1110 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.44 (3H, t, *J*=7.0 Hz), 2.29 (3H, s), 4.23 (2H, q, *J*=7.0 Hz), 6.05 (1H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.4, 20.7, 66.7, 94.5 (q, $^2J_{C-F}$=31.5 Hz), 95.0, 122.9 (q, $^1J_{C-F}$=272.8 Hz), 159.2, 167.4, 170.4 ppm; ^{19}F NMR (282 MHz, $CDCl_3$): δ −57.6 ppm; HRMS (ESI) *m*/*z* calcd for $C_9H_{10}O_3F_3$ $[(M+H)^+]$: 223.0582, found 223.0574; Anal. Calcd for $C_9H_9F_3O_3$: C, 48.66; H, 4.08%. Found: C, 48.48; H, 4.00%.

4.7.4. 6-Methyl-4-propoxy-3-(trifluoromethyl)-2H-pyran-2-one, ***18***. Yellow solid (0.035 g, 46% yield). Mp 107–109 °C. IR (KBr) ν_{max}: 1719, 1552, 1113 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.02 (3H, t, *J*=7.4 Hz), 1.77–1.86 (2H, m), 2.29 (3H, s), 4.11 (2H, t, *J*=6.3 Hz), 6.06 (1H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 8.2, 18.8, 20.4, 70.5, 92.4 (q, $^2J_{C-F}$=31.6 Hz), 93.3, 121.1 (q, $^1J_{C-F}$=272.8 Hz), 157.4, 165.6, 168.9 ppm; ^{19}F NMR (282 MHz, $CDCl_3$): δ −57.6 ppm; HRMS (ESI) m/z calcd for $C_{10}H_{12}O_3F_3$ [(M+H)$^+$]: 237.0739, found 237.0735.

4.7.5. 6-Methyl-4-phenoxy-3-(trifluoromethyl)-2H-pyran-2-one, ***19***. Yellow solid (0.103 g, 43% yield). Mp 128–129 °C. IR (KBr) ν_{max}: 1730, 1563, 1261, 1123 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.19 (3H, s), 5.62 (1H, s), 7.07–7.11 (2H, m), 7.31–7.37 (1H, m), 7.44–7.50 (2H, m) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.5, 96.3 (q, $^2J_{C-F}$=32.1 Hz), 96.9, 120.9, 122.5 (q, $^1J_{C-F}$=273.1 Hz), 126.9, 130.5, 152.4, 159.0, 167.2, 169.2 ppm; ^{19}F NMR (282 MHz, $CDCl_3$): δ −57.8 ppm; HRMS (ESI) m/z calcd for $C_{13}H_{10}O_3F_3$ [(M+H)$^+$]: 271.0582, found 271.0583; Anal. Calcd for $C_{13}H_9F_3O_3$: C, 57.79; H, 3.36%. Found: C, 57.61; H, 3.40%.

4.7.6. 3-Bromo-4-methoxy-6-(2,2,2-trifluoroethyl)-2H-pyran-2-one, ***22***. Yellow solid (0.065 g, 30% yield). Mp 146–148 °C. IR (KBr) ν_{max}: 1702, 1537, 1327 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.35 (2H, q, J=9.7 Hz), 4.02 (3H, s), 6.28 (1H, s) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.8 (q, $^2J_{C-F}$=32.0 Hz), 57.6, 91.3, 98.4, 123.5 (q, J_{C-F}=277.8 Hz), 154.0 (q, $^3J_{C-F}$=3.5 Hz), 159.7, 165.9 ppm; ^{19}F NMR (282 MHz, $CDCl_3$): δ −64.2 ppm; HRMS (ESI) m/z calcd for $C_8H_7O_3F_3Br$ [(M+H)$^+$]: 286.9531, found 286.9522.

4.7.7. 1-Benzyl-4-methoxy-6-methyl-3-(trifluoromethyl)pyridin-2(1H)-one, ***32***. Pale yellow solid (0.064 g, 62% yield). Mp 132–133 °C. IR (KBr) ν_{max}: 1656, 1557, 1396, 1108 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.33 (3H, s), 3.91 (3H, s), 5.30 (2H, s), 5.90 (1H, s), 7.15–7.18 (2H, m), 7.25–7.34 (3H, m) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.5, 47.0, 56.3, 94.5, 100.4 (q, $^2J_{C-F}$=29.4 Hz), 123.9 (q, $^1J_{C-F}$=272.9 Hz), 126.6, 127.6, 128.9, 136.0, 151.6, 160.7, 166.6 ppm; ^{19}F NMR (282 MHz, $CDCl_3$): δ −57.2 ppm; HRMS (ESI) m/z calcd for $C_{15}H_{15}NO_2F_3$ [(M+H)$^+$]: 298.1055, found 298.1047.

4.7.8. 1-(4-Fluorophenyl)-4-methoxy-6-methyl-3-(trifluoromethyl)pyridin-2(1H)-one, ***33***. White solid (0.107 g, 52% yield) mp 185–188 °C. IR (KBr) ν_{max}: 1664, 1555, 1509, 1464, 1261 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.03 (3H, s), 3.95 (3H, s), 6.03 (1H, s), 7.12–7.21 (4H, m) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.3, 56.6, 94.4, 100.5 (q, $^2J_{C-F}$=29.5 Hz), 116.8 (d, $^2J_{C-F}$=23.0 Hz), 123.7 (q, $^1J_{C-F}$=273.1 Hz), 129.9 (d, $^3J_{C-F}$=8.8 Hz), 133.6 (d, $^4J_{C-F}$=3.5 Hz), 151.4, 160.8, 162.6 (d, $^1J_{C-F}$=248.8 Hz), 167.4 ppm; ^{19}F NMR (282 MHz, $CDCl_3$): δ −112.0, −57.2 ppm; HRMS (ESI) m/z calcd for $C_{14}H_{12}NO_2F_4$ [(M+H)$^+$]: 302.0804, found 302.0796; Anal. Calcd for $C_{14}H_{11}F_4NO_2$: C, 55.82; H, 3.68; N, 4.65%. Found: C, 55.55; H, 3.61; N, 4.50%.

4.7.9. 4-Methoxy-1-(4-methoxyphenyl)-6-methyl-3-(trifluoromethyl)pyridin-2(1H)-one, ***34***. White solid (0.077 g, 46% yield). Mp 153–156 °C. IR (KBr) ν_{max}: 1662, 1552, 1514, 1251, 1108 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.04 (3H, s), 3.84 (3H, s) 3.95 (3H, s), 5.99 (1H, s), 6.98 (2H, d, J=9.0 Hz), 7.07 (2H, d, J=9.0 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.4, 55.5, 56.6, 93.9, 100.6 (q, $^2J_{C-F}$=29.3 Hz), 115.0, 123.8 (q, $^1J_{C-F}$=273.1 Hz), 129.0, 130.3, 151.9, 159.8, 161.0, 167.2 ppm; ^{19}F NMR (282 MHz, $CDCl_3$): δ −57.3 ppm; HRMS (ESI) m/z calcd for $C_{15}H_{15}NO_3F_3$ [(M+H)$^+$]: 314.1004, found 314.0997; Anal. Calcd for $C_{15}H_{14}F_3NO_3$: C, 57.51; H, 4.50; N, 4.47%. Found: C, 57.80; H, 4.47; N, 4.13%.

4.7.10. 4-Methoxy-1-methyl-3-(trifluoromethyl)quinolin-2(1H)-one, ***38***. White solid (0.120 g, 52% yield). Mp 100–102 °C. IR (KBr) ν_{max}: 1652, 1118 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.71 (3H, s), 4.05 (3H, s), 7.29–7.35 (1H, m), 7.40 (1H, d, J=8.5 Hz), 7.67–7.73 (1H, m), 7.94–7.98 (1H, m) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 29.6, 64.3 (q, $^5J_{C-F}$=2.5 Hz), 111.0 (q, $^2J_{C-F}$=29.0 Hz), 114.4, 116.7, 122.6, 122.7 (q, $^1J_{C-F}$=273.7 Hz), 125.2, 133.5, 140.8, 159.6, 165.6 (q, $^3J_{C-F}$=1.3 Hz) ppm; ^{19}F NMR (282 MHz, $CDCl_3$): δ −59.7 ppm; HRMS (ESI) m/z calcd for $C_{12}H_{11}F_3NO_2$ [(M+H)$^+$]: 258.0742, found 258.0731; Anal. Calcd for $C_{12}H_{10}F_3NO_2$: C, 56.04; H, 3.92%. Found: C, 55.82; H, 3.85%.

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