CORA Cork Open Research Archive
Cartlann Taighde Oscailte Chorcaí

Title	Synthesis and biological evaluation of novel thionucleosides
Authors	O'Donovan, Fiona P.;O'Leary, Eileen M.;O'Sullivan, Timothy P.
Publication date	2020-11-01
Original Citation	O'Donovan, F. P., O'Leary, E. M. and O'Sullivan, T. P. (2020) 'Synthesis and biological evaluation of novel thionucleosides', Current Organic Chemistry, 24(15), pp. 1717-1762. doi: 10.2174/1385272824999200608131955
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.2174/1385272824999200608131955
Rights	© 2020, Bentham Science Publishers. All rights reserved. The published manuscript is available at EurekaSelect via https://www.eurekaselect.com/node/182609/article
Download date	2026-11-06 09:20:48
Item downloaded from	https://hdl.handle.net/10468/10780



UCC
University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

Synthesis and biological evaluation of novel thionucleosides.

Fiona P. O'Donovan[a,b], Eileen M. O'Leary[c], Timothy P O'Sullivan[*a,b,d]

[a]*School of Chemistry, University College Cork, Cork, Ireland;* [b]*Analytical and Biological Research Facility, University College Cork, Cork, Ireland;* [c] *Department of Physical Sciences, Cork Institute of Technology, Cork, Ireland;* [d]*School of Pharmacy, University College Cork, Cork, Ireland.*

ARTICLE HISTORY

Received:
Revised:
Accepted:

DOI:

Abstract: The search for novel nucleosides has been a major research focus in medicinal chemistry for several decades, particularly given their proven track record in the treatment of viral infections and cancer. As bioisosteres of natural nucleosides, thionucleosides are especially attractive targets as they often display improved biological activity. Furthermore, replacement of oxygen with sulfur may sometimes be accompanied by interesting changes in pharmacological effect. This update covers recent advances in the preparation of novel thionucleosides, grouped by synthetic strategy. The biological properties of the target thionucleosides are also summarised, in addition to any reported structure activity relationships.

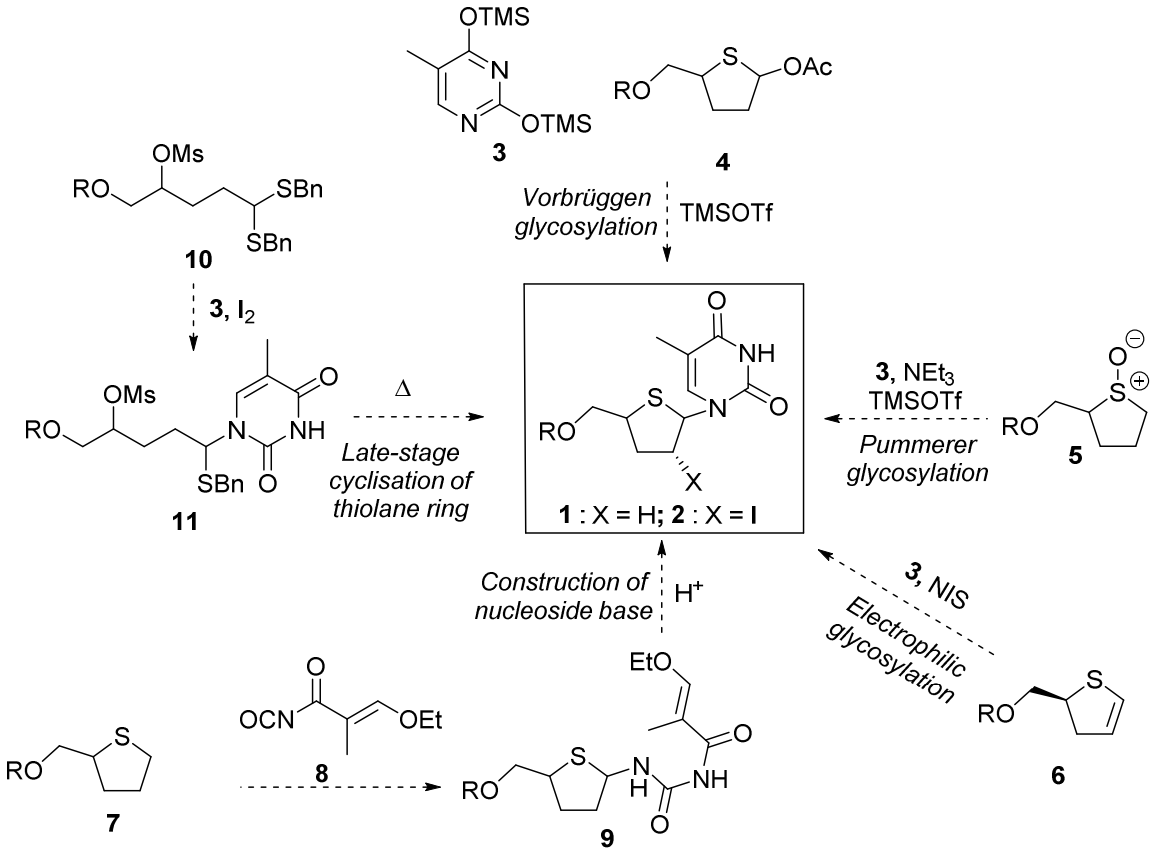
Keywords: Thionucleosides, nucleosides; sulfur, anti-virals, synthesis, glycosylation.

1. INTRODUCTION

Nucleosides have been of clinical importance for decades in the treatment of cancer and viral infections, with idoxuridine being the first drug of this class to receive approval for use in humans in the early 1960s.[1] The development of novel nucleosides has been the focus of intense research activity over the years, and has led to the emergence of several new sub-classes with improved pharmacological profiles, including nucleotides and acyclic analogues.[2-7] Likewise, thionucleosides have been attractive targets to medicinal chemists.[8] As bioisosteres of cell-native 4´-oxonucleosides, 4´-thionucleosides contain a more stable glycosyl bond between the sugar and nucleoside base, conferring increased metabolic stability to most cellular and viral enzymes.[9-13] Additionally, replacement of oxygen with sulfur is sometimes accompanied by unexpected changes in biological activity.[14-16]

Following reviews from Yokoyama[17] and Jeong[9] in the early 2000s, this update covers recent advances in the design and synthesis of novel thionucleosides and also examines their reported biological effects. This review is subdivided according to the synthetic strategy employed to synthesise the target thionucleosides, namely: 1. Vorbrüggen

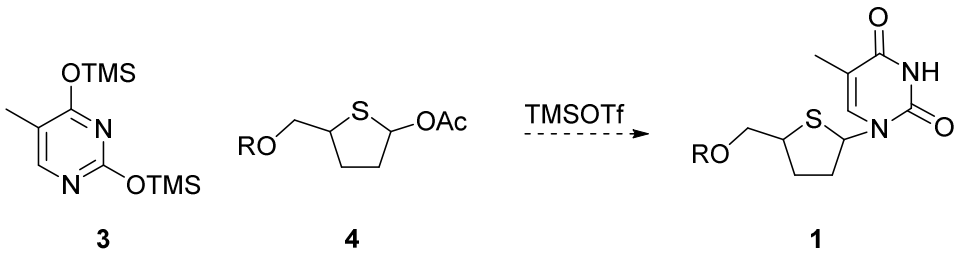
glycosylation; 2. Pummerer glycosylation; 3. Electrophilic glycosylation; 4. Late-stage construction of the nucleoside base; 5. Late-stage cyclisation of the thiolane ring (Scheme 1).



Scheme 1

2. VORBRÜGGEN GLYCOSYLATIONS

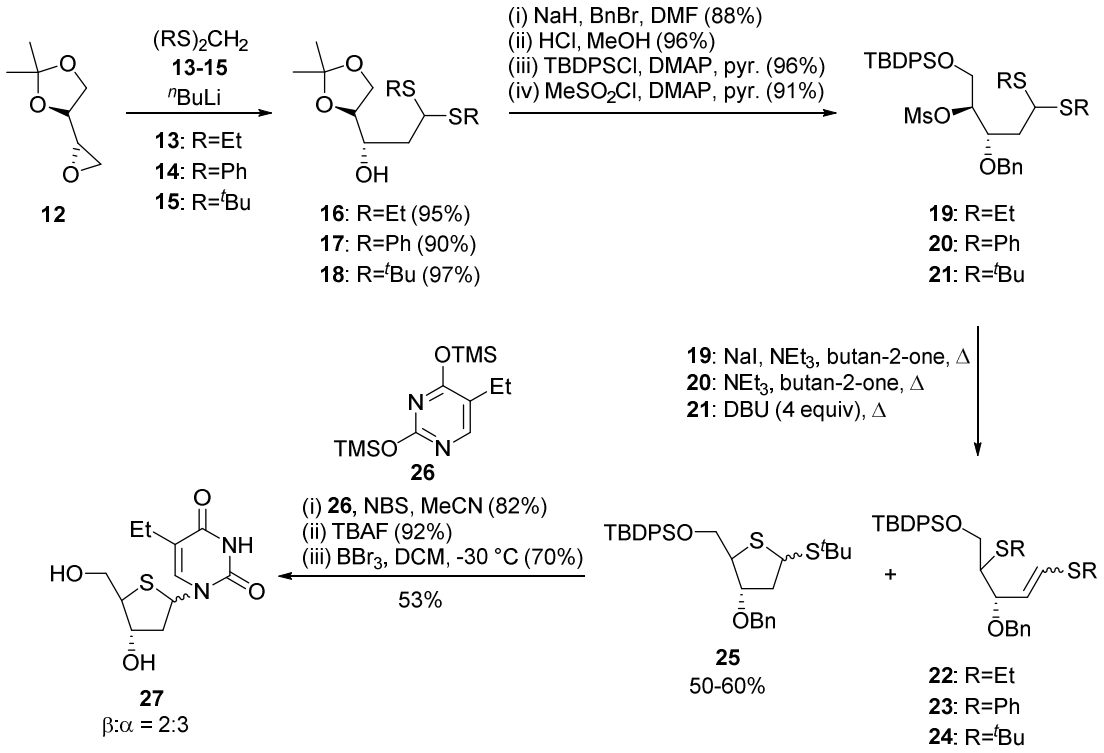
Also known as the Silyl-Hilbert-Johnson reaction, the Vorbrüggen glycosylation is probably the most commonly used method to prepare nucleosides.[18-20] This transformation is typically conducted using a Lewis acid catalyst, such as trimethylsilyltriflate, alongside a persilylated nucleobase (**3**) and acylated sugar (**4**) (Scheme 2).



3 **4** **1**

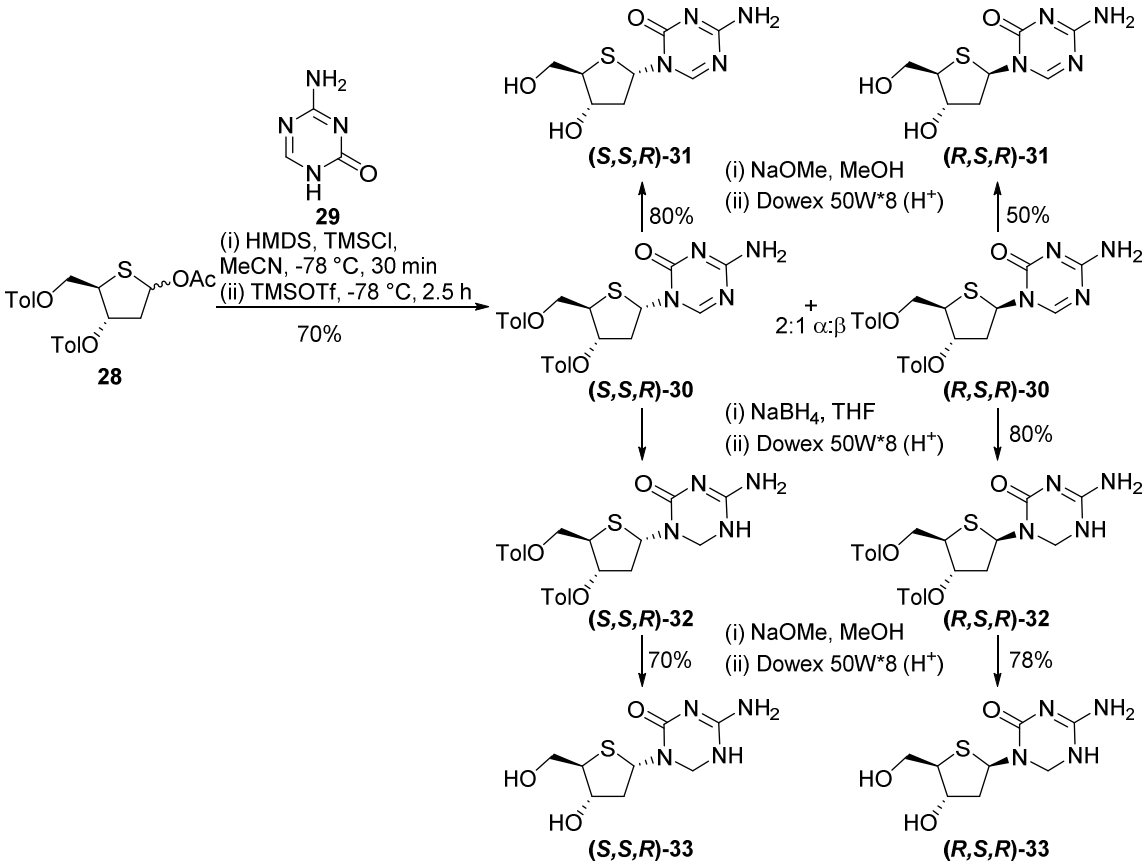
Scheme 2

Miller and colleagues exploited commercially available L-ascorbate-derived epoxide **12** to access novel 2´-deoxy-4´-thionucleosides (Scheme 3).[21] Exclusive opening of epoxide **12** at the less hindered carbon by lithiated dithianes afforded hydroxydithioacetals **16-18**.[22, 23] Manipulation of the protecting groups in **16-18**, followed by mesylation of the free hydroxyl, furnished **19-21**. Cyclisation of mesylates **19-21**, where R was a phenyl or ethyl group, was not achieved despite recourse to a range of conditions. A mixture of *E*- and *Z*-vinyl sulfides **22-24** were instead formed as a result of a 1,4-thiolate shift. However, the *tert*-butyl analogue did cyclise in the presence of DBU to form a mixture of α- and β-thiosugars **25** in 50-60% yield. The authors postulate that the kinetics of ring-closure can compete with the 1,4-thiolate shift as DBU facilitates de-*tert*-butylation *via* loss of isobutylene. Glycosylation of **25** was achieved using silylated thymine analogue **26** in the presence of *N*-bromosuccinimide (NBS) in acetonitrile. Following standard deprotection procedures, 4´-thionucleoside **27** was isolated in 53% yield as a 2:3 mixture of β- and α-isomers. The β-isomer was previously reported by Rahim *et al.* as possessing good anti-herpes activity.[24]



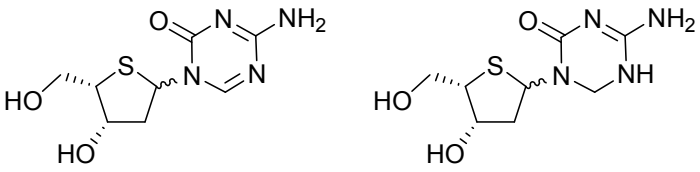
Scheme 3

In their studies on the anti-cancer activity of novel 4´-thio-5-azacytosine analogues, Secrist *et al*. exploited a Vorbrüggen-type glycosylation of 5-azacytosine **29** and glycal donor **28**[25-27] in their key step (Scheme 4). The resulting 2:1 anomeric mixture of **(*S,S,R*)-30** and **(*R,S,R*)-30** was separated by column chromatography. Following independent deprotection of the 3´- and 5´-hydroxyl groups of both the α- and β-anomers, diols **(*S,S,R*)-31** and **(*R,S,R*)-31** were isolated. However, **(*S,S,R*)-31** and **(*R,S,R*)-31** were found to be unstable in solution. By contrast, reduction of the azacytosine 5,6-double bond with sodium borohydride and subsequent methoxide-mediated deprotection afforded stable analogues **(*S,S,R*)-33** and **(*R,S,R*)-33** in high yields.



Scheme 4

This chemistry was repeated on the L-enantiomers to produce stereoisomers **(*S,S,R*)-34**, **(*R,S,R*)-34**, **(*S,S,R*)-35** and **(*R,S,R*)-35** (Figure 1). When assayed for their antitumour activity, only **(*R,S,R*)-33** displayed any cytotoxic effect on the cell lines tested (Table 1). **(*R,S,R*)-33** progressed to *in vivo* studies in female nude mice which were implanted subcutaneously with NCI-H23 non-small cell lung tumour. When 4.5 mg/kg of **(*R,S,R*)-33** was injected as a single dose on days 15-23, the lifespan of the infected mice increased by 8 days in comparison to the control models. Interestingly, when **(*R,S,R*)-33** was freshly prepared it increased mouse lifespan by an average of 10.9 days, suggesting that compound stability was an issue.



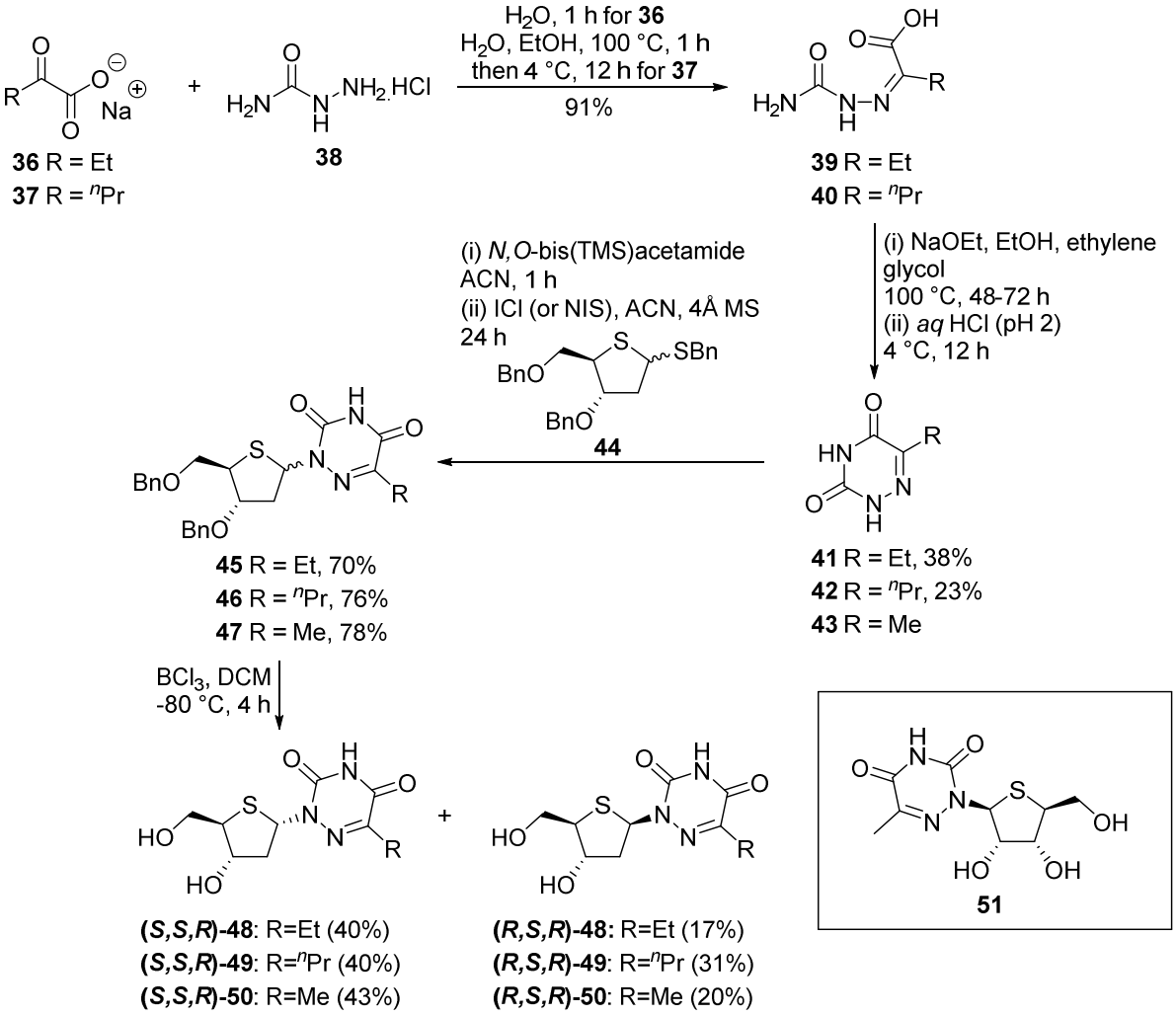
(*S,S,R*)/(*R,S,R*)-34 **(*S,S,R*)/(*R,S,R*)-35**

Figure 1. Novel azacytosine-derived thionucleosides

Table 1: Anti-cancer activity of thionucleoside **(*R,S,R*)-33**

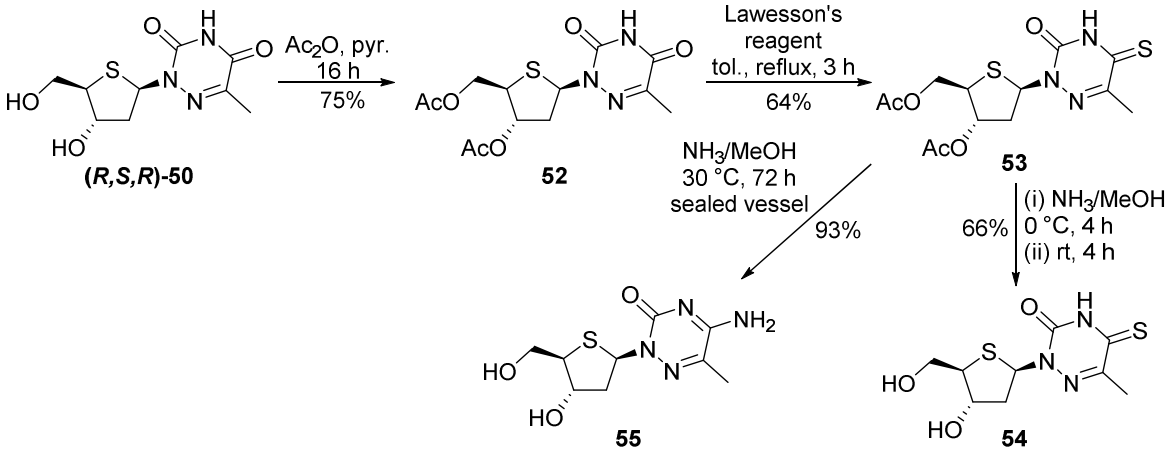
	IC_{50} (μM)					
	CCRF-CEM (leukemia)	CAKI-1 (renal)	DLD-1 (colon)	NCI-H23 (lung)	LOXIMVI (melanoma)	DNB-7 (CNS)
(*R,S,R*)-33	0.01	13.0	7.0	3.1	5.3	1.7

Several research groups have observed that 5-substituted pyrimidines display potent anti-herpes virus activity.[28, 29] In light of these findings, Simons *et al*. designed a library of novel 5-substituted-6-aza-pyrimidine-4´-thionucleosides paying particular attention to the L-thionucleosides (Scheme 5).[30] 5-Ethyl- (**41**) and 5-propyl-6-azapyrimidines (**42**) were prepared from the corresponding 2-keto-carboxylic acids **36** and **37** respectively *via* semicarbazones **39** and **40**. Pyrimidines **41** and **42**, along with commercially available 5-methyl-6-azapyrimidine (**43**), were coupled to 4´-thiosugar **44**, which was itself synthesised using Dyson's methodology.[31] A modified Vorbrüggen reaction was next employed, whereby an iodine-based reagent such as iodine monochloride or *N*-iodosuccinimide (NIS), was used in place of a Lewis acid.[32] Approximately 1:1 ratios of the α- and β-nucleoside products **45-47** were recovered. Following debenzylation with boron trichloride, α- and β-anomers **48-50** were separated by column chromatography, albeit in low yields of the desired β-products. 4´-Thioribose **51** was prepared in a similar manner by the group.



Scheme 5

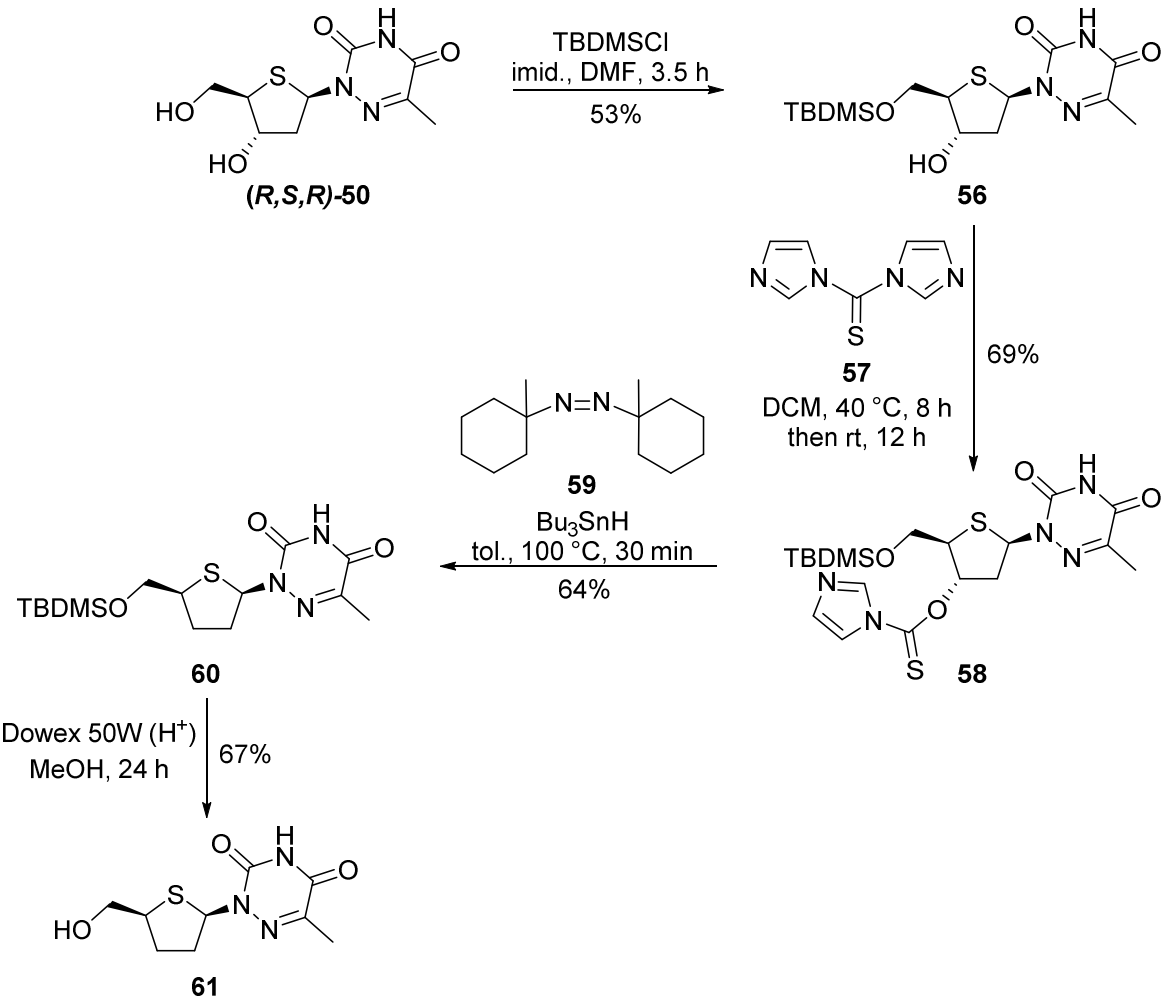
Straightforward manipulation of the 1,6-azathymine **(*R,S,R*)-50** nucleobase provided access to 4-thione **54** and 5-methylcytidine **55** derivatives in good yields (Scheme 6). Acetylation of the 5´- and 3´-hydroxyls furnished **52** which was converted to thione **53** after treatment with Lawesson's reagent in 48% overall yield. Deacetylation of **53** in ammonia solution in methanol at 0 °C produced diol **54**, while amination of **53** to form 5-methylcytidine **55** was achieved at 30 °C in a sealed vessel.



Scheme 6

The five 2-aza-4´-thionucleosides **(*R,S,R*)-48**, **(*R,S,R*)-49**, **(*R,S,R*)-50**, **54** and **55** were tested for biological activity against a range of viruses. None were found to have any activity below toxic concentrations against reovirus-1, coxsackie virus B4, sindbis virus, parainfluenza-3 virus, Punta Toro virus, vesicular stomatitis virus, respiratory syncytial virus or cytomegalovirus. Thionucleoside **(*R,S,R*)-50** was found to have activity comparable to that of acyclovir against various strains of varicella-zoster virus. **(*R,S,R*)-50** also displayed inhibitory activity towards vaccinia virus andherpes simplex virus strains HSV-1 and HSV-2.

In later work, the synthesis and antiviral activity of two related 6-azathymidine-4´-thionucleosides was described.[33] Silylation of the 5´-hydroxyl of **(*R,S,R*)-50** afforded alcohol **56** (Scheme 7). The 3´-hydroxyl was then removed over two steps *via* Barton-McCombie radical deoxygenation to form thiolane **60** in 64% yield.[34] Desilylation of **60** was achieved with an acidic resin to yield 4´-thionucleoside **61**.

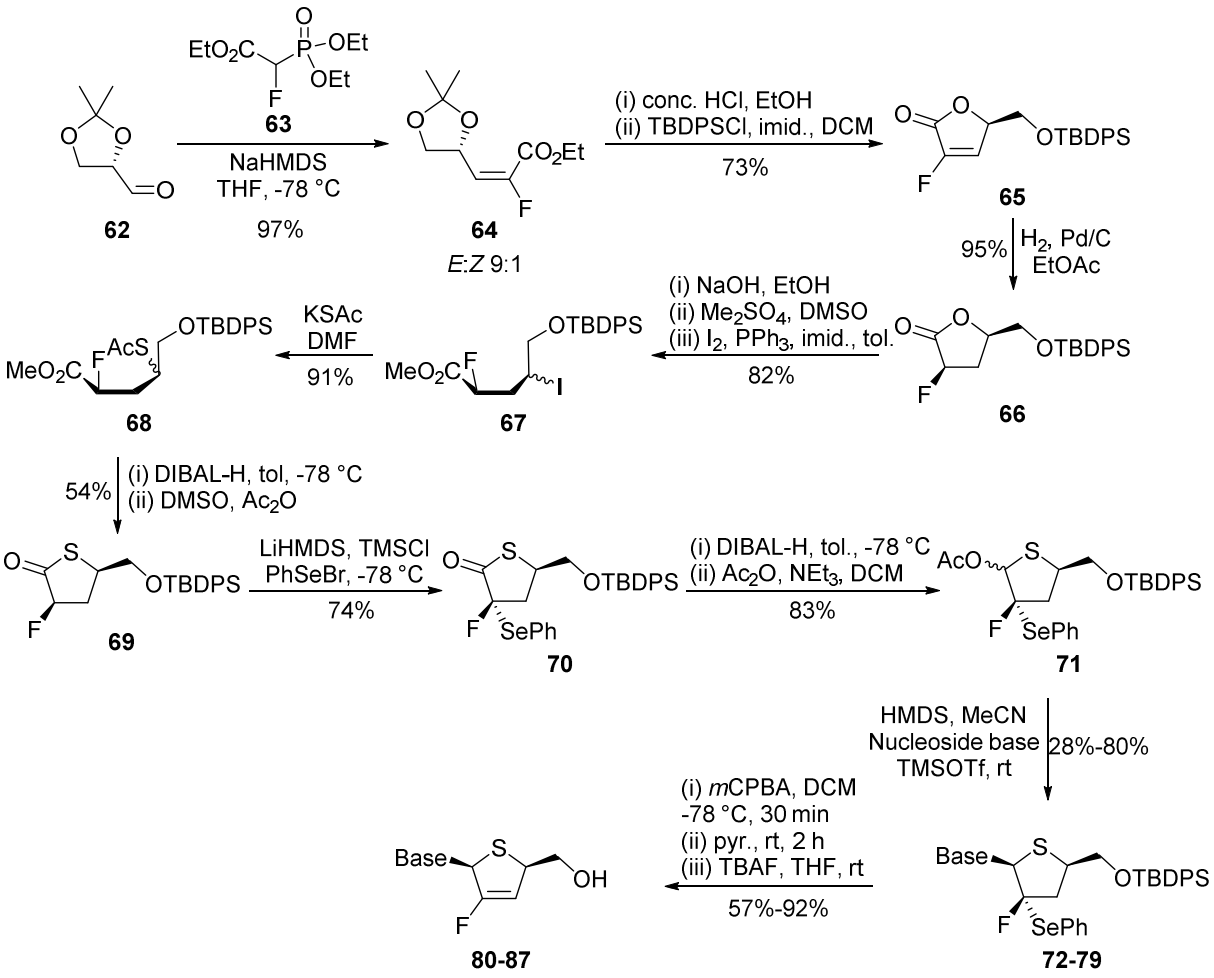


Scheme 7

Following biological testing, thiolane **61** exhibited moderate activity towards vaccinia virus, HSV-1 and HSV-2. Most interestingly, its activity was retained against the thymidine kinase-deficient HSV-1 TK^- viral strain, suggesting its activity is not dependent on viral thymidine kinase-catalysed phosphorylation.

In their search for compounds with anti-HIV activity, Chu *et al.* synthesised a range of 2´,3´-dideoxy-2´,3´-didehydro-2´-fluoro-4´-thionucleosides (Scheme 8).[35] A modified Horner-Emmons reaction was performed on aldehyde **62** using fluorinated phosphonate **63** to produce **64** in a 9:1 ratio of *E*- and *Z*-isomers. Hydrolysis of acetal **64** in concentrated hydrochloric acid was accompanied by *in situ* cyclisation. Subsequent

silylation of the 5´-hydroxyl group afforded **65**. Reduction of alkene **65** was followed by ring-opening, methylation and Garegg-Samuelsson-type iodination to afford iodo-ester **67**. Nucleophilic substitution with thioacetate allowed for the introduction of sulfur. Reduction of **68** with DIBAL-*H* promoted cyclisation to form a β-thiolactol which was subsequently oxidised to β-thiolactone **69** in a Moffatt-type reaction. The minor isomer of **69** was also formed in 6% yield and was found to be separable by column chromatography. Introduction of a 2´-phenylselenyl group was used as a means of directing β-selectivity during the glycosylation. The silyl enol ether of **69** was formed *in situ*, and subsequently reacted with phenylselenyl bromide to furnish **70**. Reduction of β-thiolactone **70** was followed by *in situ* acetylation to form glycosyl-donor **71**. Condensation of acetate **71** with various nucleobases was conducted under Vorbrüggen conditions to exclusively furnish β-anomers **72-79** (Table 2), due to the bulky α-phenylselenyl group directing stereochemistry. Selenide oxidation with *m*CPBA was followed by spontaneous *syn*-elimination of the resulting selenoxide. The 5´-silyl protecting group was removed in the usual manner to produce target nucleosides **80-87**.



Scheme 8

Table 2: Yields of selenides **72**-**79** and thionucleosides **80**-**87**

Entry	Selenide	Base	Yield	Thionucleoside	Yield
1	**72**	Cytosine	80%	**80**	88%
2	**73**	5-Fluorocytosine	73%	**81**	88%
3	**74**	Uracil	68%	**82**	92%
4	**75**	Thymine	76%	**83**	91%
5	**76**	Adenine	86%	**84**	57%

6	**77**	Hypoxanthine	86%	**85**	81%
7	**78**	Guanine	86%	**86**	61%
8	**79**	2-Fluoroadenine	28%	**87**	70%

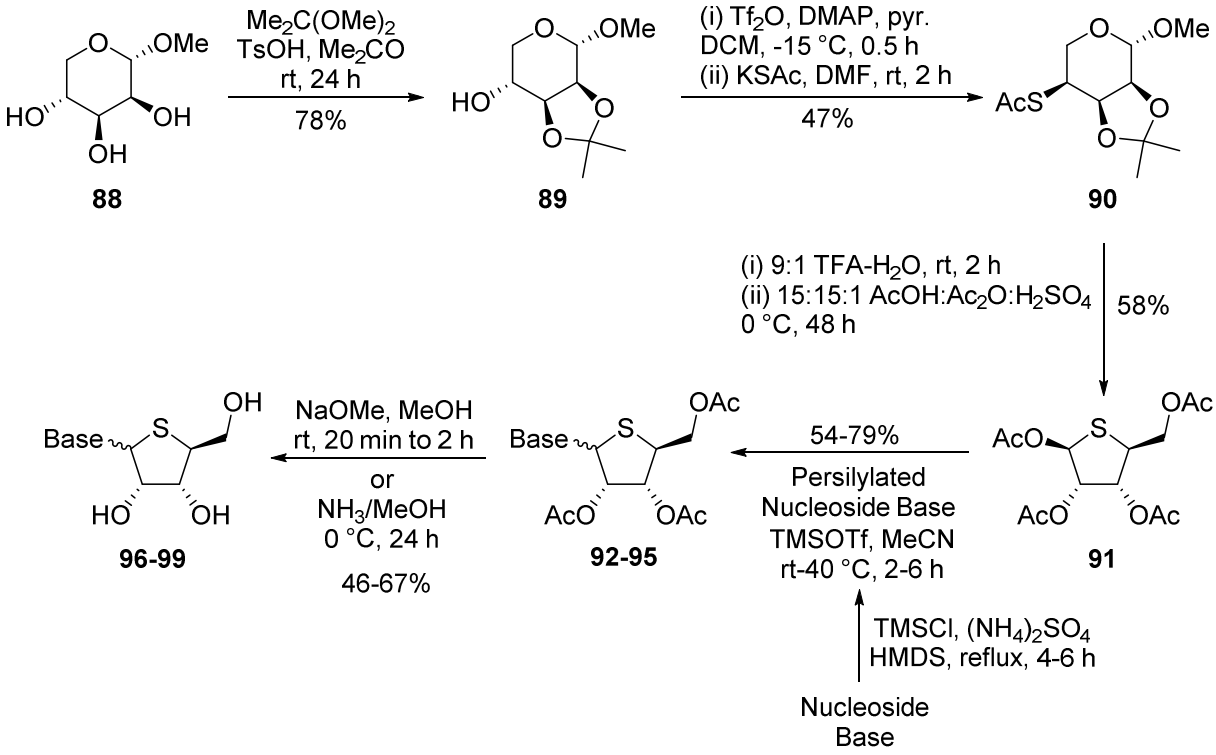
The authors also conducted molecular modelling studies to elucidate possible structure activity relationships, and found that although the 4´-sulfur is well tolerated in place of oxygen, steric hindrance is increased due to the unnatural L-configuration of the sugar. The phenomenon can be observed where the β-branched chain of valine in M184V-mutant HIV-strains confers cross-resistance to the NRTI lamivudine. From the biological data presented in Table 3, it is apparent that cytosine **80**, 5-F-cytosine **81** and adenine **84** display anti-HIV-1 activity in peripheral blood mononuclear (PBM) cells comparable to the NRTI zidovudine (AZT). These compounds were also found to be non-cytotoxic.

Table 3: Anti-HIV activity of thionucleosides **80-87** compared to AZT

Entry	Thionucleoside	Anti-HIV-1 activity (EC_{50}, μM)	Cytotoxicity (IC_{50}, μM)		
		PBM	PBM	CEM	Vero
1	**80**	0.12	>100	>100	>100
2	**81**	0.15	>100	>100	>100
3	**82**	>100	>100	>100	>100
4	**83**	>100	>100	>100	>100
5	**84**	1.7	>100	>100	>100
6	**85**	15.5	>100	>100	>100
7	**86**	43.5	>100	41.5	66.4
8	**87**	11.5	13.0	10.4	66.1

9	AZT	0.004	>100	29.0	14.3

Several novel 4´-thio-L-ribonucleosides have been synthesised by Pejanovic and co-workers, and subsequently evaluated for their anti-tumour activity.[20] α-D-Lyxopyranoside (**88**) (Scheme 9) was initially prepared using the route outlined by Reist.[8, 36] Following selective alcohol protection of **88**, acetal **89** was converted to thioacetate **90** *via* its triflic ester, using potassium thioacetate in a nucleophilic displacement reaction. Hydrolysis of the acetal protecting group afforded a mixture of products that were subjected to acetylation to form thiophene derivative **91**, exclusively as the β-anomer. Addition of a persilylated base to sugar **91** and trimethylsilyl triflate afforded the corresponding acetoxy-protected nucleosides **92-95** in yields of 48-79% (Table 4). Alcohol deprotection was achieved by stirring in sodium methoxide and methanol, or in the case of thioguanidine **95**, methanolic ammonia.



Scheme 9

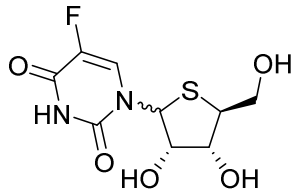
Table 4: Yields for the conversion of **91** to **92-95** and subsequent deprotection to **96-99**

Entry	Base	Protected nucleoside	Yield	Deprotected nucleoside	Yield
1	5-Fluorouracil	**92**	79%	**96**	49%
2	5-Fluorocytosine	**93**	59%	**97**	67%
3	Thymine	**94**	54%	**98**	46%
4	6-Thioguanine	**95**	48%	**99**	90%

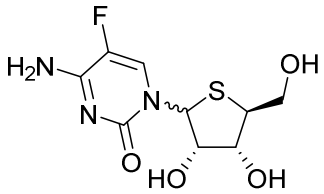
In total, four thionucleosides were prepared and tested for *in vitro* cytotoxicity to C6 rat glioma, HTB14 human glioma, HeLa human cervical carcinoma, NB4 leukemia, T47D breast cancer and normal human dermal fibroblast (NHDF) cell lines (Table 5). Analgoues **97** and **99** are believed to be the first nucleoside analogues with growth

stimulatory activity (GSA) towards NB4 and T47D cells. None of the candidates exhibited growth inhibitory effects on the NHDF cell line at any of the tested concentrations, consistent with findings that L-nucleoside analogues are ordinarily less toxic to normal cells due to native cellular enzymes not recognising them.

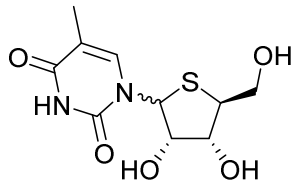
Table 5: *In vitro* cytotoxicity of thionucleosides **96-99**



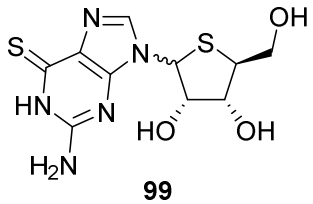
96



97



98

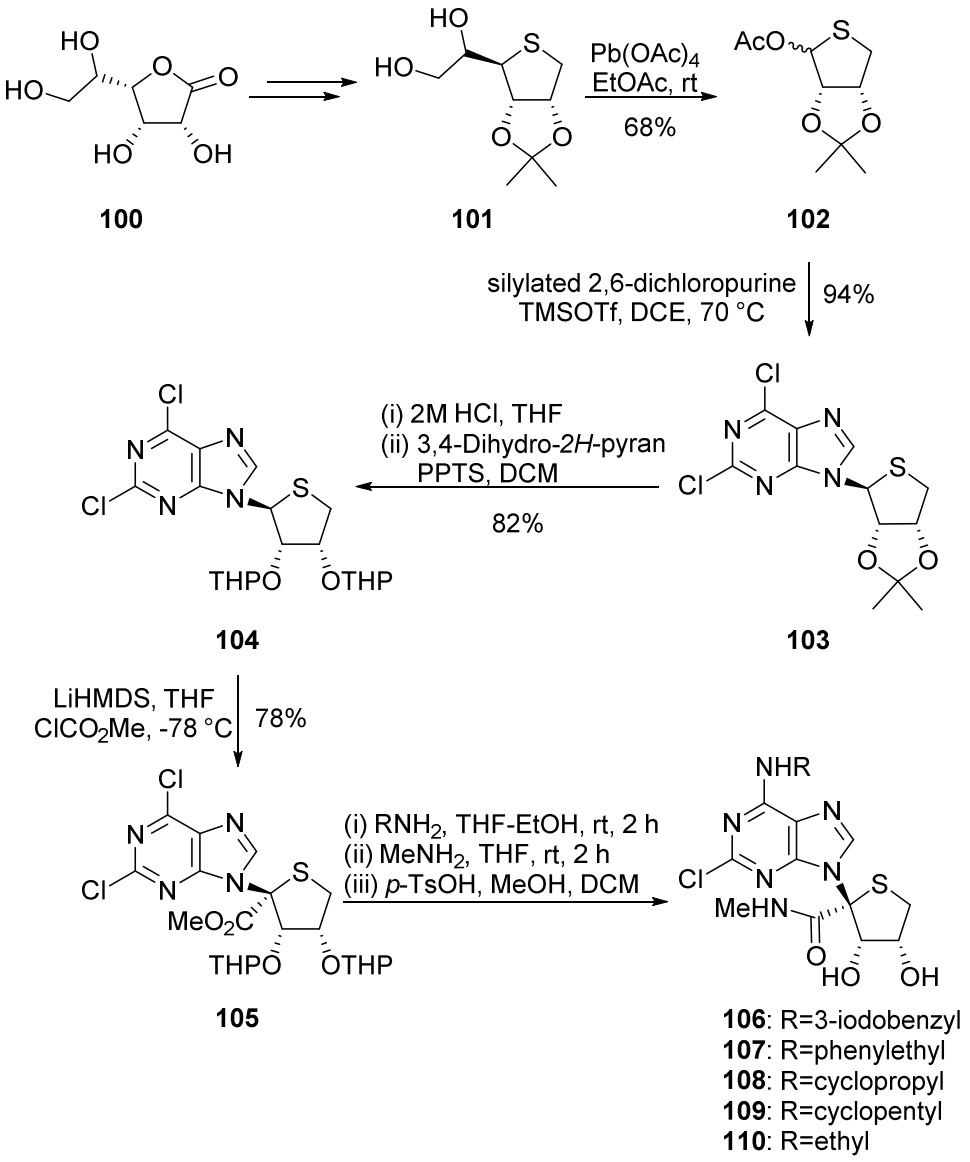


99

Entry	Thionucleoside	IC_{50} µM					
		C6	HTB14	HeLa	NB4	T47D	NHDF
1	**96**	>100	41.5	>100	>100	>100	>100
2	**97**	>100	83.3	95.9	GSA	GSA	>100
3	**98**	>100	67.8	100	>100	>100	>100
4	**99**	>100	62.1	>100	>100	GSA	>100

A library of 1´-α-substituted-4´-thionucleosides (**106-110**) was created by Jeong and colleagues and assessed for their ability to bind with the adenosine A_3 receptor (Scheme 10).[37] Diol **101** was prepared over four steps from D-gulonic-γ-lactone (**100**) using a previously published methodology.[38] Treatment of diol **101** with one equivalent of lead tetraacetate at 0 °C produced the corresponding aldehyde. By contrast, repeating the

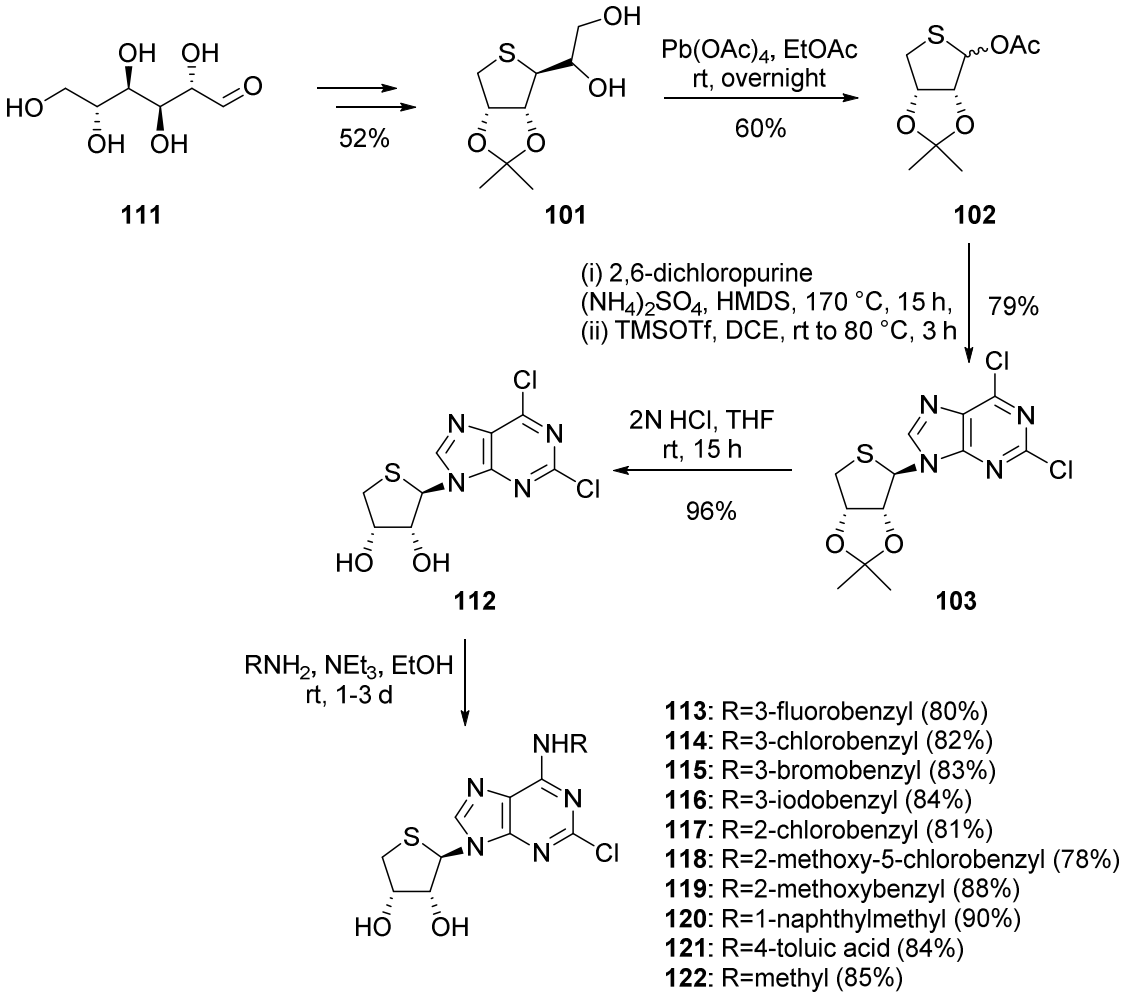
reaction with excess reagent at room temperature instead afforded acetate **102**, most likely *via* a sulfonium ion intermediate. Condensation of **102** with persilylated 2,6-dichloropurine, employing trimethylsilyl triflate as a Lewis acid catalyst, produced **103** as a single stereoisomer. Transformation of the isopropylidene group to ditetrahydropyranoether **104** was carried out to prevent deglycosylation during the final deprotection step. Deprotonation with lithium hexamethyldisilazide, followed by addition of methyl chloroformate, furnished **105** as a single stereoisomer. The authors attribute this high stereoselectivity to the formation of a stable 5-membered ring *via* 1´-lithium co-ordination to N^3, blocking β-attack by the electrophile. Finally, a range of 1´,2´-substituted-4´-thionucleosides **106-110** were synthesised *via* selective N^6-amination of **105**, followed by conversion of the methyl ester group to the methyl amide moiety and deprotection of the 2´- and 3´-alcohol groups by *para*-toluenesulfonic acid. Yields of the final products were not reported.



Scheme 10

The same group prepared a library of apiothionucleosides lacking a 5´-functionality as potential Human A_3 AR ligands.[39] Diol **101**, synthesised in good yield over five steps from D-mannose (**111**), was converted to glycosyl donor **102** with an excess of lead tetraacetate (Scheme 11). Acetal **102** underwent Vorbrüggen-type glycosylation with persilylated 2,6-dichloropurine in the presence of trimethylsilyltriflate. The acetal group of nucleoside **103** was removed to form diol **112** in excellent yield. Synthesis of target

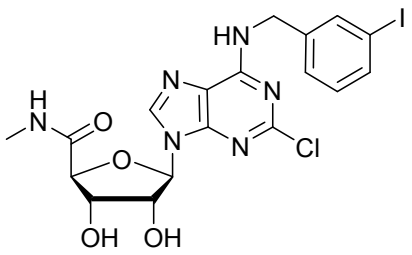
nucleosides **113-122** was achieved by treatment of **112** with various substituted alkylamines and arylalkylamines to afford the final products in good yields.



Scheme 11

The binding affinities of compounds **113-122** were measured and compared to that of known agonist Cl-IB-MECA (**123**) (Table 6). While most of the compounds exhibited high binding affinity at the human A_3 AR, 3-chlorobenzyl analogue **114** displayed the highest, and most selective, binding affinity (Entry 2). The results show that 3-substitution of the aromatic ring is preferable to either 2- or 4-substitution.

Table 6: Binding Affinities of **113-123** at three Human Adenosine Receptors subtypes

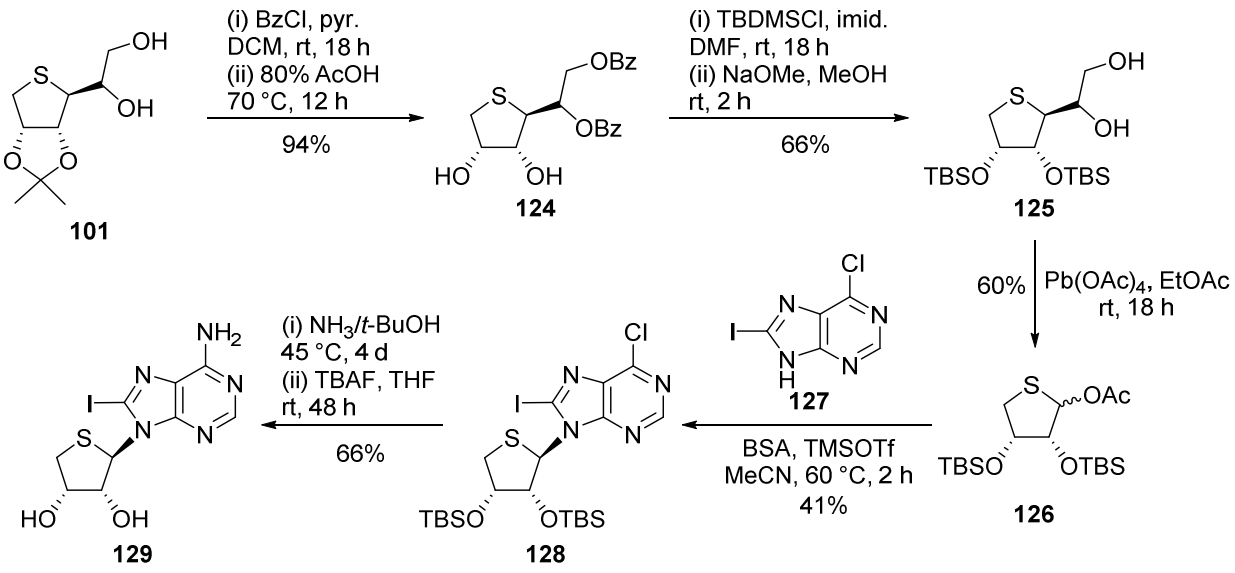


Cl-IB-MECA
123

Entry	Compound	Affinity, K_i, nM ± SEM (or % inhibition)		
		hA_1	hA_{2A}	hA_3
1	**113**	20%	48%	7.4 ± 1.3
2	**114**	38%	18%	1.66 ± 0.90
3	**115**	34%	18%	8.99 ± 5.17
4	**116**	2490 ± 940	341 ± 75	4.16 ± 0.50
5	**117**	13%	1600 ± 135	25.8 ± 6.3
6	**118**	24%	4020 ± 1750	12.7 ± 3.7
7	**119**	9%	18%	19.9 ± 7.1
8	**120**	22%	-8%	24.8 ± 8.1
9	**121**	13%	0%	42%
10	**122**	55.4 ± 1.8	45.0 ± 1.4	3.69 ± 0.25
15	**123**(Cl-IB-MECA)	222 ± 22	5360 ± 2470	1.4 ± 0.3

In a continuation of this work, the authors synthesised and tested HSP90 (heat shock protein) inhibitors for their potential application in cancer treatment.[40] Initial protection of diol **101** was followed by cleavage of the acetal to form **124** (Scheme 12). Silylation of diol **124** and subsequent debenzoylation furnished **125** in 66% yield. Treatment of diol **125** with lead tetraacetate resulted in the formation of acetate **126**. Glycal donor **126**

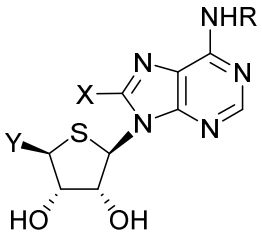
underwent Vorbrüggen-type glycosylation with *in situ* persilylated purine **127** in the presence of trimethylsilyltriflate. Amination at C-6 was achieved with ammonia in *tert*-butanol, and final desilylation with TBAF afforded **129**, an 8-iodo-adenine derivative. Analogues **130-151** were all prepared in a similar manner (Table 7).



Scheme 12

All twenty-three compounds were tested for HSP90 inhibitory activity, but only **129** was found to be active, albeit weakly so (Table 7, Entry 1). The library was also tested for anticancer activity on five different cell lines: A549 (human lung cancer), Caki-1 (human renal cancer), HCT116 (human colon cancer), MDA-MB-231 (human breast cancer) and SNU638 (human stomach cancer). Interestingly, the most potent anticancer activity across all cell lines was again seen in **129**, which lacks a 5´-hydroxyl group essential for cellular phosphorylation and therefore cannot be directly incorporated into a DNA chain. This would suggest that an alternate mechanism of action is at play. The results of the biological evaluation confirm that the 8-iodo-4´-thioadenosine analogues, in particular, have potential as anticancer agents.

Table 7: Anticancer activity of thionucleosides **129-151** against several tumour cell lines

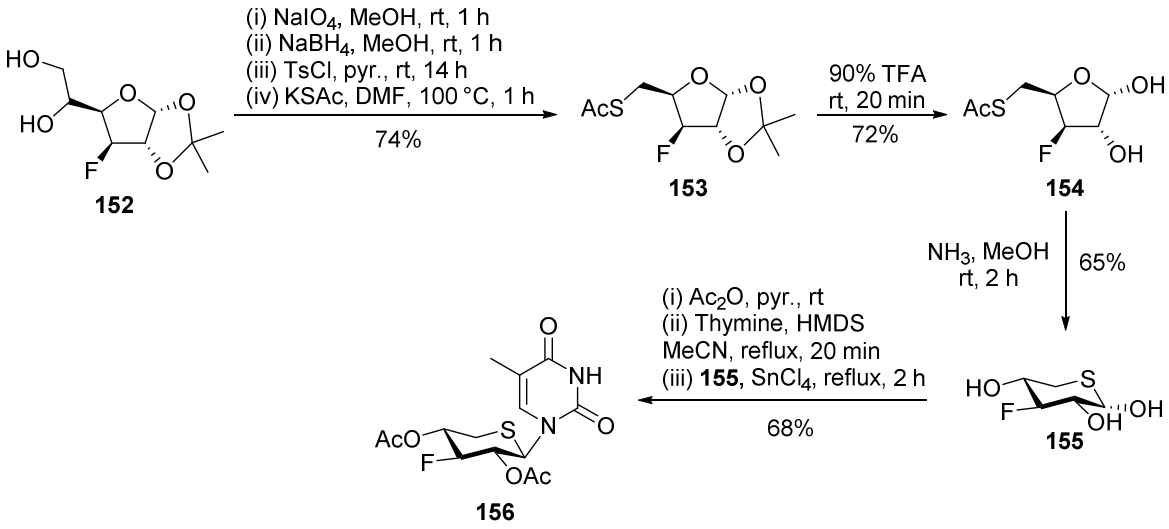


129-151

Entry	Compound	X	Y	R	IC_{50} (µM)				
					A549	Caki-1	HCT116	MDA-MB-231	SNU638
1	**129**	I	H	H	7.85	6.14	4.56	6.3	3.99
2	**130**	Furanyl	CH_2OH	H	>100	>100	>100	>100	>100
3	**131**	Thiofuranyl	CH_2OH	H	>100	66.3	>100	>100	>100
4	**132**	Piperidinyl	CH_2OH	H	>100	>100	>100	>100	>100
5	**133**	Phenyl	CH_2OH	H	>100	>100	>100	>100	>100
6	**134**	$CH_3(CH_2)_2NH$	CH_2OH	H	>100	>100	99.45	90.72	99.68
7	**135**	N_3	CH_2OH	H	>100	>100	>100	>100	>100
8	**136**	NHMe	CH_2OH	H	>100	>100	>100	>100	>100
9	**137**	I	CH_2OH	H	42.32	50.53	22.24	8.18	15.94
10	**138**	I	CH_2OBz	H	38.54	19.06	15.89	21.17	34.51
11	**139**	Furanyl	H	H	99.64	55.2	99.76	67.39	99.67
12	**140**	Furanyl	H	Me	>100	>100	>100	>100	>100
13	**141**	Thiofuranyl	H	H	>100	>100	>100	>100	>100

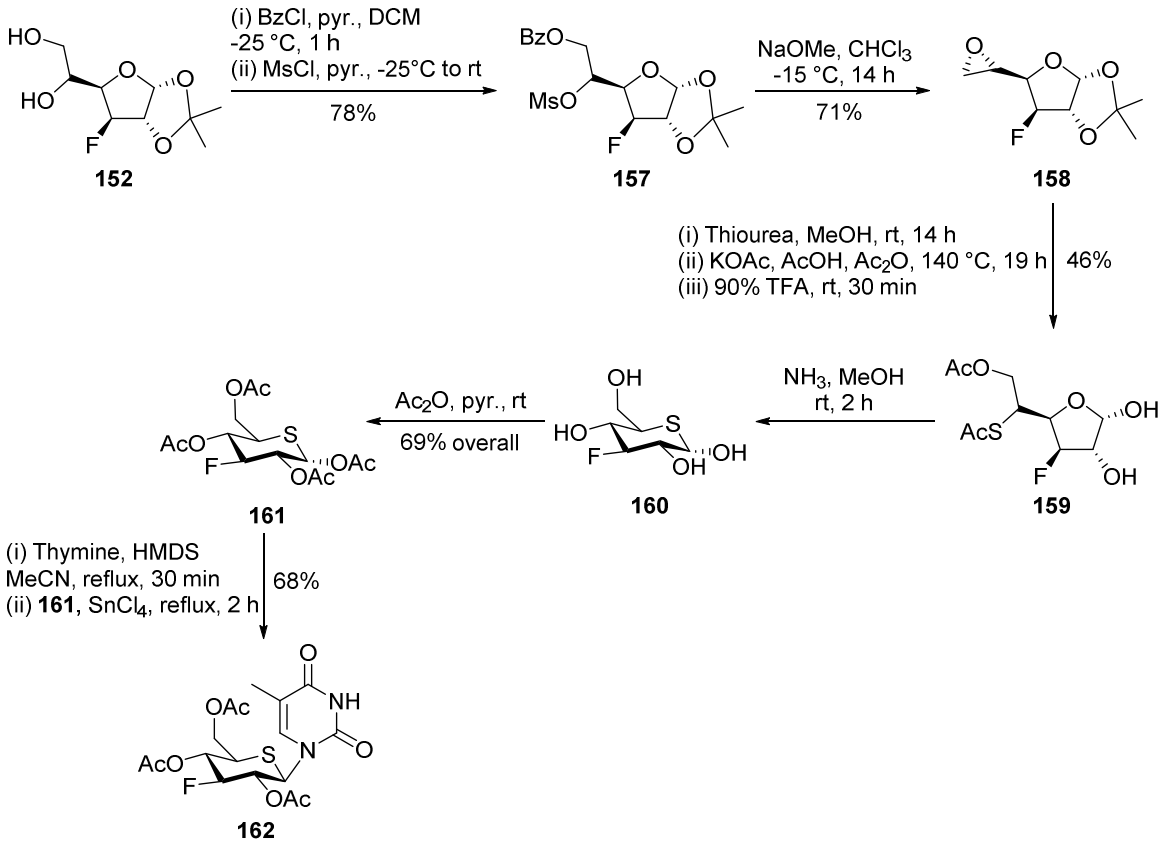
14	**142**	Thiofuranyl	H	Me	>100	>100	>100	>100	>100
15	**143**	N_3	H	H	>100	>100	>100	>100	>100
16	**144**	NHMe	H	H	>100	>100	>100	>100	>100
17	**145**	$CH_3(CH_2)_2NH$	H	H	>100	>100	>100	>100	>100
18	**146**	BnNH	H	H	>100	>100	>100	>100	>100
19	**147**	Piperonyl- amino	H	H	>100	>100	>100	>100	>100
20	**148**	Piperidinyl	H	H	>100	>100	>100	>100	>100
21	**149**	Furanyl	H	Cl	11.1	70.85	69.79	51.13	44.47
22	**150**	Thiofuranyl	H	Cl	9.35	49.92	48.9	62.39	27.78
23	**151**	N_3	H	Cl	>100	>100	>100	>100	>100

A series of novel, fluorinated thiopyranonucleosides have been prepared by Komiotis *et al*. (Scheme 13).[41] Oxidative cleavage of diol **152** was followed by reduction, tosylation and treatment with thioacetate to furnish **153** in 74% yield overall.[42] Cleavage of the acetal in **153**, followed by hydrolysis of the thioacetate, produced a thiol which underwent *in situ* cyclisation to the 6-membered thiopyranose ring **155**. Using Vorbrüggen glycosylation conditions, addition of presilylated thymine to acetylated **155** in the presence of tin tetrachloride produced nucleoside **156** solely as the β-product.



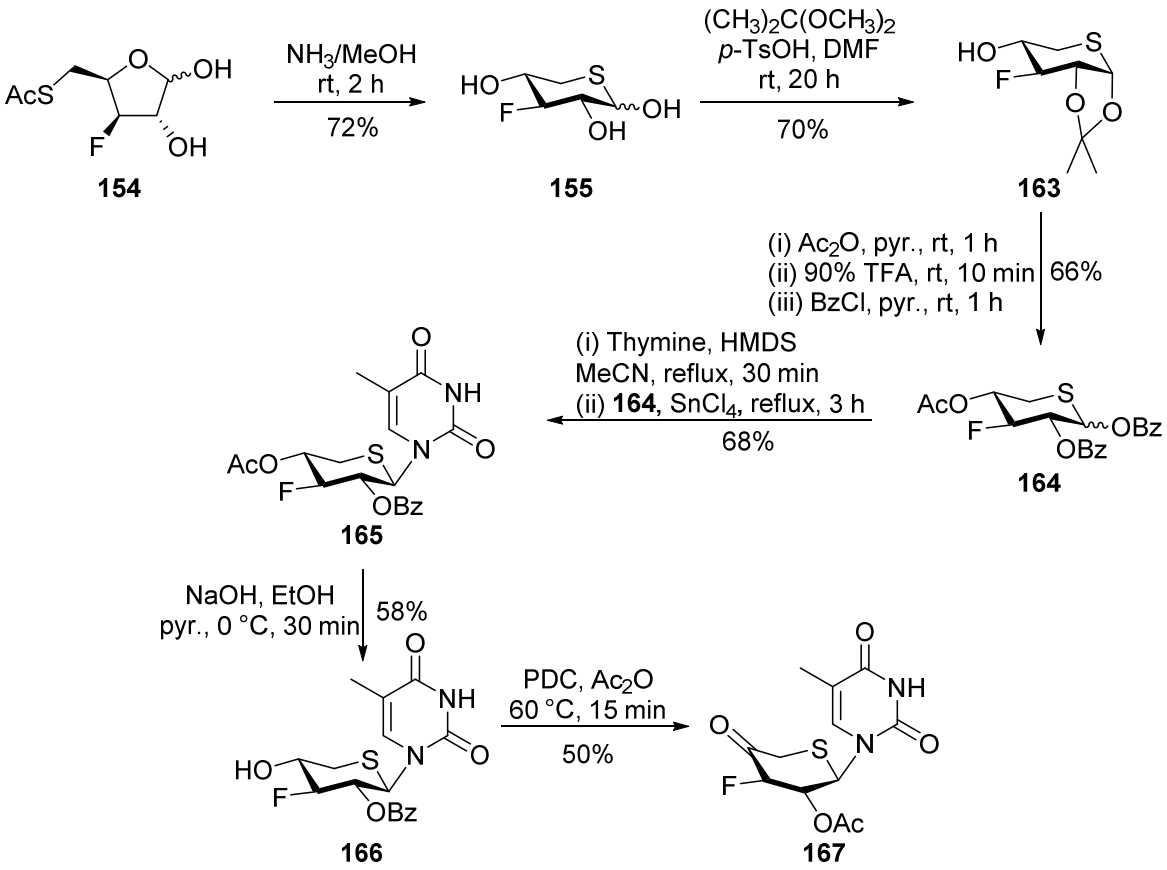
Scheme 13

Synthesis of 5-thioglucopyranose derivative **161** was achieved in a similar manner from **152** (Scheme 14). Selective benzoylation of the primary alcohol was followed by mesylation of the secondary alcohol to produce **157**. Addition of sodium methoxide promoted epoxide formation affording **158**. Epoxide **158** was converted to a thiirane intermediate, which was then subjected to acetolysis and subsequent acetal hydrolysis to furnish furanose **159** in 46% overall yield. The final steps of the synthesis are the same as for 5-thioxylopyranose **156**. 5-Thioglucopyranose **162** was isolated exclusively as the β-anomer.



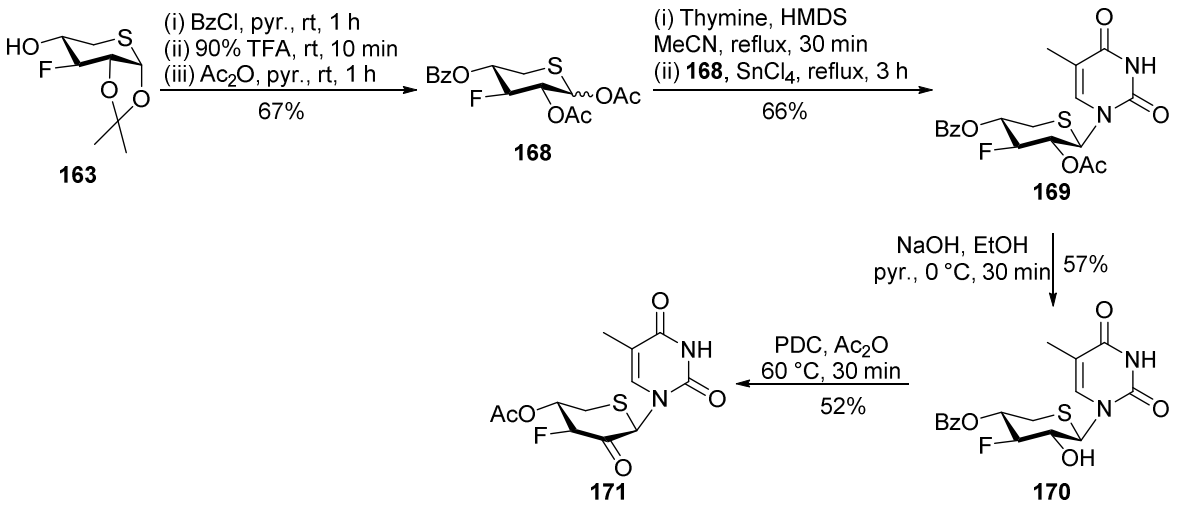
Scheme 14

In later work, Komiotis *et al.* described the synthesis of 2´-keto- and 4´-keto-xylopyranothymidine analogues (Scheme 15).[43] Deacetylation of 3´-deoxy-3´-fluoro-5´-*S*-acetyl-5´-thio-D-xylofuranose (**154**) and concomitant rearrangement to thiopyranose **155**, was followed by acetalisation at the 1´- and 2´-positions to provide **163**. Alcohol **163** was converted to its acetate, then the acetal protecting group was removed and replaced with benzoyl esters *as per* glycosyl donor **164**. Subsequent Vorbrüggen condensation was conducted on persilylated thymine and **164** in the presence of tin tetrachloride to form **165** exclusively as the β-anomer. Selective 4´-deacetylation followed by pyridinium dichromate (PDC) oxidation afforded **167** in 50% yield.



Scheme 15

Synthesis of the 2´-keto derivative **171** was achieved in a mostly similar manner from key intermediate **163** (Scheme 16). **163** was initially benzoylated at the 4´-position, and the 2´- and 3´-hydroxyl groups were acetylated following acetal deprotection. Acetate **168** then underwent Vorbrüggen-type glycosylation, followed by 2´-deacetylation and subsequent oxidation to form 2´-keto analogue **171**. The authors did not reveal results from biological testing.



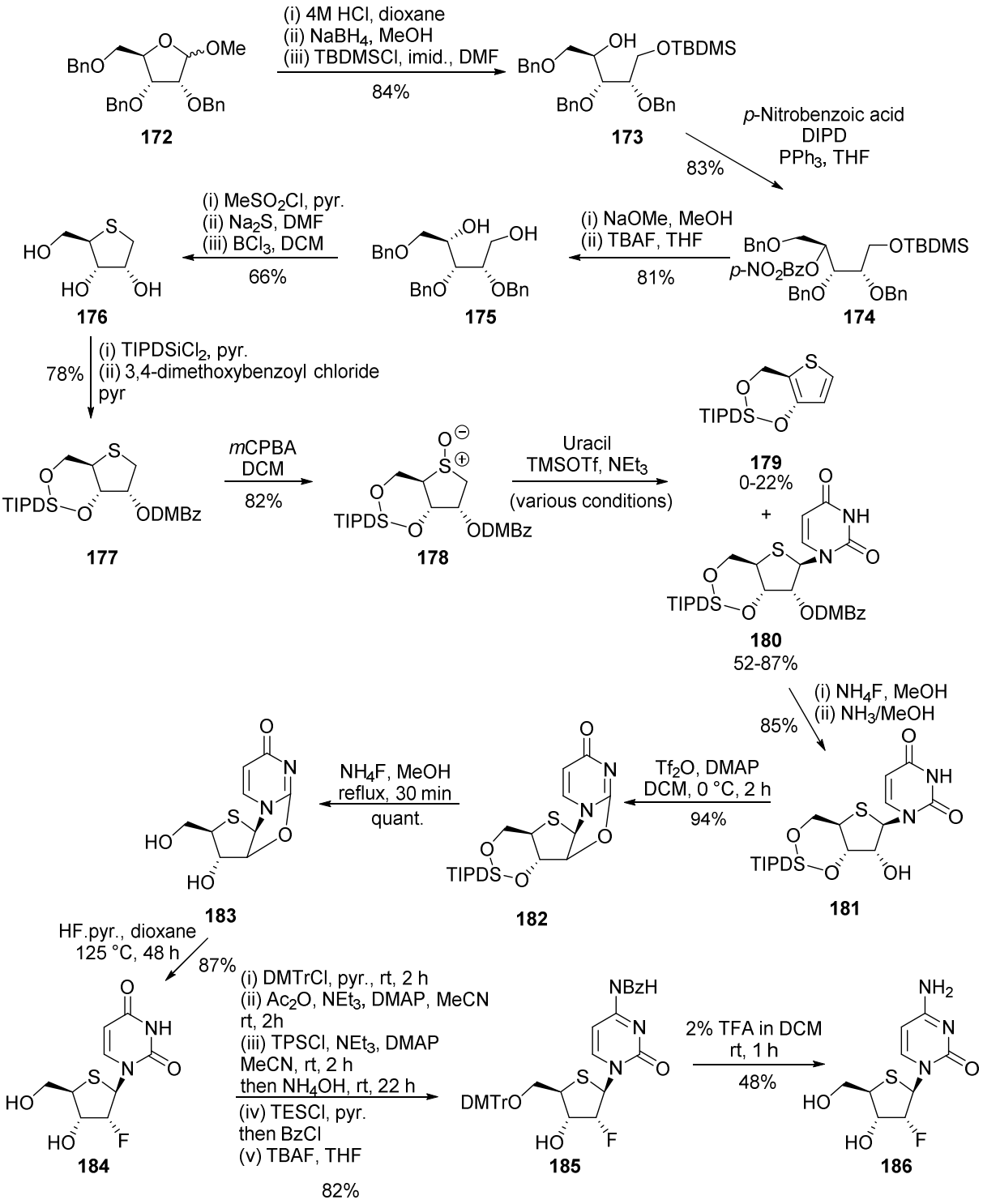
Scheme 16

The synthesis of 2´-deoxy-2´-fluoro-4´-thioribonucleosides and 2´-modified-4´-thioribonucleosides was described by Matsuda *et al*. in separate papers in 2008 and 2009 respectively.[44, 45] They employed a previously optimised methodology based on the Pummerer reaction (Scheme 17).[46] Acidic hydrolysis of ribose **172** was followed by reduction of the resultant lactol by sodium borohydride. The resulting diol was selectively protected at the primary position, leading to formation of silane **173** in 84% yield. A subsequent Mitsunobu reaction with *p*-nitrobenzoic acid afforded L-lyxose derivative **174**. Deprotection of the *p*-nitrobenzoyl group was followed by removal of the silyl group to form diol **175**, which was *bis*-mesylated and then treated with sodium sulfide to furnish thioribose analogue **176** in 53% overall yield. Protection of the 3´- and 5´-alcohols with a TIPDS ether group was followed by 2´-dimethoxybenzoyl protection to ensure β-selectivity during glycosylation. Oxidation of sulfide **177** with *m*CPBA resulted in formation of sulfoxide **178** as a mixture of diastereomers.

Glycosylation of **178** was achieved using the Pummerer method with persilylated uracil to give β-uridine analogue **180**, as well as thiophene **179**. The yield of **180**, as well as production of **179**, was dependent on reaction conditions. The best results were achieved when only ***R*-178** was employed in the reaction, resulting in an 87% yield of **180** and no by-product formation. Treatment of **180** with ammonium fluoride, followed by addition

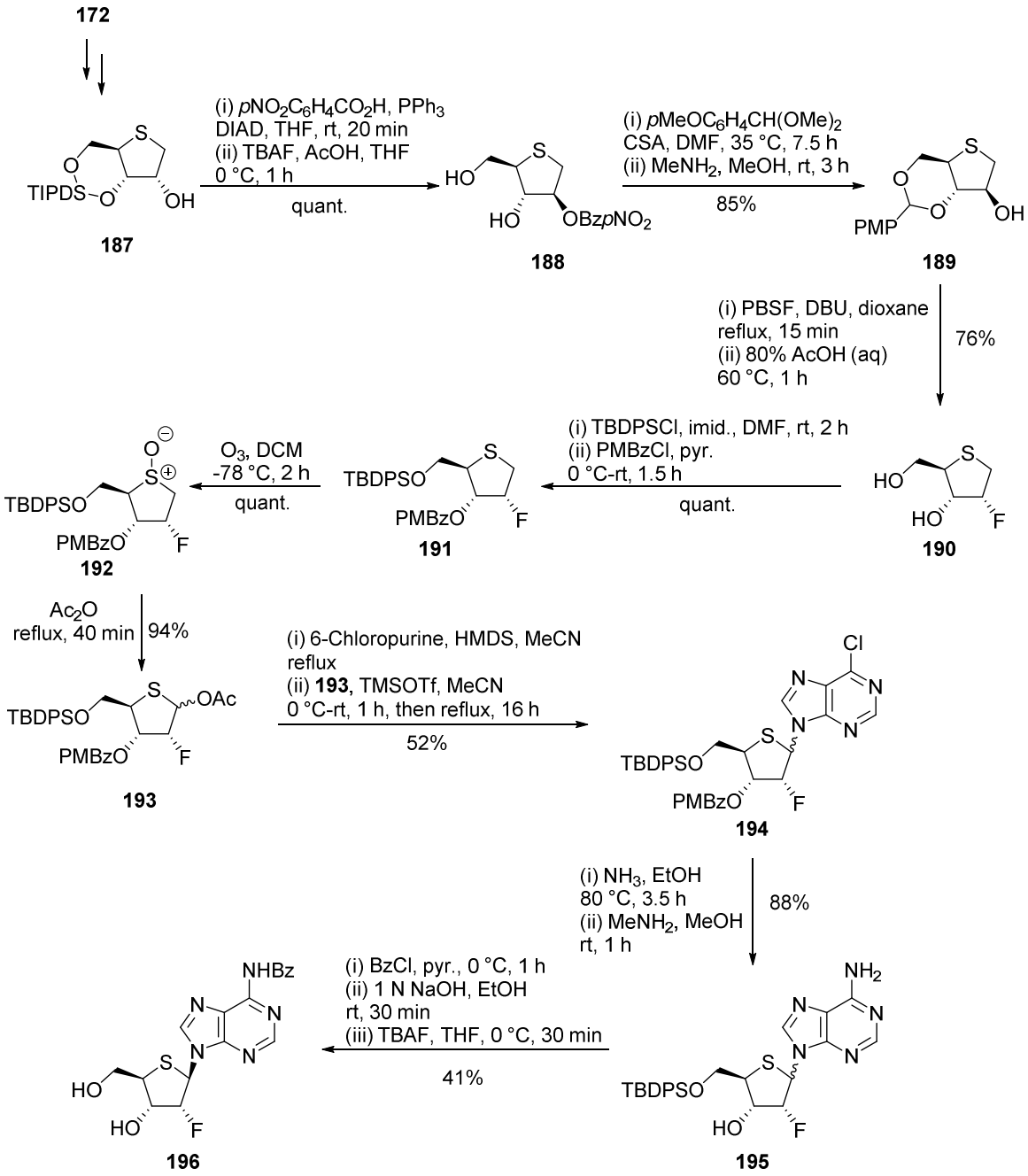
of methanolic ammonia, resulted in deprotection of the 2´-alcohol to form **181** in 85% yield. In order to incorporate a 2´-fluorine, **181** was first treated with triflic anhydride in the presence of DMAP to form imine **182** *via* loss of the 2´-hydroxyl. Removal of the silyl protecting group in ammonium fluoride afforded **183**, which was then fluorinated at the 2´-position upon addition of hydrogen fluoride pyridine, producing 2'-deoxy-2´-fluoro-4´-thiouridine (**184**) in 87% yield.

To prepare the cytidine analogue, the 5´-hydroxyl of **184** was first protected with a dimethoxytrityl group, and the 3´-hydroxyl was subsequently acetylated. Subsequent treatment with triphenylsilyl chloride and addition of ammonium hydroxide facilitated the introduction of the 6-amine and simultaneous deprotection of the 3´-hydroxyl group. The 3´-hydroxyl was next silylated to allow for selective benzoylation of the 6-amine. Removal of the 3´-silyl group was achieved in the usual manner with TBAF to form benzoylamine **185**. Finally, global deprotection of **185** with TFA afforded cytidine analogue **186** in 48% yield.


(i) 4M HCl, dioxane
(ii) NaBH4, MeOH
(iii) TBDMSCl, imid., DMF
84%
172
173
p-Nitrobenzoic acid
DIPD
PPh3, THF
83%
174
(i) NaOMe, MeOH
(ii) TBAF, THF
81%
175
(i) MeSO2Cl, pyr.
(ii) Na2S, DMF
(iii) BCl3, DCM
66%
176
78%
(i) TIPDSiCl2, pyr.
(ii) 3,4-dimethoxybenzoyl chloride
pyr
177
mCPBA
DCM
82%
178
Uracil
TMSOTf, NEt3
(various conditions)
179
0-22%
180
52-87%
85%
(i) NH4F, MeOH
(ii) NH3/MeOH
181
Tf2O, DMAP
DCM, 0 °C, 2 h
94%
182
NH4F, MeOH
reflux, 30 min
quant.
183
HF.pyr., dioxane
125 °C, 48 h
87%
184
(i) DMTrCl, pyr., rt, 2 h
(ii) Ac2O, NEt3, DMAP, MeCN
rt, 2h
(iii) TPSCl, NEt3, DMAP
MeCN, rt, 2 h
then NH4OH, rt, 22 h
(iv) TESCl, pyr.
then BzCl
(v) TBAF, THF
82%
185
2% TFA in DCM
rt, 1 h
48%
186

Scheme 17

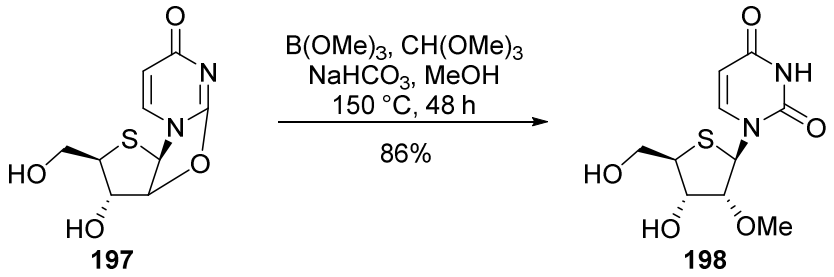
Synthesis of the adenine analogue proved more challenging. The adenine derivative of **180** could not be prepared in sufficient quantities to continue with this route. Thus, an alternate approach was taken involving the Vorbrüggen method (Scheme 18). Inversion of the 2´-hydroxyl group of **187** was achieved by way of a Mitsunobu reaction which was followed by a swap of the silyl protecting group to an acetal prior to fluorination. Alcohol **189** was then treated with perfluoro-1-butanesulfonyl fluoride (PBSF) in the presence of DBU to afford 2´-α-fluorinated **190** in 88% yield. To prepare glycosyl bond donor **193**, the 5´-hydroxyl was protected with a *tert*-butyldiphenylsilyl group, and likewise the 3´-hydroxyl as a *para*-methoxybenzoyl ester, in order to provide efficient neighbouring group participation. Sulfide **191** was oxidised to sulfoxide **192** with ozone. Acetylation of **192** afforded **192** which was subjected to Vorbrüggen conditions with silylated 6-chloropurine. N^9-Anomer **194** was initially generated in only 9% yield, with the N^7-anomer seemingly favoured in this reaction. Heating the mixture of N^9- and N^7-isomers resulted in the partial isomerisation of the N^7-isomer to the desired N^9-isomer after 16 hours. Once separated, the N^7-isomer was resubjected to the Vorbrüggen reaction conditions to afford the N^9-isomer **194** in 40% yield. The combination of these efforts resulted in an overall 52% yield of **194** from **193**. Heating of **194** in ammonia solution in ethanol effected the conversion of the 6-chloropurine to the adenosine analogue, and subsequent removal of the *para*-methoxybenzoyl group furnished **195** in 88% yield. Benzoylation of the 6-amino group enabled separation of the α- and β-anomers by flash chromatography. Finally, the β-isomer was deprotected with TBAF to yield 2´-deoxy-2´-fluoro-4´-thioadenosine analogue **196** in 41% yield.



Scheme 18

In 2009, a follow-up study by Matsuda *et al*. outlined the synthesis of very similar compounds, with a 2´-methoxy replacing the 2´-fluoro substituent.[45] The targets were synthesised in the same manner as described in Scheme 3, with the methoxy group

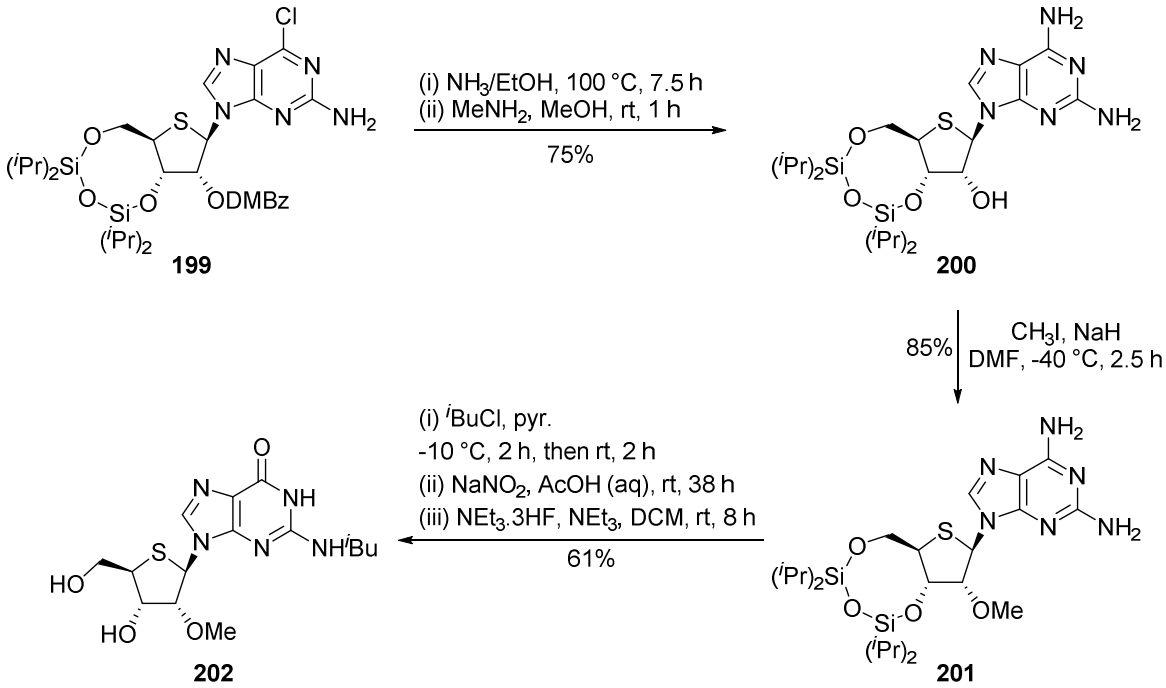
arising from the *in situ* generation of an active nucleophile by the reaction of trimethyl borate and trimethyl *ortho*-formate (Scheme 19).



Scheme 19

For the purine derivatives, direct methylation of the 2´-hydroxyl group was instead employed (Scheme 20). Methylation of **200** afforded 2´-methoxy analogue **201**. Selective protection of the 2-amino group with *iso*-butyryl chloride was carried out prior to hydrolysis *via* diazotization of the 6-amino moiety. Addition of triethylamine trihydrofluoride resulted in cleavage of the silyl ether groups, furnishing **202** in 61% yield.

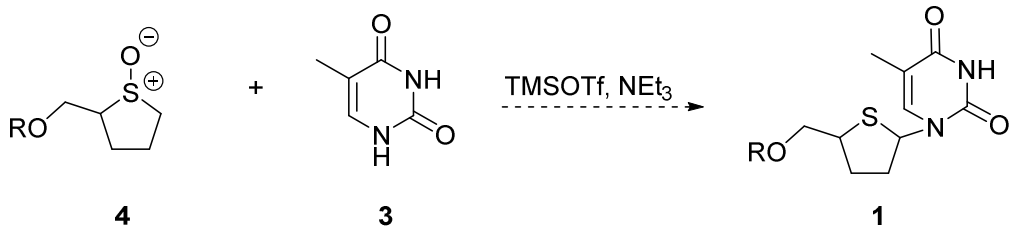
The authors did not report any biological activity data for these compounds, but instead focussed on nuclease stability.



Scheme 20

3. PUMMERER GLYCOSYLATIONS

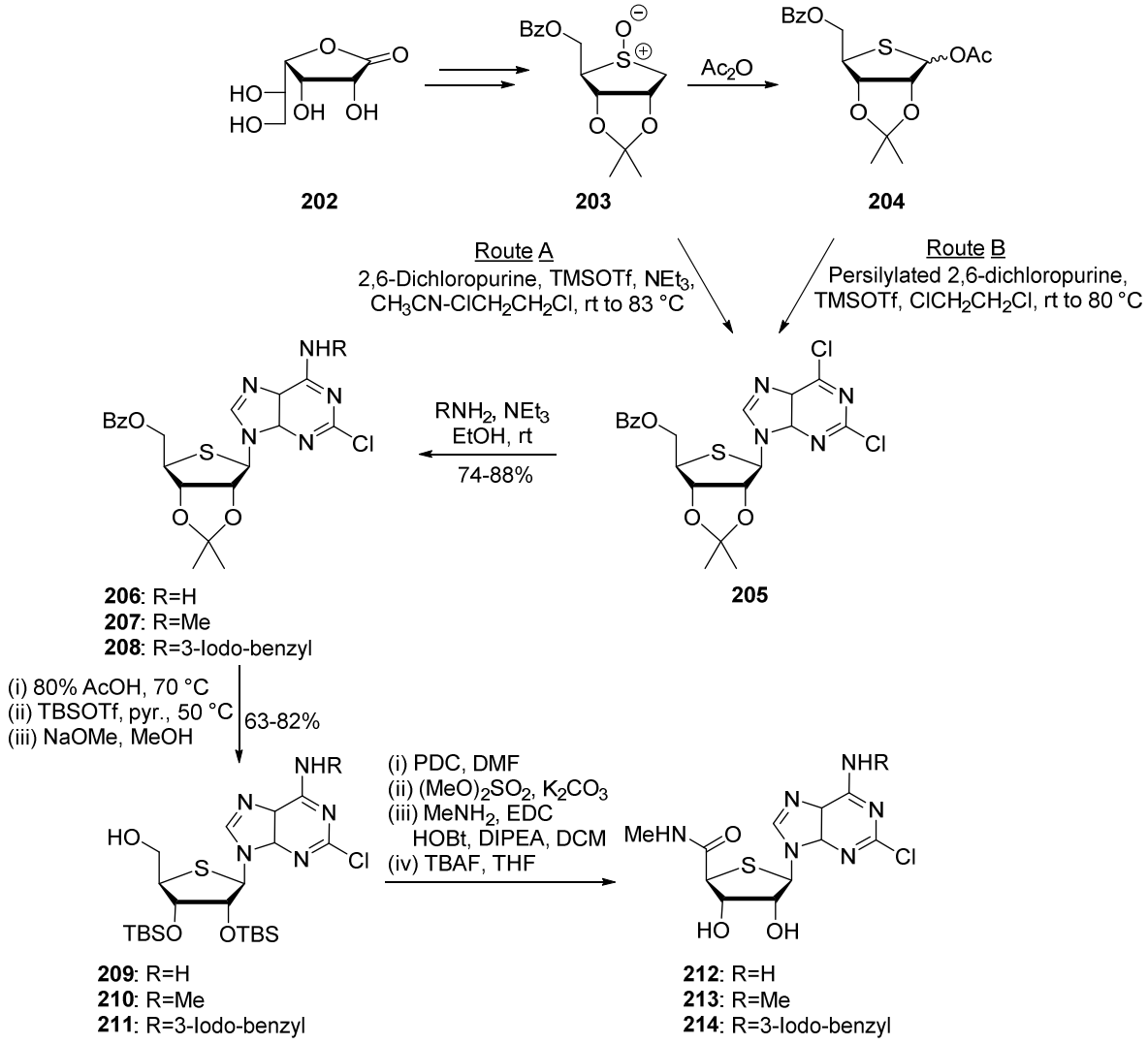
Since O'Neil's novel application of the Pummerer reaction was first published, this approach has become increasingly popular, and is extensively employed by chemists in the synthesis of thionucleosides.[47] It involves the use of trimethylsilyltriflate as a catalyst to both form the sulfenium ion and to persilylate the nucleobase (Scheme 21).



Scheme 21

The use of 4´-thioadenosine analogues as human A_3 Adenosine Receptor (A_3 AR) agonists and antagonists has been a major focus in the research literature. Many of these

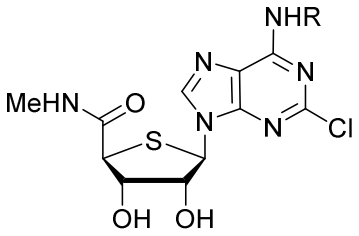
analogues have been found to be highly potent, selective agonists and a 2004 review on thionucleosides includes detailed information on the synthetic routes to such compounds.[9] Since then, several papers have been published detailing work in this area.[48-50] In each case, D-gulonic-γ-lactone (**202**) was the starting point in the preparation of one of two glycosyl donors **203** or **204** (Scheme 22).[51] While conversion of sulfoxide **203** to acetate **204** in acetic anhydride allows for glycosylation to proceed with marginally better β-selectivity than the direct glycosylation of sulfoxide **203**, the overall yield of chloropuridine **205** *via* Route B is lower than Route A (37% vs 54%).[38] Amination of 2,6-dichloropuridine **205** at the 6-position produced **206-208** in high yields. The acetal protecting group at the 2´- and 3´-positions was replaced with a *bis*-silyl ether before deprotection of the primary alcohol to furnish **209-211**. Alcohols **209-211** were finally transformed over a sequence of steps into the corresponding uronamides **212-214** which were assayed for their binding affinities to Human Adenosine Receptors.



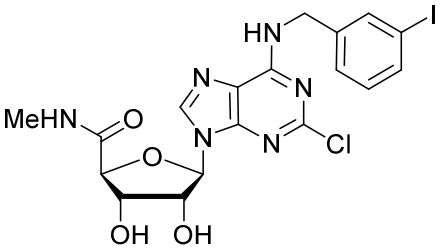
Scheme 22

The 4´-thioadenosine analogues all displayed higher binding affinity to the human A_3 AR than the known agonist Cl-IB-MECA (**215**) (K_i = 1.0 ± 0.2 nM) (Table 8).[52] The highest binding affinity was recorded for 2-chloro-N^6-methyladenosine-5´-methyluronamide (**214**) with K_i = 0.28 ± 0.09 nM. Uronamide analogue **214** was found to be selective for the human A_3 AR 4800 and 36000 fold more than the A_1 and A_{2A} receptors respectively.

Table 8: Receptor binding affinities of thionucleosides **212**-**214** for human A_3 AR



212-214

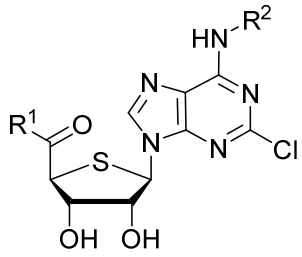


215
C1-1B-MECA

Entry	Thionucleoside	R	K_i (nM)
1	**212**	H	0.40 ± 0.06
2	**213**	CH_3	0.28 ± 0.09
3	**214**	3-iodobenzyl	0.38 ± 0.07
4	**215**		1.0 ± 0.2

A similar synthetic strategy was employed in a study of structure-activity relationships of novel 2-chloro-N^6-substituted-4´-thioadenosine-5´-uronamides as A_3 AR agonists.[38] To ensure high-affinity binding at the receptor, it was postulated that the presence of a hydrogen on the 5´-uronamide moiety is necessary for H-bonding within the binding site. It was observed that bulky amines at this position reduced binding affinity. Thionucleosides **217** and **235** were both found to exhibit higher binding affinities to the A_3 AR than their corresponding 4´-oxonucleoside analogues (Table 9, Entries 2 and 20).

Table 9: Binding affinities of thionucleosides **216-254** for the human A_3 Adenosine Receptor



216-254

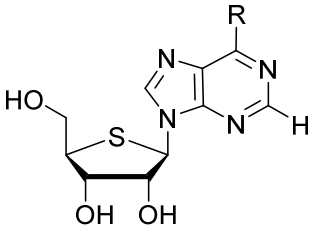
Entry	Compound	R^1	R^2	K_i (nM)
1	**216**	$NHCH_3$	H	0.4 ± 0.06
2	**217**	$NHCH_3$	Me	0.28 ± 0.09
3	**218**	$N(CH_3)_2$	Me	1500 ± 1300
4	**219**	Cyclopropyl-NH	Me	2.82 ± 1.03
5	**220**	Cyclopropylmethyl-NH	Me	1.10 ± 0.03
6	**221**	Isoamyl-NH	Me	1.63 ± 0.17
7	**222**	Morpholine	Me	3870 ± 580
8	**223**	4-Benzyl-piperidine	Me	3500 ± 340
9	**224**	4-(4-F-Phenyl)-piperazine	Me	2700 ± 880
10	**225**	3-F-Benzyl-NH	Me	17.4 ± 3.8
11	**226**	2-(3-F-Phenyl)ethyl-NH	Me	85.6 ± 35.6
12	**227**	3,3-Diphenyl-propyl-NH	Me	415 ± 16
13	**228**	$NHCH_3$	Cyclopropyl	2.24 ± 1.21
14	**229**	$NHCH_2CH_3$	Cyclopropyl	0.67 ± 0.07
15	**230**	Cyclopropyl-NH	Cyclopropyl	5.56 ± 1.77

16	**231**	4-Benzylpiperidine	Cyclopropyl	4020 ± 740
17	**232**	Morpholine	Cyclopropyl	4440 ± 160
18	**233**	$NHCH_3$	Cyclopentyl	4.27 ± 0.33
19	**234**	$NHCH_2CH_3$	Cyclopentyl	2.83 ± 0.63
20	**235**	$NHCH_3$	3-Iodobenzyl	0.38 ± 0.07
21	**236**	$NHCH_2CH_3$	3-Iodobenzyl	0.89 ± 0.18
22	**237**	Iosoamyl-NH	3-Iodobenzyl	41.9 ± 11.3
23	**238**	Cyclopropyl-NH	3-Iodobenzyl	2.96 ± 1.03
24	**239**	Cyclopropylmethyl-NH	3-Iodobenzyl	3.64 ± 0.60
25	**240**	Cyclobutyl-NH	3-Iodobenzyl	18.2 ± 13.4
26	**241**	4-Benzylpiperidine	3-Iodobenzyl	878 ± 285
27	**242**	4-(4-F-Phenyl)-piperazine	3-Iodobenzyl	1440 ± 830
28	**243**	Morpholine	3-Iodobenzyl	510 ± 69
29	**244**	3-Cl-Benzyl-NH	3-Iodobenzyl	308 ± 148
30	**245**	3-(Trifloromethyl)benzyl-NH	3-Iodobenzyl	354 ± 18
31	**246**	2-Phenylethyl-NH	3-Iodobenzyl	433 ± 141
32	**247**	3,3-Diphenylpropyl-NH	3-Iodobenzyl	343 ± 48
33	**248**	$NHCH_3$	2-Methylbenzyl	2.18 ± 0.46
34	**249**	$NHCH_2CH_3$	2-Methylbenzyl	2.50 ± 1.10
35	**250**	$N(CH_3)_2$	2-Methylbenzyl	632 ± 70
36	**251**	Cyclopropyl-NH	2-Methylbenzyl	27.8 ± 3.80

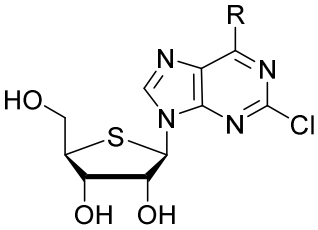
37	**252**	Cyclopropylmethyl-NH	2-Methylbenzyl	29.7 ± 11.3
38	**253**	Morpholine	2-Methylbenzyl	7670 ± 800
39	**254**	4-Benzylpiperidine	2-Methylbenzyl	49200 ± 17500

The same authors also investigated the structure-activity relationships of N^6-substituted-4´-thioadenosines as agonists at the human A_3 AR (Table 10).[38] The most effective and suitable agonist tested was the chlorinated analogue **278** (Entry 24), with a K_i value comparable to that of Cl-IB-MECA (**215**). However, this series of compounds was not as potent as the corresponding N^6-substituted-adenosines or N^6-substituted-4´thioadenosine-5´uronamides listed in Table 9.

Table 10: Binding affinities of thionucleosides **255-290** for the hA_3 AR



255-276



277-290

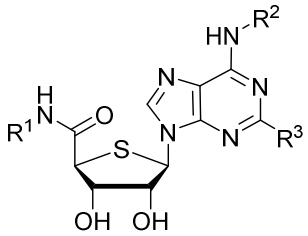
Entry	Thionucleoside	R	K_I (nM) or % displ. at 10 μM
1	**255**	NH_2	445 ± 54
2	**256**	$NHCH_3$	10.3 ± 0.7
3	**257**	Cyclopropyl-NH	45.2 ± 5.8
4	**258**	Cyclobutyl-NH	48.0 ± 4.9

5	**259**	3-Methyl-butyl-NH	65.3 ± 3.6
6	**260**	Cyclopropyl-methyl-NH	22.9 ± 0.8
7	**261**	Benzyl-NH	155 ± 33
8	**262**	3-Iodo-benzyl-NH	1.9 ± 0.4
9	**263**	3-Chloro-benzyl-NH	6.7 ± 0.4
10	**264**	3-Methyl-benzyl-NH	13.9 ± 5.7
11	**265**	3-Fluoro-benzyl-NH	57.6 ± 12.9
12	**266**	3-(Triflouromethyl)-benzyl-NH	32.7 ± 6.7
13	**267**	Naphth-1-yl-methyl-NH	42.2 ± 13.0
14	**268**	2-Phenethyl-NH	5.6 ± 1.1
15	**269**	3-Flouro-phenethyl-NH	11.3 ± 0.6
16	**270**	*trans*-2-Phenyl-cyclopropyl-NH	6.6 ± 2.9
17	**271**	1,2-Diphenyl-ethyl-NH	1080 ± 70
18	**272**	3,3-Diphenyl-propyl-NH	1650 ± 150
19	**273**	Piperidine	21%
20	**274**	4-Benzyl-piperidine	10%
21	**275**	4-(4-Flouro-benzyl)-Piperazine	22%
22	**276**	Morpholine	16%
23	**277**	NH_2	4.9 ± 1.3
24	**278**	$NHCH_3$	0.8 ± 0.1

25	**279**	Cyclopentyl-NH	94.4 ± 29.2
26	**280**	Benzyl-NH	18.2 ± 2.6
27	**281**	2-Methyl-benzyl-NH	18.9 ± 16.6
28	**282**	2-Ethyloxy-benzyl-NH	17.2 ± 2.2
29	**283**	3-Iodo-benzyl-NH	3.2 ± 0.9
30	**284**	α-Naphthylmethyl-NH	268 ± 185
31	**285**	Fluoren-9-yl-methyl-NH	50.4 ± 26.5
32	**286**	2-Phenethyl-NH	4.40 ± 0.33
33	**287**	3-Fluoro-phenethyl-NH	4.7 ± 1.6
34	**288**	1,2-Diphenylethyl	1300 ± 610
35	**289**	*trans*-2-Phenyl-cyclopropyl-NH	1.9 ± 0.4
36	**290**	3,3-Diphenyl-propyl-NH	720 ± 193

In 2009, Jeong and co-workers again focused on the 5´-uronamides as potential A_3 AR agonists.[49] They discovered that while bulky amine substituents are tolerated, smaller alkyl substituents are preferable. 5´-Methyluronamide **301** was revealed as the most effective agonist of those tested (Table 11, Entry 11). Several compounds tested were more potent than the known A_3 AR agonist IB-MECA (**291**).

Table 11: Binding affinities of thionucleosides **291-316** for A_3 AR



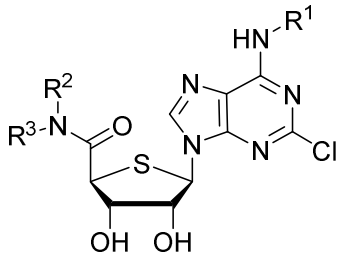
291-316

Entry	Thionucleoside	R^1	R^2	R^3	K_i (nM)
1	**291**	Me	4-Iodobenzyl	H	1.0
2	**292**	Me	Me	Cl	0.28 ± 0.09
3	**293**	Me	4-Iodobenzyl	Cl	0.38 ± 0.07
4	**294**	Me	Me	H	1.19 ± 0.23
5	**295**	Et	Me	H	0.97 ± 0.23
6	**296**	Cyclopropyl	Me	H	2.16 ± 0.24
7	**297**	Cyclopropylmethyl	Me	H	1.35 ± 0.08
8	**298**	Cyclobutyl	Me	H	1.04 ± 0.05
9	**299**	Cyclopentyl	Me	H	0.97 ± 0.007
10	**300**	3-Iodobenzyl	Me	H	15.6 ± 5.6
11	**301**	Me	4-Iodobenzyl	H	0.25 ± 0.06
12	**302**	Et	4-Iodobenzyl	H	0.42 ± 0.22
13	**303**	Cyclopropyl	4-Iodobenzyl	H	3.03 ± 0.23
14	**304**	Cyclopropylmethyl	4-Iodobenzyl	H	2.16 ± 0.29
15	**305**	Cyclobutyl	4-Iodobenzyl	H	1.17 ± 0.16

16	**306**	Cyclohexyl	4-Iodobenzyl	H	35.4 ± 10.5
17	**307**	3-Fluorobenzyl	4-Iodobenzyl	H	61.1 ± 17.6
18	**308**	3-Chlorobenzyl	4-Iodobenzyl	H	144 ± 33
19	**309**	2-Methylbenzyl	4-Iodobenzyl	H	31.0 ± 7.1
20	**310**	3-Methylbenzyl	4-Iodobenzyl	H	94.9 ± 37.3
21	**311**	4-Methylbenzyl	4-Iodobenzyl	H	135 ± 55
22	**312**	2-Methoxylbenzyl	4-Iodobenzyl	H	97.0 ± 51.2
23	**313**	2-Ethoxybenzyl	4-Iodobenzyl	H	113 ± 2
24	**314**	α-Naphthylmethyl	4-Iodobenzyl	H	1200
25	**315**	2-Phenylethyl	4-Iodobenzyl	H	433 ± 141
26	**316**	1,1-Diphenylethyl	4-Iodobenzyl	H	116 ± 48

The structure-activity relationship of a series of 2-chloro-N^6-substituted-4´-thioadenosine-5´-*N*,*N*-dialkylamides as A_3 Adenosine Receptor (A_3 AR) antagonists was outlined by Jeong and co-workers.[50] This class of compound was identified after molecular modelling of the A_3 AR indicated the *N*,*N*-dialkyl substituent destroys the hydrogen bond-donating ability of the 5´-uronamide, which is a prerequisite for the conformational change needed for receptor activation. Removal of the H-bond donors results in the complete loss of A_3 AR activation ability, allowing the *N*,*N*-dialkyl series to act as pure antagonists. The study demonstrated that steric factors of the 5´-position play an important role in the binding affinity – in general, the dimethyl derivatives were more active than the bulkier analogues (Table 12).

Table 12: Binding affinities of **317-335** for A_3 AR

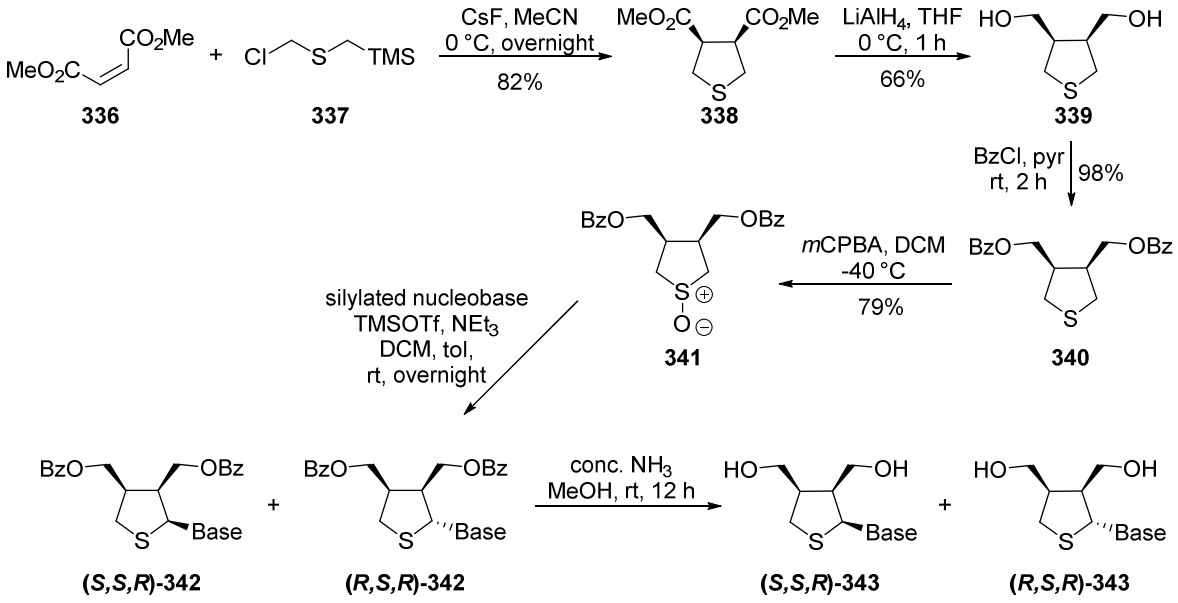


317-335

Entry	Compound	R^1	R^2	R^3	K_i (nM ± SEM)
1	**317**	3-Iodobenzyl	Me	Me	15.5 ± 3.1
2	**318**	3-Fluorobenzyl	Me	Me	121 ± 11
3	**319**	3-Chlorobenzyl	Me	Me	21.3 ± 2.1
4	**320**	3-Bromobenzyl	Me	Me	9.32 ± 3.2
5	**321**	N^6 $(CH_3)_2$	Me	Me	136
6	**322**	2-Methoxyethyl	Me	Me	425
7	**323**	Cyclopropyl	Me	Me	109 ± 16
8	**324**	Cyclopropylmethyl	Me	Me	30.7 ± 9.4
9	**325**	Cyclobutyl	Me	Me	115 ± 50
10	**326**	Cyclopentyl	Me	Me	837
11	**327**	3-Iodobenzyl	Me	Propyl	727
12	**328**	3-Iodobenzyl	Me	CH_2CH_2OH	126 ± 17
13	**329**	3-Iodobenzyl	Et	Phenyl	398
14	**330**	3-Iodobenzyl	Piperidine	-	565
15	**331**	3-Iodobenzyl	4-Methylpiperazine	-	667

16	**332**	3-Iodobenzyl	Azetidine	-	43.4 ± 2.6
17	**333**	3-Iodobenzyl	Pyrrolidine	-	117 ± 31
18	**334**	3-Iodobenzyl	4-Hydroxypiperidine	-	1530
19	**335**	3-Iodobenzyl	Thiomorpholine	-	867

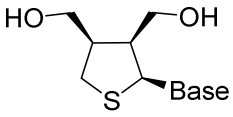
A 1,3-dipolar cycloaddition of thiocarbonyl ylides with alkenes bearing electron withdrawing substituents was exploited by Corsaro and colleagues in an effort to increase yields over previously published methods (Scheme 23).[53] Cleavage of the trimethylsilyl moiety of **337** with cesium fluoride afforded a thiocarbonyl ylide, which underwent an *in situ* 1,3-diopolar cycloaddition with methyl maleate (**336**) to produce tetrahydrothiophene **338**. Reduction of diester **338** to diol **339** was achieved with lithium aluminium hydride in 66% yield. Protection of **339** with benzoyl chloride furnished **340** which was oxidised to sulfoxide **341**. Glycosylations were achieved using a Pummerer-type reaction with a suitable silylated nucleobase, trimethylsilyl triflate and triethyl amine. The authors report that the α-anomers formed more readily, which they attributed to the presence of the bulky benzoyl groups. When the *trans*-isomer of fumarate **336** was used as the starting olefin, β-anomer **(*R,S,R*)-342** predominated. Deprotection of the hydroxyl groups was achieved in methanolic ammonia to form nucleosides **(*S,S,R*)-343** and **(*R,S,R*)-343**.



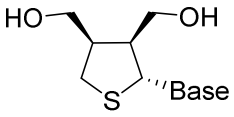
Scheme 23

A library of 4´-thionucleosides was prepared using several different glycosylation methods: the Pummerer-type seen in Scheme 23 (compound **343**), as well as Electrophilic (compound **344**) and Vorbrüggen-type (compound **345**) glycosylations.[37, 38, 48-51, 54-57] From the yields listed in Table 13, it is clear that the thiocarbonyl ylide cycloaddition strategy is a higher yielding and more convenient route to stereospecific 4´-thionucleosides. Investigations into the antiviral and antitumour activity of the synthesised nucleosides are on-going.

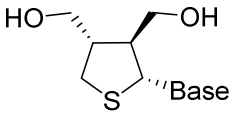
Table 13: Yields of thionucleosides **343**-**345**



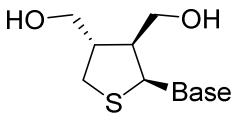
(*S,S,R*)-343



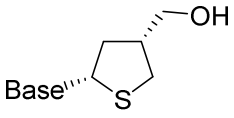
(*R,S,R*)-343



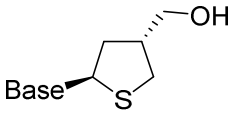
(*R,S,S*)-343



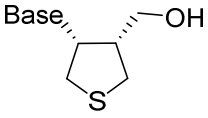
(*S,S,S*)-343



(*R,S*)-344



(*S,S*)-344

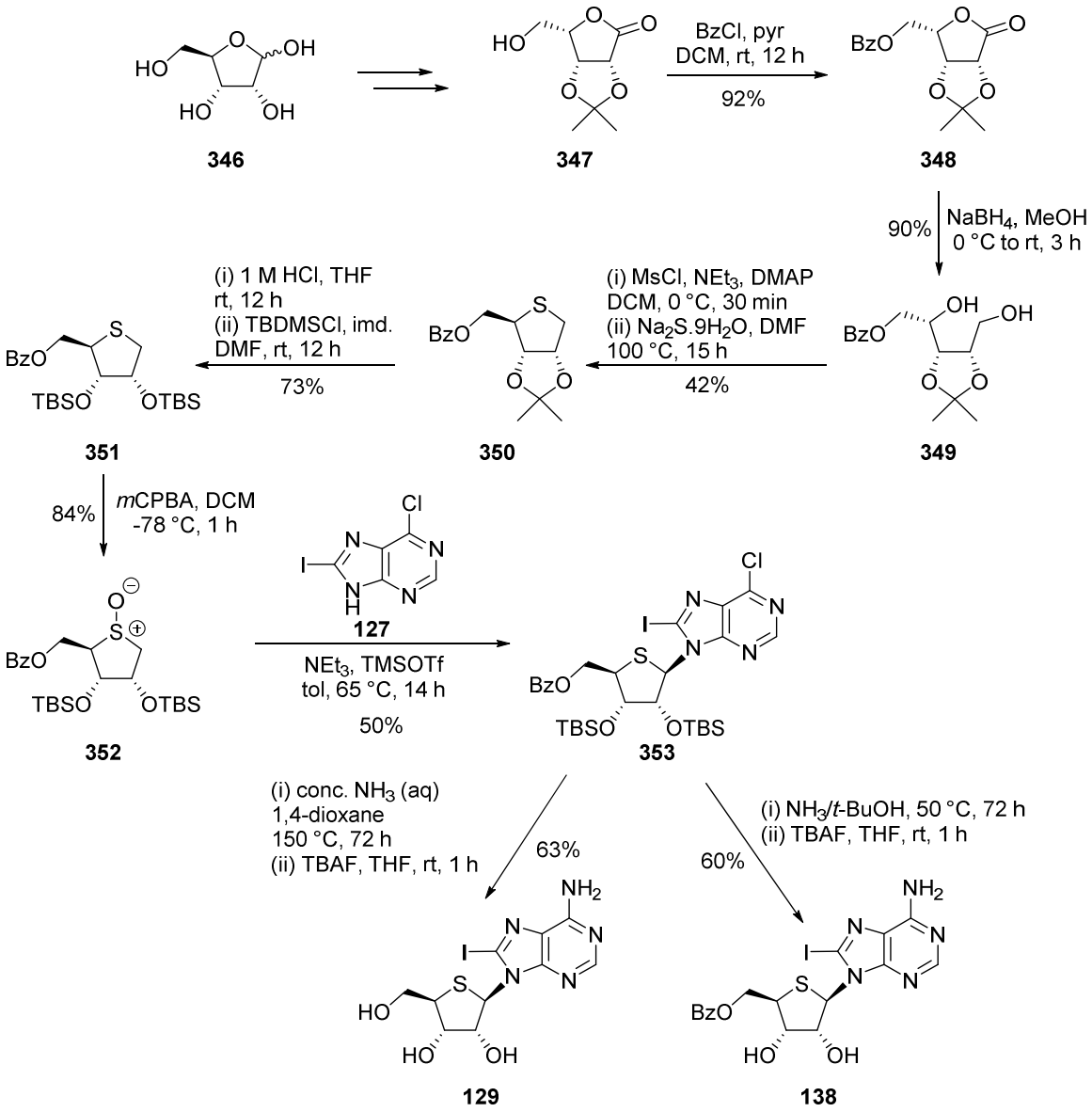


345

Entry	Nucleoside	Thymine	Cytosine	Uracil	5-F-Uracil	Adenine
1	**(*S,S,R*)-343**	75%	69%	79%	76%	n/a
2	**(*R,S,R*)-343**	4%	4%	3%	4%	n/a
3	**(*R,S,S*)-343**	73.5%	67.5%	76%	74%	62.5%
4	**(*S,S,S*)-343**	4%	4%	3%	4%	5%
5	**(*R,S*)-344**	47%	36%	52%	45%	n/a
6	**(*S,S*)-344**	4%	5%	5%	4%	n/a
7	**345**	46%	45%	38%	40%	n/a

Recently, Jeong and colleagues prepared C8-substituted-4´-thionucleosides as potential HSP90 (heat shock protein) inhibitors and investigated their anticancer properties.[40] Their analogues consisted of two main classes; 5´-substituted-4´-thionucleosides synthesised *via* the Pummerer rearrangement, and 4´-thionucleosides lacking a 5´-moiety *via* the Vorbrüggen method described previously (Scheme 12).[37] Shown here is the synthetic pathway to compounds **129** and **138**, which displayed the most promising biological effects when tested (Scheme 24).

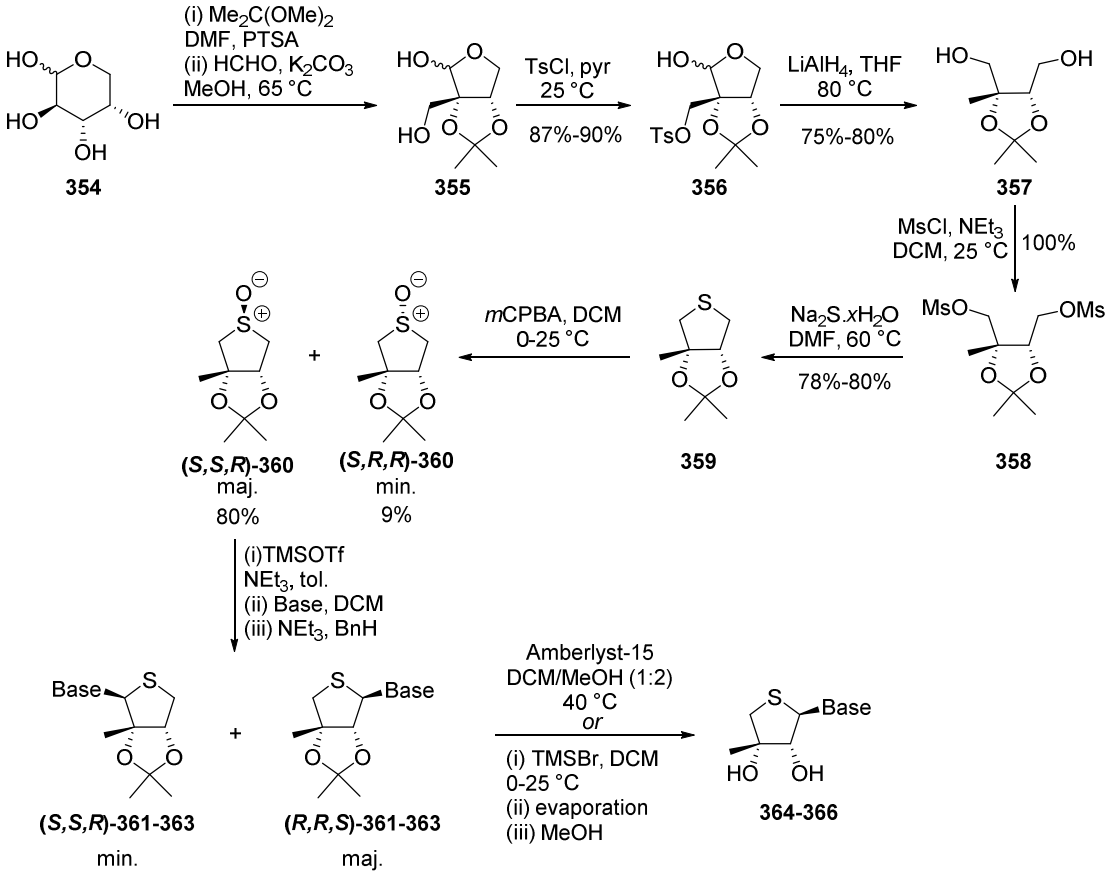
L-Lysonolactone derivative **347** was prepared from D-ribose (**346**) using Batra's previously published procedure[58], and then converted to benzoate ester **348**. Reduction of the lactone afforded diol **349** in 90% yield. Conversion of **349** to the mesylate was followed by cyclisation to thiolane **350**. Acetyl hydrolysis and subsequent silylation furnished *bis*-silyl ether **351**. Oxidation of **351** produced sulfoxide **352**, which acted as a glycosyl donor in an optimised Pummerer-type condensation with modified purine **127** to form 4´-thionucleoside **353** in 50% yield as the β-anomer, with only trace amounts of the α-product reported. Subsequent deprotections afforded alcohol **129** and benzoate ester **138** in respectable yields.



Scheme 24

Koumbis *et al.* synthesised a range of apiothionucleosides as potential anti-cancer agents (Scheme 25).[59] Lactol **355** was prepared from a mixture of L- and D-arabinose over two steps using a known methodology.[60-62] Monotosylation of **355** gave tosylate **356** which was reduced with lithium aluminium hydride to diol **357**. Conversion of **357** to *bis*-mesylate **358** was followed by sulfide substitution and subsequent cyclisation to thiolane **359** in good yields. Oxidation to sulfoxides (***S,S,R*)-360** and (***S,R,R*)-360** afforded both

diastereomers in a 10:1 ratio respectively. The authors established that Pummerer transformation proceeded with preferential β-addition. The major isomer is the C-2 substituted β-isomer **(*R,R,S*)-361**, as the C-5 position is more hindered on approach. Thymine, uracil and acetyl-cytosine analogues **361-363** were successfully prepared. Deprotection of the thymine and uracil derivatives was achieved cleanly using Amberlyst-15. The same reaction did not proceed in the case of the cytidine derivative, so trimethylsilyl bromide was instead employed. Yields are presented in Table 14.



Scheme 25

Table 14: Yields for thionucleosides **361-366**

Entry	Thionucleoside	Base	Yield

1	**(*S,S,R*)-361**	Thymine	12%
2	**(*S,S,R*)-362**	Cytosine	14%
3	**(*S,S,R*)-363**	Uracil	14%
4	**(*R,R,S*)-361**	Thymine	60%
5	**(*R,R,S*)-362**	Cytosine	56%
6	**(*R,R,S*)-363**	Uracil	56%
7	**364**	Thymine	75%
8	**365**	Cytosine	95%
9	**366**	Uracil	73%

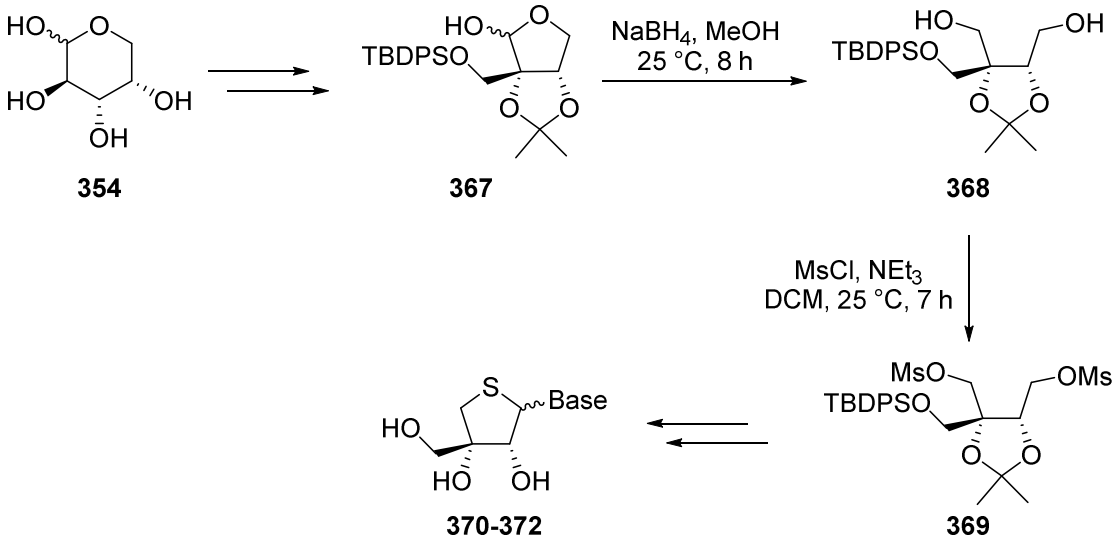
Biological evaluation of these novel apiothionucleosides indicated that some had specific cytotoxic effects on certain cancer cell lines, but none had an inhibitory effect on the normal cell line MRC-5 (Table 15). The D-analogues were found to be more active in general.

Table 15: Biological evaluation of thionucleosides **364-366** on several cancer cell lines

		IC_{50} (μM)					
Entry	Thionucleoside	A549	HeLa	MCF7	MDA-MB-231	HT29	MRC-5
1	**D-364**	41	>100	>100	3	5	>100
2	**L-364**	30	>100	>100	52	>100	>100
3	**D-365**	9	17	>100	10	>100	>100
4	**L-365**	>100	12	13	11	>100	>100
5	**D-366**	>100	>100	>100	19	8	>100

6	**L-366**	30	>100	>100	14	>100	>100

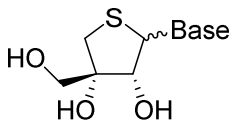
The same group later reported the synthesis of a slightly modified version of their apiothionucleosides whereby the substituent on the 3´-position is a CH_2OH moiety.[63] The route to these nucleosides was similar to an earlier approach (Scheme 26). Reduction of monosilylated lactol **367** with sodium borohydride afforded diol **368**, which was converted to the corresponding *bis*-mesylate **369**. Target nucleosides were prepared from **369** as before.



Scheme 26

These trihydroxylated apiothionucleosides displayed broader cytotoxic activity than their dihydroxylated predecessors. The L-derivatives appear to be more active, a reversal of the previous findings (Table 16). The most active candidate was L-cytidine-apiothionucleoside **L-372** (Entry 6).

Table 16: Anti-cancer activity of trihydroxylated analogues **370-372**

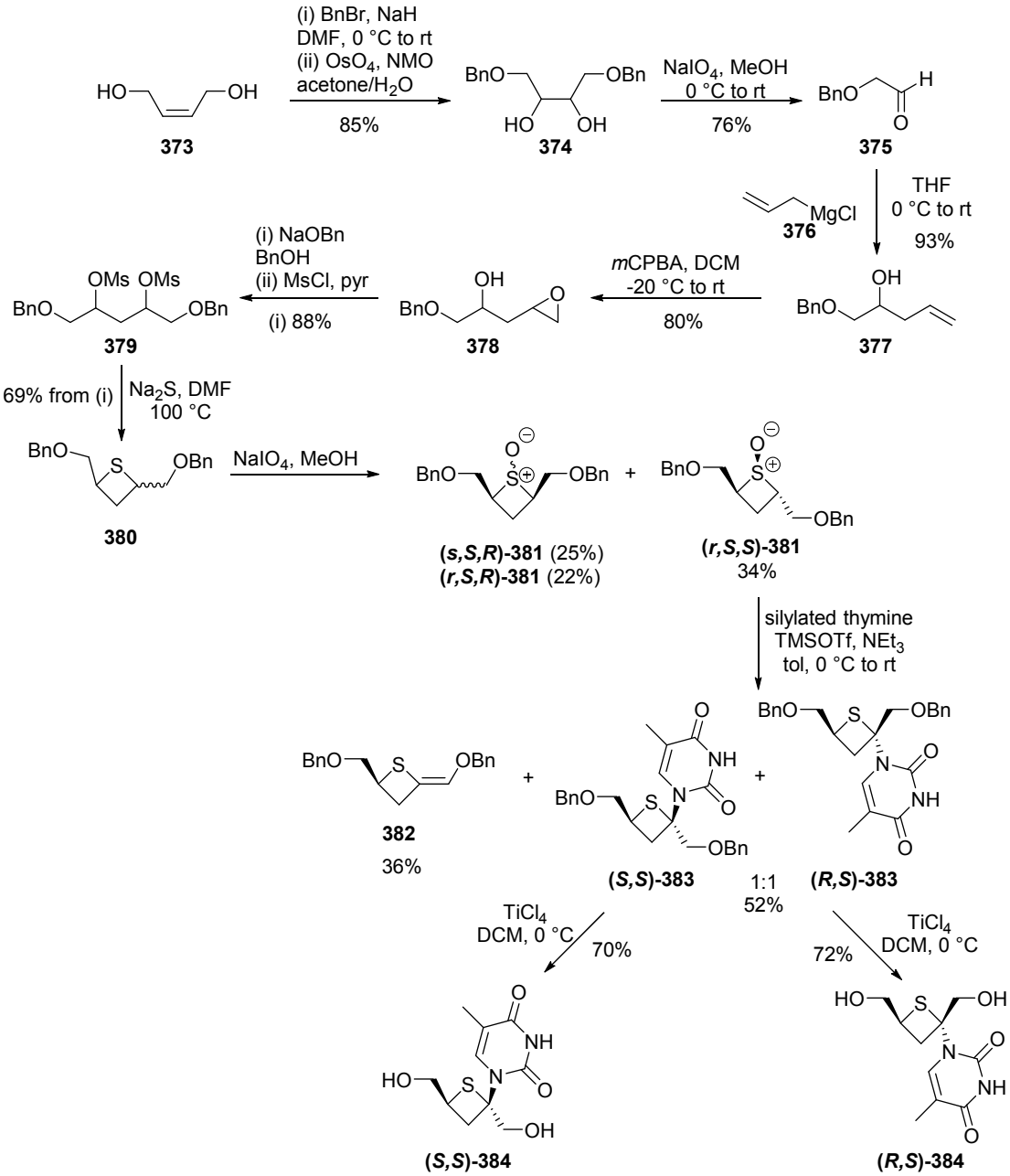


370-372

Entry	Thionucleoside	Base	IC_{50} (μM)					
			A549	HeLa	MCF7	MDA-MB-231	**HT29**	**MRC-5**
1	**D-370**	D-Thymine	24.0	91.8	>100	45.7	21.3	>100
2	**L-370**	L-Thymine	21.1	30.9	>100	39.5	16.3	>100
3	**D-371**	D-Uracil	32.7	59.3	>100	>100	28.2	86.6
4	**L-371**	L-Uracil	22.4	12.9	48.5	71.1	19.4	>100
5	**D-372**	D-Cytidine	18.7	56.12	>100	22.0	17.2	>100
6	**L-372**	L-Cytidine	29.7	5.4	>100	3.2	18.6	>100

Inspired by the oxetane-containing antibiotic Oxetanocin A[64], Nishizono *et al*. designed a series of thietane nucleoside analogues (Scheme 27).[65] Initial benzylation of **373**, followed by osmium-mediated dihydroxylation, afforded **374**. Oxidative cleavage of the diol gave aldehyde **375**, which was coupled to allylmagnesium chloride (**376**) to form alkene **377**. Epoxidation of **377** furnished **378** which was selectively benzylated at the primary alcohol and then mesylated at the remaining secondary alcohol to afford intermediate **379**. Addition of sodium sulfide to **379** furnished thietane **380** in good yield as a racemic mixture. Oxidation of **380** was achieved with sodium periodate to produce diastereomeric sulfoxides **(*s,S,R*)-381**, **(*r,S,R*)-381** and **(*r,S,S*)-381** which were subsequently separated. The outcome of the Pummerer reaction was found to be dependent on the stereochemistry of the sulfoxide, with sulfoxide **(*r,S,S*)-381** proving marginally better. Coupling of **(*r,S,S*)-381** with thymine resulted in a 1:1 mixture of protected thietane nucleosides **(*S,S*)-383** and **(*R,S*)-383**. Decoupled alkene **382** was also

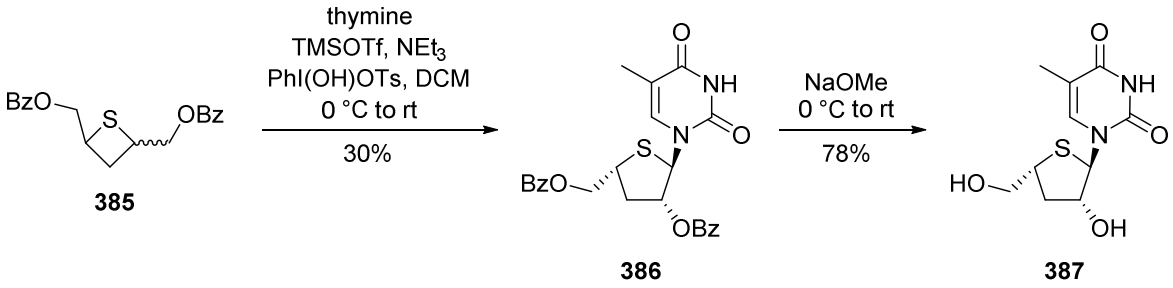
produced in the reaction 36% yield. Finally, titanium-mediated deprotection of the alcohol groups generated nucleosides (***S,S***)**-384** and (***R,S***)**-384**.



Scheme 27

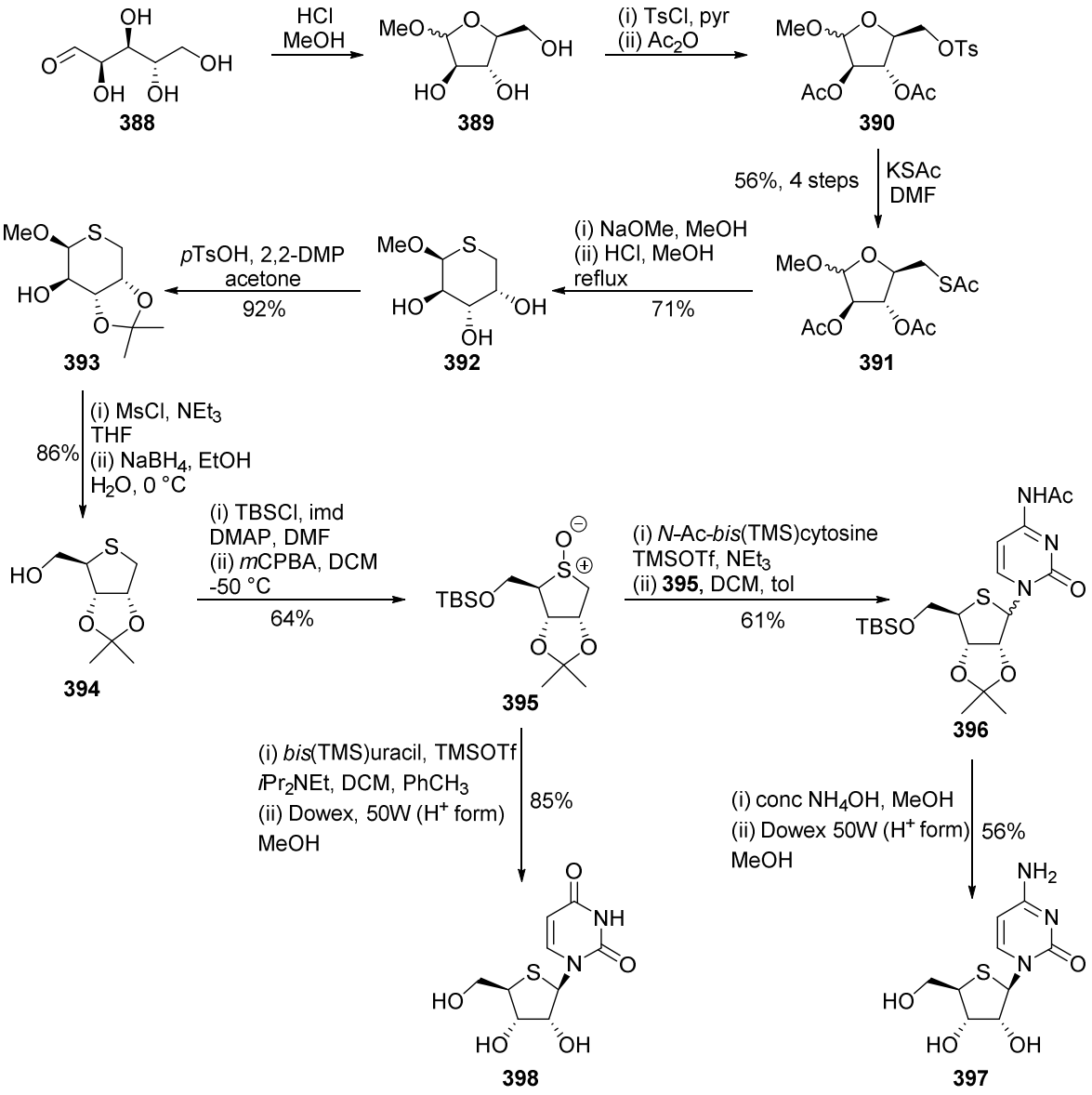
Interestingly, when the benzyl ethers in **380** were replaced with benzoyl moieties, the condensation reaction of the resulting ester **385** with thymine in the presence of silylated triflate instead afforded the ring-expanded 4´-thionucleoside **386** in 30% yield (Scheme 28). The authors postulate that the reaction proceeds *via* the elimination of a benzoyl

group *via* the formation of a thiirane, facilitated by the sulfur lone pair. Ring-opening promoted by the free benzoyl anion results in the formation of the 5-membered ring in the product. Disappointingly, biological testing revealed that neither **(*S,S*)-384** nor **(*R,S*)-384** exhibited any anti-HSV activity, even at high concentrations.



Scheme 28

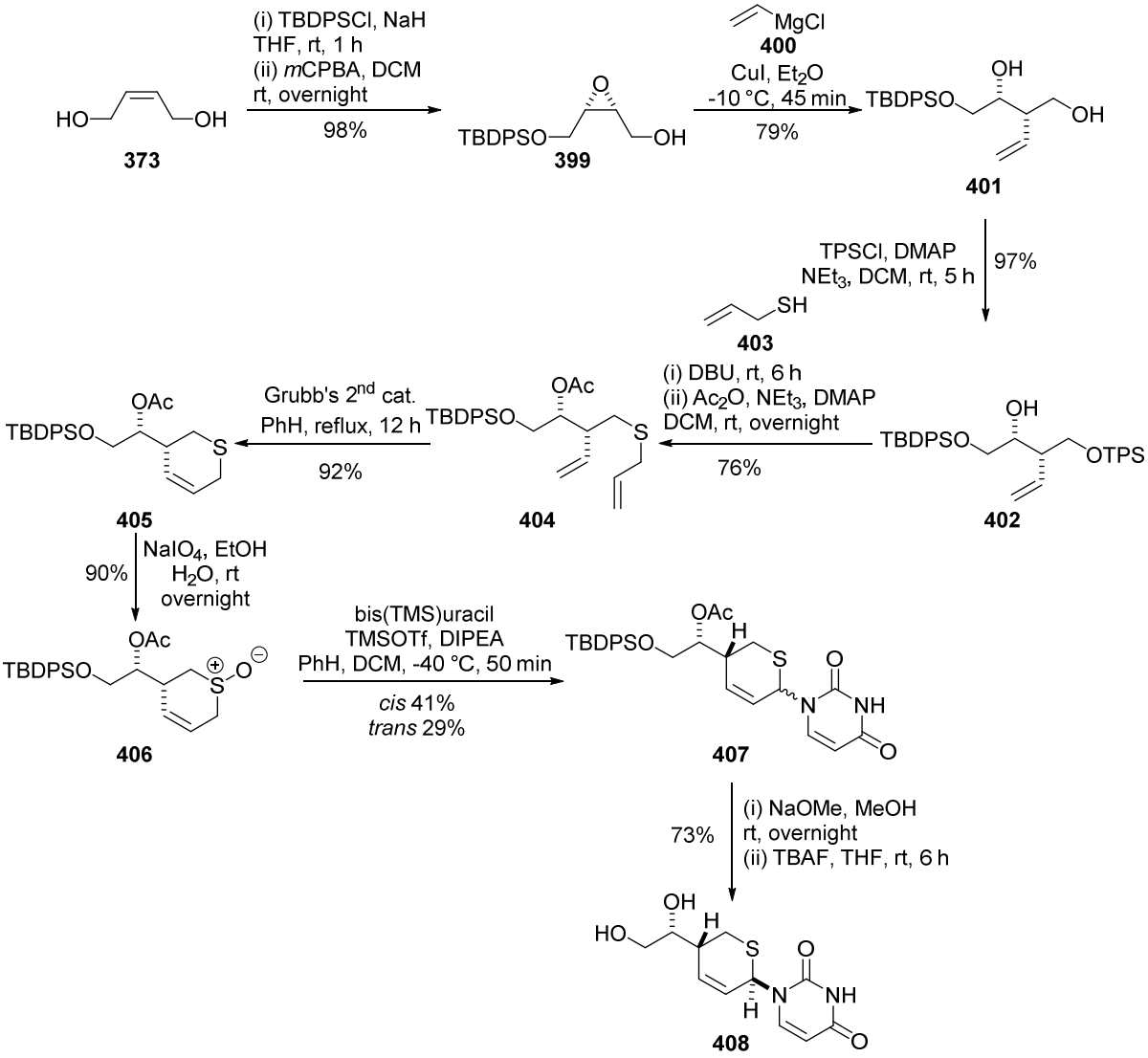
A practical route to 4´-thionucleosides from inexpensive L-arabinose (**388**) has been described by Yoshimura and colleagues (Scheme 29).[66] Acid-mediated intramolecular cyclisation of L-arabinose afforded arabinoside **389**, which was subjected to regioselective tosylation, and finally, acetylation in acetic anhydride to furnish **390**. Nucleophilic substitution of the 5´-tosyl group of **390** with potassium thioacetate gave **391** in 56% yield over 4-steps. Acetal **391** was converted to thioacetal **392** in 71% yield over two steps. Acetylation of the *cis*-diol **392**, and subsequent mesylation effected a ring-contraction to ultimately form **394**. The reaction proceeds *via* intramolecular nucleophilic attack of sulfur at the 5´-position to form an episulfonium ion, followed by ring contraction and generation of an aldehyde which is reduced *in situ* to alcohol **394**. Silylation at the 5´-position of **394** was followed by *in situ* oxidation to give sulfoxide **395** in 64% yield. Following attempts at optimising the Pummerer-type glycosylation in a model reaction, cytidine and uridine analogues **397** and **398** respectively, were prepared by reaction of **395** with the appropriate persilylated nucleoside base. Treatment with acidic resin promoted hydroxyl deprotection to afford **397** in 56% yield with a 1:5 α:β ratio, and **398** in 85% yield with a 1:6 α:β ratio. Biological activities are not reported for these molecules.



Scheme 29

In 2007, Yoshimura and co-workers redirected their attention to the synthesis of novel, ring-expanded 4´-thio-*apio*-thionucleosides.[67] Prior to forming (±)-epoxide **399** from reaction with *meta*-chloroperoxybenzoic acid (*m*CPBA), monosilylation of symmetrical diol **373** was carried out using McDougal's methodology (Scheme 30).[68] Ring-opening of the epoxide with allyl magnesium chloride in the presence of copper iodide formed vinyl diol **401**, which was subjected to selective sulfonylation at the primary hydroxyl group with bulky 2,4,6-triisopropylbenzenesulfonly chloride (TPSCl) to form **402**.

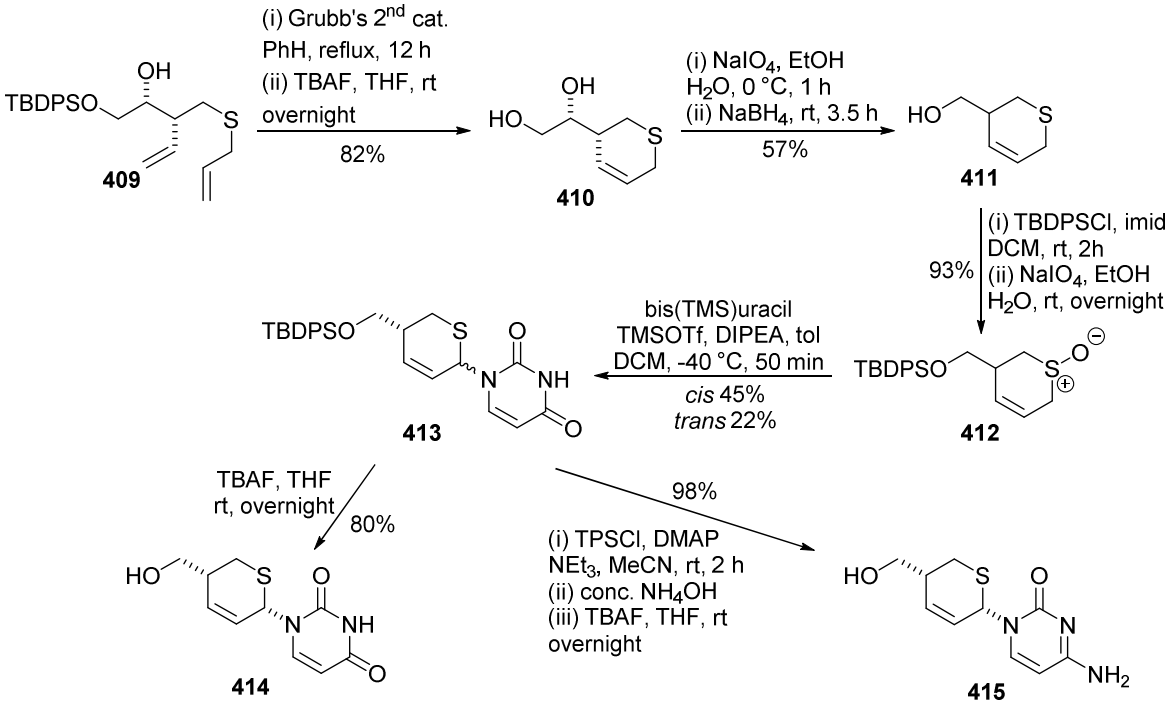
Notably, no bis-sulfonylated product was observed in the reaction mixture. Nucleophilic substitution of the leaving group with allyl mercaptan, followed by acetylation resulted in the formation of key intermediate **404** in 76% yield. Unsaturated thiane **405** was formed *via* olefin metathesis of **404** using Grubb's 2nd catalyst in 92% yield. Oxidation of **405** to sulfoxide **406** preceeded Pummerer-type glycosylation with a 41% yield of *cis*-**407** and 29% yield of *trans*-**407**. Global deprotection of *trans*-**407** produced *trans*-**408**, a thiocytidine analogue, in 73% overall yield.



Scheme 30

The preparation of compounds **414** and **415** was achieved in a similar manner (Scheme 31). Following cyclisation of **409**, the protected alcohol was desilylated to furnish **410**. Oxidative cleavage, and sodium borohydride-mediated reduction of the resulting aldehyde led to the formation of primary alcohol **411**, which was oxidised to sulfoxide **412**. Pummer-type glycosylation was subsequently carried out on **412** as previously described, giving *cis*-**413** in 45% yield and *trans*-**413** in 22% yield. Silyl deprotection of *cis*-**413** in the usual manner yielded **414**, a cytidine analogue, while conversion to the

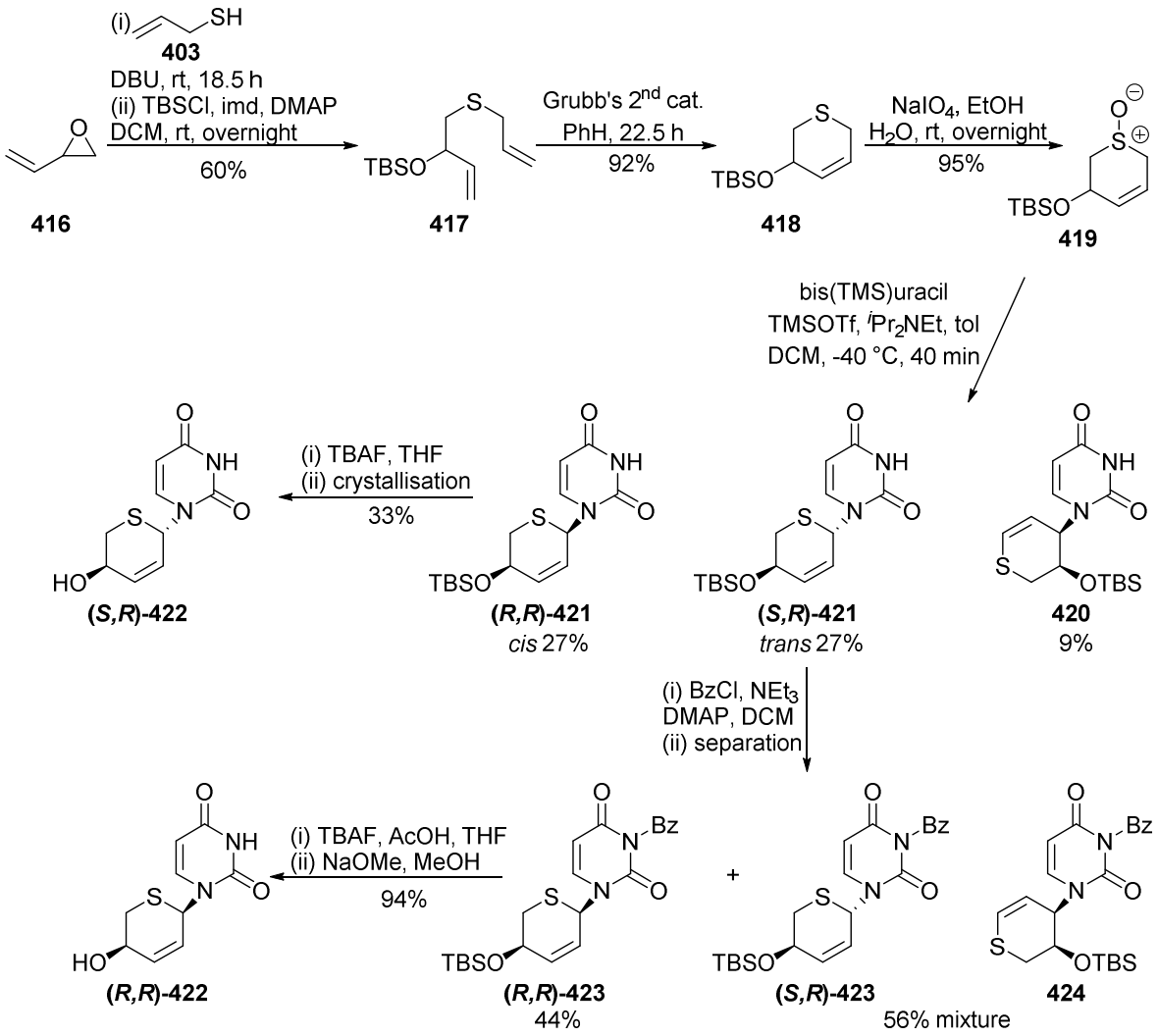
cis-uridine analogue **415** was achieved over three steps from the same precursor. Biological activity for these compounds is not reported.



Scheme 31

In a later paper, the same authors further explored the synthesis of these 6-membered thionucleosides (Scheme 32). [69] Employing a previously optimised route, sulfoxide **419** was initially prepared from allylic epoxide **416**.[67] Pummer-type glycosylation was performed with persilylated uracil on **419** to afford *trans*- and *cis*-thionucleosides **421** and **422** in 27% yield each, as well as 9% of γ-adduct **420**. Both the yield and ratio of products was found to be dependent on both reaction time and whether sulfoxide **419** was employed as a mixture of diasteromers or not. It was noted that longer reaction times promoted the generation of γ-adduct **420** alone. The authors confirmed that this outcome was due to the regeneration of an allylic sulfenium ion of the α-positioned nucleosides **(*S,R*)-421** and **(*R,R*)-421**. Silyl ether deprotection of the mixture, followed by crystallisation, saw **(*S,R*)-422** isolated in 33% yield. Alternatively, N^3-benzoylation of the mixture allowed for **(*R,R*)-423** to be isolated *via* column chromatography, which

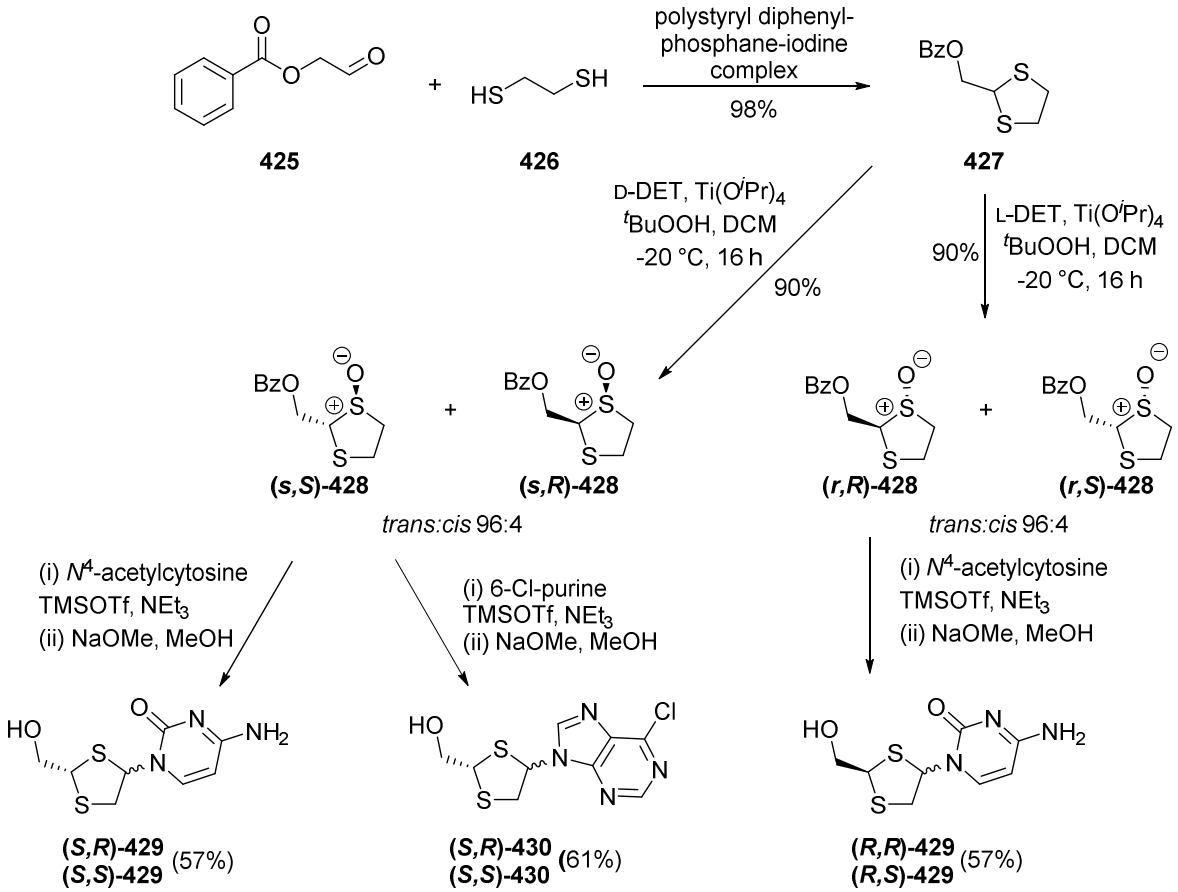
was then desilylated with TBAF to furnish **(*R,R*)-422**. Biological activity of these compounds was not reported.



Scheme 32

In an effort to mimic the oxygen-containing sugar moiety of natural nucleosides, Caputo *et al*. synthesised a range of 1,3-dithiolane nucleosides (Scheme 33).[70] Dithiolane **427** was prepared from benzoyloxyethanal[71] (**425**) and ethanethiol (**426**), in the presence of a polystyryl diphenylphosphane-iodine complex, a compound which acts as both a Lewis acid and a dehydrating agent[72]. Conversion of **427** to chiral sulfoxides **428** was achieved *via* Di Furia-Modena oxidation.[73] The *trans*-isomer was predominant in both cases. Coupling of these *trans*-sulfoxides to nucleoside bases was achieved *via* Pummerer-type

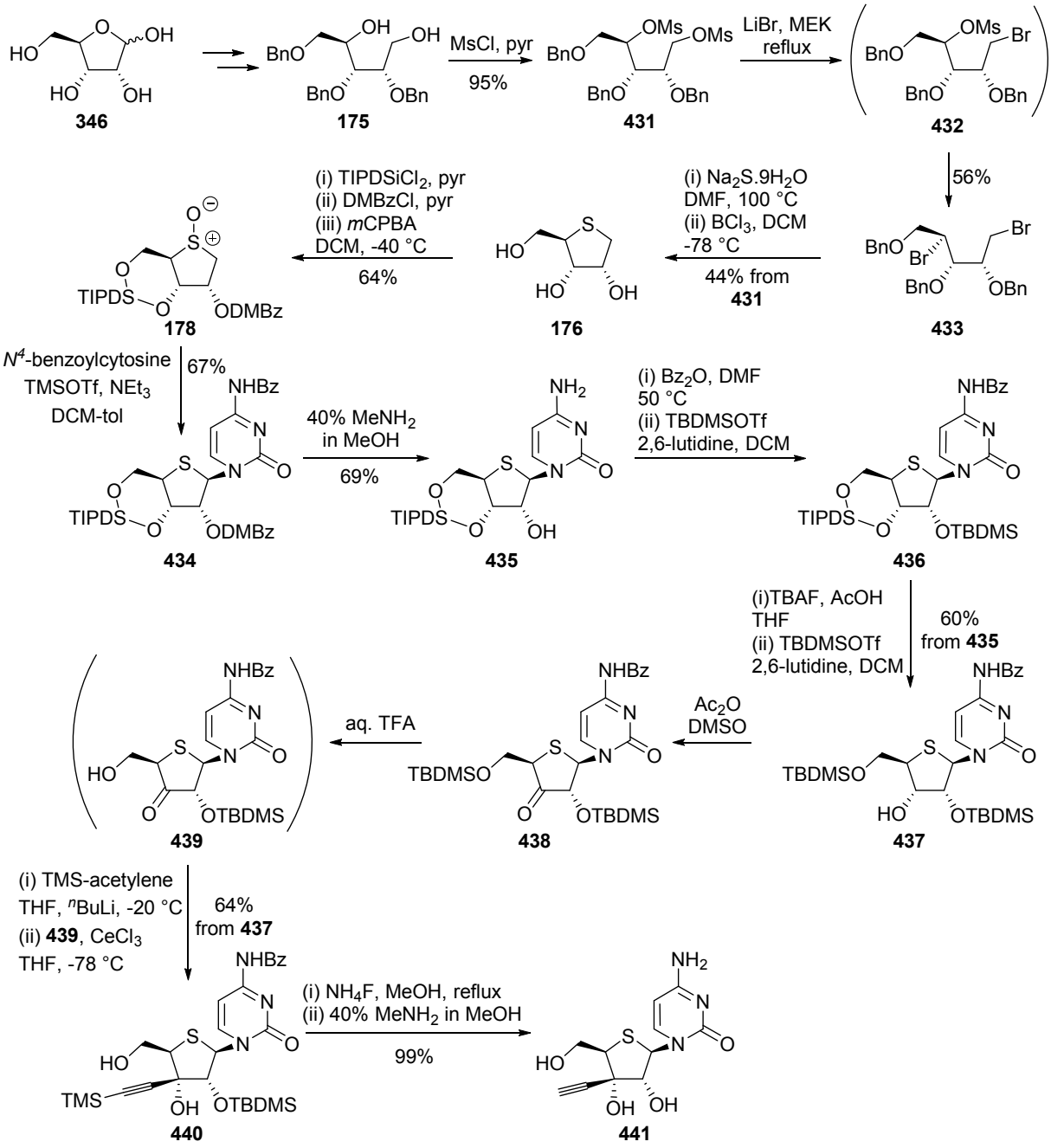
conditions in good yields. Following deprotection of **428**, **429** and **430** were isolated as their α- and β-anomers. Biological evaluation of the compounds is not reported in the paper.



Scheme 33

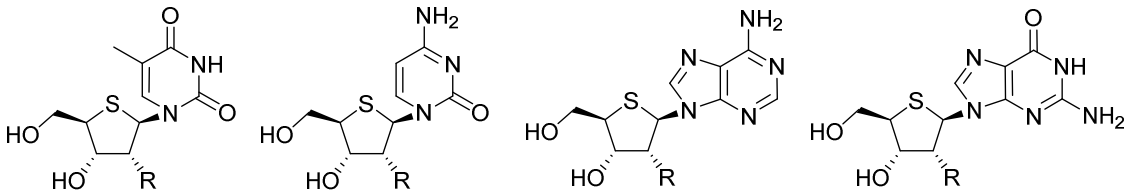
A concise synthesis of 1-(3-*C*-ethynyl-4-thio-β-D-ribofuraosyl)cytosine (**441**) was developed by Matsuda *et al*. from D-ribose (**346**) (Scheme 34).[74] Diol **175** was prepared using the previously published method and subjected to mesylation to provide **431**.[46] Optimisation of the nucleophilic bromination of **431** led to dibromide **433** in 56% yield after treatment with ten equivalents of dry lithium bromide in methyl ethyl ketone. Addition of sodium sulfide effected double nucleophilic substitution and cyclisation to the thiolane, which was debenzylated to afford **176**. Overall, this seven step route to **178** was more efficient than the previous approach, which required eleven steps.[46] Through a series of protection and oxidation steps, sulfoxide **178** was obtained in 64% overall

yield. Pummerer-type glycosylation was conducted on **178** to afford uridine analogue **434**. Incorporation of the 3´-alkyl group required several protections and deprotections to first form **437** in 41% overall yield. Oxidation of the 3´-hydroxyl group of **437** to ketone **438** was achieved *via* Swern conditions. Deprotection of 5´-hydroxy group facilitated β-faced nucleophilic addition of the alkyl group. Accordingly, hydroxy ketone **439** was treated with freshly generated cerium (trimethylsilyl)acetylide to furnish **440**. Exhaustive deprotection of **440** afforded 3´-ethynyl-4´-thiocytidine analogue **441** in 99% yield. The reported compounds were not submitted for biological testing.



Scheme 34

In a later study, the authors outlined a practical route to 4´-thioRNAs and 4´-thioDNAs: thymidine, cytidine, adenosine and guanidine analogues **442-449** respectively (Figure 2).[75-78]



442: R=OH
443: R=H

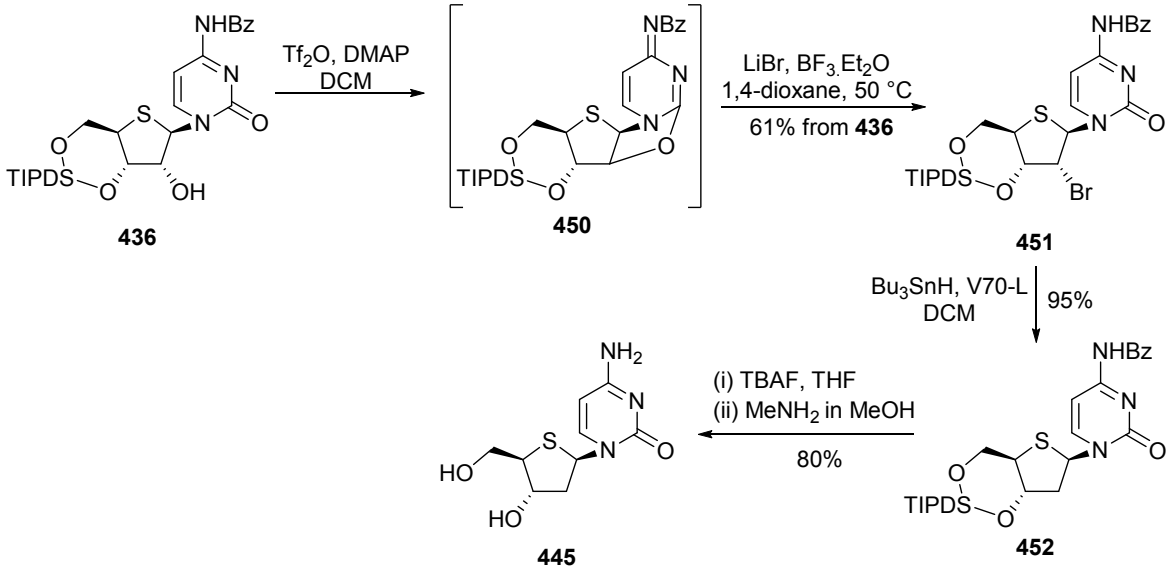
444: R=OH
445: R=H

446: R=OH
447: R=H

448: R=OH
449: R=H

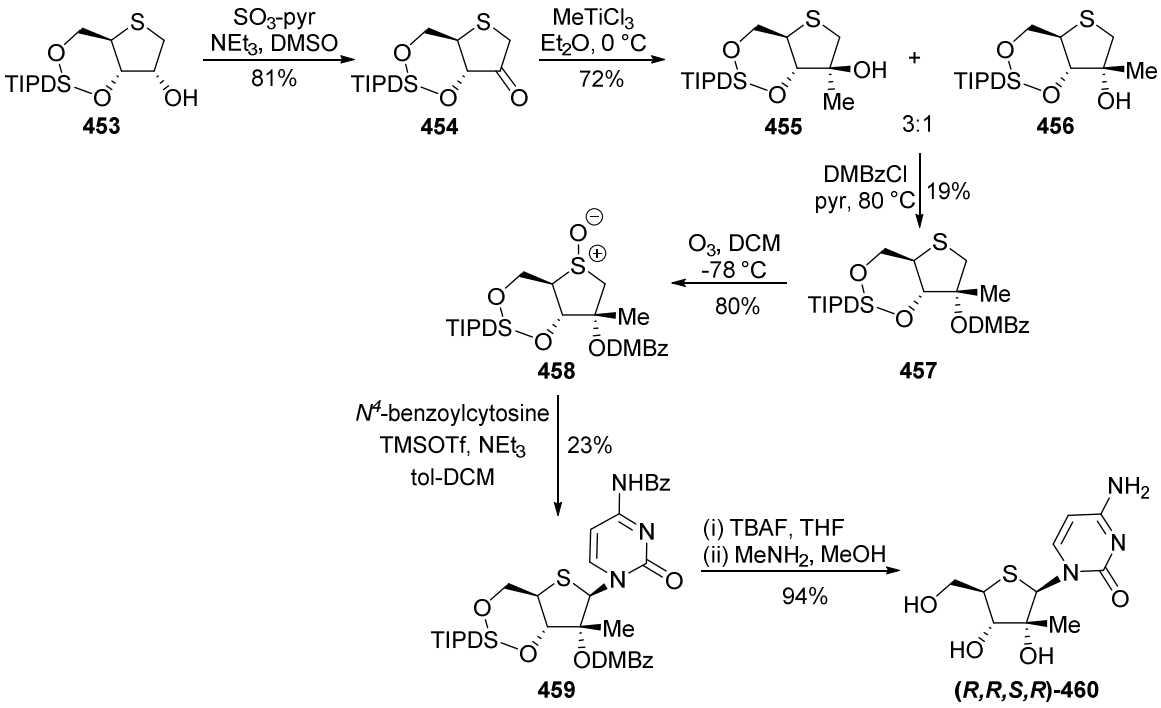
Figure 2. Novel RNA- and DNA-derived thionucleosides

In order to prepare the desired 2´-deoxy derivatives, **436** was first converted to 2,2'-*O*-anyhydro derivative **450** (Scheme 35). Crude **450** was next brominated using a combination of lithium bromide and boron trifluoride diethyl etherate. Bromide **451** was subjected to tin-mediated reduction at room temperature. Gratifyingly, no acyclic side products resulting from cleavage of the C-S bond were observed. Standard deprotection chemistry furnished nucleoside **445** in 80% yield. This synthetic method provides a robust pathway to access the 2´-deoxy derivatives on a gram scale. It was found that these 4´-thionucleoside analogues have higher resistance to 3´-exonuclease hydrolysis than cell native nucleosides, and that fully modified 4´-thioDNA behaves as an RNA-like mimic. The gene suppression ability of these derivatives was also examined and found to be present in those analogues with a 2´-fluoro moiety, *via* U1 small nuclear RNA interference machinery.



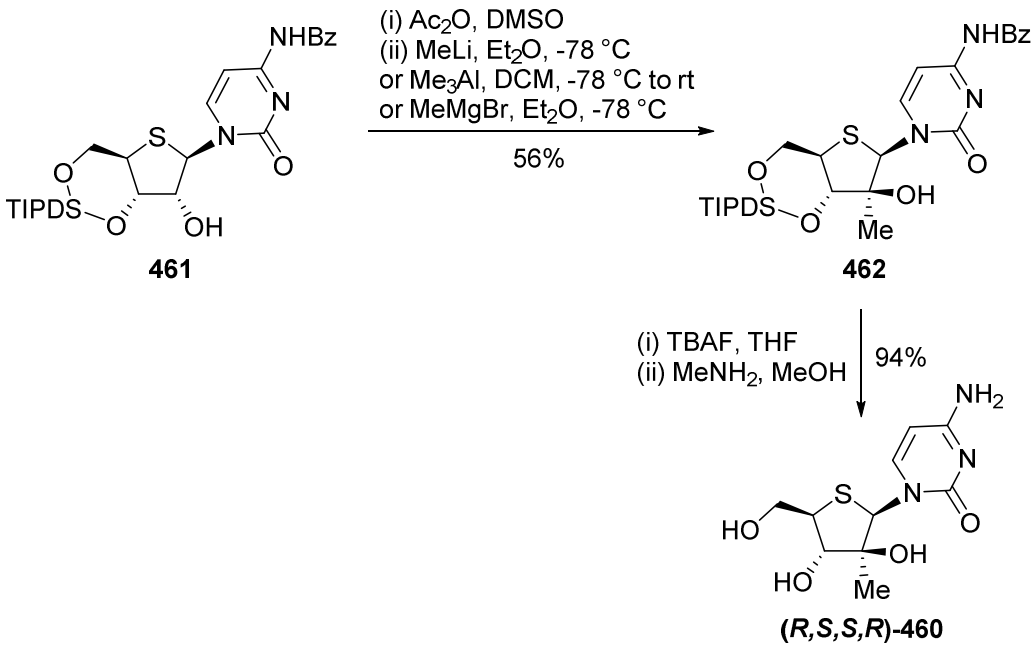
Scheme 35

In related work, Matsuda *et al.* described the synthesis of 2´-*C*-methyl-4´-thiocytidine (**460**) (Scheme 36).[79] Alcohol **453** was converted to ketone **454** *via* Parikh-Doering oxidation.[80] Methylation of **454** with methyltitanium trichloride afforded a 3:1 ratio of both α- and β-addition products **455** and **456** as an inseparable mixture. Dimethoxybenzoylation of **455** and **456** facilitated the isolation of **457** after column chromatography in 19% yield. Ozone-mediated oxidation of **457** afforded sulfoxide **458** which was subjected to Pummerer-type glycosylation with N^4-benzoylcytosine to provide protected nucleoside **459** in 23% yield. Deprotection in the usual manner produced the target cytidine nucleoside **(*R,R,S,R*)-460**.



Scheme 36

The stereoselectivity of the methylation step depends on the choice of alkylating reagent. Use of methyl lithium and trimethyl aluminium led exclusively to α-methylation in 80% yield, while use of a Grignard reagent afforded the α-product in 52% yield and β-product in 43%.[81, 82] By contrast, when 4´-thiocytidine analogue **461** was exposed to the same conditions, only the undesired α-product **462** was recovered (Scheme 37).

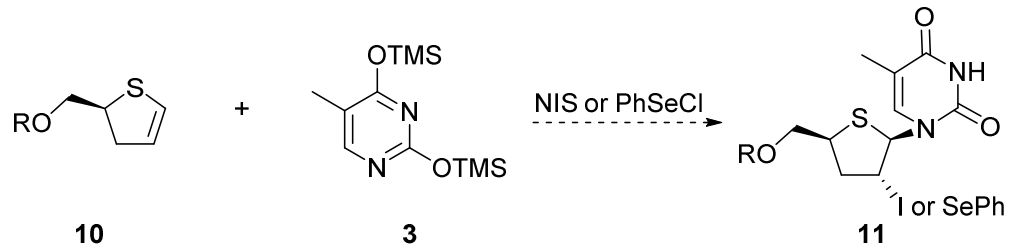


Scheme 37

Neither **(*R,R,S,R*)-460** or its α-epimer **(*R,S,S,R*)-460** were found to possess anti-leukaemia activity in L1210 cells when tested at a concentration of 100 μg/mL. The authors suggest that these results indicate that 4´-thioribocytidine derivatives are less susceptible to phosphorylation by cellular uridine-cytidine kinase.

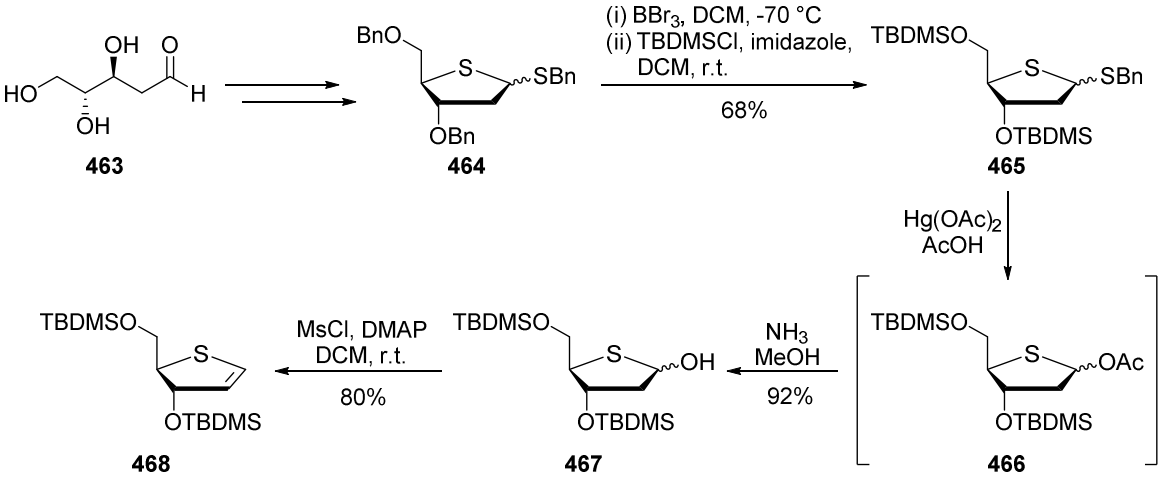
4. ELECTROPHILIC GLYCOSYLATIONS

As β-thionucleosides are superior bioisosteric matches for naturally occurring β-nucleosides, they tend to display significantly higher biologically activity than their α-counterparts, thus making face-selectivity an important consideration in their synthesis. To combat the reduced selectivity associated with Vörbruggen-type and Pummerer-type glycosylations, Haraguchi and co-workers developed an alternative methodology involving electrophilic glycosylation of thioglycals whereby β-face-selectivity could be targeted.[54] In this approach, an electrophilic glycal donor **10** is glycosylated with a persilylated nucleobase in the presence of *N*-iodosuccinimide or phenylselenylchloride, which are key to directing the β-selectivity (Scheme 38).



Scheme 38

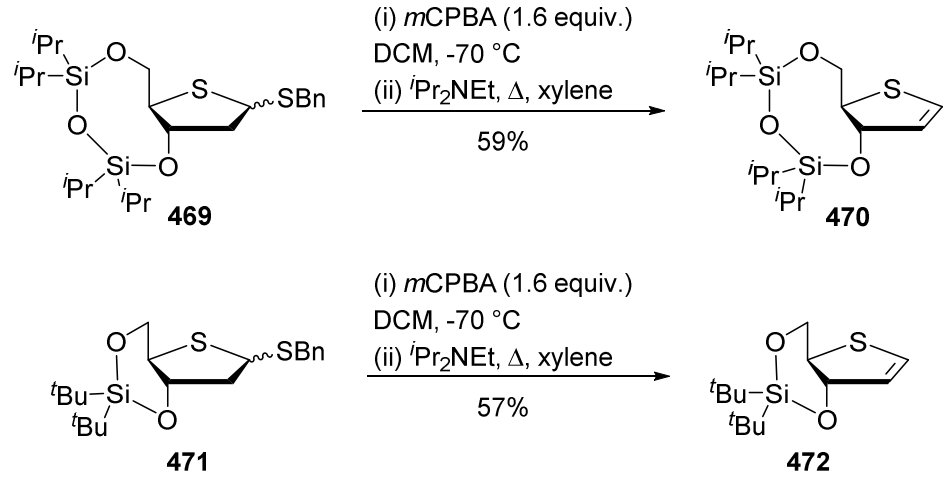
Initially, 2-deoxy-D-ribose (**463**) was converted to thiofuranose **467** over seven steps as *per* the work of Dyson *et al*. (Scheme 39).[31] Selective debenzylation of **464** was followed by silylation to furnish **465** in 68% yield. Acetolysis of **465** at the 1´-position was achieved with mercury(II) acetate and acetic acid. The resulting thioacetal **466** was deacetylated to form thiolactol **467**. Treatment of **467** with methanesulfonyl chloride and 4-dimethylaminopyridine (DMAP) promoted β-elimination of the *in situ* generated mesylate, furnishing the desired glycal **468** in 80% isolated yield.



Scheme 39

As the face-selectivity in the glycosylation step can be controlled by varying the silyl protecting groups on the 3´- and 5´-hydoxyl moieties[83, 84], several electrophilic donors were prepared in the same manner as **465**. Chemoselective oxidation of the 1´-thiobenzyl group of **469** and **471** was accomplished in the presence of *m*CPBA (Scheme

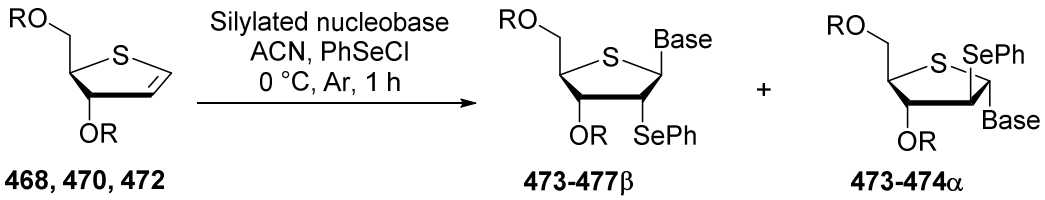
40). While several oxidation products were identified, heating the crude mixture in the presence of *N*,*N*-diisopropylethylamine afforded glycals **470** and **472** in 59% and 57% yields respectively.



Scheme 40

Installation of a phenylselenyl moiety at the 2´-position allowed for the introduction of new functionalities and further transformations at a later stage in the synthesis. Simultaneous selenation/glycosylation of **468**, **470** and **472** proceeded in a face-selective manner on varying the silyl protecting groups at the 3´- and 5´-positions. The face-selectivity of these electrophilic glycosylations could be controlled with β-approach of the electrophile favoured in all instances. The bulkier the silyl group, the higher the observed β-selectivity (Table 17).

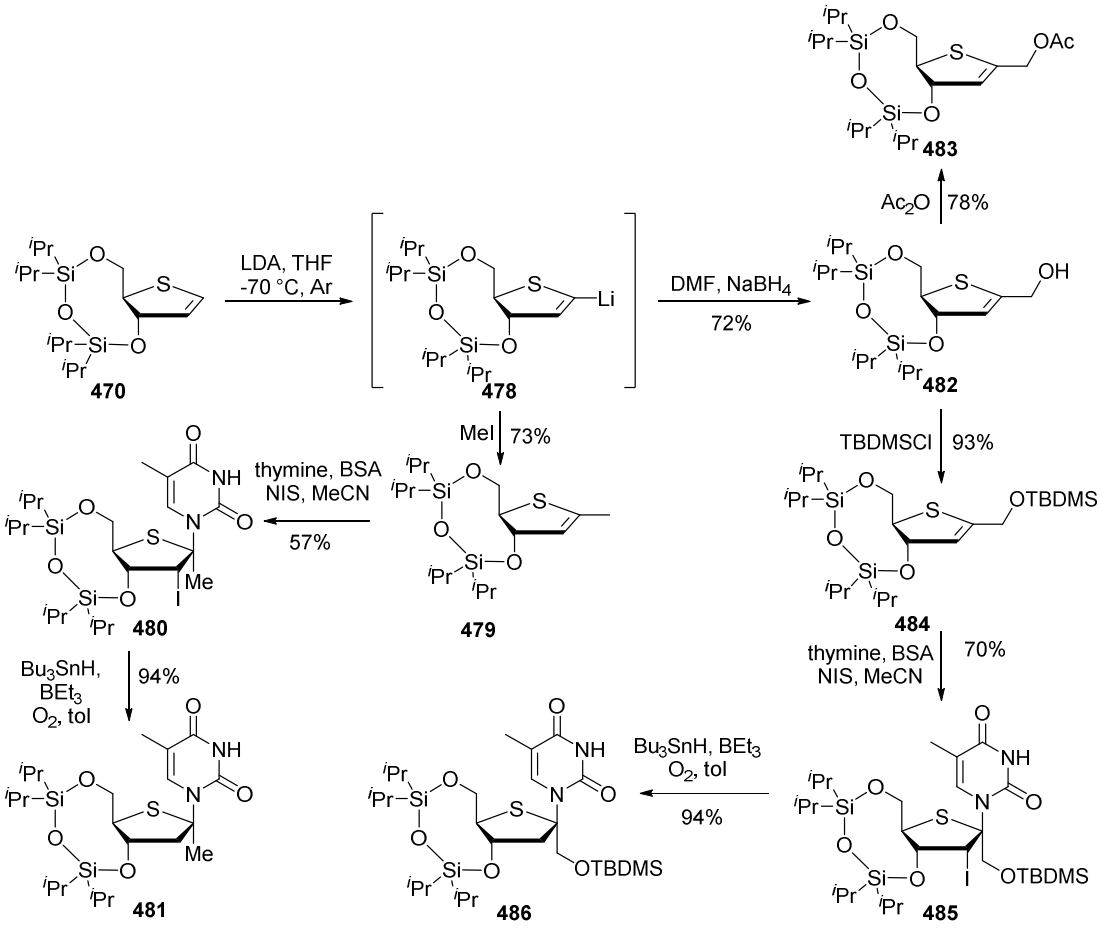
Table 17: Substituents, yields and α:β selectivities for selenides **473**-**477**



Entry	R	Base	Products	Total	Ratio β:α

	Glycal				Yield	
1	**468**	TBDMS	Uracil-1-yl	**473β**, **473α**	88%	4:1
2	**470**	-(^{i}Pr-$_2$Si)$_2$O-	Uracil-1-yl	**474β**, **474α**	87%	18:1
3	**472**	-^{t}Bu$_2$Si-	Uracil-1-yl	**475β**	88%	-
4	**472**	-^{t}Bu$_2$Si-	Thymin-1-yl	**476β**	62%	-
5	**472**	-^{t}Bu$_2$Si-	Cytosine-1-yl	**477β**	85%	-

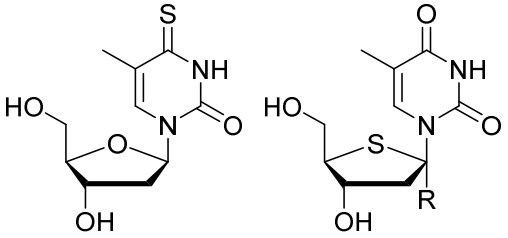
In a further elaboration of this work, Haraguchi and colleagues prepared more complex targets, again *via* electrophilic substitution.[55, 56] α-Lithiation of **470** with lithium diisopropylamide (LDA) in an argon atmosphere afforded 1´-lithiated **478** which underwent *in situ* methylation with methyl iodide to form 1´-carbosubstituted glycal **479** (Scheme 41). NIS-initiated electrophilic β-glycosylation of **479** with thymine resulted in the exclusive formation of the β-product **480** in 57% yield. Radical reduction of **480** facilitated the removal of the 2´-iodo group, employing triethyl boron as a radical initiator in the presence of tributyl tin hydride and oxygen to afford advanced intermediate **481** in excellent yield. Alternatively, reaction of **478** with DMF, followed by sodium borohydride reduction, produced alcohol **482**, which was converted to acetate **483** in 78% yield. The primary alcohol in **482** was silylated prior to electrophilic β-glycosylation to exclusively furnish **485** in 70% yield. Finally, tin-mediated dehalogenation produced **486** in an excellent 94% yield.



Scheme 41

This desilylated 1´-carbosubstituted-4´-thionucleosides were assessed for anti-HSV-1 and anti-HIV-1 activity, using 4´-thiothymidine (**487**) as a control. The data confirmed that a 1´-carbosubstituent is detrimental to the antiviral activity of the nucleoside (Table 1.18).

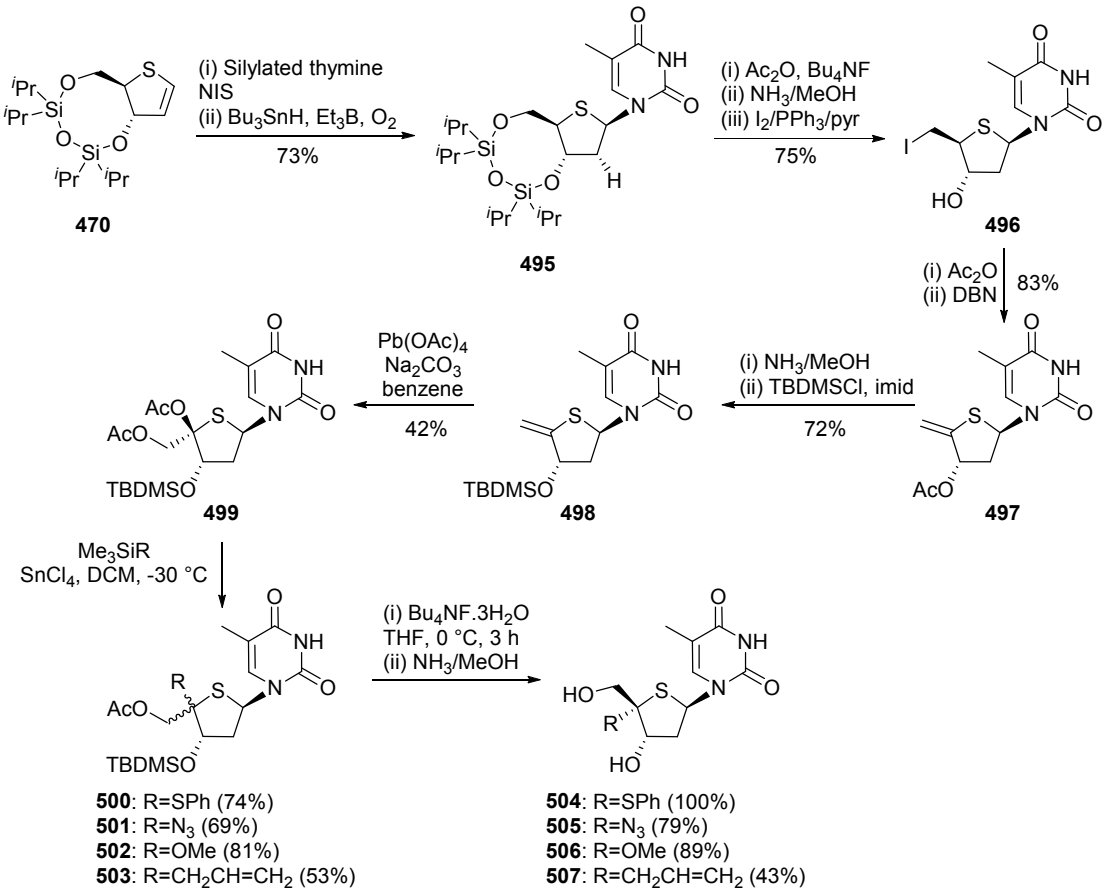
Table 18: Anti-HSV-1 activity of thionucleosides **488-494** vs 4´-thiothymidine (**487**)



487 **488-494**

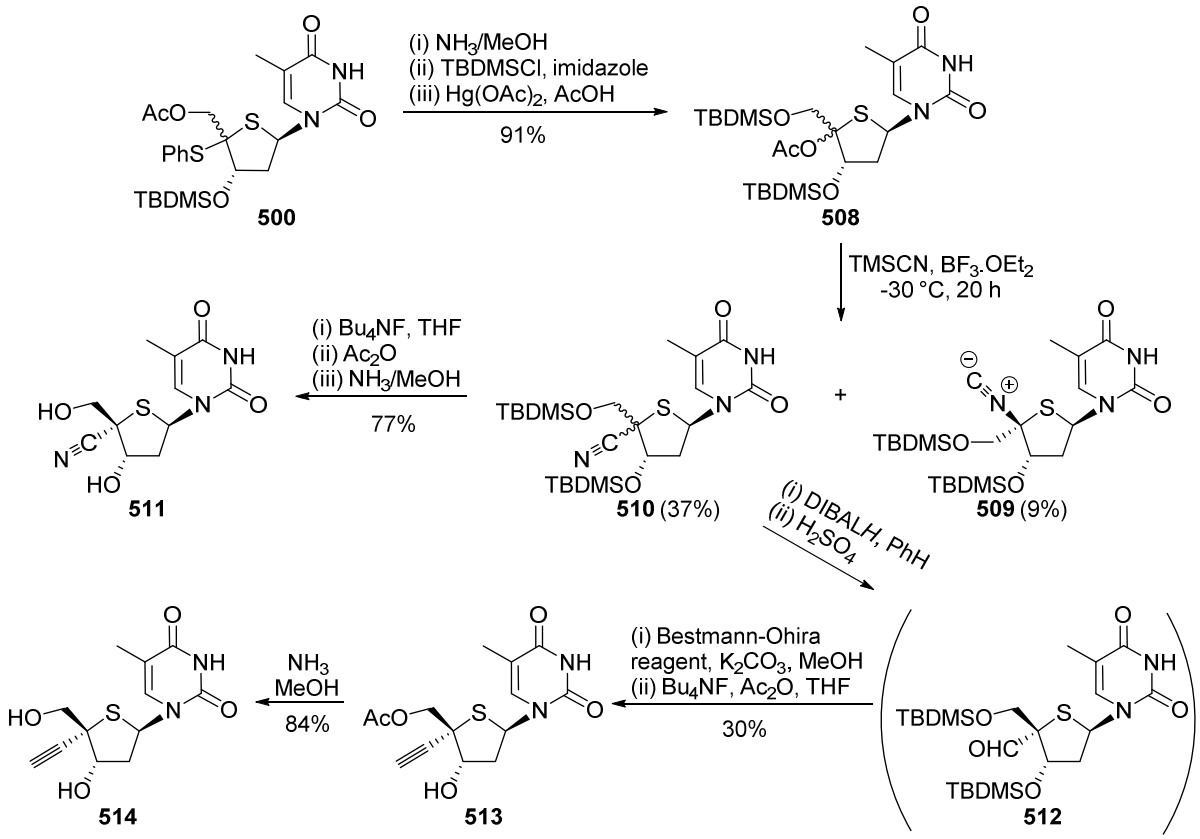
Entry	Thionucleoside	R	HSV-1 EC_{50} (µg/mL)	HIV-1 EC_{50} (µM)
1	4´-thiothymidine (**487**)	H	0.008	-
2	**488**	Me	4	>34.1
3	**489**	CH_2OH	>100	>100
4	**490**	CHO	20	>95.1
5	**492**	CH=CHCN	>100	>100
6	**493**	C(OMe)=NH	100	>100
7	**494**	CN	20	>100

The same group later turned their attention to 4´-substituted-4´-thiothymidines.[57] By exploiting the electrophilic glycosylation methodology, they proceeded to synthesise 4´,5´-unsaturated **498** over a series of nine high-yielding steps (Scheme 42). Silyl protection of the 3´-hydroxyl group allowed for selective diacetoxylation of the neighbouring alkene. Diacetoxylation of **498** was achieved in 56% yield with a mixture of lead(IV) acetate and sodium carbonate in benzene to afford **499**. Using a range of substituted trimethylsilanes and tin(IV) chloride, it was possible to displace the 4´-acetoxy groups to form **500-503** in average to good yields, with predominant formation of the D-isomer reported. Deprotection of **500-503** was achieved using TBAF and methanolic ammonia to provide 4´-substituted-4´-thionucleosides **504-507**.



Scheme 42

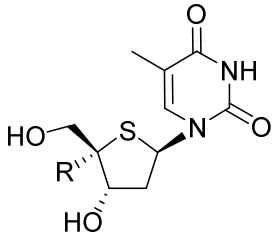
Preparation of the 4´-cyano and 4´-ethynyl derivatives proved more complex. Deacetylation of thioacetal **500**, followed by silylation and acetolysis in mercury(II) acetate furnished **508**, (Scheme 43). Reaction of 4´-acetate **508** with cyanotrimethylsilane and boron trifluorate afforded an α/β mixture of nitrile **510** (37%) and isonitrile by-product **509** (9%). Following separation, deprotection of **510** afforded 4´-thio-4´-cyano nucleoside **511**. Reduction of the same mixture with diisobutylaluminium afforded 4´-aldehyde **512**. Crude **512** was converted to ethynyl **513** using the Bestmann-Ohira reagent. Finally, deprotection furnished 4´-ethynyl-4´-thionucleoside **514**.



Scheme 43

When tested for their antiviral activity, the 4´-substituted-4´-thionucleosides exhibited some promising results (Table 19). The most potent analogues against HIV-1 and HIV-2 were found to be the 4´-azido (**505**), 4´-cyano (**511**) and 4´-ethynyl (**514**) derivatives. Compounds **511** and **514** were also found to be active against HIV-1M184V, *i.e.* wild-type HIV with a methionine to valine mutation at position-184, a well known HIV mutant resistant to a number of NRTIs, including lamivudine (3TC).

Table 19: Antiviral activity of selected thionucleosides against HIV-1 and HIV-2 cell lines

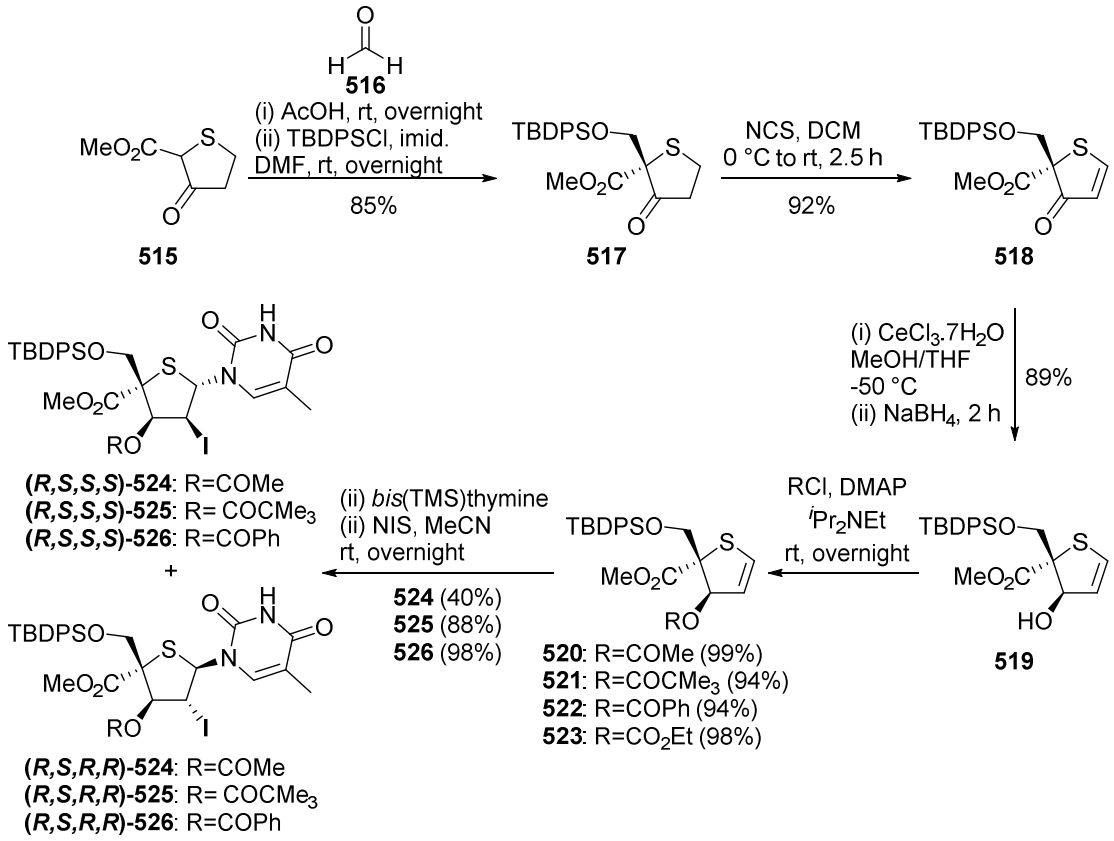


504-507, 511, 514

Entry	Nucleoside	R	HIV-1		HIV-2	
			EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
1	**504**	SPh	>100	>100	>100	>100
2	**505**	N_3	0.02	40	0.024	>10
3	**506**	OMe	>4.0	>100	1.2	>100
4	**507**	$CH_2CH{=}CH_2$	>100	>100	>100	>100
5	**511**	C≡N	0.037	>100	0.023	>10
6	**514**	C≡CH	0.31	>100	0.13	>10

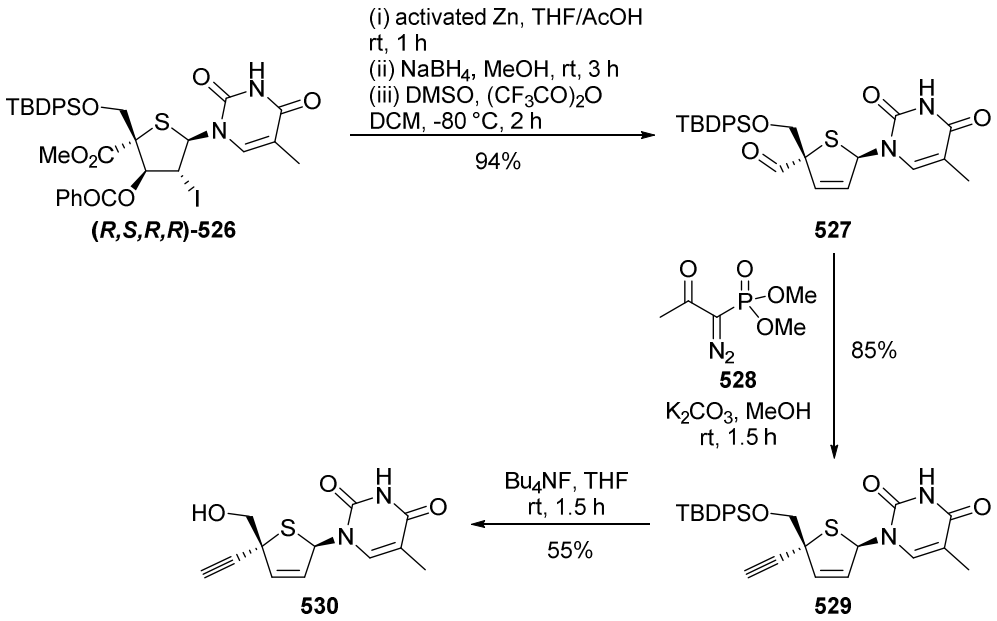
Following on from their discovery that 4´-ethynyl-stavudine is a more potent HIV inhibitor than the NRTI stavudine (d4T),[85, 86] Tanaka *et al*. developed a range of 4´-substituted-4´-thiostavudine analogues.[87] Heterocycle **515** was prepared according to chemistry pioneered by Woodward *et al*.[88] Acid-catalysed aldol addition of formaladehyde to **515** was followed by silyl-protection of the resulting alcohol affording **517** in 85% overall yield. Ketone **517** was converted to enone **518** *via* treatment with *N*-chlorosuccinimide (Scheme 44). Luche reduction of the α,β-unsaturated ketone afforded **519** and its epimer in 89% yield (ratio ~10:1).[89, 90] Acylation of **519** in the conventional manner produced esters **520-523** in excellent yields. Following several failed attempts at palladium-catalysed allylic substitution of thiofuranoid **520** with a thymine base,

electrophilic glycosylation was finally achieved using *N*-iodosuccinimide and persilylated thymine in acetonitrile.[54, 56, 91, 92] Selectivity was highest for benzoate **526** with a 1:10 ratio of α:β isomers and an overall yield of 98%. Pivalate **525** was formed in an 88% yield in a 1:1 α:β ratio.



Scheme 44

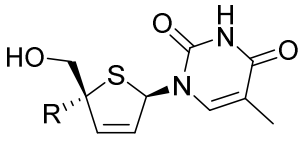
Iodide **(*R,S,R,R*)-526** readily underwent 2´-3´-elimination in the presence of activated zinc (Scheme 45). This was followed by reduction of the 4´-ester and subsequent Swern oxidation to afford aldehyde **527** in 94% yield. Conversion of **527** to alkyne **529** was achieved using the Bestmann-Ohira reagent, and finally fluoride-mediated deprotection gave target nucleoside **530**.



Scheme 45

In total, six different 4´-substituted-4´-thionucleosides were analysed for anti-HIV activity (Table 20). Alkyne **530** and nitrile **535** were found to display HIV-1 inhibitory activity (Entries 2 and 8), suggesting that an sp-hybridised 4´-carbon substituent is an important contributory factor to activity. All compounds assayed were found to be non-cytotoxic in MT-4 cells. Optical resolution of 4´-ethynyl **530** revealed the *levo*-enantiomer to be the active isomer, with an EC_{50} value comparable to that of stavudine (d4T) itself (Entry 9). It should be noted that 4´-thiostavudine has itself been found to be inactive against HIV.[93]

Table 20: Anti-HIV activity of thionucleosides **530-535**



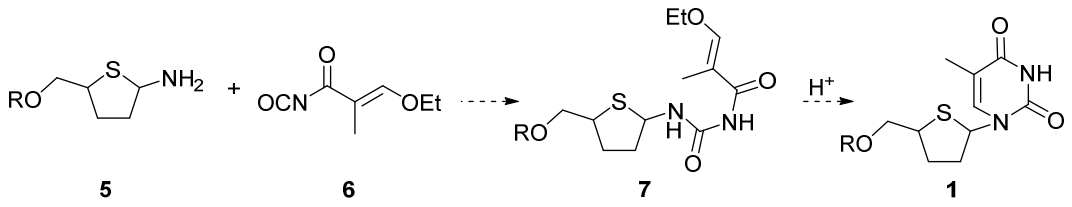
530-535

Entry	Thionucleoside	R	MT-4 cell line	
			EC_{50} (μM)	CC_{50} (μM)

1	**530**	C≡CH	0.74	>100
2	*levo*-**530**	C≡CH	0.37	>100
3	*dextro*-**530**	C≡CH	>20	>100
4	**531**	CH_2OH	>100	>100
5	**532**	CO_2Me	>100	>100
6	**533**	$CONH_2$	>100	>100
7	**534**	$CH=CH_2$	>100	>100
8	**535**	CN	7.6	>100
9	d4T	H	0.51	>100
10	4´-ethynyl-d4T	C≡CH	0.060	>100

5. LATE-STAGE CONSTRUCTION OF THE NUCLEOSIDE BASE

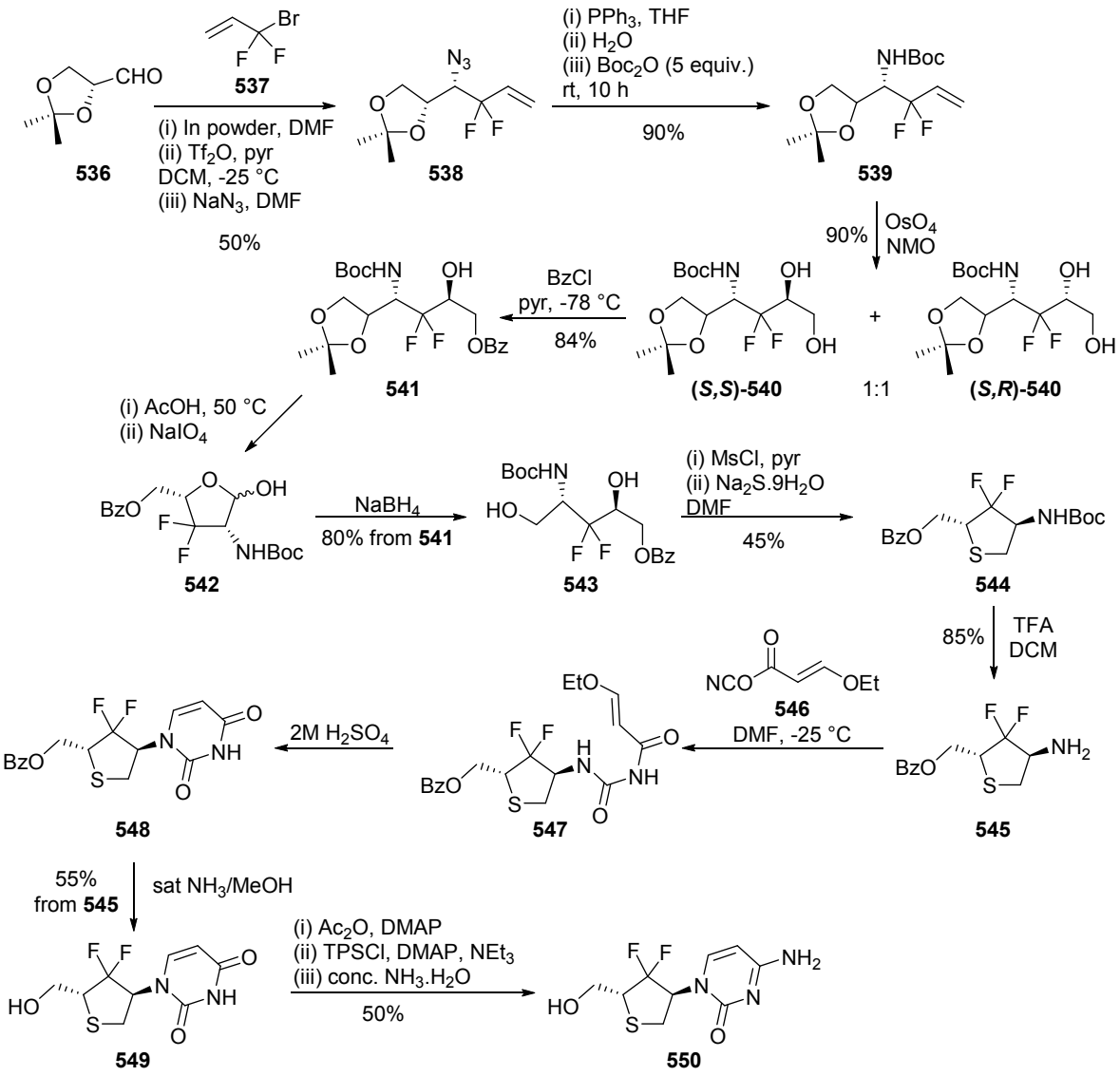
In their search for new 3TC analogues where the 1-oxygen is substituted with a difluoromethylene group, Wu *et al*. investigated novel 6´-difluoro-3´-thiopyrimidines for possible anti-HIV and anti-HBV activity.[94, 95] Their novel approach to accessing these targets involved a late-stage construction of the nucleoside base around the amine at the glycal position (Scheme 46).



Scheme 46

The reaction of 3-bromo-3,3-difluoropropene (**537**) and indium in DMF generated a *gem*-difluoroallylindium which was coupled *in situ* to aldehyde **536** to form a *gem*-

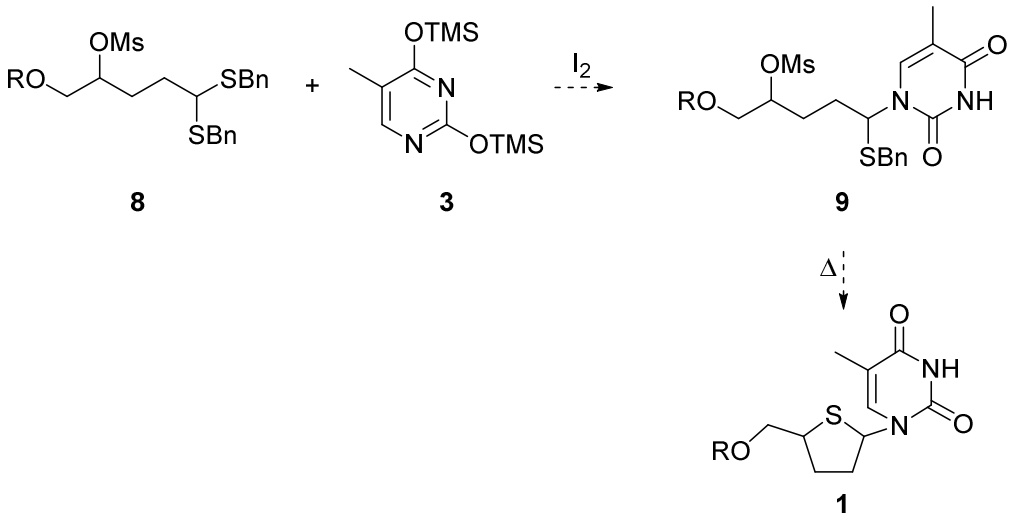
difluorohomoallyl alcohol, and then triflated and converted to azide **538** in 50% yield (Scheme 47). A Staudinger-type reduction afforded the *syn*-isomer, which was boc-protected to afford amine **539** in 90% yield. Osmium tetroxide-catalysed dihydroxylation of **539** produced a 1:1 mixture of isomeric products **(*S,R*)-540** and **(*S,S*)-540** which were separable by column chromatography. Following selective benzoylation of the primary hydroxyl in **(*S,S*)-540**, acetal hydrolysis followed by oxidative cleavage of the resulting diol revealed an aldehyde which underwent spontaneous cyclisation to **542**. Reduction of lactol **542** to diol **543** and subsequent mesylation at the 1- and 4-positions was followed by sulfide substitution with concomitant ring closure, affording thiofuranose **544** as a single stereoisomer. Deprotection with trifluoroacetic acid (TFA) produced key amine intermediate **545** in 85% yield, upon which the scaffold of the pyrimidine base could be built according to the procedure of Shaw and Warrener.[96] Condensation of 3-ethoxy-2-propenoyl isocyanate **546** with the free amine in **545** gave **547** which underwent acid-catalysed ring closure to afford protected nucleoside **548**. Hydroxyl deprotection was achieved *via* ammonolysis to furnish target uridine analogue **549** in 55% yield from **545**. **549** was converted to **550** in the usual manner. Biological evaluation of these compounds is on-going.



Scheme 47

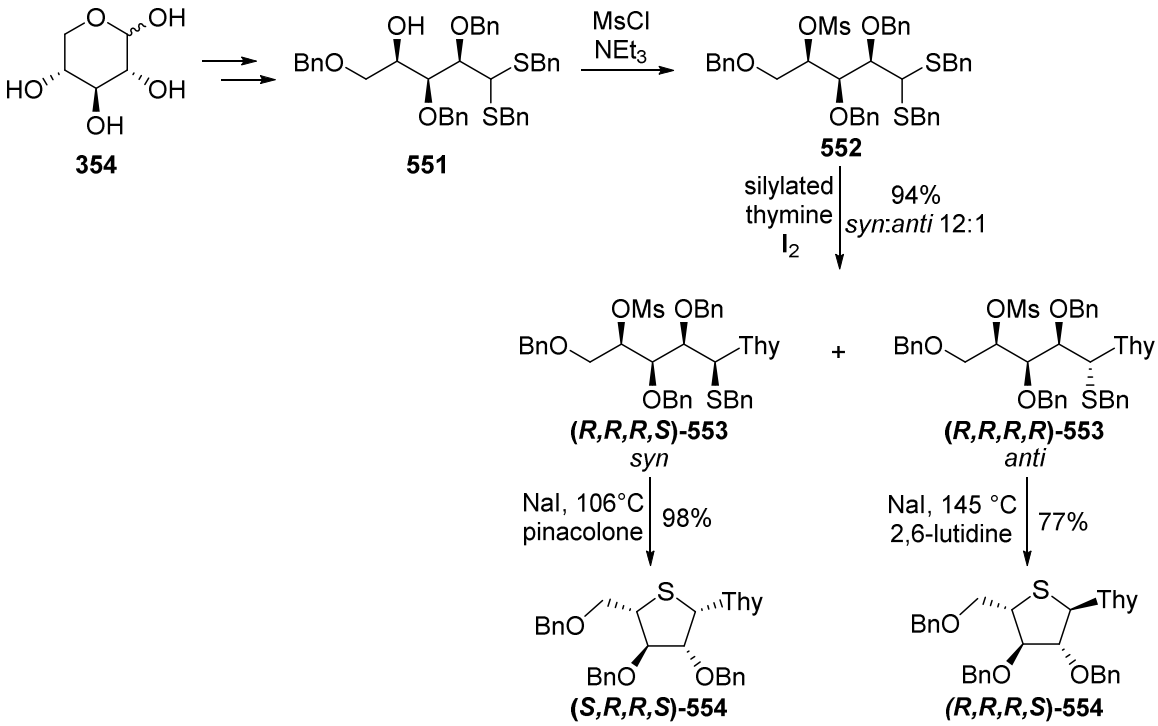
6. LATE-STAGE CONSTRUCTION OF THE THIOLANE RING

Chapdelaine *et al*. described a novel strategy to access both D- and L-series of 4´-oxo- and 4´-thionucleosides where iodine-mediated glycosylation occurs prior to cyclisation of the thiolane ring (Scheme 48).[97]



Scheme 48

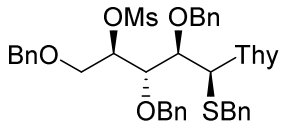
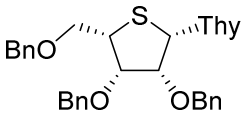
Exploiting the published procedure by Wirsching *et al.,*[98] **551** was synthesised in three steps from D-xylose (**354**) (Scheme 49). Mesylation of the 4´-hydroxyl group of **551** afforded acyclic glycal donor **552**. Glycosylation of **552** with persilylated thymine produced separable *syn* and *anti* isomers **(*R,R,R,S*)-553** and **(*R,R,R,R*)-553** in 94% overall yield, with the *syn* isomer favoured by a ratio of 12:1. Cyclisation of thioaminals **(*R,R,R,S*)-553** and **(*R,R,R,R*)-553** was achieved *via* heating at reflux with sodium iodide in the presence of pinacolone or 2,6-lutidine to furnish thionucleosides **(*S,R,R,S*)-554** and **(*R,R,R,S*)-554**.

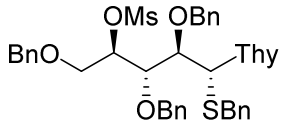
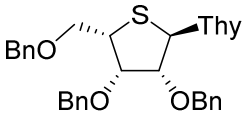
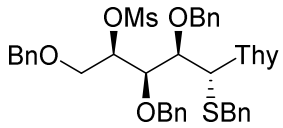
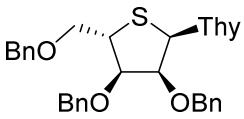
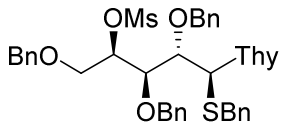
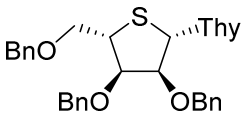
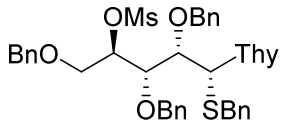
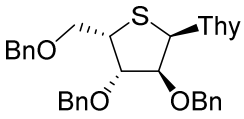
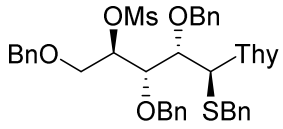
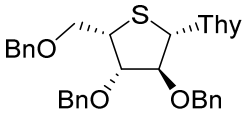


Scheme 49

This chemistry was applied to a library of isomeric analogues of **554** (Table 21). It was reported that all cyclisations were diasterospecific and products were obtained in good to excellent yields. This novel strategy for accessing thionucleosides by-passes the issues associated with complicated glycosylation steps of nucleosides with glycal donors. Biological evaluation of these compounds was not reported.

Table 21: Synthesis of thionucleoside **554**.

Entry	Substrate	Base	Temp.	Thionucleoside	Yield
1	 **(*R,S,R,S*)-553** (*syn*)	2,6-lutidine	145 °C	 **(*S,R,S,S*)-554**	99%

2	 **(*R,S,R,R*)-553** (*anti*)	2,6-lutidine	145 °C	 **(*R,R,S,S*)-554**	97%
3	 **(*R,R,R,R*)-553** (*syn*)	2,6-lutidine	145 °C	 **(*R,S,R,S*)-554**	82%
4	 **(*R,R,S,S*)-553** (*anti*)	pinacolone	106 °C	 **(*S,S,R,S*)-554**	96%
5	 **(*R,S,S,R*)-553** (*syn*)	pinacolone	106 °C	 **(*R,S,S,S*)-554**	96%
6	 **(*R,S,S,S*)-553** (*anti*)	2,6-lutidine	145 °C	 **(*S,S,S,S*)-554**	99%

7. CONCLUSION

From this review of the recent literature, it is apparent that thionucleosides remain molecules worthy of further investigation. The replacement of oxygen with sulfur produces bioisosteric analogues of cell-native oxonucleosides, compounds which are noteworthy for their increased biological activity, interesting pharmacological profiles or improved metabolic stabilities. Their potential as anti-viral agents will have been brought into sharper focus in light of the recent COVID-19 global pandemic.[99-101] The past decade has seen increased use of Pummerer-type glycosylations, in addition to the well-established Vörbruggen methodology. The medicinal chemist's synthetic toolkit

has been further augmented by the development of highly selective electrophilic glycosylation chemistry pioneered by Haraguchi. As this review demonstrates, recent advances in the preparation of thionucleosides have opened up many high-yielding synthetic routes to these valuable targets. Thus, there remains many novel thionucleoside-based structures yet to be explored, with their full potential as yet unrealised.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

FPO'D thanks the Irish Research Council for funding (GOIPG/2014/795).

REFERENCES

1. Prusoff, W. H., Synthesis and biological activities of iododeoxyuridine, an analog of thymidine. *Biochim. Biophys. Acta* **1959,** *32*, 295-296.
2. Lamberth, C., NUCLEOSIDES WITH A CARBON BRIDGE BETWEEN SUGAR AND NUCLEOBASE: THE CHEMISTRY OF 1'-HOMONUCLEOSIDES AND REVERSED NUCLEOSIDES. A REVIEW. *Org. Prep. Proc.* **2002,** *34* (2), 149-167.
3. Huang, R.-M.; Yin-Ning Chen, Y.-N.; Ziyu Zeng, Z.; Cheng-Hai Gao, C.-H.; Xiangdong Su, X.; Yan Peng, Y., Marine Nucleosides: Structure, Bioactivity, Synthesis and Biosynthesis. *Mar. Drugs* **2014,** *12* (12), 5817-5838.
4. Mathé, C.; Gosselin, G., L-Nucleoside enantiomers as antivirals drugs: A mini-review. *Antiviral Rev.* **2006,** *71* (2-3), 276-281.
5. Périgaud, C.; Gosselin, G.; Imbach, J. L., Nucleoside Analogues as Chemotherapeutic Agents: A Review. *Nucleosides Nucleotides Nucleic Acids* **1992,** *11* (2-4), 903-945.
6. De Clercq, E.; Holý, A., Acyclic nucleoside phosphonates: a key class of antiviral drugs. *Nat. Rev. Drug Discov.* **2005,** *4* (11), 928-940.
7. Ichikawa, E.; Kato, K., Sugar-Modified Nucleosides in Past 10 Years, A Review. *Curr. Med. Chem.* **2001,** *8* (4), 385-423.
8. Reist, E. J.; Gueffroy, D. E.; Goodman, L., Synthesis of 4-Thio-D- and -L-ribofuranose and the Corresponding Adenine Nucleosides. *J. Am. Chem. Soc.* **1964,** *86* (24), 5658-5663.
9. Gunaga, P.; Moon, H. R.; Choi, W. J.; Shin, D. H.; Park, J. G.; Jeong, L. S., Recent advances in 4'-thionucleosides as potential antiviral and antitumor agents. *Curr. Med. Chem.* **2004,** *11* (19), 2585-2637.

10. Madern, J. M.; Hansen, T.; van Rijssel, E. R.; Kistemaker, H. A. V.; van der Vorm, S.; Overkleeft, H. S.; van der Marel, G. A.; Filippov, D. V.; Codée, J. D. C., Synthesis, Reactivity, and Stereoselectivity of 4-Thiofuranosides. *J. Org. Chem.* **2019,** *84* (3), 1218-1227.
11. Otter, G. P.; Elzagheid, M. I.; Jones, G. D.; MacCulloch, A. C.; Walker, R. T.; Oivanen, M.; D., K. K., Synthesis and hydrolytic stability of the α and β anomers of 4'-thio-2'-deoxyuridine and their 5-substituted analogs. Competition between the acid-catalysed depyrimidination and isomerisation to a 5-thiopyranoside nucleoside. *J. Chem. Soc., Perkin Trans. 2* **1998,** *2*, 2343-2350.
12. Elzagheid, M. I.; Oivanen, M.; Walker, R. T.; Secrist III, J. A., Kinetics for the Acid-Catalyzed Hydrolysis of Purine and Cytosine 2'-Deoxy-4'-thionucleosides. *Nucleosides Nucleotides Nucleic Acids* **1999,** *18* (2), 181-186.
13. Shelton, J.; Lu, X.; Hollenbaugh, J. A.; Hyun Cho, J. H.; Amblard, F.; Schinazi, R. F., Metabolism, Biochemical Actions, and Chemical Synthesis of Anticancer Nucleosides, Nucleotides, and Base Analogs. *Chem. Rev.* **2016,** *116*, 14379−14455.
14. Meanwell, N. A., Synopsis of Some Recent Tactical Application of Bioisosteres in Drug Design. *J. Med. Chem.* **2011,** *54* (8), 2529-2591.
15. Lima, L. M.; Barreiro, E. J., Bioisosterism: A Useful Strategy for Molecular Modification and Drug Design. *Curr. Med. Chem.* **2005,** *12* (1), 23-49.
16. Patani, G. A.; LaVoie, E. J., Bioisosterism: A Rational Approach in Drug Design. *Chem. Rev.* **1996,** *96* (8), 3147-3176.
17. Yokoyama, M., Synthesis and biological activity of thionucleosides. *Synthesis* **2000,** *2000* (12), 1637-1655.
18. Boryski, J., Transglycosylation Reactions of Purine Nucleosides. A Review. *Nucleosides Nucleotides Nucleic Acids* **1996,** *15* (1-3), 771-791.
19. Manvar, A.; Shah, A., Continuous Flow and Microwave-Assisted Vorbrüggen Glycosylations: Historical Perspective to High-Throughput Strategies. *Asian. J. Org. Chem.* **2014,** *3* (11), 1134-1149.
20. Niedballa, U.; Vorbrüggen, H., A General Synthesis of Pyrimidine Nucleosides. *Angew. Chem. Int. Ed.* **1970,** *9* (6), 461-462.
21. Miller, J. A.; Pugh, A. W.; Ullah, G. M.; Gutteridge, C., A novel synthesis of 4'-thionucleosides and a potential stereospecific route to pyrimidine nucleosides. *Tetrahedron Lett.* **2000,** *41* (51), 10099-10105.
22. Trost, B. M.; Nübling, C., An asymmetric approach to 2-deoxynucleosides *via* organosulfur building blocks as chemical chameleons. *Carbohydr. Res.* **1990,** *202*, 1-12.
23. Yamagata, K.; Yamagiwa, Y.; Kamikawa, T., Perkin communications. Synthesis of chiral long-chain α-hydroxy acids from L-ascorbic acid. Useful components for the synthesis of cerebrosides. *J. Chem. Soc., Perkin Trans. 1* **1990,** (12), 3355-3357.
24. Rahim, S. G.; Trivedi, N.; Bogunovic-Batchelor, M. V.; Hardy, G. W.; Mills, G.; Selway, J. W. T.; Snowden, W.; Littler, E.; Coe, P. L.; Basnak, I.; Whale, R. F.; Walker, R. T., Synthesis and Anti-Herpes Virus Activity of 2'-Deoxy-4'-thiopyrimidine Nucleosides. *J. Med. Chem.* **1996,** *39* (3), 789-795.

25. Tiwari, K. N.; Cappellacci, L.; Montgomery, J. A.; Secrist III, J. A., Synthesis and Anti-cancer Activity of Some Novel 5-Azacytosine Nucleosides. *Nucleosides Nucleotides Nucleic Acids* **2003,** *22* (12), 2161-2170.
26. Secrist III, J. A.; Tiwari, K. N.; Riordan, J. M.; Montgomery, J. A., Synthesis and biological activity of 2'-deoxy-4'-thio pyrimidine nucleosides. *J. Med. Chem.* **1991,** *34* (8), 2361-2366.
27. Tiwari, K. N.; Montgomery, J. A.; Secrist III, J. A., The Synthesis and Biological Activity of 1-(2-Deoxy-4-Thio-α;-L-Threo-Pentofuranosyl) Thymine. *Nucleosides Nucleotides Nucleic Acids* **1993,** *12* (8), 841-846.
28. Liu, J.; Aerschot, A. V.; Luyten, I.; Wigerinck, P.; Pannecouque, C.; Balzarini, J.; Clercq, E. D.; Herdewijn, P., Synthesis and antiviral activities of some new 5-heteroaromatic substituted derivatives of 2'-deoxyuridine. *Nucleosides Nucleotides Nucleic Acids* **1995,** *14* (3-5), 525-528.
29. Clercq, E. D.; Walker, R. T., Chemotherapeutic Agents for Herpes virus Infections. In *Prog. Med. Chem.*, Ellis, G. P.; West, G. B., Eds. Elsevier: Amsterdam, Netherlands, 1986; Vol. Volume 23, pp 187-218.
30. Maslen, H. L.; Hughes, D.; Hursthouse, M.; De Clercq, E.; Balzarini, J.; Simons, C., 6-Azapyrimidine-2'-deoxy-4'-thionucleosides: Antiviral Agents against TK+ and TK− HSV and VZV Strains. *J. Med. Chem.* **2004,** *47* (22), 5482-5491.
31. Dyson, M. R.; Coe, P. L.; Walker, R. T., An improved synthesis of benzyl 3,5-di-O-benzyl-2-deoxy-1,4-dithio-D-erythro-pentofuranoside, an intermediate in the synthesis of 4'-thionucleosides. *Carbohydr. Res.* **1991,** *216*, 237-248.
32. Kim, C. U.; Misco, P. F., Facile, highly stereoselective synthesis of 2',3'-dideoxy- and 2',3'-didehydro-2',3t'-dideoxy nucleosides *via* a furanoid glycal intermediate. *Tetrahedron Lett.* **1992,** *33* (39), 5733-5736.
33. Jasamai, M.; Balzarini, J.; Simons, C., 6-Azathymidine-4'-thionucleosides: synthesis and antiviral evaluation. *J. Enzyme Inhib. Med. Chem.* **2008,** *23* (1), 56-61.
34. Barton, D. H.; McCombie, S. W., A new method for the deoxygenation of secondary alcohols. *J. Chem. Soc., Perkin Trans.1* **1975,** (16), 1574-1585.
35. Choo, H.; Chong, Y.; Choi, Y.; Mathew, J.; Schinazi, R. F.; Chu, C. K., Synthesis, Anti-HIV Activity, and Molecular Mechanism of Drug Resistance of L-2',3'-Didehydro-2',3'-dideoxy-2'-fluoro-4'-thionucleosides. *J. Med. Chem.* **2003,** *46* (3), 389-398.
36. Pejanović, V.; Stokić, Z.; Stojanović, B.; Piperski, V.; Popsavin, M.; Popsavin, V., Synthesis and biological evaluation of some novel 4′-Thio-l-ribonucleosides with modified nucleobase moieties. *Bioorg. Med. Chem. Lett.* **2003,** *13* (11), 1849-1852.
37. Jeong, L. S.; Gunaga, P.; Kim, H. O.; Tosh, D. K.; Lee, H. W.; Choe, S. A.; Moon, H. R.; Gao, Z.-G.; Jacobson, K. A.; Chun, M. W., Stereoselective Synthesis of 1'-Functionalized-4'-Thionucleosides. *Nucleosides Nucleotides Nucleic Acids* **2007,** *26* (8-9), 1011-1014.
38. Jeong, L. S.; Lee, H. W.; Jacobson, K. A.; Kim, H. O.; Shin, D. H.; Lee, J. A.; Gao, Z.-G.; Lu, C.; Duong, H. T.; Gunaga, P.; Lee, S. K.; Jin, D. Z.; Chun, M. W.; Moon, H. R., Structure−Activity Relationships of 2-Chloro-*N*6-substituted-4'-

thioadenosine-5'-uronamides as Highly Potent and Selective Agonists at the Human A3 Adenosine Receptor. *J. Med. Chem.* **2006,** *49* (1), 273-281.
39. Jeong, L. S.; Choe, S. A.; Gunaga, P.; Kim, H. O.; Lee, H. W.; Lee, S. K.; Tosh, D. K.; Patel, A.; Palaniappan, K. K.; Gao, Z.-G., Discovery of a new nucleoside template for human A3 adenosine receptor ligands: D-4'-thioadenosine derivatives without 4'-hydroxymethyl group as highly potent and selective antagonists. *J. Med. Chem.* **2007,** *50* (14), 3159-3162.
40. Qu, S.; Mulamoottil, V. A.; Nayak, A.; Ryu, S.; Hou, X.; Song, J.; Yu, J.; Sahu, P. K.; Zhao, L. X.; Choi, S.; Lee, S. K.; Jeong, L. S., Design, synthesis, and anticancer activity of C8-substituted-4'-thionucleosides as potential HSP90 inhibitors. *Bioorg. Med. Chem.* **2016,** *24* (16), 3418-3428.
41. Tsoukala, E.; Manta, S.; Tzioumaki, N.; Agelis, G.; Komiotis, D., A concise synthesis of 3-fluoro-5-thio-xylo- and glucopyranoses, useful precursors towards their corresponding pyranonucleoside derivatives. *Carbohydr. Res.* **2008,** *343* (6), 1099-1103.
42. Tsoukala, E.; Agelis, G.; Dolinšek, J.; Botić, T.; Cencič, A.; Komiotis, D., An efficient synthesis of 3-fluoro-5-thio-xylofuranosyl nucleosides of thymine, uracil, and 5-fluorouracil as potential antitumor or/and antiviral agents. *Bioorg. Med. Chem.* **2007,** *15* (9), 3241-3247.
43. Tsoukala, E.; Manta, S.; Tzioumaki, N.; Kiritsis, C.; Komiotis, D., Keto-fluorothiopyranosyl nucleosides: a convenient synthesis of 2- and 4-keto-3-fluoro-5-thioxylopyranosyl thymine analogs. *Carbohydr. Res.* **2011,** *346* (13), 2011-2015.
44. Takahashi, M.; Daidouji, S.; Shiro, M.; Minakawa, N.; Matsuda, A., Synthesis and crystal structure of 2'-deoxy-2'-fluoro-4'-thioribonucleosides: substrates for the synthesis of novel modified RNAs. *Tetrahedron* **2008,** *64* (19), 4313-4324.
45. Takahashi, M.; Minakawa, N.; Matsuda, A., Synthesis and characterization of 2'-modified-4'-thioRNA: a comprehensive comparison of nuclease stability. *Nucleic Acids Res.* **2009,** *37* (4), 1353-1362.
46. Naka, T.; Minakawa, N.; Abe, H.; Kaga, D.; Matsuda, A., The stereoselective synthesis of 4'-β-thioribonucleosides *via* the Pummerer reaction. *J. Am. Chem. Soc.* **2000,** *122* (30), 7233-7243.
47. O'Neil, I. A.; Hamilton, K. M., A Novel Method for the Coupling of Nucleoside Bases with Tetramethylene Sulphoxide. *Synlett* **1992,** *1992* (10), 791-792.
48. Jeong, L. S.; Lee, H. W.; Kim, H. O.; Jung, J. Y.; Gao, Z.-G.; Duong, H. T.; Rao, S.; Jacobson, K. A.; Shin, D. H.; Lee, J. A.; Gunaga, P.; Lee, S. K.; Jin, D. Z.; Chun, M. W.; Moon, H. R., Design, synthesis, and biological activity of *N*6-substituted-4'-thioadenosines at the human A3 adenosine receptor. *Bioorg. Med. Chem.* **2006,** *14* (14), 4718-4730.
49. Choi, W. J.; Lee, H. W.; Kim, H. O.; Chinn, M.; Gao, Z.-G.; Patel, A.; Jacobson, K. A.; Moon, H. R.; Jung, Y. H.; Jeong, L. S., Design and synthesis of *N*6-substituted-4'-thioadenosine-5'-uronamides as potent and selective human A3 adenosine receptor agonists. *Bioorg. Med. Chem.* **2009,** *17* (23), 8003-8011.
50. Jeong, L. S.; Lee, H. W.; Kim, H. O.; Tosh, D. K.; Pal, S.; Choi, W. J.; Gao, Z.-G.; Patel, A. R.; Williams, W.; Jacobson, K. A.; Kim, H.-D., Structure–activity relationships of 2-chloro-*N*6-substituted-4'-thioadenosine-5'-*N*,*N*-

dialkyluronamides as human A3 adenosine receptor antagonists. *Bioorg. Med. Chem.* **2008,** *18* (5), 1612-1616.
51. Jeong, L. S.; Jin, D. Z.; Kim, H. O.; Shin, D. H.; Moon, H. R.; Gunaga, P.; Chun, M. W.; Kim, Y.-C.; Melman, N.; Gao, Z.-G.; Jacobson, K. A., *N*6-Substituted D-4'-Thioadenosine-5'-methyluronamides: Potent and Selective Agonists at the Human A3 Adenosine Receptor. *J. Med. Chem.* **2003,** *46* (18), 3775-3777.
52. Lee, H. W.; Shin, D. H.; Jung, J. Y.; Kim, H. O.; Chun, M. W.; Melman, N.; Gao, Z. G.; Jacobson, K. A.; Jeong, L. S., D-4'-Thioadenosine Derivatives as Highly Potent and Selective Agonists at the Human A3 Adenosine Receptor. *Nucleosides Nucleotides Nucleic Acids* **2005,** *24* (5-7), 607-609.
53. Corsaro, A.; Pistarà, V.; Chiacchio, M. A.; Vittorino, E.; Romeo, R., Synthesis of 4'-thionucleosides by 1,3-dipolar cycloadditions of the simplest thiocarbonyl ylide with alkenes bearing electron-withdrawing groups. *Tetrahedron Lett.* **2007,** *48* (28), 4915-4918.
54. Haraguchi, K.; Takahashi, H.; Shiina, N.; Horii, C.; Yoshimura, Y.; Nishikawa, A.; Sasakura, E.; Nakamura, K. T.; Tanaka, H., Stereoselective Synthesis of the β-Anomer of 4'-Thionucleosides Based on Electrophilic Glycosidation to 4-Thiofuranoid Glycals. *J. Org. Chem.* **2002,** *67* (17), 5919-5927.
55. Haraguchi, K.; Takahashi, H.; Tanaka, H., 4-Thiofuranoid Glycals: Versatile Synthons for Stereoselective Synthesis of 4'-Thionucleosides. *Nucleosides Nucleotides Nucleic Acids* **2003,** *22* (5-8), 763-765.
56. Haraguchi, K.; Takahashi, H.; Tanaka, H.; Hayakawa, H.; Ashida, N.; Nitanda, T.; Baba, M., Synthesis and antiviral activities of 1'-carbon-substituted 4'-thiothymidines. *Bioorg. Med. Chem.* **2004,** *12* (20), 5309-5316.
57. Haraguchi, K.; Shimada, H.; Tanaka, H.; Hamasaki, T.; Baba, M.; Gullen, E. A.; Dutschman, G. E.; Cheng, Y. C., Synthesis and Anti-HIV Activity of 4'-Substituted 4'-Thiothymidines: A New Entry Based on Nucleophilic Substitution of the 4'-Acetoxy Group. *J. Med. Chem.* **2008,** *51* (6), 1885-1893.
58. Batra, H.; Moriarty, R. M.; Penmasta, R.; Sharma, V.; Stanciuc, G.; Staszewski, J. P.; Tuladhar, S. M.; Walsh, D. A.; Datla, S.; Krishnaswamy, S., A Concise, Efficient and Production-Scale Synthesis of a Protected L-Lyxonolactone Derivative: An Important Aldonolactone Core. *Org. Process Res. Dev.* **2006,** *10* (3), 484-486.
59. Kotoulas, S. S.; Kojić, V. V.; Bogdanović, G. M.; Koumbis, A. E., Synthesis and cytotoxic evaluation of novel pyrimidine deoxyapiothionucleosides. *Bioorg. Med. Chem. Lett.* **2013,** *23* (11), 3364-3367.
60. Kiso, M.; Hasegawa, A., Acetonation of some pentoses with 2,2-dimethoxypropane-*N*,*N*-dimethylformamide-*p*-toluenesulfonic acid. *Carbohydr. Res* **1976,** *52* (1), 95-101.
61. Koumbis, A. E.; Kaitaidis, A. D.; Kotoulas, S. S., Concise synthesis of enantiopure erythro-saccharinic acid lactone and potassium (2*R*,3*R*)-2,3,4-trihydroxy-2-methylbutanoate. *Tetrahedron Lett.* **2006,** *47* (48), 8479-8481.
62. Koumbis, A. E.; Kotoulas, S. S.; Gallos, J. K., A convenient synthesis of 2-C-methyl-d-erythritol 4-phosphate and isotopomers of its precursor. *Tetrahedron* **2007,** *63* (10), 2235-2243.

63. Kotoulas, S. S.; Kojić, V. V.; Bogdanović, G. M.; Koumbis, A. E., Synthesis of novel pyrimidine apiothionucleosides and *in vitro* evaluation of their cytotoxicity. *Tetrahedron* **2015,** *71* (21), 3396-3403.
64. Lee, W. W.; Benitez, A.; Goodman, L.; Baker, B. R., Potential Anticancer Agents. Synthesis of the β-anomer of (D-arabinofuranosyl)-adenine. *J. Am. Chem. Soc.* **1960,** *82* (10), 2648-2649.
65. Nishizono, N.; Akama, Y.; Agata, M.; Sugo, M.; Yamaguchi, Y.; Oda, K., Synthesis of thietane nucleoside with an anomeric hydroxymethyl group. *Tetrahedron* **2011,** *67* (2), 358-363.
66. Yoshimura, Y.; Kuze, T.; Ueno, M.; Komiya, F.; Haraguchi, K.; Tanaka, H.; Kano, F.; Yamada, K.; Asami, K.; Kaneko, N.; Takahata, H., A practical synthesis of 4'-thioribonucleosides. *Tetrahedron Lett.* **2006,** *47* (4), 591-594.
67. Yoshimura, Y.; Yamazaki, Y.; Kawahata, M.; Yamaguchi, K.; Takahata, H., Design and synthesis of a novel ring-expanded 4'-thio-apio-nucleoside derivatives. *Tetrahedron Lett.* **2007,** *48* (26), 4519-4522.
68. McDougal, P. G.; Rico, J. G.; Oh, Y. I.; Condon, B. D., A convenient procedure for the monosilylation of symmetric 1,n-diols. *J. Org. Chem.* **1986,** *51* (17), 3388-3390.
69. Yoshimura, Y.; Yamazaki, Y.; Saito, Y.; Takahata, H., Synthesis of 1-(5,6-dihydro-2H-thiopyran-2-yl)uracil by a Pummerer-type thioglycosylation reaction: the regioselectivity of allylic substitution. *Tetrahedron* **2009,** *65* (45), 9091-9102.
70. Caputo, R.; Guaragna, A.; Palumbo, G.; Pedatella, S., Asymmetric Synthesis of 1,3-Dithiolane Nucleoside Analogues. *Eur. J. Org. Chem.* **2003,** *2003* (2), 346-350.
71. Efimtseva, E. V.; Mikhailov, S. N.; Meshkov, S.; Hankamaki, T.; Oivanen, M.; Lonnberg, H., Dioxolane nucleosides and their phosphonate derivatives: synthesis and hydrolytic stability. *J. Chem. Soc., Perkin Trans. 1* **1995,** (11), 1409-1415.
72. Caputo, R.; Ferreri, C.; Palumbo, G., A new ready, high-yielding, general procedure for acetalization of carbonyl compounds. *Synthesis* **1987,** *1987* (04), 386-389.
73. Di Furia, F.; Licini, G.; Modena, G., Enantioselective Oxidation of Cyclic Dithioacetals and Dithioketals. *Gazz. Chim. Ital.* **1990,** *120*, 165-170.
74. Minakawa, N.; Kaga, D.; Kato, Y.; Endo, K.; Tanaka, M.; Sasaki, T.; Matsuda, A., Synthesis and structural elucidation of 1-(3-C-ethynyl-4-thio-b-D-ribofuranosyl)cytosine (4'-thioECyd). *J. Chem. Soc., Perkin Trans. 1* **2002,** (19), 2182-2189.
75. Hoshika, S.; Minakawa, N.; Matsuda, A., Synthesis and physical and physiological properties of 4'-thioRNA: application to post-modification of RNA aptamer toward NF-κB. *Nucleic Acids Res.* **2004,** *32* (13), 3815-3825.
76. Inoue, N.; Kaga, D.; Minakawa, N.; Matsuda, A., Practical Synthesis of 2'-Deoxy-4'-thioribonucleosides: Substrates for the Synthesis of 4'-ThioDNA. *J. Org. Chem.* **2005,** *70* (21), 8597-8600.
77. Inoue, N.; Minakawa, N.; Matsuda, A., Synthesis and properties of 4'-ThioDNA: unexpected RNA-like behavior of 4'-ThioDNA. *Nucleic Acids Res.* **2006,** *34* (12), 3476-3483.

78. Kikuchi, Y.; Yamazaki, N.; Tarashima, N.; Furukawa, K.; Takiguchi, Y.; Itoh, K.; Minakawa, N., Gene suppression *via* U1 small nuclear RNA interference (U1i) machinery using oligonucleotides containing 2'-modified-4'-thionucleosides. *Bioorg. Med. Chem.* **2013,** *21* (17), 5292-5296.
79. Kaga, D.; Minakawa, N.; Matsuda, A., Nucleosides and Nucleotides. 232. Synthesis of 2'-C-Methyl-4'-thiocytidine: Unexpected Anomerization of the 2'-Keto-4'-thionucleoside Precursor. *Nucleosides Nucleotides Nucleic Acids* **2005,** *24* (10-12), 1789-1800.
80. Parikh, J. R.; Doering, W. v. E., Sulfur trioxide in the oxidation of alcohols by dimethyl sulfoxide. *J. Am. Chem. Soc.* **1967,** *89* (21), 5505-5507.
81. Matsuda, A.; Itoh, H.; Takenuki, K.; Sasaki, T.; Ueda, T., Alkyl Addition Reaction of Pyrimidine 2'-Ketonucleosides : Synthesis of 2'-Branched-Chain Sugar Pyrimidine Nucleosides : Nucleosides and Nucleotides. LXXXI. *Chem. Pharm. Bull.* **1988,** *36* (3), 945-953.
82. Matsuda, A.; Takenuki, K.; Sasaki, T.; Ueda, T., Nucleosides and nucleotides. 94. Radical deoxygenation of tert-alcohols in 1-(2-C-alkylpentofuranosyl)pyrimidines: synthesis of (2'S)-2'-deoxy-2'-C-methylcytidine, an antileukemic nucleoside. *J. Med. Chem.* **1991,** *34* (1), 234-239.
83. Haraguchi, K.; Itoh, Y.; Tanaka, H.; Yamaguchi, K.; Miyasaka, T., Anomeric manipulation of nucleosides: Stereosepecific entry to 1'-C-branched uracil nucleosides. *Tetrahedron Lett.* **1993,** *34* (43), 6913-6916.
84. Itoh, Y.; Haraguchi, K.; Tanaka, H.; Gen, E.; Miyasaka, T., Divergent and Stereocontrolled Approach to the Synthesis of Uracil Nucleosides Branched at the Anomeric Position. *J. Org. Chem.* **1995,** *60* (3), 656-662.
85. Haraguchi, K.; Takeda, S.; Tanaka, H.; Nitanda, T.; Baba, M.; Dutschman, G. E.; Cheng, Y. C., Synthesis of a highly active new anti-HIV agent 2', 3'-didehydro-3'-deoxy-4'-ethynylthymidine. *Bioorg. Med. Chem. Lett.* **2003,** *13* (21), 3775-3777.
86. Dutschman, G. E.; Grill, S. P.; Gullen, E. A.; Haraguchi, K.; Takeda, S.; Tanaka, H.; Baba, M.; Cheng, Y. C., Novel 4'-substituted stavudine analog with improved anti-human immunodeficiency virus activity and decreased cytotoxicity. *Antimicrob. Agents Chemother.* **2004,** *48* (5), 1640-1646.
87. Kumamoto, H.; Nakai, T.; Haraguchi, K.; Nakamura, K. T.; Tanaka, H.; Baba, M.; Cheng, Y. C., Synthesis and Anti-Human Immunodeficiency Virus Activity of 4'-Branched (±)-4'-Thiostavudines. *J. Med. Chem.* **2006,** *49* (26), 7861-7867.
88. Woodward, R. B.; Eastman, R. H., Tetrahydrothiophene ("Thiophane") Derivatives. *J. Am. Chem. Soc.* **1946,** *68* (11), 2229-2235.
89. Luche, J. L., Lanthanides in organic chemistry. 1. Selective 1,2-reductions of conjugated ketones. *J. Am. Chem. Soc.* **1978,** *100* (7), 2226-2227.
90. Luche, J.-L.; Rodriguez-Hahn, L.; Crabbé, P., Reduction of natural enones in the presence of cerium trichloride. *J. Chem. Soc. Chem. Commun.* **1978,** (14), 601-602.
91. Haraguchi, K.; Takahashi, H.; Tanaka, H., Stereoselective entry to 1'-C-branched 4'-thionucleosides from 4-thiofuranoid glycal: synthesis of 4'-thioangustmycin C. *Tetrahedron Lett.* **2002,** *43* (32), 5657-5660.

92. Haraguchi, K.; Shiina, N.; Yoshimura, Y.; Shimada, H.; Hashimoto, K.; Tanaka, H., Novel Stereoselective Entry to 2'-β-Carbon-Substituted 2'-Deoxy-4'-thionucleosides from 4-Thiofuranoid Glycals. *Org. Lett.* **2004,** *6* (16), 2645-2648.
93. Young, R. J.; Shaw-Ponter, S.; Thomson, J. B.; Miller, J. A.; Cumming, J. G.; Pugh, A. W.; Rider, P., Synthesis and antiviral evaluation of enantiomeric 2',3'-dideoxy- and 2',3'-didehydro-2',3'-dideoxy-4'-thionucleosides. *Bioorg. Med. Chem. Lett.* **1995,** *5* (22), 2599-2604.
94. Wu, Y.-Y.; Zhang, X.; Meng, W.-D.; Qing, F.-L., Synthesis of New 2',3'-Dideoxy-6',6'-difluoro-3'-thionucleoside from *gem*-Difluorohomoallyl Alcohol. *Org. Lett.* **2004,** *6* (22), 3941-3944.
95. Yue, X.; Wu, Y.-Y.; Qing, F.-L., Synthesis of a series of novel 2',3'-dideoxy-6',6'-difluoro-3'-thionucleosides. *Tetrahedron* **2007,** *63* (7), 1560-1567.
96. Shaw, G.; Warrener, R. N., 33. Purines, pyrimidines, and glyoxalines. Part VIII. New syntheses of uracils and thymines. *J. Chem. Soc.* **1958**, 157-161.
97. Chapdelaine, D.; Cardinal-David, B.; Prévost, M.; Gagnon, M.; Thumin, I.; Guindon, Y., A Stereoselective Approach to Nucleosides and 4'-Thioanalogues from Acyclic Precursors. *J. Am. Chem. Soc.* **2009,** *131* (47), 17242-17245.
98. Wirsching, J.; Voss, J., Preparation of 2,3,5-Tri-O-benzyl-4-thio-L-arabino-furanosides and the Corresponding 4'-Thionucleoside Analogues. *Eur. J. Org. Chem.* **1999,** *1999* (3), 691-696.
99. Dong, L.; Hu, S.; Gao, J., Discovering drugs to treat coronavirus disease 2019 (COVID-19). *Drug Disc. Ther.* **2020,** *58* (1), 58-60.
100. Amiriana, E. S.; Levy, J. K., Current knowledge about the antivirals remdesivir (GS-5734) and GS-441524 as therapeutic options for coronaviruses. *One Health* **2020,** *9*, 100128.
101. Timothy P. Sheahan, T. P.; Sims, A. C.; Zhou, S.; Graham, R. L.; Pruijssers, A. J.; Agostini, M. L.; Leist, S. R.; Schäfer, A.; Dinnon, K. H.; Stevens, L. J.; Chappell, J. D.; Lu, X.; Hughes, T. M.; George, A. S.; Hill, C. S.; Montgomery, S. A.; Brown, A. J.; Bluemling, G. R.; Natchus, M. G.; Saindane, M.; Kolykhalov, A. A.; Painter, G.; Harcourt, J.; Tamin, A.; Thornburg, N. J.; Swanstrom, R.; Denison, M. R.; Baric, R. S., An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice. *Sci. Transl. Med.* **2020,** *12* (541), 5883.