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Authors	Gavin, Declan P.;Reen, F. Jerry;Rocha-Martin, J.;Abreu-Castilla, I.;Woods, David F.;Foley, Aoife M.;Sanchez-Murcia, P. A.;Schwarz, Maria;O'Neill, P.;Maguire, Anita R.;O'Gara, Fergal
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Genome mining and characterisation of a novel transaminase with remote stereoselectivity

Gavin, DP^{¥1,2}, Reen, FJ^{¥3,4}, Rocha-Martin, J^{¥3}, Abreu-Castilla, I³, Woods, DF³, Foley, AM⁵,
Sánchez-Murcia, PA⁶, Schwarz, M¹, O'Neill, P⁷, Maguire, AR^{2,5*} and O'Gara, F^{2,3,8*}

¹School of Chemistry; Analytical and Biological Chemistry Research Facility; University College
Cork, Cork, Ireland

²Synthesis and Solid State Pharmaceutical Centre, University College Cork, Cork, Ireland

³BIOMERIT Research Centre, School of Microbiology, University College Cork, Cork, Ireland

⁴School of Microbiology, University College Cork, T12 K8AF Cork, Ireland

⁵School of Chemistry, School of Pharmacy, Analytical and Biological Chemistry Research Facility,
University College Cork, Cork, Ireland

⁶Institute of Theoretical Chemistry, Faculty of Chemistry, University of Vienna, Währinger Str.
17 A-1090 Vienna, Austria

⁷ Pfizer Process Development Centre, Loughbeg, Cork, Ireland.

⁸Human Microbiome Programme, School of Pharmacy and Biomedical Sciences, Curtin Health
Innovation Research Institute, Curtin University, Perth, WA 6102, Australia and Telethon Kids
Institute, Perth, WA 6008, Australia

¥ Authors contributed equally

*Corresponding authors: a.maguire@ucc.ie; f.ogara@ucc.ie

Contents

1. Supplementary Figures	S3
2. Synthesis and characterisation of compounds	S5
3. HPLC chromatograms.....	S11
4. ^1H & ^{13}C NMR spectra.....	S19

1. Supplementary Figures

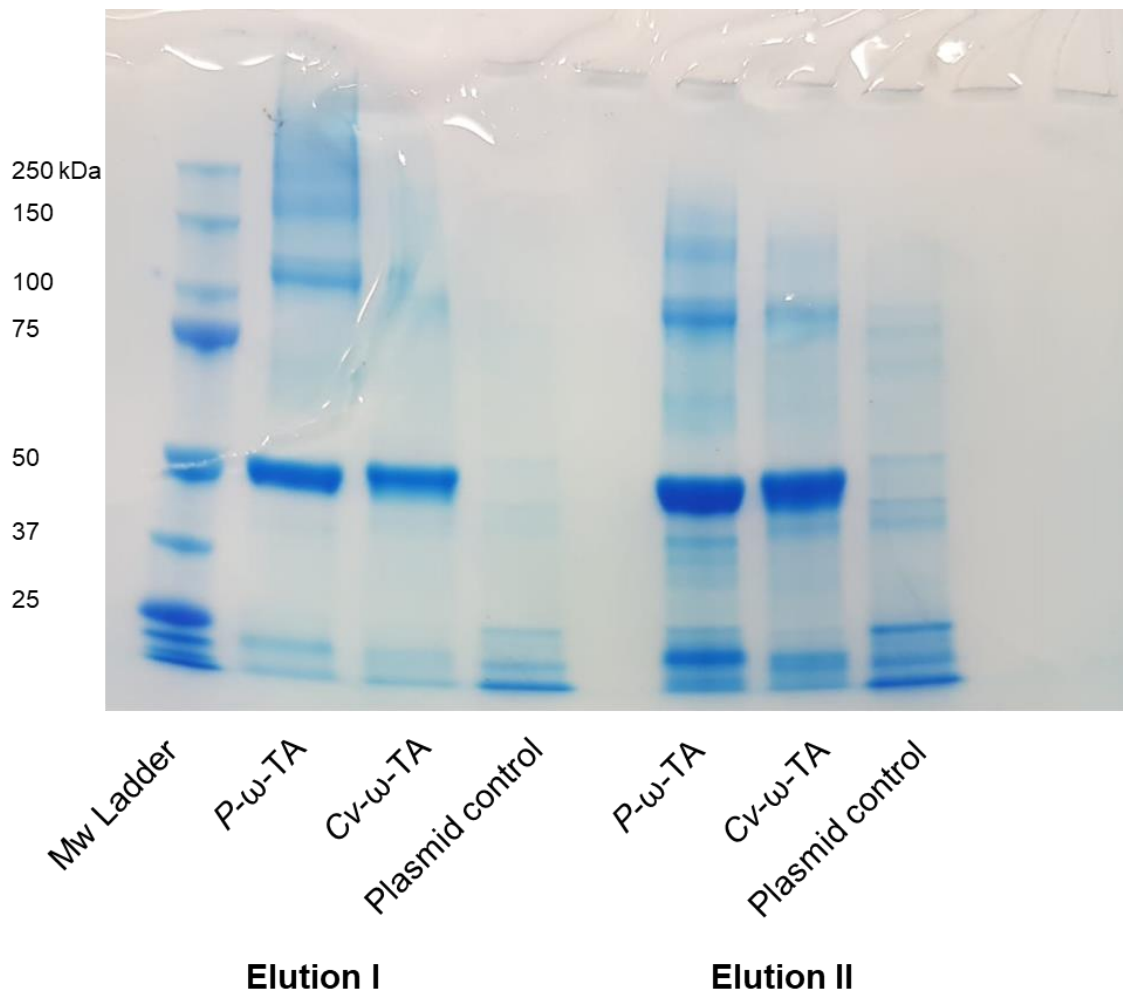
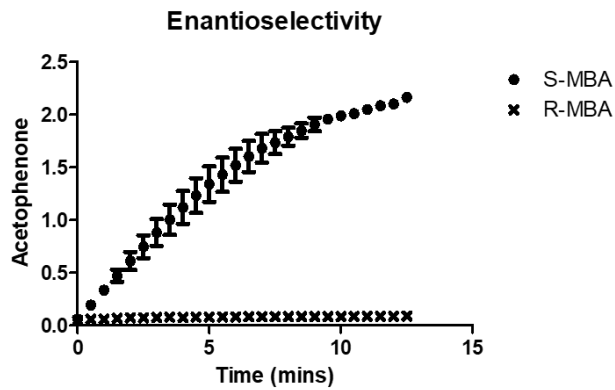
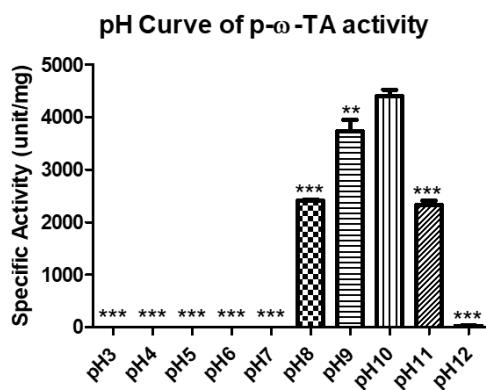


Figure S1. Purification and SDS PAGE analysis of *P- ω -TA* and *Cv- ω -TA*. Both proteins are approximately 50 kDa in size. An empty plasmid pET28a control was processed along with both recombinant constructs and is included here for validation.

(a)



(b)



(c)

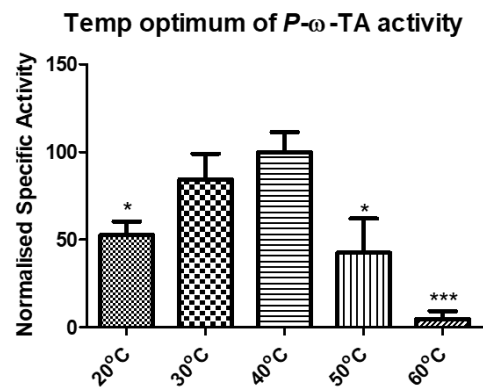


Figure S2. Biochemical characterisation of

$P-\omega$ -TA representing **a** Enantioselectivity, **b**

pH, and **c** Temperature optima. pH data is

presented for assays performed at the

optimum temperature of 40°C, while

temperature optima data is presented for

assays performed at the pH optimum of 10.

Data is normalised to the average of the

optimum value in both cases. Data

presented is the average of three

independent biological replicates and

variation is presented as standard error of

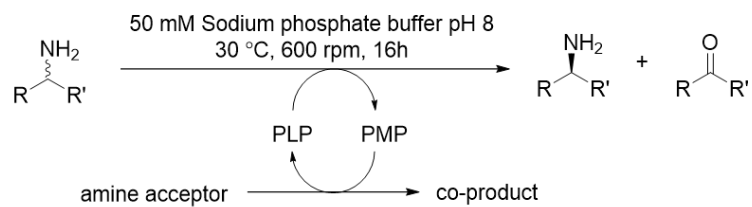
the mean. Statistical analysis was

performed using One way ANOVA with

Dunnett's multiple comparison corrective

testing to the optimum activity (* $p < 0.05$,

** $p < 0.01$, *** $p < 0.001$).



Substrate	Amine acceptor	DMSO	Conversion
		5%	71%
		10%	100%
		0	26%
		20%	25%

Figure S3. Solvent tolerance of *P*-ω-TA in the presence of a range of DMSO concentrations.

[illegible]

No:	Chain	Z	rmsd	lali	nres	%id	PDB	Description
1:	6gwi-B	53.0	1.3	450	450	59	PDB	MOLECULE: PUTRESCINE AMINOTRANSFERASE;
2:	4a6t-C	52.2	1.3	454	455	53	PDB	MOLECULE: OMEGA TRANSAMINASE;
3:	3hmu-A	51.5	1.4	454	464	57	PDB	MOLECULE: AMINOTRANSFERASE, CLASS III;
4:	5kr6-B	50.5	1.7	450	460	44	PDB	MOLECULE: 4-AMINOBUTYRATE TRANSAMINASE;
5:	6g4b-B	50.2	1.5	445	453	42	PDB	MOLECULE: ASPARTATE AMINOTRANSFERASE FAMILY PROTEIN;
6:	3gju-A	50.1	1.6	445	458	36	PDB	MOLECULE: PUTATIVE AMINOTRANSFERASE;
7:	5kqu-C	50.0	1.6	450	459	42	PDB	MOLECULE: 4-AMINOBUTYRATE TRANSAMINASE;
8:	6iol-B	49.7	1.8	448	448	41	PDB	MOLECULE: AMINOTRANSFERASE, CLASS III;
9:	6fyq-A	49.7	1.4	435	443	37	PDB	MOLECULE: AMINE TRANSAMINASE;
10:	5kr3-A	49.7	1.5	448	458	42	PDB	MOLECULE: 4-AMINOBUTYRATE TRANSAMINASE;
11:	5ghg-B	49.6	1.6	431	433	41	PDB	MOLECULE: AMINOTRANSFERASE CLASS-III;

Figure S5. Best 11 solutions of the structural alignment of our molecular model of *P*- ω -TA using the DALI server based on the Z-score value (≥ 50.0).

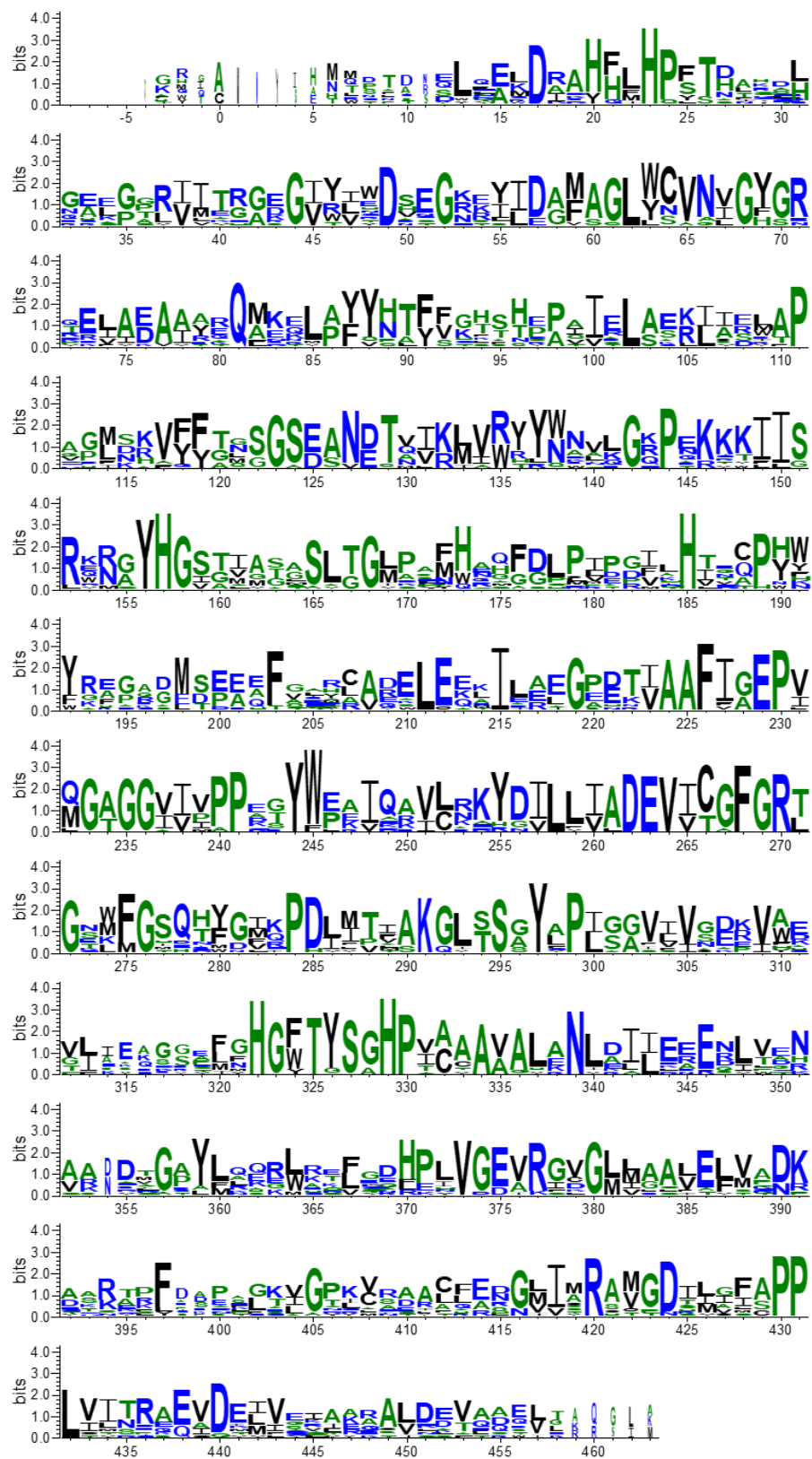


Figure S6. WebLogo of the structural alignment of the best 11 proteins shown in Figure S5. P- ω -TA is used as reference sequence.

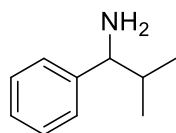
2. Synthesis and characterisation of substrates

Synthesis of model amine compounds

General procedure for reductive amination for preparation of racemic amines ¹

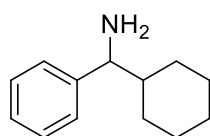
A mixture of ketone (5.0 mmol), titanium(IV) ethoxide (10.0 mmol, 2 eq.) and ammonia (2 M in methanol, 25.0 mmol, 5 eq.) was stirred under nitrogen, at ambient temperature for 15 h. Sodium borohydride (7.5 mmol, 1.5 eq.) was added and the resulting mixture was stirred at room temperature for an additional 5 h. The reaction was quenched by pouring onto ammonium hydroxide (2 M, 12.5 ml), the resulting inorganic precipitate was removed by filtration, and washed with ethyl acetate (2 × 15 ml). The layers were separated, and the aqueous solution was extracted with ethyl acetate (2 × 15 ml). The combined organic layers were extracted with aq. HCl (10%, 15 ml). The acidic aqueous extracts were washed with ethyl acetate (25 ml), then treated with aq. NaOH (2 M) to pH 10–12 and extracted with ethyl acetate (3 × 25 ml). The combined organic extracts were washed with brine (25 ml), dried (Na₂SO₄), filtered and concentrated to give the primary amine, which needed no further purification.

2-Methyl-1-phenylpropan-1-amine



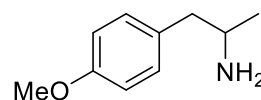
Colourless oil (yield: 49%); $\nu_{\max}$ (ATR) 2957, 2870, 1465, 1451, 701 cm⁻¹; δ_{H} (300 MHz, CDCl₃) 0.77 (d, 3H, J = 6.7, CH₃), 0.98 (d, J = 6.7, 3H, CH₃), 1.50 (br s, 2H, NH₂), 1.75–1.96 (sym m, 1H, CH(CH₃)₂), 3.60 (d, 1H, J = 7.3, CHNH₂), 7.06–7.53 (m, 5H, ArH) ppm; δ_{C} (75.5 MHz, CDCl₃) 18.9 (CH₃), 19.8, 35.5, 62.5, 126.8, 127.0, 128.2 (3 × aromatic CH), 145.5 (aromatic C) ppm; data is in agreement with previously reported data.²

Cyclohexyl(phenyl)methanamine



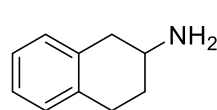
White solid (yield: 23%, m.p.: 99–102 °C); $\nu_{\max}$ (ATR) 3421, 2922, 2849, 1552, 1453, 1357, 704, 528 cm⁻¹; δ_{H} (300 MHz, CDCl₃) 0.73–1.71 (m, 12H, ring CH & NH₂), 1.71–1.83 (m, 1H, ring CH), 1.86–2.04 (m, 1H, ring CH), 3.60 (d, 1H, J = 7.5, CHNH₂), 7.10–7.48 (m, 5H, ArH) ppm; δ_{C} (75.5 MHz, CDCl₃) 26.2, 26.4, 29.5, 30.1 (4 × CH₂), 45.2, 61.7 (2 × CH), 126.8, 127.1, 128.2 (3 × aromatic CH), 145.5 (aromatic C) ppm. ¹³C signal at 145.5 ppm is very weak and was confirmed by HMBC correlation experiments. Carbon assignments were aided by DEPT experiments; data is in agreement with previously reported data.³ Melting point was not previously reported.

1-(4-Methoxyphenyl)propan-2-amine



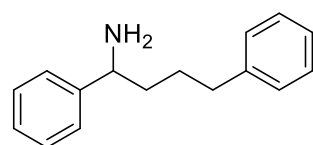
Pale yellow oil (yield: 34%); $\nu_{\max}$ (ATR) 2957, 2835, 1611, 1510, 1242, 1176, 1033, 802 cm^{-1} ; δ_{H} (300 MHz, CDCl_3) 1.10 (d, 3H, $J = 6.3$, CH_3), 2.46 (dd, 1H, $J = 13.4$, 8.0, one of CH_2), 2.65 (dd, 1H, $J = 13.4$, 5.3, one of CH_2), 3.04–3.21 (m, 1H, CHNH_2), 3.79 (s, 3H, OCH_3), 6.70–6.94 (m, 2H, ArH), 7.02–7.33 (m, 2H, ArH) ppm; δ_{C} (75.5 MHz, CDCl_3) 23.5, 45.8, 48.6, 55.3, 113.8, 130.2, 131.8, 158.1 ppm; enantiomers separated using Amylose 1 column [conditions: *n*-hexane/*i*PrOH (containing 1% DEA) = 90/10, flow rate = 0.4 ml min^{-1}], $R_t = 16.0$, $R_t = 16.8$ min, R_t (ketone) = 14.4 min; data is in agreement with previously reported data.⁴

1,2,3,4-Tetrahydronaphthalen-2-amine



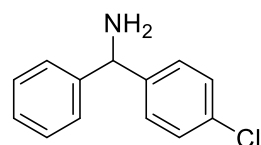
Dark green oil (yield: 48%); $\nu_{\max}$ (ATR) 3354, 2918, 2841, 741 cm^{-1} ; δ_{H} (300 MHz, CDCl_3) 1.51–1.74 [m, 3H, one of $\text{C}(3)\text{H}_2$ and NH_2 (1.66, br s)], 1.91–2.10 [m, 1H, one of $\text{C}(3)\text{H}_2$], 2.56 [dd, 1H, $J = 16.1$, 9.4, one of $\text{C}(1)\text{H}_2$], 2.77–2.94 [m, 2H, $\text{C}(4)\text{H}_2$], 3.00 [ddd, 1H, $J = 16.2$, 5.0, 1.3, one of $\text{C}(1)\text{H}_2$], 3.18 (tdd, 1H, $J = 9.5$, 5.0, 3.1, CHNH_2), 6.87–7.22 (m, 4H, ArH) ppm; δ_{C} (75.5 MHz, CDCl_3) 28.1, 33.0, 39.5 ($3 \times \text{CH}_2$), 47.3 (CH), 125.7, 125.8, 128.7, 129.3 ($4 \times$ aromatic CH), 135.3, 135.9 ($2 \times$ aromatic C) ppm; data is in agreement with previously reported data.⁵ Proton and carbon assignments were aided by 2D NMR and DEPT experiments.

1,4-Diphenylbutan-1-amine



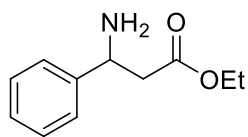
Colourless oil (yield: 16%); $\nu_{\max}$ (ATR): 2934, 1265, 732, 699 cm^{-1} ; δ_{H} (300 MHz, CDCl_3) 1.38–1.98 [m, 6H, $2 \times \text{CH}_2$ & NH_2 (1.69, s)], 2.48–2.71 (m, 2H, CH_2), 3.77–3.97 (m, 1H, CHNH_2), 6.91–7.51 (m, 10H, ArH) ppm; δ_{C} (75.5 MHz, CDCl_3) 28.4, 35.8, 39.1 ($3 \times \text{CH}_2$), 56.3 (CH), 125.7, 126.4, 127.0, 128.3, 128.4, 128.5 ($6 \times$ aromatic CH), 142.3, 146.3 ($2 \times$ aromatic C) ppm; HRMS (ES^+): $[\text{M}+\text{H}]^+$ 226.1589 (calculated: 226.1596). This compound is novel and has been fully characterised in this investigation.

(4'-Chlorophenyl)(phenyl)methanamine



Colourless oil (yield: 37%); δ_{H} (300 MHz, CDCl_3) 1.73 (v br s, 2H, NH_2), 5.12 (br s, 1H, CHNH_2), 7.49–7.09 (m, 9H, ArH) ppm; δ_{C} (75.5 MHz, CDCl_3) 59.2, 126.5, 126.8, 127.2, 127.9, 128.3, 128.56, 128.60, 132.65, 144.1, 145.2 ppm; data is in agreement with previously reported data.⁶

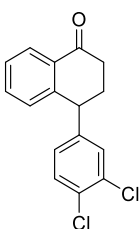
Ethyl 3-amino-3-phenylpropanoate



Sulfuric acid (conc., 2.4 ml, 4.42 g, 45.0 mmol) was added to a solution of 3-amino-3-phenylpropanoic acid (2.48 g, 15.0 mmol) in absolute ethanol (50 ml) and heated under reflux for 24 hr. Excess ethanol was evaporated under reduced pressure. The crude product was dissolved in DCM (50 ml) and washed with water (2 × 50 ml), sat. aq. NaHCO₃ (2 × 50 ml), brine (50 ml), dried, filtered and concentrated to give the ester as a colourless oil (0.999 g, 34%) which was used without further purification. $\nu_{\max}$ (ATR) 3381, 1726, 1178, 1031, 699, 539 cm⁻¹; δ_{H} (300 MHz, CDCl₃) 1.23 (t, 3H, J = 7.1, CH₂CH₃), 1.77 (br s, 2H, NH₂), 2.66 (d, 2H, J = 6.9, COCH₂), 4.14 (q, 2H, J = 7.1, OCH₂CH₃), 4.42 (dd appears as a t, 1H, J = 6.8, CHNH₂), 7.14–7.48 (m, 5H, ArH) ppm; δ_{C} (75.5 MHz, CDCl₃) 14.2, 44.2, 52.6, 60.6, 126.2, 127.4, 128.6, 144.7, 172.1 ppm; data is in agreement with previously reported data.⁷

Synthesis of Sertraline Intermediates

4-(3,4-Dichlorophenyl)-3,4-dihydronaphthalen-1(2H)-one 1



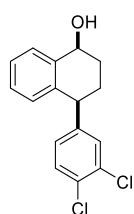
1-Naphthol (5 g, 34.68 mmol), 1,2-dichlorobenzene (32 ml) and AlCl₃ (11.56 g, 86.7 mmol) were stirred under N₂ at 110 °C for 2 h. The solution was cooled to room temperature and poured into a 1:1 mixture of ice and 1M HCl solution (30 ml). The aqueous layer was extracted with DCM (2 x 200 ml). The combined organic layer was washed with H₂O (100 ml), then stirred with Celite® (6.7 g) and activated charcoal (5 g) and filtered. The solvent was removed *in vacuo*. The crude brown liquid was purified by flash chromatography (hexane: ethyl acetate 1:0 to 17:3) and recrystallised from hexane to give the *pure* product as a colourless solid (yield: 6.20 g, 61%). $\nu_{\max}$ (ATR) 1672, 771 cm⁻¹; δ_{H} (300 MHz, CDCl₃) 2.20–2.32 (m, 1H), 2.42–2.53 (m, 1H), 2.57–2.77 (m, 2H), 4.27 (dd, J = 8.0, 4.6 Hz, 1H), 6.92–6.98 (m, 2H), 7.23 (d, J = 2 Hz, 2H), 7.36–7.42 (m, 2H), 7.47 (dt, J = 7.2, 1.5 Hz, 1H), 8.13 (dd, J = 7.9, 1.8 Hz, 1H) ppm; δ_{C} (75.5 MHz, CDCl₃) 31.7, 36.5, 44.6, 127.4, 127.7, 128.0, 129.3, 130.6, 130.5, 131.0, 132.7, 132.8, 133.9, 144.0, 144.8, 197.3; enantiomers separated using Chiralcel OJ-H [conditions: n-hexane/*i*PrOH (containing 1% DEA) 90:10, 0.5 ml min⁻¹, 25°C, λ = 235 nm], R_t = 23.8 (4*R*), 26.5 (4*S*) min; data is in agreement with previously reported data.⁸

Reduction of ketone:⁹ A solution of ketone (72 mmol) in MeOH (400 ml) was cooled to 0 °C. NaBH₄ (72 mmol) was added portion wise. The solution was allowed to warm to room temperature and stirred until TLC showed disappearance of the starting material (≈2 hr). The reaction mixture was acidified to pH 5 using 10% aqueous HCl solution. The volatiles were removed *in vacuo* and the aqueous layer was washed

with EtOAc (2 x 100 ml). The combined organic layer was washed with brine (200 ml), dried over MgSO₄, filtered and the solvent removed *in vacuo* to give a mixture of the *cis*- and *trans*- diastereomers (42:58).

Diastereomers were separated *via* repeated flash chromatography (80:20 hexane:Et₂O) to give *cis*-**7a**: 7.20 g (34%, least polar), *trans*-**7b**: 6.47 g (31%, most polar) with the remainder of the recovered material being a mixture of the two diastereomers

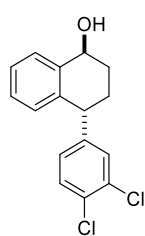
cis*-4-(3,4-Dichlorophenyl)-1,2,3,4-tetrahydronaphthalen-1-ol *cis*-**7a*



ν_{max} (ATR) 3336, 2939, 2862, 1468, 1397, 1030, 764 cm⁻¹; δ_{H} (300 MHz, CDCl₃) 1.84 (br s, 1H), 1.94–2.16 (m, 4H), 3.99 (t, *J* = 6.9 Hz, 1H), 4.86 (t, *J* = 4.2 Hz, 1H), 6.82 (d, *J* = 7.2 Hz, 1H), 6.99 (dd, *J* = 8.2, 1.9 Hz, 1H), 7.17 (dt, *J* = 7.2, 1.5 Hz, 1H), 7.21–7.31 (m, 2H), 7.37 (d, *J* = 8.2 Hz, 1H), 7.46 (dd, *J* = 7.6, 1.2 Hz, 1H) ppm; δ_{C} (75.5 MHz, CDCl₃) 28.2, 30.1, 45.1, 67.9, 127.1, 128.2, 128.3, 129.0, 129.8, 130.3, 130.4, 130.7, 132.4, 138.4, 139.0, 147.0 ppm; data

is in agreement with previously reported data.⁸

trans*-4-(3,4-Dichlorophenyl)-1,2,3,4-tetrahydronaphthalen-1-ol *trans*-**7b*



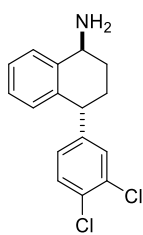
ν_{max} (ATR) 3340, 2938, 2862, 1468, 1397, 1029, 764 cm⁻¹; δ_{H} (300 MHz, CDCl₃) 1.65–1.90 (m, 2H), 1.95–2.21 (m, 2H), 2.24–2.45 (m, 1H), 4.12 (t, *J* = 6.3 Hz, 1H), 4.80–4.94 (m, 1H), 6.71–6.90 (m, 2H), 7.11 (d, *J* = 2 Hz, 1H), 7.16 (dt, *J* = 7.6, 1.4 Hz, 1H), 7.22–7.35 (m, 2H), 7.54 (d, *J* = 7.6 Hz, 1H) ppm; δ_{C} (75.5 MHz, CDCl₃) 28.9, 30.0, 44.5, 68.2, 127.2, 127.9, 128.0, 128.1, 129.9, 130.2, 130.3, 130.5, 132.3, 137.8, 139.6, 146.8 ppm; data is in agreement with previously reported data.¹⁰

General procedure for the synthesis of amines.¹¹

In a 2-necked round-bottomed flask, diphenyl phosphoryl azide (0.4 ml, 1.85 mmol, 1.2 equiv.) and the relevant alcohol (0.45g, 1.54 mmol) in dry toluene (15 ml) were stirred under N₂ at 0 °C for 10 minutes. 1,8-Diazabicyclo[5.4.0]undec-7-ene (1.85 mmol, 1.2 equiv.) was added dropwise over 20 mins. Upon addition, the orange solution turned cloudy. It was stirred at 0 °C for 2 hr, and then allowed to warm to room temperature and stirred for a further 12 hr. The solvent was removed *in vacuo* and the oily residue was passed through a silica plug to give a viscous, colourless oil. This was dissolved in THF (5 ml) and PPh₃ (0.485 g, 1.85 mmol, 1.2 equiv.) and H₂O (0.055 ml, 2 equiv.) were added. The solution was heated under reflux for 4 hr and allowed to cool. The solvent was removed under reduced pressure.

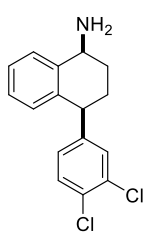
Removal of the triphenyl phosphine oxide by-product: the residue was dissolved in a 50:50 mix of hexane and Et₂O (50 ml) and stored overnight at -20°C. The white precipitate was removed by filtration and the filtrate concentrated under vacuum. The residue was dissolved in DCM (10 ml) and 5M aqueous HCl solution was added dropwise until pH 1. The resulting white precipitate was collected by filtration. The solid was resuspended in H₂O (50 ml) and the pH was adjusted to 10 with 1M aqueous NaOH solution. The mixture was stirred for 30 mins, then extracted using ethyl acetate (2 x 50 ml). The combined organic layer was washed with water (100 ml), brine (100 ml), dried (Na₂SO₄), filtered and concentrated *in vacuo* to give the *pure* product.

***trans*-4-(3,4-Dichlorophenyl)-1,2,3,4-tetrahydronaphthalen-1-amine *trans*-8a**



Prepared from alcohol *cis*-15a to give a brown oil (yield: 73%); $\nu_{\max}$ (ATR) 2929, 2857, 1464, 763 cm⁻¹; δ_{H} (300 MHz, CDCl₃) 1.52-1.68 (m, 3H), 1.74-1.86 (m, 1H), 2.04-2.16 (m, 1H), 2.24-2.36 (m, 1H), 4.06-4.15 (m, 2H), 6.80 (d, J = 7.7 Hz, 1H), 6.88 (dd, J = 8.3, 2.1 Hz, 1H), 7.08-7.16 (m, 2H), 7.21-7.28 (m, 1H), 7.33 (d, J = 8.3 Hz, 1H), 7.51 (d, J = 7.7 Hz, 1H) ppm; δ_{C} (75.5 MHz, CDCl₃) 29.9, 31.5, 45.0, 49.5, 127.0, 127.8, 128.1, 129.9, 130.1, 130.2, 130.6, 132.3, 137.6, 141.7, 147.4 ppm; enantiomers separated using Chiralcel OJ-H [conditions: n-hexane/*i*PrOH (containing 1% DEA) 90:10, 0.5 ml min⁻¹, 25°C, λ = 230 nm], R_{t} = 15.7 (1S, 4R), 18.6 (1R, 4S) min; data is in agreement with previously reported data.¹²

***cis*-4-(3,4-Dichlorophenyl)-1,2,3,4-tetrahydronaphthalen-1-amine *cis*-8b**



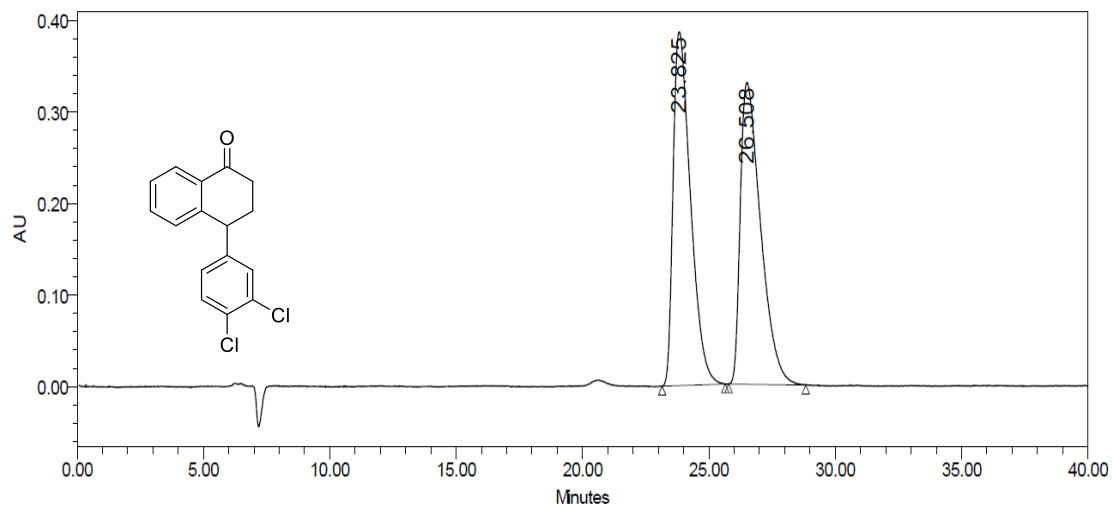
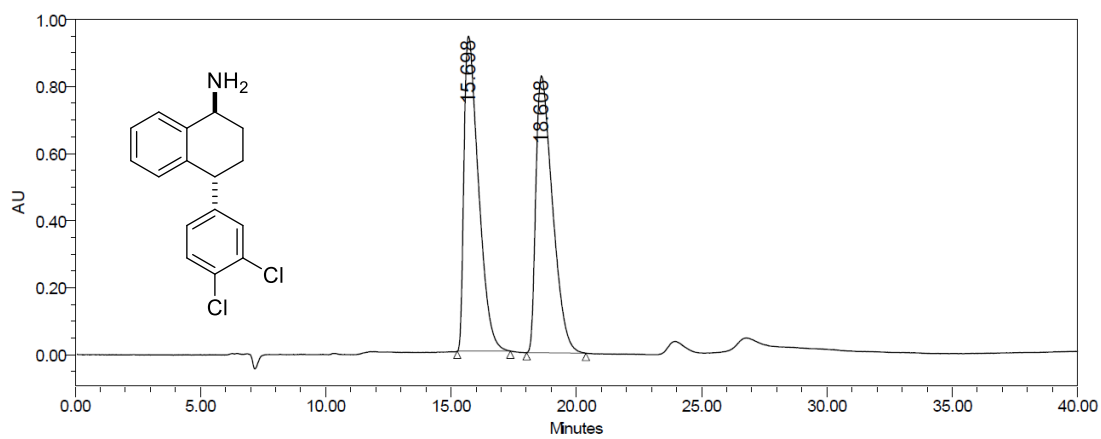
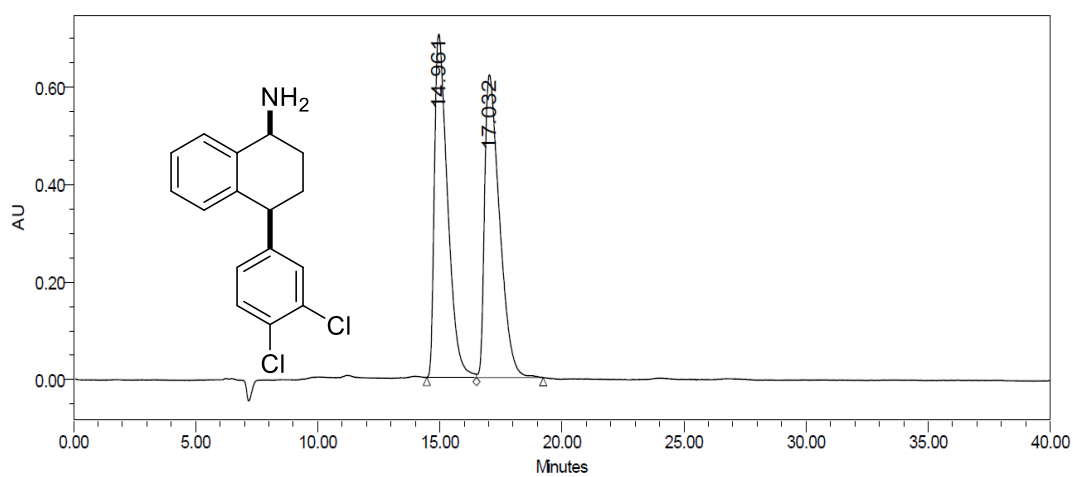
Prepared from alcohol *trans*-15b to give an off-white solid (yield: 76%); $\nu_{\max}$ (ATR) 2940, 2886, 1472, 1140, 777 cm⁻¹; δ_{H} (300 MHz, CDCl₃) 1.63 (br s, 2H), 1.71-1.82 (m, 1H), 1.92-2.14 (m, 3H), 3.99-4.04 (m, 1H), 4.04-4.11 (m, 1H), 6.80 (d, J = 7.7 Hz), 6.94 (dd, J = 8.3, 2.0 Hz), 7.11 (td, J = 7.5, 1.2 Hz, 1H), 7.20-7.26 (m, 1H), 7.35 (d, J = 8.2 Hz, 1H), 7.43 (d, J = 7.6 Hz, 1H) ppm; δ_{C} (75.5 MHz, CDCl₃) 28.7, 30.7, 45.0, 49.2, 126.9, 127.0, 128.2, 128.5, 129.8, 130.1, 130.2, 130.7, 132.3, 137.7, 141.4, 147.3 ppm; enantiomers were separated using Chiralcel OJ-H [conditions: n-hexane/*i*PrOH (containing 1% DEA) 90:10, 0.5 ml min⁻¹, 25°C, λ = 235 nm], R_{t} = 15.0 (1S, 4S), 17.0 (1R, 4R) min; data is in agreement with previously reported data.¹²

When a mix of **8a** and **8b** was used as substrate, the amine stereoisomers could not be separated but the ketone product 1 enantiomers were separated using Chiralcel IA [conditions: n-hexane/*i*PrOH (containing 1% DEA) 97:3, 0.6 ml min⁻¹, 25°C, λ = 230 nm], R_{t} = 21.8 (4R), 23.7 (4S) min.

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3. HPLC chromatograms



Chromatograms from Tables

Table 1, P- ω -TA

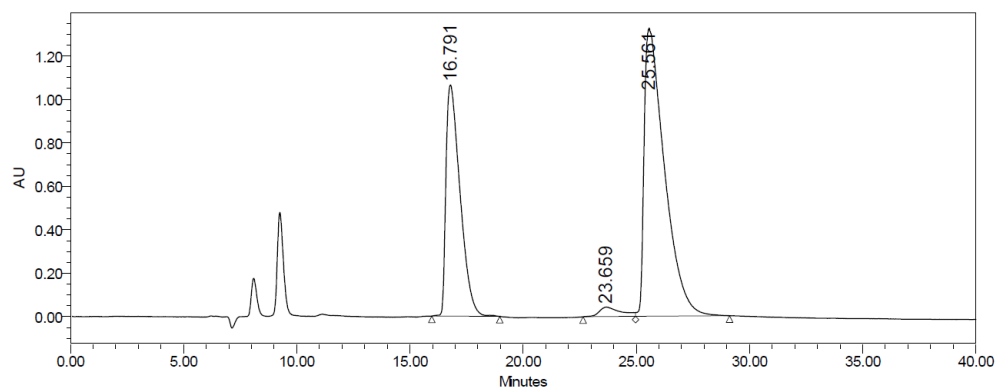


Table 1, Cv- ω -TA

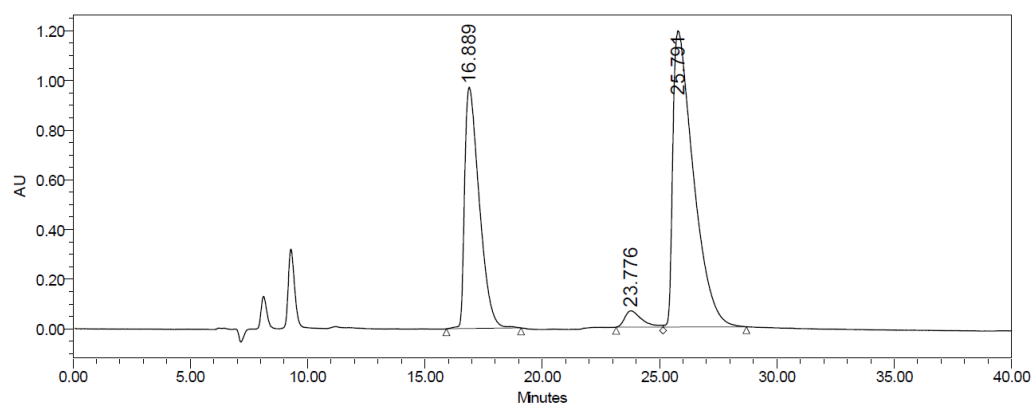


Table 2, P- ω -TA

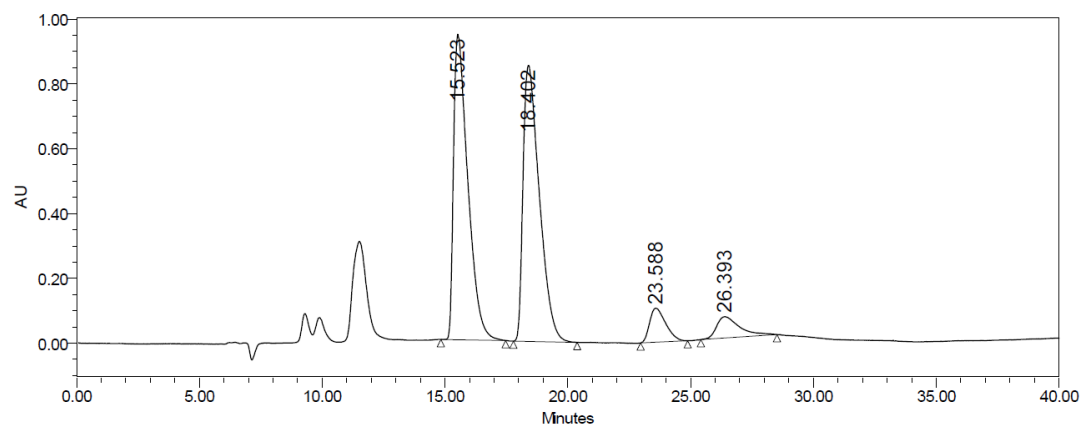


Table 2, Cv- ω -TA

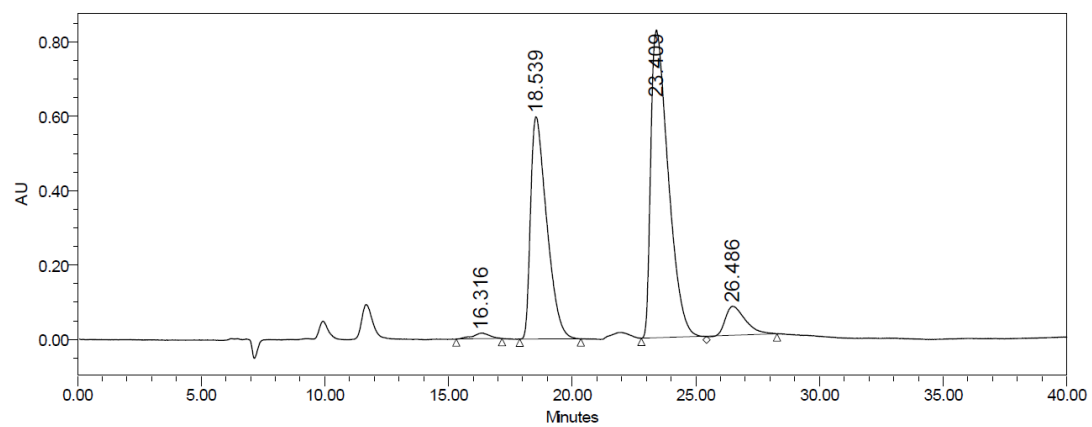


Table 3, P- ω -TA

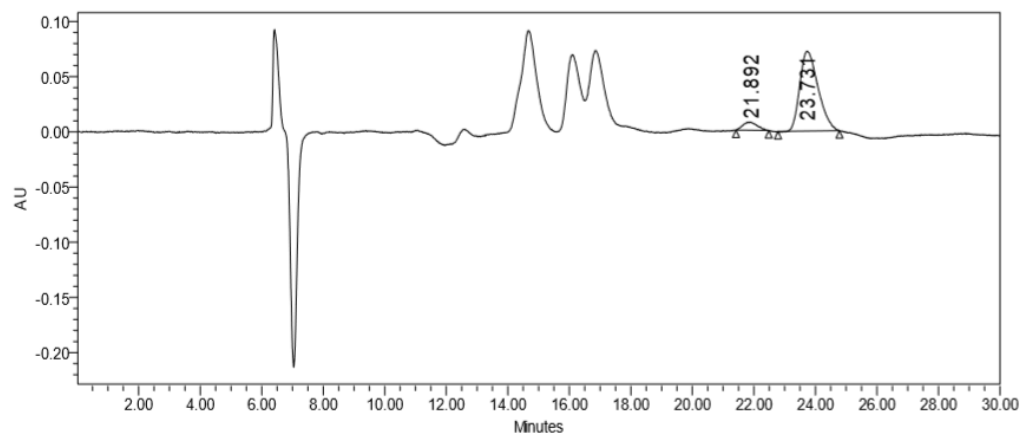
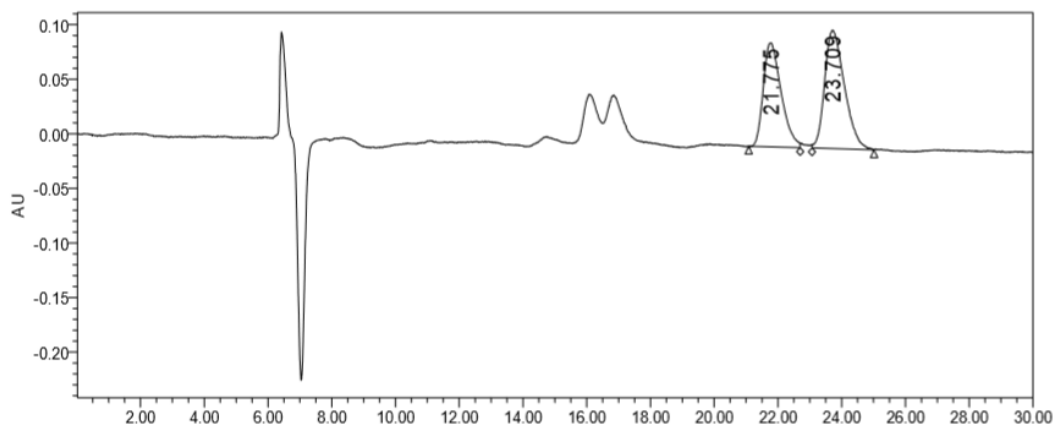


Table 3 Cv- ω -TA



4. ^1H & ^{13}C NMR spectra

