

## **The *in vitro* transport of model thiodipeptide prodrugs designed to target the intestinal oligopeptide transporter, PepT1**

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## 1. General Methods

### 1.1 Materials

Anhydrous solvents and reagents were obtained as follows: DMF was dried three times over molecular sieves (3 Å). THF was dried by distillation from sodium benzophenone ketyl, DCM and toluene by distillation from calcium hydride. All reactions were conducted at room temperature in dry glassware under a nitrogen atmosphere unless otherwise stated. All chemicals were used directly from suppliers' (Sigma-Aldrich) vessel without further purification. Protected amino acids and HBTU were supplied by Novabiochem.

### 1.2 Characterisation

$^1\text{H}$  NMR spectra were recorded at 300, 400 or 500 MHz and  $^{13}\text{C}$  NMR spectra at 75, 100 or 125 MHz on a Bruker AC300, AC400, Avance II or Varian Unity INOVA 300 spectrometer. Chemical shifts are denoted in ppm ( $\delta$ ) relative to the internal solvent standard. The splitting patterns for NMR spectra are designated as follows: s (singlet), br (broad), d (doublet), t (triplet), p (pentet), m (multiplet), or combinations thereof. Coupling constants ( $J$ ) are designated in Hz and reported to 1 decimal place.

ES-MS (and HRMS) spectra were recorded on a Micromass LCT orthogonal acceleration time-of-flight mass spectrometer (positive ion mode) with flow injection *via* a Waters 2790 separation module autosampler. IR spectra were obtained using a Nicolet-Nexus 670/680 FT-IR or ATI Mattson Genesis Series FT-IR spectrometer and are quoted in  $\text{cm}^{-1}$ . Optical rotations were measured at 589 nm in a 1 dm cell using an Optical Activity AA1000 polarimeter and are quoted in  $10^{-2} \text{ deg cm}^2 \text{ g}^{-1}$ . Melting point determinations were made using a Stuart Scientific SMP1 apparatus and are uncorrected.

Analytical TLC was performed on Merck silica gel 60 F<sub>254</sub> aluminium backed plates. The plates were visualised under UV fluorescence (254 nm) or developed using ninhydrin (0.5% w/v butanol), 2-bromocresol or acidified potassium permanganate solution with charring as necessary.  $R_f$  values are reported to the nearest 0.01. Mixed solvent system compositions are quoted as volumetric ratios. Manual column chromatography employed BDH silica gel (50-70  $\mu\text{m}$ ). Automated column chromatography was conducted on a Biotage™ SP4 purification system using appropriately sized SNAP® SP-Sil cartridges. A default setting for flow rate was used. The detection wavelength was 254 nm with the monitor wavelength set to 280 nm.

### 1.3 General Procedure for Final Deprotections

Unless otherwise stated the final acidolysis reaction of all compounds was carried out using a 33% solution of TFA in DCM at a concentration of  $15 \text{ mg mL}^{-1}$  stirred for five to six hours at room temperature. The solvent was removed *in vacuo*, the residue taken up in an equal volume of water, filtered through a pipette containing glass wool, and extracted once with a half volume of diethyl ether then lyophilised to give the final compound as a fluffy white to off-white hygroscopic solid TFA salt.

## 2. *In vitro* Biological Testing

All testing was carried out by Dr. D. Meredith and Dr. M. Pieri at the University of Oxford in the Department of Physiology, Anatomy and Genetics from 2005 to early 2008. Subsequently this testing was conducted by Dr. Meredith and Dr. Pieri at Oxford Brookes University in the School of Life Sciences.

The  $K_i$  of the compounds was determined by preparing a 10 mM stock solution of the substrate to be tested in the uptake media (at pH 5.5). A series of six dilutions, plus a blank control, were then prepared from this stock solution, between 0 and 5 mM. 50  $\mu$ L of each of these stocks was mixed with 50  $\mu$ L of radiolabelled [ $^3$ H]-D-Phe-L-Gln in the wells of a 96 well plate. PepT1-expressing oocytes with no test substrate and H<sub>2</sub>O injected oocytes were used as a control and blank respectively and five oocytes per condition were incubated for one hour. The oocytes were removed from the solution and washed sequentially five times in 1 mL of ice-cold uptake media. Each oocyte was then transferred to an individual scintillation vial, lysed and tested. The  $K_i$  is obtained from a plot of fractional uptake versus substrate concentration, using standard Michaelis-Menten kinetics as previously described.<sup>1</sup> The lower the  $K_i$  value the higher the affinity of the compound for the PepT1 transporter.

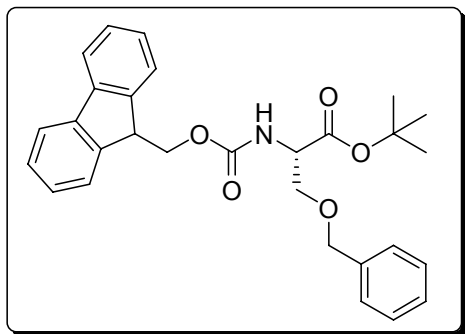
Transport of the compounds was determined using efflux studies. Five oocytes expressing PepT1 were injected with 4.6 nM of [ $^3$ H]-D-Phe-L-Gln (37.0 MBq mL<sup>-1</sup>). The oocytes were then placed in 100  $\mu$ L of a 10 mM solution of the substrate at pH 5.5 and incubated for 90 minutes. The oocytes were then washed, lysed and tested as for the inhibition studies. The amount of radioactivity remaining in the oocyte after the 90 minutes indicates the efficiency for transport of the substrate; the smaller the amount of radioactivity, the better the transport.

Transcellular transport studies were performed on Caco-2 cell monolayers grown on Corning Transwell permeable supports (0.4  $\mu$ m pore size; 0.33 cm<sup>2</sup> area polyester membrane; Appleton Woods Ltd, Birmingham, UK) until confluent (> 18 days, trans-epithelial electrical resistance (TEER) > 600  $\Omega$  cm<sup>-2</sup>). The compounds were added to the apical medium to give a final concentration of 2 mM, the cells incubated at 37 °C for one hour, and the basolateral medium assayed by HPLC. Separation was by Chromolith® Performance RP18 endcapped (3 x 100 mm) column. Varying concentrations of methanol in 21 mM aqueous KH<sub>2</sub>PO<sub>4</sub> buffer at a 1 mL min<sup>-1</sup> flow rate was used as the elutant. Retention times are quoted in minutes to one decimal place followed by the percentage methanol (v/v) in parenthesis. Detection was at 210 nm on a Jasco UV2077Plus UV/VIS detector (Jasco UK Ltd, Essex, UK). Each sample was injected in duplicate. HPLC data analysis was performed using EZChrom Elite™ software (Scientific Software Inc, Pleasanton CA, USA). The PepT1-mediated transport rate was taken to be the difference between the transport rates in the absence and presence of 20 mM GlyGln in the apical compartment during the incubation period, with at least three monolayers per compound. The rate of Phe $\Psi$ [CS-NH]-Ala was used as a control to normalise between cultures of Caco-2 cells.

### 3. Synthetic Chemistry

#### 3.1 Synthesis of thiodipeptide carriers **27** and **28**

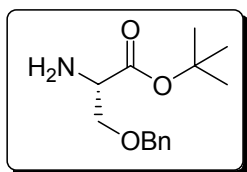
##### **(S)**-3-Benzoyloxy-2-(9H-fluoren-9-ylmethoxycarbonylamino)-propionic acid *tert*-butyl ester, **15**.



9-Fluorenylmethoxycarbonyl-*O*-benzyl-L-serine (2.15 g; 5.16 mmol) was suspended in 7.5 mL CH<sub>2</sub>Cl<sub>2</sub>:Et<sub>2</sub>O (2:1). *tert*-Butyl-2,2,2-trichloroacetimidate (1.80 mL; 10.3 mmol) was then added to the suspension. After 20 minutes at room temperature, a colourless solution was formed. After a further 20 minutes, a precipitate began to form. The reaction was stirred for 48 hours, then cooled to -78 °C and filtered through a Celite<sup>®</sup> bed. The liquor was concentrated by approximately 50% and the cooling and filtration steps repeated. On this and larger scales, the product formed was sufficiently pure to be taken forward, however further purification by flash column chromatography (CHCl<sub>3</sub>) was also possible. Yield of **15** as a colourless oil (2.40 g, 99%).

R<sub>f</sub> (CHCl<sub>3</sub>): 0.48. [α]<sub>D</sub><sup>25</sup> (CHCl<sub>3</sub>; c = 0.54): + 7.0. ν<sub>max</sub> (thin film, cm<sup>-1</sup>): 3340, 3053, 2974, 1726, 1515, 1247, 1153. δ<sub>H</sub> (CDCl<sub>3</sub>, 300 MHz): 7.81-7.76 (2H, m), 7.68-7.62 (2H, m), 7.45-7.26 (9H, m), 5.72 (1H, d, J = 6.8), 4.57 (2H, AB, J = 12.1), 4.53-4.38 (3H, m), 4.25 (1H, t, J = 7.2), 3.90 (2H, ABX, J = 9.4 & 2.6), 1.49 (9H, s). δ<sub>C</sub> (CDCl<sub>3</sub>, 75 MHz) 169.7, 156.4, 144.4-141.7, 138.0, 127.8-127.5, 125.6, 120.3, 82.7, 73.8, 70.6, 67.5, 55.3, 47.5, 28.4. MS: C<sub>29</sub>H<sub>31</sub>NO<sub>5</sub> *m/z* (ES<sup>+</sup>) 496.2 [M+Na]<sup>+</sup>. HRMS: Calculated for C<sub>29</sub>H<sub>31</sub>NNaO<sub>5</sub> 496.2100, found 496.2115.

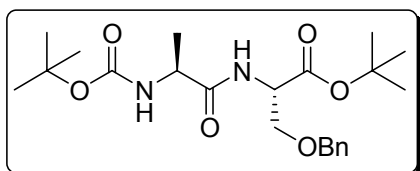
##### **(S)**-2-Amino-3-benzoyloxy-propionic acid *tert*-butyl ester, **18**.



**15** (2.44 g; 5.15 mmol) was dissolved in anhydrous THF (8 mL). After cooling to 0 °C a 1.0 M solution of TBAF in THF (5.2 mL, 5.2 mmol) was added slowly. The reaction mixture was stirred at 0 °C for 30 minutes then at room temperature for 3.5 hours. The solvent was removed *in vacuo* to yield a sticky white residue. Water (15 mL) was added and the solution was acidified to pH 2 with 2 M HCl. The aqueous phase was extracted with EtOAc (5 mL x 3) and the pH of the resultant solution was adjusted to pH 8 with 2 M NaOH. The aqueous phase was extracted with EtOAc (10 mL x 5). The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and the solvent reduced *in vacuo*. On this and larger scales, the product formed was sufficiently pure to be taken forward, however further purification by flash column chromatography (CHCl<sub>3</sub>:CH<sub>3</sub>OH, 49:1) was also possible. Yield of **18** as a colourless oil (1.07 g, 83%).

$R_f$  (95:5  $\text{CHCl}_3$ : $\text{CH}_3\text{OH}$ ): 0.35.  $[\alpha]_D^{25}$  ( $\text{CHCl}_3$ ;  $c = 1.08$ ):  $-5.9$ .  $\nu_{\max}$  (thin film,  $\text{cm}^{-1}$ ): 3382, 2988, 2924, 2864, 1729, 1601, 1377, 1242, 1164.  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ , 400 MHz) 7.40-7.28 (5H, m), 4.55 (2H, AB,  $J = 12.6$ ), 3.79-3.68 (2H, m), 3.68-3.48 (1H, m), 1.47 (9H, s).  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ , 100 MHz) 173.4, 138.4, 128.8, 128.1, 128.0, 81.7, 73.7, 72.8, 55.8, 28.4. MS:  $\text{C}_{14}\text{H}_{21}\text{NO}_3$   $m/z$  ( $\text{ES}^+$ ) 251.2  $[\text{M}+\text{H}]^+$ . HRMS: Calculated for  $\text{C}_{14}\text{H}_{22}\text{NO}_3$  252.1599, found 252.1590.

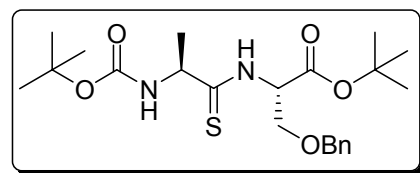
**(S)-3-Benzyloxy-2-((S)-2-tert-butoxycarbonylamino-propionylamino)-propionic acid tert-butyl ester, 21.**



*N*- $\alpha$ -tert-butoxycarbonyl-L-alanine (1.90 g, 10.0 mmol) and HOBt.H<sub>2</sub>O (1.35 g, 10.0 mmol) were dissolved in anhydrous DMF (30 mL). The solution was cooled to 0 °C then EDC.HCl (1.92 g, 10.0 mmol) and DIPEA (1.74 mL, 10.0 mmol) were added. The solution was allowed to warm to room temperature and stirred for 1 hour. After re-cooling to 0 °C, **18** (2.16 g, 10 mmol) and DIPEA (1.74 mL, 10.0 mmol) in anhydrous DMF (20 mL) were added to the activated acid solution and stirred for 2 hours at 0 °C then for 4 days at room temperature. The DMF was removed under vacuum, the resulting oil was dissolved in EtOAc (40 mL) and washed successively with saturated sodium bicarbonate (25 mL x 2), 0.6 M citric acid (25 mL x 2) and saturated NaCl (20 mL). The organic layer was dried over MgSO<sub>4</sub>, filtered and the solvent removed *in vacuo*. The resulting pale yellow oil was purified by flash column chromatography (7:1 DCM:Et<sub>2</sub>O) to give **21** as a white solid (Yield = 3.89 g; 92%).

Melting point: 109-111 °C.  $R_f$  (4:1  $\text{CH}_2\text{Cl}_2$ :Et<sub>2</sub>O): 0.65.  $[\alpha]_D^{25}$  ( $\text{CHCl}_3$ ;  $c = 0.51$ ):  $+58.2$ .  $\nu_{\max}$  (thin film,  $\text{cm}^{-1}$ ): 3342, 3295, 2984, 1738, 1719, 1665, 1526, 1373, 1253, 1157.  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ , 400 MHz) 7.41-7.26 (5H, m), 6.63 (1H, d,  $J = 6.8$ ), 5.02 (1H, br s), 4.67-4.57 (1H, m), 4.55 (2H, AB,  $J = 12.3$ ), 4.28-4.18 (1H, m), 3.89 (2H, ABX,  $J = 9.4$  & 3.0), 1.49-1.44 (18H, m), 1.39 (3H, d,  $J = 7.0$ ).  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ , 100 MHz) 172.7, 155.3, 138.0, 128.8-128.1, 83.4, 80.4, 53.5, 50.4, 28.7, 28.4, 19.3. MS:  $\text{C}_{22}\text{H}_{34}\text{N}_2\text{O}_6$   $m/z$  ( $\text{ES}^+$ ) 445.2  $[\text{M}+\text{Na}]^+$ . HRMS: Calculated for  $\text{C}_{22}\text{H}_{34}\text{N}_2\text{NaO}_6$  445.2315, found 445.2299.

**(S)-3-Benzyloxy-2-((S)-2-tert-butoxycarbonylamino-thiopropionylamino)-propionic acid tert-butyl ester, 24.**

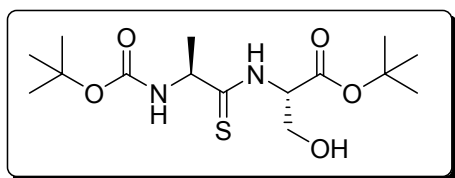


**21** (0.50 g, 1.2 mmol) and Lawesson's reagent (0.25 g, 0.6 mmol) were suspended in 10 mL of anhydrous toluene. The mixture was heated to 95 °C when a yellow solution was formed. The solution was refluxed at 115 °C for 6 hours. The toluene was removed *in vacuo* and the resulting bright orange oil was purified by flash column chromatography ( $\text{CH}_2\text{Cl}_2$ ) to yield 0.37 g (73%) of **24** as an orange oil.

$R_f$  (4:1 hexane:EtOAc): 0.32.  $[\alpha]_D^{25}$  ( $\text{CHCl}_3$ ;  $c = 0.72$ ):  $+8.9$ .  $\nu_{\max}$  (thin film,  $\text{cm}^{-1}$ ): 3329, 2977, 1734, 1705, 1692, 1516, 1496, 1249, 1158.  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ , 400 MHz): 8.52 (1H, d,  $J = 6.8$ ), 7.39-7.23 (5H, m), 5.30 (1H, br s), 5.18-5.13 (1H, m), 4.53 (2H, AB,  $J = 12.6$ ), 4.53-4.45 (1H, m), 3.94 (2H,

ABX,  $J = 9.8$  &  $3.0$ ),  $1.50$  (3H, d,  $J = 6.8$ ),  $1.46$  (9H, s),  $1.44$  (9H, s).  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ , 100 MHz) 205.6, 168.4, 155.3, 137.8, 128.8-128.1, 83.3, 80.3, 73.8, 69.0, 58.7, 54.3, 28.7, 28.4, 22.5. MS:  $\text{C}_{22}\text{H}_{34}\text{N}_2\text{O}_5\text{S}$   $m/z$  ( $\text{ES}^+$ ) 461.2 [ $\text{M}+\text{Na}$ ] $^+$ . HRMS: Calculated for  $\text{C}_{22}\text{H}_{35}\text{N}_2\text{O}_5\text{S}$  439.2266, found 439.2260.

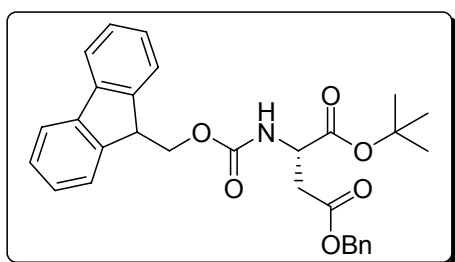
**(S)-2-((S)-2-tert-Butoxycarbonylamino-thiopropionylamino)-3-hydroxy-propionic acid tert-butyl ester, 27.**



**24** (0.94 g, 2.14 mmol) was dissolved in anhydrous THF (10 mL) and cooled to  $-78$  °C. Liquid  $\text{NH}_3$  (approx. 10 mL) was added and the mixture stirred at  $-78$  °C for 5 minutes, small pieces of sodium (0.20 g, 8.69 mmol) were added and the resulting blue coloured solution was stirred for 90 minutes, followed by addition of  $\text{NH}_4\text{Cl}$  (solid). The reaction mixture was warmed to room temperature. The solvent was removed *in vacuo* and the residue was dissolved in EtOAc (15 mL) and washed with water (5 mL x 2). The organic phase was dried ( $\text{MgSO}_4$ ), filtered and solvent removed *in vacuo*, the crude product was purified by flash column chromatography (hex:EtOAc, 3:1) to give **27** as a colourless oil (0.69 g, 92%).

$R_f$  (3:1 hexane:EtOAc): 0.31.  $[\alpha]_{\text{D}}^{25}$  ( $\text{CHCl}_3$ ;  $c = 0.37$ ): + 25.1.  $\nu_{\text{max}}$  (thin film,  $\text{cm}^{-1}$ ): 3339, 2977, 1732, 1696, 1519, 1471, 1253, 1161;  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ , 400 MHz): 8.63 (1H, d,  $J = 6.8$ ), 5.30 (1H, br s), 5.04-4.97 (1H, m), 4.42-4.33 (1H, m), 4.08 (2H, AB,  $J = 11.1$  Hz), 1.43 (9H, s), 1.40 (3H, d,  $J = 7.1$ ), 1.36 (9H, s).  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ , 75 MHz): 204.8, 167.6, 154.7, 82.1, 79.6, 60.5, 59.1, 55.9, 27.5, 27.0, 20.2. MS:  $\text{C}_{15}\text{H}_{28}\text{N}_2\text{O}_5\text{S}$   $m/z$  ( $\text{ES}^+$ ) 371.1 [ $\text{M}+\text{Na}$ ] $^+$ . HRMS: Calculated for  $\text{C}_{15}\text{H}_{28}\text{N}_2\text{NaO}_5\text{S}$  371.1617, found 371.1621.

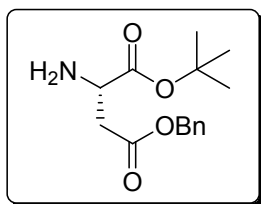
**(S)-2-(9H-Fluoren-9-ylmethoxycarbonylamino)-succinic acid 4-benzyl ester 1-tert-butyl ester, 16.<sup>2</sup>**



Proceed as for **15**, using 9-Fluorenylmethoxycarbonyl-O-benzyl-L-aspartic acid to give **16** as a white solid in 99% yield.

Melting point: 81-82 °C.  $R_f$  (4:1 DCM:Et<sub>2</sub>O): 0.86.  $[\alpha]_{\text{D}}^{25}$  ( $\text{CHCl}_3$ ;  $c = 0.75$ ): + 24.9.  $\nu_{\text{max}}$  (thin film,  $\text{cm}^{-1}$ ): 3430, 3343, 3064, 3037, 2973, 1740, 1713, 1606, 1503, 1373, 1347, 1228, 1148, 1080, 854.  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ , 400 MHz): 7.78 (2H, d,  $J = 7.5$ ), 7.62 (2H, d,  $J = 7.5$ ), 7.44-7.26 (9H, m), 5.84 (1H, d,  $J = 8.0$ ), 5.18 (2H, AB,  $J = 12.5$ ), 4.62-4.55 (1H, m), 4.53-4.38 (2H, m), 4.25 (1H, t,  $J = 7.5$ ), 3.00 (2H, ABX,  $J = 17.1$  & 4.5), 1.45 (9H, s).  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ , 100 MHz): 171.2, 167.0, 156.4, 144.3-141.7, 135.9, 129.0, 125.5, 120.4, 83.0, 67.8, 67.2, 51.4, 47.5, 37.2, 28.2. MS:  $\text{C}_{30}\text{H}_{31}\text{NO}_6$   $m/z$  ( $\text{ES}^+$ ) 524.2 [ $\text{M}+\text{Na}$ ] $^+$ . HRMS: Calculated for  $\text{C}_{30}\text{H}_{31}\text{NNaO}_6$  524.2049, found 524.2054.

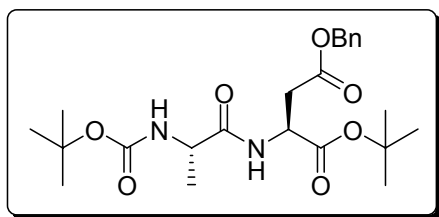
**(S)-2-Amino-succinic acid 4-benzyl ester 1-*tert*-butyl ester, 19.**<sup>3</sup>



Proceed as for **18** giving **19** as a colourless oil in 86% yield.

$R_f$  (95:5 CH<sub>3</sub>Cl:CH<sub>3</sub>OH): 0.51.  $[\alpha]_D^{25}$  (CHCl<sub>3</sub>; c = 0.57): + 10.9.  $\nu_{max}$  (thin film, cm<sup>-1</sup>): 3370, 3313, 3247, 3181, 2979, 1731, 1608, 1456, 1369, 1254, 1225, 1154, 1110, 1025, 929, 832.  $\delta_H$  (CDCl<sub>3</sub>, 400 MHz): 7.42-7.31 (5H, m), 5.15 (2H, s), 3.72-3.79 (1H, m), 2.98 (2H, ABX,  $J$  = 16.6 & 5.0), 2.06 (1H, br s), 1.40 (9H, s).  $\delta_C$  (CDCl<sub>3</sub>, 100 MHz): 173.6, 171.6, 136.0, 129.0-127.4, 82.1, 67.2, 52.1, 39.4, 28.3. MS: C<sub>15</sub>H<sub>21</sub>NO<sub>4</sub>  $m/z$  (ES<sup>+</sup>) 280.1 [M+H]<sup>+</sup>. HRMS: Calculated for C<sub>15</sub>H<sub>22</sub>NO<sub>4</sub> 280.1549, found 280.1548.

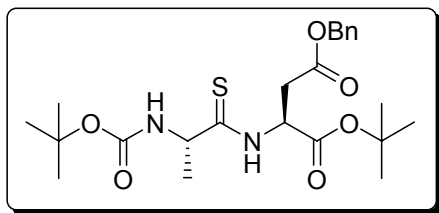
**(S)-2-((S)-2-*tert*-Butoxycarbonylamino-propionylamino)-succinic acid 4-benzyl ester 1-*tert*-butyl ester, 22.**



*N*- $\alpha$ -*tert*-butoxycarbonyl-L-alanine (3.4 g; 17.9 mmol) and **19** (4.6 g; 16.5 mmol) were dissolved in 60 mL anhydrous DMF and cooled in an ice/acetone bath. Diphenylphosphoryl azide (3.9 mL; 18.0 mmol) and triethylamine (5 mL; 36.0 mmol) were then added. The solution was stirred at -10 °C for 3 hours then allowed to slowly warm to room temperature over 16 hours. The DMF was removed under vacuum and the residue taken up in 80 mL DCM. The organic layer was washed with 0.6 M citric acid (3 x 40 mL), saturated sodium hydrogen carbonate (3 x 40 mL) and brine (40 mL). The acidic and basic aqueous layers were separately back extracted with 40 mL DCM and the combined organic layers were dried over MgSO<sub>4</sub>, filtered and concentrated under vacuum. The residue was purified by flash column chromatography (6:1 hexane:EtOAc → 1:1 hexane:EtOAc) to give **22** as a white solid (Yield = 6.5 g; 87%).

Melting point: 118-119 °C.  $R_f$  (4:1 hexane:EtOAc): 0.19.  $[\alpha]_D^{25}$  (CHCl<sub>3</sub>; c = 0.72): + 14.0.  $\nu_{max}$  (thin film, cm<sup>-1</sup>): 3317, 2982, 2929, 1745, 1669, 1526, 1463, 1394, 1368, 1253, 1166, 846;  $\delta_H$  (CDCl<sub>3</sub>, 300 MHz): 7.41-7.32 (5H, m), 6.75 (1H, d,  $J$  = 7.9), 5.06 (2H, AB,  $J$  = 12.1), 4.91 (1H, s), 4.66-4.60 (2H, m), 4.12-4.03 (1H, m), 2.88 (2H, ABX,  $J$  = 17.0 & 3.1), 1.38 (9H, s), 1.31 (9H, s), 1.27 (3H, d,  $J$  = 6.8).  $\delta_C$  (CDCl<sub>3</sub>, 75 MHz): 172.7, 171.1, 169.6, 155.5, 135.9, 129.0-128.8, 83.0, 80.3, 67.1, 50.5, 49.5, 36.8, 28.7, 28.2, 19.1. MS: C<sub>23</sub>H<sub>34</sub>N<sub>2</sub>O<sub>7</sub>  $m/z$  (ES<sup>+</sup>) 473.2 [M+Na]<sup>+</sup>. HRMS: Calculated for C<sub>23</sub>H<sub>34</sub>N<sub>2</sub>NaO<sub>7</sub> 473.2264, found 473.2250.

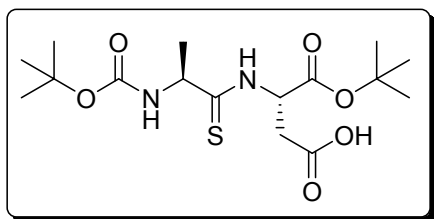
**(S)-2-((S)-2-tert-Butoxycarbonylamino-thiopropionylamino)-succinic acid 4-benzyl ester 1-tert-butyl ester, 25.**



**22** (2.8 g; 6.4 mmol) and Lawesson's reagent (1.54 g; 3.8 mmol) were suspended in anhydrous toluene (25 mL). The suspension was heated to 112 °C and refluxed for 3 hours. A yellow solution was achieved at a temperature of ~ 90 °C. After cooling (30 minutes), the toluene was removed *in vacuo* and the resulting orange oil was purified by flash column chromatography (DCM → 9:1 hexane:EtOAc → 4:1 hexane:EtOAc) to yield **25** as a yellow oil (2.6 g; 91%).

$R_f$  (9:1 hexane:EtOAc): 0.28.  $[\alpha]_D^{25}$  (CHCl<sub>3</sub>; c = 0.50): + 67.0.  $\nu_{\max}$  (thin film, cm<sup>-1</sup>): 3332, 2977, 2935, 1743, 1708, 1507, 1365, 1253, 1156, 1052;  $\delta_H$  (CDCl<sub>3</sub>, 400 MHz): 8.45 (1H, d,  $J$  = 7.1), 7.39-7.21 (5H, m), 5.28-4.95 (1H, m), 5.00 (2H, AB,  $J$  = 12.0), 4.39-4.26 (1H, m), 3.00 (2H, ABX,  $J$  = 17.3 & 4.5), 1.48-1.36 (21H, m).  $\delta_C$  (CDCl<sub>3</sub>, 75 MHz): 205.4, 170.9, 168.8, 155.2, 135.8, 129.1-128.9, 83.6, 80.6, 67.2, 57.4, 54.4, 35.3, 28.7, 28.2, 22.3. MS: C<sub>23</sub>H<sub>34</sub>N<sub>2</sub>O<sub>6</sub>S  $m/z$  (ES<sup>+</sup>) 489.2 [M+Na]<sup>+</sup>. HRMS: Calculated for C<sub>23</sub>H<sub>34</sub>N<sub>2</sub>NaO<sub>6</sub>S 489.2035, found 489.2025.

**(S)-2-((S)-2-tert-Butoxycarbonylamino-thiopropionylamino)-succinic acid 1-tert-butyl ester, 28.**



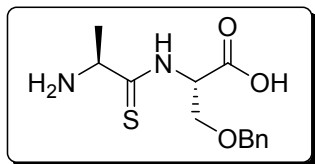
**25** (835 mg, 1.8 mmol) was dissolved in anhydrous THF (10 mL) and cooled to -78 °C. Liquid NH<sub>3</sub> (approx 10 mL) was added and the mixture stirred at -78 °C for 15 minutes, small pieces of sodium (178 mg, 7.7 mmol) were added and the resulting dark blue coloured solution was stirred for 90 minutes, the reaction mixture was quenched with the addition of NH<sub>4</sub>Cl (solid). The reaction mixture was warmed to room temperature. The solvent was removed *in vacuo* and the residue was taken up in CH<sub>3</sub>Cl (30 mL) and washed with 0.5 M HCl (10 mL x 3) then saturated aqueous NH<sub>4</sub>Cl (10 mL x 2). The cloudy organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> then filtered through Celite<sup>®</sup>. The solvent was removed under vacuum to give to give **28** as a colourless foam (668 mg, 98%).

$R_f$  (25:1 CHCl<sub>3</sub>:MeOH): 0.37.  $[\alpha]_D^{25}$  (CHCl<sub>3</sub>; c = 0.64): +132.7.  $\nu_{\max}$  (thin film, cm<sup>-1</sup>): 3316, 2977, 2933, 1740, 1716, 1702, 1509, 1401, 1377, 1238, 1157, 1055, 848;  $\delta_H$  (CDCl<sub>3</sub>, 300 MHz): 9.00 (1H, d,  $J$  = 7.9), 5.69 (1H, d,  $J$  = 9.0), 5.59-5.46 (1H, m), 5.10-4.98 (1H, m), 3.20 (2H, AB,  $J$  = 20.1), 1.53-1.35 (21H, m).  $\delta_C$  (CDCl<sub>3</sub>, 75 MHz): 206.0, 174.1, 168.5, 156.3, 83.2, 81.5, 55.3, 54.3, 35.3, 28.7, 28.2, 23.2. MS: C<sub>16</sub>H<sub>28</sub>N<sub>2</sub>O<sub>6</sub>S  $m/z$  (ES<sup>+</sup>) 399.2 [M+Na]<sup>+</sup>. HRMS: Calculated for C<sub>16</sub>H<sub>28</sub>N<sub>2</sub>NaO<sub>6</sub>S 399.1566, found 399.1560.



### 3.2 Synthesis of model thiodipeptide prodrugs 1-3

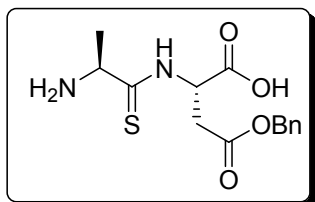
#### (S)-2-((S)-2-Amino-thiopropionylamino)-3-benzyloxy-propionic acid, **1**.



Proceed as described in Section 1.1, using **24** to give **1** as a white hygroscopic solid in 94% yield.

HPLC  $R_T$ : 3.1 (10%).  $R_f$  (1:1:1:1 Butanol:H<sub>2</sub>O:EtOAc:AcOH): 0.85.  $[\alpha]_D^{25}$  (H<sub>2</sub>O; c = 0.40): + 35.8.  $\nu_{\max}$  (thin film, cm<sup>-1</sup>): 3030, 2874, 1718, 1676, 1657, 1440, 1353, 1197, 1132, 839, 805.  $\delta_H$  (D<sub>2</sub>O, 300 MHz): 7.40-7.21 (5H, m), 5.11-5.01 (1H, m), 4.50 (2H, AB,  $J$  = 12.1), 4.48 (1H, q,  $J$  = 6.8), 3.82-3.96 (2H, m), 1.52 (2.5H, d,  $J$  = 6.8 Hz), 1.47 (0.5H, d,  $J$  = 6.8 Hz).  $\delta_C$  (D<sub>2</sub>O, 75 MHz): 201.6, 172.2, 137.3, 129.1-128.8, 73.5, 68.3, 59.5, 54.2, 19.9. MS C<sub>13</sub>H<sub>18</sub>N<sub>2</sub>O<sub>3</sub>S  $m/z$  (ES<sup>+</sup>) 283.1 [M+H]<sup>+</sup>. HRMS: Calculated for C<sub>13</sub>H<sub>19</sub>N<sub>2</sub>O<sub>3</sub>S 283.1116, found 283.1123.

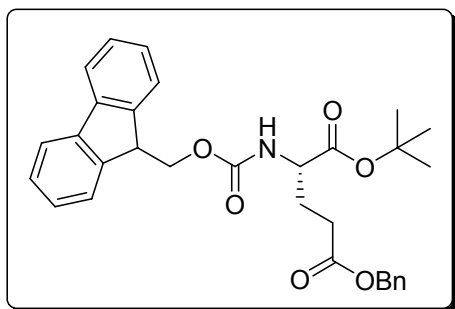
#### (S)-2-((S)-2-Amino-thiopropionylamino)-succinic acid 4-benzyl ester, **2**.



Proceed as described in Section 1.1, using **25** to give **2** as a white hygroscopic solid in 79% yield.

HPLC  $R_T$ : 3.8 (30%).  $R_f$  (1:1:1:1 Butanol:H<sub>2</sub>O:EtOAc:AcOH): 0.51.  $[\alpha]_D^{25}$  (MeOH; c = 0.68): - 13.8.  $\nu_{\max}$  (thin film, cm<sup>-1</sup>): 3206, 3031, 2980, 1731, 1673, 1443, 1389, 1361, 1198, 1139, 984, 840, 801.  $\delta_H$  (D<sub>2</sub>O, 300 MHz): 7.35-7.21 (5H, m), 5.23 (1H, t,  $J$  = 6.0), 5.03-4.99 (2H, m), 4.15 (1H, q,  $J$  = 6.8), 3.00-2.96 (2H, m), 1.47 (2.7H, d,  $J$  = 6.8), 1.24 (0.3H, d,  $J$  = 6.8).  $\delta_C$  (D<sub>2</sub>O, 75 MHz): 201.4, 172.5, 172.1, 129.2-128.7, 67.8, 59.8, 54.2, 35.0, 19.8. MS C<sub>14</sub>H<sub>18</sub>N<sub>2</sub>O<sub>4</sub>S  $m/z$  (ES<sup>+</sup>) 311.2 [M+H]<sup>+</sup>. HRMS: Calculated for C<sub>14</sub>H<sub>19</sub>N<sub>2</sub>O<sub>4</sub>S 311.1065, found 311.1059.

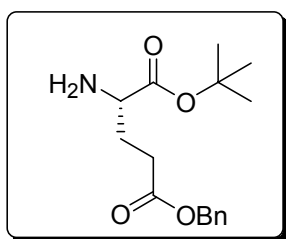
**(S)-2-(9H-Fluoren-9-ylmethoxycarbonylamino)-pentanedioic acid 5-benzyl ester 1-tert-butyl ester, 17.**<sup>4</sup>



Proceed as for **15**, using 9-Fluorenylmethoxycarbonyl-O-benzyl-L-glutamic acid to give **17** as a white solid in 95% yield.

$R_f$  (DCM): 0.20.  $[\alpha]_D^{25}$  ( $\text{CHCl}_3$ ;  $c = 0.64$ ): + 4.1.  $\nu_{\text{max}}$  (thin film,  $\text{cm}^{-1}$ ): 3371, 3354, 3331, 3070, 3031, 2976, 1735, 1521, 1447, 1373, 1260, 1221, 1155, 1054, 844.  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ , 300 MHz): 7.79 (2H, d,  $J = 7.9$ ), 7.61 (2H, d,  $J = 7.2$ ), 7.45-7.23 (9H, m), 5.48 (1H, d,  $J = 7.9$ ), 5.15 (2H, s), 4.41 (2H, d,  $J = 6.8$ ), 4.37-4.30 (1H, m), 4.25 (1H, t,  $J = 7.2$ ), 2.60-2.49 (2H, m), 2.31-1.94 (2H, m), 1.50 (9H, s).  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ , 75 MHz): 173.0, 171.4, 156.3, 144.3-144.2, 141.7, 136.2, 129.0-127.5, 125.5, 120.4, 82.9, 67.4, 66.9, 54.2, 47.5, 30.7, 28.4, 28.3. MS  $\text{C}_{31}\text{H}_{33}\text{NO}_6$   $m/z$  ( $\text{ES}^+$ ) 538.0  $[\text{M}+\text{Na}]^+$ . HRMS: Calculated for  $\text{C}_{31}\text{H}_{33}\text{NNaO}_6$  538.2206, found 538.2200.

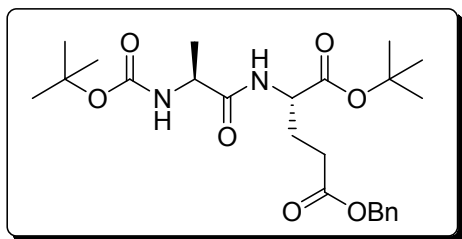
**(S)-2-Amino-pentanedioic acid 5-benzyl ester 1-tert-butyl ester, 20.**



Proceed as for **18**, using **17** to give **20** as a white hygroscopic solid in 79% yield.

$R_f$  (95:5  $\text{CHCl}_3$ :MeOH): 0.43.  $[\alpha]_D^{25}$  ( $\text{CHCl}_3$ ;  $c = 0.55$ ): - 3.8.  $\nu_{\text{max}}$  (thin film,  $\text{cm}^{-1}$ ): 3331, 2984, 2938, 2875, 1731, 1700, 1451, 1381, 1241, 1159, 1034, 1021, 848.  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ , 300 MHz): 7.32-7.16 (5H, m), 6.87 (1H, br s), 4.61 (2H, s), 4.06 (1H, m), 2.41-2.01 (4H, m), 1.45 (9H, s).  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ , 75 MHz): 178.8, 171.6, 141.6, 128.8-127.3, 82.7, 65.2, 56.6, 29.8, 28.3, 25.2.

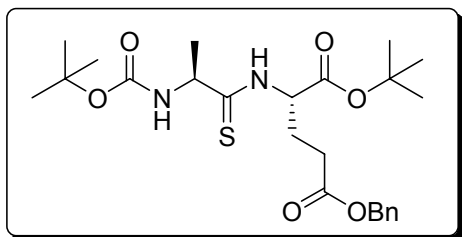
**(S)-2-((S)-2-tert-Butoxycarbonylamino-propionylamino)-pentanedioic acid 5-benzyl ester 1-tert-butyl ester, 23.**



Proceed as for **21**, using **20** to give **23** as a colourless oil in 79% yield.

$R_f$  (4:1 hexane:EtOAc): 0.11.  $[\alpha]_D^{25}$  ( $\text{CHCl}_3$ ;  $c = 0.50$ ):  $-4.4$ .  $\nu_{\text{max}}$  (thin film,  $\text{cm}^{-1}$ ): 3337, 2978, 2942, 1740, 1668, 1499, 1445, 1395, 1363, 1251, 1154, 1075, 1028, 848;  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ , 300 MHz): 7.39-7.32 (5H, m), 6.70 (1H, d,  $J = 7.9$ ), 5.14 (2H, s), 5.04 (1H, d,  $J = 5.7$ ), 4.51 (1H, dt,  $J = 7.9$  & 4.9), 4.21-4.10 (1H, m), 2.53-2.32 (2H, m), 2.29-2.16 (1H, m), 2.05-1.85 (1H, m), 1.46 (9H, s), 1.42 (9H, s), 1.35 (3H, d,  $J = 7.2$ ).  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ , 75 MHz): 173.1, 172.8, 171.0, 155.8, 136.2, 128.9-128.6, 82.8, 80.5, 66.9, 52.5, 50.5, 30.5, 28.6, 28.3, 28.0, 18.6. MS:  $\text{C}_{24}\text{H}_{36}\text{N}_2\text{O}_7$   $m/z$  ( $\text{ES}^+$ ) 487.2  $[\text{M}+\text{Na}]^+$ . HRMS: Calculated for  $\text{C}_{24}\text{H}_{36}\text{N}_2\text{NaO}_7$  487.2420, found 487.2408.

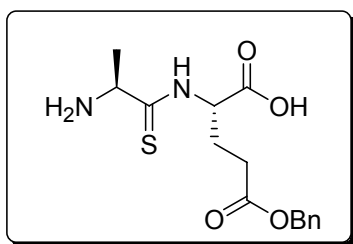
**(S)-2-((S)-2-tert-Butoxycarbonylamino-thiopropionylamino)-pentanedioic acid 5-benzyl ester 1-tert-butyl ester, 26.**



Proceed as for **24**, using **23** to give **26** as a yellow oil in 80% yield.

$R_f$  (4:1  $\text{CH}_2\text{Cl}_2$ :Et<sub>2</sub>O): 0.24.  $[\alpha]_D^{25}$  ( $\text{CHCl}_3$ ;  $c = 0.64$ ):  $-4.5$ .  $\nu_{\text{max}}$  (thin film,  $\text{cm}^{-1}$ ): 3300, 3038, 2983, 2927, 1733, 1505, 1457, 1394, 1365, 1250, 1154, 1092, 1051, 852;  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ , 300 MHz): 8.53 (1H, d,  $J = 7.2$ ), 7.30-7.20 (5H, m), 5.13 (1H, br s), 5.03 (2H, s), 4.95-4.88 (1H, m), 4.38 (1H, p,  $J = 6.8$ ), 2.21-2.50 (3H, m), 1.34-1.42 (12H, m), 2.01-2.12 (1H, m), 1.32 (9H, s).  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ , 75 MHz): 206.0, 173.1, 170.0, 155.6, 136.0, 128.9-128.7, 83.4, 80.7, 67.0, 57.5, 53.8, 30.4, 28.6, 28.3, 26.7, 18.6. MS:  $\text{C}_{24}\text{H}_{36}\text{N}_2\text{O}_6\text{S}$   $m/z$  ( $\text{ES}^+$ ) 503.1  $[\text{M}+\text{Na}]^+$ . HRMS: Calculated for  $\text{C}_{24}\text{H}_{36}\text{N}_2\text{NaO}_6\text{S}$  503.2192, found 503.2214.

**(S)-2-((S)-2-Amino-thiopropionylamino)-pentanedioic acid 5-benzyl ester, 3.**

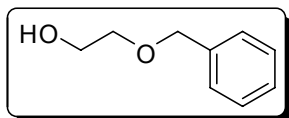


Proceed as described in Section 1.1, using **26** to give **3** as a pale yellow hygroscopic solid in 75% yield.

HPLC  $R_T$ : 2.3 (30%).  $R_f$  (1:1:1:1 Butanol:H<sub>2</sub>O:EtOAc:AcOH): 0.63.  $[\alpha]_D^{25}$  (MeOH;  $c = 0.49$ ): +14.5.  $\nu_{\max}$  (thin film,  $\text{cm}^{-1}$ ): 3230, 3035, 2993, 1722, 1672, 1435, 1389, 1354, 1197, 1144, 841;  $\delta_H$  (D<sub>2</sub>O, 300 MHz): 7.32-7.17 (5H, m), 5.03 (2H, s), 4.85-4.73 (1H, m), 4.37 (1H, q,  $J = 6.8$ ), 2.50-2.32 (2H, m), 2.27-1.91 (2H, m), 1.38 (3H, d,  $J = 6.8$ ).  $\delta_C$  (D<sub>2</sub>O, 75 MHz): 201.8, 174.9, 173.7, 135.8, 129.2-128.7, 67.6, 57.8, 54.1, 30.5, 25.2, 19.8. MS  $C_{15}H_{20}N_2O_4S$   $m/z$  (ES<sup>+</sup>) 325.0 [M+H]<sup>+</sup>. HRMS: Calculated for  $C_{15}H_{21}N_2O_4S$  325.1222, found: 325.1230.

### 3.3 Synthesis of model thiodipeptide prodrugs 5-11

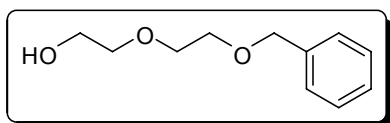
#### 2-Benzyloxy-ethanol, **32**.<sup>5,6</sup>



Ethylene glycol (500 mg; 8 mmol) in 20 mL THF was added dropwise to a stirring suspension of 60% sodium hydride in mineral oil (336 mg; 8.4 mmol) in 40 mL THF. The suspension was stirred at room temperature for 30 minutes. Benzyl bromide (0.9 mL; 7.6 mmol) in 20 mL THF was then added dropwise. Potassium iodide (670 mg; 4 mmol) was then added and the suspension heated to reflux for 48 hours over which time the medium changed from colourless to yellow. The suspension was cooled and 80 mL water was added. This was extracted with 30 mL of ether five times. The combined organic layer was washed with brine then dried over  $\text{MgSO}_4$ , filtered and concentrated. The residue was purified by flash column chromatography (9:1 hexane:EtOAc  $\rightarrow$  1:1 hexane:EtOAc) to give **32** as a pale yellow oil (Yield = 670 mg; 55%).

$R_f$  (4:1 hexane:EtOAc): 0.14.  $\delta_H$  (500 MHz,  $\text{CDCl}_3$ ): 7.43-7.28 (5H, m), 4.58 (2H, s), 3.77 (2H, t,  $J = 4.8$ ), 3.61 (2H, dt,  $J = 4.8$  & 2.2), 2.24 (1H, br s).

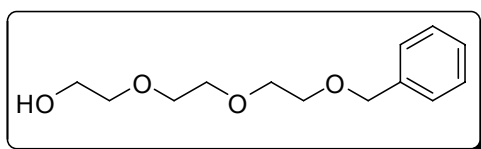
#### 2-(2-Benzyloxy-ethoxy)-ethanol, **33**.<sup>7</sup>



Diethylene glycol (2 mL; 21 mmol) was added to a stirred suspension of 60% sodium hydride in mineral oil (850 mg; 21.2 mmol) in 30 mL THF and the resultant mixture stirred for 1 hour. Benzyl bromide (2.5 mL; 21 mmol) was added to the thick white suspension formed. The mixture was stirred for 48 hours at room temperature. 100 mL DCM and 50 mL 10% (w/v)  $\text{K}_2\text{CO}_3$  was added and the organic layer was separated and washed with 50 mL water. The organic layer was dried over  $\text{MgSO}_4$ , filtered and concentrated and the residue was purified by flash column chromatography (4:1 hexane:EtOAc  $\rightarrow$  EtOAc) to give **33** as a colourless oil (Yield = 2.33 g; 56%).

$R_f$  (1:1 hexane:EtOAc): 0.30.  $\nu_{\text{max}}$  (Thin film,  $\text{cm}^{-1}$ ): 3419, 2867, 1496, 1454, 1352, 1292, 1247, 1207, 1098, 921, 889, 740.  $\delta_H$  (400 MHz,  $\text{CDCl}_3$ ): 7.35-7.28 (5H, m), 4.58 (2H, s), 3.74-3.68 (4H, m), 3.65-3.60 (4H, m), 2.68 (1H, br s).  $\delta_C$  (100 MHz,  $\text{CDCl}_3$ ): 138.3, 128.8, 128.2, 73.7, 73.0, 70.7, 69.8, 62.0. MS:  $\text{C}_{11}\text{H}_{16}\text{O}_3$   $m/z$  ( $\text{ES}^+$ ) 218.9 [ $\text{M}+\text{Na}^+$ ]. HRMS: Calculated  $\text{C}_{11}\text{H}_{16}\text{O}_3\text{Na}$  219.0992, found: 219.0994.

#### 2-[2-(2-Benzyloxy-ethoxy)-ethoxy]-ethanol, **34**.<sup>7,8</sup>

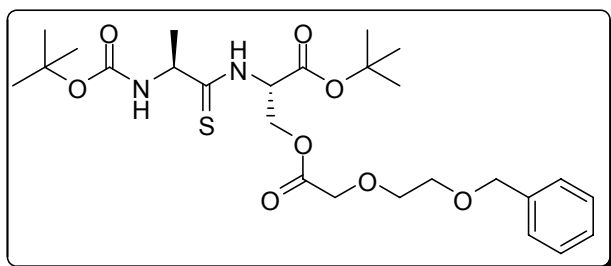


Triethylene glycol (847 mg; 5.6 mmol) was added to a stirred suspension of  $\text{Ag}_2\text{O}$  (1.96 g; 8.5 mmol) in DCM. The suspension was stirred at room temperature for 30 minutes after which time benzyl bromide (1.06 g; 6.2 mmol) was added. The reaction was stirred for 72 hours at room

temperature in the dark then filtered through a Celite<sup>®</sup> bed. The solvent was removed *in vacuo* and the residue purified by flash column chromatography (4:1 hexane:EtOAc → EtOAc) to give **34** as a pale yellow oil (Yield = 1.11 g; 82%).

R<sub>f</sub> (EtOAc): 0.35.  $\nu_{\max}$  (Thin film, cm<sup>-1</sup>): 3393, 2857, 1652, 1446, 1357, 1251, 1089.  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>): 7.39-7.21 (5H, m), 4.49 (2H, s), 3.77-3.57 (12H, m), 2.88 (1H, br s).  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>): 138.4, 128.8, 128.1, 180.0, 73.6, 73.0, 71.0, 70.6, 62.0. MS: C<sub>13</sub>H<sub>20</sub>O<sub>4</sub> *m/z* (ES<sup>+</sup>) 264.2 [M+Na<sup>+</sup>]. HRMS: Calculated C<sub>13</sub>H<sub>20</sub>O<sub>4</sub> 240.1356, found 240.1351.

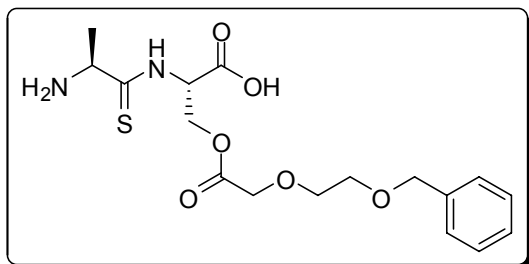
**(S)-3-[2-(2-Benzyloxy-ethoxy)-acetoxy]-2-((S)-2-tert-butoxycarbonylamino-thiopropionylamino)-propionic acid tert-butyl ester, 54.**



2 M oxalyl chloride in DCM (1.2 mL; 2.4 mmol) was diluted with 10 ml DCM and cooled to -65 °C. DMSO (0.34 mL; 4.8 mmol) was added slowly, keeping the temperature below -60 °C. This solution was stirred for 10 minutes before 2-benzyloxy-ethanol, **33** (415 mg; 2.1 mmol) in 5 mL DCM was added slowly. The solution was stirred at -65 °C for one hour then TEA (1.34 mL; 9.6 mmol) was added slowly. The solution was stirred at -65 °C for 30 minutes then warmed to room temperature. The DCM was removed under vacuum and the crude aldehyde suspended in 25 mL water, to which silver oxide (490 mg; 2.1 mmol) and sodium hydroxide (690 mg; 17.3 mmol) was added causing the temperature to rise to 40 °C. This suspension was heated to reflux for one hour. The mixture was filtered through Celite<sup>®</sup> and the orange solution extracted three times with 25 mL EtOAc. The aqueous layer was acidified with 2 M HCl then extracted five times with 25 mL EtOAc. The organic layer was dried (MgSO<sub>4</sub>), filtered and concentrated to give the crude acid (254 mg). This acid was dissolved in 4 mL DMF with HBTU (550 mg; 1.45 mmol) and DIPEA (0.1 mL; 0.55 mmol) and stirred at room temperature for 30 minutes. Protected serine thiodipeptide **27** (150 mg; 0.4 mmol) in 4 mL DMF was then added and the solution stirred at room temperature for 3 days. The DMF was removed *in vacuo* and the residue purified by flash column chromatography to give **54** as a yellow oil (Yield = 124 mg; 54%).

R<sub>f</sub> (9:1 DCM:Et<sub>2</sub>O): 0.26.  $[\alpha]_{\text{D}}^{20}$  (CHCl<sub>3</sub>; c = 2.5): 13.19.  $\nu_{\max}$  (Thin film, cm<sup>-1</sup>): 3330, 2979, 2933, 1737, 1498, 1454, 1394, 1369, 1248, 1160, 1049, 989, 913, 846, 754.  $\delta_{\text{H}}$  (500 MHz, CDCl<sub>3</sub>): 8.56 (1H, d, *J* = 6.7), 7.36-7.28 (5H, m), 5.30-5.10 (2H, m), 4.65 (1H, dd, *J* = 11.3 & 2.8), 4.62 (1H, dd, *J* = 11.3 & 3.1), 4.59 (2H, s), 4.41-4.52 (1H, m), 4.16 (2H, d, *J* = 2.5), 3.79-3.71 (2H, m), 3.69-3.65 (2H, m), 1.49-1.40 (21H, m).  $\delta_{\text{C}}$  (125 MHz, CDCl<sub>3</sub>): 206.0, 170.0, 167.2, 155.0, 137.9, 128.4, 127.7, 127.6, 83.7, 80.2, 73.3, 70.0, 69.4, 68.3, 63.0, 57.1, \* 28.7, 27.9, 21.7. MS: C<sub>26</sub>H<sub>40</sub>N<sub>2</sub>O<sub>8</sub>S *m/z* (ES<sup>+</sup>) 563.1 (M+Na<sup>+</sup>). HRMS: Calculated C<sub>26</sub>H<sub>44</sub>N<sub>3</sub>O<sub>8</sub>S 558.2844, found 558.2841. \* correlated to both  $\alpha$ -CH's.

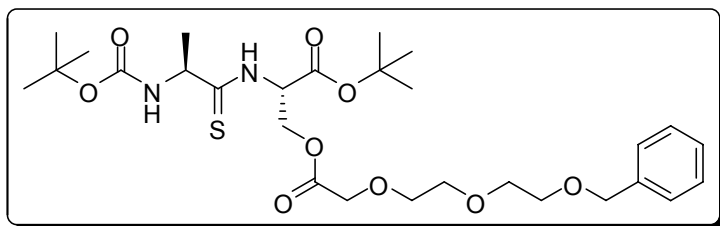
**(S)-2-((S)-2-Amino-thiopropionylamino)-3-[2-(2-benzyloxy-ethoxy)-acetoxy]-propionic acid, **7**.**



Deprotection of 120 mg of **54** as described in Section 1.1. Yield of **7** as TFA salt = 90 mg; 82%.

HPLC  $R_T$ : 1.7 (30%).  $[\alpha]_D^{20}$  (MeOH;  $c = 0.7$ ): +44.61.  $\delta_H$  (500 MHz,  $CD_3OD$ ): 7.41-7.35 (4H, m), 7.33-7.27 (1H, m), 5.49 (1H, dd,  $J = 5.0$  & 3.4), 4.69 (1H, dd,  $J = 11.7$  & 3.4), 4.61 (1H, dd,  $J = 11.7$  & 5.0), 4.58 (2H, s), 4.29 (1H, q,  $J = 6.9$ ), 4.24 (2H, s), 3.74-3.79 (2H, m), 3.66-3.71 (2H, m), 1.56 (3H, d,  $J = 6.9$ ).  $\delta_C$  (125 MHz,  $CD_3OD$ ): 202.8, 171.8, 170.3, 139.5, 129.4, 129.0, 128.8, 74.2, 72.0, 70.6, 69.1, 64.0, 58.4, 55.0, 20.7. MS:  $C_{17}H_{24}N_2O_6S$   $m/z$  ( $ES^+$ ) 385.2 ( $M+H^+$ ). HRMS: Calculated  $C_{17}H_{25}N_2O_6S$  385.1428, found 385.1429.

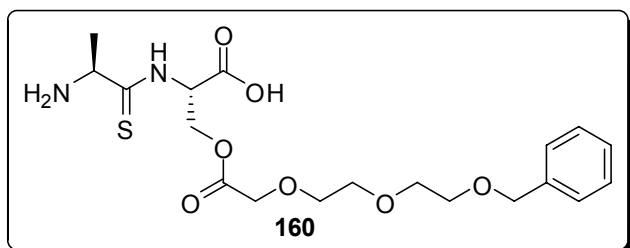
**3-{2-[2-(2-Benzyloxy-ethoxy)-ethoxy]-acetoxy}-2-(2-tert-butoxycarbonylamino-thiopropionylamino)-propionic acid tert-butyl ester, **55**.**



Protected serine carrier **27** (0.6 mmol) and 2-[2-(2-benzyloxy-ethoxy)-ethoxy]-ethanol **34** (1.4 mmol) were coupled as described for **54**, to give **55** as a yellow oil (Yield = 47%).

$R_f$  (4:1 hexane:EtOAc): 0.39.  $[\alpha]_D^{31}$  ( $CHCl_3$ ;  $c = 3.59$ ): +10.24.  $u_{max}$  (Thin film,  $cm^{-1}$ ): 3331, 3005-2871, 1736, 1498, 1454, 1393, 1368, 1248, 1158, 1123, 1048, 845, 755, 699.  $\delta_H$  (500 MHz,  $CDCl_3$ ): 8.61 (1H, d,  $J = 6.9$ ), 7.37-7.30 (5H, m), 5.26 (1H, br s), 5.23-5.21 (1H, m), 4.66-4.60 (2H, m), 4.57 (2H, s), 4.52-4.46 (1H, m), 4.17-4.13 (2H, m), 3.75-3.62 (8H, m), 1.47 (9H, s), 1.46-1.43 (12H, m).  $\delta_C$  (125 MHz,  $CDCl_3$ ): 206.9\*, 205.8, 169.2, 168.9, 154.9, 137.9, 128.2, 127.6, 127.5, 83.4, 80.0, 73.0, 70.8, 70.4, 69.1, 68.1, 62.9, 57.0, 53.3, 28.1, 27.7, 21.5, 21.4\*. MS:  $C_{28}H_{44}N_2O_9S$   $m/z$  ( $ES^+$ ) 608.0 [ $M+Na$ ]. HRMS: Calculated  $C_{28}H_{44}N_2O_9SNa$  607.2660, found 607.2653. \* minor rotamer.

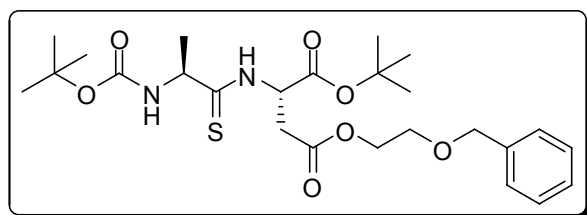
**2-(2-Amino-thiopropionylamino)-3-{2-[2-(2-benzyloxy-ethoxy)-ethoxy]-acetoxy}-propionic acid, **5**.**



Deprotection of 165 mg of **55** as described in Section 1.1. Yield of **5** as TFA salt = 106 mg; 70%.

HPLC  $R_f$ : 4.2 (30%).  $R_f$  (1:1:1:1 BuOH:EtOAc:H<sub>2</sub>O:CH<sub>3</sub>CO<sub>2</sub>H): 0.74.  $\delta_H$  (500 MHz, CD<sub>3</sub>OD): 7.36-7.26 (5H, m), 5.50 (0.3H, t,  $J = 4.1$ )\*, 5.47 (0.7H, t,  $J = 4.1$ ), 4.75 (0.3H, dd,  $J = 11.8$  & 3.4)\*, 4.66 (0.7H, dd,  $J = 11.8$  & 3.4), 4.60-4.51 (3H, m), 4.31-4.25 (1H, m), 4.19 (2H, s), 3.71-3.69 (2H, m), 3.68-3.62 (6H, m), 1.56 (2.1H, d,  $J = 6.6$ ), 1.51 (0.9, d,  $J = 6.6$ )\*.  $\delta_C$  (150 MHz, CD<sub>3</sub>OD): 202.8, 172.6, 171.4\*, 163.8, 163.5\*, 138.5, 129.8, 129.6, 129.3, 74.7, 73.9, 71.6, 70.7, 69.8, 68.9, 64.2, 58.2, 54.6, 54.3\*, 21.1, 20.4\*. MS: C<sub>19</sub>H<sub>28</sub>N<sub>2</sub>O<sub>7</sub>S  $m/z$  (ES<sup>+</sup>) 450.5 [M+Na<sup>+</sup>]. HRMS: Calculated C<sub>19</sub>H<sub>28</sub>N<sub>2</sub>O<sub>7</sub>SNa 451.1509, found 451.1511. \* minor rotamer.

**(S)-2-((S)-2-tert-Butoxycarbonylamino-thiopropionylamino)-succinic acid 4-(2-benzyloxy-ethyl) ester **1-tert-butyl ester, 56**.**

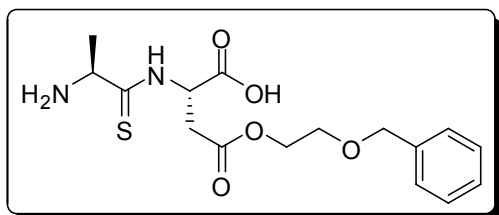


Protected aspartate thiodipeptide **28** (108 mg; 0.3 mmol) and HBTU (200 mg; 0.5 mmol) was dissolved in 5 mL DMF. DIPEA (0.06 mL; 0.4 mmol) was added and the colourless solution turned red/brown as it was stirred for 30 minutes. 2-benzyloxy-ethanol, **32** (146 mg; 1.0 mmol) was added neat and the resultant solution stirred for four days at room temperature. The DMF was removed under vacuum and the residue purified by flash column chromatography (9:1 DCM:Et<sub>2</sub>O → 4:1 DCM:Et<sub>2</sub>O) to give **56** as a yellow oil (Yield = 81 mg; 55%).

$R_f$  (4:1 DCM:Et<sub>2</sub>O): 0.71.  $\nu_{max}$  (Thin film, cm<sup>-1</sup>): 3335, 2978, 2928, 2868, 1739, 1504, 1454, 1392, 1367, 1245, 1163, 1109, 1029, 847, 743.  $\delta_H$  (500 MHz, CDCl<sub>3</sub>): 8.66 (0.1H, d,  $J = 7.2$ )\*, 8.60 (0.9H, d,  $J = 7.2$ ), 7.38-7.28 (5H, m), 5.42-5.26 (1H, m), 5.25-5.08 (1H, m), 4.58 (2H, d,  $J = 2.2$ ), 4.44 (1H, br t,  $J = 6.5$ ), 4.30 (1H, dt,  $J = 12.2$  & 4.7), 4.23 (1H, dt,  $J = 12.2$  & 4.7), 3.67 (2H, t,  $J = 4.7$ ), 3.16 (1H, dd,  $J = 17.1$  & 5.2), 3.07 (1H, dd,  $J = 17.3$  & 3.8), 1.46 (9H, s), 1.44 (9H, s), 1.41 (3H, d,  $J = 7.3$ ).  $\delta_C$  (125 MHz, CDCl<sub>3</sub>): 205.1, 170.,5 168.4, 154.9, 137.5, 128.3, 127.9, 83.1, 80.1, 77.3, 67.6, 64.0, 56.9, 54.0, 34.8, 34.7\*, 28.3, 27.8, 21.9\*, 21.8. MS: C<sub>25</sub>H<sub>38</sub>N<sub>2</sub>O<sub>7</sub>S  $m/z$  (ES<sup>+</sup>) 533.3 (M+Na<sup>+</sup>). HRMS: Calculated C<sub>25</sub>H<sub>39</sub>N<sub>2</sub>O<sub>7</sub>S 511.2472, found 511.2470. \* minor rotamer.



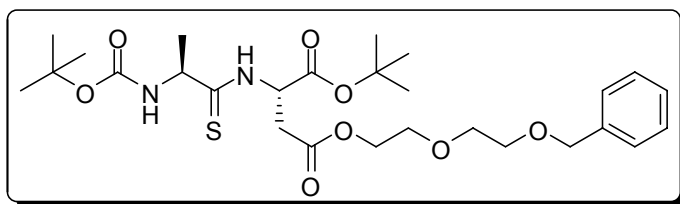
**(S)-2-((S)-2-Amino-thiopropionylamino)-succinic acid 4-(2-benzyloxy-ethyl) ester, 11.**



Deprotection of 66 mg of **56** as described in Section 1.1. Yield of **11** as TFA salt = 57 mg; 94%.

HPLC  $R_T$ : 4.0 (20%).  $\delta_H$  (500 MHz,  $CD_3OD$ ): 7.35 (2H, s), 7.34 (2H, s), 7.32-7.23 (1H, m), 5.36 (1H, s), 4.55 (2H, s), 4.27 (2H, t,  $J = 4.1$ ), 4.21 (1H, q,  $J = 6.6$ ), 3.70 (2H, t,  $J = 4.1$ ), 3.15-2.97 (2H, m), 1.54 (2.7H, d,  $J = 6.6$ ), 1.45 (0.3H, d,  $J = 6.6$ )\*.  $\delta_C$  (125 MHz,  $CD_3OD$ ): 201.9, 171.8, 139.3, 129.5, 129.0, 128.9, 74.0, 69.0, 65.2, 55.0\*\*, 36.0, 20.7. MS:  $C_{16}H_{22}N_2O_5S$   $m/z$  ( $ES^+$ ) 355.1 ( $M+H^+$ ). HRMS: Calculated  $C_{16}H_{23}N_2O_5S$  355.1322, found 355.1317. \* minor rotamer. \*\* correlated to both  $\alpha$ -CH's.

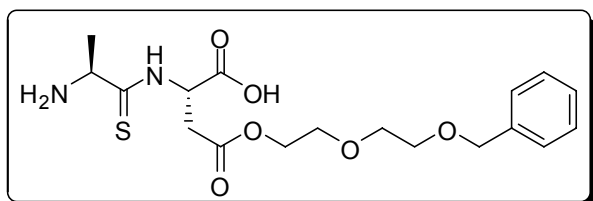
**(S)-2-((S)-2-tert-Butoxycarbonylamino-thiopropionylamino)-succinic acid 4-[2-(2-benzyloxy-ethoxy)-ethyl] ester 1-tert-butyl ester, 57.**



Protected aspartate thiodipeptide **28** (0.3 mmol) and 2-(2-benzyloxy-ethoxy)-ethanol **33** (1 mmol) were coupled as described for **56**, to give **57** as a yellow oil (Yield = 45%).

$R_f$  (4:1 DCM:Et<sub>2</sub>O): 0.47.  $u_{max}$  (Thin film,  $cm^{-1}$ ): 3335, 2978, 2933, 2871, 1740, 1513, 1453, 1393, 1369, 1247, 1163, 1097, 1050, 914, 848, 735, 699.  $\delta_H$  (500 MHz,  $CDCl_3$ ): 8.70 (0.1H, d,  $J = 7.4$ ),\* 8.63 (0.9H, d,  $J = 7.4$ ), 7.36-7.30 (5H, m), 5.44 (1H, br s), 5.17-5.26 (1H, m), 4.59 (2H, s), 4.53-4.38 (1H, m), 4.46 (1H, br t,  $J = 5.2$ ), 4.25-4.16 (1H, m), 3.73-3.68 (4H, m), 3.67-3.64 (2H, m), 3.16 (1H, dd,  $J = 17.0$  & 4.2), 3.06 (1H, dd,  $J = 17.0$  & 4.0), 1.47 (9H, s), 1.46-1.41 (12H, m).  $\delta_C$  (125 MHz,  $CDCl_3$ ): 205.2, 170.4, 168.4, 154.9, 138.0, 129.7, 128.4, 127.8, 127.7, 83.0, 80.0, 73.2, 70.5, 69.4, 68.9, 64.0, 57.0, 54.0, 34.8, 28.3, 27.9, 21.9. MS:  $C_{27}H_{42}N_2O_8S$   $m/z$  ( $ES^+$ ) 577.2 ( $M+Na^+$ ). HRMS: Calculated  $C_{27}H_{42}N_2O_8SNa$  577.2554, found 577.2559. \*minor rotamer.

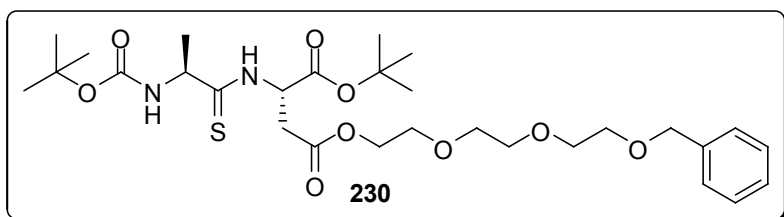
**(S)-2-((S)-2-Amino-thiopropionylamino)-succinic acid 4-[2-(2-benzyloxy-ethoxy)-ethyl] ester, **8**.**



Deprotection of 72 mg of **57** as described in Section 1.1. Yield of **8** as TFA salt = 69 mg; 99%.

HPLC  $R_T$ : 2.1 (30%).  $\delta_H$  (500 MHz,  $CD_3OD$ ): 7.40-7.22 (5H, m), 5.36 (1H, br s), 4.55 (2H, s), 4.29-4.18 (3H, m), 3.70 (2H, t,  $J = 4.5$ ), 3.68-3.65 (2H, m), 3.64-3.60 (2H, m), 3.08 (1H, dd,  $J = 16.7$  & 4.7), 3.01 (1H, dd,  $J = 16.7$  & 6.7), 1.55 (2.8H, d,  $J = 6.6$ ), 1.49 (0.2H, d,  $J = 6.6$ )\*.  $\delta_C$  (125 MHz,  $CD_3OD$ ): 201.9, 171.8, 139.5, 129.6, 128.9, 128.8, 74.2, 71.4, 70.5, 70.0, 65.2, 55.0\*\*, 36.0, 20.8\*, 20.7. MS:  $C_{18}H_{26}N_2O_6S$   $m/z$  ( $ES^+$ ) 399.1 ( $M+H^+$ ). HRMS: Calculated  $C_{18}H_{27}N_2O_6S$  399.1584, found 399.1578. \* minor rotamer \*\* correlates to both  $\alpha$ -CH's.

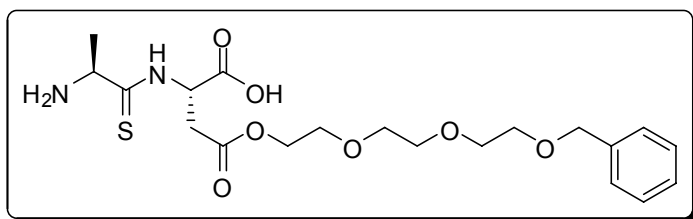
**2-(2-tert-Butoxycarbonylamino-thiopropionylamino)-succinic acid 4-{2-[2-(2-benzyloxy-ethoxy)-ethoxy]-ethyl} ester 1-tert-butyl ester, **58**.**



Protected aspartate thiodipeptide **28** (0.4 mmol) and 2-[2-(2-benzyloxy-ethoxy)-ethoxy]-ethanol **34** (1.2 mmol) were coupled as described for **56**, to give **58** as a yellow oil (Yield = 88%).

$R_f$  (1:1 hexane:EtOAc): 0.33.  $[\alpha]_D^{26}$  ( $CHCl_3$ ;  $c = 3.37$ ): +20.64.  $u_{max}$  (Thin film,  $cm^{-1}$ ): 3332, 2978-2872, 1740, 1514, 1393, 1367, 1247, 1158, 1098, 1050, 847, 752, 699.  $\delta_H$  (500 MHz,  $CDCl_3$ ): 8.74 (0.3H, d,  $J = 7.5$ )\*, 8.67 (0.7H, d,  $J = 8.8$ ), 7.38-7.28 (5H, m), 5.46 (0.7H, br s), 5.29 (0.3H, br s)\*, 5.25-5.15 (1H, m), 4.56 (2H, s), 4.51-4.40 (1H, m), 4.32-4.10 (2H, m), 3.74-3.55 (10H, m), 3.16 (1H, dd,  $J = 7.0$  & 3.5), 3.04 (1H, dd,  $J = 7.0$  & 4.0), 1.47-1.43 (21H, m).  $\delta_C$  (125 MHz,  $CDCl_3$ ): 205.1, 170.5, 168.3, 154.9, 138.0, 128.5, 128.3, 127.7, 83.1, 80.1, 73.2, 70.6, 69.3, 67.9, 68.8, 64.0, 57.0, 54.0, 34.8, 28.3, 27.8, 21.9. MS:  $C_{29}H_{46}N_2O_6S$   $m/z$  ( $ES^+$ ) 621.1 [ $M+Na^+$ ]. HRMS: Calculated  $C_{29}H_{46}N_2O_9SNa$  621.2816, found 621.2828. \* minor rotamer.

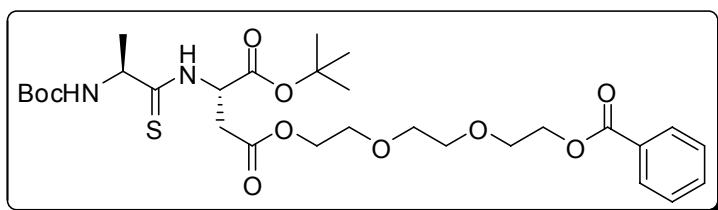
**2-(2-Amino-thiopropionylamino)-succinic acid 4-{2-[2-(2-benzyloxy-ethoxy)-ethoxy]-ethyl} ester, **6**.**



Deprotection of 162 mg of **58** as described in Section 1.1. Yield of **6** as TFA salt = 151 mg; 85%.

HPLC  $R_f$ : 4.5 (30%).  $R_f$  (1:1:1:1 BuOH:EtOAc:H<sub>2</sub>O:CH<sub>3</sub>CO<sub>2</sub>H): 0.76.  $\delta_H$  (500 MHz, CD<sub>3</sub>OD): 7.37-7.27 (5H, m), 5.41-5.38 (0.8H, dd,  $J = 4.7$  & 2.3), 5.37-5.35 (0.2H, m)\*, 4.56 (1.5H, s), 4.55 (0.5H, s)\*, 4.30-4.20 (3H, m), 3.71-3.62 (10H, m), 3.13-3.05 (1H, m), 3.03-2.95 (1H, m), 1.52 (2.4H, d,  $J = 6.7$ ), 1.49 (0.6H, d,  $J = 6.7$ )\*.  $\delta_C$  (150 MHz, CD<sub>3</sub>OD): 200.9, 171.7\*, 171.4, 162.5, 162.2\*, 137.1, 128.9, 128.6, 128.3, 127.2, 72.6, 70.3, 69.5, 69.4, 68.6, 68.1, 64.2, 53.9, 53.1, 34.4, 19.8. MS: C<sub>20</sub>H<sub>30</sub>N<sub>2</sub>O<sub>7</sub>S  $m/z$  (ES<sup>+</sup>) 443.4 [M+H<sup>+</sup>]. HRMS: Calculated C<sub>20</sub>H<sub>31</sub>N<sub>2</sub>O<sub>7</sub>S 443.1846, found 443.1846. \* minor rotamer.

**(S)-2-((S)-2-tert-Butoxycarbonylamino-thiopropionylamino)-succinic acid 4-{2-[2-(2-benzyloxy-ethoxy)-ethoxy]-ethyl} ester 1-tert-butyl ester, **59**.**

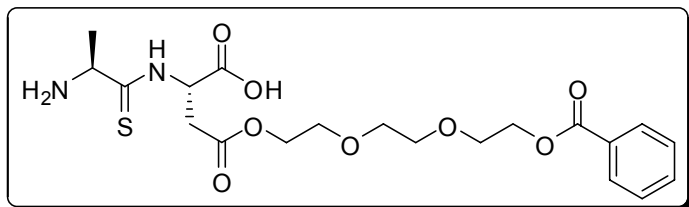


Benzoic acid (109 mg; 0.90 mmol) and triphenylphosphine (293 mg; 1.10 mmol) were suspended in 0.3 mL THF. Triethylene glycol (0.4 mL; 3.00 mmol) was then added and the suspension sonicated for 5 minutes. DIAD (0.22 mL; 1.10 mmol) was added over 10 minutes with sonication. The resultant orange solution was sonicated for 30 minutes, before initial purification using with a Biotage<sup>®</sup> column using a SNAP KP-Sil 50 g cartridge (1:1 petrol:EtOAc → EtOAc) followed by a second Biotage<sup>®</sup> column using a SNAP KP-Sil 10 g cartridge (9:1 DCM:Et<sub>2</sub>O → EtOAc) to give crude glycol benzoic acid ester **35** contaminated with triphenylphosphine oxide ( $R_f$  (EtOAc) = 0.31; 152 mg; 67% crude yield). This crude glycol (132 mg) was added neat to a solution of protected aspartate carrier **28** (89 mg; 0.24 mmol), HBTU (104 mg; 0.27 mmol) and DIPEA (50  $\mu$ L; 0.28 mmol) which had been stirred at room temperature for 30 minutes prior to alcohol addition. The reaction was stirred at room temperature for 4 days. The DMF was removed under vacuum and the residue dissolved in 5 mL CHCl<sub>3</sub>. This was washed successively with 3 mL volumes of saturated NH<sub>4</sub>Cl, saturated Na<sub>2</sub>CO<sub>3</sub> and brine. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under vacuum and the residue purified by a Biotage<sup>®</sup> column using a SNAP KP-Sil 50 g cartridge (95:5 DCM:Et<sub>2</sub>O → 4:1 DCM:Et<sub>2</sub>O) to give **59** as a yellow oil (Yield = 79 mg; 54%).

$R_f$  (9:1 DCM:Et<sub>2</sub>O): 0.13.  $[\alpha]_D^{24}$  (CHCl<sub>3</sub>;  $c = 1.58$ ): +53.80.  $u_{max}$  (Thin film, cm<sup>-1</sup>): 3336, 2977, 1721, 1700, 1695, 1616, 1553, 1512, 1452, 1369, 1275, 1158.  $\delta_H$  (300 MHz, CDCl<sub>3</sub>): 8.72 (0.2H, d,  $J = 7.1$ )\*, 8.64 (0.8H, d,  $J = 7.4$ ), 8.05 (2H, dd,  $J = 7.5$  & 1.3), 7.57 (1H, t,  $J = 7.1$ ), 7.44 (2H, t,  $J = 7.8$ ), 5.43 (0.8H, br s), 5.33-5.24 (0.2H, m)\*, 5.24-5.15 (1H, m), 4.70-4.36 (3H, m), 4.34-4.03 (2H, m), 3.84 (2H, t,  $J = 4.8$ ), 3.53-3.78 (6H, m), 3.16 (1H, dd,  $J = 17.2$  & 4.4), 3.04 (1H, dd,  $J = 17.2$  &

4.0), 1.56-1.33 (21H, m).  $\delta_c$  (75 MHz,  $CDCl_3$ ): 205.1, 204.8\*, 168.3, 166.4, 154.8, 154.5\*, 133.0, 129.9, 129.6, 128.3, 83.1, 80.0, 69.1, 69.0, 68.7, 63.8, 56.8, 53.9, 53.8\*, 34.7, 34.5\*, 28.2, 22.8, 21.9, 21.7\*. MS:  $C_{29}H_{44}N_2O_{10}S$   $m/z$  ( $ES^+$ ) 635.4 ( $M+Na^+$ ). \* minor rotamer.

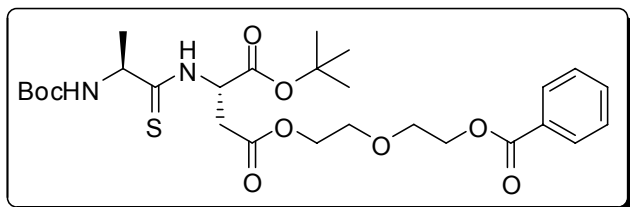
**(S)-2-((S)-2-Amino-thiopropionylamino)-succinic acid 4-[2-[2-(2-benzoyloxy-ethoxy)-ethoxy]-ethyl] ester, 9.**



Deprotection of 98 mg of **59** as described in Section 1.1. Yield of **9** as TFA salt = 77 mg; 92%.

HPLC  $R_f$ : 4.5 (30%).  $R_f$  (1:1:1:1 EtOAc:BuOH:H<sub>2</sub>O:AcOH): 0.68.  $\delta_H$  (300 MHz,  $CD_3OD$ ): 8.10-7.99 (2H, m), 7.61 (1H, tt,  $J = 7.4$  & 1.4), 7.48 (2H, dt,  $J = 7.4$  & 1.4), 5.32-5.21 (2H, m), 4.52-4.41 (2H, m), 4.27 (1H, q,  $J = 6.6$ ), 4.23-4.13 (2H, m), 3.90-3.81 (2H, m), 3.75-3.58 (6H, m), 3.13-2.88 (2H, m), 1.55 (2.5H, d,  $J = 6.6$ ), 1.49 (0.5H, d,  $J = 6.6$ )\*.  $\delta_c$  (75 MHz,  $CD_3OD$ ): 201.9, 201.8\*, 171.9, 171.4\*, 171.3, 168.7, 134.2, 131.2, 130.4, 129.4, 71.4, 71.2, 66.8, 66.7, 65.1, 65.0, 55.5, 54.8, 35.7, 35.6\*, 20.7\*, 20.5. MS:  $C_{20}H_{28}N_2O_8S$   $m/z$  ( $ES^+$ ) 457.5 ( $M+H^+$ ). \* minor rotamer.

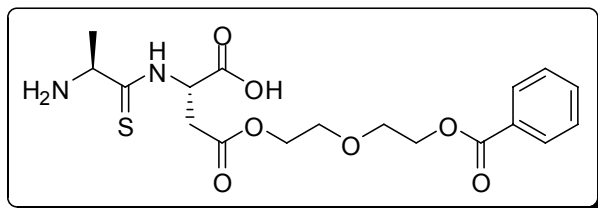
**(S)-2-((S)-2-tert-Butoxycarbonylamino-thiopropionylamino)-succinic acid 4-[2-(2-benzoyloxy-ethoxy)-ethyl] ester 1-tert-butyl ester, 60.**



Benzoic acid (132 mg; 1.08 mmol) and triphenylphosphine (325 mg; 1.24 mmol) were suspended in 0.3 mL THF. Triethylene glycol (0.28 mL; 3.00 mmol) was then added and the suspension sonicated for 5 minutes. DIAD (0.25 mL; 1.25 mmol) was added over 10 minutes with sonication. The resultant orange solution was sonicated for 30 minutes, before initial purification using a Biotage<sup>®</sup> column using a SNAP KP-Sil 50 g cartridge (1:1 petrol:EtOAc  $\rightarrow$  EtOAc) to give crude glycol benzoic acid ester **36** contaminated with an unknown impurity ( $R_f$  (1:1 petrol:EtOAc) = 0.08; 132 mg; 55% crude yield). This crude glycol (116 mg) was coupled to protected aspartate carrier (90 mg; 0.24 mmol) using the method described for **59** to give **60** as a yellow oil (Yield = 56 mg; 41%).

$R_f$  (9:1 DCM:Et<sub>2</sub>O): 0.18.  $R_f$  (9:1 DCM:Et<sub>2</sub>O): 0.13.  $[\alpha]_D^{24}$  ( $CHCl_3$ ;  $c = 1.05$ ): +40.87.  $u_{max}$  (Thin film,  $cm^{-1}$ ): 3338, 3061, 3034, 2978, 2933, 1724, 1602, 1584, 1513, 1452, 1413, 1393, 1351, 1275, 1158, 1113, 1071, 1050, 1029, 1002, 847, 714.  $\delta_H$  (300 MHz,  $CDCl_3$ ): 8.69 (0.1H, d,  $J = 7.7$ )\*, 8.61 (0.9H, d,  $J = 7.2$ ), 8.06 (2H, dd,  $J = 7.5$  & 1.5), 7.58 (1H, t,  $J = 7.5$ ), 7.45 (2H, t,  $J = 7.5$ ), 5.37 (1H, br s), 5.29-5.12 (1H, m), 4.59-4.38 (3H, m), 4.36-4.10 (2H, m), 3.85 (2H, t,  $J = 4.8$ ), 3.75 (2H, t,  $J = 4.8$ ), 3.15 (1H, dd,  $J = 17.2$  & 4.6), 3.04 (1H, dd,  $J = 17.2$  & 4.1), 1.54-1.33 (21H, m).  $\delta_c$  (75 MHz,  $CDCl_3$ ): 205.1, 204.8\*, 170.4, 168.3, 166.4, 154.8, 154.5\*, 133.0, 129.9, 129.6, 128.3, 83.0, 80.0, 69.1, 68.7, 63.8, 56.8, 53.9, 53.8\*, 34.7, 34.5\*, 28.2, 22.7, 21.9, 21.7\*. MS:  $C_{27}H_{40}N_2O_9S$   $m/z$  ( $ES^+$ ) 591.4 ( $M+Na^+$ ). \* minor rotamer.

**(S)-2-((S)-2-Amino-thiopropionylamino)-succinic acid 4-[2-(2-benzoyloxy-ethoxy)-ethyl] ester, 10.**

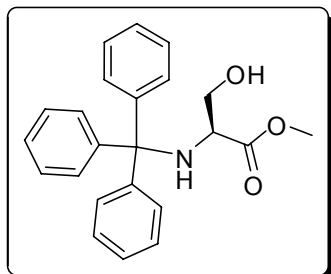


Deprotection of 84 mg of **60** as described in Section 5.1.3. Yield of **10** as TFA salt = 61 mg; 78%.

HPLC  $R_T$ : 3.2 (30%).  $R_f$  (1:1:1:1 EtOAc:BuOH:H<sub>2</sub>O:AcOH): 0.71.  $\delta_H$  (300 MHz, CD<sub>3</sub>OD): 8.097-98 (2H, m), 7.61 (1H, tt,  $J = 7.3$  & 1.6), 7.53-7.43 (2H, m), 5.42-5.31 (1H, m), 4.52-4.38 (2H, m), 4.36-4.17 (3H, m), 3.90-3.80 (2H, m), 3.80-3.70 (2H, m), 3.12-2.87 (2H, m), 1.55 (2.4H, d,  $J = 6.7$ ), 1.48 (0.6H, d,  $J = 6.7$ )\*.  $\delta_C$  (75 MHz, CD<sub>3</sub>OD): 202.2, 202.1\*, 172.2, 171.7, 171.6\*, 168.1, 134.5, 131.4, 130.7, 129.8, 70.2, 70.1, 65.3, 55.7, 55.0, 35.9, 21.0\*, 20.8.

### 3.4 Synthesis of model thiodipeptide prodrug **4**

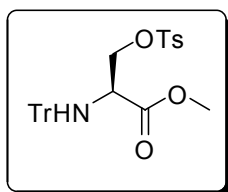
#### (S)-3-Hydroxy-2-(trityl-amino)-propionic acid methyl ester, **40**.<sup>9</sup>



A solution of L-Serine methyl ester hydrochloride **39** (1 g; 8.4 mmol) and TEA (2.3 mL; 16.8 mmol) in 5 mL CHCl<sub>3</sub> was cooled to -10 °C. Trityl chloride (2.33 g; 8.4 mmol) in a 4 mL solution of CHCl<sub>3</sub> was added and the reaction stirred at 0 °C for 24 hours. The solution was washed successively with two 10 mL volumes of 0.6 M citric acid and one 10 mL volume of water, then dried over MgSO<sub>4</sub>, filtered and concentrated. The residue was purified by flash column chromatography (9:1 petrol:EtOAc → 4:1 petrol:EtOAc) to give **40** as a white crystalline solid (Yield = 1.9 g; 63%).

Melting point: 152-154 °C. R<sub>f</sub> (DCM): 0.61. [α]<sub>D</sub><sup>24</sup> (CHCl<sub>3</sub>; c = 0.8): -6.77. u<sub>max</sub> (Thin film, cm<sup>-1</sup>): 3440, 3057, 2950, 1732, 1640, 1596, 1491, 1448, 1434, 1265, 1205, 1175, 1134, 1059, 1031, 902, 707. δ<sub>H</sub> (400 MHz, CDCl<sub>3</sub>): 7.50-7.48 (6H, m), 7.29-7.18 (9H, m), 3.73-3.70 (1H, m), 3.57-3.53 (2H, m), 3.30 (3H, s), 2.34 (1H, br s), 2.05 (1H, br s). δ<sub>C</sub> (75 MHz, CDCl<sub>3</sub>): 174.3, 146.0, 129.1, 128.3, 127.0, 71.3, 65.3, 58.2, 52.4. MS: C<sub>23</sub>H<sub>23</sub>NO<sub>3</sub> m/z (ES<sup>+</sup>) 383.7 [M+Na<sup>+</sup>]. HRMS: Calculated C<sub>23</sub>H<sub>27</sub>N<sub>2</sub>O<sub>3</sub> 379.2016, found 379.2025.

#### (S)-3-(Toluene-4-sulfonyloxy)-2-(trityl-amino)-propionic acid methyl ester, **41**.<sup>10</sup>

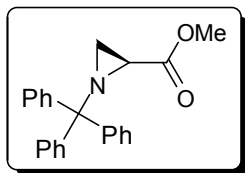


TsCl (4.9 g; 25.7 mmol) was added in batches over 10 minutes to a solution of **40** (2.8 g; 7.8 mmol) in 10 mL dry pyridine at -5 °C. The mixture was bright yellow with a white precipitate that was difficult to stir until the temperature approached 0 °C. The mixture was stirred at 0 °C for 20 hours by which time the temperature had risen to 14 °C. 20 mL EtOAc was added and the solution was washed with two 20 mL volumes of 0.6 M citric acid. The organic layer was dried over MgSO<sub>4</sub>, filtered and concentrated. The residue was purified by flash column chromatography (6:1 petrol:EtOAc → 4:1 petrol:EtOAc) to give **41** as a white solid (Yield = 3.6 g; 89%).

Melting point: 82-84 °C. R<sub>f</sub> (4:1 petrol:EtOAc): 0.21. [α]<sub>D</sub><sup>24</sup> (CHCl<sub>3</sub>; c = 1.42): +16.48. u<sub>max</sub> (Thin film, cm<sup>-1</sup>): 3058, 1739, 1597, 1491, 1448, 1364, 1190, 976, 708. δ<sub>H</sub> (400 MHz, CDCl<sub>3</sub>): 7.87 (2H, d, J = 8.0), 7.41-7.38 (6H, m), 7.33 (2H, d, J = 8.0), 7.26-7.15 (9H, m), 4.26 (1H, dd, J = 9.8 & 4.6), 4.09 (1H, dd, J = 9.8 & 6.2), 3.53-3.48 (1H, m), 3.16 (3H, s), 2.73 (1H, d, J = 9.9), 2.45 (3H, s). δ<sub>C</sub> (100 MHz, CDCl<sub>3</sub>): 172.4, 145.7, 145.3, 133.2, 130.2, 129.0, 128.5, 128.4, 127.0, 71.7, 71.4,

55.8, 52.5, 22.1. MS:  $C_{30}H_{29}NO_5S$   $m/z$  ( $ES^+$ ) 538.2 [ $M+Na^+$ ]. HRMS: Calculated  $C_{30}H_{29}NO_5SNa$  538.1659, found 538.1657.

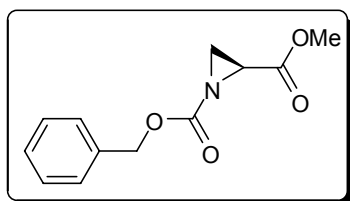
**(S)-1-Trityl-aziridine-2-carboxylic acid methyl ester, 42.**<sup>11</sup>



A solution of **41** (3.6 g; 7.0 mmol) and TEA (2 mL; 14.0 mmol) in 120 mL dry THF was refluxed at 75 °C for 48 hours. The solvent was removed and the yellow residue taken up in 30 mL EtOAc. The solution was washed with two 30 mL volumes of 0.6 M citric acid, 20 mL saturated  $NaHCO_3$  and 20 mL water. The organic layer was dried over  $MgSO_4$ , filtered and concentrated to give **42** as a cream solid (Yield = 2.3 g; 96%).

$R_f$  (4:1 petrol:EtOAc): 0.87.  $[\alpha]_D^{24}$  ( $CHCl_3$ ;  $c = 1.43$ ):  $-63.39$ .  $\nu_{max}$  (Thin film,  $cm^{-1}$ ): 3057, 2951, 1748, 1489, 1447, 1244, 1200, 1179, 1081, 1034, 907.  $\delta_H$  (400 MHz,  $CDCl_3$ ): 7.51-7.49 (6H, m), 7.30-7.22 (9H, m), 3.76 (3H, s), 2.26 (1H, dd,  $J = 3.0$  & 1.5), 1.89 (1H, dd,  $J = 6.0$  & 3.0), 1.41 (1H, dd,  $J = 6.0$  & 1.5).  $\delta_C$  (100 MHz,  $CDCl_3$ ): 170.9, 142.5, 128.3, 126.6, 125.8, 73.3, 51.1, 30.6, 27.6. MS:  $C_{23}H_{21}NO_2$   $m/z$  ( $ES^+$ ) 366.2 [ $M+Na^+$ ]. HRMS: Calculated  $C_{23}H_{21}NO_2Na$  366.1465, found 366.1470.

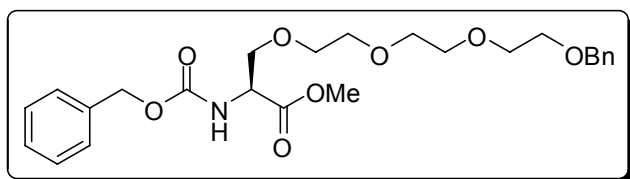
**(S)-Aziridine-1,2-dicarboxylic acid 1-benzyl ester 2-methyl ester, 43.**<sup>11</sup>



**42** (534 mg; 1.6 mmol) was dissolved in 30 mL DCM and cooled to 0 °C.  $Et_3SiH$  (1.1 mL; 6.3 mmol) and TFA (0.5 mL; 6.3 mmol) were added in succession. The solution flashed yellow upon addition of TFA then formed a colourless solution, which was stirred at 0 °C for 90 minutes. DIPEA (1.1 mL; 6.3 mmol) was then added and the solution stirred at 0 °C for a further 20 minutes. The solvent was removed under vacuum at 30 °C and the residue redissolved in 10 mL DCM. The solution was cooled to 0 °C and benzylchloroformate (0.45 mL; 3.1 mmol) was added neat. The solution was warmed to room temperature over 3 hours then stirred for 15 hours. The organic layer was washed with two 10 mL volumes of 0.7 M citric acid, 10 mL saturated  $NaHCO_3$  and 10 mL brine, then dried over  $MgSO_4$ , filtered and concentrated. The residue was purified by flash column chromatography (4:1 hexane:EtOAc) to give **43** as a pale yellow oil (Yield = 275 mg; 75%).

$R_f$  (4:1 hexane:EtOAc): 0.34.  $\delta_H$  (400 MHz,  $CDCl_3$ ): 7.50-7.30 (5H, m), 5.17 (2H, s), 3.74 (3H, s), 3.14 (1H, br t,  $J = 4.0$ ), 2.56 (1H, d,  $J = 2.0$ ), 2.51 (1H, d,  $J = 5.5$ ).  $\delta_C$  (100 MHz,  $CDCl_3$ ): 169.0, 161.1, 135.7, 129.0, 128.7, 128.0, 127.4, 69.1, 53.1, 35.2, 31.8. MS:  $C_{12}H_{13}NO_4$   $m/z$  ( $ES^+$ ) 253.2 [ $M+NH_4^+$ ]. HRMS: Calculated  $C_{12}H_{13}NO_4$  236.0917, found 236.0920.

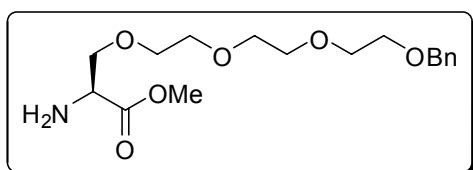
**(S)-2-Benzyloxycarbonylamino-3-{2-[2-(2-benzyloxy-ethoxy)-ethoxy]-ethoxy}-propionic acid methyl ester, **44**.**



A solution of **43** (713 mg; 3.0 mmol) and 2-(2-benzyloxy-ethoxy)-ethanol **34** (1.1 g; 4.5 mmol) in 15 mL DCM was cooled in an acetone/ice bath for 20 minutes.  $\text{BF}_3 \cdot \text{OEt}_2$  (0.2 mL; 1.5 mmol) was then added in one lot. The colourless solution was removed from the bath and stirred slowly at room temperature for 20 hours. The pale yellow solution was concentrated and purified by flash column chromatography (1:1 hexane:EtOAc) to give **44** as a pale yellow oil (Yield = 718 mg; 50%).

$R_f$  (1:1 hexane:EtOAc): 0.18.  $[\alpha]_D^{24}$  ( $\text{CHCl}_3$ ;  $c = 0.53$ ): +8.24.  $u_{\text{max}}$  (Thin film,  $\text{cm}^{-1}$ ): 2359, 2340, 1750, 1722, 1577, 1455, 1106.  $\delta_{\text{H}}$  (500 MHz,  $\text{CDCl}_3$ ): 7.37-7.26 (10H, m), 5.85 (1H, d,  $J = 8.7$ ), 5.13 (2H, s), 4.46 (1H, ddd,  $J = 8.7, 6.6 \text{ \& } 3.4$ ), 4.16 (2H, s), 3.96 (1H, dd,  $J = 9.8 \text{ \& } 6.4$ ), 3.75 (3H, s), 3.73 (1H, dd,  $J = 9.8 \text{ \& } 3.4$ ), 3.60-3.70 (12H, m).  $\delta_{\text{C}}$  (150 MHz,  $\text{CDCl}_3$ ): 169.8, 155.1, 137.2, 135.3, 127.5, 127.3, 127.1, 127.0, 126.7, 126.6, 76.2, 72.2, 70.0, 69.6, 69.3, 68.4, 66.0, 53.5, 51.5. MS:  $\text{C}_{25}\text{H}_{33}\text{NO}_8$   $m/z$  ( $\text{ES}^+$ ) 498.3 [ $\text{M}+\text{Na}^+$ ]. HRMS: Calculated  $\text{C}_{25}\text{H}_{33}\text{NO}_8\text{Na}$  498.2098, found 498.2109.

**(S)-2-Amino-3-{2-[2-(2-benzyloxy-ethoxy)-ethoxy]-ethoxy}-propionic acid methyl ester, **45**.**

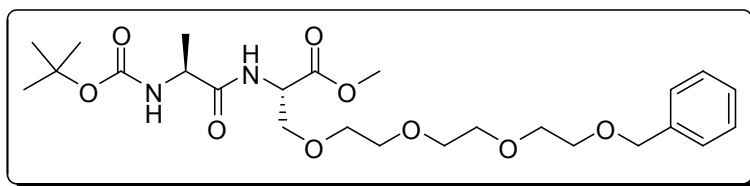


A suspension of 64 mg 10% Pd-C and **44** (200 mg; 0.6 mmol) in 15 mL EtOH was stirred under a hydrogen atmosphere for 3 hours. The catalyst was removed by filtration through a Celite<sup>®</sup> bed and the solvent removed to give **45** as a pale yellow oil (Yield = 144 mg; 99%).

$[\alpha]_D^{24}$  ( $\text{CHCl}_3$ ;  $c = 0.10$ ): +11.22.  $u_{\text{max}}$  (Thin film,  $\text{cm}^{-1}$ ): 3443-3381, 2945, 2906, 2870, 1742, 1600, 1452, 1352, 1268, 1241, 1102, 737.  $\delta_{\text{H}}$  (500 MHz,  $\text{CDCl}_3$ ): 7.36-7.29 (5H, m), 4.64-4.53 (3H, m), 3.83-3.61 (17H, m), 2.67 (2H, br s).  $\delta_{\text{C}}$  (125 MHz,  $\text{CDCl}_3$ ): 172.3, 138.0, 128.4, 127.9, 127.8, 127.7, 73.3, 72.6, 72.4, 71.0, 70.9, 70.6, 70.3, 69.5, 54.6, 52.3. MS:  $\text{C}_{17}\text{H}_{27}\text{NO}_6$   $m/z$  ( $\text{ES}^+$ ) 342.3 [ $\text{M}+\text{H}^+$ ]. HRMS: Calculated  $\text{C}_{17}\text{H}_{28}\text{NO}_6$  342.1911, found 342.1916.



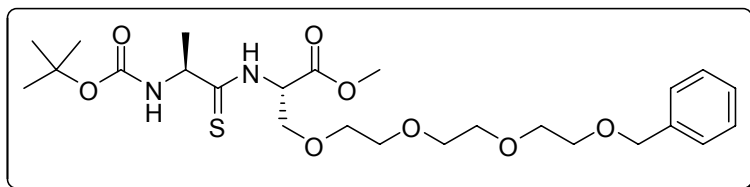
**(S)-3-{2-[2-(2-Benzyloxy-ethoxy)-ethoxy]-ethoxy}-2-((S)-2-*tert*-butoxycarbonylamino-propionylamino)-propionic acid methyl ester, 46.**



A solution of *N*- $\alpha$ -*tert*-butoxycarbonyl-L-alanine (256 mg; 1.4 mmol) and **45** (353 mg; 1.0 mmol) in 5 mL DMF was cooled to  $-10$  °C. Diphenylphosphoryl azide (0.3 mL; 1.4 mmol) was added and the solution stirred at  $-10$  °C for 10 minutes. TEA (0.4 mL; 2.8 mmol) was then added. The solution was stirred at  $< 0$  °C for one hour then warmed to room temperature and stirred for 24 hours. The DMF was removed under high vacuum and the residue purified by flash column chromatography (4:1 DCM:Et<sub>2</sub>O, 98:2 CHCl<sub>3</sub>:MeOH  $\rightarrow$  95:5 CHCl<sub>3</sub>:MeOH) to give **46** as a yellow oil (Yield = 500 mg; 95%).

R<sub>f</sub> (95:5 CHCl<sub>3</sub>:MeOH) 0.36.  $[\alpha]_D^{26}$  (CHCl<sub>3</sub>; c = 0.46): +5.65.  $u_{max}$  (Thin film, cm<sup>-1</sup>): 3337, 2973, 2872, 1747, 1712, 1680, 1517, 1493, 1453, 1365, 1289, 1247, 1210, 1167, 1141, 1108, 969, 916, 854, 812, 733, 709.  $\delta_H$  (300 MHz, CDCl<sub>3</sub>): 7.37-7.28 (5H, m), 6.99 (1H, d, *J* = 8.1), 5.25 (1H, br s), 4.69 (1H, ddd, *J* = 8.1, 4.5 & 3.5), 4.55 (2H, s), 3.94 (1H, dd, *J* = 3.4 & 7.1), 3.79-3.57 (17H, m), 1.44 (9H, s), 1.36 (3H, d, *J* = 7.1).  $\delta_C$  (75 MHz, CDCl<sub>3</sub>): 173.0, 170.7, 155.5, 138.5, 129.1, 128.6, 128.1, 126.4, 80.1, 73.5, 71.6, 71.2, 70.9, 70.8, 69.8, 66.8, 53.3, 53.0, 52.8, 28.6, 19.0. MS: C<sub>25</sub>H<sub>40</sub>N<sub>2</sub>O<sub>9</sub> *m/z* (ES<sup>+</sup>) 535.4 [M+Na<sup>+</sup>]. HRMS: Calculated C<sub>25</sub>H<sub>40</sub>N<sub>2</sub>O<sub>9</sub>Na: 535.2626, found 535.2625.

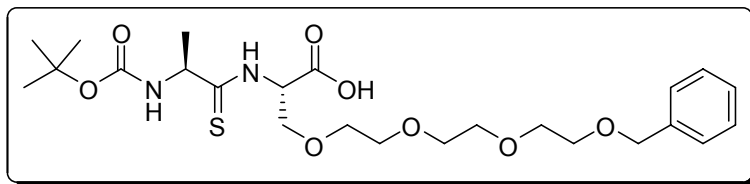
**(S)-3-{2-[2-(2-Benzyloxy-ethoxy)-ethoxy]-ethoxy}-2-((S)-2-*tert*-butoxycarbonylamino-thiopropionylamino)-propionic acid methyl ester, 47.**



A suspension of **46** (500 mg; 1.0 mmol) and Lawesson's reagent (207 mg; 0.5 mmol) in 8 mL dry toluene was heated to reflux. The resulting yellow solution was refluxed for 3 hours. After cooling to room temperature (30 minutes), the toluene was removed under high vacuum and the residue purified by flash column chromatography (9:1 DCM:Et<sub>2</sub>O  $\rightarrow$  95:5 CHCl<sub>3</sub>:MeOH) to give **47** as a yellow oil (Yield = 360 mg; 70%).

R<sub>f</sub> (95:5 CHCl<sub>3</sub>:MeOH) 0.49.  $[\alpha]_D^{26}$  (CHCl<sub>3</sub>; c = 0.77): +20.73.  $u_{max}$  (Thin film, cm<sup>-1</sup>): 3347, 3340, 2390, 1746, 1699, 1693, 1514, 1503, 1494, 1453, 1366, 1245, 1164, 1104, 1048, 880.  $\delta_H$  (300 MHz, CDCl<sub>3</sub>): 8.84 (1H, d, *J* = 8.5), 7.36-7.21 (5H, m), 5.54 (1H, br s), 5.30-5.27 (1H, m), 4.60-4.40 (3H, m), 4.03 (1H, dd, *J* = 10.2 & 3.0), 3.88 (1H, dd, *J* = 10.2 & 3.1), 3.77 (3H, s), 3.74-3.56 (12H, m), 1.56-1.28 (12H, m).  $\delta_C$  (75 MHz, CDCl<sub>3</sub>): 206.3, 169.7, 155.2, 138.4, 129.0, 128.6, 128.2, 128.0, 127.9, 80.3, 73.5, 71.4, 71.3, 70.8, 70.6, 69.7, 58.4, 57.0, 53.0, 28.6, 22.2. MS: C<sub>25</sub>H<sub>40</sub>N<sub>2</sub>O<sub>8</sub>S *m/z* (ES<sup>+</sup>) 551.5 [M+Na<sup>+</sup>]. HRMS: Calculated C<sub>25</sub>H<sub>40</sub>N<sub>2</sub>O<sub>8</sub>SNa 551.2398, found 551.2390.

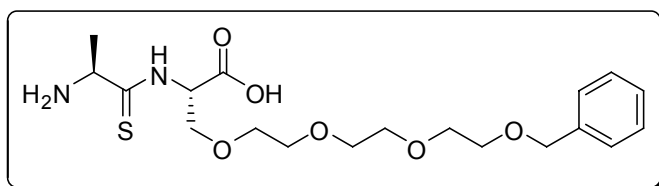
**(S)-3-{2-[2-(2-Benzyloxy-ethoxy)-ethoxy]-ethoxy}-2-((S)-2-tert-butoxycarbonylamino-thiopropionylamino)-propionic acid, 61.**



A solution of **47** (290 mg; 0.6 mmol) and LiOH.H<sub>2</sub>O (27 mg; 0.6 mmol) in 10 mL 3:1 THF:H<sub>2</sub>O was stirred at room temperature for 22 hours. 10 mL EtOAc was added and the organic layer was washed with two 5 mL volumes of 2M HCl. The aqueous layer was back extracted with three 10 mL volumes of EtOAc and the combined yellow organic layer dried over MgSO<sub>4</sub>, filtered and concentrated to give **61** as a yellow oil (Yield = 272 mg; 96%).

$[\alpha]_D^{26}$  (CHCl<sub>3</sub>; c = 0.41): +28.40.  $u_{max}$  (Thin film, cm<sup>-1</sup>): 3334, 2920, 2871, 1705, 1514, 1496, 1366, 1247, 1163, 1111, 915, 857, 734.  $\delta_H$  (300 MHz, CDCl<sub>3</sub>): 8.77 (1H, d,  $J$  = 7.3), 7.42-7.24 (5H, m), 5.51 (1H, br s), 5.28-5.25 (1H, m), 4.63-4.48 (3H, m), 4.03 (1H, dd,  $J$  = 10.1 & 2.7), 3.92 (1H, dd,  $J$  = 10.1 & 3.3), 3.54-3.75 (12H, m), 1.44 (9H, s), 1.38 (1H, d,  $J$  = 8.5)\*, 1.25 (2H, d,  $J$  = 8.5).  $\delta_C$  (75 MHz, CDCl<sub>3</sub>): 206.3, 170.1, 137.6, 128.7, 128.6, 128.2, 128.0, 127.9, 80.3, 73.4, 70.9, 70.7, 70.6, 70.4, 69.8, 69.7, 69.3, 62.3, 58.0, 28.6, 22.3. MS: C<sub>24</sub>H<sub>38</sub>N<sub>2</sub>O<sub>8</sub>S  $m/z$  (ES<sup>+</sup>) 537.4 [M+Na<sup>+</sup>]. HRMS: Calculated C<sub>24</sub>H<sub>38</sub>N<sub>2</sub>O<sub>8</sub>SNa 537.2241, found 537.2239. \* minor rotamer.

**(S)-2-((S)-2-Amino-thiopropionylamino)-3-{2-[2-(2-benzyloxy-ethoxy)-ethoxy]-ethoxy}-propionic acid, 4.**

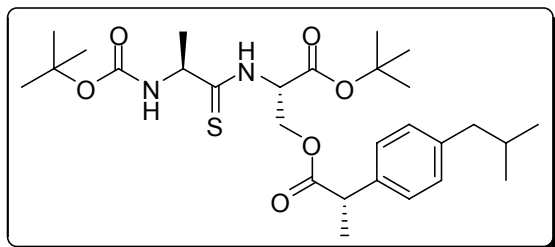


Deprotection of 275 mg of **61** as described in Section 1.1. Yield of **4** as TFA salt = 273 mg; 99% (yellow oil).

HPLC R<sub>T</sub>: 3.2 (30%).  $\delta_H$  (300 MHz, CD<sub>3</sub>OD): 7.38-7.24 (5H, m), 5.24 (0.67H, dd,  $J$  = 5.0 & 3.4), 4.63 (0.33H, dd,  $J$  = 5.0 & 3.4)\*, 4.55 (2H, s), 4.53-4.47 (0.33H, m)\*, 4.35-4.25 (0.67H, m), 4.05-3.83 (2H, m), 3.79-3.56 (12H, m), 1.56-1.48 (2H, m), 1.40-1.36 (1H, m)\*.  $\delta_C$  (75 MHz, CD<sub>3</sub>OD): 200.3, 170.0, 138.2, 128.3, 127.8, 72.5, 70.3, 69.0, 53.2, 52.5, 19.8. MS: C<sub>19</sub>H<sub>30</sub>N<sub>2</sub>O<sub>6</sub>S  $m/z$  (ES<sup>+</sup>) 415.3 [M+H<sup>+</sup>]. HRMS: Calculated C<sub>19</sub>H<sub>31</sub>N<sub>2</sub>O<sub>6</sub>S 415.1897, found 415.1895. \* minor rotamer.

### 3.5 Synthesis of thiodipeptide ibuprofen prodrugs **48-53**

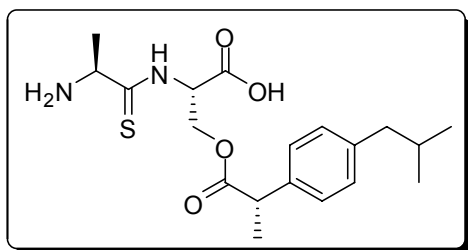
#### (S)-2-((S)-2-tert-Butoxycarbonylamino-thiopropionylamino)-3-[(S)-2-(4-isobutyl-phenyl)-propionyloxy]-propionic acid tert-butyl ester, **62**.



Ibuprofen (136 mg; 0.7 mmol) and HBTU (305 mg; 0.8 mmol) were dissolved in 3 mL DMF. DIPEA (0.12 mL; 0.7 mmol) was added and the solution stirred at room temperature for 30 minutes. A yellow solution of protected serine thiodipeptide **27** (134 mg; 0.4 mmol) in 2 mL DMF was then added and the reaction stirred at room temperature for three days. The DMF was removed and the residue purified by flash column chromatography (DCM → 9:1 DCM:Et<sub>2</sub>O) to give **62** as a yellow oil (Yield = 193 mg; 95%).

R<sub>f</sub> (95:5 DCM:Et<sub>2</sub>O): 0.55. [α]<sub>D</sub><sup>26</sup> (CHCl<sub>3</sub>; c = 2.02): +5.10. u<sub>max</sub> (Thin film, cm<sup>-1</sup>): 3338, 2978, 2933, 2870, 1739, 1513, 1455, 1394, 1368, 1337, 1247, 1163, 1094, 1051, 1031, 986, 912, 846, 777, 734. δ<sub>H</sub> (300 MHz, CDCl<sub>3</sub>): 8.28-8.19 (1H, m), 7.23-7.06 (4H, m), 5.25-4.85 (2H, m), 4.70-4.30 (3H, m), 3.74-3.60 (1H, m), 2.49-2.44 (2H, m), 1.89-1.78 (1H, m), 1.55-1.20 (24H, m), 0.91-0.86 (6H, m). δ<sub>C</sub> (75 MHz, CDCl<sub>3</sub>): 205.7\*, 205.5, 174.5\*, 174.4, 167.6\*, 167.5, 155.3\*, 155.2, 141.2\*, 141.1, 137.7\*, 137.4, 129.9\*, 129.8, 127.7, 127.5, 83.8, 80.1, 63.4, 63.2\*, 57.6, 57.5\*, 45.4, 45.3\*, 45.2, 30.6, 28.7, 28.1, 22.8, 22.2\*, 22.0, 18.8\*, 18.3. MS: C<sub>28</sub>H<sub>44</sub>N<sub>2</sub>O<sub>6</sub>S m/z (ES<sup>+</sup>) 559.0 [M+Na<sup>+</sup>]. HRMS: Calculated C<sub>28</sub>H<sub>44</sub>N<sub>2</sub>O<sub>6</sub>SNa 559.2813, found 559.2812. \* minor rotamer.

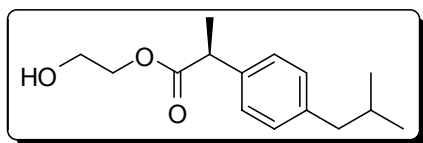
#### (S)-2-((S)-2-Amino-thiopropionylamino)-3-[(S)-2-(4-isobutyl-phenyl) propionyloxy]-propionic acid, **48**.



Deprotection of 223 mg of **62** as described in Section 5.1.3. Yield of **48** as TFA salt = 177 mg; 86%.

HPLC R<sub>T</sub>: 7.2 (50%). [α]<sub>D</sub><sup>29</sup> (CH<sub>3</sub>OH; c = 0.14): -29.90. u<sub>max</sub> (Thin film, cm<sup>-1</sup>): 3213, 2956, 2867, 1725, 1710, 1688, 1670, 1641, 1631, 1462, 1441, 1383, 1204, 1139, 1069, 836, 797, 719. δ<sub>H</sub> (400 MHz, CD<sub>3</sub>OD): 7.23-7.17 (2H, m), 7.15-7.09 (2H, m), 5.50-5.13 (1H, m), 4.75-4.25 (3H, m), 3.80-3.65 (1H, m), 2.49-2.44 (2H, m), 1.93-1.80 (1H, m), 1.60-1.44 (6H, m), 0.92 (6H, d, J = 6.0). δ<sub>C</sub> (100 MHz, CD<sub>3</sub>OD): 203.3, 176.,3 170.9, 142.5, 139.6, 131.0, 128.9, 64.7, 59.3, 55.6, 46.7, 32.1, 28.8, 23.4, 21.5, 19.9. MS: C<sub>19</sub>H<sub>28</sub>N<sub>2</sub>O<sub>4</sub>S m/z (ES<sup>+</sup>) 381.1 [M+H<sup>+</sup>]. HRMS: Calculated C<sub>19</sub>H<sub>29</sub>N<sub>2</sub>O<sub>4</sub>S 381.1843, found 381.1842.

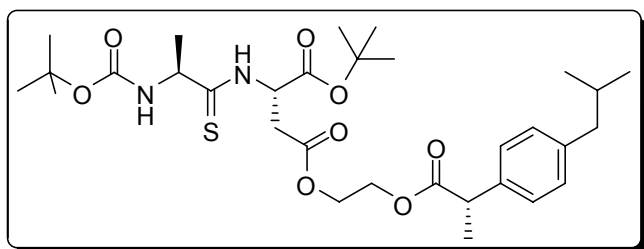
**(S)-2-(4-Isobutyl-phenyl)-propionic acid 2-hydroxy-ethyl ester, 63.**



Ibuprofen (0.1525 g, 0.74 mmol), EDC.HCl (0.17 g, 0.86 mmol) and DMAP (0.1084 g, 0.86 mmol) were dissolved in CH<sub>2</sub>Cl<sub>2</sub> (7 mL) and the mixture stirred at room temperature for 20 mins. Ethylene glycol (0.1836 g, 2.96 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and added slowly to the activated acid solution, the reaction mixture was stirred at room temperature for 2 days. The solvent was removed *in vacuo*. The residue was taken up in EtOAc (10 mL) and washed with saturated aqueous NaHCO<sub>3</sub>, 0.6 M citric acid and saturated aqueous NaCl. The organic phase was dried (MgSO<sub>4</sub>), filtered and the solvent removed *in vacuo*. The residue was purified by flash column chromatography (CHCl<sub>3</sub>:MeOH, 49:1) to yield **63** as a colourless oil (0.1792 g, 97%).

R<sub>f</sub> (CHCl<sub>3</sub>): 0.26. δ<sub>H</sub> (CDCl<sub>3</sub>, 300 MHz) 7.18 (4H, d, *J* = 7.9), 4.23 (2H, t, *J* = 4.5), 3.81-3.63 (3H, m), 2.45 (2H, d, *J* = 7.2), 1.85 (1H, septet, *J* = 6.8), 1.70 (1H, br s), 1.50 (3H, d, *J* = 7.2), 0.91 (6H, d, *J* = 6.8).

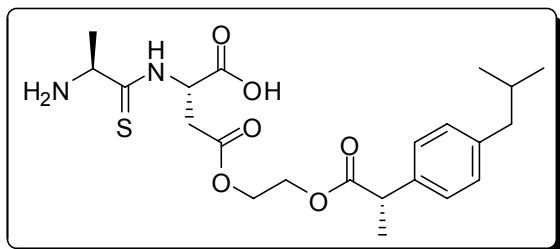
**(S)-2-((S)-2-tert-Butoxycarbonylamino-thiopropionylamino)-succinic acid 1-tert-butyl ester 4-{2-[(S)-2-(4-isobutyl-phenyl)-propionyloxy]-ethyl} ester, 64**



**63** (0.23 mmol) and **28** (0.21 mmol) were coupled as described for **63** over 3 days to give **64** as a colourless oil (62% yield).

R<sub>f</sub> (2:1 hexane:EtOAc): 0.33. [α]<sub>D</sub><sup>26</sup> (CHCl<sub>3</sub>; *c* = 0.82): +34.6. *u*<sub>max</sub> (Thin film, cm<sup>-1</sup>): 3324, 2974, 2934, 1735, 1506, 1451, 1360, 1247, 1163, 1047, 842, 777. δ<sub>H</sub> (CDCl<sub>3</sub>, 400 MHz): 8.72-8.59 (1H, m), 7.20 (4H, AB, *J* = 8.0), 5.48 (1H, br s), 5.32-5.18 (1H, m), 4.54-4.47 (1H, m), 4.40-4.18 (4H, m), 3.78 (1H, q, *J* = 7.0), 3.08 (2H, ABX, *J* = 17.1 & 4.0), 2.45 (2H, d, *J* = 7.0), 1.83 (1H, septet, *J* = 6.5), 1.55-1.47 (21H, m), 1.52 (3H, d, *J* = 7.0 Hz), 0.91 (6H, d, *J* = 6.2). δ<sub>C</sub> (CDCl<sub>3</sub>, 75 MHz): 205.8, 175.1, 170.8, 168.7, 155.3, 141.1, 137.7, 129.7, 127.5, 83.6, 80.5, 63.2, 62.4, 57.2, 54.3, 45.4, 45.3, 35.1, 30.6, 28.7, 28.2, 22.8, 22.3, 22.1, 18.8. MS: C<sub>31</sub>H<sub>48</sub>N<sub>2</sub>O<sub>8</sub>S *m/z* (ES<sup>+</sup>) 630.8 [M+Na]<sup>+</sup>. HRMS: Calculated for C<sub>31</sub>H<sub>48</sub>N<sub>2</sub>NaO<sub>8</sub>S 631.3024, found 631.3032.

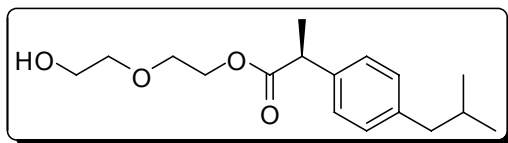
**(S)-2-((S)-2-Amino-thiopropionylamino)-succinic acid 4-{2-[(S)-2-(4-isobutyl-phenyl)-propionyloxy]-ethyl} ester, **49**.**



Deprotection of 75 mg **64** as described in Section 1.1. Yield of **49** as a TFA salt = 44 mg (63%).

$R_f$  (17:2:1  $\text{CHCl}_3$ :MeOH: $\text{CH}_3\text{COOH}$ ): 0.37.  $[\alpha]_D^{26}$  ( $\text{CH}_3\text{OH}$ ;  $c = 0.88$ ): +40.3.  $\nu_{\text{max}}$  (Thin film,  $\text{cm}^{-1}$ ): 3206, 2958, 2595, 1749, 1687, 1523, 1460, 1403, 1208, 1135, 1080, 879, 861.  $\delta_{\text{H}}$  ( $\text{CD}_3\text{OD}$ , 300 MHz): 7.18 (4H, AB,  $J = 7.9$ ), 5.32 (1H, t,  $J = 5.7$ ), 4.38-4.18 (5H, m), 3.78 (1H, q,  $J = 7.2$ ), 3.07-2.90 (2H, m), 2.45 (2H, d,  $J = 7.2$ ), 1.85 (1H, septet,  $J = 6.8$ ), 1.57 (3H, d,  $J = 6.8$ ), 1.47 (3H, d,  $J = 7.2$ ), 0.91 (6H, d,  $J = 6.4$ ).  $\delta_{\text{C}}$  ( $\text{CD}_3\text{OD}$ , 75 MHz): 202.1, 176.7, 172.0, 142.2, 139.6, 130.8, 128.7, 64.2, 63.9, 56.2, 55.4, 46.6, 46.4, 36.2, 31.8, 23.1, 23.0, 21.0, 19.3. MS:  $\text{C}_{22}\text{H}_{32}\text{N}_2\text{O}_6\text{S}$   $m/z$  ( $\text{ES}^+$ ) 453.2  $[\text{M}+\text{H}]^+$ . HRMS: Calculated for  $\text{C}_{22}\text{H}_{33}\text{N}_2\text{O}_6\text{S}$  453.2054, found 453.2064.

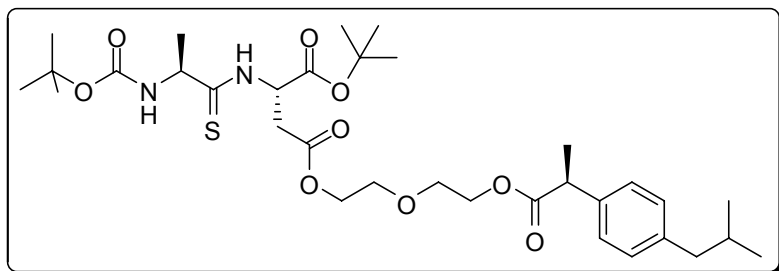
**(S)-2-(4-Isobutyl-phenyl)-propionic acid 2-(2-hydroxy-ethoxy)-ethyl ester, **65**.**



Ibuprofen (0.66 mmol) and diethylene glycol (2.64 mmol) were coupled as described for **63** over 3 days to give **65** as a colourless oil (97% yield).

$R_f$  ( $\text{CHCl}_3$ ): 0.25.  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ , 300 MHz): 7.18 (4H, AB  $J = 7.9$ ), 4.28-4.23 (2H, m), 3.73 (1H, q,  $J = 7.2$ ), 3.69-3.63 (4H, m), 3.48-3.40 (2H, m), 2.47 (2H, d,  $J = 7.2$ ), 1.92-1.81 (2H, m), 1.49 (3H, d,  $J = 7.2$ ), 0.90 (6H, d,  $J = 6.4$ ).

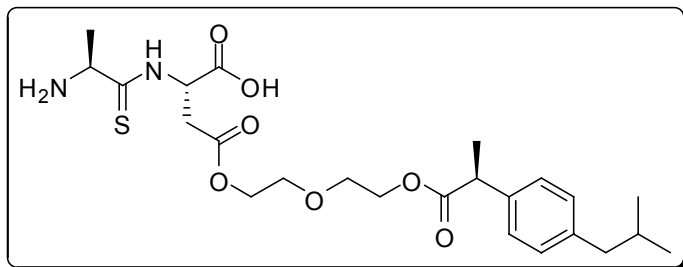
**(S)-2-((S)-2-tert-Butoxycarbonylamino-thiopropionylamino)-succinic acid 1-tert-butyl ester 4-(2-{2-[(S)-2-(4-isobutyl-phenyl)-propionyloxy]-ethoxy}-ethyl) ester, **66**.**



**65** (0.23 mmol) and **28** (0.21 mmol) were coupled as described for **63** over 3 days to give **66** as a colourless oil (59% yield).

$R_f$  (3:2 hexane:EtOAc): 0.36.  $[\alpha]_D^{25}$  ( $\text{CHCl}_3$ ;  $c = 0.46$ ): +46.9.  $u_{\text{max}}$  (Thin film,  $\text{cm}^{-1}$ ): 3334, 2976, 2953, 2927, 1731, 1510, 1454, 1368, 1245, 1159, 1051, 849.  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ , 300 MHz): 8.65-8.58 (1H, m), 7.15 (4H, AB,  $J = 7.9$ ), 5.39 (1H, br s), 5.21-5.12 (1H, m), 4.44-4.38 (1H, m), 4.28-4.16 (4H, m), 3.71 (1H, q,  $J = 7.2$ ), 3.61-3.48 (4H, m), 3.08 (2H, ABX,  $J = 17.0$  & 4.5), 2.44 (2H, d,  $J = 7.2$ ), 1.82 (1H, septet  $J = 6.8$ ), 1.55-1.42 (24H, m), 0.89 (6H, d,  $J = 6.4$ ).  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ , 75 MHz): 205.6, 175.0, 170.9, 168.8, 155.3, 141.0, 138.0, 129.7, 127.6, 83.5, 80.6, 69.4, 69.1, 64.2, 64.1, 57.3, 54.4, 45.4, 45.3, 35.2, 30.6, 28.7, 28.2, 22.7, 22.6, 22.4, 18.9. MS:  $\text{C}_{33}\text{H}_{52}\text{N}_2\text{O}_9$   $m/z$  ( $\text{ES}^+$ ) 674.8  $[\text{M}+\text{Na}]^+$ .

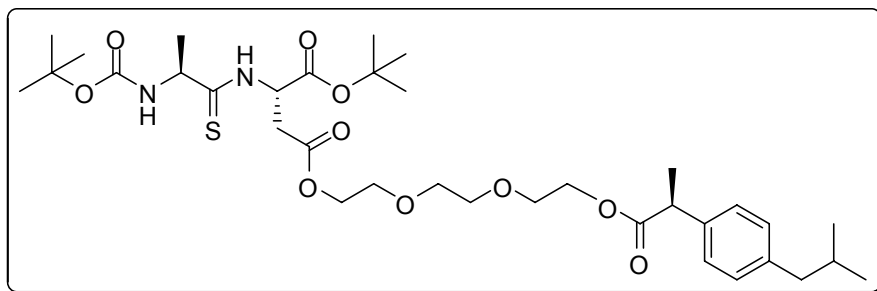
**(S)-2-((S)-2-Amino-thiopropionylamino)-succinic acid 4-(2-{2-[(S)-2-(4-isobutyl-phenyl)-propionyloxy]-ethoxy}-ethyl) ester, **50**.**



Deprotection of 45 mg **66** as described in Section 1.1. Yield of **50** as a TFA salt = 30 mg (72%).

$R_f$  (17:2:1  $\text{CHCl}_3$ :MeOH: $\text{CH}_3\text{COOH}$ ): 0.34.  $[\alpha]_D^{25}$  ( $\text{CH}_3\text{OH}$ ;  $c = 0.45$ ): +51.5.  $u_{\text{max}}$  (Thin film,  $\text{cm}^{-1}$ ): 3200, 2966, 2870, 1750, 1691, 1546, 1481, 1401, 1225, 1187, 1137, 1088, 899, 859, 790.  $\delta_{\text{H}}$  ( $\text{CD}_3\text{OD}$ , 300 MHz): 7.16 (4H, AB,  $J = 7.9$ ), 5.32-5.15 (1H, m), 4.33-4.09 (5H, m), 3.78 (1H, q,  $J = 6.8$ ), 3.68-3.52 (4H, m), 3.03 (2H, ABX,  $J = 16.6$  & 6.4), 2.45 (2H, d,  $J = 7.2$ ), 1.85 (1H, septet,  $J = 6.8$ ), 1.58 (3H, d,  $J = 6.4$  Hz), 1.45 (3H, d,  $J = 7.2$  Hz), 0.90 (6H, d,  $J = 6.8$  Hz).  $\delta_{\text{C}}$  ( $\text{CD}_3\text{OD}$ , 75 MHz): 201.4, 176.7, 174.1, 172.5, 142.2, 139.7, 130.8, 128.7, 70.4, 70.3, 65.4, 65.3, 57.2, 55.5, 46.7, 46.4, 36.6, 31.9, 23.1, 23.0, 21.0, 19.4. MS:  $\text{C}_{24}\text{H}_{36}\text{N}_2\text{O}_7\text{S}$   $m/z$  ( $\text{ES}^+$ ) 497.2  $[\text{M}+\text{H}]^+$ . HRMS: Calculated for  $\text{C}_{24}\text{H}_{37}\text{N}_2\text{O}_7\text{S}$  497.2316, found 497.2320

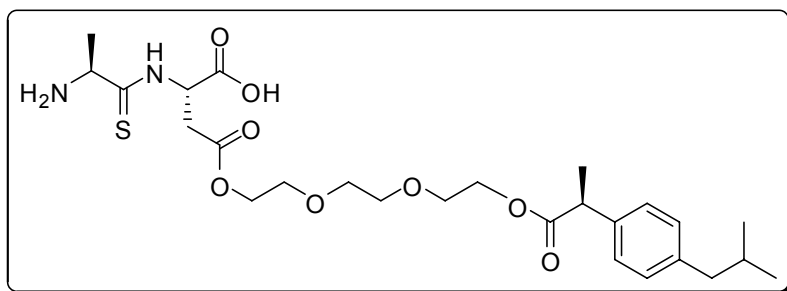
**(S)-2-((S)-2-tert-Butoxycarbonylamino-thiopropionylamino)-succinic acid 1-tert-butyl ester 4-[2-(2-{2-[(S)-2-(4-isobutyl-phenyl)-propionyloxy]-ethoxy}-ethoxy)-ethyl] ester, 67.**



Ibuprofen (0.1826 g, 0.89 mmol), EDC.HCl (0.1706 g, 0.89 mmol) and DMAP (0.1087 g, 0.89 mmol) were dissolved in CH<sub>2</sub>Cl<sub>2</sub> (7 mL) and the mixture stirred at room temperature for 20 mins. Ethylene glycol (0.1136 g, 0.89 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and added slowly to the activated acid solution, the reaction mixture was stirred at room temperature for 5 days. The solvent was removed *in vacuo*. The residue was taken up in EtOAc (10 mL) and washed with saturated aqueous NaHCO<sub>3</sub>, 0.6 M citric acid and saturated aqueous NaCl. The organic phase was dried (MgSO<sub>4</sub>), filtered and the solvent removed *in vacuo*. The residue was purified by flash column chromatography (9:1 DCM:Et<sub>2</sub>O → MeOH) to yield crude monoester in 65% yield. This was coupled to **29** as described for **63** over 4 days to give **67** as a colourless oil in 80% yield.

R<sub>f</sub> (2:1 hexane:EtOAc): 0.36. [α]<sub>D</sub><sup>25</sup> (CHCl<sub>3</sub>; c = 0.96): +20.7. u<sub>max</sub> (Thin film, cm<sup>-1</sup>): 2981, 2955, 2928, 1730, 1510, 1455, 1370, 1245, 1158, 1055, 890, 849. δ<sub>H</sub> (CDCl<sub>3</sub>, 400 MHz): 8.77-8.65 (1H, m), 7.18 (4H, AB, J = 8.0), 5.48 (1H, br s), 5.32-5.24 (1H, m), 4.55-4.48 (1H, m), 4.31-4.20 (4H, m), 3.75 (1H, q, J = 7.0), 3.72-3.58 (8H, m), 3.12 (2H, ABX, J = 17.1 & 3.5), 2.46 (2H, d, J = 7.5), 1.90-1.81 (1H, m), 1.52-1.43 (24H, m), 0.90 (6H, d, J = 6.5). δ<sub>C</sub> (CDCl<sub>3</sub>, 100 MHz): 205.6, 175.1, 170.9, 168.8, 155.4, 140.9, 138.0, 129.7, 127.6, 83.5, 80.5, 70.9, 70.8, 69.5, 69.3, 64.4, 64.2, 57.3, 54.4, 45.4, 45.3, 35.2, 30.6, 28.7, 28.3, 22.8, 22.7, 22.4, 19.0. MS: C<sub>35</sub>H<sub>56</sub>N<sub>2</sub>O<sub>10</sub>S m/z (ES<sup>+</sup>) 719.2 [M+Na]<sup>+</sup>. HRMS: Calculated for C<sub>35</sub>H<sub>56</sub>N<sub>2</sub>NaO<sub>10</sub>S 719.3548, found 719.3548.

**(S)-2-((S)-2-Amino-thiopropionylamino)-succinic acid 4-[2-(2-{2-[(S)-2-(4-isobutyl-phenyl)-propionyloxy]-ethoxy}-ethoxy)-ethyl] ester, 51**

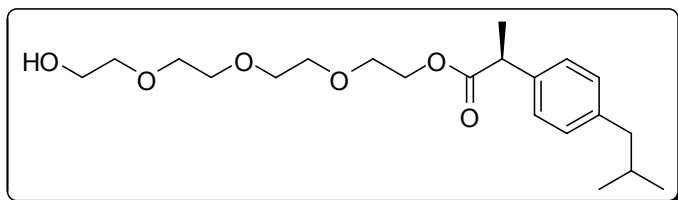


Deprotection of 96 mg **67** as described in Section 1.1. Yield of **51** as a TFA salt = 84 mg (93%).

R<sub>f</sub> (1:1:1:1 EtOAc:BuOH:H<sub>2</sub>O:AcOH): 0.60. [α]<sub>D</sub><sup>25</sup> (CH<sub>3</sub>OH; c = 1.02): +23.8. u<sub>max</sub> (Thin film, cm<sup>-1</sup>): 3225, 2958, 2868, 2603, 1750, 1691, 1534, 1454, 1410, 1366, 1214, 1155, 904, 855, 793. δ<sub>H</sub> (CD<sub>3</sub>OD, 400 MHz): 7.16 (4H, AB, J = 8.0), 5.40 (1H, t, J = 6.5), 4.31-4.21 (5H, m), 3.78 (1H, q, J = 7.0), 3.71-3.63 (4H, m), 3.59-3.52 (4H, m), 3.03 (2H, ABX, J = 17.0 & 6.5), 2.47 (2H, d, J = 7.0), 1.86 (1H, septet, J = 7.0), 1.59 (3H, d, J = 7.0), 1.48 (3H, d, J = 7.0), 0.92 (6H, d, J = 6.5). δ<sub>C</sub> (CD<sub>3</sub>OD, 75 MHz): 201.3, 176.7, 172.2, 171.8, 141.2, 138.2, 129.4, 127.5, 70.1, 68.8, 68.7, 68.6,

64.7, 64.4, 56.2, 54.2, 45.0, 44.9, 34.8, 30.2, 22.4, 22.3, 20.0, 18.5. MS: C<sub>26</sub>H<sub>40</sub>N<sub>2</sub>O<sub>8</sub>S *m/z* (ES<sup>+</sup>) 541.1 [M+H]<sup>+</sup>. HRMS: Calculated for C<sub>26</sub>H<sub>41</sub>N<sub>2</sub>O<sub>8</sub>S 541.2578, found 541.2578.

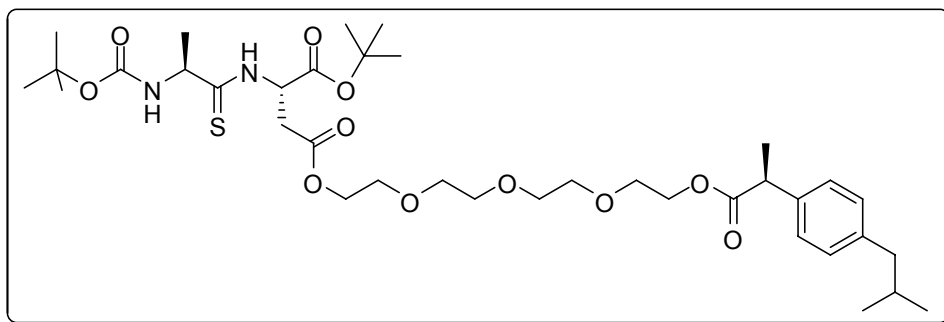
**(S)-2-(4-Isobutyl-phenyl)-propionic acid 2-{2-[2-(2-hydroxy-ethoxy)-ethoxy]-ethoxy}-ethyl ester, 68.**



Ibuprofen (0.71 mmol) and tetraethylene glycol (0.85 mmol) were coupled as described for **63** over 4 days to give **68** as a colourless oil (67% yield).

R<sub>f</sub> (CHCl<sub>3</sub>:MeOH): 0.39. δ<sub>H</sub> (CDCl<sub>3</sub>, 300 MHz): 7.15 (4H, AB, *J* = 7.9), 4.32-4.20 (2H, m), 3.80-3.25 (15H, m), 2.47 (2H, d, *J* = 7.2), 2.30 (1H, br s), 1.84 (1H, septet, *J* = 6.4), 1.51 (3H, d, *J* = 7.2), 0.90 (6H, d, *J* = 6.8).

**(S)-2-((S)-2-tert-Butoxycarbonylamino-thiopropionylamino)-succinic acid 1-tert-butyl ester 4-{2-[2-(2-{2-[(S)-2-(4-isobutyl-phenyl)-propionyloxy]-ethoxy}-ethoxy)-ethoxy]-ethyl} ester, 69.**

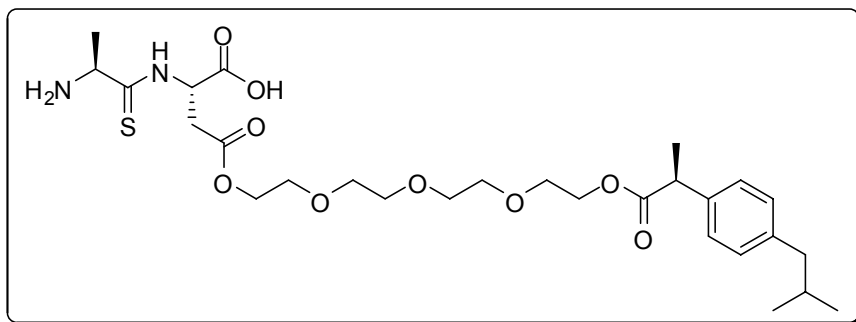


**68** (0.20 mmol) and **28** (0.24 mmol) were coupled as described for **63** over 3 days to give **69** as a colourless oil (59% yield).

R<sub>f</sub> (2:1 hexane:EtOAc): 0.31. [α]<sub>D</sub><sup>25</sup> (CHCl<sub>3</sub>; *c* = 0.58): +30.7. *u*<sub>max</sub> (Thin film, cm<sup>-1</sup>): 3338, 2975, 2927, 2868, 1727, 1514, 1451, 1370, 1253, 1168, 1044, 845; δ<sub>H</sub> (CDCl<sub>3</sub>, 400 MHz): 8.76-8.69 (1H, m), 7.15 (4H, AB, *J* = 8.0), 5.49 (1H, br s), 5.26-5.20 (1H, m), 4.52-4.43 (1H, m), 4.31-4.18 (4H, m), 3.78-3.55 (13H, m), 3.13 (2H, ABX, *J* = 17.6 & 4.0), 2.43 (2H, d, *J* = 7.0), 1.85 (1H, septet, *J* = 6.5), 1.52-1.41 (24H, m), 0.91 (6H, d, *J* = 6.5). δ<sub>C</sub> (CDCl<sub>3</sub>, 75 MHz): 205.6, 175.1, 168.8, 155.3, 140.9, 138.0, 129.7, 127.6, 83.5, 80.5, 71.0-64.2, 57.2, 54.4, 45.4 45.3, 35.2, 30.6, 28.7, 28.2, 22.8, 22.7, 22.4, 19.0. MS C<sub>37</sub>H<sub>60</sub>N<sub>2</sub>O<sub>11</sub>S *m/z* (ES<sup>+</sup>) 763.8 [M+Na]<sup>+</sup>.



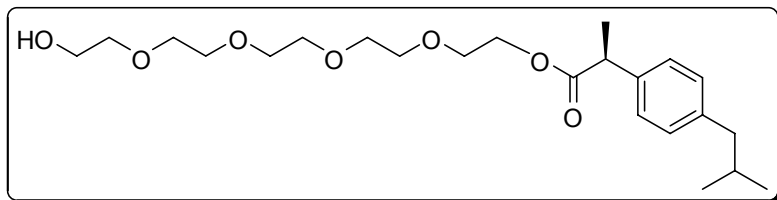
**(S)-2-((S)-2-Amino-thiopropionylamino)-succinic acid 4-{2-[2-(2-{2-[(S)-2-(4-isobutyl-phenyl)-propionyloxy]-ethoxy]-ethoxy)-ethoxy]-ethyl} ester, **52**.**



Deprotection of 57 mg **69** as described in Section 1.1. Yield of **52** as a TFA salt = 44 mg (81%).

$R_f$  (CHCl<sub>3</sub>:MeOH:CH<sub>3</sub>COOH 17:2:1): 0.32.  $[\alpha]_D^{25}$  (MeOH; c = 0.50): +24.2.  $u_{max}$  (Thin film, cm<sup>-1</sup>): 3212, 1968, 1874, 1749, 1689, 1640, 1387, 1335, 1212, 1132, 960, 884, 834, 793.  $\delta_H$  (CD<sub>3</sub>OD, 300 MHz): 7.16 (4H, AB,  $J$  = 7.9), 5.38-5.24 (1H, m), 4.30-4.15 (5H, m), 3.78 (1H, q,  $J$  = 6.8), 3.72-3.50 (12H, m), 3.03 (2H, ABX,  $J$  = 17.6 & 7.2), 2.45 (2H, d,  $J$  = 7.2), 1.85 (1H, septet,  $J$  = 6.8), 1.58 (3H, d,  $J$  = 6.8), 1.45 (3H, d,  $J$  = 7.2), 0.91 (6H, d,  $J$  = 6.4).  $\delta_C$  (CD<sub>3</sub>OD, 75 MHz): 202.1, 176.7, 172.8, 172.1, 142.1, 139.7, 130.8, 128.7, 71.9-65.3, 55.5, 53.5, 46.7, 46.4, 36.5, 31.9, 23.1, 23.0, 21.0, 19.5. MS C<sub>28</sub>H<sub>44</sub>N<sub>2</sub>O<sub>9</sub>S  $m/z$  (ES<sup>+</sup>) 585.3 [M+H]<sup>+</sup>. HRMS: Calculated for C<sub>28</sub>H<sub>45</sub>N<sub>2</sub>O<sub>9</sub>S 585.2840, found 585.2848.

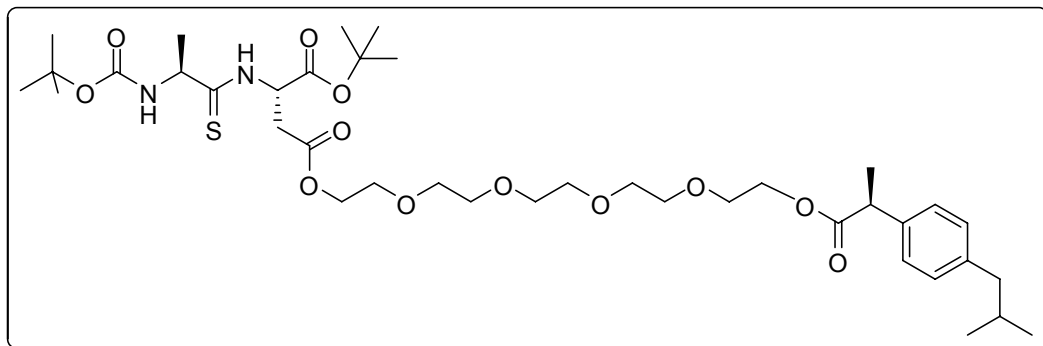
**(S)-2-(4-Isobutyl-phenyl)-propionic acid 2-(2-{2-[2-(2-hydroxy-ethoxy)-ethoxy]-ethoxy}-ethoxy)-ethyl ester, **70**.**



Ibuprofen (0.71 mmol) and pentaethylene glycol (0.85 mmol) were coupled as described for **63** over 4 days to give **70** as a colourless oil (62% yield).

$R_f$  (CHCl<sub>3</sub>:MeOH): 0.16.  $\delta_H$  (CDCl<sub>3</sub>, 300 MHz): 7.18 (4H, AB,  $J$  = 7.9), 4.32-4.05 (2H, m), 3.80-3.52 (19H, m), 2.55 (1H, br s), 2.42 (2H, d,  $J$  = 7.2), 1.85 (1H, septet,  $J$  = 6.4), 1.51 (3H, d,  $J$  = 7.2), 0.90 (6H, d,  $J$  = 6.4).

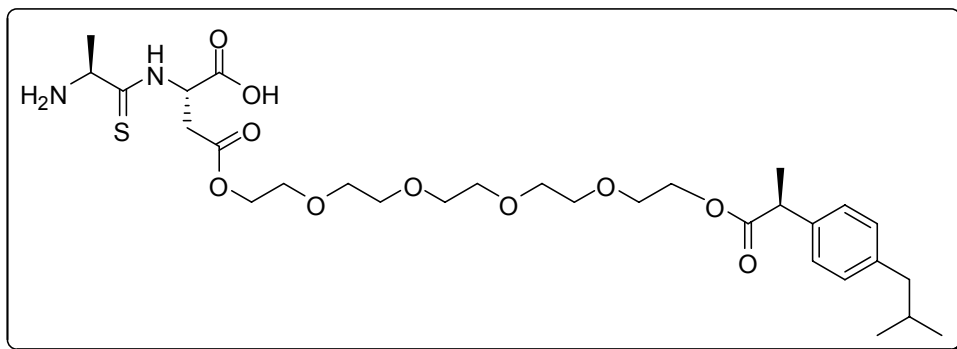
**(S)-2-((S)-2-tert-Butoxycarbonylamino-thiopropionylamino)-succinic acid 1-tert-butyl ester 4-(2-{2-[2-(2-{2-[(S)-2-(4-isobutyl-phenyl)-propionyloxy]-ethoxy}-ethoxy)-ethoxy]-ethoxy}-ethyl) ester, **71**.**



**70** (0.16 mmol) and **28** (0.19 mmol) were coupled as described for **63** over 3 days to give **71** as a colourless oil (71% yield).

$R_f$  (2:1 hexane:EtOAc): 0.15.  $[\alpha]_D^{25}$  (CHCl<sub>3</sub>;  $c = 0.87$ ): +12.6.  $u_{max}$  (Thin film, cm<sup>-1</sup>): 3347, 2976, 2950, 2865, 1734, 1507, 1455, 1363, 1248, 1166, 1044, 843, 773.  $\delta_H$  (CDCl<sub>3</sub>, 300 MHz): 8.77-8.61 (1H, m), 7.10 (4H, AB,  $J = 7.9$ ), 5.42 (1H, br s), 5.28-5.11 (1H, m), 4.49-4.38 (1H, m), 4.21-4.05 (4H, m), 3.71-3.40 (17H, m), 3.10 (2H, ABX,  $J = 17.0$  & 3.8), 2.38 (2H, d,  $J = 7.2$ ), 1.78 (1H, septet,  $J = 6.8$ ), 1.43-1.30 (24H, m), 0.85 (6H, d,  $J = 6.4$ ). (CDCl<sub>3</sub>, 75 MHz): 205.6, 175.1, 170.9, 168.8, 155.3, 140.9, 138.0, 129.7, 127.6, 83.5, 80.4, 70.9-64.2, 57.3, 54.4, 45.4, 45.3, 35.0, 30.6, 28.7, 28.2, 22.8, 22.4, 22.3, 19.0. MS C<sub>39</sub>H<sub>64</sub>N<sub>2</sub>O<sub>12</sub>S  $m/z$  (ES<sup>+</sup>) 806.8 [M+Na]<sup>+</sup>.

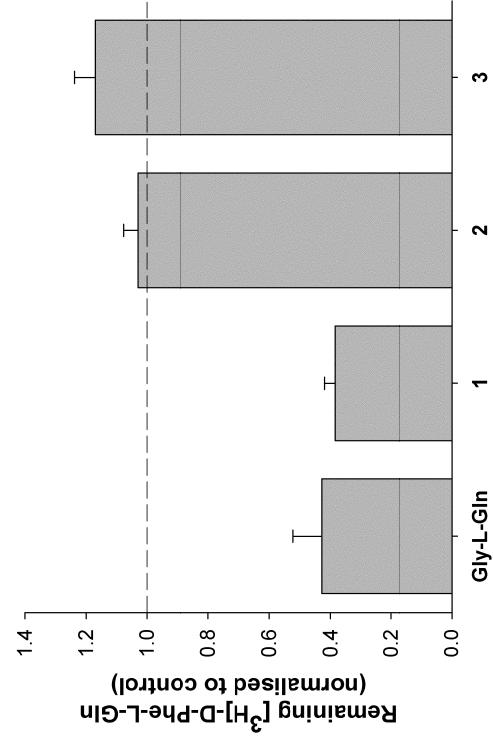
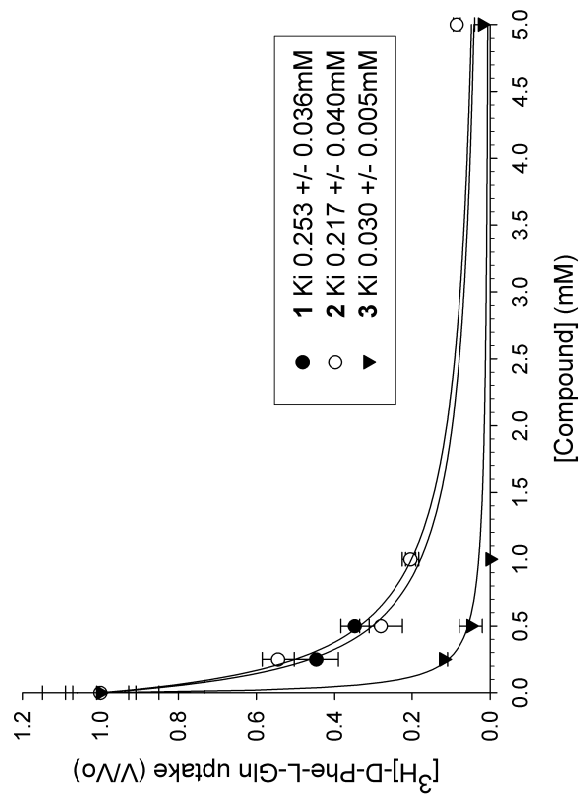
**(S)-2-((S)-2-Amino-thiopropionylamino)-succinic acid 4-(2-{2-[2-(2-{2-[(S)-2-(4-isobutyl-phenyl)-propionyloxy]-ethoxy}-ethoxy)-ethoxy]-ethoxy}-ethyl) ester, **53**.**

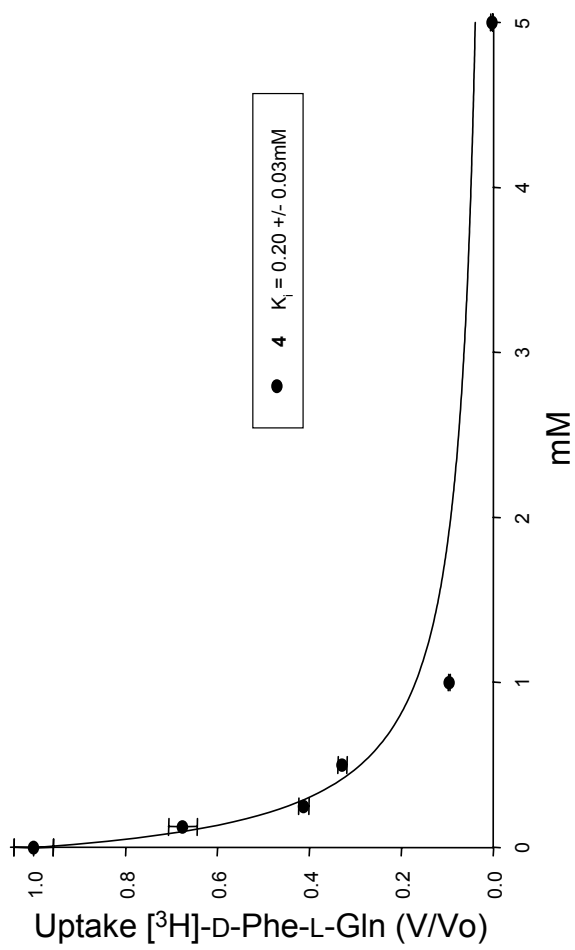
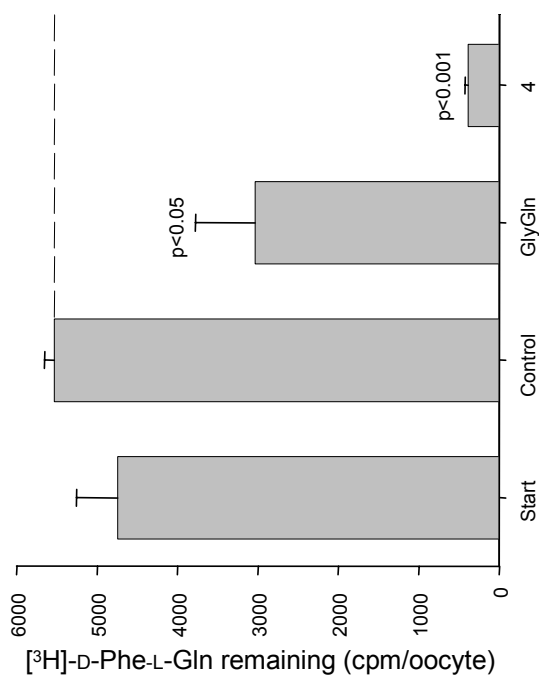


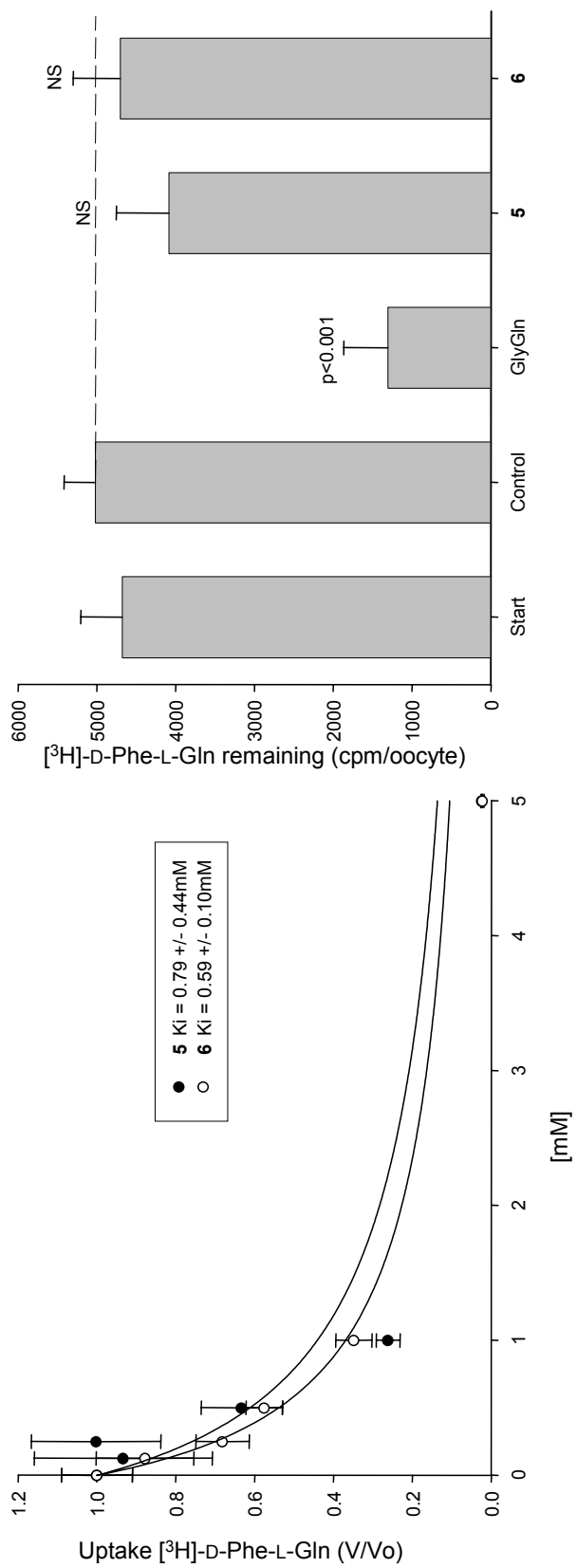
Deprotection of 87 mg **71** as described in Section 1.1. Yield of **53** as a TFA salt = 70 mg (85%).

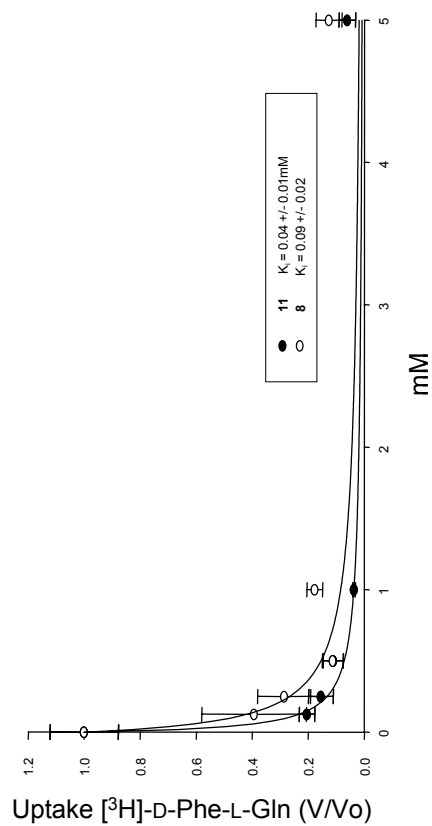
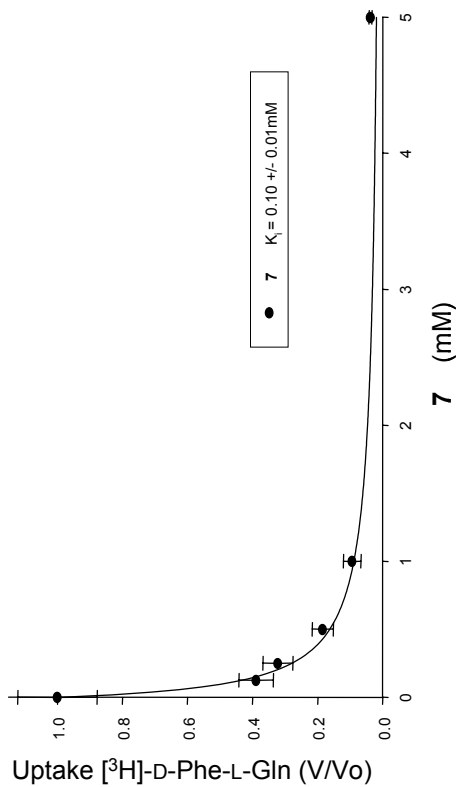
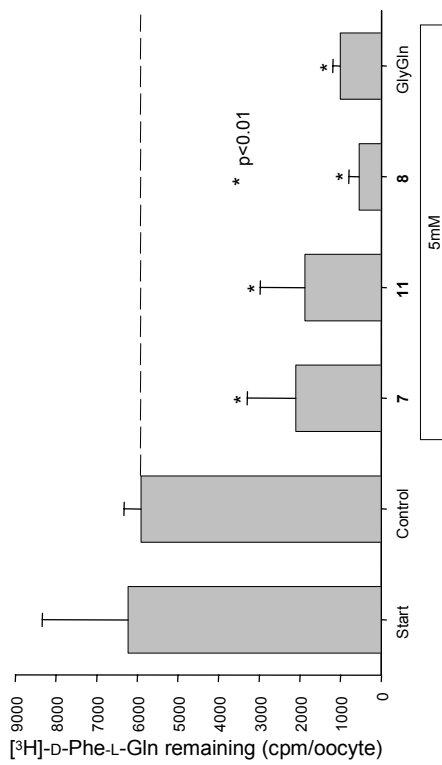
$R_f$  (CHCl<sub>3</sub>:MeOH:CH<sub>3</sub>COOH 17:2:1): 0.31.  $[\alpha]_D^{25}$  (MeOH;  $c = 0.73$ ): +18.6.  $u_{max}$  (Thin film, cm<sup>-1</sup>): 3170, 2968, 2937, 2866, 1749, 1691, 1529, 1456, 1368, 1227, 1143, 976, 884, 850, 782.  $\delta_H$  (CD<sub>3</sub>OD, 300 MHz): 7.16 (4H, AB,  $J = 7.9$ ), 5.41-5.32 (1H, m), 4.30-4.15 (5H, m), 3.83-3.50 (17H, m), 3.06 (2H, ABX,  $J = 16.6$  & 7.5), 2.45 (2H, d,  $J = 7.2$ ), 1.85 (1H, septet,  $J = 6.8$ ), 1.58-1.52 (3H, m), 1.45 (3H, d,  $J = 7.2$ ), 0.90 (6H, d,  $J = 6.4$ ).  $\delta_C$  (CD<sub>3</sub>OD, 75 MHz): 202.6, 176.7, 172.3, 171.8, 142.1, 139.7, 130.8, 128.7, 71.8-65.2, 56.0, 55.5, 46.7, 46.4, 36.5, 31.9, 23.1, 23.0, 21.1, 19.5. MS: C<sub>30</sub>H<sub>48</sub>N<sub>2</sub>O<sub>10</sub>S  $m/z$  (ES<sup>+</sup>) 629.3 [M+H]<sup>+</sup>. HRMS: Calculated for C<sub>30</sub>H<sub>49</sub>N<sub>2</sub>O<sub>10</sub>S 629.3102, found 629.3108.

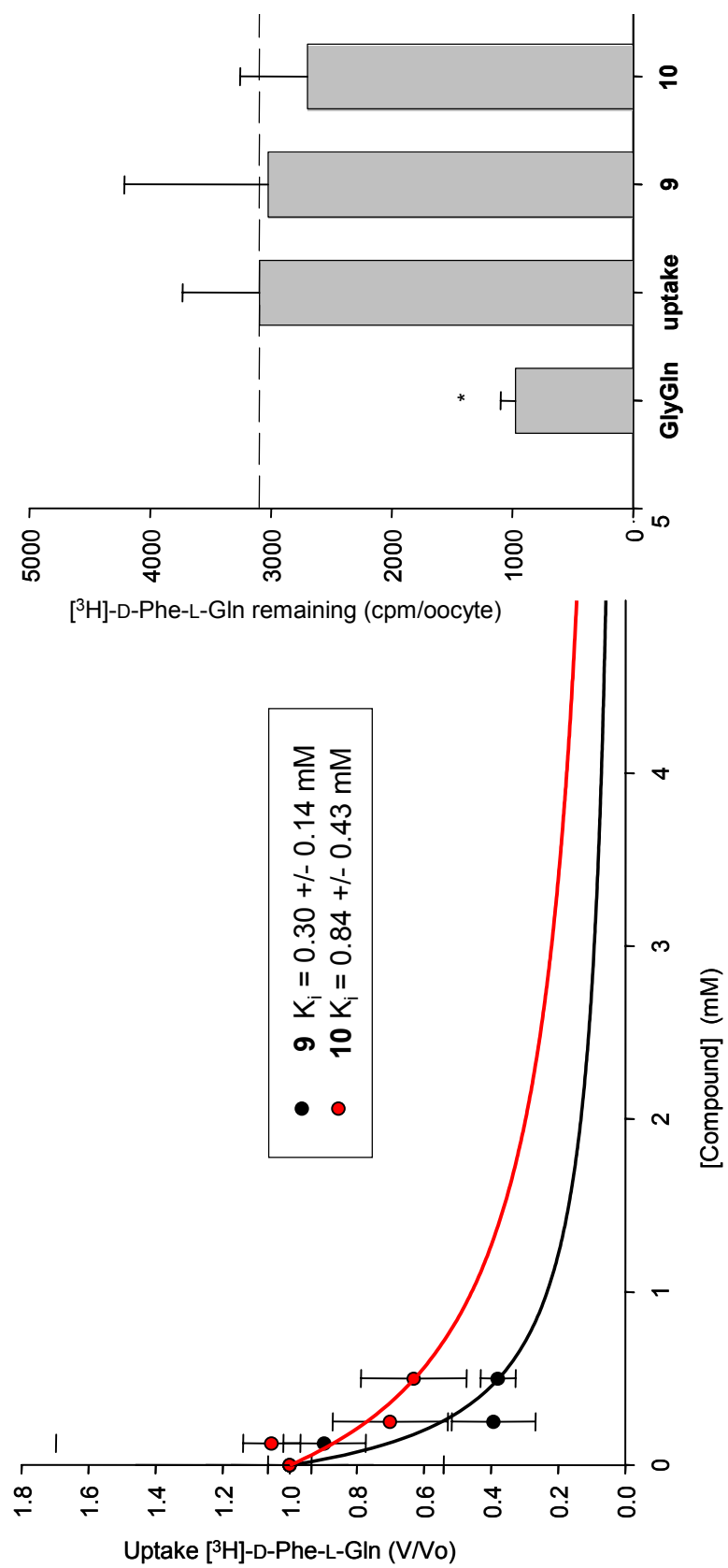
#### 4. Affinity and *trans*-stimulated efflux data

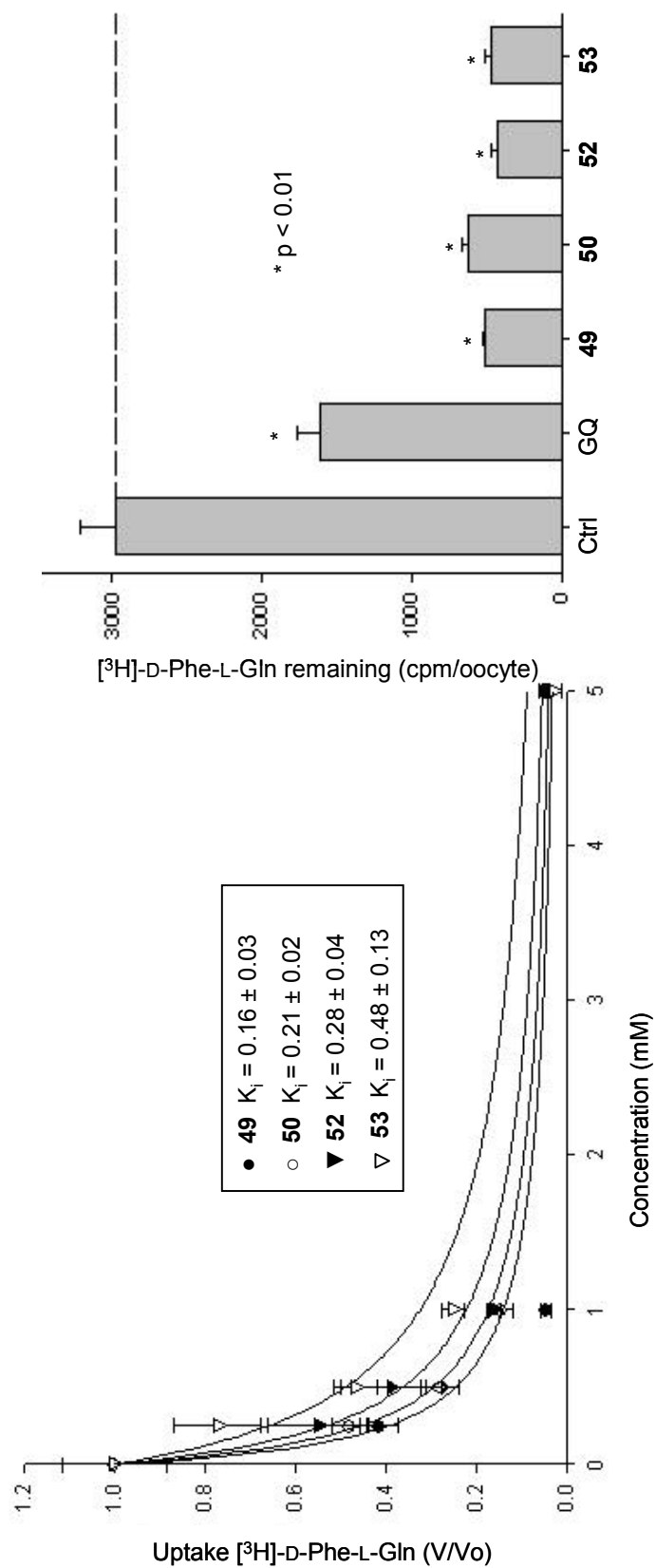




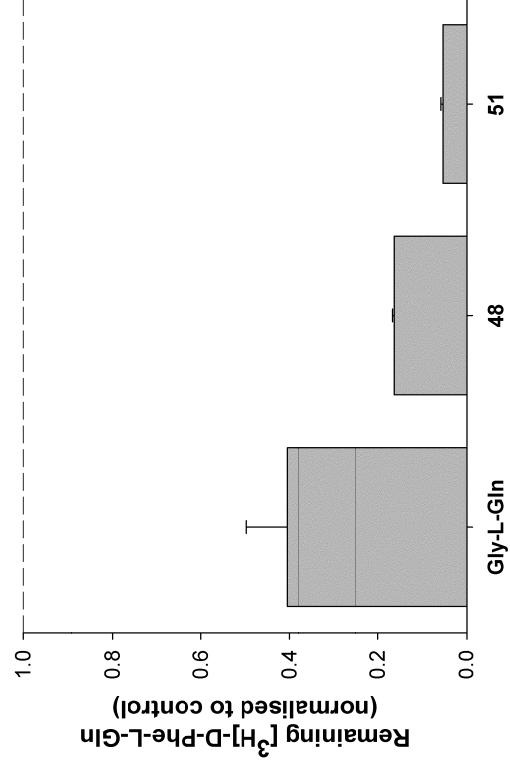
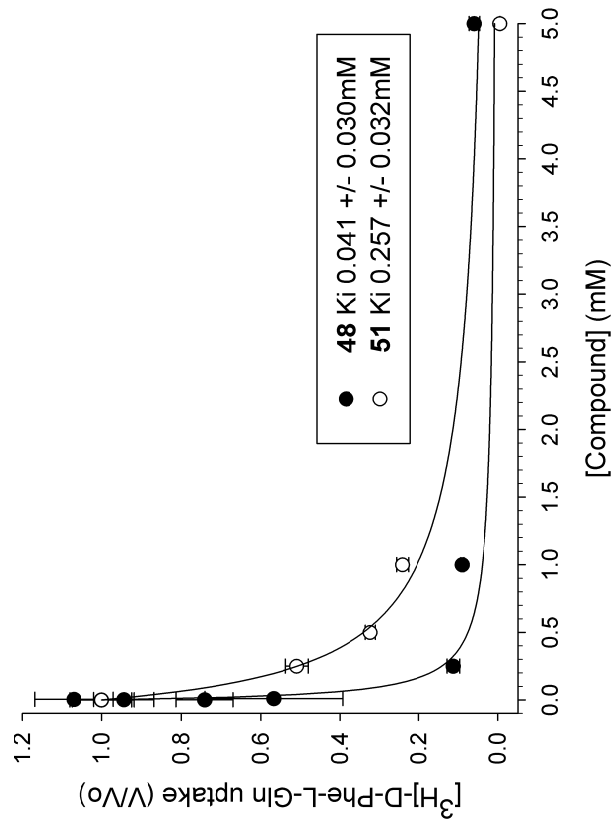












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