

## **Supporting Information**

### **Design of Next Generation Polymyxins with Lower Toxicity: The Discovery of SPR206**

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## Table of Contents

<b>1. Synthesis of Carboxylic acids .....</b>	<b>S2</b>
<b>1.1 Alpha substituted 4-aminobutanoic acids .....</b>	<b>S3</b>
<b>1.2 Beta substituted 4-aminobutanoic acids .....</b>	<b>S9</b>
<b>1.3 Amino propionate.....</b>	<b>S14</b>
<b>1.4 Piperidine-3-carboxylic acids .....</b>	<b>S16</b>
<b>1.5 Piperazine-2-carboxylic acids.....</b>	<b>S22</b>
<b>2 Chiral separation of 3-[[tert-butoxycarbonyl]amino]methyl}-4-phenylbutanoic acid and confirmation of stereochemistry by small molecule X-ray crystallography. .</b>	<b>S23</b>
<b>2.1 Separation of enantiomers of 4-((tert-butoxycarbonyl)amino)-3-(3-chlorophenyl)butanoic acid.....</b>	<b>S24</b>
<b>2.2 Confirmation of stereochemistry .....</b>	<b>S25</b>
<b>3 Synthesis of dipeptides.....</b>	<b>S27</b>
<b>4 Characterisation of final compounds (2) - (43) and (46) - (47).....</b>	<b>S28</b>
<b>5 Mouse renal toxicity of compounds 20, 22 and 24.....</b>	<b>S44</b>
<b>6 References .....</b>	<b>S45</b>

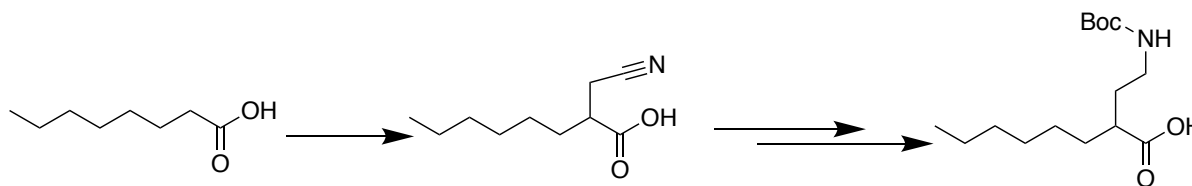
### 1. Synthesis of Carboxylic acids

Where not commercially available, synthesis of the required amine-containing N-terminal acids was in the racemic form. <sup>1</sup>H nmr spectra were recorded at 400 MHz on a Mercury 400 NMR spectrometer (Agilent Technologies) or at 300 MHz on a DPX300 spectrometer (Bruker). Chemical shifts ( $\delta$ ) are reported in ppm downfield from TMS. Coupling constants *J* are recorded in Hertz (Hz). Mass spectra were recorded on an LCQ DecaXP mass spectrometer with +ve ion electrospray ionisation. High resolution nanoelectrospray analyses (HRMS (+ve nESI)) were performed on the Thermo LTQ Orbitrap XL using the Advion TriVersa NanoMate.

## 1.1 Alpha substituted 4-aminobutanoic acids

### 2-{2-[(*tert*-Butoxycarbonyl)amino]ethyl}octanoic acid

Prepared using the methodology of Gil *et al*<sup>S1</sup> (Scheme S1).



**Scheme S1.**

#### (i) (2-Cyanomethyl)octanoic acid

THF (60 mL) was added to a stirred solution of *n*-butyl lithium (2.5 M in hexane, 24 mL, 60.9 mmol) at  $-78^{\circ}\text{C}$ . To this solution was added diethylamine (0.5 mL, 4.8 mmol) over 2 minutes. The solution was warmed to  $0^{\circ}\text{C}$  for 15 minutes then cooled to  $-78^{\circ}\text{C}$ . A solution of octanoic acid (4.4 mL, 27.7 mmol) in THF (40 mL) was added over 25 minutes. The resulting mixture was warmed to  $0^{\circ}\text{C}$  for 30 minutes to give a solution. The solution was cooled to  $-78^{\circ}\text{C}$  and then to this stirred mixture was added a solution of bromoacetonitrile (1.94 mL, 27.7 mmol) in THF (20 mL) over 25 minutes. The mixture was stirred at  $-78^{\circ}\text{C}$  for 30 minutes then warmed to ambient temperature and left to stir for 21 h. After this time, the mixture was quenched by the slow addition of water (400 mL) to give a mixture with pH 14. The mixture was washed with diethyl ether (2 x 200 mL). The aqueous phase from the extraction was acidified to pH 1 with conc. hydrochloric acid (approx. 5 mL) and extracted with ethyl acetate (3 x 200 mL). The emulsion formed during the extraction was clarified by

filtration through Celite. The organic extracts were dried ( $\text{MgSO}_4$ ) and evaporated *in vacuo* to a brown oil. The oil was purified by column chromatography over silica gel eluting with a gradient of acetone/toluene to give (2-Cyanomethyl)octanoic acid as an off-white solid (0.946 g, 5.2mmol, 19%),  $m/z$  184  $[\text{MH}]^+$ .  $^1\text{H NMR}$  (300MHz,  $\text{CDCl}_3$ ):  $\delta$  0.91 (3H, t), 1.31-1.37 (8H, m), 1.71-1.89 (2H, m), 2.56-2.74 (2H, m), 2.77-2.86 (1H, m).

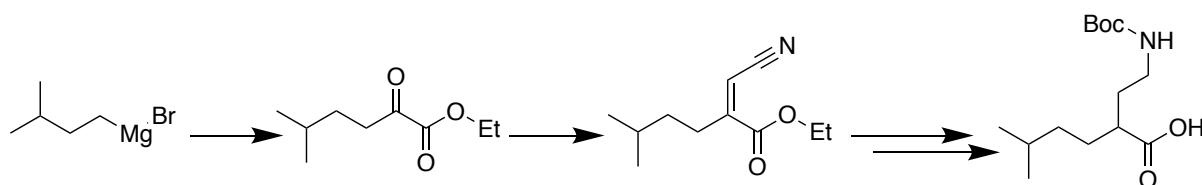
(ii) Title compound

Nickel chloride hexahydrate (1.21g, 5.1 mmol) was added to a stirred solution of (2-Cyanomethyl)octanoic acid (930 mg, 5.1 mmol) in methanol (100 mL) at ambient temperature to give a solution. To this solution under  $\text{N}_2$ , cooled in an ice bath, was added sodium borohydride (1.35g, 35.7 mmol) in portions over 10 minutes (caution: effervescence!) The black mixture was stirred at ambient temperature for 22h then filtered under suction through Celite and washed through with methanol (2 x 15mL). The filtrate and washings were evaporated to a pale green solid which was stirred for 10 minutes with a mixture of dichloromethane/methanol (3:1) (50 mL) and re-filtered. The filtrate was evaporated to give a pale green solid which was dried under vacuum for 3h (4.5g). This solid was dissolved in methanol (60 ml) and to the stirred solution was added triethylamine (1.42 mL) and then a solution of di-tert butyl dicarbonate (1.22 g, 5.58 mmol) in methanol (10 mL) and the mixture stirred for 21h. After this time the mixture was evaporated and the residue dissolved in water (100ml) to give a solution with pH 11. The solution was extracted with diethyl ether (2x50 mL) and the extract discarded. The aqueous phase was acidified to pH 3 by addition of 1M hydrochloric acid and extracted with ethyl acetate (2 x 60 mL) and dichloromethane (60ml). The combined extracts were dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated to an oil. This material was purified by column chromatography

over silica gel eluting with a gradient of methanol in DCM to give 2-{2-[(*tert*-butoxycarbonyl)amino]ethyl}octanoic acid as a colourless oil (522 mg, 36% over 2 steps),  $m/z$  288 [MH]<sup>+</sup>, 232 [M-<sup>t</sup>Bu]<sup>+</sup>. <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>): δ 0.90 (3H, t), 1.25-1.36 (9H, m), 1.50 (9H, s), 1.66 – 1.95 (3H, m), 3.17-3.26 (2H, m), 4.71 (1H, br. s).

### 2-{2-[(*tert*-Butoxycarbonyl)amino]ethyl}-5-methylhexanoic acid

Prepared according to Scheme S2



#### Scheme S2

##### (i) Ethyl-5-methyl-2-oxohexanoate

Isopentylmagnesium bromide (2M in diethyl ether, 8mL, 10.0 mmol) was added dropwise to a solution of diethyl oxalate (2.1 mL, 15.5 mmol) in dry THF (10mL) cooled to -50°C. The reaction mixture was warmed to -15°C and stirred for 2h. The mixture was quenched by the addition of 1M hydrochloric acid (10mL). The organic layer was separated and the aqueous phase further extracted with diethyl ether (x2). The combined organic phases were dried over anhydrous magnesium sulphate, filtered and concentrated to give a yellow oil (2.5g), <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>): δ 0.94-1.37 (6H, m), 1.39 (3H, t,  $J$ = 6.0 Hz), 1.51-1.62 (3H, m), 2.82-2.87 (2H, m), 4.34 (2H, q,  $J$ =-6.0 Hz).

##### (ii) Ethyl-2-(cyanomethylidene)-5-methylhexanoate

To a solution of ethyl-5-methyl-2-oxohexanoate (1.0g, 5.81 mmol) in toluene (30mL) at 0°C was added (triphenylphosphoranylidene)acetonitrile (1.75g, 5.81 mmol). The reaction mixture was allowed to warm to room temperature, and stirred overnight. A further aliquot

of (triphenylphosphoranylidine)acetonitrile (250mg) was added and the mixture stirred for a further 18h. The mixture was concentrated under reduced pressure, then applied to a column of silica (120g) and eluted with a gradient of 10-100% dichloromethane in cyclohexane. The product was obtained as a pale yellow oil as a mixture of (E) and (Z) isomers (983 mg, 87%), <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>): δ 0.94-1.00 (6H, m), 1.33-1.46 (5H, m), 1.62-1.66 (1H, m), 2.43-2.46 (m, 2H, minor isomer), 2.61-2.67 (m, 2H, major isomer), 4.30 (q *J*= 6.0 Hz, 2H major isomer), 4.36-4.41 (m, 2H, minor isomer), 5.66 (s, 1H minor isomer), 6.29 (s, 1H major isomer).

(iii) Ethyl 2-{2-[(*tert*-butoxycarbonyl)amino]ethyl}-5-methylhexanoate

To a solution of ethyl-2-(cyanomethylidene)-5-methylhexanoate (200mg, 1.03 mmol) in methanol (10mL) was added cobalt chloride (265 mg, 2.04 mmol). The mixture was cooled to 0°C and then treated portionwise with sodium borohydride (390 mg, 10.2 mmol). After the addition was complete, the mixture was warmed to room temperature and stirred for 1.5h. The mixture was quenched with 1M HCl and stirred for 2 mins. The pH was adjusted to 11 with saturated aqueous ammonia and the product extracted into dichloromethane. The organic extracts were combined, dried over anhydrous magnesium sulphate, filtered and concentrated to give the corresponding amino ethyl compound as a yellow oil (202mg, 97%), *m/z* 202 [MH]<sup>+</sup>.

This material (200mg, 0.99 mmol) was dissolved in dichloromethane (10ml) and treated with di-*tert* butyl dicarbonate (300mg, 1.37 mmol). The reaction mixture was stirred at room temperature overnight then concentrated at reduced pressure. The product was purified by silica gel chromatography eluting with 0-20% ethyl acetate in hexane to afford the BOC protected ester as a colourless oil. (164mg, 55%). *m/z* 202 [M-BOC]<sup>+</sup>. <sup>1</sup>H NMR (300MHz,

CDCl<sub>3</sub>): δ 0.94-0.99 (7H, m), 1.15-1.18 (2H, m), 1.28 (3H, t), 1.45 – 1.85 (13H, m, incl 1.45, s, BOC), 2.30-2.52 (1H, m), 3.08-3.21 (2H, m), 4.17 (2H, q), 4.61 (1H, br.s).

(iv) Title compound

Ethyl 2-{2-[(*tert*-butoxycarbonyl)amino]ethyl}-5-methylhexanoate (160 mg, 0.53 mmol) dissolved in 1,4-dioxane (6mL) and water (3mL) was treated with lithium hydroxide hydrate (66mg, 1.57 mmol). The mixture was stirred at room temperature overnight, then partially concentrated. The pH was adjusted to approx. pH1 and the product extracted into diethyl ether (x3). The combined organic phases were dried over anhydrous magnesium sulphate, filtered and evaporated to give 2-{2-[(*tert*-butoxycarbonyl)amino]ethyl}-5-methylhexanoate as a colourless oil (155mg, quant). *m/z* 218 [M-*t*Bu]<sup>+</sup>. <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>): δ 0.89-0.91 (7H, m), 1.21-1.26 (2H, m), 1.47 (9H, s), 1.47-1.57 (1H, m), 1.62-1.90 (3H, m), 2.36-2.42 (1H, m), 3.15-3.25 (2H, m), 4.75(1H, br.s).

The following acids were prepared by the same route (Scheme S2)

**4-[(*tert*-Butoxycarbonyl)amino]-2-(3-chlorophenyl)-butanoic acid**

*m/z* (+ve ion ESI) 336 [M+Na]<sup>+</sup>, (-ve ion ESI) 312 (M-H)<sup>-</sup>. <sup>1</sup>H NMR (300MHz CD<sub>3</sub>OD), 1.44 (9H, s), 1.86-1.96 (1H, m), 2.19-2.30 (1H, m), 2.95-3.07 (2H, m), 3.62 (t, 1H), 7.27-7.36 (4H, m).

**4-[(*tert*-Butoxycarbonyl)amino]-2-(4-chlorophenyl)-butanoic acid**

*m/z* (-ve ion ESI) 312 (M-H)<sup>-</sup>. <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>) 1.45 (9H, s), 1.82-1.96 (1H, m), 2.25-2.40 (1H, m), 3.10-3.20 (2H, m), 3.63 (t, 1H), 4.60 (1H, br s), 7.26-7.34 (4H, m).

**4-[(*tert*-Butoxycarbonyl)amino]-2-(naphthalene-2-yl)-butanoic acid**

$m/z$  (+ve ion ESI) 352  $[M+Na]^+$ , (-ve ion ESI) 328  $(M-H)^-$ .  $^1H$  NMR (300MHz,  $CDCl_3$ ), 1.43 (9H, s), 2.00-2.12 (1H, m), 2.38-2.50 (1H, m), 3.15-3.28 (2H, m), 3.83(t, 1H,  $J=7.5$  Hz), 4.60 (1H, br s), 7.28-7.50 (3H, m), 7.78-7.74 (4H, m).

#### 4-[(*tert*-Butoxycarbonyl)amino]-2-[(4-chlorophenyl)methyl]butanoic acid

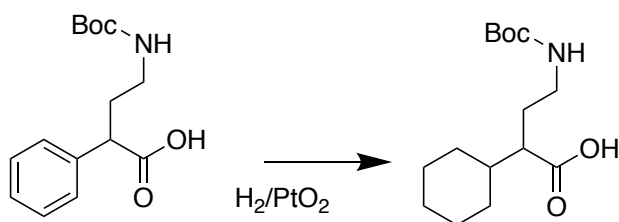
$m/z$  (-ve ion ESI) 326  $(M-H)^-$ .  $^1H$  NMR (300MHz,  $CDCl_3$ ), 1.4 (9H, s), 1.68-1.80 (1H, m), 1.81-1.92(1H, m), 2.62-2.78 (2H, m), 3.00-3.10 (m, 1H), 3.18-3.30( 2H, m), 4.70 (1H, br s), 7.12-7.28 (4H, m).

#### 4-[(*tert*-Butoxycarbonyl)amino]-2-(naphthalene-2-ylmethyl)-butanoic acid

$m/z$  (-ve ion ESI) 342  $(M-H)^-$ .  $^1H$  NMR (300MHz,  $CDCl_3$ ), 1.44 (9H, s), 1.68-1.81 (1H, m), 1.85-1.98 (1H, m), 2.85-3.00 (2H, m), 3.20-3.27 (3H, m), 4.70 (1H, br s), 7.45-7.48 (3H, m), 7.77-7.83 (4H, m).

#### 4-[(*tert*-Butoxycarbonyl)amino]-2-cyclohexylbutanoic acid

Prepared by reduction of the corresponding aromatic system (Scheme S3)



**Scheme S3**

A suspension of platinum oxide (60 mg, 0.21 mmol) in acetic acid (2 mL) was added to a stirred solution of 4-[(*tert*-butoxycarbonyl)amino]-2-phenylbutanoic acid (185 mg, 0.66 mmol) in acetic acid (20 mL). The reaction was hydrogenated for 24h at ambient temperature

and atmospheric pressure. The reaction mixture was filtered through Celite and the filter pad washed with acetic acid (20 mL). The filtrate was evaporated at reduced pressure and azeotroped with toluene to leave a semi solid product (189 mg, quant).  $m/z$  286  $[M+H]^+$ ,  $^1H$  NMR (400MHz,  $CD_3OD$ ), 0.84-1.31 (6H, m), 1.45 (9H, s), 1.48-1.59 (1H, m), 1.59-1.83 (6H, m), 2.10-2.21 (1H, m), 2.85-3.13 (2H, m).

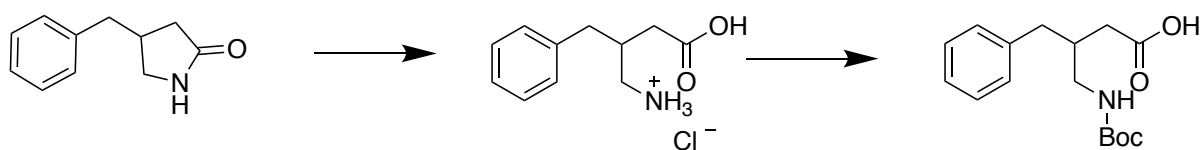
#### 4-((*tert*-Butoxycarbonyl)amino)-2-(cyclohexylmethyl)butanoic acid

prepared by reduction of the 2-benzyl-4-[(*tert*-butoxycarbonyl)amino]-butanoic acid using the methodology shown in Scheme S3.  $m/z$  300  $[M+H]^+$ ,  $^1H$  NMR (300MHz,  $CDCl_3$ ), 0.84-0.91 (2H, m), 1.18-1.29 (5H, m), 1.45 (9H, s), 1.58-1.80 (8H, m), 2.49-2.56 (1H, m), 3.08-3.25(2H, m), 4.86 (1H, br. s), 10.10 (br s).

## 1.2 Beta substituted 4-aminobutanoic acids

### 3-[[(*tert*-Butoxycarbonyl)amino]methyl]-4-phenylbutanoic acid

Prepared from the corresponding pyrrolidinone (Scheme S4)



**Scheme S4**

#### (i) 4-Amino-3-benzylbutanoic acid hydrochloride

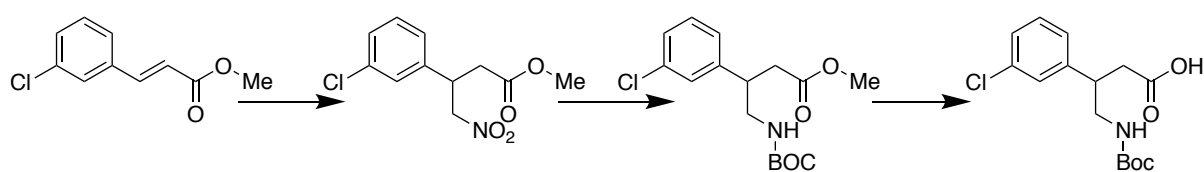
A mixture of 4-benzyl-2-pyrrolidinone (1.0 g), water (10 ml) and concentrated hydrochloric acid (37%) (10 ml) was heated at 100°C for 17h. The mixture was evaporated to dryness, then co-evaporated with methanol and then dichloromethane. The residues were dried under

vacuum, producing the desired product as the hydrochloride salt as a cream solid (1.30 g),  $m/z$  (+ve ESI) 194.0  $[M+H]^+$ .

## (ii) Title compound

4-Amino-3-benzylbutanoic acid hydrochloride (1.30 g), was dissolved in 1,4-dioxane (15ml) and treated with 1M NaOH(aq) (15 ml) and di-tert-butyl dicarbonate (1.25 g), and the mixture stirred at room temperature for 17h. The mixture was evaporated to dryness. The residues were partitioned between water and ethyl acetate and neutralised with 1M HCl(aq). The aqueous layer was separated and extracted with ethyl acetate (x2). The organic extracts were combined, dried with magnesium sulphate, filtered and evaporated to dryness, producing 3-[[tert-butoxycarbonyl]amino]methyl}-4-phenylbutanoic acid as an orange oil (1.66 g).  $m/z$  (+ve ESI) 294  $[M+H]^+$ .  $^1H$  NMR (300MHz,  $CDCl_3$ ) 1.46 (9H, s), 2.38-2.41 (3H, m), 2.66-2.70 (2H, m), 3.10-3.18 (1H, m), 3.24-3.32 (1H, m), 4.71 (1H, br.s), 7.18-7.31 (5H, m).

## 4-[(tert-Butoxycarbonyl)amino]-3-(3-chlorophenyl)butanoic acid



**Scheme 5. Reagents and conditions:** (i) nitromethane, DBU, 74%; (ii), Zn, acetic acid, 0°C – RT, 56%; (iii)  $BOC_2O$ ,  $CH_2Cl_2$ , 34%; (v) LiOH,  $H_2O$ , dioxane, quant.

## (i) Methyl (2E)-3-(3-chlorophenyl)prop-2-enoate

A mixture of 3-chlorocinnamic acid (10 g), methanol (100 ml) and concentrated sulphuric acid (4 ml) was stirred at room temperature for 20h. The mixture was evaporated to dryness

and the residues partitioned between dichloromethane (DCM) and water. The aqueous phase was separated and extracted with additional DCM. The organic extracts were combined, dried with magnesium sulphate, filtered and evaporated to dryness, producing the desired product as a white solid (10.19 g),  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3$ ) 3.81(3H, s), 6.45(1H, d,  $J=16.0$  Hz), 7.27-7.68 (5H, m).

(ii) Methyl 3-(3-chlorophenyl)-4-nitrobutanoate

A mixture of methyl (2E)-3-(3-chlorophenyl)prop-2-enoate (10.19 g), nitromethane (32 ml) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (8.5 ml) was stirred at room temperature for 20h. The mixture was evaporated to dryness and the residues partitioned between 0.5M HCl(aq) and diethyl ether. The aqueous phase was separated and extracted with additional diethyl ether. The organic extracts were combined, washed with brine, dried with magnesium sulphate, filtered and evaporated to dryness. The residues were purified on silica, eluting with hexane and ethyl acetate (0-100%). The appropriate fractions were combined and evaporated to dryness, producing the desired product as a yellow oil (9.93 g).  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3$ ) 2.76 (2H, dd,  $J=1.7, 7.3$  Hz), 3.65 (3H, s), 3.93-4.01 (1H, m), 4.63(1H, dd,  $J= 8.0, 12.8$  Hz), 4.73 (1H, dd,  $J = 6.7, 12.8$  Hz), 7.11-7.28 (4H, m).

(iii) Methyl 4-[(*tert*-butoxycarbonyl)amino]-3-(3-chlorophenyl)butanoate

To a solution of methyl 3-(3-chlorophenyl)-4-nitrobutanoate (9.93 g) in acetic acid (90 ml) stirred at  $\sim 0^\circ\text{C}$  was added zinc powder (20.1 g) portion wise (caution delayed exotherm). The mixture was allowed to warm to room temperature, stirring for 19h. The mixture was evaporated to dryness and the residues partitioned between  $\text{NaHCO}_3$ (aq) and ethyl acetate. The mixture was then filtered through celite and the aqueous and organic phases separated. The aqueous phase was re-extracted with additional ethyl acetate. The organic extracts were

combined, washed with brine, dried with magnesium sulphate, filtered and evaporated to dryness, producing the desired product as an orange oil (4.80 g). This was dissolved in dichloromethane (100 ml), and treated with di-*tert*-butyl dicarbonate (5.28 g) and the mixture stirred at room temperature for 18h. The mixture was evaporated to dryness and the residues purified on silica, eluting with 0-100% ethyl acetate in hexane. The appropriate fractions were combined and evaporated to dryness, producing the desired product as a cream solid (2.59 g). <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) 1.40 (9H, s), 2.56 (1H, dd, *J*=7.9, 15.8 Hz), 2.69 (dd, *J*= 6.3, 15.8 Hz), 3.31-3.34 (2H, m), 3.38-3.44 (1H, m), 3.60 (3H, s), 4.52 (1H, br s), 7.08 - 7.26 (4H, m).

(iv) Title compound

A mixture of methyl 4-[(*tert*-butoxycarbonyl)amino]-3-(3-chlorophenyl)butanoate (2.49 g), lithium hydroxide (546 mg), 1,4-dioxane (40 ml) and water (40 ml) was stirred at room temperature for 64h. The mixture was evaporated to dryness. The residues were dissolved in water, neutralised with 1M HCl(aq) and extracted with ethyl acetate (x2). The organic extracts were combined, washed with brine, dried with magnesium sulphate, filtered and evaporated to dryness, producing 4-[(*tert*-butoxycarbonyl)amino]-3-(3-chlorophenyl)butanoic acid as a yellow oil (2.51 g), *m/z* [+ve ion ESI] 313.8 [MH]<sup>+</sup>. <sup>1</sup>H NMR (400MHz, CD<sub>3</sub>OD) 1.38 (9H, s), 2.56 (1H, dd, *J*=8.8, 15.8 Hz), 2.71 (dd, *J*= 4.4, 15.6 Hz), 3.24 -3.31 (3H, m), 7.17 -7.29 (4H, m).

**3-[(*tert*-Butoxycarbonyl)amino]methyl}octanoic acid**

(i) Methyl 3-(nitromethyl)octanoate

To a solution of methyl (E)-oct-2-enoate (10.0 g, 64 mmol) in nitromethane (20 mL) was added DBU (9.55 mL, 64 mmol) over 10 mins with ice-bath cooling. The reaction mixture

was stirred at room temperature overnight, concentrated under vacuum and the residue partitioned between 0.5M HCl (aq) and diethyl ether. The aqueous layer was separated and extracted with additional diethyl ether. The organic extracts were combined, washed with brine, dried with MgSO<sub>4</sub>, filtered and evaporated to dryness. The residues were purified on silica, eluting with pet ether 40-60 and ethyl acetate (0-100%). The appropriate fractions were combined and evaporated to dryness, producing 10.58g of colourless oil (76%), *m/z* 218 [M+H]<sup>+</sup>

(ii) 4-Pentylpyrrolidin-2-one

Zinc dust (27g, 219mmol) was added portionwise to a solution of methyl 3-(nitromethyl)octanoate (10.58g, 48.7mmol) in acetic acid (100 mL), stirred at 0-5 °C (caution – exothermic). The mixture was allowed to warm to room temperature, stirring for 17h. The mixture was evaporated to dryness and the residue partitioned between ethyl acetate and saturated NaHCO<sub>3</sub> (aq). The mixture was filtered through Celite. The aqueous phase was separated and extracted with additional ethyl acetate. The organic extracts were combined, dried with MgSO<sub>4</sub>, filtered and evaporated to dryness, to afford methyl 3-(aminomethyl)octanoate (10.2g), which was used directly in the next reaction. The crude methyl ester was dissolved in toluene (500 ml) and heated to reflux for 4h. The mixture was cooled, and the solvent evaporated, afford 4-pentylpyrrolidin-2-one (8.25g, contains 20% toluene, 80% yield) , *m/z* 156 [M+H]<sup>+</sup>, <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) 0.88 (3H, *t*, *J*=6.6 Hz), 1.28-1.32 (6H, *m*), 1.42-1.46 (2H, *m*), 1.97-2.06 (3H, *m*), 2.38-2.46 (2H, *m*), 3.03 (1H, *dd*, *J*=6.4, 9.4Hz), 3.49-3.52 (1H, *m*), 6.50 (1H, *br s*).

(iii) Title compound

A mixture of 4-pentylpyrrolidin-2-one (1.0g, 6.45mmol), and 6M HCl(aq) (30 mL) was heated at 100 degC for 16h. The mixture was evaporated to dryness, then azeotroped with toluene, producing the desired ring-opened product, which was converted to the BOC-protected amine without further purification. Half of the material from the ring-opening (3.2 mmol) was dissolved in 1,4-dioxane (10mL) and 1M NaOH (10mL), and treated with di-*tert*-butyl dicarbonate (738 mg, 1.08 eq) with ice-bath cooling. The mixture was concentrated under reduced pressure, then partitioned between ethyl acetate and water. The aqueous layer was adjusted to pH3 with 1M HCl, and the product extracted into ethyl acetate. The organic extract was dried and purified by chromatography on silica eluting with 0-100% ethyl acetate in hexane. 3-[[*tert*-butoxycarbonyl]amino]methyl}octanoic acid was obtained as a colourless oil (305 mg, 35 % over 2 steps) which crystallised on standing.  $m/z$  (ESI<sup>+</sup>)273.7, [M+H]<sup>+</sup>. <sup>1</sup>H NMR indicates the presence of rotamers (400MHz, CDCl<sub>3</sub>) 0.88 (3H, t,  $J=6.1$  Hz), 1.19-1.31 (8H, m), 1.39-1.44 (9H, m), 2.00-2.40 (3H, m), 2.90-3.21(2H, m), 4.76 and 6.14 (total 1H, br s).

#### **4-[(*tert*-Butoxycarbonyl)amino]-3-cyclohexylbutanoic acid**

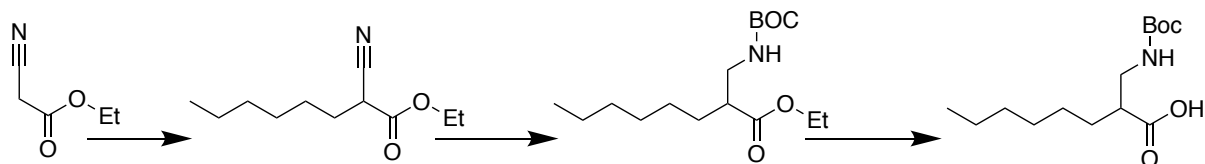
Prepared by reduction of the corresponding aromatic system, as in Scheme S3.

$m/z$  (+ve ion ESI) 285.9 [M+H]<sup>+</sup>. <sup>1</sup>H NMR indicates the presence of rotamers (400MHz, CDCl<sub>3</sub>) 0.93-1.37 (6H, m), 1.41 and 1.45 (total of 9H, 2 x s), 1.61-1.86 (5H, m), 1.84-2.24 (2H, m), 2.33-2.39 (1H, m), 2.90-3.24 (2H, m), 4.84 and 6.17(total of 1H, 2 x br s).

### **1.3 Amino propionate**

#### **2-[(*tert*-Butoxycarbonylamino)methyl]octanoic acid**

Prepared according to Scheme S6



**Scheme S6**

(i) Ethyl 2-cyano-octanoate

A mixture of ethyl cyanoacetate (2 mL, 19.0 mmol), 1-iodohexane (3.0 mL, 20.0 mmol) and potassium carbonate (2.76 g, 20.0 mmol) in acetone (100 mL) was heated to reflux for 4 h. After cooling to room temperature, the reaction mixture was filtered through Celite and washed with acetone. The filtrate was evaporated at reduced pressure and then partitioned between dichloromethane and saturated ammonium chloride solution. After separation of the layers, the organic phase was washed with brine, dried over magnesium sulphate, filtered and concentrated at reduced pressure. The product was purified by silica gel chromatography eluting with 0 – 30% ethyl acetate in iso-hexane to yield the title compound as a colourless oil (2.29 g, 61%).  $m/z$  (+ve ion ESI) 198  $[M+H]^+$ ,  $^1H$  NMR (300MHz,  $CDCl_3$ ) 0.94 (3H, t), 1.24-1.39 (9H, m), 1.50-1.56 (2H, m), 1.96 (2H, q), 2.60-2.68 (1H, m), 3.50 (1H, t), 4.29 (2H, q).

(ii) Ethyl 2-[(*tert*-butoxycarbonylamino)methyl]octanoate

To a solution of ethyl 2-cyano-octanoate (880 mg, 4.47 mmol) in methanol (40 mL) was added cobalt chloride (1.14 g, 8.76 mmol). The mixture was cooled to 0°C and then treated portion-wise with sodium borohydride (1.67 g, 44 mmol). After the addition was complete, the reaction mixture was warmed to room temperature and stirred for a further 30 minutes. The mixture was quenched with 1 M hydrochloric acid and stirred for 2 minutes. The mixture was then basified to ~ pH 11 by the addition of .880 ammonia and the product extracted into

dichloromethane ( $\times 4$ ). The combined organic phases were dried over magnesium sulphate, filtered and concentrated to give the crude ethyl 2-(aminomethyl)octanoate as a pale brown oil (775 mg, 86%).  $m/z$  (+ve ion ESI) 202 [M+H]<sup>+</sup>.

To a solution of this material (775 mg, 3.86 mmol) in dichloromethane (50 ml) was added di-*tert*-butyl dicarbonate (1.0 g, 4.58mmol). The reaction mixture was stirred at room temperature for 16h and then concentrated at reduced pressure. The product was purified by silica gel chromatography eluting with 0 – 20% ethyl acetate in isohexane to yield BOC protected amine as a colourless oil (818 mg, 71%).  $m/z$  (+ve ion ESI) 246 [M-BOC]<sup>+</sup>, <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>) 0.87-1.95 (3H, m), 1.19-1.57 (11H, m), 1.29-1.57 (11H, m, includes 9H, s), 2.60-2.68 (1H, m), 3.21-3.42 (2H, m), 4.18 (2H, q, J=6.0 Hz), 5.32 (br. s).

### (iii) Title compound

To a solution of ethyl 2-[(*tert*-butoxycarbonylamino)methyl]octanoate (816 mg, 2.71 mmol) in dioxane (12 mL) and water (6 mL) was added lithium hydroxide (350 mg, 8.33 mmol). The reaction mixture was stirred at room temperature for 16 h and then partially concentrated at reduced pressure. The residue was partitioned between diethyl ether and water and the pH 20 adjusted to 1 by the addition of 1 M hydrochloric acid. After separation of the layers, the aqueous phase was re-extracted with diethyl ether ( $\times 2$ ). The combined organic layers were dried over magnesium sulphate, filtered and concentrated to give 2-[(*tert*-butoxycarbonylamino)methyl]octanoic acid as a colourless oil (715 mg, 97%).  $m/z$  (-ve ion ESI) 272 [M-H]<sup>-</sup>, <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>) 0.87-1.95 (3H, m), 1.19-1.66 (22H, m, includes 9H, s), 2.60-2.68 (1H, m), 3.15-3.48 (2H, m), 4.98 (br. s).

## 1.4 Piperidine-3-carboxylic acids

### ***cis-* and *trans*-1-(*tert*-Butoxycarbonyl)-5-phenylpiperidine-3-carboxylic acid**

Prepared by chromatographic separation of the corresponding methyl ester.

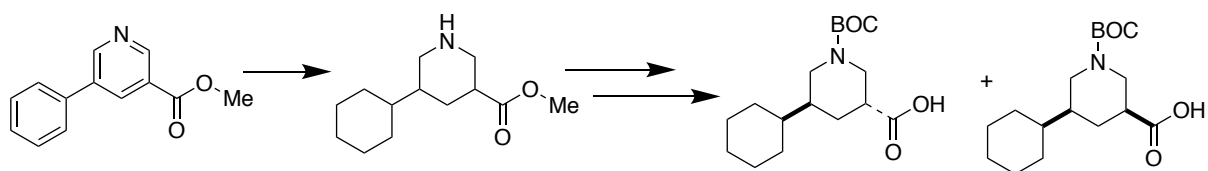
1-(*tert*-Butoxycarbonyl)-5-phenylpiperidine-3-carboxylic acid (commercial mixture of *cis* and *trans*) was dissolved in methanol (6 mL) and diethyl ether (6 mL) and treated with a solution of TMS diazomethane (2M in hexane, 2.1 mL). The reaction mixture was stirred at room temperature for 16h, then evaporated under reduced pressure. The residue was chromatographed on silica eluting with 10% dichloromethane in hexane to separate the two components.

The faster eluting component (130 mg) was hydrolysed with lithium hydroxide to afford *trans*-1-(*tert*-butoxycarbonyl)-5-phenylpiperidine-3-carboxylic acid (78 mg). <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) 1.45 (9H, s), 1.85-1.88 (1H, m, appears as br. t), 2.38 (br.d J=14 Hz), 2.61 – 3.01 (3H, m), 3.19 (1H, br.d J 11.5 Hz), 4.42(1H, br. d, J=11.2 Hz), 7.22-7.34 (5H, m). The relative stereochemistry was assigned *trans* by comparison to the *cis* isomer below, which was consistent with the published literature.<sup>S2</sup>

The slower eluting compound (40mg) was hydrolysed with lithium hydroxide to afford *cis*-1-(*tert*-butoxycarbonyl)-5-phenylpiperidine-3-carboxylic acid (28mg). <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) 1.47 (9H, s), 1.81 (1H, q, J=12.5 Hz), 2.36 (br.d J=13.2 Hz), 2.60 – 2.90 (4H, m), 4.27 (1H, br.s), 4.46 (1H, br.s), 7.21-7.34 (5H, m). <sup>1</sup>H Nmr was in agreement with the published data for this compound.<sup>S2</sup>

### ***cis* - and *trans* - 1-(*tert*-Butoxycarbonyl)-5-cyclohexylpiperidine-3-carboxylic acid**

Prepared by a modification of the method of Diaz *et al*<sup>S2</sup> (Scheme S7)



### Scheme S5.

#### (i) Methyl 5-cyclohexylpyridine-3-carboxylate

Methyl 5-phenylpyridine-3-carboxylate (703 mg, 3.3 mmol) was dissolved in acetic acid (120ml) and treated with a slurry of platinum oxide (265 mg) in acetic acid (5ml). The mixture was hydrogenated at room temperature and atmospheric pressure for 4h. LCMS showed the presence of the partially reduced product. A further aliquot (250mg) of platinum oxide was added and the mixture hydrogenated at room temperature and atmospheric pressure for a further 16h. The reaction mixture was filtered through Celite and the filter pad washed with acetic acid (20mL) followed by dichloromethane (20mL). The filtrates were combined and evaporated. The residue was dissolved in water and the pH adjusted to 10 by the addition of concentrated aqueous ammonia. The resultant mixture was extracted with dichloromethane (3 x 20ml) and the combined organic extracts dried (MgSO<sub>4</sub>) and evaporated to a straw-coloured oil (665 mg, 89%) consisting of a mixture of *cis* and *trans* isomers of the desired product,  $m/z$  226 [M+H]<sup>+</sup>.

#### (ii) Title compounds

Methyl 5-cyclohexylpyridine-3-carboxylate (mixture of *cis* and *trans*) (645 mg, 2.9 mmol) was dissolved in dichloromethane (50mL) and treated with triethylamine (0.6 mL, 4.3 mmol) followed by di-tert-butyl dicarbonate (937 mg, 4.3mmol). The reaction mixture was stirred at room temperature for 16h and then concentrated at reduced pressure. The mixture was poured into a solution of saturated aqueous ammonium chloride (250mL) and extracted with diethyl ether (3 x 50mL). The combined extracts were dried (MgSO<sub>4</sub>) and evaporated. The residue

was chromatographed on silica gel (100g) eluting with 0 – 35% diethyl ether in isohexane, to separate the *cis* and *trans* isomers.

Early eluting fractions were combined and evaporated to an oil (479 mg, 50%), *m/z* (+ve ion ESI) 326 [M+H]<sup>+</sup>, 270 [M+H-<sup>t</sup>Bu]<sup>+</sup> (100%)

Later-eluting fractions were combined and evaporated to an oil (400 mg, 42%) *m/z* (+ve ion ESI) 270 [M+H-<sup>t</sup>Bu]<sup>+</sup> (100%)

Combined early eluting fractions (470 mg) were hydrolysed with sodium hydroxide to afford *cis* - 1-(*tert*-butoxycarbonyl)-5-cyclohexylpiperidine-3-carboxylic acid as a white crystalline solid (390 mg). *m/z* 312 [M+H]<sup>+</sup>, <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>) 1.01-1.45 (8H, m), 1.45 (9H, s), 1.62-1.80 (5H, m), 2.21-2.25 (1H, m), 2.30-2.41(1H, m), 2.42-2.55(1H, m), 2.62 (1H, t), 4.21 (1H, br s), 4.37 (1H, br s). <sup>13</sup>C NMR (75MHz, CDCl<sub>3</sub>) 26.5, 26.5, 28.4, 29.9, 30.1, 31.4, 40.8, 41.7, 45.6, 47.7, 79.9, 154.7, 179.2.

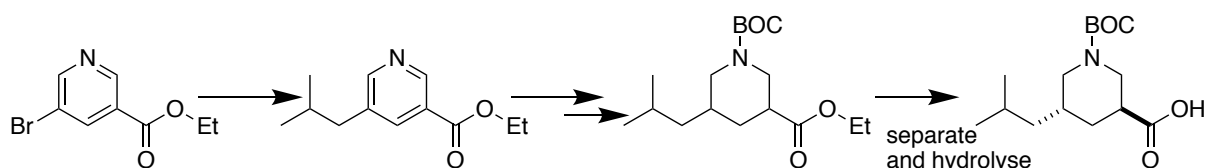
Structural assignment was consistent with the literature values reported for the *cis* isomer.<sup>S2</sup>

Combined later eluting fractions (390 mg) were hydrolysed to afford *trans*- 1-(*tert*-butoxycarbonyl)-5-cyclohexylpiperidine-3-carboxylic acid as a colourless oil which solidified on standing (316 mg) *m/z* 312 [M+H]<sup>+</sup>, <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>) 0.90-1.36 (8H, m), 1.47 (9H, s), 1.65-2.15 (7H, m), 2.66 (1H, t), 3.40-4.10 (4H, br. m). <sup>13</sup>C NMR (75MHz, CDCl<sub>3</sub>) 26.9, 26.5, 28.4, 29.3, 30.0, 30.6, 37.9, 38.4, 45.0, 46.6, 79.7, 154.8, 179.2

Structural assignment was by comparison with the previously reported *cis* isomer.

### ***trans*- 1-(*tert*-Butoxycarbonyl)-5-(2-methylpropyl)piperidine-3-carboxylic acid**

Prepared according to Scheme S8



### Scheme S8.

#### (i) Ethyl 5-(2-methylpropyl)pyridine-3-carboxylate

Isobutylmagnesium chloride (2 M solution in THF, 9.25 mL, 18.52 mmol) was added dropwise to a solution of zinc bromide (4.17 g, 18.52 mmol) in THF (30 mL) at 0°C under nitrogen 30 and the resulting suspension was stirred for 30 minutes. The mixture was cooled to -78°C and [1,1'-Bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane (252 mg, 0.309 mmol) was added followed by a solution of 5-bromonicotinic acid ethyl ester (1.42 g, 6.17 mmol) in THF (5 mL). After 5 minutes the reaction was allowed to warm to room temperature and stirring was continued for 3 hours. The reaction mixture was diluted with water and ethyl acetate and the phases were separated. The aqueous layer was extracted with ethyl acetate and the combined organic layers were dried (MgSO<sub>4</sub>) and the solvent evaporated to afford a crude oil. This was purified by Biotage SP4, 50 g SNAP cartridge eluting with 0 to 50% EtOAc / i-hexane to afford the 5-isobutyl-nicotinic acid isobutyl ester (365 mg) followed by the 5-isobutyl-nicotinic acid ethyl ester (576mg). Ethyl 5-(2-methylpropyl)pyridine-3-carboxylate: <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>) 0.95 (6H, d), 1.45 (3H, t), 1.90 (1H, sept), 2.55 (2H, d), 4.45 (2H, q), 8.10(1H, s), 8.55 (1H, s), 9.05 (1H, s).

#### (ii) Ethyl- *trans*-1-(*tert*-butoxycarbonyl)-5-(2-methylpropyl)piperidine-3-carboxylate

To a solution of ethyl 5-(2-methylpropyl)pyridine-3-carboxylate (5.0 g, 24.1 mmol) in acetic acid (100 mL) was added platinum oxide (400 mg). The reaction mixture was hydrogenated for 16 h., then filtered under suction through Celite and washed through with acetic acid followed by DCM. The filtrate was evaporated at reduced pressure and then the residue was partitioned, dissolved in ethyl acetate (200ml) and washed with saturated aqueous NaHCO<sub>3</sub> solution. The aqueous phase was extracted with ethyl acetate, and the organic phases

combined, dried (anhydrous  $\text{MgSO}_4$ ) and evaporated to give ethyl 5-(2-methylpropyl)piperidine-3-carboxylate as a colourless oil (5.1g, 99%).

To a solution of ethyl 5-(2-methylpropyl)piperidine-3-carboxylate (3.0g, 14.1 mmol) in dichloromethane (70 mL) was added a solution of di-*tert*-butyl dicarbonate (3.99 g, 18.3 mmol) in dichloromethane (15mL) (effervescence). The reaction mixture was stirred at room temperature for 16 hours and then concentrated at reduced pressure. The residue was purified by silica gel chromatography (340g column) eluting with 0-30% diethyl ether in iso-hexane. The first product to elute was assigned *cis* stereochemistry by comparison to the 5-cyclohexyl analogue (1.13g, 26%)  $m/z$  (+ve ion ESI) 314 (M-*t*Bu)<sup>+</sup>. Further elution of the column gave the *trans* derivative (2.6g, 59%).  $m/z$  314 (M-*t*Bu)<sup>+</sup>. <sup>1</sup>H NMR (300MHz,  $\text{CDCl}_3$ ) (6H, d, J= 6 Hz), 1.05-1.22 (2H, m), 1.27 (3H, t, J=6.5 Hz), 1.47 (9H, s), 1.61-1.78 (1H, m), 1.80-2.21 (2H, m), 2.50-2.60 (1H, m), 3.27-3.92 (4H, m), 4.10-4.22 (2H, m).

(iii) *trans*-1-(*tert*-Butoxycarbonyl)-5-(2-methylpropyl)piperidine-3-carboxylic acid

To a solution of ethyl *trans*-1-(*tert*-butoxycarbonyl)-5-(2-methylpropyl)piperidine-3-carboxylate (slow-running isomer) (1.0g, 3.2 mmol) in methanol (30mL) was added a solution of lithium hydroxide monohydrate (402mg, 9.6 mmol in water (8mL)). The reaction mixture was stirred at room temperature for 24 hours. The reaction mixture was concentrated at reduced pressure and the residue dissolved in water (50mL), washed with diethyl ether (50mL) and the organic phase discarded. The aqueous phase was acidified by the addition of 1M hydrochloric acid and the product extracted into ethyl acetate. The organic phase was dried ( $\text{MgSO}_4$ ) and the solvent evaporated at reduced pressure to yield the title compound as an oil which solidified on standing, (895 mg, 98%).  $m/z$  (-ve ion ESI) 284 (M-H). <sup>1</sup>H NMR (500MHz,  $\text{CDCl}_3$ ) 0.89-0.91 (6H, m,  $\text{CH}_3$ ), 1.05 -1.08 (1H, m,

$\text{CH}_2\text{CH}(\text{CH}_3)_2$ ), 1.16-1.18 (1H, m,  $\text{CH}_2\text{CH}(\text{CH}_3)_2$ ), 1.47 (9H, s), 1.46-1.61 (1H, m, 4- $\text{CH}_A$  piperidine), 1.66-1.71 (1H, m,  $\text{CH}(\text{CH}_3)_2$ ), 1.70-1.84 (1H, m, 5- $\text{CH}$  piperidine), 2.03-2.08 (1H, m, 4- $\text{CH}_B$  piperidine), 2.66-2.71 (1H, m, 3- $\text{CH}$  piperidine), 2.90-3.05 (1H, m, 6- $\text{CH}_A$  piperidine), 3.52-3.58 (3H, m, 6- $\text{CH}_B$  and 2- $\text{CH}_A$  piperidine), 3.78-3.82 (1H, m, 2- $\text{CH}_B$  piperidine).

The *trans* geometry of the compound was confirmed from NMR analysis of the deprotected form, *trans*-5-(2-methylpropyl)piperidine-3-carboxylic acid.

#### ***trans*-5-(2-Methylpropyl)piperidine-3-carboxylic acid.**

*trans*-1-(*tert*-Butoxycarbonyl)-5-(2-methylpropyl)piperidine-3-carboxylic acid was deprotected with TFA to afford *trans*-5-(2-methylpropyl)piperidine-3-carboxylic acid.  $^1\text{H}$  NMR experiments enables a full assignment, showing 3- $\text{CH}$  of the piperazine ring to be equatorial, and 5- $\text{CH}$  of the piperazine ring to be axial.  $^1\text{H}$  NMR (500MHz, MeOD) 0.89-0.91 (6H, m,  $\text{CH}_3$ ), 1.13 -1.20 (2H, m,  $\text{CH}_2\text{CH}(\text{CH}_3)_2$ ), 1.53 (appears as dt J = 5, 10 Hz 4- $\text{CH}_{\text{ax}}$  piperidine), 1.66-1.71 (1H, m,  $\text{CH}(\text{CH}_3)_2$ ), 1.70-1.84 (1H, m, 5- $\text{CH}$  piperidine), 2.25 (1H, appears as br d, J = 15 Hz, 4- $\text{CH}_{\text{eq}}$  piperidine), 2.65 (1H, appears as t, J = 10 Hz, 6- $\text{CH}_{\text{ax}}$  piperidine), 2.99 (1H, appears as br s, 3- $\text{CH}$  piperidine), 3.05 (1H, dd, J 5, 15 Hz, 2- $\text{CH}_A$ ), 3.21 (1H, appears as br d, J=10 Hz, 6- $\text{CH}_{\text{eq}}$  piperidine), 3.60 (1H, appears as br d, J = 15 Hz, 2- $\text{CH}_B$  piperidine)

### **1.5 Piperazine-2-carboxylic acids**

Prepared by reductive alkylation of the commercially available (2*S*)-4-BOC piperazine-2-carboxylic acid

### **(2S)-4-(tert-Butoxycarbonyl)-1-(2-methylpropyl)piperazine-2-carboxylic acid**

A solution of 2-methylpropanal (0.392 mL, 4.3 mmol) in tetrahydrofuran (2 mL) was added to a stirred solution of (2S)-4-(tert-butoxycarbonyl)-piperazine-2-carboxylic acid (1.0 g, 4.3 mmol) in tetrahydrofuran (130 mL). Acetic acid (0.9 mL, 15.8 mmol) was added to the solution. After stirring the reaction mixture for 30 minutes, sodium triacetoxyborohydride (1.37 g, 6.5 mmol) was added in portions over 5 minutes and the mixture stirred for 18h. The mixture was then filtered through Celite and the filtrate evaporated to an oil (1.47 g). The oil was stirred with diethyl ether (20 mL) and the insoluble solid was filtered off. The filtrate was evaporated and the residue was purified by column chromatography over silica gel, eluting with a gradient of methanol/ethyl acetate to give the title compound as a white solid (528 mg, 43%),  $m/z$  (+ve ion ESI) 287  $[M+H]^+$ ,  $^1H$  NMR (300MHz, MeOD) 1.03 (3H, d,  $CH_3$ ), 1.14 (3H, d,  $CH_3$ ), 1.50 (9H, s, BOC), 2.10-2.25 (1H, m,  $CH(CH_3)_2$ ), 2.86 (1H, dd), 2.98-3.06 (1H, m), 3.13 (1H, dd), 3.45-3.64 (4H, m), 4.00 (1H, appears as br. d), 4.28 (1H, appears as br.d).

### **(2S)-1-Benzyl-4-(tert-butoxycarbonyl)piperazine-2-carboxylic acid**

The title compound was prepared using the methodology described for (2S)-4-(tert-butoxycarbonyl)-1-(2-methylpropyl)piperazine-2-carboxylic acid.  $m/z$  (-ve ion ESI) 321  $[M-H]^-$ .

## **2 Chiral separation of 3-[(tert-butoxycarbonyl)amino]methyl-4-phenylbutanoic acid and confirmation of stereochemistry by small molecule X-ray crystallography.**

Small molecular crystallographic data will be submitted upon publication to the Cambridge Structural Database ([www.ccdc.cam.ac.uk](http://www.ccdc.cam.ac.uk)).

## **2.1 Separation of enantiomers of 4-((*tert*-butoxycarbonyl)amino)-3-(3-chlorophenyl)butanoic acid.**

4-((*tert*-Butoxycarbonyl)amino)-3-(3-chlorophenyl)butanoic acid was purified by supercritical fluid chromatography using the preparative separation conditions described below. After separation, the fractions were evaporated under reduced pressure at bath temperature 40°C to obtain the desired separated enantiomers. The slower-running enantiomer was then further purified on the same column eluting with 20% B. Enantiomeric purity was confirmed using the chiral analytical chromatography method below.

Obtained the separated enantiomers:

4-{{*tert*-Butoxycarbonyl}amino}-3-(3-chlorophenyl)butanoic acid (enantiomer 1), 29.72g, retention time 2.796 mins (analytical method), 99.1%ee.

4-{{*tert*-Butoxycarbonyl}amino}-3-(3-chlorophenyl)butanoic acid (enantiomer 2), 31.73g, retention time 3.264 mins (analytical method). Assigned the (3*S*)-stereochemistry by small molecule crystallography of the BOC-protected material, as described below.

Chiral preparative chromatography conditions:

Instrument: Thar 200 preparative SFC (SFC-10)

Column: ChiralPak AY, 300 × 50 mm I.D., 10 μm

Mobile phase A: CO<sub>2</sub> and Mobile phase B: IPA

Gradient: A:75%, B 25%

Flow rate: 200 mL /min

Back pressure: 100 bar

Column temperature: 38°C

Wavelength: 220 nm

Cycle time: ~4.5 min

Chiral analytical chromatography conditions:

Instrument: Waters UPC2 analytical SFC (SFC-H)

Column: ChiralPak AY, 150 × 4.6 mm I.D., 3 μm

Mobile phase: A: CO<sub>2</sub> and Mobile phase B: IPA (0.05% DEA)

Gradient: B 5-40%

Flow rate: 2.5 mL/min

Back pressure: 100 bar

Column temperature: 40°C

Wavelength: 220 nm

## 2.2 Confirmation of stereochemistry

### **Trifluoroacetate salt of (3S)-4-amino-3-(3-chlorophenyl)butanoic acid**

To an ice water cooled solution of 4-(*tert*-butoxycarbonylamino)-3-(3-chlorophenyl)butanoic acid isomer 2 (Retention time 3.264 mins on chiral HPLC) (1.5 g, 4.78 mmol) in dichloromethane (20 ml) was added trifluoroacetic acid (5 mL). After addition, the mixture was stirred at this temperature for 4 hours. The reaction mixture was then concentrated. The crude mixture was dissolved in water (10 mL) and lyophilized to afford the product; TFA salt of (3S)-4-amino-3-(3-chlorophenyl)butanoic acid (1.5 g, 95% yield). *m/z* 214 (M+H)<sup>+</sup>

### **(3S)-4-Amino-3-(3-chlorophenyl)butanoic acid**

To a solution of the trifluoroacetic acid salt of (3S)-4-amino-3-(3-chlorophenyl)butanoic acid (0.5 g, 1.53 mmol) in water (10 mL), was added diluted *aq.* ammonium hydroxide until the pH reached 7. The resulting solution was stored in an open 25 mL flask and stood at room temperature for 1 day. Upon crystallization, the mother liquor was decanted, and the solid crystals were submitted to X-ray diffraction studies. X-ray diffraction studies showed the compound to have the (*S*) stereochemistry ( **Figure S1**)

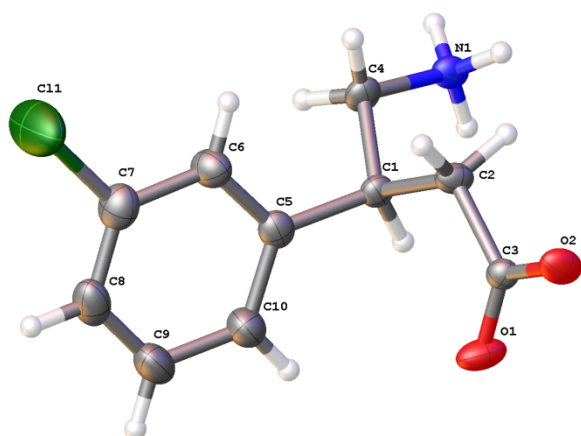
X-ray diffraction conditions:

#### **Experimental**

A suitable crystal of C<sub>10</sub>H<sub>12</sub>ClNO<sub>2</sub> was selected and mounted on a Bruker APEX-II CCD diffractometer. The crystal was kept at 302.71 K during data collection. Using Olex2<sup>S3</sup>, the structure was solved with the ShelXT<sup>S4</sup> structure solution program using Intrinsic Phasing and refined with the ShelXL<sup>S5</sup> refinement package using Least Squares minimisation.

#### **Crystal structure determination of (3S)-4-amino-3-(3-chlorophenyl)butanoic acid**

Crystal Data for C<sub>10</sub>H<sub>12</sub>ClNO<sub>2</sub> (*M* = 213.66 g/mol): monoclinic, space group P2<sub>1</sub> (no. 4), *a* = 7.707(2) Å, *b* = 6.821(3) Å, *c* = 10.508(4) Å, β = 106.963(17)°, *V* = 528.3(3) Å<sup>3</sup>, *Z* = 2, *T* = 302.71 K, μ(GaKα) = 1.986 mm<sup>-1</sup>, *D*<sub>calc</sub> = 1.343 g/cm<sup>3</sup>, 10593 reflections measured (7.652° ≤ 2Θ ≤ 121.154°), 2364 unique (*R*<sub>int</sub> = 0.0491, *R*<sub>sigma</sub> = 0.0393) which were used in all calculations. The final *R*<sub>1</sub> was 0.0766 (*I* > 2σ(*I*)) and *wR*<sub>2</sub> was 0.2284 (all data).



**Figure S1. (3*S*)-4-amino-3-(3-chlorophenyl)butanoic acid**

### **3 Synthesis of dipeptides**

#### **CBZThr (O-*t*Bu)-Dab(BOC)OH**

To a stirred suspension of (*S*)-2-(benzyloxycarbonyl)amino-3-(*tert*-butoxy)butyric acid DHCA salt (3.65g, 7.4mmol) and (*S*)-2-amino-4-[(*tert*-butoxycarbonyl)amino]butyric acid methyl ester HCl salt (2.0g, 7.4mmol) in a mixture of DCM (60ml) and DMF (120ml) was added *N,N*-diisopropylethylamine (3.85ml, 22.1mmol). To this stirred mixture was added 1-hydroxy-7-azabenzotriazole (1.0g, 7.3mmol) followed by *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide HCl salt (1.42g, 7.4mmol). The mixture was stirred for 17h at ambient temperature then filtered under suction to remove the insoluble by-product, which was discarded. The filtrate was concentrated to a yellow oil which was partitioned between a solvent mixture of EtOAc/Et<sub>2</sub>O (1:1) (250ml) and 0.5M hydrochloric acid (200ml). The aqueous phase was re-extracted with fresh solvent mixture (100 ml) and the combined organic extracts were successively washed with water (150ml) and sat. NaHCO<sub>3</sub> solution (150ml), dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated to a colourless oil (3.72g). This oil was purified by silica gel chromatography, eluting with a solvent gradient of EtOAc/iso-hexane (0-70%).

Fractions containing the product (Rf 0.26 in EtOAc/iso-hexane 3:7, visualized with KMnO<sub>4</sub> spray) were pooled and concentrated to give the protected dipeptide as a colourless foam (3.58g, 6.8mmol, 92%), *m/z* 524 [M+H]<sup>+</sup>.

This material was dissolved in methanol (64ml), treated with a solution of lithium hydroxide monohydrate (0.861g, 20.5mmol) in water (16ml) and the mixture stirred at ambient temperature for 19h. To this solution was added 1M HCl (24ml) resulting in a milky mixture (pH 1) which was quickly extracted with DCM (3 x 135ml). The combined organic extract was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated to give the title compound as a colourless foam (3.27g, 6.4mmol, 86%), *m/z* 1041[2M+Na]<sup>+</sup>, 532[M+Na]<sup>+</sup>, 510 [M+H]<sup>+</sup>.

<sup>1</sup>H nmr (300 MHz, CDCl<sub>3</sub>) 1.06 – 1.08 (4H, m), 1.23 (9H, s), (1.46, 9H, s), 1.86-2.15 (2H, m), 3.0 -3.20 (1H, m), 3.35-3.50 (1H, m), 4.15-4.26 (2H, m), 4.48-4.63 (1H, m), 5.09 – 5.19 (3H, m), 5.94 (3H, br.s, 1H), 7.33-7.37 ( 5H, m), 7.88 (br.s, 1H ).

#### **CBZThr (O-<sup>t</sup>Bu)-Dap(BOC)OH**

Prepared as described above for CBZ Thr(O-<sup>t</sup>Bu)-Dab(BOC)OH on 9 mmol scale in 73% overall yield. *m/z* 496 [M+H]<sup>+</sup>. <sup>1</sup>H nmr (300 MHz, CDCl<sub>3</sub>) 1.08 – 1.10 (4H, m), 1.28 (9H, s), (1.44, 9H, s), 3.0 -3.20 (1H, m), 3.48-3.68 (2H, m), 4.15-4.26 (2H, m), 4.50 (1H, br s), 5.09 – 5.19 (3H, m), 5.84 (3H, br.s, 1H), 7.36-7.38 ( 5H, m), 8.18 (1H, br.s)

#### **4 Characterisation of final compounds (2) - (43) and (46) - (47)**

Analytical HPLC methods:

Method (1)

Column: Phenomenex Hyperclone C18 BDS 5 μm × 4.6 mm × 150 mm

Mobile phase: A: water/acetonitrile 90/10, v/v, 0.15% TFA.

B: acetonitrile/water 90/10, v/v, 0.15% TFA

Flow rate: 1 mL/min

Gradient:	Time (mins)	% mobile phase A
	0	100%
	20	0%
	11	0%
	11.2	100%

Cycle time: 15 mins

Detection: 210, 254 nm

Injection volume: 20  $\mu$ L

#### Method (2)

Column: Phenomenex Hyperclone C18 BDS 5  $\mu$ m  $\times$  4.6 mm  $\times$  150 mm

Mobile phase: A: water/acetonitrile 90/10, v/v, 0.15% TFA.

B: acetonitrile/water 90/10, v/v, 0.15% TFA

Flow rate: 1 mL/min

Gradient:	Time (mins)	% mobile phase A
	0	100%
	20	40%
	21	0%
	23	0%
	23.5	100
	25	100

Detection: 210, 254 nm

Injection volume: 20  $\mu$ L

All mass spectrometry was carried out by +ve ion ESI unless otherwise stated.

**(2) Octanoyl-Thr-Dab-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] acetate salt.** HPLC rt

(method 1) 6.38 min.  $m/z$  1090  $[M+H]^+$ , 546  $[M+2H]^{2+}$ .  $^1H$  NMR (400MHz,  $D_2O$ ) 0.52

(3H, d,  $J = 6.1$  Hz), 0.59 (3H, d,  $J = 6.2$  Hz), 0.64 - 0.71 (4H, m), 0.98– 1.15 (14H, m, incl

1.01 (d,  $J=6.1$  Hz), 1.10 (d,  $J = 6.4$  Hz)), 1.20 – 1.35 (2H, m), 1.38 – 1.46 (2H, m), 1.65-2.25

(24H, m, incl 1.73, s, OAc), 2.55 – 2.72 (2H, m), 2.85-3.00 (9H, m), 3.14 – 3.22 (1H, m),

4.03 – 4.16 (8H, m), 4.28 – 4.32 (2H, m) 4.41 (1H, t,  $J = 8.1$  Hz), 7.08 – 7.25 (5H, m).

**(3) Octanoyl-Dab-Thr-Dab-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] TFA salt.**

HPLC rt (method 1) 5.85 min.  $m/z$  1190  $[M+H]^+$ , 596  $[M+2H]^{2+}$ , 397 $[M+3H]^{3+}$ .  $^1H$  NMR

(300MHz,  $D_2O$ ) 0.59 (3H, d), 0.70 (3H, d), 0.75 - 0.80 (4H, m), 1.08 – 1.27 (14H, m), 1.31 –

1.42 (2H, m), 1.45 – 1.58 (2H, m), 1.75-2.28 (14H, m), 2.64 – 2.83 (2H, m), 2.95-3.14 (11H,

m), 3.20 – 3.30 (1H, m), 4.12 – 4.25 (7H, m), 4.28 (1H, d), 4.38 – 4.48 (3H, m) 4.52 (1H, t),

7.15 – 7.32 (5H, m).

**(4) Octanoyl-Gly-Thr-Dab-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] TFA salt.**  $m/z$  1147

$[M+H]^+$ , 574  $[M+2H]^{2+}$ .  $^1H$  NMR (300MHz,  $D_2O$ ) 0.58 (3H, d), 0.67 (3H, d), 0.70 - 0.82

(4H, m), 1.10 – 1.25 (14H, m), 1.32 – 1.42 (2H, m), 1.45 – 1.58 (2H, m), 1.76-2.28 (14H, m),

2.65 – 2.85 (2H, m), 2.95-3.15 (11H, m), 3.20 – 3.35 (1H, m), 3.90 ( 2H, ABq), 4.10 – 4.28

(8H, m), 4.35 – 4.41 (2H, m) 4.53 (1H, t), 7.18 – 7.32 (5H, m).

**(5) Octanoyl-DDab-Thr-Dab-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] TFA salt.** HPLC

rt (method 1) 5.82 min.  $m/z$  1190  $[M+H]^+$ . HRMS (+ve nESI)  $[M + H]^+$  calcd for

$C_{55}H_{97}N_{16}O_{13}$ , 1189.7416; found, 1189.7410;  $[M + 2H]^{2+}$ ; calcd for  $C_{55}H_{98}N_{16}O_{13}$ ,

595.3744; found, 595.3738;  $[M + 3H]^{3+}$  calcd for  $C_{55}H_{99}N_{16}O_{13}$ , 397.2520; found, 397.2522.  $^1H$  NMR (400MHz,  $D_2O$ ) 0.64 (3H, d,  $J = 6.3$  Hz), 0.71 (3H, d,  $J = 6.4$  Hz), 0.72 - 0.84 (4H, m), 1.08 - 1.29 (14H, m, incl 1.13 (d,  $J=6.3$  Hz), 1.15 (d,  $J = 6.5$  Hz)), 1.29 - 1.56 (4H, m), 1.76-2.27 (14H, m), 2.66 - 2.83 (2H, m), 2.95-3.10 (11H, m), 3.23 - 3.29 (1H, m), 4.13 - 4.28 (8H, m), 4.37 - 4.45 (4H, m) 4.53 (1H, t,  $J = 8.2$  Hz), 7.19 - 7.35 (5H, m).

**(6) [2-(2-Aminoethyl)octanoyl]-Thr-Dab-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr]**

**diastereomer 1, TFA salt.** HPLC rt (method 1) 5.53 min.  $m/z$  1133  $[M+H]^+$ , 567  $[M+2H]^{2+}$ .

HRMS (+ve nESI)  $[M + H]^+$  calcd for  $C_{53}H_{94}N_{15}O_{12}$ , 1132.7201; found, 1132.7187;  $[M + 2H]^{2+}$  calcd for  $C_{53}H_{95}N_{15}O_{12}$ , 566.8637; found, 566.8627,  $[M + 3H]^{3+}$  calcd for  $C_{53}H_{96}N_{15}O_{12}$ , 378.2449; found, 378.2450.  $^1H$  NMR (300MHz,  $D_2O$ ) 0.64 (3H, d), 0.68 - 0.86 (7H, m), 1.13 - 1.22 (14H, m), 1.38 - 1.52 (4H, m), 1.72- 2.22 (12H, m), 2.48 - 2.52 (1H, m), 2.68 - 3.15 (13H, m), 3.23 - 3.32 (1H, m), 4.08 - 4.30 (8H, m), 4.30 (1H, d), 4.38- 4.50 (2H, m), 4.55 (1H, t), 7.18 - 7.36 (5H, m).

**(7) [2-(2-Aminoethyl)octanoyl]-Thr-Dab-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr]**

**diastereomer 2, TFA salt.** HPLC rt (method 1) 5.90 min.  $m/z$  1133  $[M+H]^+$ , 567

$[M+2H]^{2+}$ . HRMS (+ve nESI)  $[M + H]^+$  calcd for  $C_{53}H_{94}N_{15}O_{12}$ , 1132.7201;

found, 1132.7187;  $[M + 2H]^{2+}$  calcd for  $C_{53}H_{95}N_{15}O_{12}$ , 566.8637; found, 566.8627,  $[M + 3H]^{3+}$  calcd for  $C_{53}H_{96}N_{15}O_{12}$ , 378.2449; found, 378.2450.  $^1H$  NMR (300MHz,  $D_2O$ ) 0.63 (3H, d), 0.71 (3H, d), 0.73-0.82 (4H, m), 1.10 - 1.24 (14H, m), 1.38 - 1.55 (4H, m), 1.75- 2.24 (12H, m), 2.50 - 2.60 (1H, m), 2.68 - 3.18 (13H, m), 3.24 - 3.34 (1H, m), 4.12 - 4.28 (7H, m), 4.30 (1H, d), 4.41- 4.50 (2H, m), 4.58 (1H, t), 7.20 - 7.46 (5H, m).

**(8) [3-(Aminomethyl)octanoyl]-Thr-Dab-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr]**

**diastereomer 1, acetate salt.** HPLC rt (method 1) 5.54 min  $m/z$  1117  $[M+H]^+$ , 559

$[M+2H]^{2+}$ .  $^1H$  NMR (400MHz,  $D_2O$ ) 0.54 – 0.70 (10 H, m, incl 0.55 (d)), 1.00-1.32(16H, m, incl 1.02 (d), 1.04 (d), 1.56-1.80 (18H, m, incl 1.72, s, OAc), 2.18 (1H, d), 2.50-2.72 (2H, m), 2.75-3.00 (11H, m), 3.08 – 3.10 (1H, m), 4.01 – 4.08 (8Hz, m), 4.28 – 4.38 (2H,m), 4.47 (1H, t), 7.08 – 7.25 (5H, m).

**(9) [3-(Aminomethyl)octanoyl]-Thr-Dab-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr]**

**diastereomer 2. TFA salt.** HPLC rt (method 1) 5.62 min  $m/z$  1117  $[M+H]^+$ , 559

$[M+2H]^{2+}$ .  $^1H$  NMR (400MHz,  $D_2O$ ) 0.63 (3H, d,  $J = 6.0$  Hz), 0.69 - 0.80 (7H, m), 1.12 – 1.47 (16H, m, incl 1.13 (d,  $J=6.4$  Hz), 1.16 (d,  $J = 6.4$  Hz)), 1.75-2.24 (11H, m), 2.34 – 2.45 (2H, m), 2.68-2.85 (2H, m), 2.90-3.12 (11H, m), 3.24 – 3.31 (1H, m), 4.12 – 4.29 (8H, m), 4.47 – 4.45 (2H, m) 4.51 (1H, t,  $J = 8.2$  Hz), 7.18 – 7.33(5H, m).

**(10) [2-(Aminomethyl)octanoyl]-Thr-Dab-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr]**

**diastereomer 1, acetate salt.** HPLC rt (method 1) 5.50 min.  $m/z$  1119  $[M+H]^+$ , 560

$[M+2H]^{2+}$ . HRMS (+ve nESI)  $[M + H]^+$  calcd for  $C_{52}H_{92}N_{15}O_{12}$ , 1118.7044;

found,1118.7041;  $[M + 2H]^{2+}$  calcd for  $C_{52}H_{93}N_{15}O_{12}$ , 559.8559; found, 559.8553,  $[M +$

$3H]^{3+}$  calcd for  $C_{52}H_{94}N_{15}O_{12}$ , 373.5730; found, 373.5734.  $^1H$  NMR (300MHz,  $D_2O$ ) 0.63

(3H, d), 0.68 - 0.82 (7H, m), 1.10 – 1.25 (14H, m), 1.28 – 1.60 (5H, m), 1.75- 2.20 (21H, m,

incl 1.80 (s, OAc)), 2.68-2.82 (3H, m), 2.96 - 3.38 (12H,m), 4.08 – 4.42 (8H, m), 4.40- 4.58

(3H, m), 7.20 – 7.38 (5H, m).

**(11) [2-(Aminomethyl)octanoyl]-Thr-Dab-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr]**

**diastereomer 2, acetate salt.** HPLC rt (method 1) 5.90 min.  $m/z$  1119  $[M+H]^+$ , 560

$[M+2H]^{2+}$ .  $^1H$  NMR (300MHz,  $D_2O$ ) 0.62 (3H, d), 0.68 - 0.82 (7H, m), 1.11 - 1.30 (14H, m), 1.32 - 1.60 (5H, m), 1.75- 2.20 (21H, m, incl 1.82 (s, OAc)), 2.65-2.82 (3H, m), 2.96 - 3.38 (12H,m), 4.10 - 4.26 (7H, m), 4.32 (1H, d), 4.40- 4.53 (3H, m), 7.18 - 7.40 (5H, m).

**(12) [(2R)-2-Amino-2-cyclohexylacetyl]-Thr-Dab-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-**

**Thr] TFA salt.** HPLC rt (method 1) 5.24 min.  $m/z$  1103  $[M+H]^+$ , 552  $[M+2H]^{2+}$ .  $^1H$  NMR (400MHz,  $D_2O$ ) 0.49 - 0.74 (7H, m), incl 0.52, d, J 6.0, 0.58, d, J = 6.0)), 0.91 - 1.17 (11H, m, incl 1.02, d, J = 6.4, 1.06, d, J = 6.4)), 1.22 - 1.37 (2H, m), 1.46 - 2.12 (15H, m), 2.56- 2.75 (2H, m), 2.83 - 3.24 (11H, m), 3.76 (1H, d, J = 6.2), 4.00 - 4.14 (7H, m), 4.20 (1H, d, J = 4.4 Hz), 4.30 - 4.37 (2H, m), 4.41 (1H, appears as t, J 8.0), 7.08 - 7.31 (5H, m).

**(13) [(2S)-2-Amino-2-cyclohexylacetyl]-Thr-Dab-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-**

**Thr] TFA salt.** HPLC rt (method 1) 5.00 min.  $m/z$  1217  $[M+H+TFA]^+$ , 1103  $[M+H]^+$ , 552  $[M+2H]^{2+}$ . HRMS (+ve nESI)  $[M + H]^+$  calcd for  $C_{51}H_{88}N_{15}O_{12}$ , 1102.6731; found, 1102.6723;  $[M + 2H]^{2+}$  calcd for  $C_{51}H_{89}N_{15}O_{12}$ , 551.8402; found, 551.8394;  $[M + 3H]^{3+}$  calcd for  $C_{51}H_{90}N_{15}O_{12}$ , 368.2292; found, 368.2294.  $^1H$  NMR (400MHz,  $D_2O$ ) 0.61 - 0.82 (7H, m), 1.02 - 1.25 (11H, m, incl 1.13, d, J = 6.4, 1.15, d, J = 6.4)), 1.33 - 1.45 (2H, m), 1.57 - 2.21 (15H, m), 2.67-2.88 (2H, m), 2.91 - 3.16 (11H, m), 3.22 - 3.31 (1H, m), 3.83 (1H, d, J = 6.2), 4.08 - 4.26 (7H, m), 4.33 (1H, d, J = 5.5 Hz), 4.39 - 4.52 (3H, m), 7.13 - 7.34 (5H, m).

**(14) [(3S)-Piperidine-3-carbonyl]-Thr-Dab-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr]**

**TFA salt.** HPLC rt (method 1) 5.24 min.  $m/z$  1187 [M+TFA]<sup>+</sup>, 1074.5 [M+H]<sup>+</sup>, 595

[M+TFA+2H]<sup>2+</sup>. HRMS (+ve nESI) [M + H]<sup>+</sup> calcd for C<sub>49</sub>H<sub>84</sub>N<sub>15</sub>O<sub>12</sub>, 1074.6418;

found, 1074.6403; [M + 2H]<sup>2+</sup> calcd for C<sub>49</sub>H<sub>85</sub>N<sub>15</sub>O<sub>12</sub>, 537.8246; found, 537.8232, [M +

3H]<sup>3+</sup> calcd for C<sub>49</sub>H<sub>85</sub>N<sub>15</sub>O<sub>12</sub>, 358.8855; found, 358.8854. <sup>1</sup>H NMR (300MHz, D<sub>2</sub>O) 0.62

(3H, d), 0.67 (3H, d) 0.70 – 0.85 (1H, m), 1.08 – 1.13 (6H, m), 1.28 – 1.48 (2H, m), 1.65 -

2.18 (14H, m), 2.62 – 2.82 (2H, m), 2.85 -3.10 (11H, m), 3.20 – 3.34 (4H, m), 4.10 – 4.22

(7H, m), 4.25 (1H, d), 4.38 - 4.48 (2H, m), 4.50 (1H, t), 7.15 – 7.38 (5H, m).

**(15) [(3R)-Piperidine-3-carbonyl]-Thr-Dab-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr]**

**TFA salt.** HPLC rt (method 1) 5.00 min.  $m/z$  1074 [M+H]<sup>+</sup>, 538 [M+2H]<sup>2+</sup>. HRMS (+ve

nESI) [M + H]<sup>+</sup> calcd for C<sub>49</sub>H<sub>84</sub>N<sub>15</sub>O<sub>12</sub>, 1074.6418; found, 1074.6412; [M + 2H]<sup>2+</sup> calcd

for C<sub>49</sub>H<sub>85</sub>N<sub>15</sub>O<sub>12</sub>, 537.8246; found, 537.8239, [M + 3H]<sup>3+</sup> calcd for C<sub>49</sub>H<sub>85</sub>N<sub>15</sub>O<sub>12</sub>,

358.8855; found, 358.8859. <sup>1</sup>H NMR (300MHz, D<sub>2</sub>O) 0.61 (3H, d), 0.67 (3H, d) 0.70 – 0.85

(1H, m), 1.08 – 1.13 (6H, m), 1.28 – 1.45 (2H, m), 1.58 - 2.20 (14H, m), 2.62-3.12 (13H, m),

3.20 – 3.38 (4H, m), 4.05 – 4.22 (8H, m), 4.38 - 4.45 (2H, m), 4.50 (1H, t), 7.15 – 7.35 (5H,

m).

**(16) (Piperidine-4-carbonyl)-Thr-Dab-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] TFA**

**salt.**  $m/z$  1187 [M+TFA]<sup>+</sup>, 1075 [M+H]<sup>+</sup>, 594 [M+TFA]<sup>2+</sup>. <sup>1</sup>H NMR (400MHz, D<sub>2</sub>O) 0.50

– 0.70 (7H, m), 1.10 – 1.18 (6H, m), 1.24 – 1.45 (2H, m), 1.73 - 2.28 (14H, m), 2.66-3.11

(14H, m), 3.26 – 3.44 (3H, m), 4.07 – 4.27 (8H, m), 4.34 - 4.51 (3H, m), 7.19 – 7.39 (5H,

m).

**(17) [(*cis*)-5-Cyclohexylpiperidine-3-carbonyl]-Thr-Dab-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] diastereomer 1, TFA salt.** HPLC rt (method 1) 5.37 min. *m/z* 1157

[M+H]<sup>+</sup>, 579 [M+2H]<sup>2+</sup>. <sup>1</sup>H NMR (300MHz, D<sub>2</sub>O) 0.59 (3H, d), 0.66 (3H, d) 0.70 – 0.82 (1H, m), 0.89 – 1.04 (2H, m), 1.05 – 1.23 (10H, m), 1.32 – 1.45 (3H, m), 1.50-1.70 (6H, m), 1.75 – 2.20 (11H, m), 2.62-2.88 (5H, m), 2.95 – 3.12 (9H, m), 3.20 – 3.30 (1H, m), 3.35 – 3.48 (2H, m), 4.08 – 4.25 (8H, m), 4.38 - 4.44 (2H, m), 4.52 (1H, t), 7.18 – 7.38 (5H, m).

**(18) [(*cis*)-5-Cyclohexylpiperidine-3-carbonyl]-Thr-Dab-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] diastereomer 2, TFA salt.** HPLC rt (method 1) 5.79 min. *m/z* 1157

[M+H]<sup>+</sup>, 579 [M+2H]<sup>2+</sup>. <sup>1</sup>H NMR (300MHz, D<sub>2</sub>O) 0.61 (3H, d), 0.68 – 0.80 (4H, m, incl 0.72, d), 0.90 – 1.04 (2H, m), 1.05 – 1.22 (10H, m), 1.32 – 1.48 (3H, m), 1.54-1.68 (6H, m), 1.72 – 2.20 (11H, m), 2.62-2.93 (5H, m), 2.98 – 3.11 (9H, m), 3.20 – 3.30 (1H, m), 3.35 – 3.42 (2H, m), 4.10 – 4.28 (8H, m), 4.40 - 4.48 (2H, m), 4.48 (1H, t), 7.18 – 7.36 (5H, m).

**(19) [(*trans*)-5-Cyclohexylpiperidine-3-carbonyl]-Thr-Dab-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] diastereomer 2, TFA salt.** HPLC rt (method 1) 5.70 min. *m/z* 1157 [M+H]<sup>+</sup>,

579 [M+2H]<sup>2+</sup>. <sup>1</sup>H NMR (300MHz, D<sub>2</sub>O) 0.61 (3H, d), 0.63 – 0.70 (4H, m, incl 0.64, d), 0.76 – 0.94 (2H, m), 1.00 – 1.20 (10H,m), 1.32 – 1.43 (2H, m), 1.50-1.62 (7H, m), 1.72 – 2.21 (11H, m), 2.62-2.80 (3H, m), 2.92 – 3.11 (11H, m), 3.20 – 3.42 (2H, m), 3.45 – 3.50 (1H,m), 4.08 – 4.25 (7H, m), 4.36 (1H.), 4.38 - 4.42 (2H,m), 4.46 (1H, t), 7.13 – 7.30 (5H, m).

**(20) [(trans)-5-(2-Methylpropyl)piperidine-3-carbonyl]-Thr-Dab-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] diastereomer 2, acetate salt.** HPLC rt (method 1) 5.58 min.  $m/z$  1130  $[M+H]^+$ , 566  $[M+2H]^{2+}$ .  $^1H$  NMR (400MHz,  $D_2O$ ) 0.49 – 0.77 (13H, m, incl 0.68, d,  $J = 6.4$  Hz, 0.71, d,  $J = 6.6$  Hz), 1.00 (3H, d,  $J = 6.3$  Hz), 1.05 (3H, d,  $J = 6.1$  Hz), 1.81 – 1.34 (2H, m), 1.43-1.58 (2H, m), 1.62 – 2.20 (27H, m, incl 1.72, s (OAc)), 2.33 – 2.40 (1H, m), 2.54-2.71 (2H, m), 2.84 – 3.27 (17H, m), 4.00 – 4.11 (7H, m), 4.15 – 4.18 (1H, m), 4.29-4.35 (2H,m), 4.38 (1H, appears as t,  $J = 8.5$  Hz), 7.07 – 7.26 (5H, m).

**(21) [(trans)-5-Phenylpiperidine-3-carbonyl]-Thr-Dab-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] diastereomer 2, TFA salt.** HPLC rt (method 1) 5.93 min.  $m/z$  1151  $[M+H]^+$ , 577  $[M+2H]^{2+}$ .  $^1H$  NMR (400MHz,  $D_2O$ ) 0.53 (3H, d,  $J = 6.3$  Hz), 0.56 - 0.71 (4 H, incl d,  $J = 6.2$  Hz), 1.03 (3H, d,  $J = 6.3$  Hz), 1.06 (3H, d,  $J = 6.5$  Hz), 1.23 – 1.37 ( 2H, m), 1.62-2.18 (12H, m), 2.56 – 2.76 (2H, m), 2.87 – 3.20 (15H, m), 3.33 – 3.37 (1H, m), 3.52 (1H, d,  $J = 12.9$  Hz), 4.03 – 4.17 (7H, m), 4.31 – 4.36 (2H,m), 4.42 (1H, appears as t,  $J = 8.6$  Hz), 4.67 – 4.81 (m, under  $D_2O$ ), 7.09 – 7.32 (10H, m).

**(22) [(2S)-1-(2-Methylpropyl)piperazine-2-carbonyl]-Thr-Dab-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] acetate salt.** HPLC rt (method 1) 5.28 min.  $m/z$  1132  $[M+H]^+$ , 567  $[M+2H]^{2+}$ .  $^1H$  NMR (400MHz,  $D_2O$ ) 0.55 - 0.68 (7H, m), 0.75 (3H, d,  $J = 6.6$  Hz), 0.77 (3H, d,  $J = 6.5$  Hz), 1.06 (3H, d,  $J = 6.2$  Hz), 1.11 (3H, d,  $J = 6.3$  Hz), 1.23 – 1.32 ( 2H, m), 1.68-2.26 (27H, m, incl 1.76 (s, OAc)), 2.38 – 2.46 (1H, m), 2.60-2.77 (2H, m), 2.89 – 3.24 (13H, m), 3.26 – 3.32 (3H, m), 4.05 – 4.17 (7H, m), 4.21 (1H, d,  $J 4.8$  Hz), 4.36 – 4.39 (2H,m), 4.44 (1H, appears as t,  $J = 8.1$  Hz), 7.12 – 7.30 (5H, m).

**(23) [(2S)-1-Benzylpiperazine-2-carbonyl]-Thr-Dab-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] TFA salt.** HPLC rt (method 1) 5.45 min.  $m/z$  1166  $[M+H]^+$ , 583  $[M+2H]^{2+}$ .  $^1H$  NMR (300MHz,  $D_2O$ ) 0.62 (3H, d), 0.70 (3H, d), 0.71-0.85 (1H, m), 1.10 (3H, d), 1.18 (3H, d), 1.30 – 1.45 (2H, m), 1.71– 2.20 (10H, m), 2.50 – 2.58 (1H, m), 2.61 – 2.82 (2H, m), 3.00– 3.10 (11H, m), 3.16 - 3.40 (3H, m), 3.42 – 3.50 (3H, m), 3.88 (1H, d), 4.10 – 4.22 (7H, m), 4.31(1H, d), 4.38 – 4.44 (2H, m), 4.50 (1H, t), 7.18 – 7.38 (10H, m).

**(24) [(trans)-5-(2-Methylpropyl)piperidine-3-carbonyl]-Thr-Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] diastereomer 2, acetate salt.** HPLC rt (method 1) 5.45 min.  $m/z$  1117  $[M+H]^+$ , 559  $[M+2H]^{2+}$ .  $^1H$  NMR (400MHz,  $D_2O$ ) 0.52 (3H, d,  $J = 6.1$  Hz), 0.56 – 0.71 (10H, m), 1.03 (3H, d,  $J = 6.5$  Hz), 1.07 (3H, d,  $J = 6.5$  Hz), 1.21 – 1.36 (2H, m), 1.43-1.58 (2H, m), 1.65 – 1.89 (19H, m, incl 1.75, s (OAc)), 1.95 – 2.11 (5H, m), 2.54 (1H, t,  $J = 12.0$  Hz), 2.59-2.75 (2H, m), 2.87 – 3.03 (10H, m), 3.09 – 3.22 (3H, m), 3.28 (1H, dd,  $J = 3.2$ , 13.5 Hz), 3.38 (1H, d,  $J = 13.0$  Hz), 4.03 – 4.21 (7H, m), 4.29 (1H, d,  $J = 3.7$  Hz), 4.33 (1H, dd,  $J = 8.8$ , 5.4 Hz), 4.43 (1H, appears as t,  $J = 8.4$  Hz), 4.58 – 4.83 (m, under  $D_2O$  peak), 7.10 – 7.26 (5H, m).

**(25) [(trans)-5-Cyclohexylpiperidine-3-carbonyl]-Thr-Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] diastereomer 2, acetate salt.** HPLC rt (method 1) 5.92 min.  $m/z$  1143  $[M+H]^+$ , 572  $[M+2H]^{2+}$ .  $^1H$  NMR (400MHz,  $D_2O$ ) 0.54 (3H, d,  $J = 6.0$  Hz), 0.61 – 0.72 (4H, m, incl 0.62, d,  $J = 6.0$  Hz), 0.77 – 0.89 (2H, m), 1.05 (3H, d,  $J = 6.3$  Hz), 1.08 (3H, d,  $J = 6.3$  Hz), 1.22 – 1.38 (2H, m), 1.45-1.58 (6H, m), 1.62 – 1.91 (17H, m, incl 1.76, s (OAc)), 1.95 – 2.16 (5H, m), 2.59-2.76 (3H, m), 2.88 – 3.09 (11H, m), 3.16 – 3.29 (3H, m), 3.40 (1H,

d, J = 12.0 Hz), 4.04 – 4.22 (7H, m), 4.31 (1H, d, J 3.6 Hz), 4.34 (1H, dd, J = 8.8, 5.5 Hz), 4.43 (1H, appears as t, J = 8.1 Hz), 4.51 (1H, dd, J – 5.0, 8.8 Hz), 7.11 – 7.27 (5H, m).

**(26) [(*cis*)-5-Phenylpiperidine-3-carbonyl]-Thr-Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] diastereomer 2, acetate salt.** HPLC rt (method 1) 5.55 min.  $m/z$  1137 [M+H]<sup>+</sup>, 569 [M+2H]<sup>2+</sup>. <sup>1</sup>H NMR (400MHz, D<sub>2</sub>O) 0.54 (3H, d, J = 6.2 Hz), 0.61 – 0.74 (4H, m, incl 0.62, d, J = 6.3 Hz), 1.03 (6H, d, J = 6.3 Hz), 1.21 – 1.37 (2H, m), 1.70-1.93 (5H, m), 1.96 – 2.12 (4H, m), 2.20 (1H, d, J = 12.8 Hz), 2.57-2.75 (2H, m), 2.91 – 3.05 (11H, m), 3.15 – 3.22 (2H, m), 3.34 – 3.47 (3H, m), 4.04 – 4.17 (7H, m), 4.27 (1H, d, J 3.8 Hz), 4.35 (1H, dd, J = 9.0, 5.5 Hz), 4.43 (1H, appears as t, J = 8.2 Hz), 4.58 - 4.68 (m, partially obscured by D<sub>2</sub>O), 7.10 – 7.31 (10H, m).

**(27) [(2*S*)-1-(2-Methylpropyl)piperazine-2-carbonyl]-Thr-Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] Acetate salt.** HPLC rt (method 1) 5.20 min.  $m/z$  1118 [M+H]<sup>+</sup>, 559 [M+2H]<sup>2+</sup>. <sup>1</sup>H NMR (400MHz, D<sub>2</sub>O) 0.56 (3H, d, J = 6.1 Hz), 0.63 (3H, d, J = 6.2 Hz), 0.66-0.78 (7H, m), 1.05 (3H, d, J = 6.3 Hz), 1.11 (3H, d, J = 6.4 Hz), 1.23 – 1.39( 2H, m), 1.67-1.92 (19H, m, incl 1.78 (s, OAc)), 1.98 – 2.15 (4H, m), 2.18 – 2.28 (2H, m), 2.44 – 2.53 (1H, m), 2.60-2.78 (2H, m), 2.90 – 3.26 (13H, m), 3.31 – 3.37 (3H, m), 4.05 – 4.18 (7H, m), 4.29 (1H, d, J 4.1 Hz), 4.36 (1H, dd, J = 8.8, 5.5 Hz), 4.44 (1H, appears as t, J = 8.3 Hz), 4.58 -4.68 (m, partially obscured by D<sub>2</sub>O), 7.12 – 7.28 (5H, m).

**(28) [(2*S*)-1-Benzylpiperazine-2-carbonyl]-Thr-Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] TFA salt.** HPLC rt (method 1) 5.41 min.  $m/z$  1152 [M+H]<sup>+</sup>, 576 [M+2H]<sup>2+</sup>, 384 [M+3H]<sup>3+</sup>. <sup>1</sup>H NMR (300MHz, D<sub>2</sub>O) 0.61 (3H, d), 0.70 (3H, d), 0.71-0.85 (1H, m), 1.10

(3H, d), 1.20 (3H, d), 1.38 – 1.45 (2H, m), 1.71- 2.00 (4H, m), 2.05 – 2.20 (4H, m), 2.48 – 2.56 (1H, m), 2.60 – 2.85 (2H, m), 3.00– 3.20 (9H, m), 3.18 - 3.35 (4H,m), 3.40 – 3.50 (4H, m), 3.88 (1H, d), 4.10 – 4.24 (8H, m), 4.38 – 4.45 (2H, m), 4.50 (1H, t), 7.13 – 7.40 (10H, m).

**(29) [2-(Aminomethyl)octanoyl]-Thr-Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr]**

**diastereomer 2, acetate salt.** HPLC rt (method 1) 6.16 min.  $m/z$  1105  $[M+H]^+$ , 553

$[M+2H]^{2+}$ .  $^1H$  NMR (300MHz,  $D_2O$ ) 0.63 (3H, d), 0.71 (3H, d), 0.75-0.82 (4H, m), 1.12 – 1.26 (14H, m), 1.32 –1.65 (5H, m), 1.80- 2.00 (14H, m, incl 1.88 (s, OAc)), 2.06 – 2.22 (5H, m), 2.68-2.88 (3H, m), 3.00 - 3.35 (12H,m), 4.10 – 4.28 (7H, m), 4.35 (1H, d), 4.40- 4.62 (3H, m), 7.20 – 7.38 (5H, m).

**(30) [2-(2-Aminoethyl)octanoyl]-Thr-Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr]**

**diastereomer 1, acetate salt.** HPLC rt (method 1) 5.84 min.  $m/z$  1119  $[M+H]^+$ , 560

$[M+2H]^{2+}$ , 374  $[M+3H]^{3+}$ .  $^1H$  NMR (300MHz,  $D_2O$ ) 0.62 (3H, d), 0.71 (3H, d), 0.75-0.82 (4H, m), 1.10 – 1.24 (14H, m), 1.32 –1.48 (5H, m), 1.70- 2.25 (21H, m, incl 1.85 (s, OAc)), 2.40 – 2.51 (1H, m), 2.68 – 3.20 (14H, m), 3.30 - 3.40 (1H,m), 4.10 – 4.30 (8H, m), 4.38 – 4.50 (2H, m), 4.50- 4.60 (1H, m), 7.20 – 7.40 (5H, m).

**(31) [2-(2-Aminoethyl)octanoyl]-Thr-Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr]**

**diastereomer 2, acetate salt.** HPLC rt (method 1) 6.17 min.  $m/z$  1119  $[M+H]^+$ , 560

$[M+2H]^{2+}$ , 374  $[M+3H]^{3+}$ .  $^1H$  NMR (300MHz,  $D_2O$ ) 0.63 (3H, d), 0.71 (3H, d), 0.73-0.82 (4H, m), 1.10 – 1.22 (14H, m), 1.32 –1.50 (5H, m), 1.68- 2.18 (21H, m, incl 1.85 (s, OAc)),

2.40 – 2.50 (1H, m), 2.58 – 3.18 (14H, m), 3.28 - 3.38 (1H,m), 4.10 – 4.28 (7H, m), 4.30 (1H, d), 4.40- 4.55 (3H, m), 7.20 – 7.42 (5H, m).

**(32) [2-(2-Aminoethyl)-5-methylhexanoyl]-Thr-Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] diastereomer 2, acetate salt.** HPLC rt (method 1) 5.91 min.  $m/z$  1104 [M+H]<sup>+</sup>, 553 [M+2H]<sup>2+</sup>. <sup>1</sup>H NMR (400MHz, D<sub>2</sub>O) 0.58 (3H, d, J = 6.1 Hz), 0.65 (3H, d, J = 6.2 Hz), 0.72-0.74 (7H, m), 0.93 – 1.04 (1H, m), 1.07(3H, d, J = 6.3 Hz), 1.12 (3H, d, J = 6.4 Hz), 1.23 – 1.48( 5H, m), 1.66-1.94 (20H, m, incl 1.80 (s, OAc)), 1.98 – 2.19 (4H, m), 2.40 – 2.47 (1H, m), 2.61 – 3.06 (12H, m), 3.14 – 3.25 (2H, m), 3.34 (1H, dd, J 4.9, 13.5 Hz), 4.07 – 4.20 (7H, m), 4.30 (1H, d, J 4.0 Hz), 4.38 (1H, dd, J = 8.8, 5.5 Hz), 4.48(1H, appears as t, J = 8.4 Hz), 4.62 -4.72 (1H, m, partially obscured by D<sub>2</sub>O), 7.14 – 7.29 (5H, m).

**(33) [4-Amino-2-(cyclohexylmethyl)butanoyl]-Thr-Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] diastereomer 2, acetate salt.** HPLC rt (method 1) 5.61 min.  $m/z$  1117 [M+H]<sup>+</sup>, 559 [M+2H]<sup>2+</sup>. <sup>1</sup>H NMR (400MHz, D<sub>2</sub>O) 0.58 (3H, d, J = 6.1 Hz), 0.65 (3H, d, J = 6.2 Hz), 0.68-0.78 (1H, m), 0.82 -1.15 (12H, m, incl 1.07(d, J = 6.3 Hz), 1.12 (d, J = 6.4 Hz)), 1.25-1.93 (29H, m, incl 1.81 (s, OAc)), 1.99 – 2.26 (4H, m), 2.61 – 3.07 (12H, m), 3.14 – 3.25 (2H, m), 3.37 (1H, dd, J 4.9, 13.5 Hz), 4.07 – 4.30 (7H, m), 4.37 (1H, d, J 4.1 Hz), 4.38 (1H, dd, J = 8.5, 5.3 Hz), 4.46 (1H, appears as t, J = 8.4 Hz), 4.71 (1H, dd, J = 4.7, 8.7 Hz)), 7.14 – 7.30 (5H, m).

**(34) [4-Amino-2-(cyclohexylmethyl)butanoyl]-Thr-Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] diastereomer 2, TFA salt** HPLC rt (method 1) 5.73 min.  $m/z$  1244[M+TFA]<sup>+</sup>, 1131 [M+H]<sup>+</sup>, 586 [M+2H]<sup>2+</sup>. <sup>1</sup>H NMR (300MHz, D<sub>2</sub>O) 0.60 (3H, d), 0.66-0.91 (6H, m,

incl 3H, d), 1.05 -1.20 (10H, m, incl 1.15(d), 1.18 (d)), 1.22-1.98 (15H, m), 2.05 – 2.25 (4H, m), 2.60 – 3.12 (12H, m), 3.18 – 3.32 (2H, m), 3.45 (1H, dd), 4.18 – 4.28 (7H, m), 4.38 (1H, d), 4.40 – 4.50 (2H, m), 4.74 – 4.80 (1H, m), 7.15 – 7.30 (5H, m).

**(35) [4-Amino-2-(4-chlorophenyl)-butanoyl]-Thr-Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] diastereomer 2, acetate salt.** HPLC rt (method 1) 5.53 min.  $m/z$  1145 [M+H]<sup>+</sup>, 573 [M+2H]<sup>2+</sup>. <sup>1</sup>H NMR (400MHz, D<sub>2</sub>O) 0.55 (3H, d, J = 6.2 Hz), 0.61 – 1.76 (4H, m incl d, J = 6.3 Hz), 0.81 (3H, d, J = 6.4 Hz), 1.04 (3H, d, J = 6.4 Hz), 1.22-1.38 (2H, m), 1.66 – 1.90 (18H, m, incl 1.76 (s, OAc)), 1.90 – 2.23 (6H, m), 2.57 – 3.22 (14H, m), 3.30 (1H, dd, J = 5.1, 13.4 Hz), 3.72( 1H, dd, J = 6.9, 8.5 Hz), 4.00 – 4.17 (7H, m), 4.22(1H, d, J = 3.7 Hz), 4.34 (1H, dd, J = 5.4, 8.7 Hz), 4.43 (1H, appears as t, J = 8.1 Hz), 7.09 – 7.32 (9H, m).

**(36) [4-Amino-2-(4-chlorophenyl)-butanoyl]-Thr-Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] diastereomer 2, acetate salt.** HPLC rt (method 1) 5.55 min.  $m/z$  1145 [M+H]<sup>+</sup>, 573 [M+2H]<sup>2+</sup>. <sup>1</sup>H NMR (400MHz, D<sub>2</sub>O) 0.50 (3H, d, J = 5.9 Hz), 0.57 – 1.66 (4H, m incl d, J = 5.9 Hz), 0.76 (3H, d, J = 6.5 Hz), 1.00, ( 3H, d, J = 6.4 Hz), 1.18-1.35 (2H, m), 1.62 – 1.86 (19H, m, incl 1.73 (s, OAc)), 1.91 – 2.22 (6H, m), 2.53 – 3.26 (15H, m), 3.67 – 3.71 (1H, m), 4.00 – 4.14 (7H, m), 4.19(1H, d, J = 3.7 Hz), 4.31 (1H, dd, J = 5.5, 8.8 Hz), 4.39 (1H, appears as t, J = 8.4 Hz), 7.06 – 7.29 (9H, m).

**(37) [4-Amino-2-(naphthalene-2-yl)-butanoyl]-Thr-Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] diastereomer 2, acetate salt.** HPLC rt (method 1) 5.67 min.  $m/z$  1161 [M+H]<sup>+</sup>, 581 [M+2H]<sup>2+</sup>. <sup>1</sup>H NMR (400MHz, D<sub>2</sub>O) 0.50 – 0.73 ( 10H, m, incl 0.50 (d, J = 6.1 Hz), 0.58 (d, J = 6.1 Hz), 0.68 (d, J = 6.5 Hz), 0.99 (3H, d, J = 6.3 Hz), 1.35-1.65 (2H, m), 1.86 –

1.92 (19H, m, incl 1.73 (s, OAc)), 1.95 – 2.32 (6H, m), 2.54 – 3.19 (13H, m), 3.29 (1H, dd, J = 4.9, 13.4 Hz), 3.87 – 3.91 (1H, m), 3.99 – 4.13 (7H, m), 4.22 (1H, d, J = 3.6 Hz), 4.30 (1H, d, J = 5.8, 9.3 Hz), 4.40 (1H, appears as t, J = 8.2 Hz), 7.05 – 7.45 (8H, m), 7.73 – 7.87 (4H, m).

**(38) {4-Amino-2-[(4-chlorophenyl)methyl]butanoyl}-Thr-Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] diastereomer 2, acetate salt.** HPLC rt (method 1) 5.69 min.  $m/z$  1159  $[M+H]^+$ , 580  $[M+2H]^{2+}$ .  $^1H$  NMR (400MHz,  $D_2O$ ) 0.52-0.72 (10H, m, incl 0.53 (d, J = 6.1 Hz), 0.56 (J= 6.4 Hz), 1.03 (3H, d, J = 6.4 Hz), 1.21-1.37 (2H, m), 1.63- 1.91 (22H, m, incl 1.75, s, OAc), 1.94-2.12 (4H, m), 2.52 – 3.19 (17H, m), 3.28 (1H, dd, J = 5.0, 13.3 Hz), 3.75- 3.82 (1H, m), 4.02 – 4.15 (7H, m), 4.33 (1H, dd, J = 5.6, 9.4 Hz), 4.41 (1H, t, J = 8.5 Hz), 4.59 – 4.65 (1H, m, partially obscured by HOD), 7.02 – 7.25 (9H, m).

**(39) {4-Amino-2-[(naphthalene-2-yl)methyl]butanoyl}-Thr-Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] diastereomer 2, TFA salt.** HPLC rt (method 1) 6.00 min  $m/z$  1176  $[M+H]^+$ , 586  $[M+2H]^{2+}$ .  $^1H$  NMR (300MHz,  $D_2O$ ) 0.15 (3H, d), 0.65-0.88 (7H, m, incl 0.66 (d), 0.70 (d)), 1.08 (3H, d), 1.26-1.48 (2H, m), 1.68 – 2.30 (10H, m), 2.65 – 3.38 (17H, m), 3.58 – 3.68 (1H, m), 4.05-4.22 (8H, m), 4.42 (1H, dd), 4.50 (1H, t), 7.15-7.62 (9H, m), 7.80 - 7.90 (3H, m).

**(40) [3-(Aminomethyl)octanoyl]-Thr-Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] diastereomer 1, acetate salt.** HPLC rt (method 1) 5.72 min  $m/z$  1105  $[M+H]^+$ , 553  $[M+2H]^{2+}$ .  $^1H$  NMR (400MHz,  $D_2O$ ) 0.58 (3H, d, J = 6.1 Hz), 0.65 (3H, d, J = 6.3 Hz), 0.67 – 0.76 (4H, m), 1.05-1.41(17H, m, incl 1.07, (d, J= 6.9 Hz), 1.11(d, J=6.4 Hz)), 1.74-1.91

(19H, m, incl 1.82, s, OAc), 1.95-2.17 (5H, m), 2.38 (2H, d, J= 6.8 Hz), 2.61-2.79 (2H, m), 2.84-3.05 (10H, m), 3.16 – 3.26 (2H, m), 3.38 (1H, dd, J=5.1, 13.5 Hz), 4.06 – 4.21(8H, m), 4.28 (1H, d, J = 4.1 Hz), 4.37 (1H, dd, J = 5.5, 9.0 Hz), 4.47 (1H, t, J = 8.1 Hz), 7.12 – 7.31(5H, m).

**(41) [3-(Aminomethyl)octanoyl]-Thr- Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr]**

**diastereomer 2, TFA salt.** HPLC rt (method 1) 5.78 min  $m/z$  1105  $[M+H]^+$ , 553

$[M+2H]^2+$ .  $^1H$  NMR (400MHz,  $D_2O$ ) 0.50 (3H, d), 0.57 - 0.69 (7H, m), 1.00-1.34 (17H, m), 1.65 -1.87 (4H, m), 1.95-2.10 (5H, m), 2.22 – 2.35 (2H, m), 2.54-2.71 (2H, m), 2.77-3.00 (10H, m), 3.10 – 3.19 (2H, m), 3.32 (1H, dd, J=4.0, 13.5 Hz), 4.00 – 4.14(8H, m), 4.22 (1H, d, J = 4.0 Hz), 4.29 – 4.33 (1H, m), 4.39 (1H, t, J = 8.0 Hz), 7.06 – 7.23 (5H, m).

**(42) [4-Amino-3-cyclohexylbutanoyl]-Thr- Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr]**

**diastereomer 1, TFA salt** HPLC rt (method 1) 5.34 min.  $m/z$  1117  $[M+H]^+$ , 559

$[M+2H]^2+$ .  $^1H$  NMR (400MHz,  $D_2O$ ) 0.51 (3 H, d, J = 6.0 Hz), 0.58 – 0.69 ( 4H, m, incl 0.59 (d, J = 6.2 Hz), 0.80-1.17 (11H, m, incl 1.01 (d, J = 6.3Hz), 1.05(d, J = 6.4 Hz)), 1.20-1.60 (8H, m), 1.63 – 2.10 (10H, m), 2.19-2.43 (2H, m), 2.61-2.18 (3H, m), 2.79 – 2.99 (9H, m), 3.10-3.20 (2 H, m), 3.33 (1H, dd, J 5.0, 13.3 Hz), 4.01-4.15 (8H, m), 4.22 (1H, d, J = 4.1 Hz), 4.32 (1H, dd, J -= 5.8, 8.6 Hz), 4.41 (1 H, t, J 8.5 Hz), 7.09 – 7.29 (5 H, m).

**(43) [4-Amino-3-cyclohexylbutanoyl]-Thr- Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr]**

**diastereomer 2, TFA salt .** HPLC rt (method 1) 5.42 min.  $m/z$  1117  $[M+H]^+$ , 559

$[M+2H]^2+$ .  $^1H$  NMR (300MHz,  $D_2O$ ) 0.63 (3 H, d, J = 6 Hz), 0.64 - 0.77 (4H, m, incl 0.70 (d)), 0.90-1.20 (9H, m, incl 1.13 (d, J = 6 Hz), 1.17(d, J = 6 Hz)), 1.22-2.35 (22H, m), 2.55 –

3.20 (10H, m), 3.25-3.32 (2 H, m), 3.46 (1H, dd), 4.13-4.32 (8H, m), 4.34 (1H, d), 4.35-4.46 (1H, m), 4.52 (1 H, appears as t), 7.15 – 7.35 (5 H, m)

**(46) (4-Amino-3-benzylbutanoyl)-Thr- Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr]**

**diastereomer 1, acetate salt.** HPLC rt (method 2) 10.70 min.  $m/z$  1125  $[M+H]^+$ , 563

$[M+2H]^{2+}$ .  $^1H$  NMR (400MHz,  $D_2O$ ) 0.58 (3 H, d,  $J = 5.8$  Hz), 0.65 (3 H, d,  $J = 5.7$  Hz), 0.64 - 0.77 (1H, m), 1.05 (3H, d,  $J = 6.1$  Hz), 1.10 (3H, d,  $J = 6.3$  Hz), 1.22-1.40 (2H, m), 1.62-1.80 (17 H, m, includes 1.78, s, OAc), 2.03-2.33 (4 H, m), 2.40-2.46 (3H, m), 2.47-3.06 (13H, m), 3.07-3.18 (2H, m), 3.27-3.36 (1H, m), 4.05-4.22 (9H, m), 4.33-4.36 (1H, m), 4.46 (1 H, appears as t,  $J$  7.9 Hz), 7.10 – 7.35 (10 H, m)

**(47) (4-Amino-3-benzylbutanoyl)-Thr-Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr]**

**diastereomer 2, acetate salt.** HPLC rt (method 2) 11.00 min.  $m/z$  1125  $[M+H]^+$ , 563

$[M+2H]^{2+}$ .  $^1H$  NMR (400MHz,  $D_2O$ ) 0.57 (3 H, d,  $J = 6.1$  Hz), 0.64 (3 H, d,  $J = 6.2$  Hz), 0.64 - 0.78 (1H, m), 1.04-1.12 (6H, m), 1.22-1.44 (3 H, m), 1.65-1.90 (17 H, m, includes 1.77, s, OAc), 2.00-2.14 (4 H, m), 2.32-2.81 (7 H, m), 2.98-3.04 (9 H, m), 3.08-3.35 (3H, m), 3.99-4.20 (8H, m), 4.32-4.39 (1H, m), 4.45 (1 H, appears as t,  $J$  8.3 Hz), 7.11 – 7.28 (10 H, m)

## **5 Mouse renal toxicity of compounds 20, 22 and 24.**

Urinary biomarkers of kidney injury for compounds 20 and 22 (Table S1) and compound 24 (Table S2) compared to Polymyxin B in the same experiment, determined in male CD-1 mice ( $n = 6$ ) after dosing SC three times per day at 17.2 mg/kg (at 4h intervals) for 4 consecutive days, with urine collection over the following 24 hours.

Table S1.

	<b>PMB</b>	<b>22</b>	<b>20</b>
	17.2 mg/kg	17.2 mg/kg	17.2 mg/kg
KIM-1 (ng/24h)	9	8	26
Cystatin C (ng/24h)	1,525	1,185	4,748
Albumin ( $\mu$ g/24h)	1,126	1,252	nd

None significantly different at  $p=0.05$  (Mann Whitney)

Table S2

	<b>PMB</b>	<b>PMB</b>	<b>PMB</b>	<b>Colistin</b>	<b>24</b>
	4.3 mg/kg	8.6 mg/kg	17.2 mg/kg	17.2 mg/kg	17.2 mg/kg
KIM-1 (ng/24h)	0.4*	0.6*	10	42*	86*
Cystatin C (ng/24h)	nd	nd	1,400	1,678	6,426*
Albumin ( $\mu$ g/24h)	130*	345*	1,912	2,548	4,998*

\* significantly different ( $p= 0.05$ ) to PMB at 17.2 mg/kg

## 6 References

(S1) Gil, S., Parra, M, and Rodriguez, P. (2007) A simple synthesis of  $\gamma$ -aminoacids.

*Tetrahedron Lett.* 48, 3451–3453.

(S2) Díaz, J. D., Fernández-Forner, D., Bach, J., and Lavilla, R. (2008) Fast and Efficient

Access to a Family of Multifunctional 1,3,5- Trisubstituted Piperidines. *Synth. Commun.*, 38,

2799-2813, DOI: 10.1080/00397910701877608

- (S3) Dolomanov, O.V., Bourhis, L. J., Gildea, R. J, Howard, J.A.K. & Puschmann, H. (2009) OLEX2: a complete structure solution, refinement and analysis program. *J. Appl. Cryst.* 42, 339-341.
- (S4) Sheldrick, G.M. (2015). *Acta Cryst.* A71, 3-8.
- (S5) Sheldrick, G.M. (2015). *Acta Cryst.* C71, 3-8