Supporting Information:

Influence of lipophilicity on the antibacterial activity of polymyxin derivatives and on their ability to act as potentiators of rifampicin

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1. Synthesis of suitably protected Carboxylic acids

Where not commercially available, synthesis of the required amine-containing N-terminal acids was in the racemic form. ^{1}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). Chemicals shifts (δ) 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.

1.1. Alpha substituted 4-aminobutanoic acids

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

2-{2-[(*tert*-Butoxycarbonyl)amino]ethyl}undecanoic acid was prepared using the conditions described for 2-{2-[(*tert*-butoxycarbonyl)amino]ethyl}octanoic acid (Brown *et al* . *ACS Infect. Dis.* 5 (10), 1645 – 1656). The title compound was obtained as a colourless oil *m/z* 330 $[M+H]^+$. ^{1}H NMR (300MHz,D_6-DMSO): 0.83-0.90 (3H, m), 1.20 - 1.28 (14H, m, CH_2), 1.48 – 1.50 (12H, m, BOC + CH_2), 1.55 – 1.67 (1H, m, CH_2), 2.18 – 2.28 (1H, m, CHCO_2H), 2.86 – 2.95 (2H, m, CH_2NHBOC), 6.73-6.82 (1H, m, NHBOC), 12.05 (1H, s, CO_2H)

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

2-{2-[(*tert*-Butoxycarbonyl)amino]ethyl}hexanoic acid was prepared using the conditions described for 2-{2-[(*tert*-butoxycarbonyl)amino]ethyl}-5-methylhexanoic acid (Brown *et al* . *ACS Infect. Dis.* 5 (10), 1645 – 1656). The title compound was obtained as a colourless oil. ^{1}H NMR (300MHz,$CDCl_3$): 0.83-0.95 (3H, m), 1.28 - 1.36 (4H, m, CH_2), 1.45 – 1.50 (10H, m, BOC + CH_2), 1.65 – 1.92 (3H, m, CH_2), 2.42 – 2.50 (1H, m, CHCO_2H), 3.12 – 3.30 (2H, m, CH_2NHBOC), 4.75 (1H, br. s., NHBOC)

4- *tert*-Butoxycarbonyl)amino-2-(2-cyclohexylethyl)butanoic acid

4- *tert*-Butoxycarbonyl)amino-2-(2-cyclohexylethyl)butanoic acid was prepared using the conditions described for 2-{2-[(*tert*-butoxycarbonyl)amino]ethyl}-5-methylhexanoic acid (Brown *et al* . *ACS Infect. Dis.* 5 (10), 1645 – 1656). The title compound was obtained as a colourless oil ^{1}H NMR (300MHz,$CDCl_3$): 0.85-0.95 (2H, m, CH_2), 1.18 - 1.26 (6H, m, CH_2), 1.45 – 1.50 (10H, m, BOC + CH_2), 1.64 – 1.92 (8H, m, CH_2), 2.35 – 2.45 (1H, m, CHCO_2H), 3.15 – 3.28 (2H, m, CH_2NHBOC), 4.75 (1H, br. s., NHBOC)

1.2. Beta substituted 4-aminobutanoic acids

3-({[(Benzyloxy)carbonyl]amino}methyl)hexanoic acid

A mixture of 4-propylpyrrolidine-2-one (250mg, 1.97 mmol) and 6M HCl (8.5 mL) was heated to 100 deg C for 17h. The mixture was evaporated to dryness, then co-evaporated from dichloromethane to afford the ring-opened amino acid as the hydrochloride salt, as a yellow oil. The material was dissolved in water (3ml) and 1,4-dioxane (3ml), treated with sodium bicarbonate (522 mg, 2.5 equiv) and cooled to 0 deg C. A solution of N-(benzyloxycarbonyloxy)succinimide (541 mg, 1.1 equiv) in 1,4-dioxane (1.5 mL) was added dropwise. The mixture was allowed to warm to room temperature and stirred for 16h. The mixture was evaporated to dryness and the residue partitioned between ethyl acetate and saturated aq sodium bicarbonate. The aqueous phase was separated and washed with additional ethyl acetate. The organic phase was discarded. The aqueous phase was acidified to pH4 with citric acid (20% aqueous) and then extracted with ethyl acetate (x3). The organic extracts were combined, dried (MgSO4) and evaporated . The residue was chromatographed in silica eluting with 0-100% ethyl acetate in hexane to afford the title compound as a colourless oil (325 mg, 54%) m/z 280$[M+H]^+$, ^{1}H NMR (400MHz,CD_3OD): 0.90 (3H, t, *J* = 7.2 Hz), 1.20 - 1.38 (4H, m, CH_2), 1.98 – 2.05 (1H, m, CH), 2.18 (1H, dd, *J* = 7.1, 15.5 Hz), 2.30 (1H, dd, , *J* = 6.3, 15.5 Hz), 3.04 (1H, dd, *J* = 7.1, 13.7 Hz), 3.17 (1H, dd, *J* = 5.8, 13.7 Hz), 5.05 (2H, s), 7.24 – 7.38 (5H, m).

3-({[(benzyloxy)carbonyl]amino}methyl)octanoic acid

The title compound was prepared from 4-pentylpyrrolidine-2-one (Brown *et al* . ACS Infect. Dis. 5 (10), 1645 – 1656) using the method described for 3-({[(Benzyloxy)carbonyl]amino}methyl)hexanoic acid. *m/z* 308 $[M+H]^+$, ^1H NMR (400MHz,CD_3OD): 0.91 (3H, t, *J* = 7.1 Hz), 1.22 - 1.37 (8H, m, CH_2), 1.97 – 2.03 (1H, m, C*H*), 2.18 (1H, dd, *J* = 7.1, 15.4 Hz), 2.29 (1H, dd, , *J* = 6.2, 15.4 Hz), 3.05 (1H, dd, *J* = 6.9, 13.5 Hz), 3.17 (1H, dd, *J* = 5.8, 13.5 Hz), 5.06 (2H, s), 7.23 – 7.46 (5H, m).

The racemic product (1.05g) was subjected to chiral separation by supercritical fluid chromatography using the preparative separation conditions described below. Enantiomeric purity was confirmed using the chiral analytical chromatography method below.

Preparative chiral supercritical fluid chromatography conditions:

Column: Phenomenex LuxA2, 250 × 21.2 mm I.D., 5 µm
Mobile phase: A: CO2 and Mobile phase B: EtOH
Isochratic conditions: 25% B
Flow rate: 50 mL/min
Column temperature: 40°C
Wavelength: 210 nm

Chiral analytical chromatography conditions:

Column: Phenomenex LuxA2, 250 × 4.6 mm I.D., 5 µm
Mobile phase: A: CO2 and Mobile phase B: EtOH (0.2% DEA)
Isochratic conditions: 25% B
Flow rate: 4 mL/min
Back pressure: 125 bar
Column temperature: 40°C
Wavelength: 210 - 400 nm

This afforded the faster-eluting enantiomer: (0.464g) retention time 1.75 min, 100% ee. Slower eluting entantiomer (0.473g): retention time: 1.99 min, 99% ee. The slower-eluting enantiomer was used in the preparation of compound 24 and shown to correspond to authentic material which had been prepared by separation of the diastereomers of the final product (Brown *et al* . ACS Infect. Dis. 5 (10), 1645 – 1656).

The slower-eluting enantiomer was subsequently used in the preparation of compounds 37, 40, 41 and 45. By analogy with the corresponding nonanoic acid derivative below, the slower enantiomer was assigned the (*R*) stereochemistry.

3-({[(benzyloxy)carbonyl]amino}methyl)nonanoic acid

(i) Methyl 3-(nitromethyl)nonanoate

To a solution of methyl (E)-non-2-enoate (1.46 g, 6.8 mmol) in nitromethane (10 mL) was added DBU (4.99 mL, 30 mmol) at over 15 mins with ice-bath cooling. The reaction mixture was stirred at room temperature over the weekend, 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 MgSO4, filtered and evaporated to dryness. The residues were purified on silica, eluting with pet ether 40-60 and ethyl acetate (0-50%). The appropriate fractions were combined and evaporated to dryness, producing 5.50g of colourless oil (82%). ^{1}H NMR (400MHz, $CDCl_3$): 0.87 (3H, t, *J*= 7.0 Hz), 1.17-1.44 (10H, m), 2.44 (2H, d, *J* = 6.6Hz), 2.55 – 2.69 (1H, m), 3.69 (3H, s), 4.46(2H, dABq, *J* = 14.7, 6.1 Hz).

(ii) Methyl 3-(benzyloxycarbonylaminomethyl)nonanoate

Zinc dust (14.3g, 219mmol) was added portion wise to a solution of methyl 3-(nitromethyl)nonanoate (5.5g, 24mmol) in acetic acid (65mL), stirred at 0-5 degC (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 $NaHCO_3$ (aq). The mixture was filtered through celite. The aqueous phase was separated and extracted with additional with ethyl acetate. The organic extracts were combined, dried with $MgSO_4$, filtered and evaporated to dryness, producing 2.81g of orange oil (58%). Mass spectroscopy gave evidence of the desired product together with the corresponding lactam. m/z 202 $[M+H]^+$ (desired product), 170 $[M+H]^+$ (lactam). The material was used directly in the next reaction.

A mixture of the crude methyl 3-(aminomethyl)nonanoate (2.81g, 13.96mmol) , water (18ml), sodium bicarbonate (1.76g, 21mmol) , and 1,4-dioxane (9ml) was cooled in an ice bath. A solution of N-benzyloxycarbonyloxy)succinimide (3.83g, 15.35mmol) in 1,4-dioxane (9 ml) was added drop wise. The mixture was stirred at 0-5 degC for 30 minutes and then allowed to warm to room temperature, stirring for 18h. The mixture was evaporated to dryness and the residues partitioned between diethyl ether and 0.5M HCl(aq). The aqueous layer was separated and extracted with additional diethyl ether. The organic extracts were combined, washed with brine, dried with MgSO4, filtered and evaporated to dryness. The residues were purified on silica, eluting with pet ether 40-60 and ethyl acetate. The appropriate fractions were combined and evaporated to dryness, producing 820mg of colourless oil (2.44 mmol 18% yield); m/z 336 $[M+H]^+$

(iii) Title compound

A mixture of methyl 3-(benzyloxycarbonylaminomethyl)nonanoate (0.82g, 2.44mmol), lithium hydroxide (0.18g, 7.33mmol), Water (10mL) and 1,4-Dioxane (10mL) was stirred at room temperature for 6h. The mixture was evaporated to dryness, the residues were suspended in water and extracted with ethyl acetate. The organic layer was discarded. The aqueous was acidified with 1M HCl(aq), then extracted with ethyl acetate (x2). The organic extracts were combined, dried with MgSO4, filtered and evaporated to dryness, producing 419 mg of colourless oil (53%); m/z 322 $[M+H]^+$. ^{1}H NMR (400MHz, CD_3OD): 0.89 (3H, t, *J*= 6.6 Hz), 1.21-1.39 (10H, m), 1.94-2.06 (1H, m), 2.24 (2H, dd, *J* = 7.0, 14.7 Hz), 3.11 (2H, dd, *J* = 6.6, 14.0 Hz), 5.04 – 5.11 (2H, m), 7.24 – 7.43 (5H, m).

Alternative synthesis:

Methyl 3-(nitromethyl)nonanoate was converted to 4-hexylpyrrolidine-2-one as described in the synthesis of 4-pentylpyrrolidine-2-one (Brown *et al* . ACS Infect. Dis. 5 (10), 1645 – 1656). 4-hexylpyrrolidine-2-one was converted to 3-({[(benzyloxy)carbonyl]amino}methyl)nonanoic acid as described for 3-({[(benzyloxy)carbonyl]amino}methyl)octanoic acid. m/z 322 $[M+H]^+$.

(3*R*)-3-({[(benzyloxy)carbonyl]amino}methyl)nonanoic acid

The racemic product was subjected to chiral separation by supercritical fluid chromatography using the preparative separation conditions described below. Enantiomeric purity was confirmed using the chiral analytical chromatography method below.

Preparative chiral supercritical fluid chromatography conditions:

Instrument: Thar 350 preparative SFC (SFC-6)
Column: ChiralPak AY, 300×50 mm I.D., 10 µm.
Mobile phase: A: CO_2 B: EtOH
Isochratic method: B 25%
Flow rate: 200 mL /min
Back pressure: 100 bar
Column temperature: 38℃
Wavelength: 220 nm
Cycle time: 5 min

Sample preparation: Compound was dissolved in ~15000mL EtOH/DCM
Injection: 6 ml per injection.

Work up: After separation, the fractions were dried off via rotary evaporator at bath temperature 40℃ to get the desired isomers.
Enantiomer A, faster eluting isomer by SFC on Analytical column 2.6g 97.1%ee
Enantiomer B, slower eluting isomer by SFC on Analytical column 1.17g 99.1%ee

Chiral analytical chromatography conditions:

Column: Phenomenex LuxA2, 250 × 4.6 mm I.D., 5 µm
Mobile phase: A: CO2 and Mobile phase B: EtOH (0.2% DEA)
Isochratic conditions: 20% B
Flow rate: 4 mL/min
Back pressure: 125 bar
Column temperature: 40°C
Wavelength: 210 - 400 nm

Fast isomer: retention time on analytical system: 1.90 min
Slow isomer: retention time on analytical system: 2.16 min.
The slower-eluting enantiomer was used in the preparation of compound 34, and shown to correspond to authentic material which had been prepared by separation of the diasteromers of the final product.

Confirmation of stereochemistry

Ethyl 3-(nitromethyl)octanoate was suspended in water and treated with Novozyme 435, using the procedure of *Tetrahedron Asymmetry* 2008, 19, 945-955, which has been demonstrate to hydrolyse the (*S*) enantiomer of the closely related ethyl 3-(nitromethyl)-5-methylhexanoate. The reaction was allowed to proceed to 50% conversion. The unreacted ester was then converted to **3-({[(benzyloxy)carbonyl]amino}methyl)nonanoic acid** as described above. Chiral HPLC on the analytical conditions above gave a retention time of 2.17 min, corresponding to the slower of the two enantiomers. This material was thus assigned the (*R*) stereochemistry.

3-({[(benzyloxy)carbonyl]amino}methyl)decanoic acid

Prepared as described for 3-({[(Benzyloxy)carbonyl]amino}methyl)hexanoic acid.

m/z 336 $[M+H]^+$, ^{1}H NMR (400MHz,CD_3OD): 0.90 (3H, t, *J* = 7.1 Hz), 1.22 - 1.33 (12H, m, CH_2), 1.99 – 2.03 (1H, m, C*H*), 2.19 (1H, dd, *J* = 7.2, 15.5 Hz), 2.30 (1H, dd, , *J* = 6.3, 15.5 Hz), 3.06 (1H, dd, *J* = 7.0, 13.6 Hz), 3.19 (1H, dd, *J* = 5.8, 13.6 Hz), 5.07 (2H, s), 7.28 – 7.34 (5H, m).

3-({[(*tert*-Butoxy)carbonyl]amino}methyl)undecanoic acid

(i) - 3-Nitromethyl undecanoic acid ethyl ester

To a solution of (E)-Undec-2-enoic acid ethyl ester (1.46 g, 6.8 mmol) in acetonitrile (5.84 mL) were added nitromethane (1.86 mL, 34.3 mmol) and DBU (1.05 mL, 7.0 mmol) at room temperature. The reaction mixture was heated at 65 °C for 4h, cooled and concentrated. The residue was partitioned between ethyl acetate (20 mL) and water (20 mL). The aqueous layer was extracted twice with ethyl acetate (20 mL). The combined organic extracts were washed further with 0.1M HCl (30 mL), water (30 mL) and brine (30 mL). The organic extract was dried over magnesium sulphate, filtered and concentrated. The crude material was purified by automated flash column chromatography eluting with 0-80% ethyl acetate in hexane. Fractions were isolated and concentrated to leave a colourless

oil. Yield 0.48 g, 26%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.82 (t, 3H, CH_3), 1.25 (m, 16H, CH_2), 1.42 (m, 3H, CH_3), 2.42 (m, 2H, CH_2), 2.62 (m, 1H, CH), 4.19 (m, 2H, CH_2), 4.46 (m, 2H, CH_2).

(ii) 3-Aminomethyl undecanoic acid ethyl ester

To a solution of 3-nitromethyl undecanoic acid ethyl ester (200 mg, 0.73 mmol) in acetic acid (5 mL) were added at room temperature THF (10.52 mL), water (2.1 mL), concentrated HCl (820 uL), and portionwise Zn dust (600 mg, 9.2 mmol). The reaction mixture was vigorously stirred for 2.5 h. The suspension was then filtered and the filtrate was concentrated *in vacuo*. The residue was diluted with DCM (20 mL) washed with water (20 mL). The organic layer was isolated and dried over magnesium sulphate, filtered and the filtrate was concentrated in vacuo to leave the acetate salt. Yield 150 mg, 88%. *m/z* 244.2 $[M+H]^+$.

(iii) - Tert-butoxycarbonyl-3-aminomethyl undecanoic acid ethyl ester

To a solution of 3-aminomethyl undecanoic acid ethyl ester (150 mg, 0.6 mmol) in THF (20 mL), triethyl amine (170 µL, 1.2 mmol) was added. After 10 min di-*tert*-butyl dicarbonate (147 mg, 0.67 mmol) was added and the reaction mixture was stirred for 6 h at room temperature. The reaction mixture was evaporated to dryness; the crude material was re-dissolved in water (20 mL) and extracted with DCM (20 mL). The organic layer was isolated, dried over magnesium sulphate, filtered and concentrated *in vacuo*. The crude material was purified by automated flash column chromatography eluting with 0-80% ethyl acetate in hexane. Fractions were isolated and evaporated to leave a colourless oil. Yield 70 mg, 33%. *m/z* 344 $[M+H]^+$.

(iv) - Tert-butoxycarbonyl-3-aminomethyl undecanoic acid

Tert-butoxycarbonyl-3-Aminomethyl undecanoic acid ethyl ester (70mg, 0.2 mmol) was dissolved in THF (600 µL). To the solution was added 1M NaOH (4 mL) and the reaction was stirred at room temperature for 16 h. The reaction mixture was then concentrated, re-dissolved in water (20 mL)

and extracted with DCM (20 mL). The aqueous layer was isolated, acidified to pH 1 using 1M HCl and extracted with DCM (20 mL). The organic layer was isolated, dried over magnesium sulphate, filtered and concentrated to dryness to leave a yellow oil. Yield 40 mg, (63%). *m/z* 316.6 $[M+H]^+$.

3-({[(benzyloxy)carbonyl]amino}methyl)undecanoic acid

(i) Ethyl 3-({[(benzyloxy)carbonyl]amino}methyl)undecanoate

3-Aminomethyl undecanoic acid ethyl ester (1.65g, 6.78 mmol) was dissolved in a mixture of water (11mL) and 1,4-dioxane (11mL) and treated with sodium bicarbonate (0.857 g, 10.2 mmol), and the mixture cooled in an ice bath. A solution of benzyl chloroformate (1.06mL, 7.46 mmol) in 1,4-dioxane (6mL) was added dropwise and the mixture stirred at 10degC for 1hr, then allowed to warm to room temperature and stirred for a further 17h.

The mixture was evaporated to dryness and the residue partitioned between diethyl ether and 0.5M HCl. The aqueous layer was separated and extracted with diethyl ether. The organic extracts were combined, washed with brine, dried (MgSO4) and evaporated to dryness. The residue was chromatographed on silica eluting with 0-80% ethyl acetate in hexane. Product-containing fractions were combined and evaporated to a colourless oil (0.81g, 32%). m/z 378 $[M+H]^+$

(ii) Title compound

Ethyl 3-({[(benzyloxy)carbonyl]amino}methyl)undecanoate (0.80g) was dissolved in 1,4-dioxane (8 mL) and water (8mL), and treated with lithium hydroxide (153 mg, 3.0 equiv. The reaction was stirred at room temperature for 17 h. The reaction mixture was then evaporated, suspended in water, acidified with 1M HCl, then extracted twice with ethyl acetate. The organic extracts were combined, dried over magnesium sulphate, filtered and evaporated to dryness to give the title compound as a colourless oil (724 mg, 97%) *m/z* 350 $[M+H]^+$.

3-{[(*tert*-Butoxycarbonyl)amino]methyl}-4-(3-chlorophenyl)butanoic acid

Prepared as described for 3-{[(*tert*-Butoxycarbonyl)amino]methyl}-4-phenylbutanoic acid (Brown *et al* . ACS Infect. Dis. 5 (10), 1645 – 1656). m/z 327 [M^+]

2. Beta branched amino propionates: assignment of stereochemistry:

Compound 36 [(3*R*)-3-(aminomethyl)-5-methylhexanoyl]- Thr-Dap-cyclo[Dab-Dab-DLeu-Leu-Dab-Dab-Thr]

[(3*R*)-3-(aminomethyl)-5-methylhexanoyl]- Thr-Dap-cyclo[Dab-Dab-DLeu-Leu-Dab-Dab-Thr] was prepared from by reaction of the corresponding nonapeptide (Brown et al , ACS infectious dis) with commercially-available racemic 3-{[(*tert*-butoxycarbonyl)amino]methyl}-5-methylhexanoic acid using the general procedure given in Brown et al. The diastereomers were separated by preparative HPLC, and the separated diastereomers analysed by HPLC using Analytical method 2.

Faster diastereomer m/z 1057$[M+H]^+$, 529$[M+2H]^{2+}$ retention time 9.43 mins assigned to compound 36.

Slower diastereomer m/z 1057$[M+H]^+$, 529$[M+2H]^{2+}$ retention time 9.69 mins.

In a separate experiment, commercially available (3*S*)- 3-{[(*tert*-butoxycarbonyl)amino]methyl}-5-methylhexanoic acid was used in the same procedure, to afford a single product which corresponded to the slower diastereomer m/z 1057$[M+H]^+$, 529$[M+2H]^{2+}$ retention time 9.69 mins.

Thus, compound 36 was assigned the (*R*) stereochemistry.

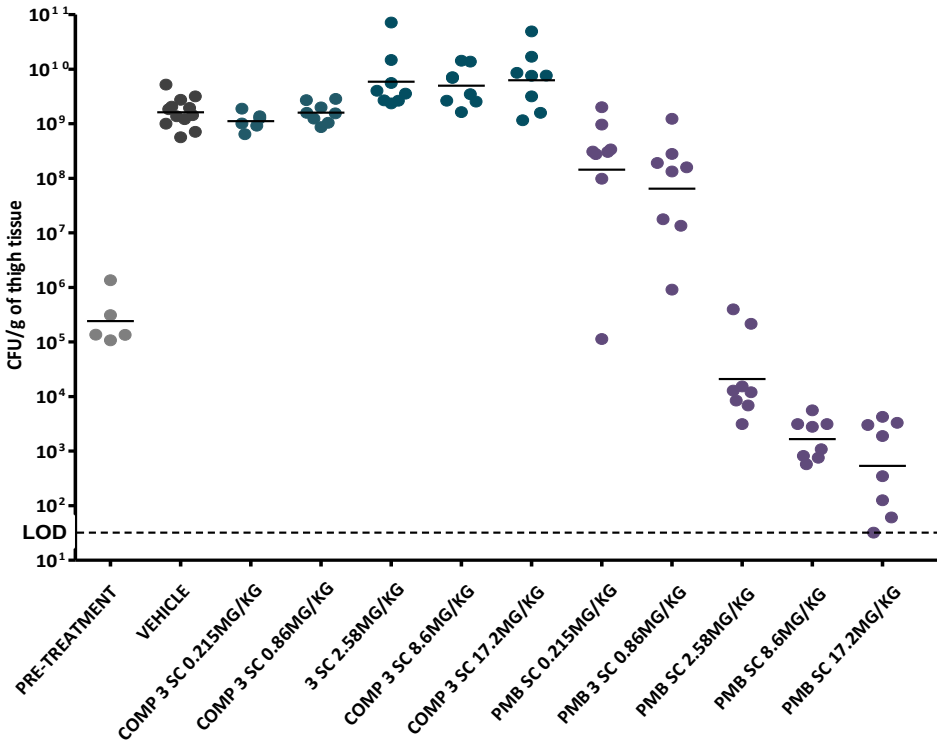
By analogy, and also by analogy with the enzymic hydrolysis of the precursor to compound (34), the faster diastereomer in the beta branched amino propionate series of compounds was assigned the (R) stereochemistry.

3. Table S1. Effect of microtitre plate material on MIC value

Compound	*E. coli* ATCC25922	*E. coli* IHMA558090	*K. pneumoniae* ATCC13882	*K. pneumoniae* IHMA 580884	*P. aeruginosa* ATCC27853	*A. baumannii* NCTC13424
PMB	0.03/0.25	4/4	ND/0.25	32/8	0.06/0.5	0.03/0.25
3 (1033)	0.125/2	0.25/2	0.25/2	0.125/2	0.125/2	0.125/2
26 (1263)	0.03/0.125	8/8	0.125/0.125	32/16	0.125/0.25	0.03/0.06

The data show MIC values (µg/ml) determined in polystyrene-NBS (non-binding surface) microtitre plates (Corning 3641) compared with polypropylene plates as used in all other work presented here. Format: MIC in low binding plates/MIC in polypropylene plates.

4. Efficacy of compound 3 in a neutropenic mouse thigh model versus *A. baumannii* NCTC 13301



Male CD-1 mice (n=4) were rendered neutropenic (cyclophosphamide *i.p.* 150mg/kg d-4, 100mg/kg d-1) then inoculated in each thigh with 1.5 x 10^5 cfu of *A. baumannii* NCTC 13301. Mice were dosed *s.c.* with PMB sulphate or Compound 3 at 2, 6 and 10h post inoculation. One group (pre-treatment) was sacrificed just prior to dosing and the remainder were harvested after 16h and thighs prepared for colony counts. Both thighs were treated separately to generate two data points per animal. LOD = limit of detection. (Evotec Ltd.).

Figure S1. Efficacy of compound 3 in a neutropenic mouse thigh model versus *A. baumannii* NCTC 13301

5. Table S2. Biomarker data from mouse renal toxicity model and plasma exposure from single dose PK

Parameter	Duration	Vehicle	PMB 12.5mg/kg	PMB 25mg/kg	Comp 34 25mg/kg	Comp 34 50mg/kg	Comp 34 75mg/kg
Cystatin C (x 10^{-3})	24hr	21.1	28.4	53.8	29.7	40.6	34.9
	4 days	55.8	87.5	141	88.9	106	153
Beta 2 microglobulin (x 10^{-3})	24hr	0.05	0.13	2.78	0.04	7.98	29.6
	4 days	0.12	0.10	18.0	0.48	5.1	5.6
KIM-1 (x 10^{-6})	24hr	6.5	12.8	836	6.3	86.1	349
	4 days	4.7	11.4	462	18.3	123	2,034
NGAL (x 10^{-3})	24hr	0.0	0.0	2.6	0.0	0.0	0.34
	4 days	0.0	0.0	2.2	0.0	0.0	2.3
Albumin	24hr	3.4	3.7	7.4	4.4	7.1	6.9
	4 days	0.7	5.3	8.6	5.5	7.1	9.1
Plasma AUC_{inf} (µg.h/ml)			23	57	41	113	215

Doses refer to mg free base/kg

6. Characterisation of tested compounds

Compounds were assessed for purity by HPLC, nmr and mass spec. Mass spectra were recorded on an LCQ DecaXP mass spectrometer with +ve ion electrospray ionisation. HPLC retention time is reported using the conditions of Analytical HPLC method 1 below. Purity was determined by analytical HPLC method (2).

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 μL

Method (2)

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	40%
21	0%
23	0%
23.5	100
25	100

Detection: 210, 254 nm

Injection volume: 20 μL

	name	HPLC RT (min)	AlogP	Purity %	formula	Mass	m/z
3	[2-(2-Aminoethyl)undecanoyl]-Thr-Dap-Cyclo[Dab-Dab-DBip-Leu-Dab-Dab-Thr] Isomer 2	7.46	-2.7	91	C61H101N15O12	1235.8	1237 $[M+H]^+$, 619 $[M+2H]^{2+}$
4	[2-(2-Aminoethyl)undecanoyl]-Thr-Dab-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] Isomer 2	6.88	-4.2	>95	C56H99N15O12	1173.8	1175$[M+H]^+$, 588 $[M+2H]^{2+}$
5	[(3*R*)-3-(Aminomethyl)undecanoyl]-Thr-Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr]	6.46	-4.8	84	C54H95N15O12	1145.7	1146.8$[M+H]^+$
6	[(3*R*)-4-Amino-3-(cyclohexylmethyl)butanoyl]- Thr-Dab-Cyclo[Dab-Dab-DCha-Leu-Dab-Dab-Thr]	6.25	-5.1	>95	C54H99N15O12	1149.8	1151$[M+H]^+$
7	[4-Amino-2-cyclohexylbutanoyl]- Thr-Dab-Cyclo[Dab-Dab-DCha-Leu-Dab-Dab-Thr] Isomer 2	6.04	-5.4	90	C53H97N15O12	1135.7	1136.6 $[M+H]^+$
8	[2-(2-Aminoethyl)octanoyl]-Thr-Dab-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] Isomer 2	6.06	-5.5	>95	C53H93N15O12	1131.7	1133$[M+H]^+$
9	[4-Amino-2-cyclohexylbutanoyl]- Thr-Dap-Cyclo[Dab-Dab-DCha-Leu-Dab-Dab-Thr] Isomer 2	6.10	-5.5	>95	C52H95N15O12	1121.7	1123 $[M+H]^+$
10	[(3*R*)-4-Amino-3-cyclohexylbutanoyl]- Thr-Dab-cyclo[Dab-Dab-DCha-Leu-Dab-Dab-Thr]	5.97	-5.6	>95	C53H97N15O12	1135.7	1137 $[M+H]^+$
11	[4-Amino-2-(2-cyclohexylethyl)butanoyl]-Thr-Dap-cyclo[Dab-Dab-DLeu-Leu-Dab-Dab-Thr] Isomer 2	6.15	-5.6	>95	C51H95N15O12	1109.7	1110.6 $[M+H]^+$, 556$[M+2H]^{2+}$
12	[(3*R*)-4-Amino-3-cyclohexylbutanoyl]- Thr-Dap-cyclo[Dab-Dab-DCha-Leu-Dab-Dab-Thr]	6.08	-5.7	>95	C52H95N15O12	1121.7	1122.7 $[M+H]^+$
13	[(3*R*)-3-(aminomethyl)nonanoyl]- Thr-Dap-cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr]	5.93	-5.7	>95	C52H91N15O12	1117.7	1118.6$[M+H]^+$, 560$[M+2H]^{2+}$

	name	HPLC RT (min)	AlogP	Purity %	formula	Mass	m/z
14	[2-(2-Aminoethyl)hexanoyl]- Thr-Dab-cyclo[Dab-Dab-DCha-Leu-Dab-Dab-Thr] isomer 2	5.94	-5.8	>95	C51H95N15O12	1109.7	1111$[M+H]^+$, 556$[M+2H]^{2+}$
15	[2-(2-Aminoethyl)hexanoyl]- Thr-Dap-cyclo[Dab-Dab-DCha-Leu-Dab-Dab-Thr] isomer 2	5.98	-5.8	>95	C50H93N15O12	1095.7	1097 $[M+H]^+$,549$[M+2H]^{2+}$
16	[(3*R*)-4-Amino-3-benzylbutanoyl]- Thr-Dab-cyclo[Dab-Dab-DCha-Leu-Dab-Dab-Thr]	5.77	-5.8	90	C54H93N15O12	1143.7	1146$[M+H]^+$, 573$[M+2H]^{2+}$
17	[(3*R*)-3-(Aminomethyl)undecanoyl]-Thr-Dap-Cyclo[Dab-Dab-DLeu-Abu-Dab-Dab-Thr]	6.11	-5.9	96	C49H93N15O12	1083.7	1084.7$[M+H]^+$, 543$[M+2H]^{2+}$
18	[(3*R*)-4-Amino-3-benzylbutanoyl]- Thr-Dap-cyclo[Dab-Dab-DCha-Leu-Dab-Dab-Thr]	6.01	-5.9	>95	C53H91N15O12	1129.7	1131 $[M+H]^+$, 566 $[M+2H]^{2+}$
19	[(3*R*)-4-Amino-3-(3-chlorobenzyl)butanoyl]-Thr-Dap- cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr]	5.80	-5.9	>95	C53H84ClN15O12	1157.6	1159$[M+H]^+$, 580 $[M+2H]^{2+}$
20	[(3*R*)-3-(Aminomethyl)decanoyl]-Thr-Dap-Cyclo[Dab-Dab-DPhe-Abu-Dab-Dab-Thr]	5.84	-6.0	>95	C51H89N15O12	1103.7	1104.4 $[M+H]^+$, 553$[M+2H]^{2+}$
21	4-Amino-2-phenylbutanoyl]- Thr-Dab-Cyclo[Dab-Dab-DCha-Leu-Dab-Dab-Thr] Isomer 2	5.89	-6.1	>95	C53H91N15O12	1129.7	1130.5 $[M+H]^+$
22	[4-Amino-2-cyclohexylbutanoyl]- Thr-Dab-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] Isomer 2	5.69	-6.1	>95	C53H91N15O12	1129.7	1243 $[M+TFA]^+$, 1131 $[M+H]^+$
23	[4-Amino-2-cyclohexylbutanoyl]- Thr-Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr] Isomer 2	5.70	-6.2	>95	C52H89N15O12	1115.7	See published data[1]
24	[(3*R*)-3-(aminomethyl)octanoyl]- Thr-Dap-cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr]	5.67	-6.2	99	C51H89N15O12	1103.7	See published data[1]
25	[(3*R*)-4-Amino-3-cyclohexylbutanoyl]- Thr-Dap-cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr]	5.60	-6.3	>95	C52H89N15O12	1115.7	See published data[1]

	name	HPLC RT (min)	AlogP	Purity %	formula	Mass	m/z
26	[(3S)-4-Amino-3-(3-chlorophenyl)butanoyl]-Thr-Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr]	5.48	-6.3	100	C52H82ClN15O12	1143.6	See published data[1]
27	[(3*R*)-3-(Aminomethyl)decanoyl]-Thr-Dap-Cyclo[Dab-Dab-DLeu-Abu-Dab-Dab-Thr]	5.79	-6.3	98	C48H91N15O12	1069.7	536$[M+2H]^{2+}$
28	[(3*R*)-3-(Aminomethyl)decanoyl]-Thr-Dap-Cyclo[Dab-Dab-DAbu-Leu-Dab-Dab-Thr]	5.85	-6.3	97	C48H91N15O12	1069.7	1183.7 $[M+TFA]^{+}$, 1071$[M+H]^{+}$, 536 $[M+2H]^{2+}$
29	[4-Amino-3-cyclohexylbutanoyl]- Thr-Dab-cyclo[Dab-Dab-DLeu-Leu-Dab-Dab-Thr] Isomer 2	5.56	-6.4	86	C50H93N15O12	1095.7	1210$[M+TFA]^{+}$, 1097 $[M+H]^{+}$
30	[(3*R*)-4-amino-3-benzylbutanoyl] -Thr- Dap-Cyclo[Dab-Dab-DPhe-Leu-Dab-Dab-Thr].	5.49	-6.5	92	C53H85N15O12	1123.7	See published data[1]
31	[(3*R*)-3-(Aminomethyl)undecanoyl]-Thr-Dap-Cyclo[Dab-Dab-DAbu-Abu-Dab-Dab-Thr]	5.85	-6.6	100	C47H89N15O12	1055.7	585$[M+TFA+H]^{2+}$, 529$[M+2H]^{2+}$
32	[(3*R*)-3-(Aminomethyl)decanoyl]-Thr-Dap-Cyclo[Dab-Dab-DnorLeu-Ala-Dab-Dab-Thr]	5.71	-6.6	95	C47H89N15O12	1055.7	1169$[M+TFA]^{+}$, 1056.5$[M+H]^{+}$, 529$[M+2H]^{2+}$
33	[(3*R*)-3-(aminomethyl)nonanoyl]- Thr-Dap-cyclo[Dab-Dab-DnorVal-norVal-Dab-Dab-Thr]	5.50	-6.6	95	C47H89N15O12	1055.7	1056.5$[M+H]^{+}$, 529$[M+2H]^{2+}$
34	[(3*R*)-3-(Aminomethyl)nonanoyl]-Thr-Dap-Cyclo[Dab-Dab-DLeu-Abu-Dab-Dab-Thr]	5.43	-6.8	92	C47H89N15O12	1055.7	529$[M+2H]^{2+}$
35	[(3*R*)-3-(aminomethyl)octanoyl]- Thr-Dap-cyclo[Dab-Dab-DPhe-Abu-Dab-Dab-Thr]	5.23	-6.9	>95	C49H85N15O12	1075.7	1076.5$[M+H]^{+}$, 539$[M+2H]^{2+}$
36	[(3*R*)-3-(aminomethyl)-5-methylhexanoyl]-Thr-Dap-cyclo[Dab-Dab-DLeu-Leu-Dab-Dab-Thr]	5.35	-7.2	99	C47H89N15O12	1055.7	1057$[M+H]^{+}$, 529$[M+2H]^{2+}$
37	[(3*R*)-3-(aminomethyl)octanoyl]- Thr-Dap-cyclo[Dab-Dab-DLeu-Abu-Dab-Dab-Thr]	5.15	-7.2	>98	C46H87N15O12	1041.7	1155$[M+TFA]^{+}$, 1042.6 $[M+H]^{+}$,

	name	HPLC RT (min)	AlogP	Purity %	formula	Mass	m/z
38	[(3*R*)-3-(aminomethyl)hexanoyl]- Thr-Dap-cyclo[Dab-Dab-DLeu-Leu-Dab-Dab-Thr]	5.12	-7.4	85	C46H87N15O12	1041.7	1043$[M+H]^+$, 522$[M+2H]^{2+}$
39	[(3*R*)-3-(Aminomethyl)undecanoyl]-Thr-Dap-Cyclo[Dab-Dab-DAbu-Thr-Dab-Dab-Thr]	5.79	-7.6	100	C47H89N15O13	1071.7	1072.4$[M+H]^+$, 537$[M+2H]^{2+}$
40	[(3*R*)-3-(aminomethyl)octanoyl]- Thr-Dap-cyclo[Dab-Dab-DLeu-Ala-Dab-Dab-Thr]	5.00	-7.8	100	C45H85N15O12	1027.7	1028.4 $[M+H]^+$, 515$[M+2H]^{2+}$
41	[(3*R*)-3-(aminomethyl)octanoyl]- Thr-Dap-cyclo[Dab-Dab-DPhe-Thr-Dab-Dab-Thr]	5.11	-7.9	>95	C49H85N15O13	1091.7	1092.4 $[M+H]^+$, 547 $[M+2H]^{2+}$
42	[(3*R*)-3-(aminomethyl)hexanoyl]- Thr-Dap-cyclo[Dab-Dab-DLeu-Abu-Dab-Dab-Thr]	4.74	-8.1	90	C44H83N15O12	1013.6	1014.8$[M+H]^+$, 508$[M+2H]^{2+}$
43	[(3*R*)-3-(aminomethyl)hexanoyl]- Thr-Dap-cyclo[Dab-Dab-DPhe-Thr-Dab-Dab-Thr]	4.70	-8.9	85	C47H81N15O13	1063.6	1065$[M+H]^+$, 533 $[M+2H]^{2+}$
44	[4-aminobutanoyl]- Thr-Dap-cyclo[Dab-Dab-DLeu-Leu-Dab-Dab-Thr]	4.99	-8.7	>99	C43H81N15O12	999.6	1114 $[M+TFA]^+$, 1000.4 $[M+H]^+$, 501$[M+2H]^{2+}$
45	[(3*R*)-3-(aminomethyl)hexanoyl]- Thr-Dap-cyclo[Dab-Dab-DNorVal-Ala-Dab-Dab-Thr]	3.95	-8.9	84	C42H79N15O12	985.6	1099$[M+TFA]^+$, 986.5 $[M+H]^+$, 494$[M+2H]^{2+}$
46	[(3*R*)-3-(aminomethyl)hexanoyl]- Thr-Dap-cyclo[Dab-Dab-DLeu-Thr-Dab-Dab-Thr]	4.09	-9.2	84	C44H83N15O13	1029.6	1030.6 $[M+H]^+$, 516$[M+2H]^{2+}$
47	[3-aminopropanoyl]- Thr-Dap-cyclo[Dab-Dab-DLeu-Thr-Dab-Dab-Thr]	3.55	-10.7	85	C40H75N15O13	973.6	974.5 $[M+H]^+$,488 $[M+2H]^{2+}$

*DBip: D-(4-Phenyl)phenylalanine, Cha: cyclohexyl alanine

Reference:

(1) Brown, P., Abbott, E., Abdulle, O., Boakes, S., Coleman, S., Divall, N., Duperchy, E., Moss, S., Rivers, D., Simonovic, M., Singh, J., Stanway, S., Wilson, A., Dawson, M. J. (2019) Design of next generation polymyxins with lower toxicity: the discovery of SPR206. *ACS Infect. Dis. 5* (10), 1645 – 165