Developing an anion host for lipid A binding and antibacterial activity

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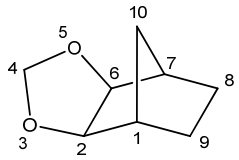
SUPPLEMENTARY INFORMATION

Naming

Compounds **2a** and **2b** are named as substituted versions of the following parent framework.

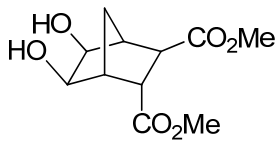


3,5-dioxatricyclo[5.2.1.$0^{2,6}$]decane

General Experimental

All ^{1}H and ^{13}C NMR spectra were recorded on a Jeol JNM-EX 270 MHz or Eclispe 400 MHz FT-NMR spectrometer as indicated. Samples were dissolved in deuterated chloroform ($CDCl_3$), with the residual solvent peak used as an internal reference (7.26 (^{1}H NMR) and 77.10 (^{13}C NMR)). Proton spectra are reported as follows: chemical shift δ (ppm), (integral, multiplicity (s=singlet, br s = broad singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet), coupling constant J (Hz). Carbon spectra are reported as chemical shift δ (ppm). Fourier transform infrared (FT-IR) spectra were recorded on an Excalibur Series Biorad FT-IR spectrometer. High Resolution Mass Spectra (HRMS) were recorded on a 6210 MSD TOF mass spectrometer (Agilent Technologies, Australia) with the following conditions: drying gas nitrogen (7.0 L/min, 325 ºC); nebuliser gas nitrogen (15 psi); capillary voltage 3.0 kV; vaporiser temperature 29 ºC; and cone voltage 40 V. Methanol was used as the mobile phase. Samples were dissolved in methanol, with less than 1 mg of sample per mL of solvent. Thin Layer Chromatography (TLC) was performed using aluminium-backed Merck TLC Silica gel 60 F_{254} plates, and samples were visualised using 254 nm ultraviolet (UV) light, and potassium permanganate/potassium carbonate oxidising dip (1:1:100$KMnO_4$:K_2CO_3:H_20 w/w). HPLC analysis was carried out on an Agilent Technologies 1200 series liquid chromatography system, equipped with a quaternary pump, solvent degasser system, autosampler and diode array detector (Agilent Technologies, Forest Hill, Victoria, Australia). A reverse phase Agilent Eclipse XDB-C18 analytical column (150 mm × 4.6 mm i.d., particle size 5 µm, Agilent Technologies) operating at room temperature with an injection volume of 10 µL and a flow rate of 1 mL/min (60% acetonitrile, 40% Water).

Synthesis

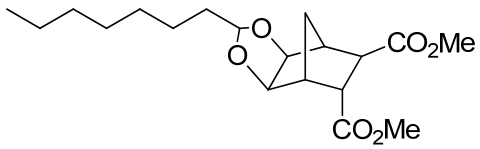


4

Dimethyl-5,6-dihydroxybicyclo[2.2.1]heptane-2,3-dicarboxylate (**4**)

Osmium tetroxide (0.5 mL, 2.5% solution in *t*-butanol) was added to a solution of dimethyl bicyclo[2.2.1]hept-5-ene-2-*endo*,3-*exo*-dicarboxylate **3**[1] (690 mg, 4.5 mmol) in acetone/water (4:1, 10 mL) and the solution stirred overnight at room temperature. Sodium metabisulfite (2.0 mL, sat) was added and the solution stirred for 10 minutes. Water (30 mL) was added and the aqueous phase extracted with ethyl acetate (3 × 30 mL), the combined organic phases were washed with brine (3 × 30 mL), dried ($MgSO_4$) and solvent removed *in vacuo* to give an orange solid. Purification by silica

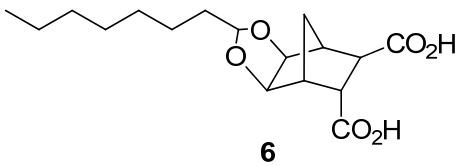
gel chromatography (pet. spirit:ethyl acetate, 1:1) of the crude solid gave diol **4** as a white powder (790 mg, 71%). m.p. 81 – 84 ^{o}C; δ_H (400 MHz, $CDCl_3$) 3.83 (1H, t, *J* = 5.0 Hz), 3.73 (1H, t, *J* = 5.0 Hz), 3.69 (3H, s, OCH_3), 3.68 (3H, s, OCH_3), 3.17 (1H, dd, *J* 5.6, 5.6 Hz), 2.47 – 2.55 (3H, m), 1.85 (1H, d, *J* = 11.0 Hz), 1.40 (1H, d, *J* = 11.0 Hz); δ_C (100 MHz, $CDCl_3$) 174.2, 173.1, 73.1, 70.0, 52.4, 52.2, 48.0, 46.3, 46.1, 44.7, 31.7; HRMS (+ve) calced for $C_{11}H_{16}O_6Na$ 267.0840 $[M + H]^+$, found 267.0845.



5

Dimethyl 4-heptyl-3,5-dioxatricyclo[5.2.1.$0^{2,6}$]decane-8,9-dicarboxylate **(5)**

Trifluoroacetic acid (5 drops) and $MgSO_4$.anhydrous (2 g) was added to a solution of diol **4** (420 mg, 1.74 mmol) and octanal (244 mg, 1.91 mmol) in $CHCl_3$ (5 mL) and the solution stirred at room temperature for 16 hrs. The suspension filtered, the filtrate collected and reduced to dryness *in vacuo* to give a pale yellow oil. Purification by silica gel chromatography (CH_2Cl_2) gave diester **5** as a colourless oil (470 mg, 76%). υ_{max} (thin film, cm^{-1}) 2952.6, 2926.1, 2856.9, 1734.6, 1436.6, 1299.6, 1242.8, 1195.2, 1028.5; δ_H (270 MHz, $CDCl_3$) 4.66 (1H, t, *J* 4.9 Hz), 4.01 (1H, d, *J* 5.5 Hz), 3.89 (1H, d, *J* 5.5 Hz), 3.69 (6H, br s), 3.21 (1H, t, *J* 5.1 Hz), 2.70 - 2.64 (3H, m), 1.77 (1H, d, *J* 10.6 Hz), 1.60 (2H, m), 1.40 – 1.20 (11H, m), 0.85 (3H, m); δ_C (67.5 MHz, $CDCl_3$) 174.0, 172.8, 104.3, 81.3, 78.9, 52.4, 52.2, 45.4, 45.1, 43.7, 43.3, 32.8, 31.8, 31.7, 29.6, 29.2, 24.3, 22.7, 14.1; HRMS (+ ve) calcd for $C_{19}H_{31}O_6$ 355.2115 $[M+H]^+$, found 355.2118.

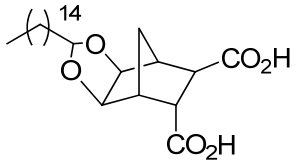


6

4-Heptyl-3,5-dioxatricyclo[5.2.1.$0^{2,6}$]decane-8,9-dicarboxylic acid **(6)**

THF (1 mL) was added to a solution of diester **5** (248 mg, 7.0 mmol) in sodium hydroxide (2 M, 15 mL) and the solution stirred at 50 ^{o}C for 16 hrs. Water (30 mL) was added and the aqueous phase extracted with ethyl acetate (3 × 25 mL), the aqueous phase was then acidified (HCl, 2 M) to pH 1 followed by extraction with ethyl acetate (3 × 30 mL). The combined organic phases (from the acidic aqueous wash) were dried ($MgSO_4$) and the solvent removed to give diacid **6** as white plates (193 mg, 84%). Analysis by ^{1}H NMR spectroscopy showed the desired compound in > 95% purity. m.p. 153 -

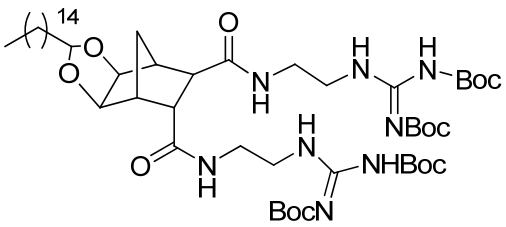
154 °C; υ_{max} (KBr/cm^{-1}) 3158.1, 3153.9, 2945.1, 2860.3, 1733.1, 1710.4, 1421.9, 1295.3, 1200.4, 1118.5, 1042.8, 710.3; δ_H (270 MHz, d_6-DMSO) 12.42 (2H, br s, COOH), 4.63 (1H, t, *J* 4.7 Hz), 3.97 (1H, d, *J* 5.6 Hz), 3.90 (1H, d, *J* 5.6 Hz), 2.99 (1H, dd, *J* 5.2, 5.2 Hz), 2.35 - 2.50 (3H, m), 1.54 (3H, m), 1.24 (11H, m), 0.85 (3H, m); δ_C (67.5 MHz, d_6-DMSO) 175.1, 173.8, 103.8, 81.2, 78.7, 45.6, 45.0, 43.8, 43.1, 32.8, 31.7, 29.5, 29.5, 24.2, 22.6, 21.6, 14.5; HRMS (+ve) calcd $C_{17}H_{27}O_6Na$ 349.1621 $[M+Na]^+$, found 349.1620.



9

4-Pentadecyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8,9-dicarboxylic acid **(9)**

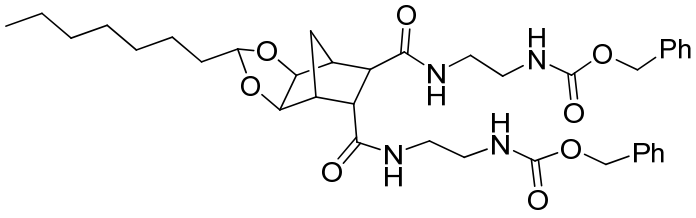
Trifluoroacetic acid (5 drops) and $MgSO_4$.anhydrous (2 g) was added to a solution of dodecanal (550 mg, 2.4 mmol) and diol **4** (373 mg, 1.53 mmol) in $CHCl_3$ (10 mL) and the suspension stirred overnight at 50 °C. Analysis of the solution by TLC showed complete consumption of diol **4**, the solution was filtered and the filtrate reduced to dryness to give a beige powder. The crude powder was then taken up in sodium hydroxide (18 mL) and THF (2 mL) was added. The suspension was then stirred at 50 °C for 16 hrs. Water (30 mL) was added and the aqueous phase extracted with ethyl acetate (3 × 25 mL), the aqueous phase was then acidified (HCl, 2 M) to pH 1 followed by extraction with ethyl acetate (3 × 30 mL). The combined organic phases (from the acidic aqueous wash) were dried ($MgSO_4$) and the solvent removed to give diacid **9** as an off white solid (492 mg, 73% - over two steps). m.p. 125 – 127 °C; υ_{max} (thin film/cm^{-1}) 3365.1, 2943.7, 2924.4, 2834.4, 1697.1, 1648.8, 1443.2, 1027.0; δ_H (270 MHz, d_4-MeOH) 4.66 (1H, t, *J* 4.7 Hz), 4.00 (2H, m), 3.16 (1H, dd, *J* 4.9, 4.9 Hz), 2.67 – 2.50 (3H, m), 1.75 (1H, d, *J* 9.4 Hz), 1.59 (2H, m), 1.55 – 1.15 (24H, m), 0.90 (3H, t, *J* 6.9 Hz) ; δ_C (100 MHz, d_4-MeOH) 177.7, 176.3, 106.0, 83.5, 81.0, 44.0, 43.8, 42.3, 41.8, 31.3, 30.5, 29.8, 28.1 (8 × C), 28.0, 27.9, 26.0, 24.6, 15.3; HRMS $C_{25}H_{41}O_6$ (-ve) $[M - H]^-$ calcd 437.2908, found 437.2848



11

8,9-Di[2'-(2'',3''di*tert*butoxycarbonylguanidino)ethylcarbamoyl] 4-pentadecyl-3,5-dioxatricyclo[5.2.1.$0^{2,6}$]decane **(11)**

EDCI (143 mg, 0.75 mmol), HOBt (7 mg, 30 μmol) were added to a solution of diacid **9** (132 mg, 0.3 mmol) in $CHCl_3$ (8 mL) and the solution stirred for 5 minutes at room temperature. A solution of amine **10** (227 mg, 0.75 mmol) in $CHCl_3$ (3 mL) was added and the solution stirred at room temperature overnight. Water (25 mL) was added and the aqueous phase extracted with EtOAc (3 × 30 mL), the combined organic phases were washed with sodium hydroxide (2 M, 1 × 40 mL), brine (1 × 30 mL), dried ($MgSO_4$) and the solvent removed to give a white resin. Purification of the resin by silica gel chromatography (EtOAc/Petrol/MeOH, 48/48/4) gave the desired compound as colourless gum (166 mg, 53%). υ_{max} (thin film/ cm^{-1}) 3320.9, 2974.8, 2925.5, 2845.3, 1723.1, 1644.0, 1617.2, 1414.2, 1366.2, 1137.6, 1051.2; δ_H (400 MHz, $CDCl_3$) 11.47 (1H, br s), 11.44 (1H, br s), 8.62 (1H, br t, *J* 6.6 Hz, NH), 8.50(1H, br t, *J* 5.9 Hz, NH), 8.00 (1H, br s, NH), 6.87 (1H, br t, *J* 4.8 Hz, NH), 4.60 (1H, t, *J* 4.8 Hz), 3.58 – 3.48 (4H, m), 3.43 – 3.30 (4H, m), 2.94 (1H, dd, *J* 4.8 Hz), 2.70 – 2.42 (3H, m), 1.77 (1H, d, *J* 9.9 Hz), 1.62 – 1.55 (2H, m), 1.52 – 1.45 (36H, s), 1.42 (2H, m), 1.24 (24H, s), 0.86 (3H, t, *J* 7 Hz); δ_C (100 MHz, $CDCl_3$) 172.7, 170.6, 162.0, 161.6, 156.5, 155.7, 151.8 (2 × C), 102.7, 82.3, 82.0, 80.2, 78.5, 78.2, 77.6, 46.3, 43.0, 42.9, 41.6, 40.8, 38.8, 38.6, 31.6, 30.6, 28.4 (12 × C), 28.3, 28.1, 27.1, 27.0, 26.8 (8 × C), 22.9, 21.4, 12.8; HRMS (+ve) predicted for $C_{51}H_{90}N_9O_{12}$ $[M+H]^+$ 1007.675, found 1007.686.

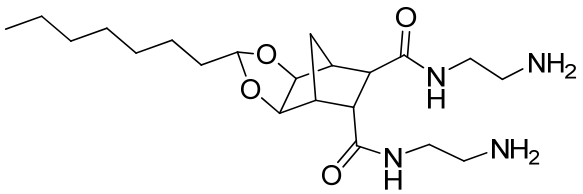


7

8,9-Di[2'-(benzyloxycarbonylamino)ethylcarbamoyl] 4-heptyl-3,5-dioxatricyclo[5.2.1.$0^{2,6}$]decane **(7)**

EDCI (260 mg, 1.35 mmol), HOBt (8 mg, 61 μmol) were added to a solution of diacid **6** (200 mg, 0.613 mmol) in $CHCl_3$ (8 mL) and the solution stirred for 5 minutes at room temperature. A solution of benzyl (2-aminoethyl)carbamate (262 mg, 1.35 mmol) in $CHCl_3$ (2 mL) was added and the solution stirred at room temperature overnight. Water (25 mL) was added and the aqueous phase extracted with EtOAc (3 × 30 mL), the combined organic phases were washed with brine (1 × 30 mL), dried ($MgSO_4$) and the solvent removed to give a white resin. Purification by silica gel chromatography (EtOAc/Petrol 1:1 → EtOAc/MeOH 9.5:0.5) gave diamide **7** (261 mg, 63%) as a white gum. υ_{max} (thin film/cm^{-1}) 3341.8, 3067.4, 2926.3, 2855.1, 1703.8, 1644.3, 1542.5, 1455.3, 1263.5, 1145.6, 1034.5; δ_H (270 MHz, $CDCl_3$) 7.56 (2H, br s, NH), 7.40 (1H, br s, NH), 7.20 – 7.35 (10H, m), 6.00

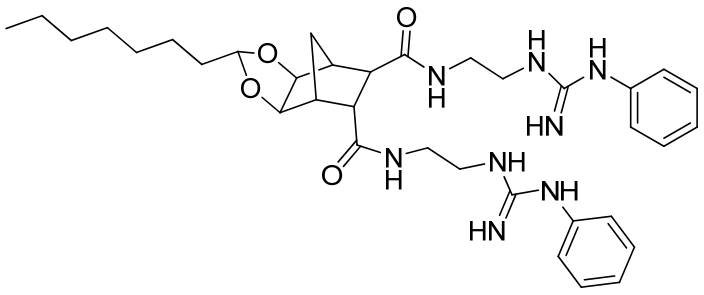
(2H, br s, NH), 5.02 (4H, br s), 4.59 (1H, t, *J* 4.7 Hz), 4.03 (1H, d, *J* 4.8 Hz), 3.97 (1H, d, *J* 4.8 Hz), 3.10 – 3.42 (8H, m), 2.45 - 2.60 (3H, m), 1.73 (1H, d, *J* 10.0 Hz), 1.50 – 1.60 (2H, m, OCH_2) 1.49 (1H, d, *J* 10 Hz), 1.23 (10H, m), 0.85 (3H, m); δ_C (67.5 MHz, $CDCl_3$) 174.7, 172.6, 157.2 (× 2), 136.5, 128.6 (× 5), 128.2 (× 2), 128.1 (× 5), 104.0, 81.6. 78.8, 66.9, 66.8, 46.6, 46.3, 44.0 (× 2), 40.7, 39.9, 39.7, 33.0, 32.1, 31.9, 29.6, 29.3, 24.3, 22.7, 14.2; HRMS (+ve) Calcd for $C_{37}H_{51}N_4O_8$ 679.3701 $[M+H]^+$, found 679.3724.



8

8,9-Di[2'-(amino)ethylcarbamoyl] 4-heptyl-3,5-dioxatricyclo[5.2.1.$0^{2,6}$]decane **(8)**

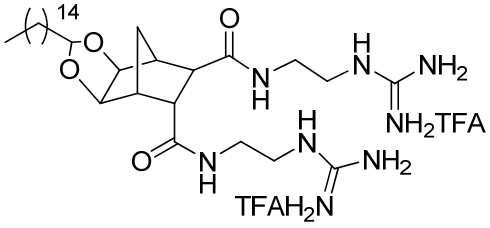
Pd/C (5% Pd loading on Carbon) (67 mg, 20% w/w) was added to a solution of protected amide **7** (366 mg, 0.89 mmol) in methanol (20 mL). The system was flushed with hydrogen gas (balloon) and the solution stirred for 16 hours at room temperature. The system was then flushed with nitrogen gas three times, celite added to the reaction mixture and the resulting slurry filtered through a pad of celite. The filtrate was collected and the solvent removed *in vacuo* to give a diamine **8** as a white gum (266 mg, 96%). υ_{max} (thin film/ cm^{-1}) 3400.5, 2955.3, 2929.0, 2859.0, 2522.4, 2236.2, 2077.8, 1646.4 (broad), 1455.3, 1212.9, 1116.4, 103635, 971.9; δ_H (270 MHz, d_4-methanol) 4.65 (1H, t, *J* 4.6 Hz), 3.20 – 3.60 (8H, m), 3.21 (1H, t, *J* 4.7 Hz), 2.40 – 2.65 (3H, m), 1.73 (1H, d, *J* 10 Hz), 1.58 (3H m), 1.46 (1H, d, *J* 10 Hz), 1.28 (10H, m), 0.88 (3H, m) (amine and amide hydrogens not observed due to deuterium exchange); δ_C (67.5 MHz, d_4-methanol) 175.5, 173.6, 103.7, 81.5, 78.7, 46.0, 45.9, 43.8, 43.7, 39.7 (× 2), 37.9 (× 2), 32.5, 31.6, 31.4, 29.4, 29.3, 23.9, 22.3, 11.5; HRMS (+ve) calculated $C_{21}H_{38}N_4O_4$ 411.2971 $[M+H]^+$, found 411.2944.



2a

8,9-di[2'-(3''phenylguanidino)ethylcarbamoyl] 4-heptyl-3,5-dioxatricyclo[5.2.1.$0^{2,6}$]decane **(2a)**

Diamine **8** (226 mg, 0.55 mmol) in $CHCl_3$ (3 mL) was added to a solution phenylcyanamide (143 mg, 1.2 mmol) in $CHCl_3$ (5 mL) and the sealed vessel heated at 90 ^oC for 48 hours. The solvent was then removed *in vacuo* to give a pale yellow solid. Recrystallisation of the solid (CH_2Cl_2/Petrol) gave a white precipitate which was collected by vacuum filtration and air dried to give guanidine **2a** as white blocks (264 mg, 73%). Analysis of the solid by ^1H NMR spectroscopy confirmed the presence of the desired compound and HPLC R_t = 7.9 min; m.p. 158 - 160 ^oC; ν_{max} (thin film) 3311.4, 3088.4, 2932.4, 1711.6, 1641.1, 1593.7, 1453.0, 1263.8, 1073.5; δ_H (270 MHz, d_4-MeOH) 6.79 – 7.07 (10H, m), 4.62 (1H, m), 4.0 (2H, m), 3.00 – 3.50 (9H, m), 2.42 -2.71 (4H, m), 1.71 (1H, d, *J* 10.9 Hz), 1.57 (2H, m), 1.26 (11H, m), 0.88 (3H, t, *J* 5.9 Hz); δ_C (100 MHz, d_6-DMSO) 172.1, 170.2, 154.8, 151.5, 129.1 (2 × C) , 128.1 (2 × C), 128.0 (2 × C), 127.5 (2 × C), 127.2, 121.2, 119.2, 117.2, 112.9, 102.1, 80.3, 77.4, 34.8, 31.6, 30.4, 28.1, 27.8, 22.9, 21.3, 13.2; HRMS (+ve) predicted for $C_{35}H_{51}N_5O_4^+$ $[M+H]^+$ 647.40274, found 647.4020.



2b

8,9-di[2'-(guanidino)ethylcarbamoyl] 4-pentadecyl-3,5-dioxatricyclo[5.2.1.$0^{2,6}$]decane ditrifluoroacetate **(2b)**

Guanidine **11** (189 mg, 0.19 mmol) was taken up in TFA/CH_2Cl_2 (1:4, 5 mL) and stirred over night at room temperature. The solvent was removed *in vacuo* and the solution repeatedly taken up in $CHCl_3$ (10 mL) and solvent removed (to remove residual TFA) followed by high vacuum. The product was obtained as a light yellow resin (151 mg, quant). ^1H NMR analysis of the resin showed complete absence of *t*-butyl peaks. HRMS (+ve) predicted for $C_{39}H_{51}N_8O_4^+$ $[M+H]^+$ 607.4659, found 607.4621.

1. Compound **3** is known. See: H. Koch, *Monatshefte fuer Chemie*, 1962, **93**, 1343-1347.

Time lapse NMR study

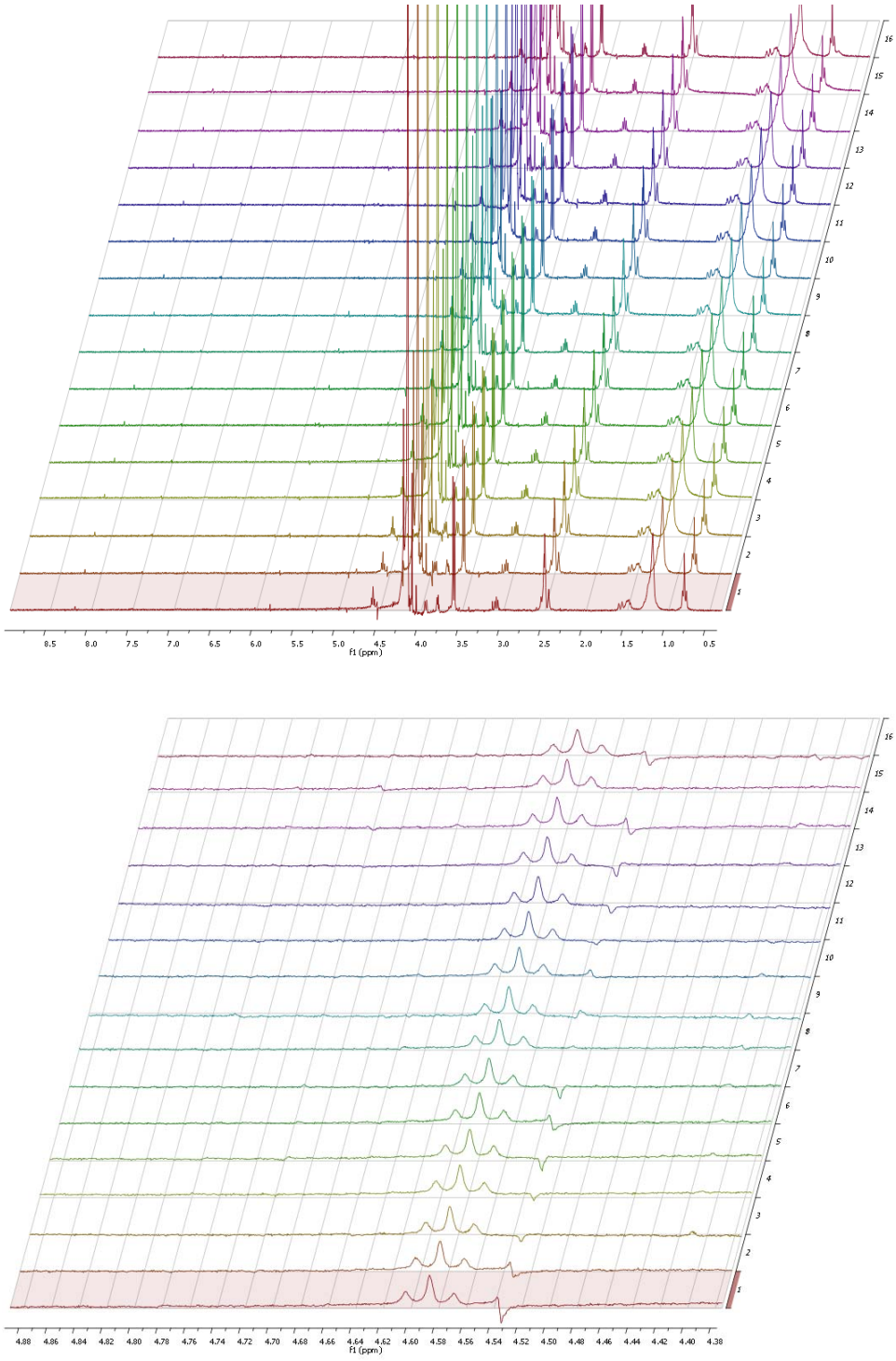


Fig. S1. Stack plot of compound **5** in DMSO acidified to pH 1 with aq. HCl. Top image shows the whole spectrum and the bottom is a zoom to the acetal C-H proton. Scale on the right hand side corresponds to the time after the experiment was started (eg 3 corresponds to t = 3 hr).

Molecular modelling of the 2a-lipopolysacchaide (LPS) complex[2]

The model of **2a** in complex with two LPS molecules was constructed using Accelrys Discovery Studio V2.1 (Accelrys, San Diego, CA). Initially a diverse conformational search was performed on **2a**. The top scoring conformation of **2a** was then manually docked between the structures of two LPS molecules and the complex was energy minimized in vacuum. The modeling process took into account the electrostatic interactions between the positively charged amine groups of **2a** and the negatively charged phosphoester groups on the lipid A, and in addition maximized the reduction of solvent exposed hydrophobic area on all molecules. The coordinates of *E.coli* LPS were derived from the crystal structure of FhuA, the receptor for ferrichrome-iron in *E.coli* with bound LPS (PDB code:1QFF).

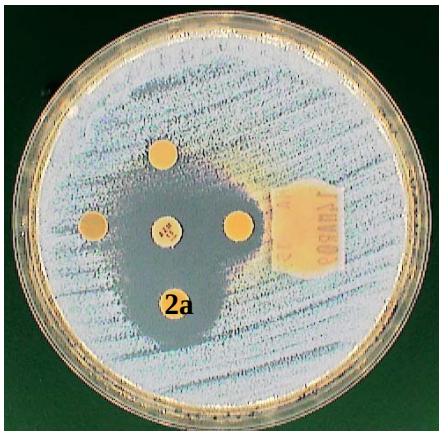
2. For a similar approach see: A. D. Ferguson, W. Welte, E. Hofmann, B. Lindner, O. Holst, J. W. Coulton, K. Diederichs, *Structure Fold. Des*., 2000, **8**, 585-592.

Whilst NMR studies were trialled in order to elicit the exact points of host:guest interaction unfortunately the amphiphilic compounds **2a** and **2b** form aggregates in solution (as does colistin) and as such their NMR spectrum was complex. Simple NMR titrations were attempted but no useful information could be gleaned. Further studies for the elucidation of the exact binding mode are currently being pursued.

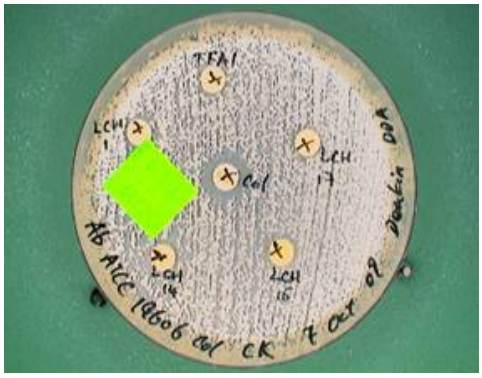
Disk Diffusion

The procedures followed the guidelines of *BSAC Methods for Antimicrobial Susceptibility Testing* (http://www.bsac.org.uk/_db/_documents/Version_8_-_January_2009.pdf), except that blank discs (Oxoid) containing 50 µg compound **2a** (or 30 µg of **2b)** were employed. Nutrient agar (Oxoid) plates were incubated at 35°C for 20 hours before the inhibition zones were measured. Colistin was employed as a control.

Compound **2a**:
A. baumannii ATCC 19606



Compound **2b** (LCH17)
Acinetobacter baumannii ATCC 19606 in the presence of 10 mg/L colistin sulphate



Compound **2b** (LCH17)
Pseudomonas aeruginosa ATCC 27853

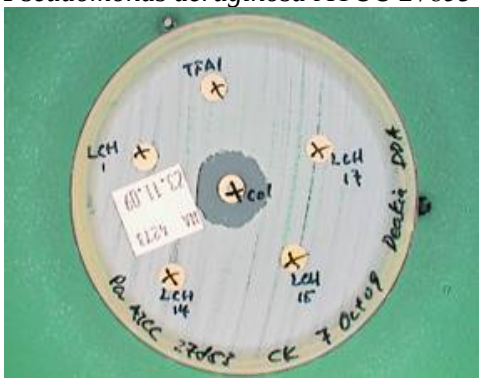


Fig S2. Additional results from disk diffusion assays.

Haemolysis[3]

Total haemolysis was assessed using healthy human red blood cells. Concentrations of compound **2a** were 0.5 to 128 mg/L. A 2% solution of Triton X-100 was employed as a positive control and red blood cells in phosphate buffer saline was a negative control. Haemolysis was calculated as: % Haemolysis = (Abs_{450nm} sample – Abs_{450nm} blank) / (Abs_{450nm} Triton-X100 – Abs_{450nm} of negative control) × 100.

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