Supplementary Material

Supplementary Information for

Positive Ion Pair Cooperativity Exhibited under Physiological Conditions.

Patrick T. Gunning
University of Glasgow, Joseph Black Building, Dept. of Chemistry, Glasgow, UK. Fax: 203 432 5570; Tel: 203 432 5218; E-mail: patrick.gunning@yale.edu

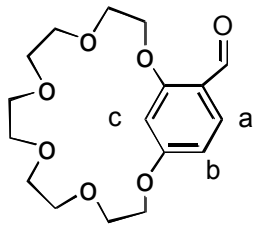
Experimental Procedures.

General Procedures and Methods. All chemicals were purchased from commercial sources and used without further purification. Solvents were dried by standard literature methods, distilled before use and stored over molecular sieves. All ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrophotometer using the solvent as internal standard. Mass spectra were obtained using a Jeol "The Mstation" JMS 700 Mass Spectrometer using CI, EI, and FAB ionization techniques. Elemental analysis was carried out using in-house facilities.

Isothermal Titration Calorimetry ITC. Isothermal titration calorimetry (ITC) experiments were used to measure the binding of **ZnL** to various substrates, and were performed at 25 ^{o}C (298 K) using Microcal MCS and VP-ITC titration microcalorimeters. In order to minimize mixing heat effects caused by differences in solution composition, the substrates and **ZnL** were both dissolved in freshly prepared HEPES buffer (pH = 7.4) before each titration experiment. All solutions prior to experiments were degassed before being added to the calorimeter cell. The substrates, at a concentration of approximately 5.0 mM, were injected in 10μL increments into the reaction cell (cell volume 1.31–1.41 mL) containing **ZnL** at a concentration of *ca* 0.25 mM, until there occurred a saturation of the macrocyclic cavity. A 250 μL injection syringe with 310–400 rpm stirring was used to give a series of 10 μL injections at 3-minute intervals. Control experiments for heats of mixing and dilution were performed under identical conditions and used for data correction in subsequent analysis. Data acquisition and subsequent non-linear regression analysis were done in terms of a simple binding model using the Microcal ORIGIN software package.

Supplementary Material

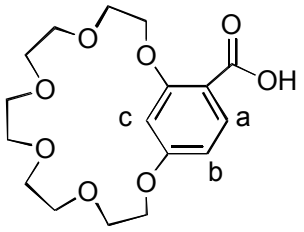
Syntheis of ZnL.(OTf_2



Preparation of 2,5,8,11,14,17-hexaoxa-bicyclo[16.3.1]docosa-1(21),18(22), 19-triene-19-carbaldehyde.

To a stirred mixture of K_2CO_3 (3.15 g, 22.1 mmol) in DMF (30 mL) was added penta(ethylene glycol)di-*p*-toluene sulphonate (5.00 g, 9.11 mmol) in DMF (45 mL) and 2,4-dihydroxybenzaldehyde (1.26 g, 9.11 mmol) in DMF (45 mL) at 90 ^oC under an N_2 atmosphere via a twin drive syringe pump over 48 hrs and then left for a further 24hrs. The reaction solution was left to cool for 1 hr before it was quenched with water (100 mL) and extracted with diethyl ether repeatedly (3 x 100 mL). The organic solutions were then combined and washed with brine (3 x 150 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure to yield crude product which was further purified using silica gel chromatography (5:1, ethyl acetate: petroleum ether) to give pure product as a colourless oil (0.78 g, 25 %): HRMS (FAB^+), calcd. for $C_{17}H_{24}O_7$ [M^+] *m/z* = 340.1522, fnd. 340.1523; ν_{max} (KBr)/cm^{-1}; 2983, 2884, 2260, 1668 (C=O), 1607, 1579, 1353, 1257, 1184, 1106, 1056, 941, 800, 767; ^1H NMR ($CDCl_3$) δ 3.62-3.78 (12H, m, CH_2), 3.87-3.90 (4H, m, CH_2), 4.27 (2H, t, *J* = 4.4 Hz, CH_2), 4.42 (2H, t, *J* = 4.4 Hz, CH_2), 6.57 (1H, dd, *J* = 8.8 and 2.4 Hz, Ar-H_b), 6.97 (1H, d, *J* = 2.4 Hz, Ar-H_c), 7.78 (1H, d, *J* = 8.4 Hz, Ar-H_a), 10.32 (1H, s, CHO); ^{13}C NMR ($CDCl_3$) δ 68.0 (CH_2), 69.2 (CH_2), 69.7 (CH_2), 70.5 (CH_2), 70.6 (CH_2), 70.8 (2 x CH_2), 70.9 (CH_2), 71.2 (CH_2), 71.4 (CH_2), 101.3 (CH), 108.0 (CH), 119.5 (C), 129.9 (CH), 163.7 (C), 165.5 (C), 188.6 (CHO).

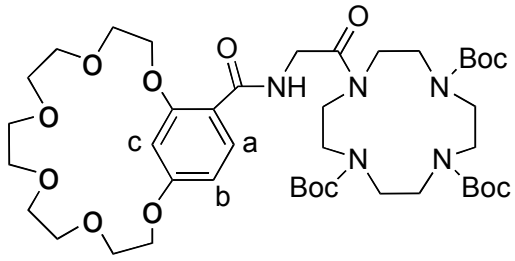
Supplementary Material



2,5,8,11,14,17-Hexaoxa-bicyclo[16.3.1]docosa-1(21),18(22),19-triene-19-carboxylic acid.

To a solution of macrocyclic aldehyde (0.17 g, 0.52 mmol) in an acetone/water solution (3:1 ratio, 2.5 mL), was added slowly sulphamic acid (0.152 g, 1.56 mmol) followed by sodium chlorite (0.052 g, 0.57 mmol) at room temperature. The reaction solution was allowed to stir continuously for 3 hrs before being quenched with the addition of CH_2Cl_2 (40 mL) and washed several times with brine (3 x 40 mL). The organic layer was then dried over $MgSO_4$ and the solvent removed under reduced pressure to yield impure compound that was purified by column chromatography (5:1, ethyl acetate: petroleum ether) to give pure product as a colourless oil (0.17 g, 91 %): Elemental analysis calcd. for $C_{17}H_{24}O_8$: C, 57.30; H, 6.79 %, fnd. C, 57.40; H, 6.85 %; HRMS (FAB^+), calcd. for $C_{17}H_{24}O_8$ [M^+] *m/z* = 356.1471, fnd. 356.1473; ν_{max} (KBr)/cm^{-1}; 3531, 3312, 2920, 2359, 2094, 1697, 1606, 1541, 1502, 1461, 1294, 1236, 1184, 1144, 1105, 1091, 983, 887, 801; ^{1}H NMR ($CDCl_3$) δ 3.45-3.65 (12H, m, CH_2), 3.78 (2H, t, *J* = 4.4 Hz, CH_2), 3.84 (2H, t, *J* = 4.8 Hz, CH_2), 4.20 (2H, t, *J* = 4.4 Hz, CH_2), 4.45 (2H, t, *J* = 4.4 Hz, CH_2), 6.60 (1H, dd, *J* = 8.8 and 2.4 Hz, Ar-H_b), 6.95 (1H, d, *J* = 2.0 Hz, Ar-H_c), 7.99 (1H, d, *J* = 8.8 Hz, Ar-H_a), 10.75 (1H, s (broad), COOH); ^{13}C NMR ($CDCl_3$) δ68.0 (CH_2), 69.9 (CH_2), 70.0 (CH_2), 70.5 (2 x CH_2), 70.7 (CH_2), 70.8 (CH_2), 71.1 (CH_2), 71.2 (CH_2), 71.5 (CH_2), 102.0 (CH), 109.0 (CH), 110.9 (C), 134.6 (CH), 159.4 (C), 164.5 (C), 165.5 (COOH).

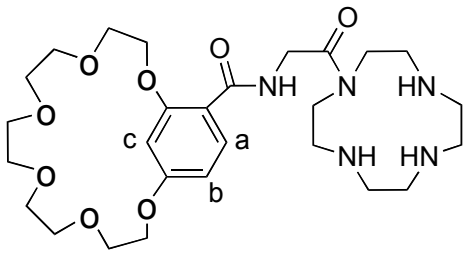
Supplementary Material



Preparation of 10-{2-[(2,5,8,11,14,17-Hexaoxa-bicyclo[16.3.1]docosa-1(21),18(22),19-triene-19-carbonyl)-amino]-acetyl}-1,4,7,10tetraaza-cyclododecane-1,4,7-tricarboxylic acid tri-*tert*-butyl ester.

To a stirred solution of **4** (0.18 g, 0.50 mmol) in CH_2Cl_2 (20 mL) under an N_2 atmosphere at room temperature was added slowly DCC (0.11 g, 0.55 mmol) followed by the drop-wise addition of **3** (0.11 g, 0.50 mmol) in CH_2Cl_2 (10 mL) and DMAP (0.07 g, 0.55 mmol). The solution was left to stir overnight at room temperature before being filtered through glass fibre paper and the solvent removed under reduced pressure to give a yellow oil which was purified using silica gel chromatography (5:1, ethyl acetate:petroleum ether) to furnish pure product as a white oil (0.31 g, 79 %): mp 95-97 ^{o}C; HRMS (FAB^{+}), calcd. for $C_{42}H_{70}O_{14}N_5$ [M^{+}] *m/z* = 868.4919, fnd. 868.4908; ν_{max} (KBr)/cm^{-1}; 3361, 3256, 2971, 2109, 1684, 1643, 1601, 1463, 1365, 1243, 1159, 1109, 937, 855, 777; ^{1}H NMR ($CDCl_3$) δ 1.46 (9H, s, CH_3), 1.48 (9H, s, CH_3), 1.53 (9H, s, CH_3), 3.35-3.78 (28H, m, CH_2) 3.86 (2H, t, *J* = 4.4 Hz, CH_2), 3.96 (2H, t, *J* = 4.8 Hz, CH_2), 4.24 (2H, t, *J* = 4.4 Hz, CH_2), 4.28 (2H, d, *J* = 4.0 Hz, GLY-CH_2), 4.49 (2H, t, *J* = 4.4 Hz, CH_2), 6.60 (1H, dd, *J* = 8.8 and 2.4 Hz, Ar-H_b), 7.01 (1H, d, *J* = 2.0 Hz, Ar-H_c), 8.10 (1H, d, *J* = 8.8 Hz, Ar-H_a), 8.86 (1H, s (broad), NH); ^{13}C NMR ($CDCl_3$) δ68.0 (CH_2), 69.9 (CH_2), 70.0 (CH_2), 70.5 (2 x CH_2), 70.7 (CH_2), 70.8 (CH_2), 71.1 (CH_2), 71.2 (CH_2), 71.5 (CH_2), 102.0 (CH), 109.0 (CH), 110.9 (C), 134.6 (CH), 159.4 (C), 164.5 (C), 165.5 (COOH).

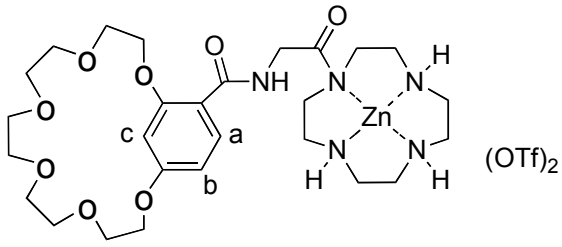
Supplementary Material



2,5,8,11,14,17-Hexaoxa-bicyclo[16.3.1]docosa-1(21),18(22),19-triene-19-carboxylic acid[2-oxo-2-(1,4,7,10tetraaza-cyclododec-1-yl)-ethyl]-amide(L).

To a stirred solution of **5** (0.17 g, 0.22 mmol) in CH_2Cl_2 (20 mL) was added TFA (3 mL) under an N_2 atmosphere and was left to stir at room temperature for 4 hrs before the solution was quenched with 3M NaOH (20 mL) and washed several times with brine (3 x 30 mL). The organic layer was then dried over $NaSO_4$ and the solvent removed under reduced pressure to yield the pure compound as a colourless oil (0.095 g, 75 %): HRMS (FAB^+), calcd. for $C_{27}H_{45}O_8N_5$ [M^+] *m/z* = 567.3268, fnd. 567.3275; ν_{max} (KBr)/cm^{-1}; 3381, 2923, 2347, 2245, 1633, 1604, 1531, 1469, 1355, 1294, 1256, 1184, 1121, 989, 911, 827; ^{1}H NMR ($CDCl_3$) δ 2.60 (t, 4H, *J* = 5.6 Hz, CH_2), 2.66 (t, 2H, *J* = 5.6 Hz, CH_2), 2.72 (t, 2H, *J* = 4.4 Hz, CH_2), 2.79 (t, 2H, *J* = 5.6 Hz, CH_2), 2.82 (t, 2H, *J* = 5.2 Hz, CH_2), 3.46 (t, 2H, *J* = 4.8 Hz, CH_2), 3.52-3.66 (m, 14H, CH_2), 3.75 (t, 2H, *J* = 4.4 Hz, CH_2), 3.86 (t, 2H, *J* = 4.8 Hz, CH_2), 4.13 (t, 2H, *J* = 4.4 Hz, CH_2), 4.28 (d, 2H, *J* = 4.0 Hz, CH_2), 4.39 (t, 2H, *J* = 4.8 Hz, CH_2), 6.50 (dd, 1H, *J* = 8.8 and 2.4 Hz, Ar-H_b), 6.92 (d, 1H, *J* = 2.4 Hz, Ar-H_c), 8.00 (d, 1H, *J* = 8.8 Hz, Ar-H_a), 8.82 (t, 1H, *J* = 4.0 Hz, NH); ^{13}C NMR ($CDCl_3$) δ □□□ (CH_2), 44.9 (CH_2), 46.5 (CH_2), 47.4 (CH_2), 47.9 (CH_2), 48.1 (CH_2), 48.3 (CH_2), 49.1 (CH_2), 49.4 (CH_2), 67.9 (CH_2), 69.6 (CH_2), 69.8 (CH_2), 70.5 (2 x CH_2), 70.8 (3 x CH_2), 71.2 (CH_2), 71.3 (CH_2), 102.3 (CH), 107.6 (CH), 114.7 (C), 133.0 (CH), 159.2 (C), 162.7 (C), 165.0 (C=O), 170.3 (C=O).

Supplementary Material



ZnL.(OTf)$_2$.

To a stirred solution of **6** (0.05 g, 0.088 mmol) in anhydrous MeOH (1 mL) was added Zn(OTf)$_2$ (0.032 g, 0.088 mmol) under an N_2 atmosphere and was left to stir at room temperature for 4 hrs. The solution was filtered and the solvent removed under reduced pressure to yield the pure compound as a colourless oil (0.08g, 97 %); HRMS (FAB$^+$), calcd. for $C_{27}H_{44}O_8Zn$ [M^+] *m/z* = 630.2481, fnd. 630.2448; ν_{max} (KBr)/cm^{-1}; 3222, 2870, 2356, 1603 (C=O), 1545, 1456, 1361, 1242, 1152, 1025, 978, 844, 815, 716, 663 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ2.60-3.30 (16H, m, CH_2), 3.45-3.53 (6H, m, CH_2), 3.57 (4H, t, *J* = 4.4 Hz, CH_2), 3.73 (2H, t, *J* = 4.4 Hz, CH_2), 3.82 (4H, t, *J* = 4.4 Hz, CH_2), 4.18 (2H, t, *J* = 4.4 Hz, CH_2), 4.29 (2H, s, CH_2), 4.41 (2H, t, *J* = 4.4 Hz, CH_2), 6.55 (dd, 1H, *J* = 8.8 and 2.4 Hz, Ar-H_b), 6.95 (d, 1H, *J* = 2.0 Hz, Ar-H_c), 7.87 (1H, d, *J* = 8.8 Hz, Ar-H_a).

ITC graphs

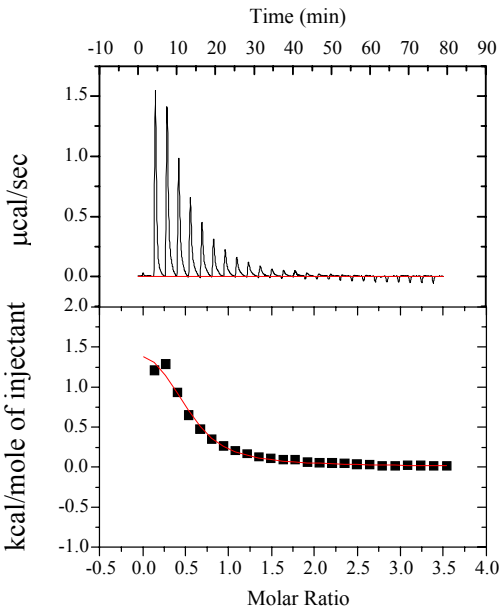


Figure 1. ITC graph for the binding of $Li[H_2PO_4]$ by $ZnL.(OTf)_2$.

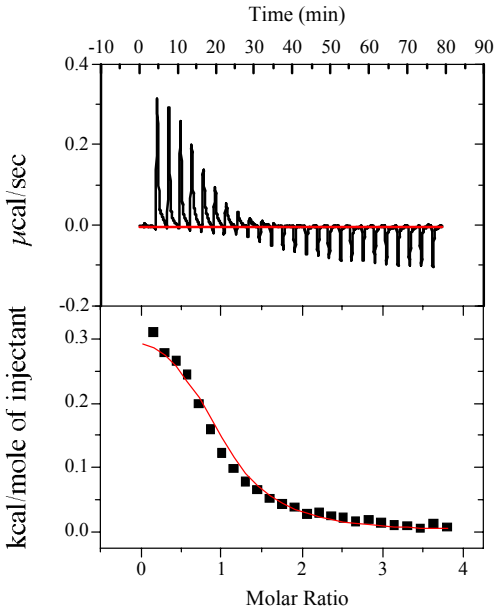


Figure 2. ITC graph for the binding of $K[H_2PO_4]$ by $ZnL.(OTf)_2$.

Supplementary Material

^{1}H NMR Spectrum of ZnL.(OTf)$_2$