ELECTRONIC SUPPLEMENTARY MATERIAL

Sulfate templated synthesis of a triply interlocked capsule

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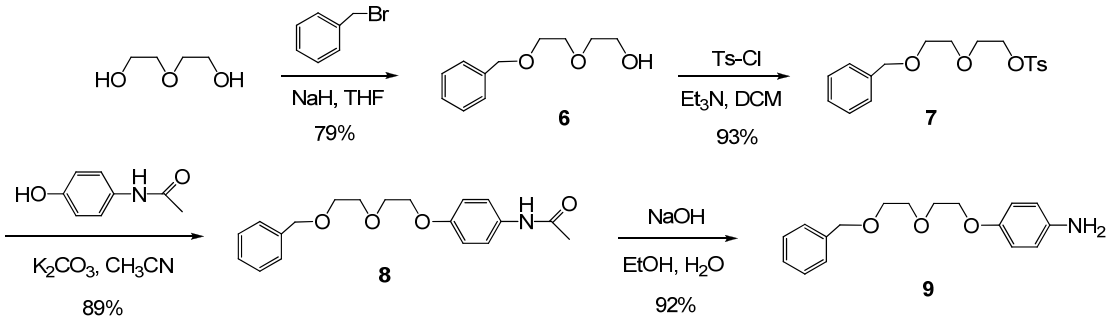
EXPERIMENTAL METHODS

General considerations

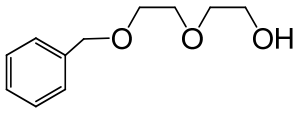
Dry solvents were obtained by purging with nitrogen and then passing through an MBraun MPSP-800 column. H_2O was de-ionised and microfiltered using a Milli-Q® Millipore machine. All tetrabutylammonium salts were stored in a vacuum desiccator over phosphorus pentoxide prior to use. All other solvents and commercial grade reagents were used without further purification unless otherwise noted.

Column chromatography was performed on silica gel (160-200 mesh), and thin-layer chromatography (TLC) was performed on preparative silica gel GF plates with UV254 (1000 microns, Analtech, USA). ^1H and ^{13}C NMR spectra were recorded on a Varian Mercury VX300 or Varian Unity Plus 500 spectrometer. High resolution electrospray ionization (ESI) mass spectrometry was performed on a Micromass LCT (ESMS) instrument.

Synthetic procedures

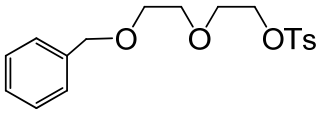


Synthesis of 2-(2-(benzyloxy)ethoxy)ethanol (6)



To a suspension of NaH (1.2 g, 49 mmol) in dry THF (30 mL) was added a solution of diethylene glycol (20 g, 190 mmol) in THF (40 mL) dropwise at 0 ^{0}C. The resulting mixture was stirred for 30 minutes, after which time benzyl bromide (6.2 g, 38 mmol) in THF (30 mL) was added dropwise over 30 minutes. The reaction mixture was then stirred under N_2 overnight at room temperature. After this time H_2O (10 mL) was added carefully to quench excess NaH and the solvent was removed *in vacuo*. The residue was dissolved in ethyl acetate (200 mL) and washed with H_2O (3 × 100 mL). The organic layer was then dried over $MgSO_4$ and the solvent was evaporated. The residue was then dissolved hexane (200 mL) and extracted with H_2O (3 × 100 mL). The aqueous layer was evaporated and then dried under high vacuum to give **6** as a colourless oil (5.8 g, 79 %); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ 7.37-7.30 (5H, m, Ar*H*), 4.59 (2H, s, Ar-C*H*$_2$-O), 3.76-3.70 (4H, m, -C*H*$_2$C*H*$_2$OH), 3.67-3.61 (4H, m, -OC*H*$_2$C*H*$_2$O-); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ 137.9, 128.4, 127.8, 127.7, 73.3, 72.4, 70.5, 69.4, 61.8. ESI-MS (*m*/*z*): $[M + Na]^+$ 219.0989, $C_{11}H_{16}O_3Na$ (calc. 219.0992).

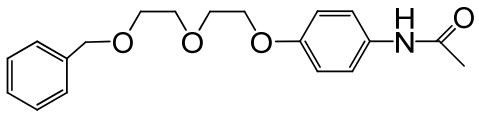
Synthesis of 2-(2-(benzyloxy)ethoxy)ethyl-*p*-toluenesulfonate (7)



2-(2-(benzyloxy)ethoxy)ethanol, **6**, (5.7 g, 29 mmol) and *p*-toluenesulfonyl chloride (5.5 g, 29 mmol) were dissolved in dry DCM (150 mL). Triethylamine (3 mL) and a catalytic amount of DMAP (~20 mg) were then added and the solution stirred under N_2 at room temperature for 16 hours. Water (100 mL) was then added and the reaction mixture acidified with aqueous 10% citric acid to pH 7. The organic layer was washed with water (3×100 mL) and sat. NaCl $_{(aq)}$ (100 mL). The organic layer was then dried over $MgSO_4$ and the solvent removed to give the product as a clear viscous oil (9.2 g, 93 %); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ 7.80 (2H, d, ^3J = 8.2 Hz, HQ*H*), 7.36-7.29 (7H, m, Ar*H* and HQ*H*), 4.54 (2H, s, Ar-C*H*$_2$-O), 4.20-4.16 (2H, m, -C*H*$_2$-),

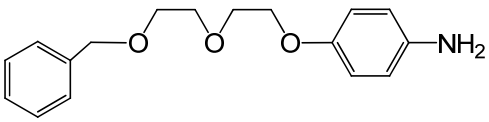
3.72-3.69 (2H, m, -CH_2-), 3.64-3.61 (2H, m, -CH_2-), 3.59-3.56 (2H, m, -CH_2-), 2.44(3H, s, -CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ 144.8, 138.1, 132.9, 129.8, 128.4, 128.0, 127.7, 127.7, 73.3, 70.8, 69.3, 69.3, 68.7, 21.6; ESI-MS (*m*/*z*): $[M + Na]^+$ 373.1070, $C_{18}H_{22}O_5SNa$ (calc. 373.1080).

Synthesis of *N*-(4-(2-(2-(benzyloxy)ethoxy)ethoxy)phenyl)acetamide (8)



A solution of **7** (6.17 g, 17.6 mmol), *N*-(4-hydroxyphenyl)acetamide (2.66 g, 17.6 mmol) and K_2CO_3 (2.92 g, 21.1 mmol) in dry acetonitrile (100 mL) was refluxed under N_2 for 16 hours. The reaction mixture was then allowed to cool to room temperature, filtered and the solvent removed *in vacuo*. The crude product was purified by column chromatography using 2 % MeOH/DCM as the eluent to give the pure product as a colourless viscous oil (5.13 g, 89 %); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ 7.40-7.28 (7H, m, Ar*H* and HQ*H*), 7.11 (1H, s, -N*H*-), 6.88 (2H, d, ^3J = 9.1 Hz, HQ*H*), 4.59 (2H, s, Ar-CH_2-O), 4.14-4.11 (2H, m, -CH_2-), 3.88-3.85 (2H, m, -CH_2-), 3.77-3.74 (2H, m, -CH_2-), 3.69-3.65 (2H, m, -CH_2-), 2.16 (3H, s, -CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ 168.2, 155.5, 131.2, 128.5, 127.8, 127.6, 121.7, 114.9, 73.3, 70.8, 69.7, 69.4, 67.7, 24.3; ESI-MS (*m*/*z*): $[M + Na]^+$ 352.1523, $C_{19}H_{23}NO_4Na$ (calc. 352.1519).

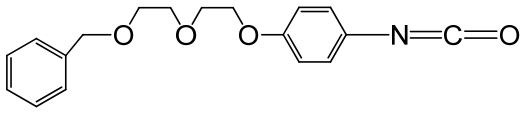
Synthesis of 4-(2-(2-(benzyloxy)ethoxy)ethoxy)aniline (9)



A solution of **8** (5.13 g, 15.6 mmol) and NaOH (12 g, 0.30 mol) in 5:1 water: ethanol (180 mL) was refluxed under N_2 for 48 hours. After this time the reaction mixture was allowed to cool to room temperature, filtered and the solvent removed *in vacuo*. The residue was dissolved water (150 mL) and extracted with DCM (3×150 mL). The organic layer was dried over $MgSO_4$ the solvent was evaporated *in vacuo* to give the product as a brown viscous oil (4.12 g, 92 %); ^{1}H NMR (300 MHz, DMSO-d_6, 298 K): δ 7.37-7.24 (5H, m, Ar*H*), 6.67-6.62 (2H, m, HQ*H*), 6.52-6.46 (2H, m, HQ*H*), 4.60 (2H,

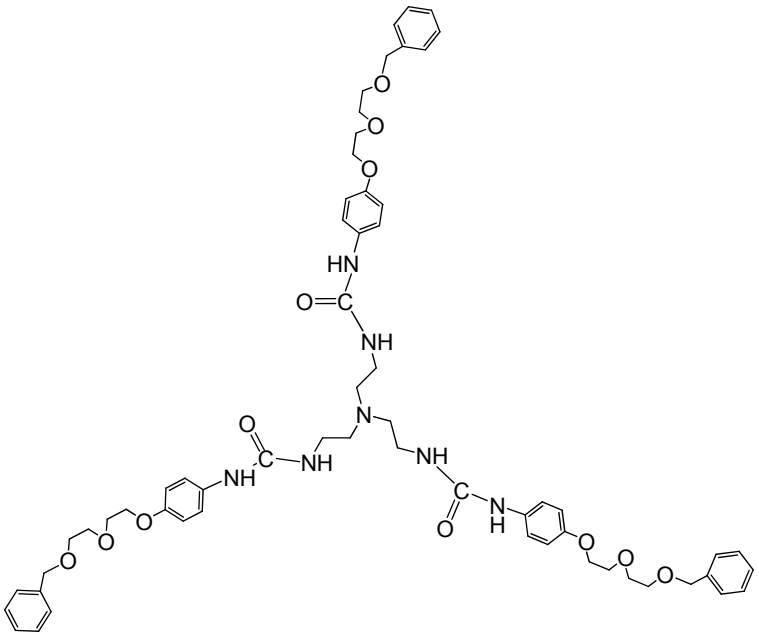
s, -N*H*$_2$), 4.49 (2H, s, Ar-C*H*$_2$-O), 3.94-3.91 (2H, m, -C*H*$_2$-), , 3.69-3.66 (2H, m, -C*H*$_2$-), 3.63-3.60 (2H, m, -C*H*$_2$-), 3.58-3.55 (2H, m, -C*H*$_2$-); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ157.4, 143.6, 138.6, 128.4, 127.8, 127.6, 116.3, 115.9, 73.3, 70.8, 69.9, 69.4, 68.2; ESI-MS (*m*/*z*): $[M + Na]^+$ 310.1417, $C_{17}H_{21}NO_3Na$ (calcd. 310.1414).

Synthesis of 1-(2-(2-(benzyloxy)ethoxy)ethoxy)-4-isocyanatobenzene (1)



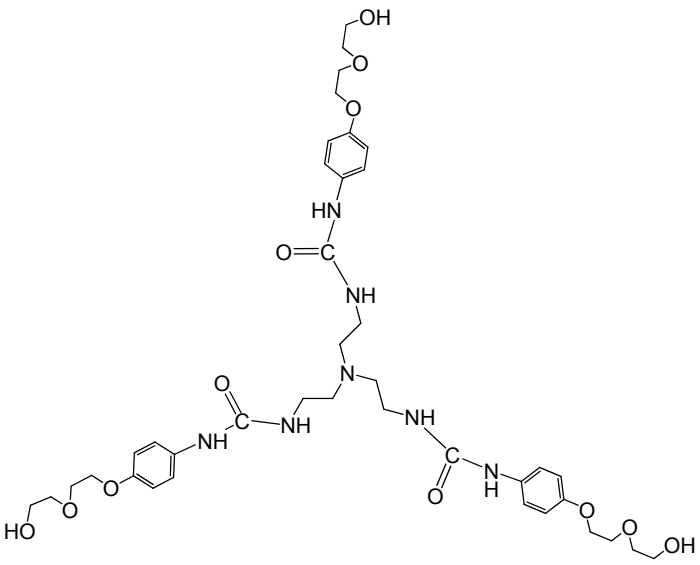
Compound **9** (1.0 g, 3.5 mmol), triphosgene (0.62 g, 2.1 mmol) and triethylamine (0.51 mL, 3.6 mmol) were dissolved in toluene (75 mL) and heated at 70 ^{0}C for 5 hours. The reaction mixture was then cooled to room temperature, filtered and the solvent evaporated to give **1** as a pale white solid in quantitative yield. This product was used immediately in the next step without any further purification or characterisation.

Synthesis of 1,1',1''-(nitrilotriethane-2,1-diyl)tris(3-(4-(2-(2-(benzyloxy)ethoxy) ethoxy)phenyl)urea) (2)



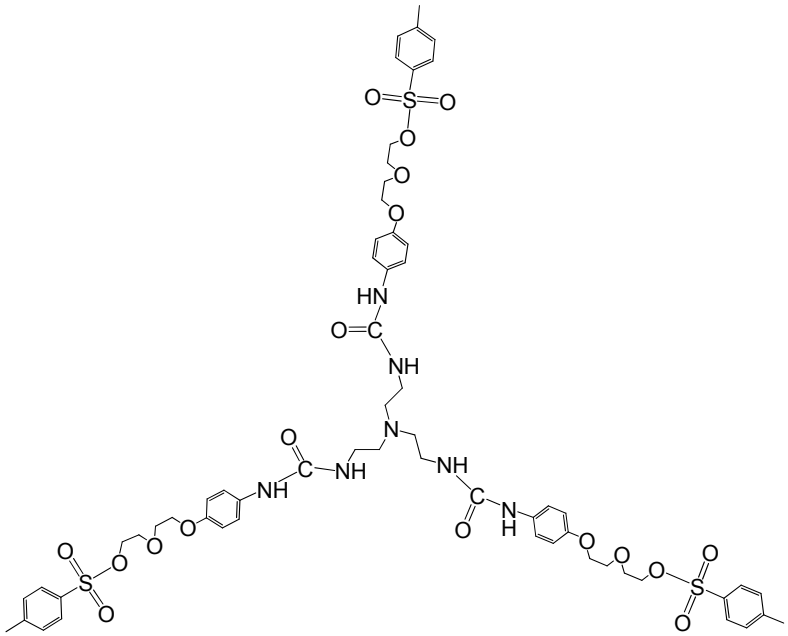
Compound **1** (1.09 g, 3.40 mmol), *N*,*N*-bis(2-aminoethyl)ethane-1,2-diamine (0.154 g, 1.05 mmol) and dibutyltin dilaurate (0.33 g, 0.52 mmol) were dissolved in dry DCM (50 mL). The mixture was stirred under N_2 at room temperature for 48 hours. After this time the solvent was evaporated and the crude product was purified by column chromatography using 3 % MeOH/DCM as the eluent to give the pure product as a pale pink solid (0.95 g, 83 %); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ 7.58 (3H, s, -CH_2N*H*CO), 7.25-7.16 (15H, m, Ar*H*), 6.96 (6H, d, ^3J = 8.8 Hz, HQ*H*), 6.59 (6H, d, ^3J = 8.8 Hz, HQ*H*), 6.15 (3H, s, -CON*H*HQ), 4.47 (6H, s, Ar-CH_2-O), 3.91-3.88 (6H, m, -CH_2-), 3.72-3.69 (6H, m, -CH_2-), 3.64-3.61 (6H, m, -CH_2-), 3.56-3.53 (6H, m, -CH_2-), 3.02 (6H, s, -CH_2-), 2.24 (6H, s, -CH_2-); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ157.1, 154.5, 138.1, 132.3, 128.3, 127.7, 127.6, 121.4, 114.8, 73.2, 70.7, 69.7, 69.3, 67.6, 54.3, 37.9; ESI-MS (*m*/*z*): $[M + H]^+$ 1086.5526, $C_{60}H_{76}N_7O_{12}$ (calc. 1086.5546).

Synthesis of 1,1',1''-(nitrilotriethane-2,1-diyl)tris(3-(4-(2-(2-(hydroxyethoxy) ethoxy)phenyl)urea) (10)



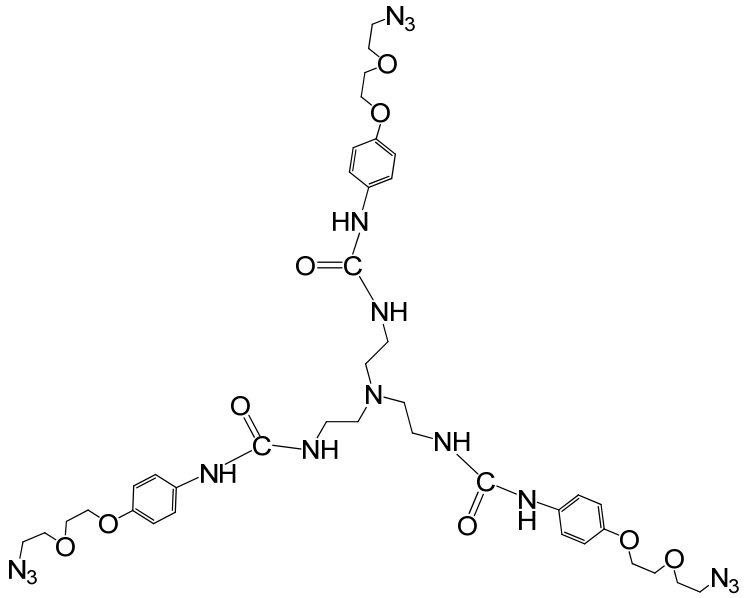
The benzyl protected tren **2** (0.61 g, 0.56 mmol) and Et_3N (20 mL, 0.14 mol) were dissolved in THF (100 mL). 10 % Pd on C (0.50 g, 10 % by weight) was added and the reaction mixture was stirred vigorously under H_2 for 3 days. After this time the solvent was filtered through celite and the solvent was removed *in vacuo*. The crude product was purified by column chromatography using 20 % MeOH/DCM as the eluent to give the pure product as a colourless viscous oil (0.31 g, 68 %); ^{1}H NMR (300 MHz, CD_3OD, 298 K): δ 7.10 (6H, d, ^3J = 8.9 Hz, HQ*H*), 6.63 (6H, d, ^3J = 8.9 Hz, HQ*H*), 3.92-3.89 (6H, m, -CH_2-), 3.69-3.67 (6H, m, -CH_2-), 3.58-3.56 (6H, m, -CH_2-), 3.52-3.50 (6H, m, -CH_2-), 3.24 (6H, s, -CH_2-), 2.69 (6H, s, -CH_2-). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ 158.8, 155.8, 133.9, 122.5, 115.8, 73.8, 70.8, 68.9, 62.2, 55.6, 38.3; ESI-MS (*m*/*z*): $[M + H]^+$ 816.4138, $C_{39}H_{58}N_7O_{12}$ (calc. 816.4138).

Synthesis of *p*-toluenesulfonyl-1,1',1''-(nitrilotriethane-2,1-diyl)tris(3-(4-(2-(2-(hydroxyethoxy)ethoxy)phenyl)urea) (11)



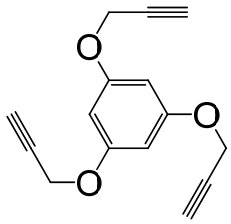
Compound **10** (0.30 g, 0.368 mmol) and *p*-toluenesulfonyl chloride (0.42 g, 2.2 mmol) were dissolved in dry DCM (50 mL). Et_3N (2 mL) and a catalytic amount of DMAP were (~20 mg) were then added and the reaction mixture was stirred for 48 hours. After this time the solvent was concentrated *in vacuo* then the crude residue was purified by column chromatography using 10 % MeOH/DCM as the eluent to give **11** as a pale yellow solid (0.33 g, 71 %); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ7.76 (6H, d, ^3J = 8.3 Hz, CH_3Ar*H*S), 7.42 (3H, s, -CH_2N*H*CO), 7.29 (6H, d, ^3J = 8.3 Hz, CH_3Ar*H*S), 6.98 (6H, d, ^3J = 8.9 Hz, HQ*H*), 6.63 (6H, d, ^3J = 8.9 Hz, HQ*H*), 6.11 (3H, s, -CON*H*HQ), 4.17-4.14 (6H, m, -CH_2-), 3.90-3.87 (6H, m, -CH_2-), 3.73-3.70 (12H, m, -CH_2-), 3.13 (6H, s, -CH_2-), 2.39 (9H, s, -CH_3-), 2.36 (6H, s, -CH_2-); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ157.0, 154.0, 145.0, 133.2, 132.7, 129.9, 127.9, 121.0, 114.7, 69.8, 69.4, 68.8, 67.7, 54.0, 46.2, 21.6; ESI-MS (*m*/*z*): $[M + Na]^+$ 1300.4247, $C_{60}H_{76}N_7O_{18}S_3Na$ (calcd. 1300.4223).

Synthesis of 1,1',1''-(2,2',2''-nitrilotris(ethane-2,1-diyl))tris(3-(4-(2-(2-azido ethoxy)ethoxy)phenyl)urea) (3)



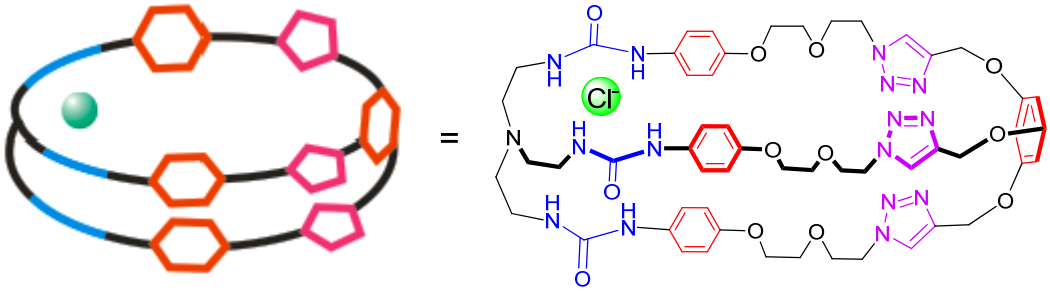
A solution of **11** (0.26 g, 0.20 mmol) and sodium azide (0.13 g, 2.0 mmol) in dry DMF (50 mL) were stirred at room temperature under N_2 for 48 hours. The solvent was then removed *in vacuo* and the residue was then stirred in water (40 mL) and ethyl acetate (40 mL) for 30 minutes. The aqueous layer was then extracted with ethyl acetate (3 × 25 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed to give **3** as a pale yellow solid (0.16 g, 89 %); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ7.44 (3H, s, -CH_2N*H*CO), 6.96 (6H, d, ^3J = 8.9 Hz, HQ*H*), 6.66 (6H, d, ^3J = 8.9 Hz, HQ*H*), 6.15 (3H, s, -CON*H*HQ), 4.00-3.97 (6H, m, -CH_2-), 3.82-3.78 (6H, m, -CH_2-), 3.74-3.71 (6H, m, -CH_2-), 3.42-3.39 (6H, m, -CH_2-), 3.11 (6H, s, -CH_2-), 2.32 (6H, s, -CH_2-); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ157.1, 154.6, 132.2, 121.7, 115.0, 71.5, 70.2, 69.8, 67.7, 50.7, 42.8; ESI-MS (*m*/*z*): $[M + Na]^+$ 913.4158, $C_{39}H_{54}N_{16}O_9Na$ (calc. 913.4152).

Synthesis of 1,3,5-tris(prop-2-ynyloxy)benzene (12)[1]



Benzene-1,3,5-triol (0.50 g, 3.1 mmol), propargyl bromide (1.3 g, 11 mmol), K_2CO_3 (1.5 g, 11 mmol) and 18-crown-6 (0.060 g, 0.60 mmol) were suspended in dry CH_3CN (30 mL). This mixture was then refluxed under N_2 for 16 hours. The reaction mixture was diluted with EtOAc (50 mL) and then washed with sat. NaCl (aq) (3 × 20 mL). The organic phase was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The residue was then purified by crystallisation from EtOAc/hexane followed by column chromatography (EtOAc/hexane, 1: 6) to give **12** as a white solid (0.49 g, 66 %); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ6.27 (3H, s, Ar*H*), 4.65 (6H, d, ^3J = 2.4 Hz, -CH_2-), 2.55-2.53 (3H, m, -CH); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ159.3, 95.4, 78.2, 75.7, 55.9; ESI-MS (*m*/*z*): $[M + Na]^+$ 263.0678, $C_{15}H_{12}O_3Na$ (calc. 263.0679).

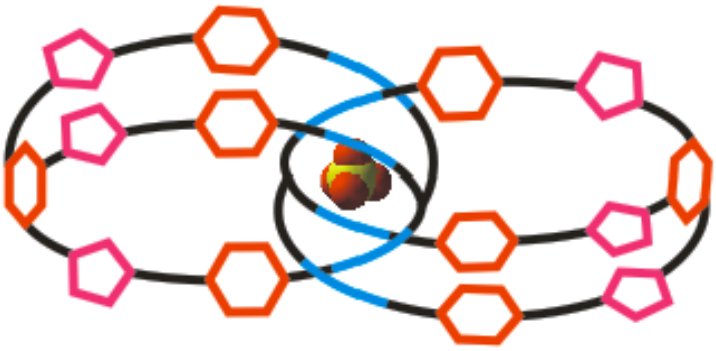
Synthesis of Macrobicycle-Cl$^-$ (4-Cl$^-$)



The azide substituted TREN **3** (85 mg, 0.095 mmol), **12** (23 mg, 0.095 mmol), N, N'-diisopropyethylamine (46 mg, 0.062 mL, 0.36 mmol), Tris(1-benyl-1H-1,2,3-triazol-4-yl)methyl)amine (TBTA) (5 mg) and TBACl (27 mg, 0.095 mmol) were dissolved in dry DCM (40 mL). $Cu(MeCN)_4PF_6$ (16 mg, 0.043 mmol) was added and the reaction mixture was stirred under N_2 at room temperature for 48 hours. After this time the reaction mixture was filtered and the solvent was removed *in vacuo*. The crude residue was purified by preparative thin layer chromatography (TLC) using 12:1 DCM/MeOH as the eluent to give the pure product

as a pale white solid (0.34 g, 32 %); ^{1}H NMR (500 MHz, DMSO-d_6, 298 K): δ8.98 (3H, s, -N*H*-), 8.11 (3H, s, triazole-C*H*), 7.28 (6H, d, ^{3}J = 8.8 Hz, HQ-*H*), 6.71 (6H, d, ^{3}J = 9.3 Hz, HQ-*H*), 6.50 (3H, s, -N*H*-), 6.41 (3H, s, Ar-C*H*-), 5.11 (6H, s, triazole-CH_2-O), 4.55-4.53 (6H, m, -CH_2-), 3.96-3.95 (6H, m, -CH_2-), 3.81-3.79 (6H, m, -CH_2-), 3.67-3.65 (6H, m, -CH_2-), 3.19 (6H, s, -CH_2-), 2.41 (6H, s, -CH_2-); ^{13}C NMR (125 MHz, $CDCl_3$: CD_3OD = 1:1, 298 K): δ161.3, 158.9, 155.7, 144.8, 134.2, 126.4, 122.7, 116.3, 96.7, 71.1, 70.5, 68.6, 62.7, 55.6, 51.7, 39.0; ESI-MS (*m*/*z*): $[M + H]^+$ 1131.5023, $C_{54}H_{67}N_{16}O_{12}$ (calc. 1131.5119).

Synthesis of Capsule-SO_4^{2-} (5-SO_4^{2-}):



The azide substituted TREN **3** (85 mg, 0.095 mmol), **12** (23 mg, 0.095 mmol), N, N'-diisopropyethylamine (46 mg, 0.062 mL, 0.36 mmol), Tris(1-benyl-1H-1,2,3-triazol-4-yl)methyl)amine (TBTA) (5 mg) and TBA_2SO_4 (28 mg, 0.048 mmol) were dissolved in dry DCM (40 mL). $Cu(MeCN)_4PF_6$ (16 mg, 0.043 mmol) was added and the reaction mixture was stirred under N_2 at room temperature for 48 hours. After this time the reaction mixture was filtered and the solvent evaporated *in vacuo*. The residue was purified by column chromatography (100:25:1 DCM: MeOH: Et_3N) to give the pure product as a pale white solid (0.23 g, 21%); ^{1}H NMR (500 MHz, DMSO-d_6, 298 K): δ 8.63 (2H, s, -N*H*-), 8.57 (1H, s, -N*H*-), 8.16-8.14 (3H, m, triazole-C*H*), 7.25-7.22 (6H, m, HQ-*H*), 6.72-6.71 (6H, m, HQ-*H*), 6.34 (1H, s, -N*H*-), 6.31 (3H, s, Ar-C*H*-), 6.28 (1H, s, -N*H*-), 5.05 (6H, s, triazole-CH_2-O), 4.54-4.52 (6H, m, -CH_2-), 3.90-3.88 (6H, m, -CH_2-), 3.82-3.80 (6H, m, -CH_2-), 3.65-3.63 (6H, m, -CH_2-), 3.12 (6H, s, -CH_2-), 2.44 (6H, s, -CH_2-); ^{13}C NMR (125 MHz, DMSO-d_6, 298 K): δ159.8, 155.4, 152.9, 142.5, 133.8, 124.9, 119.0,

114.5, 94.7, 68.7, 67.1, 62.5, 61.2, 52.0, 49.4, 36.5. ESI-MS (*m*/*z*): $[M + SO_4]^{2-}$ 1178.9656, $C_{108}H_{132}N_{32}O_{28}S/2$ (calc. 1178.9824).

^{1}H NMR titration data

Titration protocols

All titrations were conducted on an Oxford Instruments Varian Unity Plus 500 MHz spectrometer, at 298 K. Initial sample volumes were 600 μL. The starting concentration of the host was 2 mM for all titrations. All anions were added as their TBA salts. 22 aliquots of the TBAX solutions were added until a total of 10 equivalents of the anion had been added. Spectra were recorded after each addition, and the sample shaken thoroughly before measurement.

Stability constants were obtained by analysis of the resulting titration data using the WinEQNMR[2] computer program. Estimates for each binding constant, the limiting chemical shifts and the complex stoichiometry were also added to the input file. The various parameters were refined by non-linear least-squares analysis to achieve the best fit between observed and calculated chemical shifts. The parameters were varied until the values for the stability constants converged. Comparison of the calculated binding isotherm with that obtained experimentally demonstrated that the model used was appropriate.

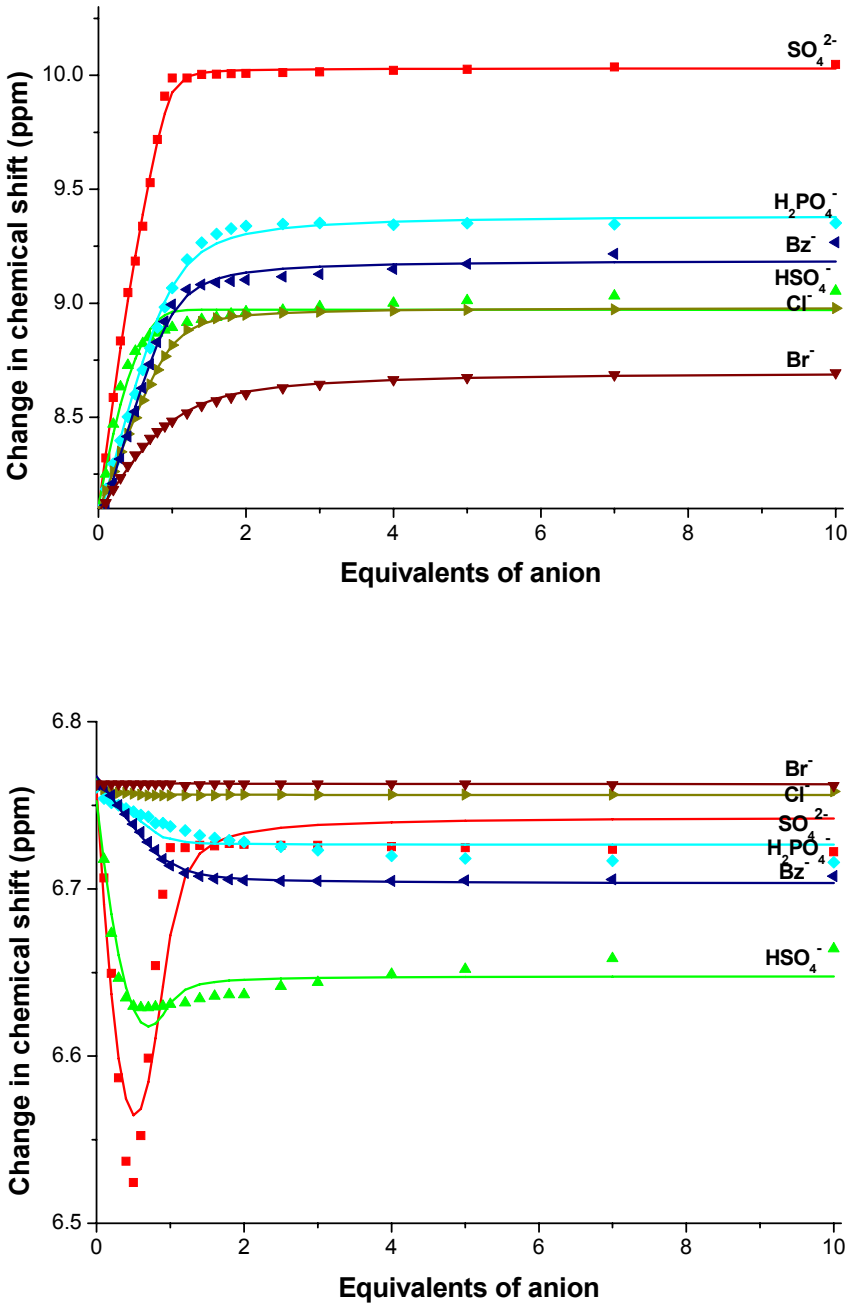


Figure S1. Change in the chemical shift of urea proton *a* (top) and hydroquinone proton *d* (bottom) on addition of anions to a 2 mM solution of **2** in 5: 1 CD_3CN: DMSO-d_6 at 298 K. Symbols represent experimental data points; Continuous lines represent calculated curves. All anions were added as their TBA salts.

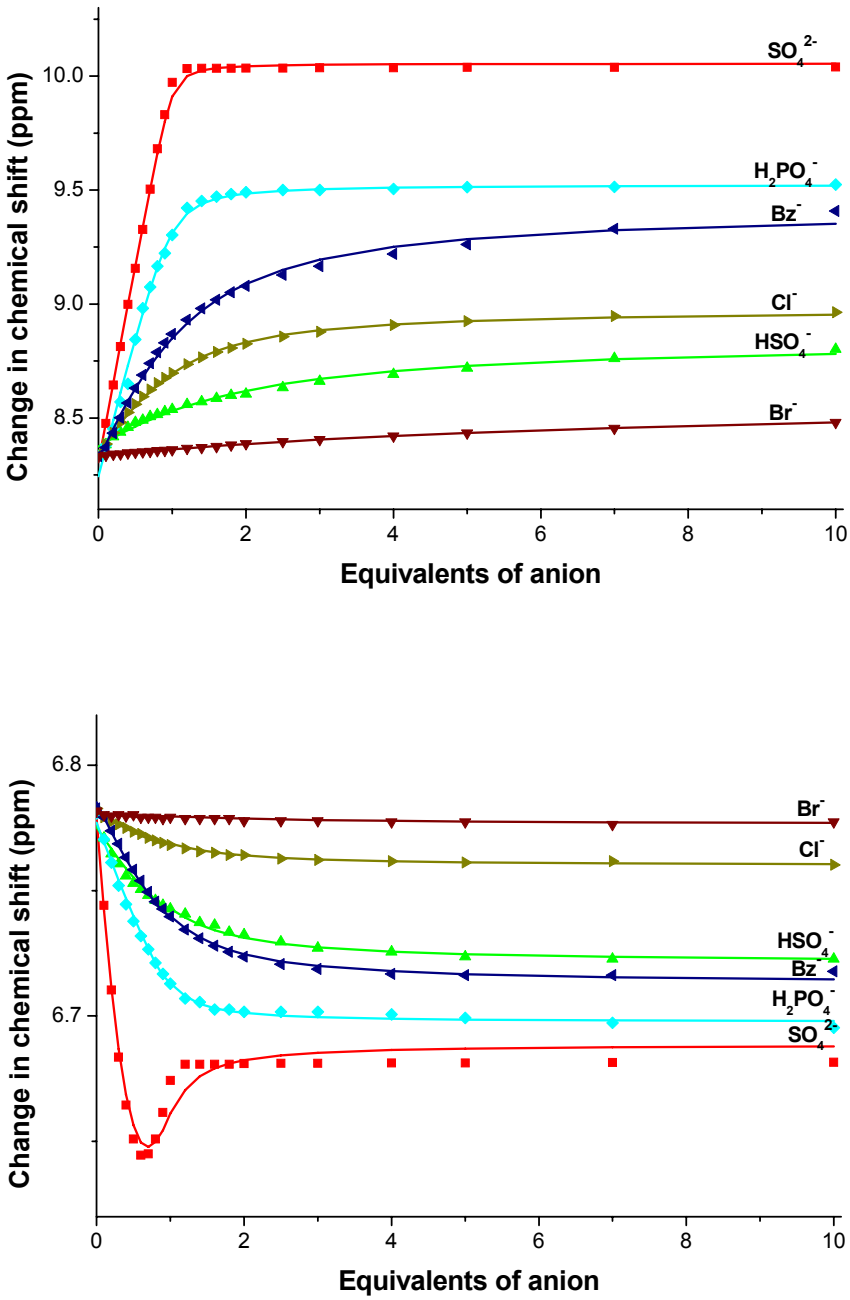


Figure S2. Change in the chemical shift of urea proton *a* (top) and hydroquinone proton *d* (bottom) on addition of anions to a 2 mM solution of **2** in DMSO-d_6 at 298 K. Symbols represent experimental data points; Continuous lines represent calculated curves. All anions were added as their TBA salts.

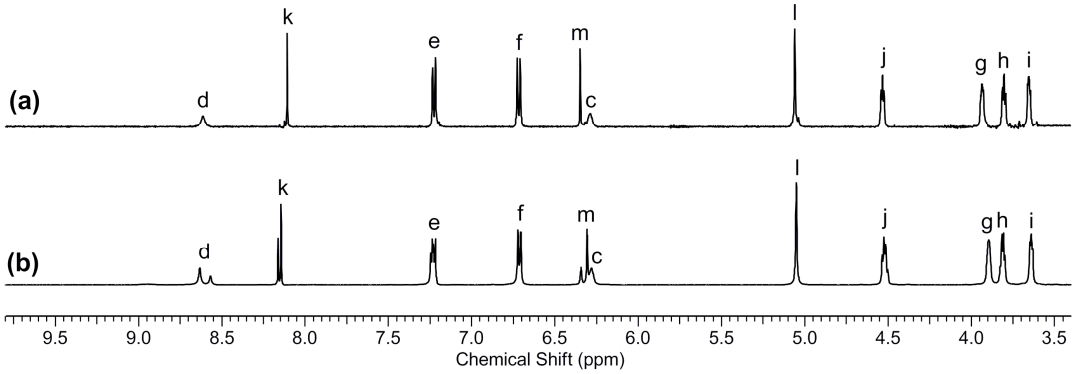


Figure S3. ^{1}H NMR spectra of a) **4** and b) **5**-SO_4^{2-} in d_6-DMSO at 298 K.

2D DOSY experiment

All DOSY experiments were conducted on an Bruker AVII 500 MHz spectrometer, at 298 K and in triplicate. Data were collected with the BPP-LED sequence using diffusion times (Δ) of 200 ms, encoding gradient pulses of total duration (δ) of 4 ms (applied as half-sine shaped bipolar pairs) and an LED period of 5 ms. 16 data sets were collected per experiment with gradient strengths up to 27 G $^{cm-}$1. Data analyses were performed using tools within TOPSPIN 2.1 software. Sample volumes were 600 μL and the concentration of the samples was 6 mM. Sulfate was added as their TBA salts. **4,** **4**-SO_4^{2-} and **5**-SO_4^{2-} are prepared with 6×10^{-3} M in DMSO-d_6 at 298 K. Diffusion coefficients and hydrodynamic radii are correlated theoretically by the Stokes-Einstein relation (equation 1).

$$D = \frac{kT}{6\pi\eta r_s} \Rightarrow r_s = \frac{kT}{6\pi\eta D}$$

where D is the diffusion coefficient, k is the Boltzmann constant ($1.3807\text{x}10^{-23}$ $m^2Kgs^{-2}K^{-1}$), T is the temperature in Kelvin (298 K), η is the viscosity of the solution (DMSO $1.991\text{x}10^{-2}$ $gcm^{-1}s^{-1}$), and rs is the radius of the molecular sphere.

Table 1 2-D DOSY evidence for capsule formation

Samples	D ($\times10^{-10}$ m^2s^{-1})	rs ($\times10^{-10}$ m)
4	1.376 ± 0.0014	7.97 ± 0.008
4 + SO_4^{2-}	1.234 ± 0.040	8.90 ± 0.290
5 + SO_4^{2-}	0.915 ± 0.00015	12.16 ± 0.002

Samples are prepared with 6×10^{-3} M in DMSO-d_6 at 298 K

Molecular dynamic simulations

Computational Details

All molecular dynamics simulations were carried out with Amber 10 molecular modeling suite.[3] Force Field parameters for **4**, **4**-SO_4^{2-}, **5**-SO_4^{2-} and TBA^+ were taken from GAFF.[4] RESP fitted charges[5] for the individual macrocycle **4**, TBA^+, and SO_4^{2-} were obtained at HF/6-31G* level calculations using the Gaussian03.[6] Dimethyl sulfoxide solvent molecules (DMSO) were described with an explicit full atom model using parameters and atomic charges taken from Kollman *et al.*[7]

Conformational analysis of **4**, **4**-SO_4^{2-} and **5**-SO_4^{2-} was performed in the gas-phase trough quenched molecular dynamics methods. Each molecule was submitted to a 10 ns molecular dynamics run at 2000 K, using a 1 fs time step. A trajectory file composed of 100000 structures was saved. Subsequently all these structures were minimized by molecular mechanics, through 1000 steps of the steepest descent method, followed by the conjugate gradient method until a convergence criterion of 0.0001 kcal mol^{-1} was achieved. The minimized structures were sorted by molecular mechanics energy and the lowest energy one was used as starting point for the molecular dynamics simulations in solution.

The lowest energy conformations of **4**, **4**-SO_4^{2-} and **5**-SO_4^{2-} were solvated with 778, 1736 or 2080 DMSO molecules, respectively. The neutrality of the **4**-SO_4^{2-} and **5**-SO_4^{2-} systems was obtained adding two TBA^+ as counter-ions to their cubic periodic boxes. In all cases, the solvent was minimized with the solute under positional restraints, in order to remove eventual bad contacts. Afterwards, the restraints were removed and the whole system was minimized. The systems were heated to 300 K in the NVT ensemble during 50 ps, followed by a 750 ps equilibration period with an isothermal-isobaric ensemble (NPT). Subsequently, the resulting equilibrated systems were used in the data collection runs of 20 ns in NPT. The SHAKE[8] algorithm was employed in all condensed phase simulations to constrain all bonds involving hydrogen atoms, allowing the use of 2 fs time steps. Non-bonded

van der Waals interactions were restrained to a 10 Å cutoff, while the particle mesh Ewald method was used to describe the long range electrostatic interactions. The temperature of the systems was controlled by the Langevin thermostat, using a collision frequency of 1.0 ps^{-1}.

The radius of gyration (R_g), defined as

$$R_g = \left(\frac{\sum m_i r_i^2}{\sum m_i} \right)^{\frac{1}{2}}$$

were m_i is the mass of the i^{th} atom in the molecule and r_i is the distance of i from the centre of mass of the molecule, was calculated for each frame and averaged over the 20 ns of the NPT simulation. A similar property can also be calculated: instead of considering the sum of all m_i r_i^2, for each frame we only take into account the maximum value of the all distances from the center of mass (r_i^{max}). Each r_i^{max} is averaged over the 20 ns trajectory yielding R_{max}.

Since the temperature control methods tend to distort the dynamics of the system, the diffusion coefficients (D), for **4**, **4**-SO_4^{2-}, **5**-SO_4^{2-}, were estimated from MD simulations carried out with a microcanonical ensemble (NVE) as follows: the previously NPT equilibrated systems were subject to 500 ps NVE simulation followed of 10 ns data collection run.

The self diffusion coefficients (D) were then determined by the slope of a mean square displacement (MSD) *vs.* time plot via the Einstein relation

$$D = \lim_{t \to \infty} \frac{1}{6t} \left\langle \left| r_i(t) - r_i(0) \right|^2 \right\rangle$$

where t is the time and r_i is the center of mass of **4** or **5**. In order to increase the statistics, the 10 ns trajectories were divided in 10 x 1 ns trajectories and the values of D were calculated by fitting the 10 resultant curves in the interval of 50 to 150 ps following a strategy similar to Chitra and Yashonath.[9] The final reported D values correspond to the average.

Molecular Modelling Discussion

Additional information about the interlocked capsule formation was gathered from molecular dynamics simulations carried out with **4, 4**-SO_4^{2-} and **5**-SO_4^{2-} in NPT periodic conditions. Firstly, lowest energy conformations in the gas-phase of **4**, **4**-SO_4^{2-} and **5**-SO_4^{2-} were determined by conformational analysis and they are shown in **Fig. S4**.

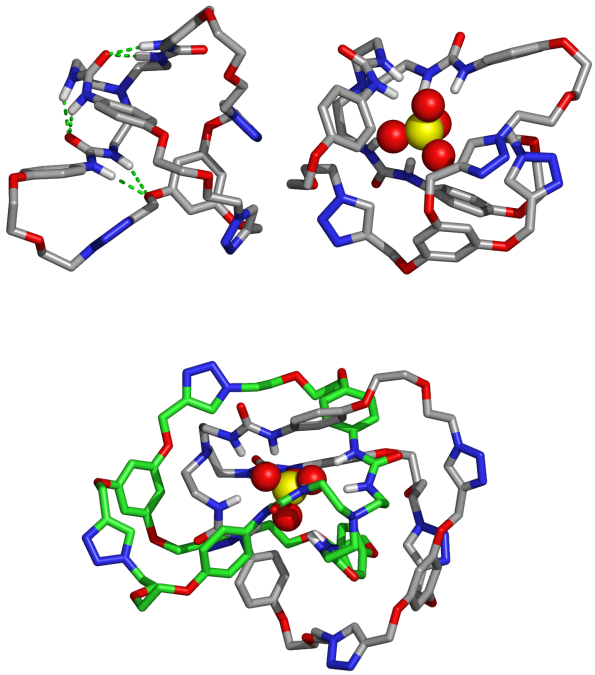


Fig. S4 Gas-phase lowest energy structures of **4** (top, left), **4**-SO_4^{2-} (top, right) and **5**-SO_4^{2-} (bottom). Only urea N-H hydrogen binding groups are shown for clarity.

The macrocycle **4** coils around itself due to the presence of six intramolecular hydrogen interactions (depicted with green dashes in Fig. S4) between both N-H bonds of the urea groups and the carbonyl groups or a polyether oxygen from adjacent linkages. This hydrogen bonding network is not present in the low-energy structure of **4**-SO_4^{2-}, since the sulfate anion is encapsulated into the cage and hydrogen bonded to all urea's N-H binding groups. Interestingly, due to the rigid tetrahedral geometry of the sulfate anion, the N-H···O=S bonds, with H···O distances, ranging from 1.79 to 2.13 Å, are not all equivalent. In addition, one sulfate oxygen atom is not involved in any hydrogen bonding interactions. The lowest energy conformation of **5**-SO_4^{2-} shows

the sulfate anion trapped into the interlocked **5** cage, establishing hydrogen bonds with the three urea groups from both macrocyclic entities. All sulfate oxygen atoms are involved in N-H···O hydrogen bonds, but some of them are shared with more than one urea group.

These lowest energy structures were immersed in DMSO cubic boxes in order to evaluate the stability of the binding arrangements described in the gas phase and to estimate other relevant properties such as the diffusion coefficients by means of conventional molecular dynamics simulations in an NPT ensemble. For **4**, only two N-H···O=C hydrogen bonds between two adjacent urea groups, with average distances of 1.96 Å, are maintained along the 20 ns data collection period, (see Fig. S5, top). The remaining four N-H···O=C and the N-H···O(ether) bonding interactions, seen in the gas-phase, are broken during the earlier stages of the equilibration period and they are not restored over the long data collection time (see middle and bottom of Fig. S5). These four N-H binding groups, not involved in the intramolecular hydrogen bonds, form four N-H···O=S$(CH_3)_2$ interactions with the DMSO solvent molecules, as illustrated by the snapshot taken at the end of simulation (Fig. S6).

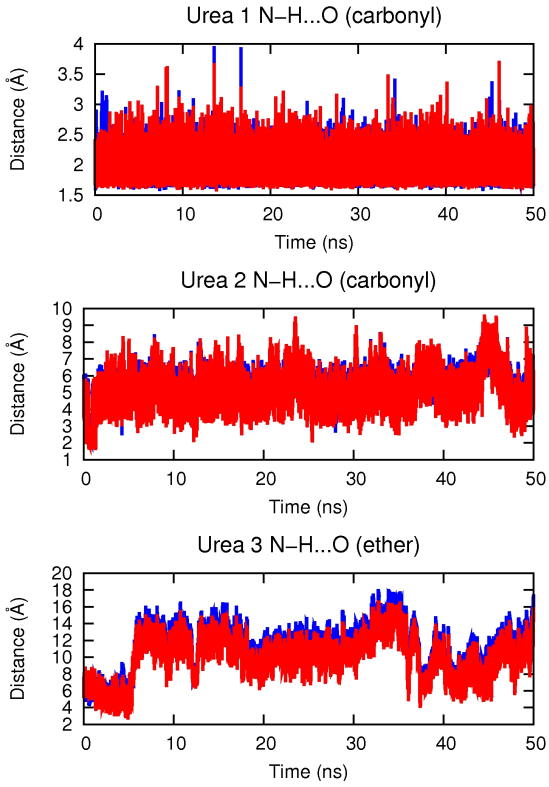


Fig. S5 – Time evolution of the intramolecular N-H···O=C (carbonyl, top and middle) and N-H···O (bottom) for both N-H binding groups (red and blue lines) of each urea group for 20 ns of data collection in macrocycle **4**.

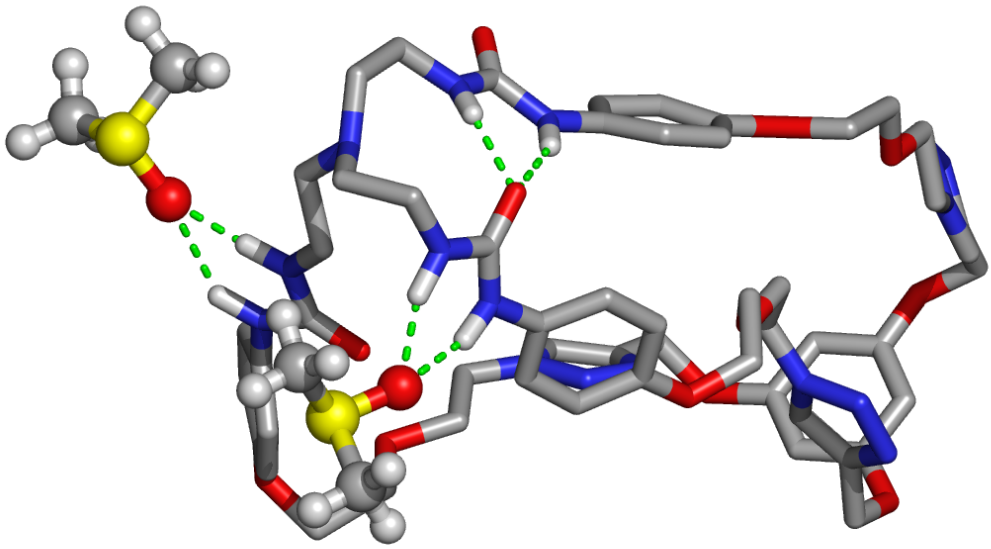


Fig. S6 – Snapshot of **4** in DMSO solution after 20 ns of simulation, showing two intramolecular N-H···O=C bonds and four N-H···O=S (green dashes) established between the receptor and two DMSO solvent molecules.

For **4**-SO_4^{2-} in DMSO solution, one important structural feature is whether the sulfate anion remains inside the cage, hydrogen bonded. Due to the rotation of the anion, the N-H···O=S bonds with one urea binding group are broken and formed with another one. Therefore, the monitoring of a specific N-H···O=S distance over the simulation time would sometimes lead to large values, even when the sulfate is still bonded. A better way to check whether the sulfate remains encapsulated into the cage over the 20 ns simulation is to follow the N-H···S distances (see Fig S7). The N-H···S distances for the three urea groups, ranging from 2.09 Å to 3.79 Å, indicates that the SO_4^{2-} is trapped in the cage **4**.

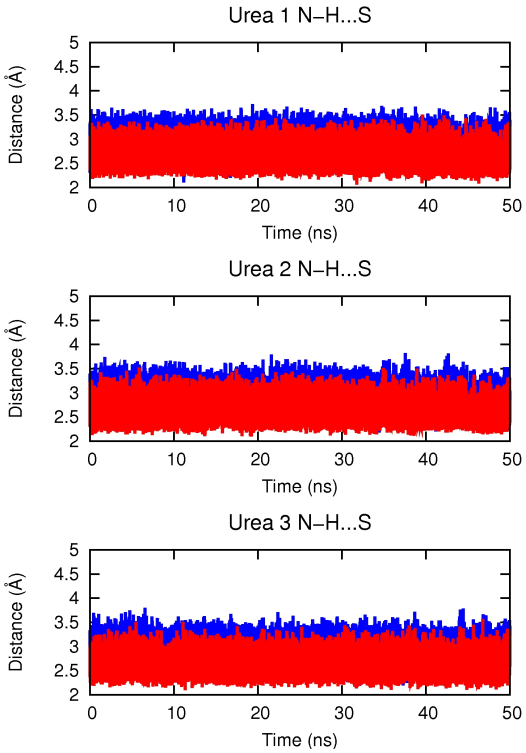


Fig. S7 – Time evolution of the two N-H···S distances (red and blue) of each urea-binding group for **4**-SO_4^{2-} during the long 20 ns of the collection period.

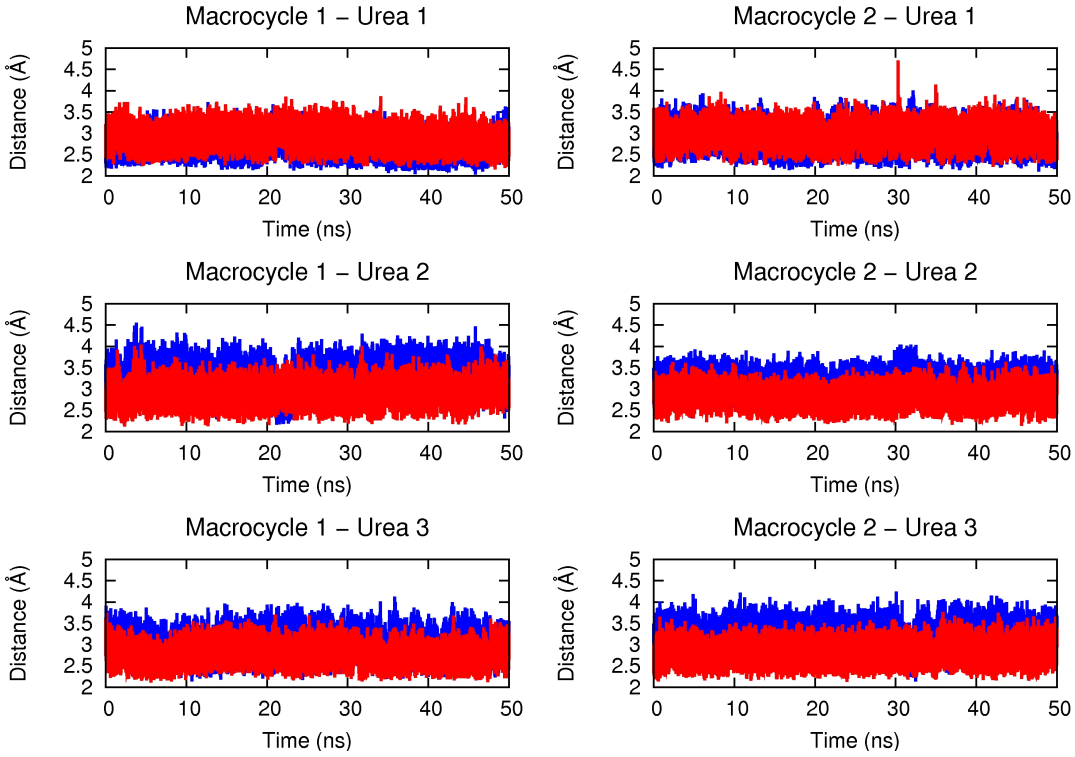


Fig. S8 – Variations in two N-H···S distances (red and blue) of the six urea binding groups from two macrocyclic entities of **5**-SO_4^{2-} capsule for the 20 ns of molecular dynamics data collection.

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