Supporting information

Nitrate anion templated assembly of a [2]rotaxane for selective nitrate recognition in aqueous solvent media

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All reagents and solvents were purchased from commercial sources and used without further purification. Where necessary, solvents were dried by passing through a MBraun MPSP-800 column and degassed with nitrogen. Column chromatography was carried out on Merck® silica gel 60 under a positive pressure of nitrogen. Size exclusion chromatography was carried out using Biobeads SX-3, with CHCl$_3$ as the eluent. Where mixtures of solvents were used, ratios reported are by volume. Triethylamine was distilled from and stored over potassium hydroxide. NMR spectra were recorded on Varian Mercury 300, Bruker AVII 500 (with cryoprobe) and Bruker AVIII 500 spectrometers. Mass spectra were carried out on a Waters Micromass LCT and Bruker microTOF spectrometers.
Section 1 – Synthesis and characterisation

Scheme SI-1 – Synthesis of thread 2PF₆
Scheme SI-2 – Synthesis of axle precursor 3NO₃
General procedure for acid chloride synthesis.

To convert carboxylic acids to their acid chloride derivatives the following general procedure was used. To a suspension of acid (1 mmol) in dry CH₂Cl₂ (10 mL) was added oxalyl chloride (2 mmol) dropwise under N₂. A drop of DMF (~0.01 mL, cat.) was added and the reaction refluxed at 40 °C under N₂ until the solution became homogeneous. The solvent was removed in vacuo to leave a yellow solid. This was immediately re-dissolved in CH₂Cl₂ and reacted on as desired. The yield was assumed to be quantitative.

Compounds SI-4,1 SI-1,2 SI-5,3 SI-111 and stopper-alkyne 44 were prepared according to literature procedures.

Compound SI-2:

Acid SI-1 (2.00 g, 11.1 mmol) was converted to its acid chloride using the general procedure. Assuming quantitative conversion, the crude acid chloride was immediately re-dissolved in CH₂Cl₂ (150 mL) and a solution of hexylamine (1.61 mL, 12.2 mmol), dry triethylamine (2.32 mL, 16.7 mmol) in CH₂Cl₂ (50 mL) was added dropwise at 0 °C. The reaction mixture was stirred at room temperature under N₂ for 16 hrs and then washed with 10% citric acid (2 × 50 mL) and NaHCO₃ (2 × 50 mL). The organic layer was dried over MgSO₄ and solvent removed in vacuo. Purification by silica gel column chromatography (99:1 CH₂Cl₂/CH₃OH) to give a pale yellow oil. (1.31 g, 45 %).

1H NMR (500 MHz, CDCl₃) δ (ppm): 8.35 (t, 4J = 1.7 Hz, 1H, ArH), 8.15 (m, 1H, ArH), 8.04 (m, 1H, ArH), 7.52 (t, 3J = 7.8 Hz, 1H, ArH), 6.30 (br s, 1H, NH), 3.94 (s, 3H, OCH₃), 3.46 (m, 2H, CH₂NH), 1.63 (quintet, 3J = 6.9 Hz, 2H, CH₂CH₂NH), 1.38 (m, 2H, hexyl CH₂), 1.32 (m, 4H, hexyl CH₂), 0.89 (t, 3J = 7.2 Hz, 3H, CH₃). 13C NMR (125 MHz, CDCl₃) δ (ppm): 166.4, 135.1, 132.2, 131.8, 130.4, 128.9, 127.4, 52.3, 40.2, 31.5, 29.6, 26.6, 22.5, 14.0, One peak missing, presumed overlapped. HRMS (ES +ve) m/z: 264.1601 ([M + H]+, C₁₅H₂₂NO₃ requires 264.1594).

Compound SI-3

Ester SI-2 (607 mg, 2.31 mmol) was dissolved with KOH (143 mg, 2.54 mmol) in MeOH (35 mL). The reaction mixture was stirred under N₂ for 16 hrs. The solvent removed in vacuo and re-dissolved in H₂O (50 mL) and washed with CH₂Cl₂ (2 × 20 mL). The aqueous layer was neutralised with 10% citric acid and the resulting precipitate was collected by vacuum filtration and washed with H₂O (10 mL), CH₂Cl₂ (10 mL) and dried to give a white solid. (445 mg, 77 %). 1H NMR (500 MHz, DMSO-d₆) δ (ppm): 8.65 (t, 3J = 5.3 Hz, 1H, CONH), 8.42 (s, 1H, ArH), 8.07 (dd, 3J = 7.7 Hz, 2J = 1.8 Hz, 2H, ArH), 7.59 (t, 3J = 7.6 Hz, 1H, ArH), 3.26 (m, 2H, CH₂NH), 1.53 (m, 2H, CH₂CH₂NH), 1.29 (m, 6H, CH₃) 0.88 (t, 3J = 7.0 Hz, 3H, CH₃). 13C NMR (125 MHz, DMSO-d₆) δ (ppm): 167.0, 165.3, 135.0, 131.6, 131.4, 131.0, 128.7, 128.0, 31.0, 30.0, 26.2, 22.1, 13.9, One peak missing, presumed overlapped. HRMS (ES +ve) m/z: 272.1256 ([M + Na]+, C₁₃H₁₉NO₃Na requires 272.1257).
Acid SI-4 (500 mg, 2.00 mmol) was converted to its corresponding acid chloride using the general procedure. Assuming quantitative conversion, the crude acid chloride was immediately re-dissolved in CH₂Cl₂ (40 mL). A solution of SI-5 (380 mg, 2.20 mmol) and dry triethylamine (0.42 mL, 3.00 mmol) in CH₂Cl₂ (10 mL) was added dropwise at 0 °C. The reaction mixture was stirred at room temperature under N₂ for 16 hrs and then washed with 10% citric acid (2 × 30 mL) and NaHCO₃ (2 × 30 mL). The organic layer was dried over MgSO₄ and solvent removed in vacuo. Purification by silica gel column chromatography (95:5 CH₂Cl₂/CH₃OH) afforded the product as a pale yellow solid (490 mg, 60%).

**Compound SI-6**

![Chemical Structure](image)

1H NMR (500 MHz, CDCl₃) δ (ppm): 9.19 (br s, 2H, pyridine ArH), 8.57 (s, 1H, pyridine ArH), 7.98 (br s, 1H, CONH), 6.78 (br s, 1H, CONH), 4.96 (br s, 1H, CH₂NH), 3.53 (m, 2H, C₆H₂NH), 3.47 (m, 2H, C₆H₂NH), 3.26 (m, 2H, CH₂CH₂NH₂), 1.97 (m, 2H, CH₂), 1.35 (m, 9H, C(C₆H₃)), 1.38 (m, 2H, C₆H₂), 1.31 (m, 4H, C₆H₂), 0.89 (t, 3J = 6.6 Hz, 3H, C₃H₃). 13C NMR (125 MHz, CDCl₃) δ (ppm): 164.7, 164.6, 157.2, 150.7, 150.1, 133.5, 130.1, 129.9, 80.0, 40.3, 37.0, 36.1, 31.4, 29.8, 29.5, 28.4, 26.6, 22.5, 14.0. HRMS (ES+ve) m/z: 429.2476 [M + Na⁺], C₂₁H₃₄N₄O₄Na requires 429.2472.

**Compound SI-7**

To a solution of SI-6 (490 mg, 1.21 mmol) in CH₂Cl₂ (30 mL) at 0 °C was added trifluoroacetic acid (5 mL) dropwise. The reaction mixture was allowed to warm to room temperature and stirred under N₂. The reaction was monitored using thin layer chromatography (9:1 CH₂Cl₂/CH₃OH) until all the amine had been deprotected. The solvent was removed in vacuo and re-dissolved in CH₃OH which was subsequently again removed in vacuo. This was repeated until all the excess TFA had been removed to give the trifluoroacetate salt of the product (423 mg, 83%).

1H NMR (500 MHz, CD₃OD) δ (ppm): 9.16 (br s, 2H, pyridine ArH), 8.70 (s, 1H, pyridine ArH), 3.57 (t, 3J = 6.2 Hz, 2H, CH₂NH₃), 3.45 (t, 3J = 6.8 Hz, 2H, CH₂NH₂), 3.06 (t, 3J = 7.6 Hz, 2H, CH₂NH₂), 2.01 (quintet, 3J = 7.0 Hz, 2H, CH₂CH₂NH₂), 1.66 (quintet, 3J = 7.7 Hz, 2H, CH₂CH₂NH₂), 1.43 (m, 2H, hexyl-CH₂CH₃), 1.37 (m, 4H, CH₂), 0.94 (t, 3J = 6.9 Hz, 3H, CH₃). 13C NMR (125 MHz, CD₃OD) δ (ppm): 167.8, 166.9, 161.4, 159.0 (quartet, 1J = 41.9 Hz, CF₃), 151.2, 151.1, 136.1, 117.2, 115.0, 41.4, 38.4, 37.8, 32.8, 30.5, 28.9, 28.0, 23.9, 14.6. HRMS (ES+ve) m/z: 307.2126 ([M + H⁺], C₁₆H₂₇N₄O₂ requires 307.2129).
Compound SI-8

To a suspension of acid SI-3 (68 mg, 0.272 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (10 mL) was added EDC·HCl (62.7 mg, 0.327 mmol), HOBt (50 mg, 0.327 mmol) and DMAP (cat. ~1 mg). This was stirred for 24 hrs under N\textsubscript{2} until the solution became homogeneous. To this was added dry triethylamine (0.19 mL, 1.36 mmol) and a solution of amine salt SI-7 (100 mg, 0.327 mmol) in DMF (3 mL). The reaction mixture was stirred for 2 days under N\textsubscript{2} and washed with 10 \% citric acid (2 \times 5 mL) and NaHCO\textsubscript{3} (2 \times 5 mL). The organic layer was dried over MgSO\textsubscript{4} and the solvent removed \textit{in vacuo}. The product was purified using silica gel column chromatography (95:5 CH\textsubscript{2}Cl\textsubscript{2}/CH\textsubscript{3}OH) to afford a yellow solid (79 mg, 54 \%). \textsuperscript{1}H NMR (500 MHz, 1:1 CDCl\textsubscript{3} / CD\textsubscript{3}OD) \(\delta\) (ppm): 9.12 (s, 1H, pyridine Ar\textsubscript{H}), 9.10 (s, 1H, pyridine Ar\textsubscript{H}), 8.64 (s, 1H, pyridine Ar\textsubscript{H}), 7.98 (m, 2H, Ar\textsubscript{H}), 7.54 (t, \(^3\)J = 7.73, 1H, Ar\textsubscript{H}), 3.52 (m, 4H, propyl CH\textsubscript{2}NH), 3.41 (m, 4H, hexyl CH\textsubscript{2}NH), 1.94 (m, 2H, propyl CH\textsubscript{2}CH\textsubscript{2}NH), 1.64 (m, 4H, hexyl CH\textsubscript{2}CH\textsubscript{2}NH), 1.39 (m, 4H, hexyl CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}NH), 1.33 (m, 8H, hexyl CH\textsubscript{2}), 0.90 (m, 6H, hexyl CH\textsubscript{3}). \textsuperscript{13}C NMR (125 MHz, 1:1 CDCl\textsubscript{3} / CD\textsubscript{3}OD) \(\delta\) (ppm): 169.6, 169.3, 167.3, 166.9, 151.7, 151.5, 136.4, 135.8, 135.8, 131.7, 131.3, 130.1, 127.1, 118.1, 41.6, 41.6, 38.5, 38.4, 32.8, 32.8, 30.6, 30.5, 28.0, 28.0, 23.8, 15.0. Four peaks missing, presumed overlapped. HRMS (ES +ve) \(m/z\): 560.3211 ([M + Na]\(^+\), \(C_{30}H_{43}N_5O_4\)Na requires 560.3207).

\textsuperscript{1}H NMR (500 MHz, 1:1 CDCl\textsubscript{3} / CD\textsubscript{3}OD)

\textsuperscript{13}C NMR (125 MHz, 1:1 CDCl\textsubscript{3} / CD\textsubscript{3}OD)
Methyl-iodide (2 mL) was added to a solution of SI-8 (70 mg, 0.130 mmol) in DMF (2 mL) and stirred under N$_2$ for 16 hrs. The solvent was removed in vacuo to give compound 2 as the iodide salt. Ion exchange to the PF$_6$- salt was achieved by washing a solution of 2I in CH$_2$Cl$_2$ (20 mL) with a solution of with a solution of aqueous ammonium hexafluorophosphate solution (8 × 12.5 mL of 0.1 M) and water (2 × 10 mL). The organic layer was dried over MgSO$_4$ and the solvent removed in vacuo to give 2PF$_6$. (70.0 mg, 77 %).

$^1$H NMR (500 MHz, 1:1 CDCl$_3$ / CD$_3$OD) δ (ppm): 9.48 (s, 1H, pyridinium ArH), 9.43 (s, 1H, pyridine ArH), 9.39 (s, 1H, pyridinium ArH), 8.28 (t, $^4J = 1.7$ Hz, 1H, ArH), 7.97 (m, 2H, ArH), 7.54 (t, $^3J = 7.7$ Hz, 1H, ArH), 4.53 (s, 3H, N+CH$_3$), 3.58 (t, $^3J = 6.4$ Hz, 2H, propyl CH$_2$NH), 3.46 (t, $^3J = 7.7$ Hz, 2H, hexyl CH$_2$NH), 3.40 (t, $^3J = 7.2$ Hz, 2H, hexyl CH$_2$NH), 1.99 (quintet, $^3J = 6.1$ Hz, 2H, propyl CH$_2$NH), 1.65 (m, 4H, hexyl CH$_2$NH), 1.33 (m, 12H, hexyl CH$_2$), 0.89 (t, $^3J = 7.2$ Hz, 6H, hexyl CH$_3$). $^{13}$C NMR (125 MHz, 1:1 CDCl$_3$/CD$_3$OD) δ (ppm): 169.7, 169.1, 162.6, 162.3, 148.0, 147.8, 142.6, 136.3, 136.2, 136.0, 135.7, 131.6, 131.5, 130.2, 127.2, 42.1, 41.6, 38.9, 38.3, 32.9, 32.8, 30.6, 30.4, 29.7, 28.0, 28.0, 23.9, 23.8, 15.0. Two peaks missing, presumed overlapped. $^{19}$F NMR (282.5 MHz, 1:1 CDCl$_3$/CD$_3$OD) δ (ppm): -72.2 (d, $^1J = 717$ Hz, PF$_6$). HRMS (ES +ve) m/z: 552.3544 ([M$^+$], C$_{31}$H$_{46}$N$_5$O$_4$ requires 552.3544).
Compound SI-9

Mono-acid SI-1 (1.50 g, 8.33 mmol) was converted to its respective acid chloride via the general procedure. Assuming quantitative yield the acid chloride was redissolved in dry CH$_2$Cl$_2$ (150 mL) and cooled to 0 °C. To this was added dry triethylamine (5.80 mL, 41.7 mmol) dropwise before adding 3-bromopropylamine hydrobromide (3.64 g, 16.7 mmol). The reaction mixture was allowed to warm to room temperature and stirred under N$_2$ for 16 hrs. The solution was washed with 10 % citric acid (2 × 30 mL) and NaHCO$_3$ (2 x 30 mL) and the organic layer dried over MgSO$_4$ before removing the solvent in vacuo to give a pale yellow oil. This was dissolved in DMF (50 mL) before adding sodium azide (1.08 g, 16.7 mmol) to the solution. The reaction mixture was stirred under N$_2$ and refluxed at 70 °C for 16 hrs. The solution was allowed to cool to room temperature before pouring into H$_2$O (100 mL). The product was extracted into CH$_2$Cl$_2$ (3 × 40 mL) and the combined organic layers dried over MgSO$_4$. The solvent was removed in vacuo and the resulting oil purified via silica gel column chromatography (98:2 CH$_2$Cl$_2$/CH$_3$OH) to afford a pale yellow oil (1.89 g, 87 %).

$^1$H NMR (500 MHz, CDCl$_3$) δ (ppm): 8.37 (t, $^4$J = 1.7 Hz, 1H, ArH), 8.18 (m, 1H, ArH), 8.04 (m, 1H, ArH), 7.55 (t, $^3$J = 7.8 Hz, 1H, ArH), 6.51 (br s, 1H, CONH), 3.95 (s, 3H, OC$_3$H$_3$), 3.59 (app quartet, $^3$J = 6.5 Hz, 2H, C$_2$H$_2$N$_3$), 3.47 (t, $^3$J = 6.5 Hz, 2H, C$_2$H$_2$N$_3$), 1.94 (quintet, $^3$J = 6.7 Hz, C$_2$H$_2$CH$_2$N$_3$).

$^{13}$C NMR (125 MHz, CDCl$_3$) δ (ppm): 166.5, 166.3, 134.7, 132.5, 131.8, 130.5, 129.0, 127.4, 52.4, 49.5, 37.9, 28.7. HRMS (ES +ve) m/z: 285.0960 ([M + Na]$^+$, C$_{12}$H$_{14}$N$_4$O$_3$Na requires 285.0958).

Compound SI-10

Ester SI-9 (1.04 g, 3.97 mmol) and KOH (0.24 g, 4.37 mmol) were dissolved in CH$_3$OH (55 mL) was stirred for 16 hrs under N$_2$. The solvent was removed in vacuo and the residue was dissolved in H$_2$O (100 mL) and washed with CH$_2$Cl$_2$ (3 × 50 mL) The aqueous layer was neutralised by addition of 10 % citric acid dropwise and the white precipitate was collected through vacuum filtration and washed with H$_2$O (10 mL) and CH$_2$Cl$_2$ (10 mL). The product was dried under vacuum to afford a white solid (536 g, 54 %).

$^1$H NMR (500 MHz, DMSO- $^{d_6}$) δ (ppm): 8.73 (app t, $^3$J = 5.4 Hz, 1H, CONH), 8.43 (t, $^4$J = 1.6 Hz, 1H, ArH), 8.08 (m, 2H, ArH), 7.16 (t, $^3$J = 7.9 Hz, 1H, ArH), 3.43 (t, $^3$J = 6.9 Hz, 2H, C$_2$H$_2$NH), 3.36 (t, $^3$J = 6.7 Hz, 2H, C$_2$H$_2$N$_3$), 1.80 (quintet, $^3$J = 6.5 Hz, 2H, C$_2$H$_2$CH$_2$NH).

$^{13}$C NMR (125 MHz, DMSO-$^{d_6}$) δ (ppm): 166.9, 165.5, 134.8, 131.8, 131.5, 131.0, 128.7, 128.0, 48.5, 36.7, 28.3. HRMS (ES +ve) m/z: 271.0810 ([M + Na]$^+$, C$_{11}$H$_{12}$N$_2$O$_3$Na requires 271.0802).

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Compound SI-12

Acid SI-10 (1.41 g, 7.79 mmol) was converted to its respective acid chloride by the general method. Assuming quantitative yield, the acid chloride was immediately re-dissolved in dry CH$_2$Cl$_2$ (150 mL) and cooled to 0 °C. To this was added dry triethylamine (5.43 mL, 39.0 mmol) dropwise before adding 3-bromopropylamine hydrobromide (3.42 g, 15.6 mmol). The reaction mixture was allowed to warm to room temperature and stirred under N$_2$ for 16 hrs. The solution was washed with 10 % citric acid (2 × 50 mL) and NaHCO$_3$ (2 × 50 mL) and the organic layer dried over MgSO$_4$ before removing the solvent in vacuo to give a pale yellow oil. This was dissolved in DMF (50 mL) before adding sodium azide (1.01 g, 15.6 mmol) to the solution. The reaction mixture was stirred under N$_2$ and refluxed at 70 °C for 16 hrs. The solution was allowed to cool to room temperature before pouring into H$_2$O (100 mL). The product was extracted into CH$_2$Cl$_2$ (3 × 40 mL) and the combined layers dried over MgSO$_4$. The solvent was removed in vacuo and the resulting oil purified via silica gel column chromatography (98:2 CH$_2$Cl$_2$/CH$_3$OH) to afford a pale yellow oil (0.85 g, 41 %). 1H NMR (500 MHz, DMSO-d$_6$) δ (ppm): 9.22 (d, $^4$J = 2.2 Hz, 1H, pyridine ArH), 9.21 (d, $^4$J = 2.1 Hz, 1H, pyridine ArH), 8.94 (br s, 1H, CO$_2$NH), 8.69 (t, $^3$J = 2.2 Hz, 1H, pyridine ArH), 3.94 (s, 3H, OC$_3$H$_3$), 3.44 (t, $^3$J = 6.7 Hz, 2H, N$_3$C$_2$H$_2$), 3.38 (m, 2H, NHC$_2$H$_2$), 1.81 (quintet, $^3$J = 6.7 Hz, 2H, C$_2$H$_2$NH). 13C NMR (125 MHz, DMSO-d$_6$) δ (ppm): 164.8, 163.8, 152.3, 151.9, 135.4, 129.8, 125.3, 52.7, 48.5, 36.8, 28.1. HRMS (ES +ve) m/z: 286.0902 ([M + Na]$^+$, C$_{11}$H$_{13}$N$_5$O$_3$Na requires 286.0911).

Compound SI-13

Ester SI-12 (850 mg, 3.23 mmol) and KOH (199 g, 3.55 mmol) were dissolved in CH$_3$OH (48 mL) and stirred for 16 hrs under N$_2$. The solvent was removed in vacuo and the resulting oil re-dissolved in H$_2$O (75 mL). This solution was washed with CH$_2$Cl$_2$ (3 × 30 mL) and the aqueous layer neutralised by addition of 10 % citric acid dropwise. The resulting white precipitate was collected through vacuum filtration, washed with H$_2$O (10 mL) and CH$_2$Cl$_2$ (10 mL). It was dried under vacuum to afford a white solid (547 g, 68 %). 1H NMR (500 MHz, DMSO-d$_6$) δ (ppm): 9.19 (d, $^4$J = 2.2 Hz, 1H, pyridine ArH), 9.17 (d, $^4$J = 2.2 Hz, 1H, pyridine ArH), 8.94 (br s, 1H, CONH), 8.69 (t, $^3$J = 2.2 Hz, 1H, pyridine ArH), 3.44 (t, $^3$J = 6.6 Hz, 2H, N$_3$CH$_2$), 3.38 (m, 2H, NHCH$_2$), 1.81 (quintet, $^3$J = 6.8 Hz, 2H, CH$_2$CH$_2$NH). 13C NMR (125 MHz, DMSO-d$_6$) δ (ppm): 165.8, 164.0, 152.2, 151.0, 135.5, 129.7, 126.5, 48.5, 36.8, 28.1. HRMS (ES +ve) m/z: 272.0750 ([M + Na]$^+$, C$_{10}$H$_{11}$N$_5$O$_3$Na requires 272.0754).
Mono-acid **SI-13** (200 mg, 0.803 mmol) was converted to an acid chloride via the general method. Assuming quantitative yield the acid chloride was immediately re-dissolved in CH₂Cl₂ (25 mL) and a solution of protected amine **SI-5** (209 mg, 1.21 mmol), dry triethylamine (0.168 mL, 1.21 mmol) in CH₂Cl₂ (5 mL) was added dropwise at 0°C. The reaction mixture was stirred at room temperature under N₂ for 16 hrs and then washed with 10 % citric acid (2 × 10 mL) and NaHCO₃ (2 × 10 mL). The organic layer was dried over MgSO₄ and solvent removed in vacuo. Silica gel column chromatography (98:2 CH₂Cl₂/CH₃OH then 96:4) was used to purify giving a pale yellow solid (224 mg, 69 %).

**1H NMR** (500 MHz, CDCl₃) δ (ppm): 9.16 (br s, 2H, pyridine Ar H), 8.55 (br s, 1H, pyridine Ar H), 8.04 (br s, 1H, CON), 7.44 (br s, 1H, CON), 5.02 (br s, 1H, CON), 3.56 (m, 2H, C₃H₂NH), 3.50 (m, 2H, C₃H₂NH), 3.44 (t, 3J = 6.6 Hz, 2H, C₃H₂N₃), 3.23 (m, 2H, C₃H₂NH), 1.92 (quintet, 3J = 6.6 Hz, 2H, CH₂NH₂NH), 1.72 (m, 2H, CH₂CH₂NH), 1.44 (s, 9H, C(C₃H₃))₃).

**13C NMR** (125 MHz, CDCl₃) δ (ppm): 165.2, 164.8, 157.2, 150.8, 150.4, 133.5, 129.9, 129.8, 79.9, 53.4, 37.8, 37.1, 36.3, 30.9, 29.8, 28.7.


**Compound SI-15**

To a solution of **SI-14** (220 mg, 0.543 mmol) in CH₂Cl₂ (10 mL) at 0 °C, was added trifluoroacetic acid (2 mL) dropwise. The reaction mixture was allowed to warm to room temperature and stirred under N₂. The reaction was monitored using thin layer chromatography (9:1 CH₂Cl₂/CH₃OH) until all the amine had been deprotected. The solvent was removed in vacuo and re-dissolved in CH₃OH. The CH₃OH was removed in vacuo and this was repeated until all the excess TFA had been removed to give the trifluoroacetate salt of the product in a quantitative yield (228 mg).**1H NMR** (500 MHz, CD₃OD) δ (ppm): 9.13 (br s, 2H pyridine Ar H), 8.67 (t, 3J = 1.9 Hz, 1H, pyridine Ar H), 8.00 (bs, 1H, CONH), 3.56 (t, 3J = 6.7 Hz, 2H, CH₂), 3.52 (t, 3J = 6.9 Hz, 2H, CH₂), 3.45 (t, 3J = 6.6 Hz, 2H, CH₂), 3.06 (t, 3J = 7.5 Hz, 2H, CH₂), 2.02 (quintet, 3J = 7.2 Hz, 2H, CH₂CH₂CH₂), 1.92 (quintet, 3J = 6.7 Hz, 2H, CH₂CH₂CH₂). **13C NMR** (125 MHz, CD₃OD) δ (ppm): 167.7, 167.2, 164.9 (quartet, 1J = 34.7 Hz, CF₃), 162.9, 151.5, 151.4, 135.9, 131.8, 131.4, 50.2, 38.6, 38.4, 37.7, 29.7, 28.8. **HRMS (ES +ve)** m/z: 306.1662 ([M + H]⁺ requires 306.1673).
Compound SI-16

To a suspension of acid SI-10 (64 mg, 0.257 mmol) in CH₂Cl₂ (10 mL) was added EDC HCl (59 mg, 0.308 mmol), HOBt (47 mg, 0.308 mmol) and DMAP (cat. ~ 1 mg). This was stirred for 2 hrs under N₂ until the solution became homogeneous. To this was added dry triethylamine (0.18 mL, 1.29 mmol) and a solution of amine SI-15 (140 mg, 0.344 mmol) in DMF (3 mL). The reaction mixture was stirred for 4 days under N₂ and then the solvent removed in vacuo. The solution was washed with 10% citric acid (2 × 5 mL) and NaHCO₃ (2 × 5 mL). The organic layer was dried with MgSO₄ and the solvent removed in vacuo. The product was purified using silica gel column chromatography (96:4 CH₂Cl₂/CH₃OH) to afford a white solid (52 mg, 38%).

**1H NMR (500 MHz, 1:1 CDCl₃/CD₃OD)** δ (ppm): 9.14 (br s, 1H, pyridine ArH), 9.11 (br s, 1H, pyridine ArH), 8.67 (t, J = 2.0 Hz, 1H, pyridine ArH), 8.28 (t, J = 1.7 Hz, 1H, ArH), 7.98 (m, 2H, ArH), 7.55 (t, J = 7.7 Hz, 1H, ArH), 3.52 (m, 8H, CH₂N), 3.43 (m, 4H, CH₂N₃), 1.92 (m, 6H, CH₂CH₂NH). **13C NMR (125 MHz, 1:1 CDCl₃/CD₃OD)** δ (ppm): 169.6, 169.5, 167.1, 167.0, 152.5, 151.4, 136.1, 136.0, 131.6, 131.4, 130.2, 50.4, 50.3, 38.8, 38.7, 38.5, 38.4, 30.2, 29.9, 29.8, Three peaks missing, presumed overlapped. HRMS (ES +ve) m/z: 558.2287, ([M + Na]⁺, C₂₄H₂₉N₁₁O₄Na requires 558.2296).

**1H NMR (500 MHz, 1:1 CDCl₃/CD₃OD)**

![1H NMR spectrum](image)

**13C NMR (125 MHz, 1:1 CDCl₃/CD₃OD)**

![13C NMR spectrum](image)
Methyl-iodide (2 mL) was added to a solution of SI-16 (48 mg, 0.089 mmol) in DMF (2 mL) and stirred under N₂ for 16 hrs. The solvent was removed in vacuo. Ion exchange to the nitrate salt was achieved by passing down a nitrate loaded Amberlite® column in 9:1 acetone/water. The solvent was removed in vacuo to leave a pale yellow oil (52 mg, 94 %).

**1H NMR (500 MHz, 1:1 CDCl₃/d₆-acetone)** δ (ppm): 9.79 (br s, 1H, pyridinium Ar H), 9.65 (bs, 1H, pyridinium Ar H), 9.57 (br s, 1H, pyridinium Ar H), 9.19 (t, 3J = 5.4 Hz, 1H, CON H), 9.04 (t, 3J = 5.3 Hz, 1H, CON H), 8.47 (br s, 1H, Ar H), 8.37 (t, 3J = 5.9 Hz, 1H, CON H), 8.20 (t, 3J = 5.3 Hz, 1H, CON H), 8.05 (d, 3J = 7.8 Hz, 1H, Ar H), 8.02 (d, 3J = 7.8 Hz, 1H, Ar H), 7.49 (t, 3J = 7.8 Hz, 1H, Ar H), 4.75 (s, 3H, N⁺CH₃), 3.56 (m, 4H, CH₂NH), 3.52 (m, 4H, CH₂NH), 3.46 (m, 4H, CH₂N₃), 1.94 (m, 6H, CH₂CH₂NH).

**13C NMR (125 MHz, 1:1 CDCl₃/d₆-acetone)** δ (ppm): 167.3, 167.3, 161.6, 161.5, 148.2, 147.9, 141.6, 135.6, 135.1, 135.1, 135.1, 135.1, 135.1, 131.2, 131.2, 129.4, 126.1, 50.3, 50.0, 49.9, 49.8, 38.5, 38.1, 37.4, 29.7, 29.3, 28.7. HRMS (ES +ve) m/z: 550.2613 ([M]+, C₂₅H₃₂N₁₁O₄ requires 550.2633).

**1H NMR (500 MHz, 1:1 CDCl₃/d₆-acetone)**

**13C NMR (125 MHz, 1:1 CDCl₃/d₆-acetone)**
Compound 3PF₆

Anion exchange of a further sample of 3NO₃ to the PF₆⁻ salt was achieved by passing down a PF₆⁻ loaded Amberlite® column in 9:1 acetone/water. ¹H NMR (500 MHz, 1:1 CDCl₃/CD₃OD) δ (ppm): 9.61 (s, 1H, pyridinium ArH), 9.56 (s, 1H, pyridinium ArH), 9.49 (s, 1H, ArH), 8.38 (s, 1H, ArH), 8.05 (d, ³J = 7.8 Hz, 1H, ArH), 8.00 (d, ³J = 7.8 Hz, 1H, ArH), 7.61 (t, ³J = 7.8 Hz, 1H, ArH), 4.56 (s, 3H, N⁺CH₃), 3.55 (m, 4H, CH₂), 3.51 (m, 4H, CH₂), 3.45 (m, 4H, CH₂N₃), 3.57 (m, 4H, CH₂), 2.40 (m, 6H, CH₂). ¹⁹F NMR (282.5 MHz, 1:1 CDCl₃ / CD₃OD) δ (ppm): -73.5 (d, ³J = 712 Hz, PF₆⁻).

Rotaxane 5NO₃

A solution of thread 3NO₃ (26 mg, 0.0425 mmol) and macrocycle 1 (25.1 mg, 0.0386 mmol) was stirred for 30 mins under N₂ in dry 4:1 CH₂Cl₂/Acetone (2 mL. A solution of stopper alkyne 4 (46.1 mg, 0.0849 mmol) in dry 4:1 CH₂Cl₂/Acetone (0.5 mL) was prepared. Half of this solution, Cu(CH₃CN)₄PF₆ (4.85 mg, 0.0155 mmol) and TBTA (5.19 mg, 0.0155 mmol) was added to reaction mixture and allowed to stir for a further 30 mins. The remaining stopper alkyne 4 solution was added and the reaction mixture stirred under N₂ for 3 days. The reaction was then washed with 0.1 M ETDA solution (3 mL), H₂O (3 mL) and the organic layer dried over MgSO₄. The solvent was removed in vacuo. Size exclusion chromatography (CHCl₃) was used to remove any remaining macrocycle 1 followed by silica gel chromatography (97:3 CH₂Cl₂/CD₃OD). A final preparative thin layer chromatography (Acetone) was used to afford rotaxane 3NO₃ as a yellow solid (22 mg, 24%).
$^1$H NMR (500 MHz, CDCl$_3$) δ (ppm): 9.53 (br s, 1H, pyridinium Ar$H$), 9.08 (br s, 1H, isophthalamide macrocycle Ar$H$), 8.84 (br s, 2H, pyridinium Ar$H$), 8.76 (br s, 1H, CON$H$), 8.63 (br s, 1H, CON$H$), 8.50 (br s, 1H, CON$H$), 8.39 (br s, 1H, CON$H$), 8.31 (s, 2H, isophthalamide macrocycle Ar$H$), 8.22 (br s, 1H, isophthalamide Ar$H$), 8.12 (m, 2H, isophthalamide Ar$H$), 7.82 (s, 1H, triazole CH), 7.80 (s, 1H, triazole CH), 7.51 (t, 1H, $^3$J = 7.7 Hz, isophthalamide Ar$H$), 7.23 (m, 12H, stopper Ar$H$), 7.09 (m, 4H, stopper Ar$H$), 7.07 (m, 12H, stopper Ar$H$), 6.87 (d, $^3$J = 8.8 Hz, 2H, stopper Ar$H$), 6.82 (d, $^3$J = 9.0 Hz, 2H, stopper Ar$H$), 6.37 (d, $^3$J = 8.7 Hz, 4H, hydroquinone Ar$H$), 6.10 (d, $^3$J = 7.7 Hz, 4H, hydroquinone Ar$H$), 5.19 (s, 2H, CH$_2$-C triazole), 5.11 (s, 2H, CH$_2$-C triazole), 4.66 (s, 3H, N$^+$C$H$_3$), 4.52 (br m, 2H, CH$_2$-N triazole), 4.45 (br m, 2H, CH$_2$-N triazole) 4.09 (br m, 2H, CH$_2$NH macrocycle), 4.01 (br m, 2H, CH$_2$NH macrocycle), 3.82 – 3.64 (br m, 20H, CH$_2$O macrocycle), 3.63 – 2.97 (br m, 8H, CH$_2$NH), 2.04 – 1.41 (br m, 8H, CH$_2$CH$_2$NH). $^{13}$C NMR (125 MHz, CDCl$_3$) δ (ppm): 167.1, 166.9, 166.2, 160.1, 159.4, 156.2, 153.6, 153.0, 151.8, 148.3, 144.7, 144.4, 144.2, 144.0, 140.2, 140.1, 132.3, 132.3, 130.7, 129.2, 129.1, 124.0, 123.4, 122.9, 121.5, 114.7, 114.3, 113.2, 70.6, 70.6, 70.0, 68.4, 65.2, 63.0, 62.0, 61.8, 58.4, 50.9, 49.9, 48.3, 47.9, 41.1, 37.6, 37.4, 37.2, 37.1, 36.9, 35.1, 34.4, 34.3, 31.0, 30.3, 30.0, 29.7, 29.5, 29.3. Thirteen peaks missing, presumed overlapped due to apparent symmetry.

$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (125 MHz, CDCl$_3$)
Anion exchange to the hexafluorphosphate salt was achieved by passing $3\text{NO}_3$ down a hexafluorphosphate loaded Amberlite® column (9:1 Acetone/H$_2$O). The solvent was removed in vacuo to afford $3\text{PF}_6$ in quantitative yield. $^1$H NMR (500 MHz, 1:1 CDCl$_3$/CD$_2$OD) δ (ppm): 8.98 (s, 1H, pyridinium ArH), 8.93 (s, 1H, pyridinium ArH), 8.82 (s, 1H, isophthalamide macrocycle ArH), 8.32 (t, $^3$J = 1.4 Hz, 1H, isophthalamide ArH), 8.12 (d, $^4$J = 1.6 Hz, 2H isophthalamide macrocycle ArH), 7.97 (s, 1H, CH triazole), 7.96 (m, 2H, isophthalamide ArH), 7.94 (s, 1H, CH triazole), 7.53 (t, $^3$J = 7.9 Hz, 1H, isophthalamide ArH), 7.22 (m, 12H, stopper ArH), 7.11-7.07 (m, 4H, stopper ArH), 7.06 (m, 12H, stopper ArH), 6.85 (d, $^3$J = 9.1 Hz, 2H, stopper ArH), 6.82 (d, $^3$J = 9.1 Hz, 2H, stopper ArH), 6.47 (d, $^3$J = 9.0 Hz, 4H, hydroquinone ArH), 6.25 (d, $^3$J = 9.1 Hz, 4H, hydroquinone ArH), 5.14 (s, 2H, CH$_2$-C triazole), 5.09 (s, 2H, CH$_2$-C triazole), 4.50 (s, 3H, N$^+$C$_3$H$_3$), 4.49 (t, $^3$J = 7.0 Hz, 2H, CH$_2$-N triazole), 4.30 (t, $^3$J = 7.0 Hz, 2H, CH$_2$-N triazole), 4.14 – 4.02 (m, 4H, CH$_2$-NH macrocycle), 3.81 – 3.65 (m, 20H, CH$_2$O macrocycle), 3.49 – 3.34 (m, 8H, CH$_2$NH), 2.25 (quintet, $^3$J = 6.5 Hz, 2H, CH$_2$CH$_2$NH), 2.12 (quintet, $^3$J = 6.5 Hz, 2H, CH$_2$CH$_2$NH), 1.86 (quintet, $^3$J = 6.5 Hz, 2H, CH$_2$CH$_2$NH). $^{13}$C NMR (125 MHz, 1:1 CDCl$_3$/CD$_2$OD) δ (ppm): 169.9, 169.4, 169.4, 161.9, 161.7, 157.4, 157.5, 157.5, 154.5, 154.2, 153.4, 149.7, 145.5, 141.7, 135.9, 135.5, 133.6, 132.0, 131.7, 130.2, 129.7, 127.3, 125.4, 125.2, 123.7, 116.2, 116.1, 114.5, 72.0, 71.9, 71.4, 69.4, 67.4, 64.4, 62.7, 62.7, 41.6, 39.1, 38.7, 38.5, 38.4, 36.4, 35.5, 33.2, 32.4, 32.1, 31.3, 30.9, 30.6. Twenty peaks missing, presumed overlapped due to apparent symmetry. $^{19}$F NMR (282.5 MHz, 1:1 CDCl$_3$/CD$_2$OD) δ (ppm): -73.2 (d, $^1$J = 710 Hz, PF$_6$). HRMS (ES +ve) m/z: 2286.2915 ([M]$^+$, C$_{141}$H$_{170}$N$_{13}$O$_{15}$ requires 2286.2966).

$^1$H NMR (500 MHz, 1:1 CDCl$_3$ / CD$_2$OD)

$^{13}$C NMR (125 MHz, 1:1 CDCl$_3$ / CD$_2$OD)
Figure SI-1 $^1$H ROESY NMR spectrum of 5NO$_3$ (1:1 CDCl$_3$/CD$_3$OD). Selected cross-peaks indicating through space interactions between the interlocked macrocycle and axle components are highlighted.
Figure SI-2 Electrospray mass spectrum of SI-8 (top) with theoretical isotope model for [M+Na]+ (bottom)

Figure SI-3 Electrospray mass spectrum of 2PF₆ (top) with theoretical isotope model for [M–PF₆]⁺ (bottom)
**Figure SI-4** Electrospray mass spectrum of SI-16 (top) with theoretical isotope model for [M+Na]^+ (bottom)

**Figure SI-5** Electrospray mass spectrum of 3NO_3 (top) with theoretical isotope model for [M−NO_3]^+ (bottom)
Section 2 – Additional pseudorotaxane studies

The lack of formation of a pseudorotaxane in the absence of the nitrate anion template is demonstrated by a $^1$H NMR study in which the thread and pyridinium protons are not perturbed upon mixing (Figure SI-2).

Figure SI-7 $^1$H NMR spectra (a) Macrocycle 1, (b) 1:1 mixture of 1 and thread 2PF$_6$ and (c) Thread 2PF$_6$ in $d_6$-acetone (500 MHz).
Section 3 – $^1$H NMR titrations

$^1$H NMR spectra were recorded on a Bruker AVIII 500 spectrometer. A solution of the anion guest, as the non-complexing tetrabutylammonium (TBA) salt (or in the case of hydrogen carbonate, as the triethylammonium (TEA) salt), was added to a solution of the host at 298 K. The chemical shift of the axle pyridinium proton $I$ was monitored for seventeen titration points (for 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 4.0, 5.0, 7.0 and 10.0 equivalents of guest added). The resulting data were analysed using the WinEqNMR2$^5$ computer program. (In all experiments the association of guest and host was fast on the NMR timescale).

The anion guest was titrated into a solution of host species [2]rotaxane $^{8 \text{PF}_6}$ in 45:45:10 CDCl$_3$/CD$_3$OD/D$_2$O. A 75 mM solution of anion was added to 0.5 mL of a 1.5 mM solution of [3]rotaxane. The volumes of salt solution added were 10 x 2 μL, 2 x 5 μL, 2 x 10 μL, 1 x 20 μL, and 1 x 30 μL. The values of the observed chemical shift and the guest concentration were entered into winEQNMR2 for every titration point and estimates for the binding constant and limiting chemical shifts were made. The parameters were refined using non-linear squares analysis to obtain the best fit between observed and calculated chemical shifts for a 1:1 host-guest binding stoichiometry. The input parameters were varied until the best-fit values of the stability constants, and their errors, converged.

Figure SI-8. Plots of change in chemical shift against anion concentration in 45:45:10 CDCl$_3$/CD$_3$OD/D$_2$O, at 298 K. Observed data (solid points) and fitted isotherms (lines), monitoring proton $I$.

1) Spence, G. T.; Chan, C.; Szemes, F.; and Beer, P. D.; *Dalton Trans.*, **2012**, *41*, 13474-13485