Supplementary Information for: Neutral [2]rotaxane host systems that recognise halide anions in aqueous solvent mixtures

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S1 Experimental

S1.1 Instrumental methods

NMR spectra were recorded on Varian Mercury 300 MHz, Varian Unity Plus 500 MHz or Bruker Avance III 500 MHz spectrometers at 298 K. Low resolution electrospray ionisation mass spectrometry (ESI-MS) was performed using a Waters LCT Premier spectrometer, high resolution ESI-MS using Bruker μTOF and 9.4 T FT-ICR-MS spectrometers and MALDI-MS using a Waters MALDI Micro MX spectrometer. Melting points were recorded on a Gallenkamp capillary melting point apparatus and are uncorrected.

S1.2 Solvents and reagents

Commercially available solvents and chemicals were used without further purification unless specified. Dry solvents were obtained by purging with nitrogen and then passing through a MBraun MPSP-800 column. Water was de-ionised and microfiltered using a Milli-Q Millipore machine. NEt_3 was distilled and stored over KOH. All TBA salts, TBTA and $Cu(MeCN)_4PF_6$ were stored in vacuum desiccators prior to use.

S1.3 Compound characterisation

Novel compounds were characterised by ^{1}H and ^{13}C NMR spectroscopy, low and high resolution mass spectrometry and, where necessary, melting points. If required, ^{1}H NMR assignments were aided by 2D ^{1}H-^{1}H COSY and ROESY NMR spectroscopies.

S1.4 Synthetic procedures

Compounds **1**[1], **2**[2], **5**[3], **6**[4], **11**[5], **12**[6] and **13**[7] were prepared as previously reported.

Bis-azide macrocycle precursor (3)

Amine **1** (3.13 g, 14.1 mmol), NEt_3 (3.54 mL, 25.4 mmol) and a catalytic amount of DMAP were dissolved in dry DCM (100 mL) and then the solution was cooled to 0 ^oC. 5-*Tert*-butylisophthaloyl dichloride **2** (1.66 g, 6.40 mmol) in dry DCM (75 mL) was then added dropwise before the reaction mixture was left to stir at room temperature for two hours under N_2. After this time, the reaction mixture was washed with 10% $HCl_{(aq)}$ (50 mL) and water (2 x 50 mL), dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The resulting brown solid was then purified by silica gel column chromatography (6:4 EtOAc/ hexane) to give **3** as a white solid (2.77 g, 73%). **MS** (ESI-MS) *m/z* 635.24 ($[M + Na]^+$, $C_{32}H_{38}N_8NaO_6$, calc. 653.28); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.96 (2H, s, Ar*H*), 7.94 (1H, s, Ar*H*), 6.86 (8H, s, Ar*H* hydroquinone), 6.80-6.72 (2H, m, -CON*H*CH_2), 4.15-4.07 (8H, m, -OCH_2), 3.90-3.81 (4H, m, -CONHCH_2), 3.57 (4H, t, ^3J = 5.0 Hz, -CH_2N_3), 1.36 (9H, s, -^{t}Bu); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 167.3, 165.6, 134.5, 122.3, 115.7, 115.5, 67.6, 67.3, 50.2, 39.7, 35.0, 31.2.

Bis-amine macrocycle prescursor (4)

Bis-azide **3** (276 mg, 0.438 mmol) was dissolved in EtOH (50 mL). 20% by weight Pd/C (55.2 mg) was then added to the solution before it was stirred under 5 atmospheres of $H_{2(g)}$ overnight. After this time, the reaction mixture was filtered through celite and the solvent removed *in vacuo* to give the product as a white solid in quantitative yield which was used immediately in the next step without further purification or characterisation.

***Tert*-butyl functionalised macrocycle (7)**

Bis-amine **4** (254 mg, 0.438 mmol) and NEt_3 (0.130 mL, 0.963 mmol) were dissolved in dry DCM (20 mL) and loaded into a 20 mL syringe. 5-*Tert*-butylisophthaloyl dichloride **2** (113 mg, 0.438 mmol) was then also dissolved in dry DCM (20 mL) and loaded into another 20

mL syringe. The contents of both syringes were then added dropwise to a stirred flask of dry DCM (600 mL) under N_2 over 6 hours using a syringe pump. The mixture was then left to stir overnight under N_2. The solvent volume was then reduced (~ 100 mL) and the reaction mixture washed with 1 M $HCl_{(aq)}$ (2 x 75 mL), H_2O (2 x 75 mL) and brine (2 x 75 mL) before being dried over $MgSO_4$, filtered and concentrated. The crude product was purified *via* silica gel column chromatography (97:3 DCM/MeOH) to give the product as a white solid (166 mg, 50%). **Mp** > 250 °C; **MS** (ESI-MS) *m/z* 787.3649 ($[M + Na]^+$, $C_{44}H_{52}N_4NaO_8$, calc. 787.3677); **^{1}H NMR** (300 MHz, 1:1 $CDCl_3/CD_3OD$) δ 8.05 (4H, d, ^4J = 1.78 Hz, Ar*H* *tert*-butyl isophthalamide), 7.99 (2H, t, ^4J = 1.77 Hz, Ar*H* *tert*-butyl isophthalamide), 6.80-6.78 (8H, m, Ar*H* hydroquinone), 4.06 (8H, t, ^3J = 4.94 Hz, -OCH_2), 3.76 (8H, t, ^3J = 4.99 Hz, -CONHCH_2), 1.34 (18H, s, -^{t}Bu); **^{13}C NMR** (75 MHz, 1:1 $CDCl_3/CD_3OD$) δ 168.2, 152.6, 152.1, 133.6, 133.4, 127.6, 121.9, 115.1, 114.9, 114.7, 66.5, 39.4, 34.5, 30.4.

Nitro-functionalised macrocycle (8)

Bis-amine **4** (252 mg, 0.435 mmol) and NEt_3 (0.243 mL, 1.74 mmol) were dissolved in dry DCM (20 mL) and loaded into a 20 mL syringe. Acid chloride **5** (107 mg, 0.435 mmol) was also dissolved in dry DCM (20 mL) and loaded into another 20 mL syringe. The contents of both syringes were then added dropwise to a stirred flask of dry DCM (750 mL) under N_2 over a 4 hour period using a syringe pump. The resulting mixture was then stirred overnight under N_2. The solvent volume was reduced *in vacuo* (~150 mL) and the organic phase washed with 10% citric $acid_{(aq)}$ (3 x 75 mL), water (3 x 75 mL) and brine (3 x 75 mL) before being dried over $MgSO_4$ and filtered. The solvent was then removed to give a yellow solid which was purified *via* silica gel column chromatography (98:2 DCM/MeOH increasing to 95:5 DCM/MeOH) to yield **8** as an off-white solid (76.0 mg, 23%). **Mp** 166 °C; **MS** (ESI-MS) *m/z* 788.2706 ($[M + Cl]^-$, $C_{40}H_{43}N_5O_{10}Cl$, calc. 788.2704); **^{1}H NMR** (500 MHz, 1:1

$CDCl_3/CD_3OD$) δ 8.80 (2H, s, Ar*H* nitro isophthalamide), 8.77-8.70 (2H, m, -CON*H* nitro isophthalamide), 8.60 (1H, s, Ar*H* nitro isophthalamide), 8.37-8.29 (2H, m, -CON*H* *tert*-butyl isophthalamide), 8.04 (2H, s, Ar*H* *tert*-butyl isophthalamide), 8.02 (1H, s, Ar*H* *tert*-butyl isophthalamide), 6.76 (8H, s, Ar*H* hydroquinone), 4.10-4.00 (8H, m, -CONHCH_2), 3.81-3.71 (8H, m, -OCH_2), 1.34 (9H, s, -^{t}Bu); **^{13}C NMR** (126 MHz, 1:1 $CDCl_3/CD_3OD$) δ 168.0, 165.1, 152.6, 152.5, 152.0, 148.1, 135.7, 133.5, 130.3, 127.4, 124.7, 121.8, 114.8, 66.5, 66.3, 39.6, 39.3, 34.4, 30.2, 29.1.

Pyridine-functionalised macrocycle (9)

Bis-amine **4** (254 mg, 0.438 mmol) and NEt_3 (0.130 mL, 0.963 mmol) were dissolved in dry DCM (20 mL) and loaded into a 20 mL syringe. Acid chloride **6** (88.9 mg, 0.438 mmol) was then also dissolved in dry DCM (20 mL) and loaded into another 20 mL syringe. The contents of both syringes were then added dropwise to a stirred flask of dry DCM (600 mL) under N_2 over 6 hours using a syringe pump. The mixture was then left to stir overnight under N_2. The solvent volume was then reduced (~ 100 mL) and the reaction mixture washed with 10% citric $acid_{(aq)}$ (2 x 75 mL), H_2O (2 x 75 mL) and brine (2 x 75 mL) before being dried over $MgSO_4$, filtered and concentrated. The crude product was purified *via* silica gel column chromatography (98:2 DCM/MeOH increasing to 95:5 DCM/MeOH) to give the product as a white solid (76.3 mg, 25%). **Mp** 203 °C (dec.); **MS** (ESI-MS) *m/z* 732.3007 ([M + Na]$^+$, $C_{39}H_{43}N_5NaO_8$, calc. 732.3004); **^{1}H NMR** (300 MHz, 1:1 $CDCl_3/CD_3OD$) δ 9.10-9.07 (2H, m, Ar*H* pyridine isophthalamide), 8.60 (2H, t, ^3J = 5.5 Hz, -CON*H* pyridine isophthalamide), 8.58-8.55 (1H, m, Ar*H* pyridine isophthalamide), 8.23, (2H, t, ^3J = 5.4 Hz, -CON*H* *tert*-butyl isophthalamide), 8.06-8.03 (2H, m, Ar*H* *tert*-butyl isophthalamide), 8.01-7.98 (1H, m, Ar*H* *tert*-butyl isophthalamide), 6.81-6.75 (8H, m, Ar*H* hydroquinone), 4.10-4.01 (8H, m, -CONHCH_2), 3.80-3.73 (8H, m, -OCH_2), 1.35-1.32 (9H, m, -^{t}Bu); **^{13}C NMR**

(75 MHz, 1:1 $CDCl_3/CD_3OD$) δ 168.0, 165.4, 152.6, 152.5, 152.0, 150.2, 133.5, 133.2, 129.4, 127.4, 121.8, 114.9, 66.5, 66.3, 39.3, 39.2, 34.4, 30.2, 29.0.

Pyridine N-oxide functionalised macrocycle (10)

Pyridine functionalised macrocycle **9** (50.0 mg, 0.0704 mmol) and $NaHCO_3$ (177 mg, 2.11 mmol) were dissolved in 1:1 2-butanone/H_2O (20 mL). Oxone (130 mg, 0.423 mmol) was then added and the reaction mixture left to stir at room temperature overnight. After this time, NaCl (408 mg, 7.04 mmol) was added before the product was extracted with EtOAc (6 x 50 mL). The organic phase was then dried over $MgSO_4$, filtered and the solvent removed to give macrocycle **10** as an off-white solid (36.4 mg, 71%). **Mp** 173 °C (dec.); **MS** (ESI-MS) *m/z* 748.2925 ($[M + Na]^+$, $C_{39}H_{43}N_5NaO_9$, calc. 748.2953); **^{1}H NMR** (300 MHz, 1:1 $CDCl_3/CD_3OD$) δ 8.88 (1H, s, Ar*H* pyridine N-oxide isophthalamide), 8.72 (2H, s, Ar*H* pyridine N-oxide isophthalamide), 8.28 (1H, s, Ar*H* *tert*-butyl isophthalamide), 8.05 (2H, s, Ar*H* *tert*-butyl isophthalamide), 6.85-6.74 (8H, m, Ar*H* hydroquinone), 4.13-4.00 (4H, m, 3.80-3.69, -OCH_2), (4H, m, -CONHCH_2), 1.37-1.32 (9H, m, -^{t}Bu); **^{13}C NMR** (126 MHz, DMSO-d^6) δ 166.5, 162.4, 152.7, 151.1, 139.3, 134.3, 133.3, 126.7, 123.6, 122.5, 115.5, 79.2, 66.6, 66.3, 34.7, 31.0.

Tert-butyl functionalised rotaxane (14)

Macrocycle **7** (40.6 mg, 0.0531 mmol), N-oxide axle precursor **12** (23.9 mg, 0.0690 mmol), stoppered alkyne **13** (74.9 mg, 0.138 mmol), TBA·Cl (19.2 mg, 0.0690 mmol), TBTA (14.6 mg, 0.0276 mmol) and DIPEA (0.04 mL, 0.207 mmol) were dissolved in $CHCl_3$ (5 mL). $Cu(MeCN)_4PF_6$ (10.3 mg, 0.0276 mmol) was then added and the reaction stirred for 48 hours under nitrogen. After this time, the reaction mixture was washed with H_2O (10 x 10 mL), dried over $MgSO_4$ and filtered before the solvent was removed *in vacuo*. The resulting solid was then purified by preparative thin layer chromatography (97:3 DCM/MeOH then 95:5

EtOAc/MeOH) to give rotaxane **14** as an off-white solid (10.7 mg, 9%). **Mp** 182 °C; **MS** (ESI-MS) *m/z* 1121.0921 ($[M + 2Na]^{2+}$, $C_{137}H_{161}N_{13}Na_2O_{13}$, calc. 1121.1061); **^{1}H NMR** (500MHz, 1:1 $CDCl_3/CD_3OD$) δ 8.49-8.44 (2H, m, Ar*H* *tert*-butyl isophthalamide), 8.43 (2H, s, Ar*H* pyridine N-oxide), 8.14 (1H, s, Ar*H* pyridine N-oxide), 8.12 (4H, s, Ar*H* *tert*-butyl isophthalamide), 7.87 (2H, s, triazole-*H*), 7.19 (12H, d, ^3J = 8.54 Hz, Ar*H* stopper), 7.07 (4H, d, ^3J = 8.70 Hz, Ar*H* stopper), 7.03 (12H, d, ^3J = 8.40 Hz, Ar*H* stopper), 6.81 (4H, d, ^3J = 8.69 Hz, Ar*H* stopper), 6.36-6.33 (8H, m, Ar*H* hydroquinone), 5.09 (4H, s, -OCH_2 axle), 4.20 (4H, t, ^3J = 7.10 Hz, -CH_2CH_2-triazole), 4.00-3.94 (8H, m, -CONHCH_2 macrocycle), 3.75-3.70 (8H, m, -CONHCH_2 macrocycle), 3.29-3.24 (4H, m, -CONHCH_2 axle), 2.08-1.99 (4H, m, -$CONHCH_2CH_2$ axle), 1.34 (9H, s, -^{t}Bu macrocycle), 1.26 (54H, s, -^{t}Bu axle); **^{13}C NMR** (126 MHz, 1:1 $CDCl_3/CD_3OD$) δ 140.2, 133.8, 127.7, 124.3, 124.3 (sic), 120.0, 115.8, 112.0, 111.1, 105.6, 103.9, 102.3, 99.9, 95.7, 95.5, 94.3, 88.5, 84.8, 38.2, 34.7, 33.0.

Nitro functionalised rotaxane (15)

Nitro-functionalised macrocycle **8** (30.0 mg, 0.0398 mmol), N-oxide axle precursor **12** (18.0 mg, 0.0518 mmol), stoppered alkyne **13** (57.0 mg, 0.105 mmol), TBA·Cl (14.4 mg, 0.0518 mmol), TBTA (11.0 mg, 0.0207 mmol) and DIPEA (0.03 mL, 0.155 mmol) were dissolved in $CHCl_3$ (5 mL). $Cu(MeCN)_4PF_6$ (7.72 mg, 0.0207 mmol) was then added and the reaction stirred for 48 hours under nitrogen. After this time, the organic phase was washed with H_2O (10 x 15 mL), dried over $MgSO_4$ and filtered before the solvent was removed *in vacuo*. The resulting solid was then purified by silica gel column chromatography (96:4 DCM/MeOH) followed by preparative thin layer chromatography (95:5 EtOAc/MeOH followed by 9:1 EtOAc/MeOH followed by 3:1 MeCN/DCM) to give the product as an off-white solid (15.0 mg, 17%). **Mp** 151 °C; **MS** (MALDI-MS) *m/z* 2209.61 ($[M + Na]^+$, $C_{133}H_{152}N_{14}NaO_{15}$, calc. 2209.15); **^{1}H NMR** (500 MHz, 1:1 $CDCl_3/CD_3OD$) δ 8.99 (1H, s, Ar*H* nitro isophthalamide),

8.87 (2H, s, Ar*H* nitro isophthalamide), 8.45 (2H, s, Ar*H* pyridine N-oxide), 8.41 (1H, s, Ar*H* *tert*-butyl isophthalamide), 8.10 (2H, s, Ar*H* *tert*-butyl isophthalamide), 8.09 (1H, s, Ar*H* pyridine N-oxide), 7.84 (2H, s, triazole-C*H*), 7.20 (12H, d, ^{3}J = 8.4 Hz, Ar*H* stopper), 7.07 (4H, d, ^{3}J = 8.8 Hz, Ar*H* stopper), 7.04 (12H, d, ^{3}J = 7.9 Hz, Ar*H* stopper), 6.81 (4H, d, ^{3}J = 8.8 Hz, Ar*H* stopper), 6.41-6.35 (8H, m, Ar*H* hydroquinone), 5.07 (4H, s, -OCH_2 axle), 4.24 (4H, t, ^{3}J = 7.1 Hz, -CH_2CH_2-triazole), 4.01-3.93 (8H, m, -OCH_2 macrocycle), 3.76-3.70 (8H, m, -CONHCH_2 macrocycle), 3.27 (4H, t, ^{3}J = 7.1 Hz, -CONHCH_2 axle), 2.10-2.01 (4H, m, -CONHCH_2CH_2 axle), 1.33 (9H, s, -^{t}Bu macrocycle), 1.27 (54H, s, -^{t}Bu axle); **^{13}C NMR** (126 MHz, 1:1 $CDCl_3$/CD_3OD) δ 169.3, 166.6, 162.9, 156.9, 153.6, 153.3, 149.1, 144.9, 141.1, 140.3, 136.9, 134.7, 133.4, 133.0, 131.4, 129.2, 128.6, 128.2, 126.3, 124.8, 116.7, 116.2, 115.8, 115.6, 114.0, 71.5, 67.3, 63.8, 62.2, 37.9, 35.7, 34.9, 31.8, 31.6, 30.3.

Pyridine functionalised rotaxane (16)

Macrocycle **9** (28.2 mg, 0.0398 mmol), N-oxide axle precursor **12** (18.0 mg, 0.0518 mmol), stoppered alkyne **13** (57.0 mg, 0.105 mmol), TBA·Cl (14.4 mg, 0.0518 mmol), TBTA (11.0 mg, 0.0207 mmol) and DIPEA (0.03 mL, 0.155 mmol) were dissolved in $CHCl_3$ (5 mL). $Cu(MeCN)_4PF_6$ (7.72 mg, 0.0207 mmol) was then added and the reaction stirred for 48 hours under nitrogen. After this time, the organic phase was washed with H_2O (10 x 15 mL), dried over $MgSO_4$ and filtered before the solvent was removed *in vacuo*. The resulting solid was then purified by silica gel column chromatography (95:5 DCM/MeOH) followed by preparative thin-layer chromatography (9:1 EtOAc/MeOH) to yield the product as a white solid (18.0 mg, 16%). **Mp** 196 °C; **MS** (ESI-MS) *m/z* 1093.5700 ($[M + 2Na]^{2+}$, $C_{123}H_{152}N_{14}Na_2O_{13}$, calc. 1093.5724); **^{1}H NMR** (500 MHz, 1:1 $CDCl_3$/CD_3OD) δ 9.19 (2H, s, Ar*H* pyridine isophthalamide), 9.00 (1H, s, Ar*H* pyridine isophthalamide), 8.49 (2H, s, Ar*H* pyridine N-oxide), 8.41 (1H, s, Ar*H* *tert*-butyl isophthalamide), 8.13 (2H, s, Ar*H* *tert*-butyl

isophthalamide), 8.08 (1H, s, Ar*H* pyridine N-oxide), 7.87 (2H, s, triazole-C*H*), 7.21 (12H, d, ^{3}J = 8.3 Hz, Ar*H* stopper), 7.09 (4H, d, ^{3}J = 8.7 Hz, Ar*H* stopper), 7.06 (12H, d, ^{3}J = 8.3 Hz, Ar*H* stopper), 6.82 (4H, d, ^{3}J = 8.7 Hz, Ar*H* stopper), 6.43-6.38 (8H, m, Ar*H* hydroquinone), 5.08 (4H, s, -OCH_2 axle), 4.24 (4H, t, ^{3}J = 7.1 Hz, -CH_2CH_2-triazole), 3.99-3.92 (8H, m, -OCH_2 macrocycle), 3.76-3.69 (8H, m, -CONHCH_2 macrocyle), 3.26 (4H,t, ^{3}J = 6.3 Hz, -CONHCH_2 axle), 2.08-2.01 (4H, m, -CONHCH_2CH_2 axle), 1.32 (9H,s, -^{t}Bu macrocycle), 1.25 (54H, s, -^{t}Bu axle); **^{13}C NMR** (75 MHz, 1:1 $CDCl_3$/CD_3OD) δ 169.3, 163.0, 156.8, 153.5, 153.4, 149.1, 144.9, 141.1, 140.2, 134.8, 133.4, 133.1, 131.4, 124.8, 123.6, 115.8, 115.7, 113.9, 67.3, 63.8, 62.1, 40.9, 38.0, 34.9, 31.8, 31.6, 30.5.

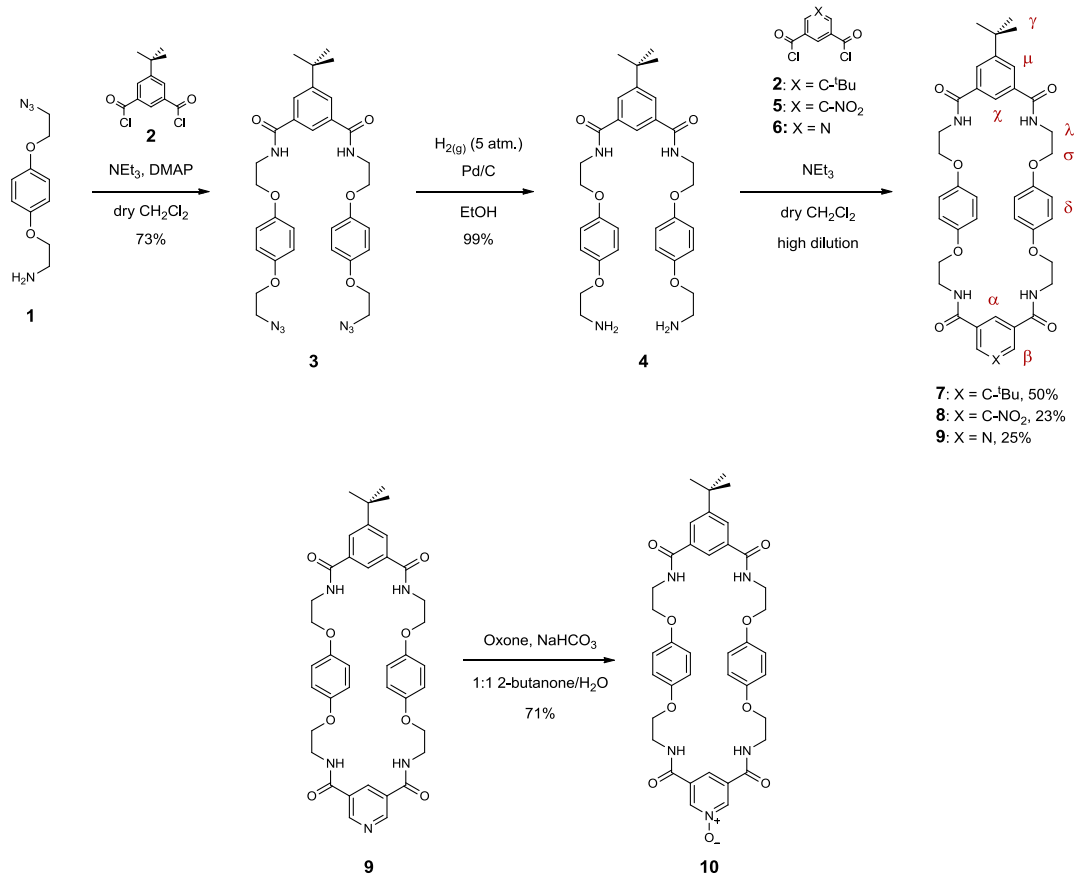
Pyridine N-oxide functionalised rotaxane (17)

Macrocycle **10** (36.4 mg, 0.0502 mmol), N-oxide axle precursor **12** (22.6 mg, 0.0652 mmol), stoppered alkyne **13** (70.8 mg, 0.130 mmol), TBA·Cl (18.1 mg, 0.0652 mmol), TBTA (13.8 mg, 0.0261 mmol) and DIPEA (0.03 mL, 0.196 mmol) were dissolved in $CHCl_3$ (5 mL). $Cu(MeCN)_4PF_6$ (9.72 mg, 0.0261 mmol) was then added and the reaction stirred for 48 hours under nitrogen. After this time, the reaction mixture was washed with H_2O (10 x 15 mL), dried over $MgSO_4$ and filtered before the solvent was removed *in vacuo*. The resulting solid was then purified by silica gel column chromatography (95:5 DCM/MeOH) followed by preparative thin layer chromatography (96:4 DCM/MeOH) to give rotaxane **17** an off-white solid (17.7 mg, 16%). **Mp** 165 °C (dec.); **MS** (MALDI-MS) *m/z* 2159.36 ($[M]^+$, $C_{132}H_{152}N_{14}O_{14}$, calc. 2159.17); **^{1}H NMR** (500 MHz, 1:1 $CDCl_3$/CD_3OD) δ 8.94 (1H, s, Ar*H* macrocycle pyridine N-oxide isophthalamide), 8.82 (2H, s, Ar*H* macrocycle pyridine N-oxide isophthalamide), 8.61 (1H, s, Ar*H* *tert*-butyl isopthalamide), 8.48 (2H, s, Ar*H* axle pyridine N-oxide isophthalamide), 8.27 (1H, s, Ar*H* macrocycle pyridine N-oxide isophthalamide), 8.12 (2H, s, Ar*H* *tert*-butyl isopthalamide), 7.93 (2H, s, triazole-C*H*), 7.19 (12H, d, ^{3}J = 8.3

Hz, Ar*H* stopper), 7.07 (4H, d, ^{3}J = 8.7 Hz, Ar*H* stopper), 7.03 (12H, d, ^{3}J = 8.1 Hz, Ar*H* stopper), 6.82 (4H, d, ^{3}J = 8.5 Hz, Ar*H* stopper), 6.38-6.28 (8H, m, Ar*H* hydroquinone), 5.11 (4H, s, -OCH_2 axle), 4.28 (4H, t, ^{3}J = 7.2 Hz, -CH_2CH_2-triazole), 4.07-4.03 (4H, m, -CONHCH_2 macrocycle), 3.94-3.90 (4H, m, -CONHCH_2 macrocycle), 3.78-3.69 (8H, m, -OCH_2 macrocycle), 3.35-3.32 (4H, m, -CONHCH_2 axle), 2.12-2.05 (4H, m, -CONHCH_2CH_2 axle), 1.33 (9H, s, -^{t}Bu macrocycle), 1.26 (54H, s, -^{t}Bu stopper); **^{13}C NMR** (126 MHz, 1:1 $CDCl_3$/CD_3OD) δ 169.3, 163.9, 162.9, 156.9, 153.7, 153.3, 149.1, 145.0, 141.2, 140.4, 134.7, 133.1, 131.5, 129.1, 124.8, 115.7, 114.0, 67.1, 67.0, 63.9, 62.1, 43.5, 41.3, 41.1, 38.0, 37.2, 35.8.

S2 Novel bis-isophthalamide macrocycle syntheses

Novel bis-isophthalamide macrocycles **7**-**10** were synthesised as shown in Scheme **S1.1** (detailed synthetic procedures can be found in Section **S1.4**). Two equivalents of mono-amine **1**[1] were reacted with acid chloride **2**[2] to give bis-azide **3** in 73% yield. Reduction of the azide functionality of **3** using Pd/C (10% by weight) and hydrogen gas (5 atm) afforded bis-amine macrocycle precursor **4** (99%). Target macrocycles **7**, **8** and **9** were then synthesised by condensation reactions of **4** with acid chlorides **2**[2], **5**[3] or **6**[4] under high dilution conditions in 50%, 23% and 25% yields respectively. Oxidation of macrocycle **9** with Oxone, in the presence of $NaHCO_3$ afforded pyridine N-oxide functionalised macrocycle **10** in 71% yield.



Scheme S1.1 – Synthesis of novel bis-isophthalamide macrocycles **7**-**10**.

S3 ^{1}H NMR titration protocols

Titrations were conducted on either an Oxford Instruments Varian Unity Plus or a Bruker Avance III 500 MHz spectrometer, at 298 K. Initial sample volumes were 500 μL. The starting concentration of the host was 2 mM for all titrations. All anions were added as their TBA salts. 17 aliquots of the guest were added until a total of 10 equivalents 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 WinEQNMR2[8] computer program. Estimates for each binding constant, the limiting chemical shifts and the complex stoichiometry were 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. Comparison of the calculated binding isotherm with that obtained experimentally demonstrated that the model used was appropriate.

S4 Pseudorotaxane studies for macrocycles 7-9 with thread 11

S4.1 ^{1}H NMR evidence

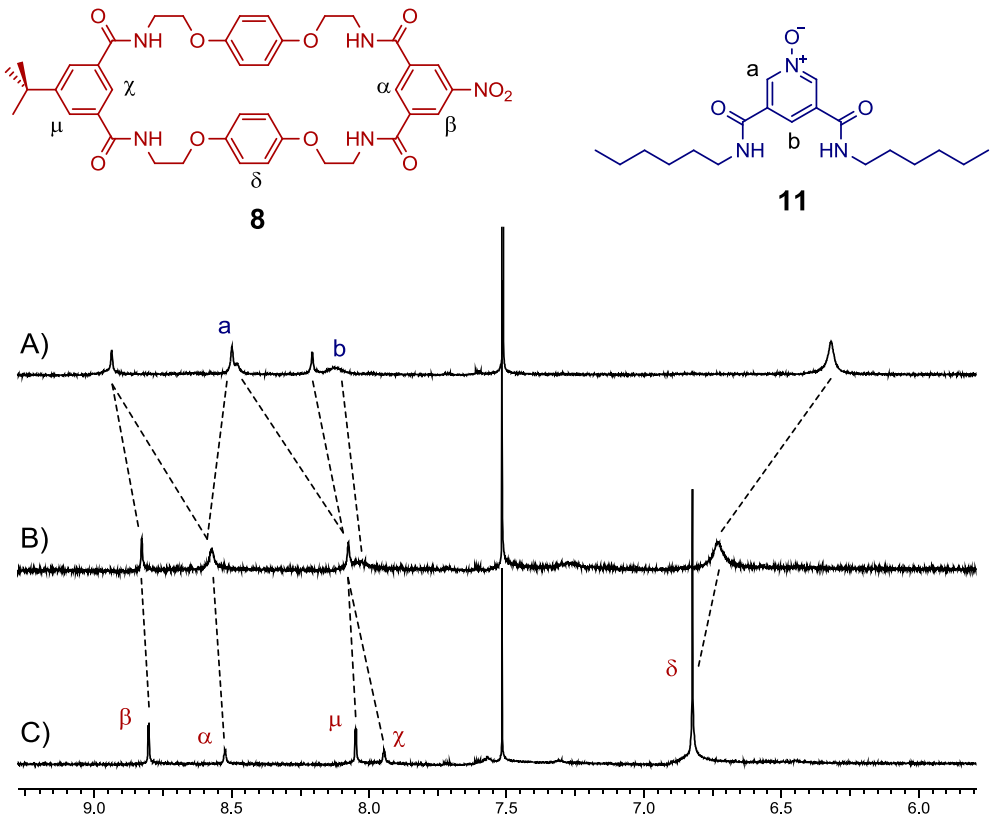


Figure S4.1 – ^{1}H NMR spectra of A) a 1:1:1 mixture of macrocycle **8**, thread **11** and TBA·Cl, B) a 1:1 mixture of macrocycle **8** and thread **11**, and C) macrocycle **8** (500 MHz, 1:1 $CDCl_3/CD_3CN$, 298 K).

S4.2 Titration data

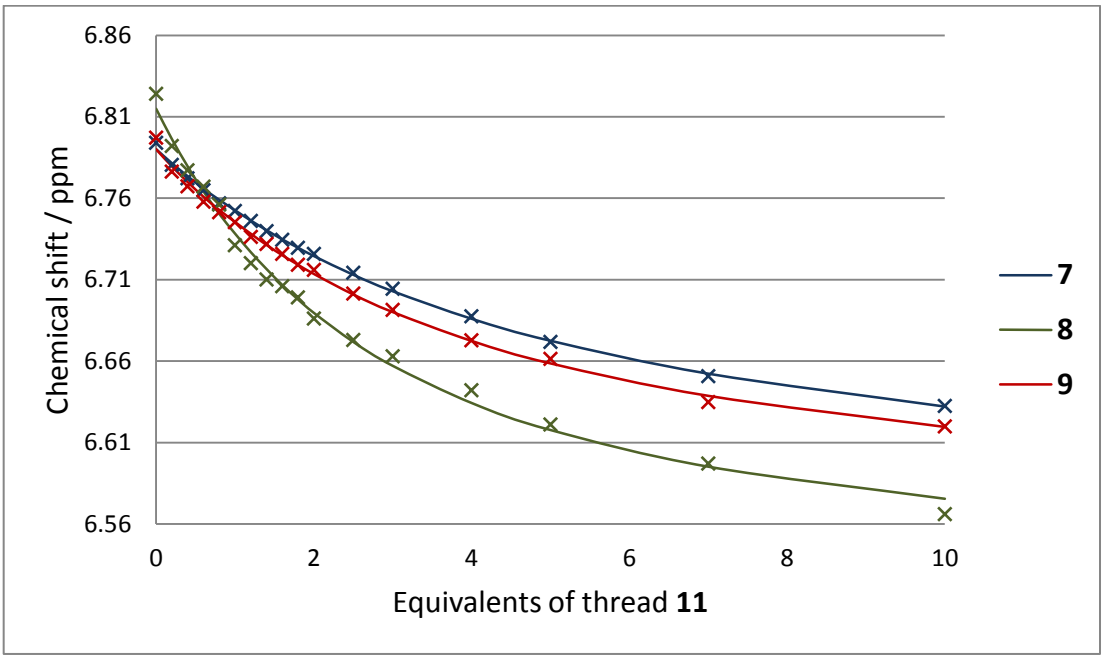


Figure S4.2 – Changes in chemical shift of hydroquinone protons λ and φ of macrocycles **7**, **8** and **9** upon addition of increasing amounts of pyridine N-oxide thread **11** (1:1 $CD_3CN/CDCl_3$, 298 K, crosses represent data points, continuous lines represent the calculated curves).

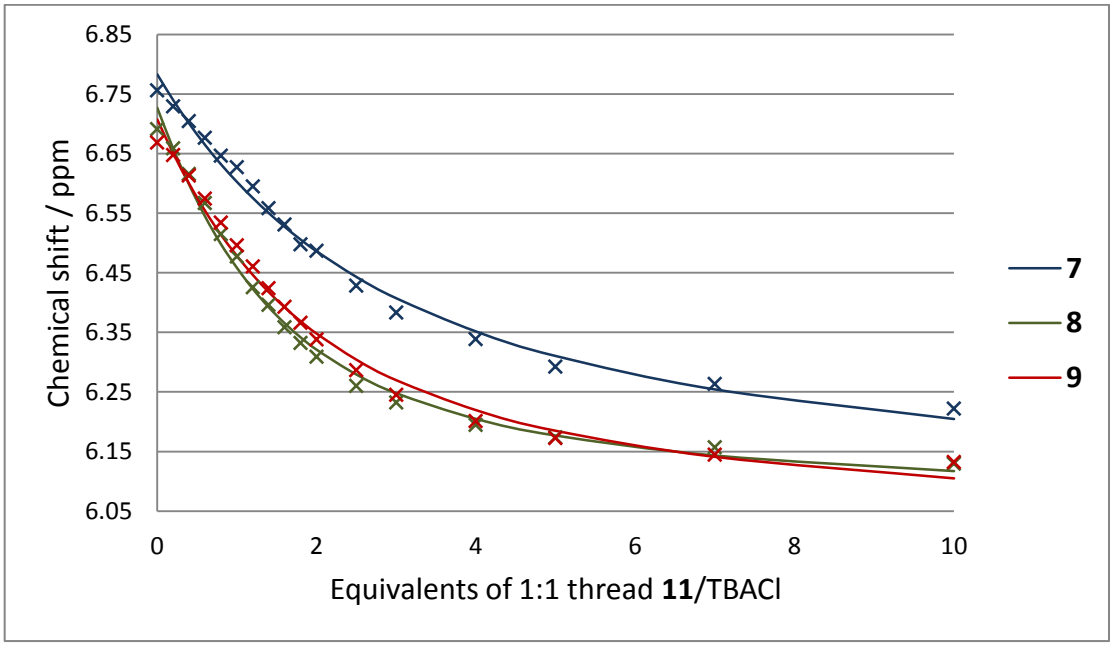


Figure S4.3 – Changes in chemical shift of hydroquinone protons λ and φ of macrocycles **7**, **8** and **9** upon addition of increasing amounts of 1:1 pyridine N-oxide thread **11**/TBACl (9:1 $CDCl_3/CD_3OD$, 298 K, crosses represent data points, continuous lines represent the calculated curves).

S4.3 Table of association constants

	7	**8**	**9**
*Ka (**11**)*	93(6)	216(15)	120(12)
*Kapp (Cl⁻/**11**)*	-	> 10^4	> 10^4

Table S4.1 – 1:1 Stoichiometric association and apparent association constants for macrocycles **7**-**9** with pyridine N-oxide thread **11** and a 1:1 mixture of TBACl/thread **11** (1:1 $CDCl_3/CD_3CN$, 298 K, values quoted as M^{-1}, errors in parentheses). Macrocycle **10** was insoluble in the solvent mixture used.

S5 Characterisation data for [2]rotaxanes 14-17

S5.1 [2]Rotaxane 14

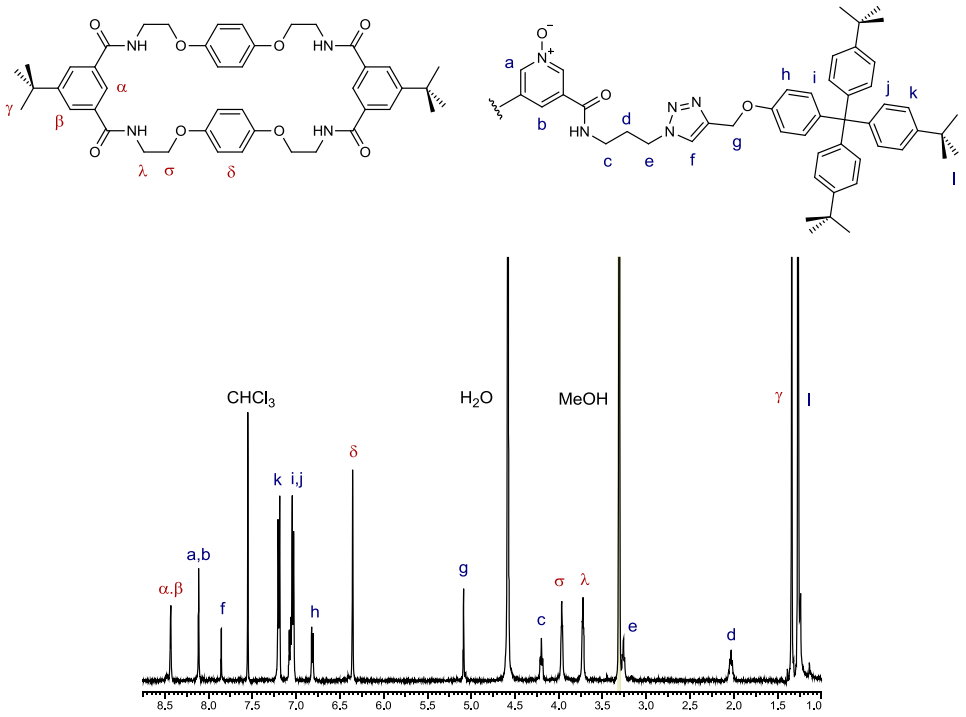


Figure S5.1 – ^{1}H NMR spectrum of [2]rotaxane **14** (1:1 $CDCl_3/CD_3OD$, 500 MHz, 298 K).

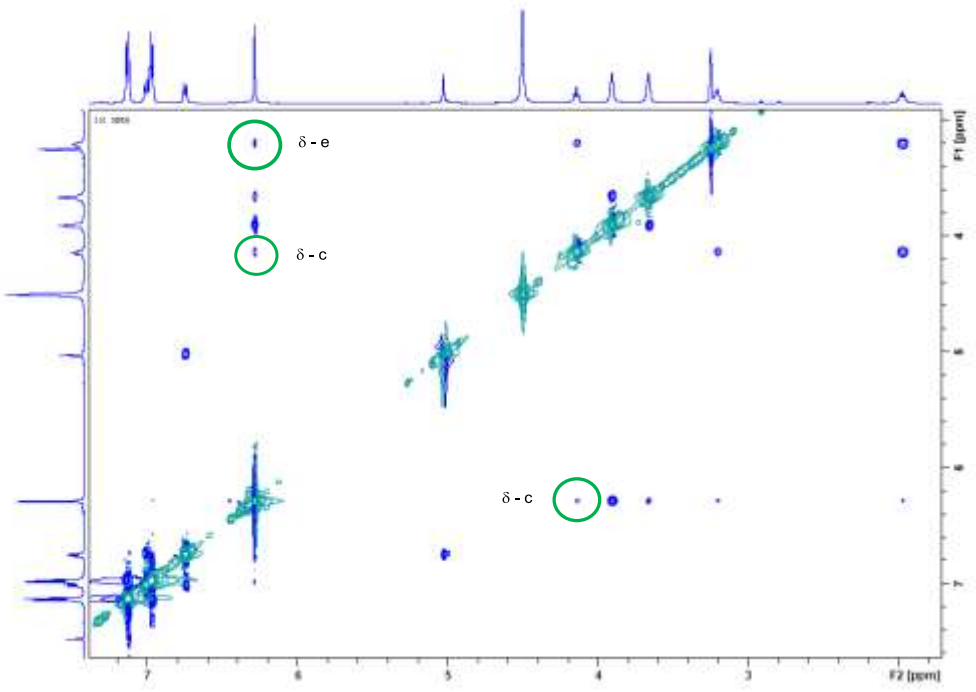


Figure S5.2 – 2D ROESY NMR spectrum of [2]rotaxane **14** (1:1 $CDCl_3/CD_3OD$, 500 MHz, 298 K).

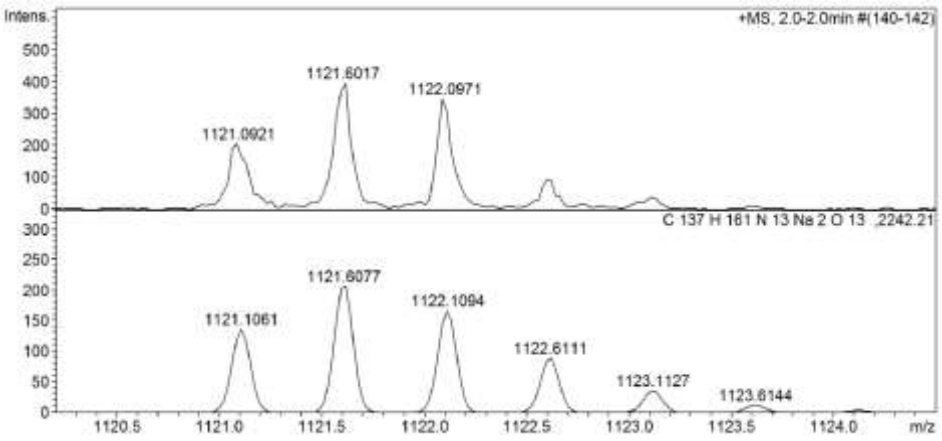


Figure S5.3 – High resolution ESI-MS spectrum of [2]rotaxane **14**.

S5.2 [2]Rotaxane 15

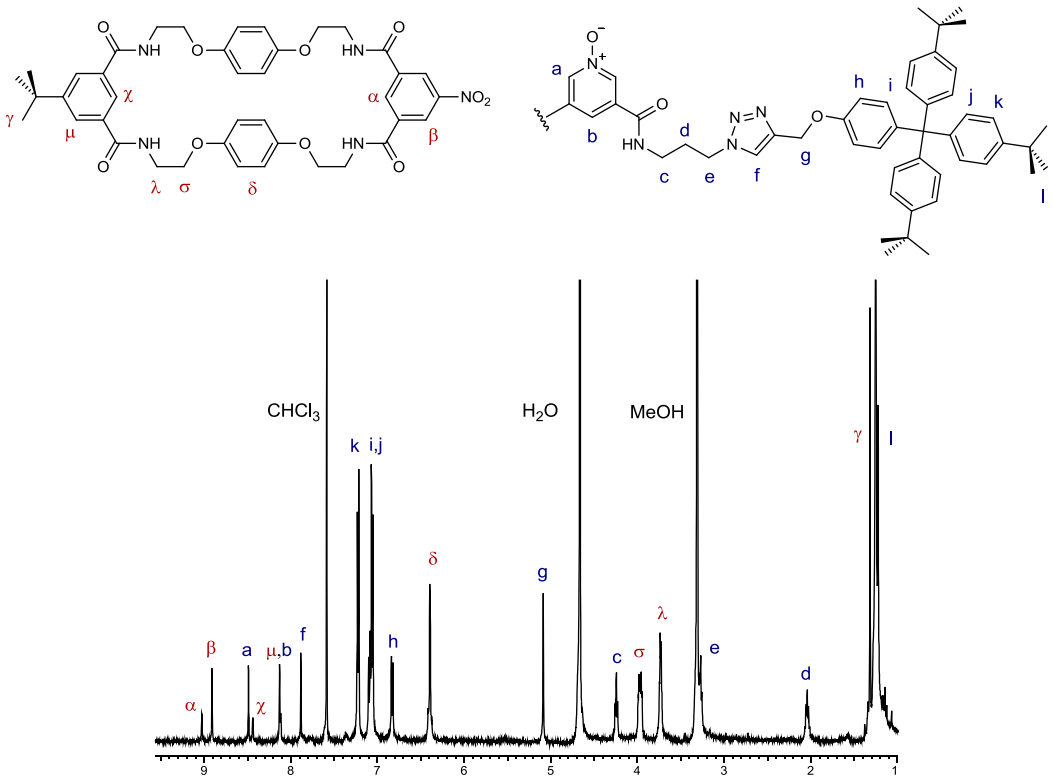


Figure S5.4 - ^{1}H NMR spectrum of [2]rotaxane **15** (1:1 $CDCl_3/CD_3OD$, 500 MHz, 298 K).

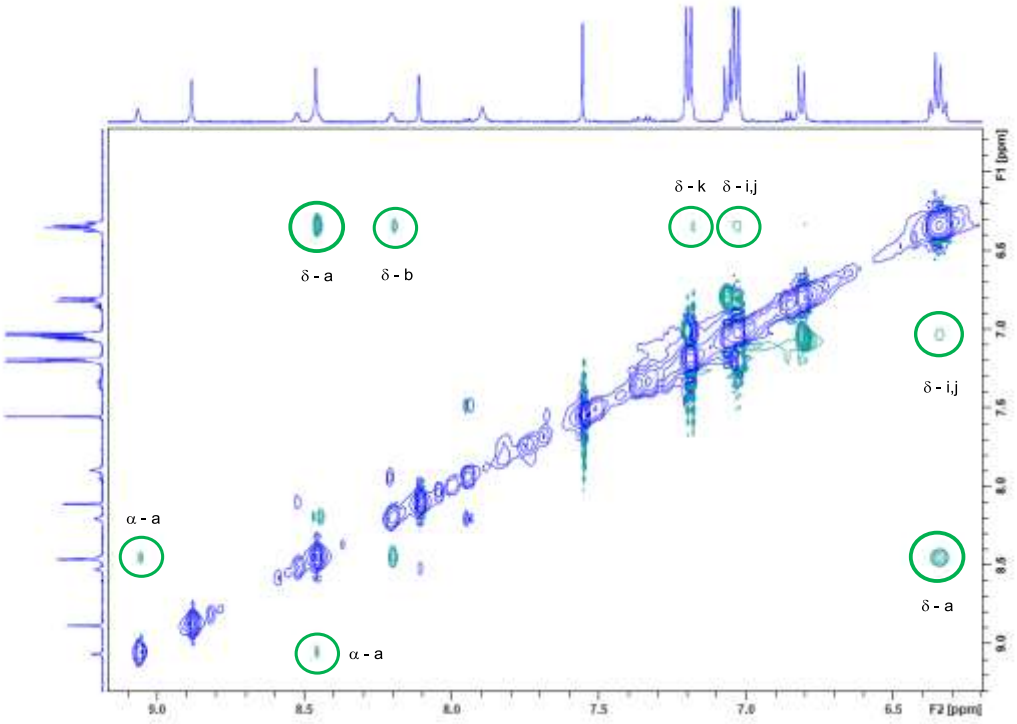


Figure S5.5 - 2D ROESY NMR spectrum of [2]rotaxane **15** (1:1 $CDCl_3/CD_3OD$, 500 MHz, 298 K).

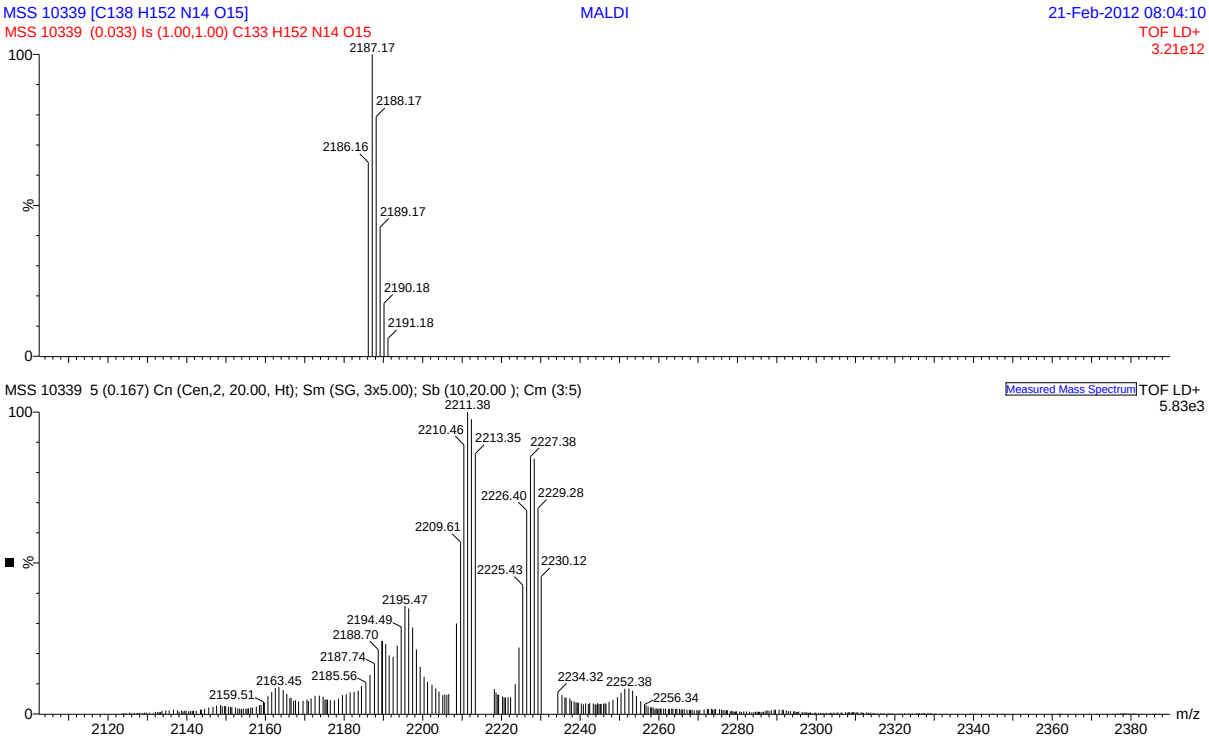


Figure S5.6 – MALDI-MS spectrum of [2]rotaxane **15**.

S5.3 [2]Rotaxane 16

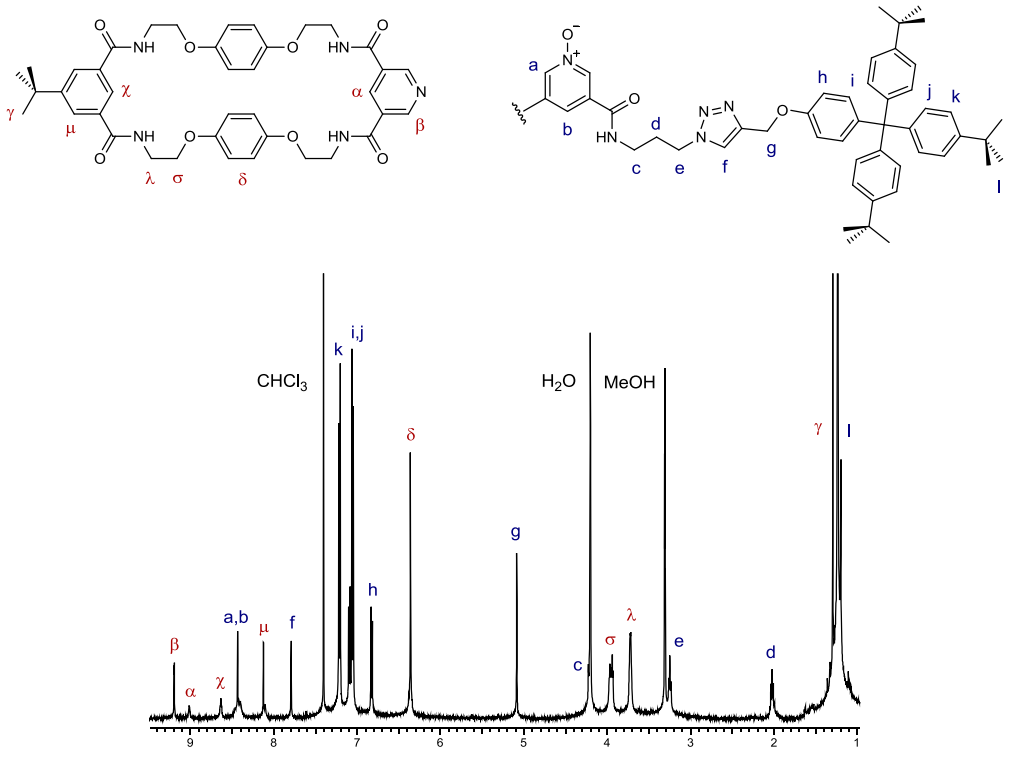


Figure S5.7 - ^{1}H NMR spectrum of [2]rotaxane **16** (1:1 $CDCl_3$/CD_3OD, 500 MHz, 298 K).

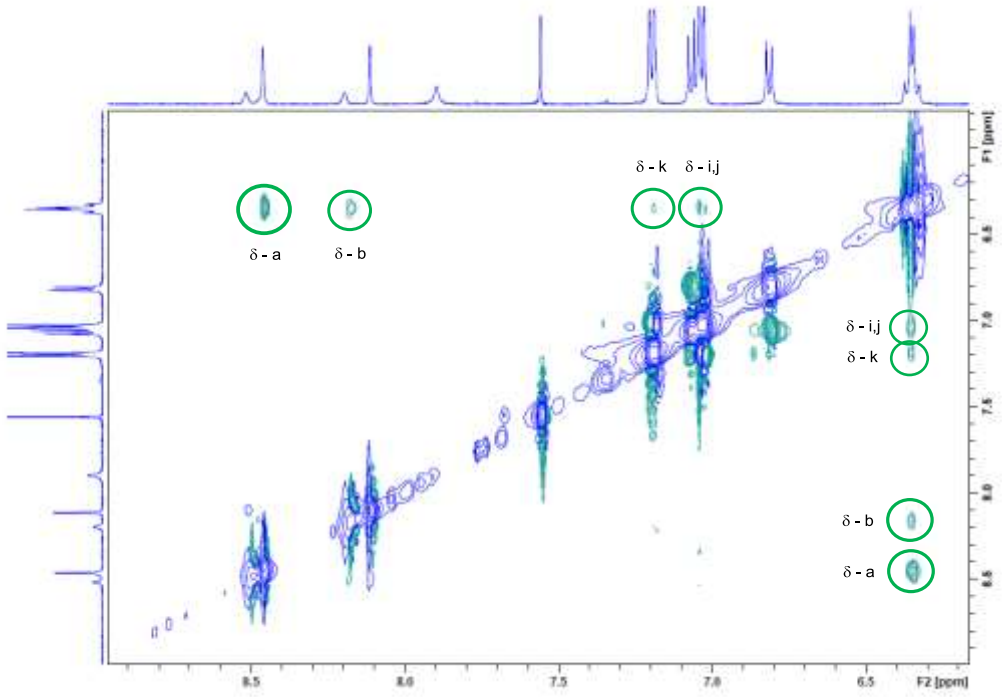


Figure S5.8 - 2D ROESY NMR spectrum of [2]rotaxane **16** (1:1 $CDCl_3/CD_3OD$, 500 MHz, 298 K).

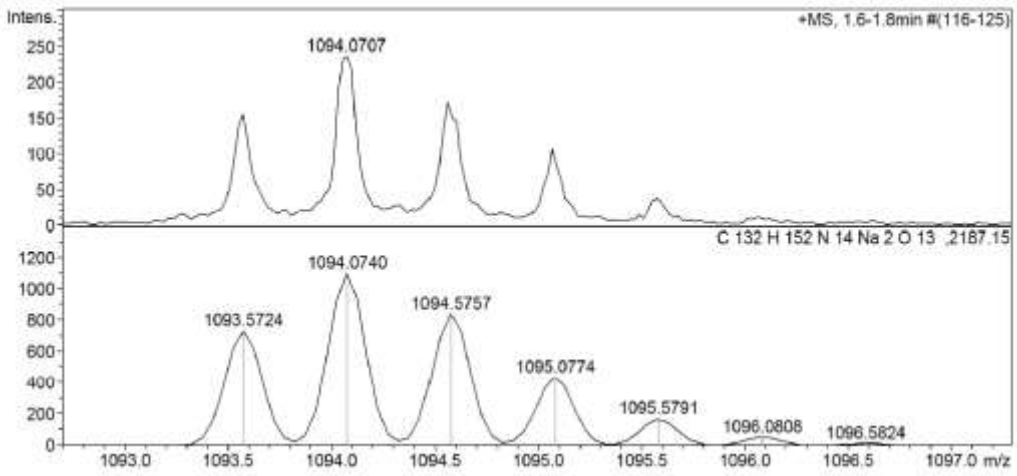


Figure S5.9 – High resolution ESI-MS spectrum of [2]rotaxane **16**.

S5.4 [2]Rotaxane 17

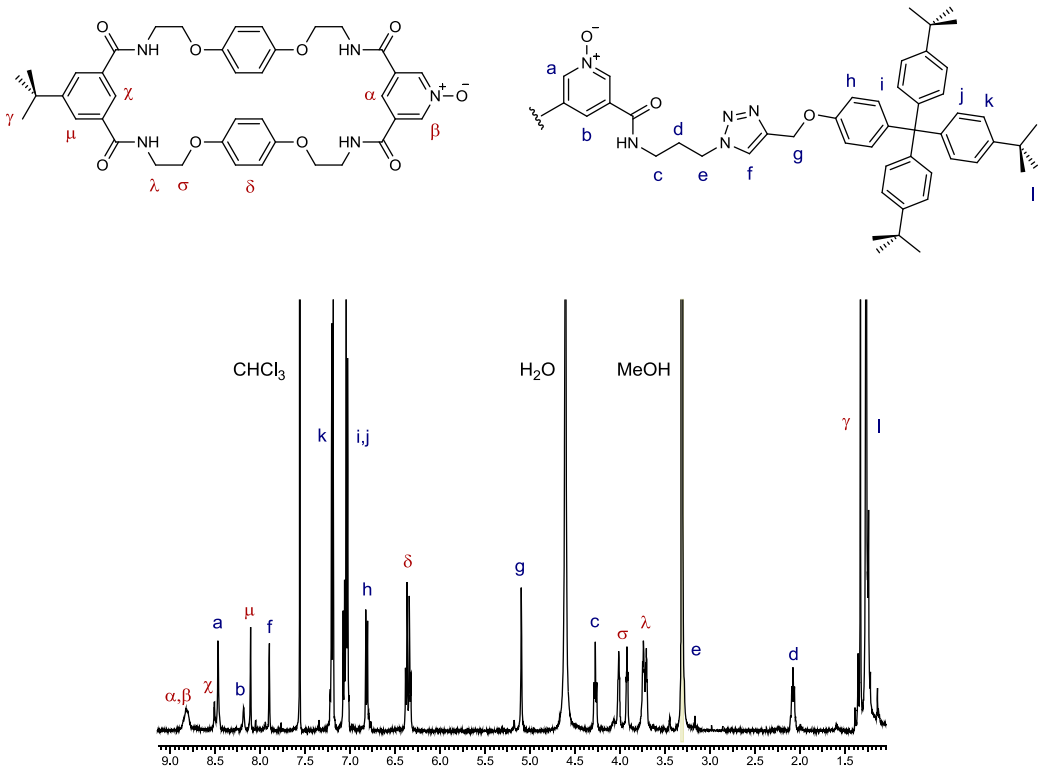


Figure S5.10 - ^{1}H NMR spectrum of [2]rotaxane **17** (1:1 $CDCl_3/CD_3OD$, 500 MHz, 298 K).

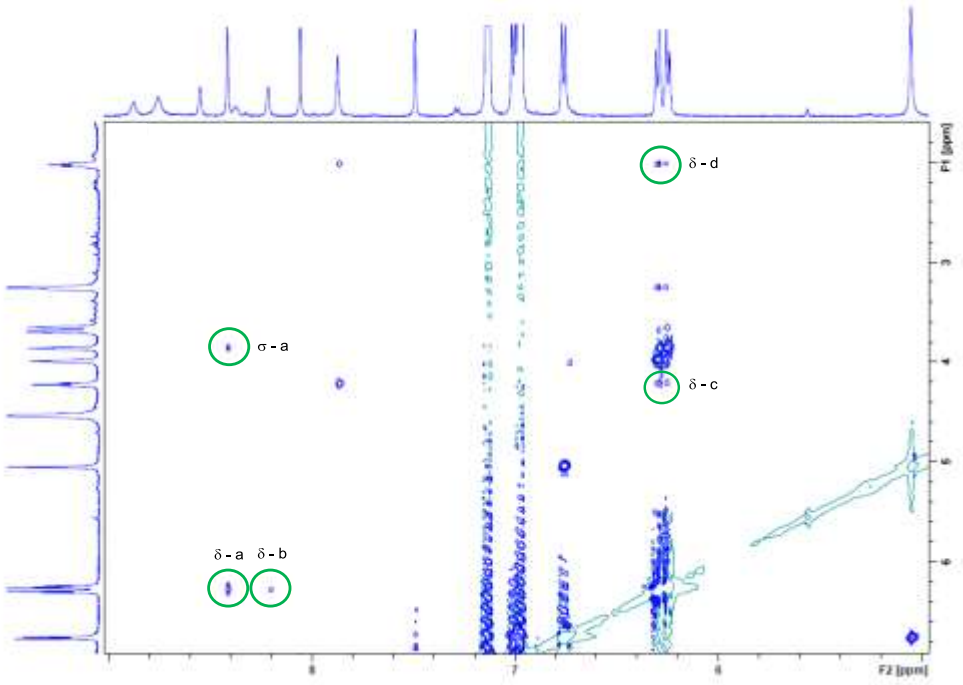


Figure S5.11 - 2D ROESY NMR spectrum of [2]rotaxane **17** (1:1 $CDCl_3/CD_3OD$, 500 MHz, 298 K).

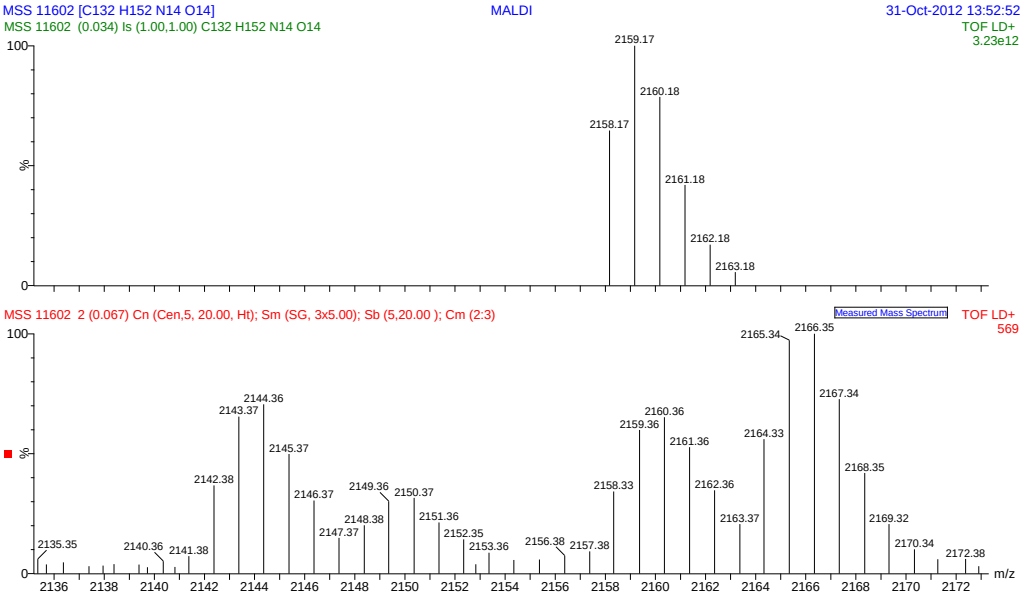


Figure S5.12 – MALDI-MS spectrum of [2]rotaxane **17**.

S6 Low temperature VT ^{1}H NMR studies of [2]rotaxane 15

S6.1 [2]Rotaxane 15

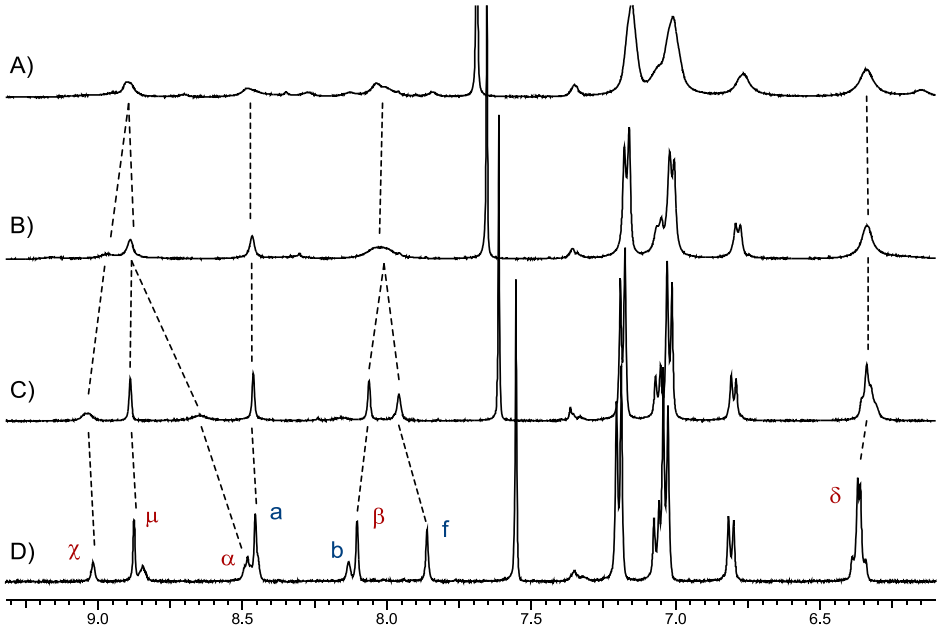


Figure S6.1 – ^{1}H NMR spectra of [2]rotaxane **15** at A) 198 K, B) 223 K, C) 248 K and D) 298 K (500 MHz, 1:1 $CDCl_3/CD_3OD$, proton assignments taken from Section **S4.2**).

S6.2 1:1 [2]Rotaxane 15/TBACl

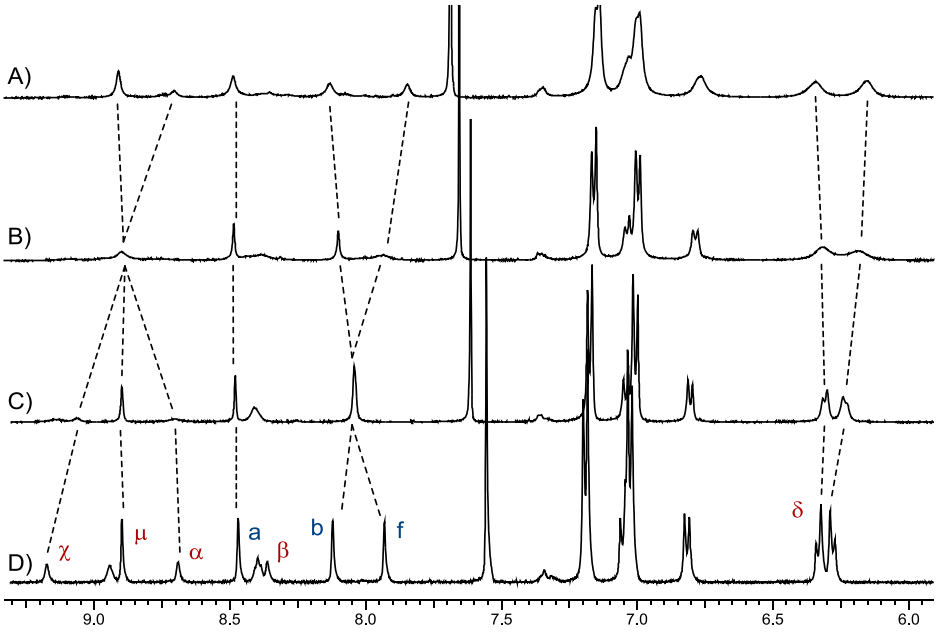


Figure S6.2 - ^{1}H NMR spectra of [2]rotaxane **15** plus 1 equivalent of TBACl at A) 198 K, B) 223 K, C) 248 K and D) 298 K (500 MHz, 1:1 $CDCl_3/CD_3OD$, proton assignments taken from Section **S4.2**).

S7 Titration data for [2]rotaxanes 14-17 (1:1 $CDCl_3/CD_3OD$)

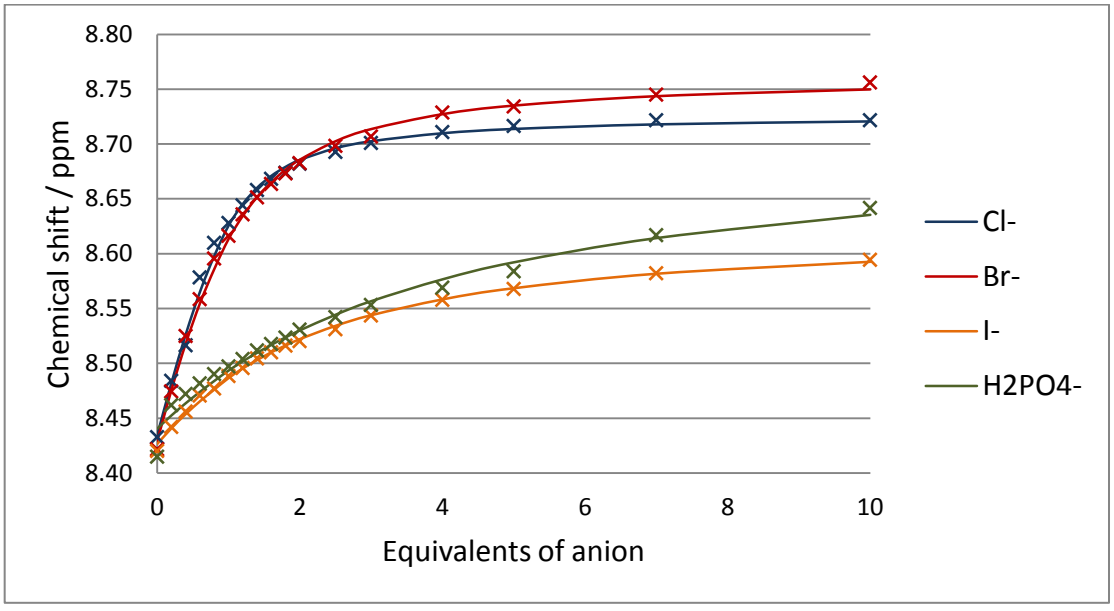


Figure S7.1 – Changes in chemical shift of proton α upon addition of increasing amounts of different anions to rotaxane **14** (1:1 $CDCl_3/CD_3OD$, 298 K, crosses represent data points, continuous lines represent the calculated curves).

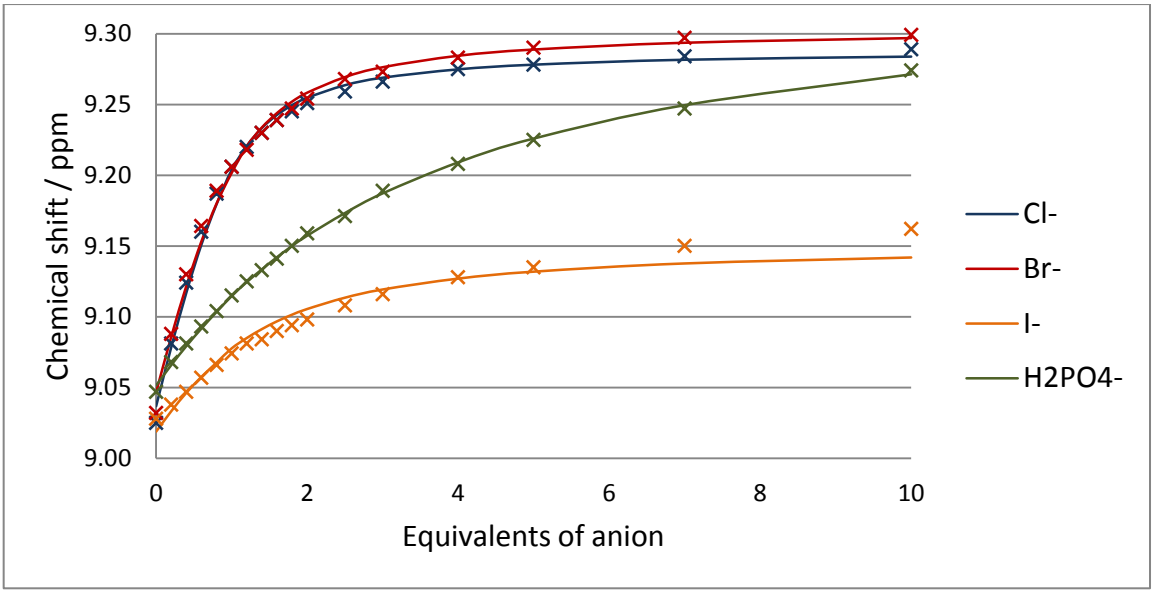


Figure S7.2 - Changes in chemical shift of proton α upon addition of increasing amounts of different anions to rotaxane **15** (1:1 $CDCl_3/CD_3OD$, 298 K, crosses represent data points, continuous lines represent the calculated curves).

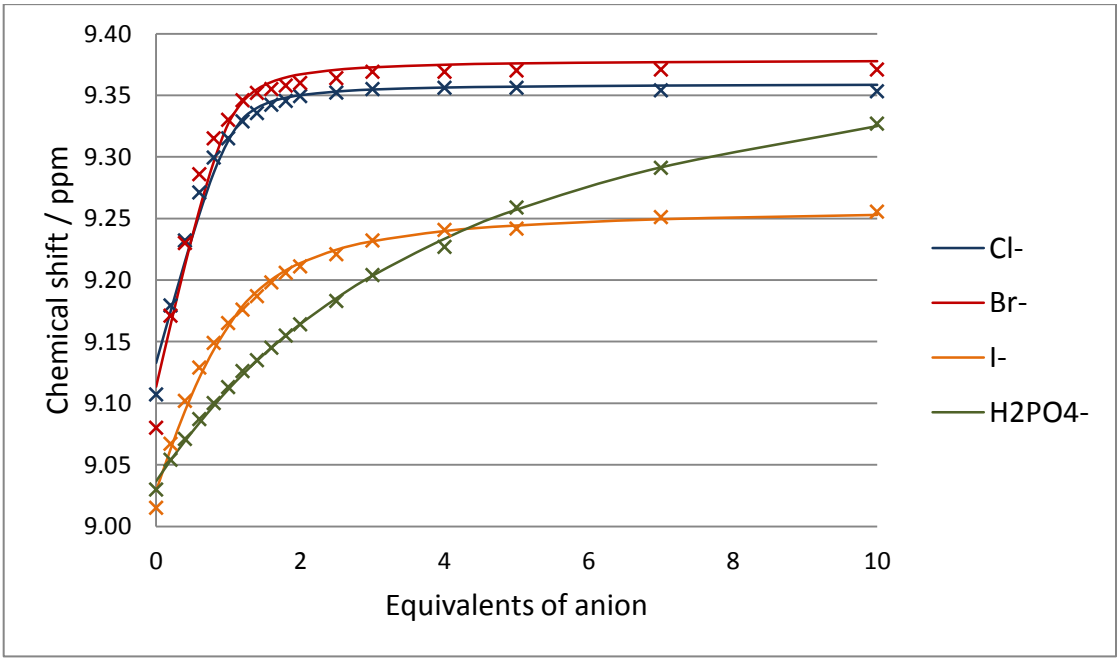


Figure S7.3 - Changes in chemical shift of proton α upon addition of increasing amounts of different anions to rotaxane **16** (1:1 $CDCl_3/CD_3OD$, 298 K, crosses represent data points, continuous lines represent the calculated curves).

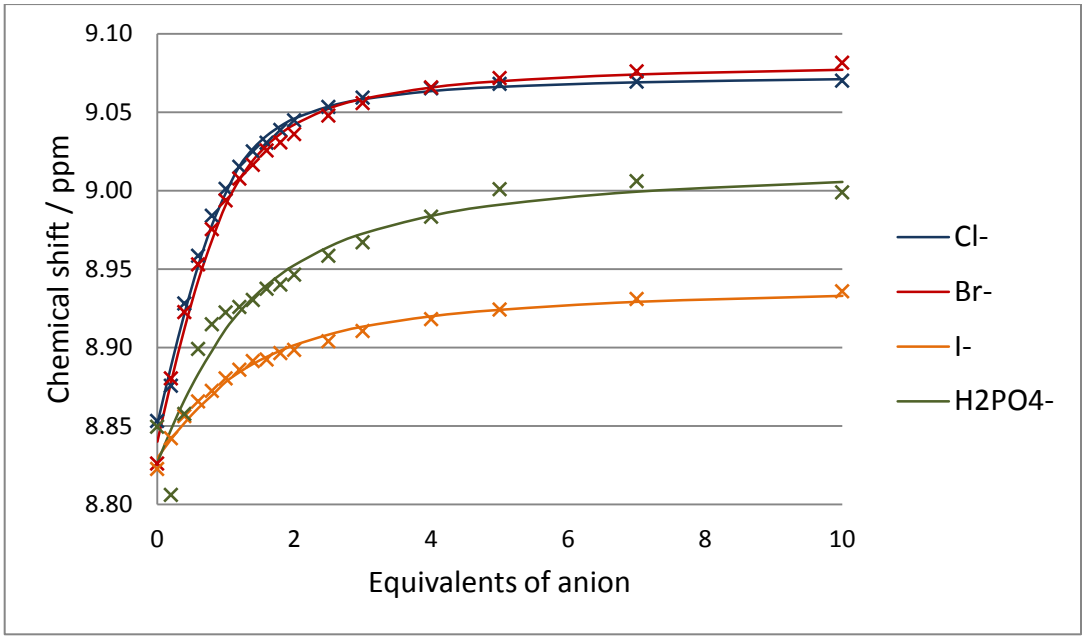


Figure S7.4 - Changes in chemical shift of proton α upon addition of increasing amounts of different anions to rotaxane **17** (1:1 $CDCl_3/CD_3OD$, 298 K, crosses represent data points, continuous lines represent the calculated curves).

S8 Titration data for [2]rotaxanes 14-17 (45:45:10 $CDCl_3/CD_3OD/D_2O$)

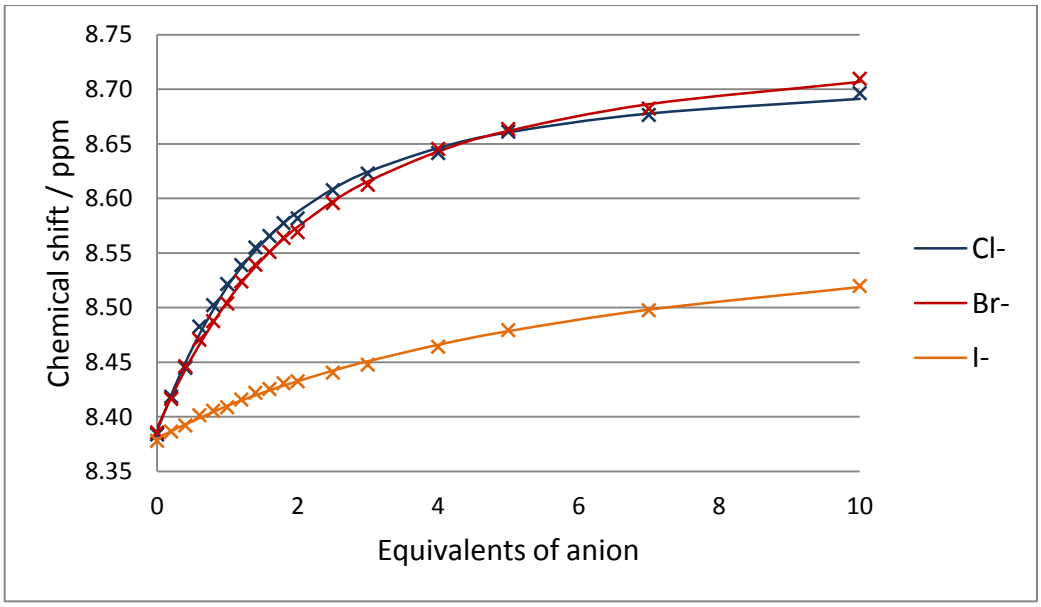


Figure S8.1 - Changes in chemical shift of proton α upon addition of increasing amounts of different anions to rotaxane **14** (45:45:10 $CDCl_3/CD_3OD/D_2O$, 298 K, crosses represent data points, continuous lines represent the calculated curves).

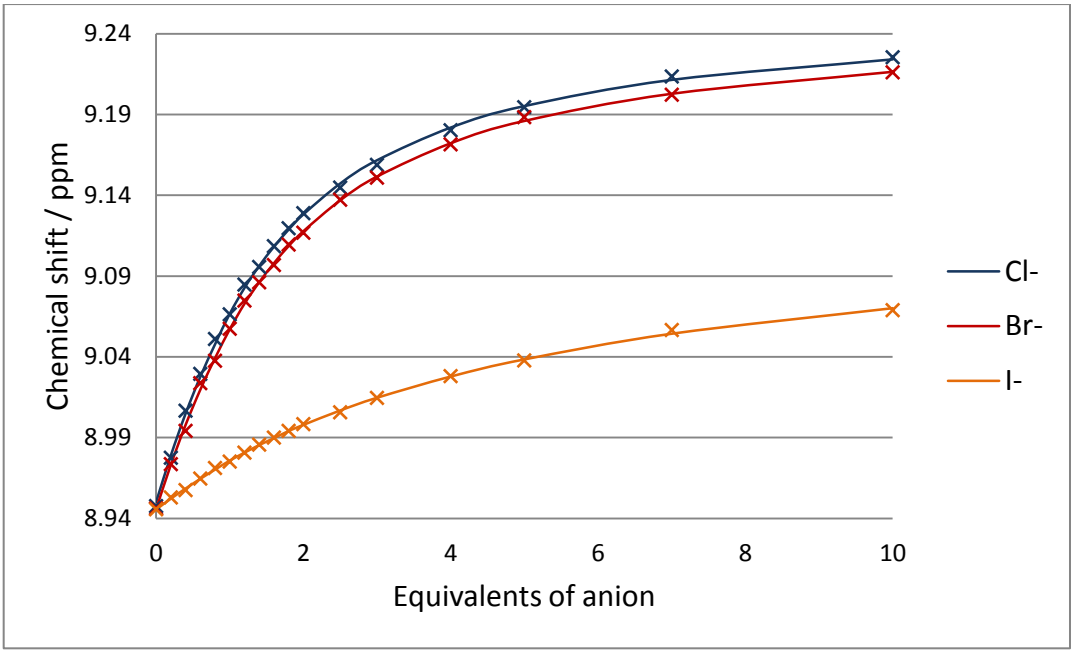


Figure S8.2 - Changes in chemical shift of proton α upon addition of increasing amounts of different anions to rotaxane **15** (45:45:10 $CDCl_3/CD_3OD/D_2O$, 298 K, crosses represent data points, continuous lines represent the calculated curves).

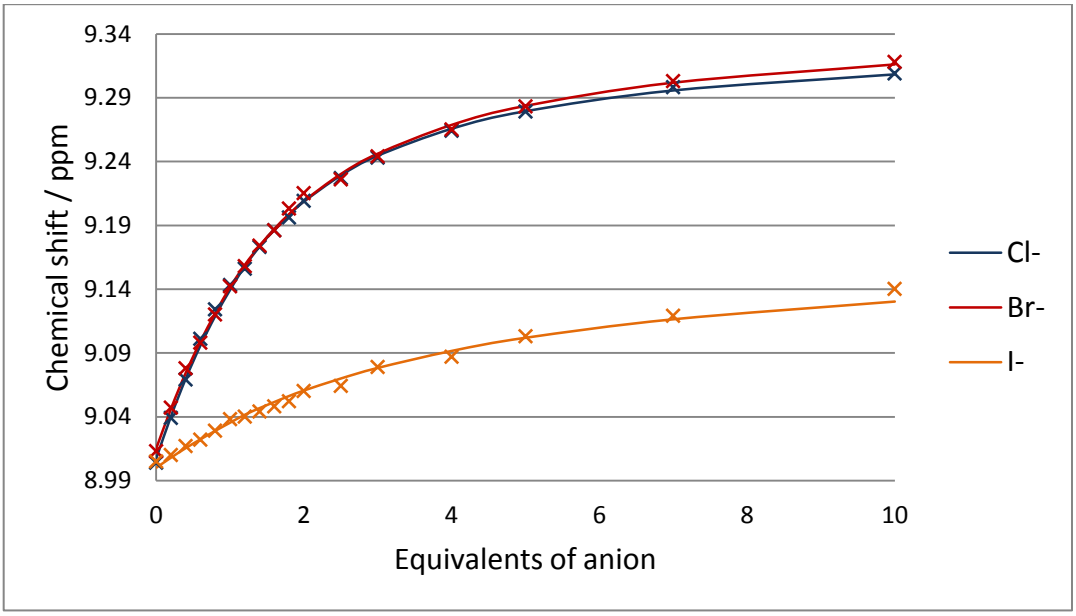


Figure S8.3 - Changes in chemical shift of proton α upon addition of increasing amounts of different anions to rotaxane **16** (45:45:10 $CDCl_3/CD_3OD/D_2O$, 298 K, crosses represent data points, continuous lines represent the calculated curves).

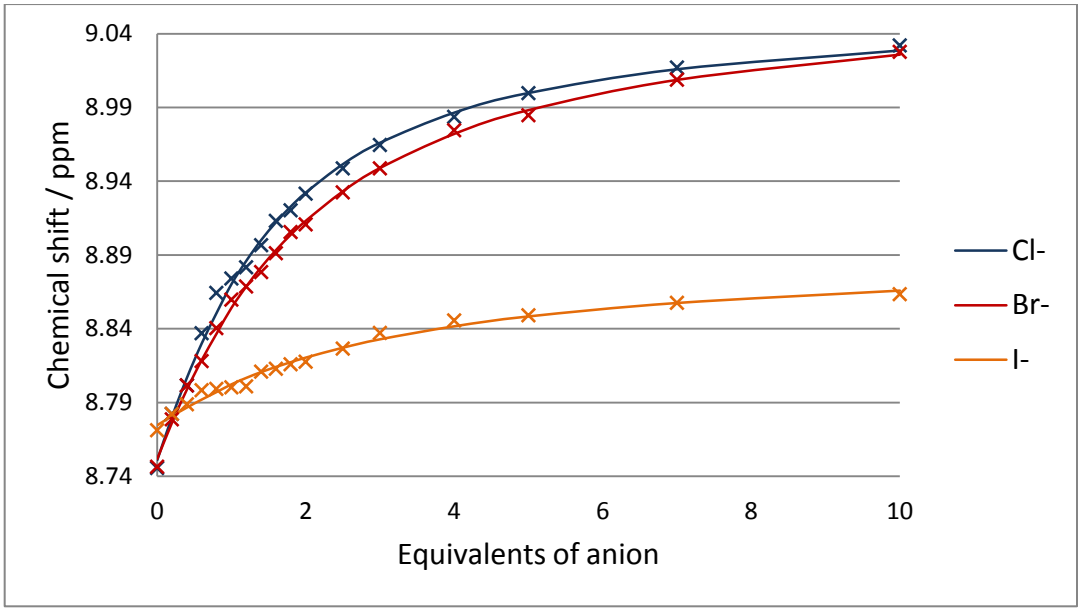


Figure S8.4 - Changes in chemical shift of proton α upon addition of increasing amounts of different anions to rotaxane **17** (45:45:10 $CDCl_3/CD_3OD/D_2O$, 298 K, crosses represent data points, continuous lines represent the calculated curves).

S9 References

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