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Electronic Supplementary Information

An amide hydrogen bond templated [1]rotaxane displaying a peptide motif – demonstrating an expedient route to synthetic mimics of lasso peptides

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NB: Experimental Procedures for:
[2]Rotaxane **4**, Boc-Peptide [2]Rotaxane **5** & [1]Rotaxane **6**
are to be found in the main article.

Experimental Procedures & Spectral Characterization for Novel Compounds

General information

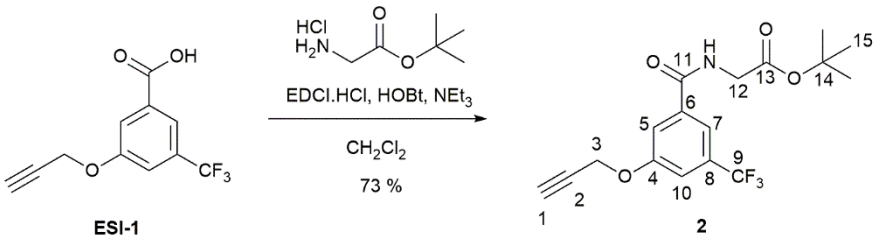
Commercially available solvents and chemicals were used without further purification unless stated. Dry solvents, NEt_3 and DIPEA were purchased dry and stored under an inert atmosphere. $Cu(CH_3CN)_4BF_4$ was stored in desiccator over P_4O_{10}. Deionised water was used in all cases.

Azide **1**,[1] 3-(prop-2-yn-1-yloxyl)-5-(trifluoromethyl)benzoic acid **ESI-1**,[2] bis-amine **ESI-2**[3] and template **ESI-3**[4] were prepared based upon previously reported procedures. 5-Nitro-isophthaloyl chloride was prepared by refluxing 5-nitro-isophthalic acid in $SOCl_2$ overnight (in the presence of a catalytic drop of dry DMF), then removing excess $SOCl_2$ *in vacuo* and using resulting solid assuming 100% conversion.

Silica gel with a 60Å particle size was used as the stationary phase for column chromatography. Analytical TLC was used to monitor the progress of column chromatography and analytical TLC plates were examined under short wavelength (λ = 254 nm) UV light. Preparatory TLC was carried out on silica gel possessing a fluorescent indicator to allow for examination with short wavelength UV light.

IR spectra were recorded on an Agilent Technologies Cary 630 FTIR spectrometer. NMR spectra were recorded on a Bruker AVANCE III 400 spectrometer at 298 K (unless otherwise stated). Mass spectra (ES and APC) were recorded on a Shimadzu LCMS IT ToF instrument. Melting points were recorded on a Gallenkamp capillary melting point apparatus and are uncorrected.

Alkyne Stopper **2**



*Scheme ESI-1: Preparation of alkyne stopper **2***

Compound **ESI-1** (60 mg, 0.25 mmol) was dissolved in dry CH_2Cl_2 (5 mL), and cooled to 0 °C under an Ar (g) atmosphere. Then dry NEt_3 (75 mg, 103 μL, 0.74 mmol), glycine *tert*-butyl ester hydrochloride (41 mg, 0.25 mmol), HOBt.hydrate (49 mg, 0.32 mmol) and EDCI.HCl (61 mg, 0.32 mmol) were added, maintaining the temperature at 0 °C. The reaction was stirred at r.t. for 16 h, then diluted with CH_2Cl_2 (15 mL), and then washed with H_2O (1 × 20 mL), 1M HCl (aq) (2 × 20 mL), saturated $NaHCO_3$ (aq) (2 × 20 mL) and brine (1 × 20 mL). The organic layer was dried ($MgSO_4$), and the solvent removed *in vacuo* to yield the product as a yellow oil that solidified to a white film on standing (64 mg, 73%).

ν_{max}/cm^{-1} (neat): 3370 (N–H), 3240 (C–H), 3090 (C–H), 2990 (C–H), 2940 (C–H), 2130 (alkyne C-C), 1710 (C=O ester), 1660 (C=O amide), 1600, 1550, 1450, 1370, 1350, 1290, 1270, 1230, 1160, 1130, 1110, 1040.

δ_H (400 MHz; $CDCl_3$): 7.63-7.65 (2H, m, C^7H & C^5H), 7.37 (1H, br s, $C^{10}H$), 6.69 (1H, br s, $C^{11}NH$), 4.79 (2H, d, ^4J = 2.4 Hz, C^3H), 4.15 (2H, d, ^4J = 4.9 Hz, $C^{12}H$), 2.58 (1H, t, ^4J = 2.4 Hz, C^1H), 1.52 (9H, s, $C^{15}H$).

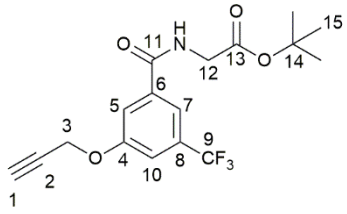
δ_C (100 MHz; $CDCl_3$): 168.9 (C^{13}), 165.5 (C^{11}), 157.9 (C^4), 136.3 (C^6), 132.4 (q, ^2J = 33 Hz, C^8), 123.4 (q, ^1J = 271 Hz, C^9), 116.9 (C^5), 116.5 (q, ^3J = 3.8 Hz, C^7), 115.5 (q, ^3J = 3.9 Hz, C^{10}), 82.9 (C^{14}), 77.0 (C^2), 76.8 (C^1), 56.3 (C^3), 42.6 (C^{12}), 28.1 (C^{15}).

δ_F (377 MHz; $CDCl_3$): –62.8.

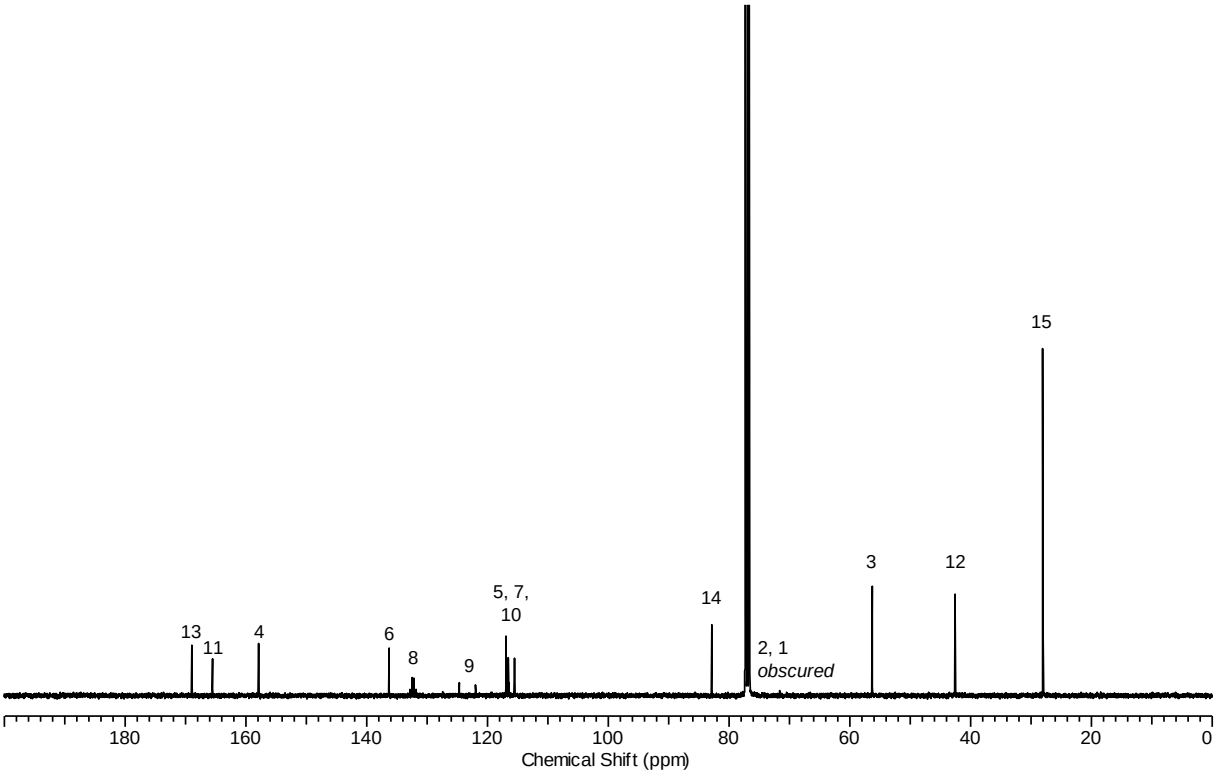
***m/z* (APC):** 356.1107 ($[M - H]^-$ $C_{17}F_6H_{17}NO_4$ requires 356.1115).

Alkyne Stopper **2**

^{1}H NMR ($CDCl_3$, 400 MHz)
Inset: ^{19}F NMR ($CDCl_3$, 377 MHz)

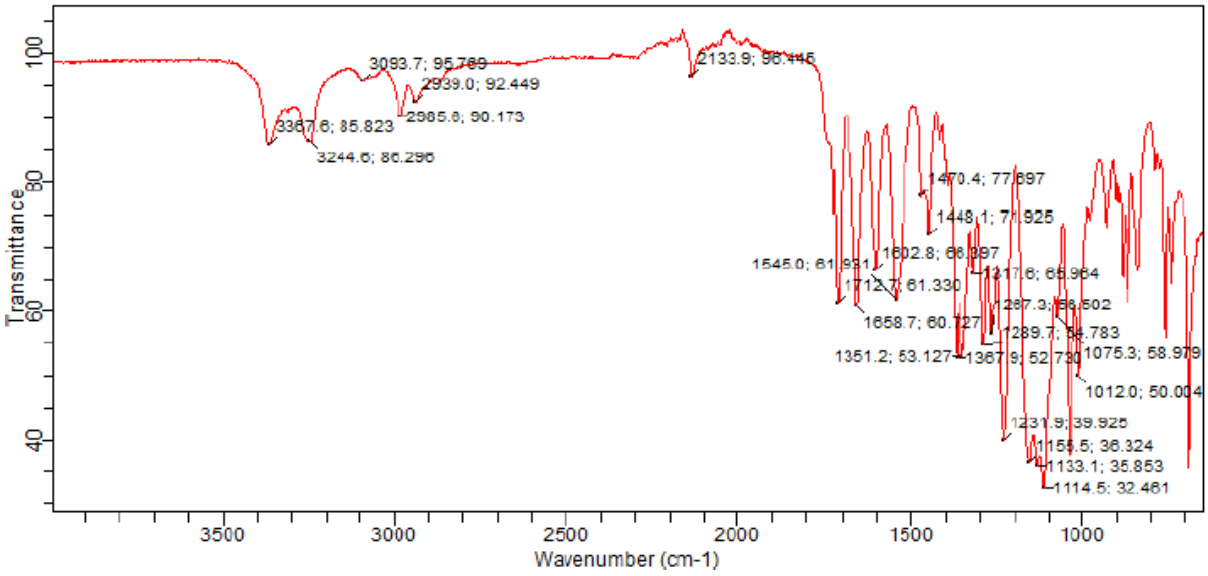


^{13}C NMR ($CDCl_3$, 100 MHz)

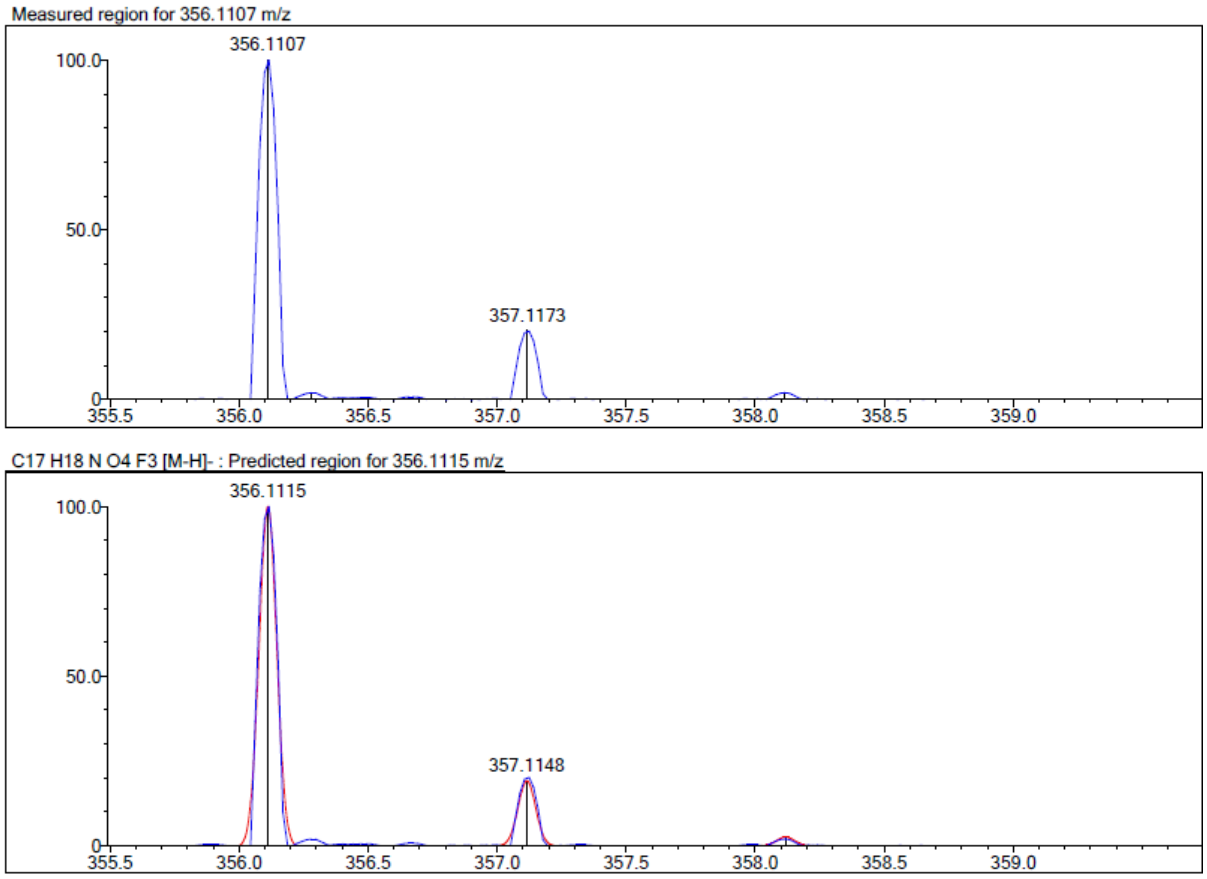


Alkyne Stopper **2**

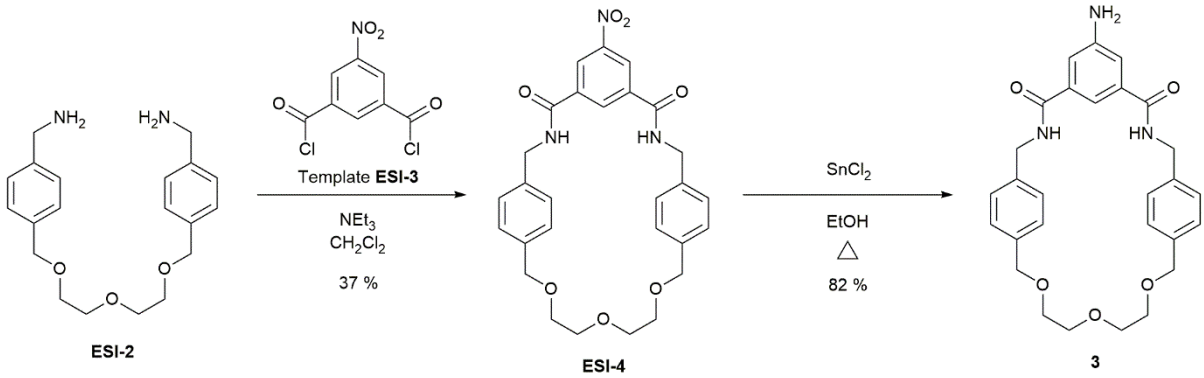
IR Spectrum (neat)



Mass Spectrum (APC –ve)

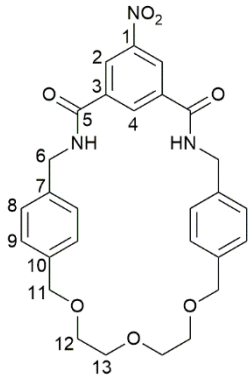


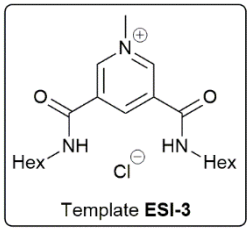
Nitro Macrocycle **ESI-4** & Amino Macrocycle **3**



*Scheme ESI-2: Preparation of amino macrocycle **3***

Nitro Macrocycle **ESI-4**





A solution of 5-nitro-isophthaloyl chloride (863 mg, 3.48 mmol) in dry CH_2Cl_2 (25 mL) was added dropwise to a solution of bis-amine **ESI-2** (1.20 g, 3.48 mmol), methyl pyridinium template **ESI-3** (1.34 g, 3.48 mmol) dry NEt_3 (881 mg, 1.21 mL, 8.71 mmol) in dry CH_2Cl_2 (50 mL). This reaction mixture was stirred for 1 h under an Ar (g) atmosphere at r.t., and then was washed with 1M HCl (aq) (2 × 50 mL) and brine (1 × 50 mL). The organic layer was dried ($MgSO_4$) and the solvent removed *in vacuo* to leave a foaming yellow film. The crude material was submitted to silica gel chromatography (EtOAc/CH_2Cl_2 9:1), yielding pure product as a white solid (677 mg, 37%). *NB: Further contaminated product material was also isolated.*

***R*f** 0.58 (EtOAc/CH_2Cl_2 9:1).

Mp 266-268 °C.

ν_{max}/cm^{-1} (neat): 3400 (N–H), 3280 (C–H), 3060 (C–H), 2870 (C–H), 1680 (C=O), 1640 (C=O), 1540, 1520 (NO_2 asymmetric), 1450, 1350 (NO_2 symmetric), 1310, 1270, 1110, 1080.

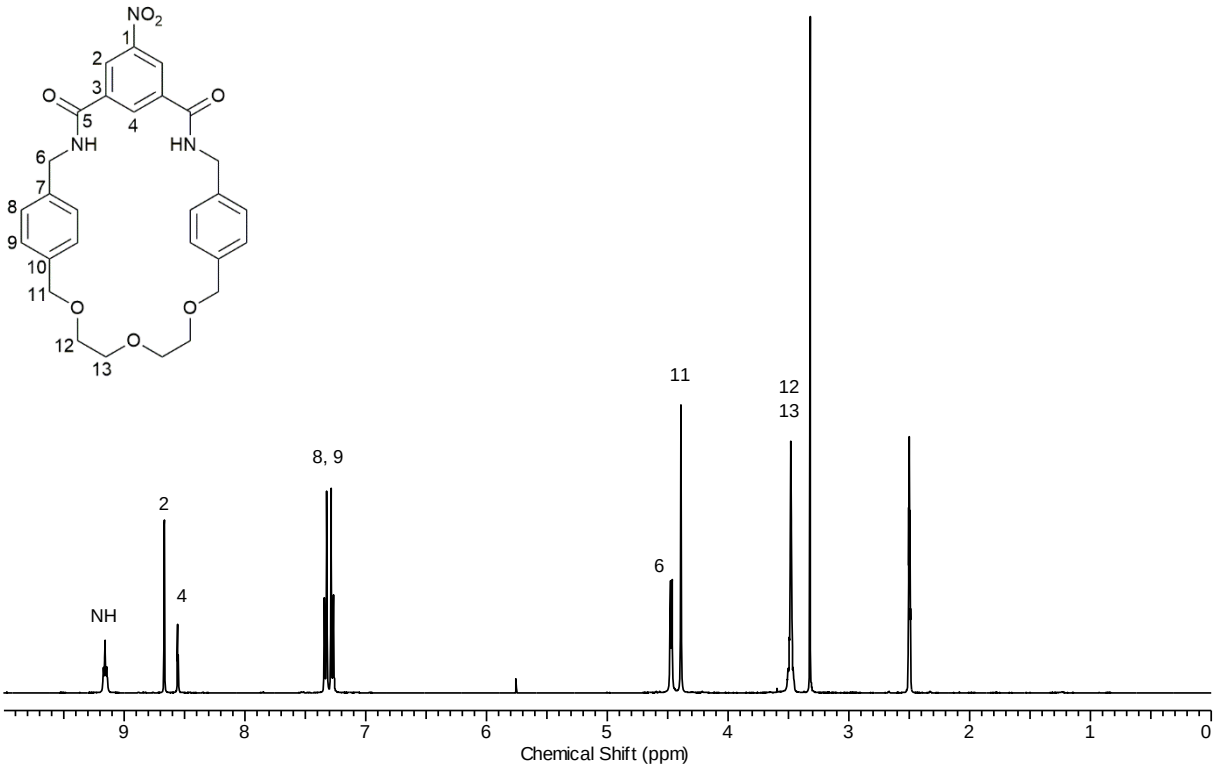
δ_H (400 MHz; D$_6$-DMSO): 9.16 (2H, t, ^3J = 5.4 Hz, C^{5}NH), 8.67 (2H, d, ^4J = 1.5 Hz, C^{2}H), 8.56 (1H, t, ^4J = 1.5 Hz, C^{4}H), 7.32-7.34 (2H, m, C^{8}H), 7.27-7.29 (2H, m, C^{9}H), 4.47 (4H, d, ^3J = 5.4 Hz, C^{6}H), 4.39 (4H, s, C^{11}H), 3.46-3.51 (8H, m, C^{12}H & C^{13}H).

δ_C (100 MHz; D$_6$-DMSO): 164.1 (C^5), 147.9 (C^1), 138.0 (C^7), 137.1 (C^{10}), 136.4 (C^3), 131.5 (C^4), 128.5 (C^9), 128.2 (C^8), 124.4 (C^2), 72.1 (C^{11}), 69.8 (C^{12} or C^{13}), 68.8 (C^{12} or C^{13}), 43.2 (C^6).

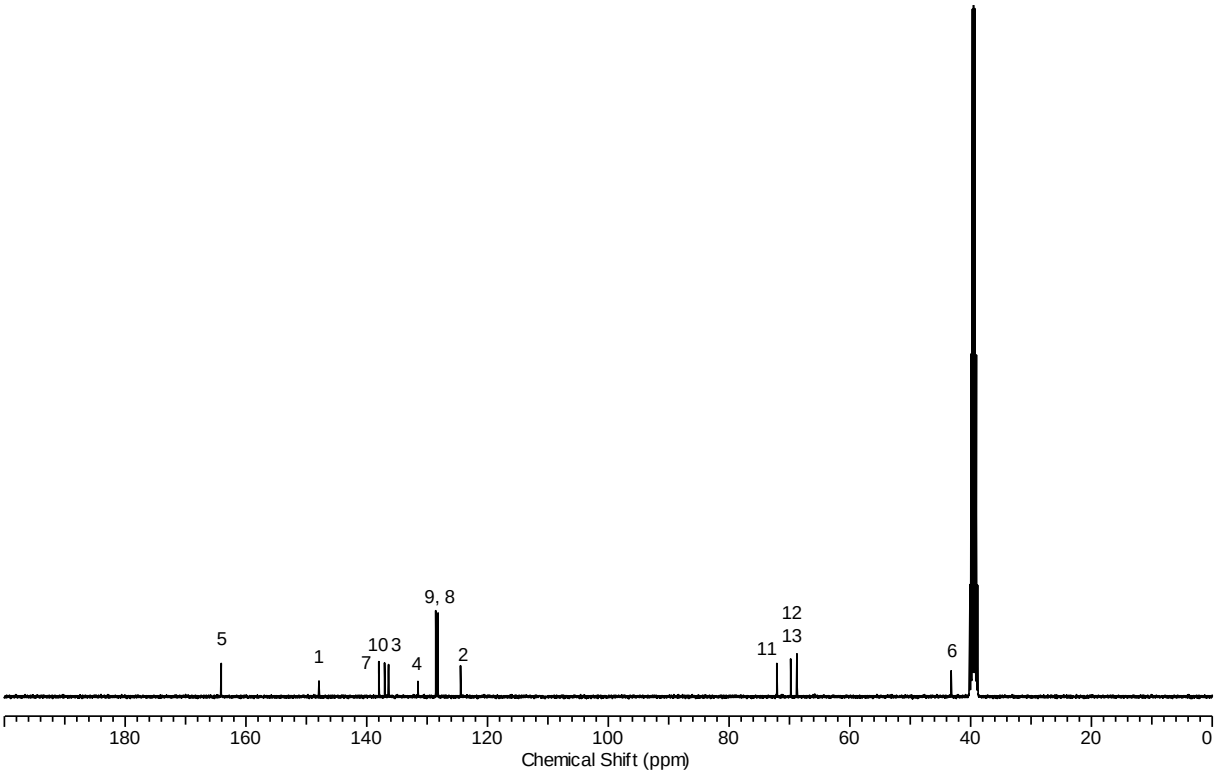
***m/z* (ES):** 542.1873 ([M + Na]$^+$ $C_{28}H_{29}N_3NaO_7$ requires 542.1898).

Nitro Macrocycle **ESI-4**

^{1}H NMR (D_6-DMSO, 400 MHz)

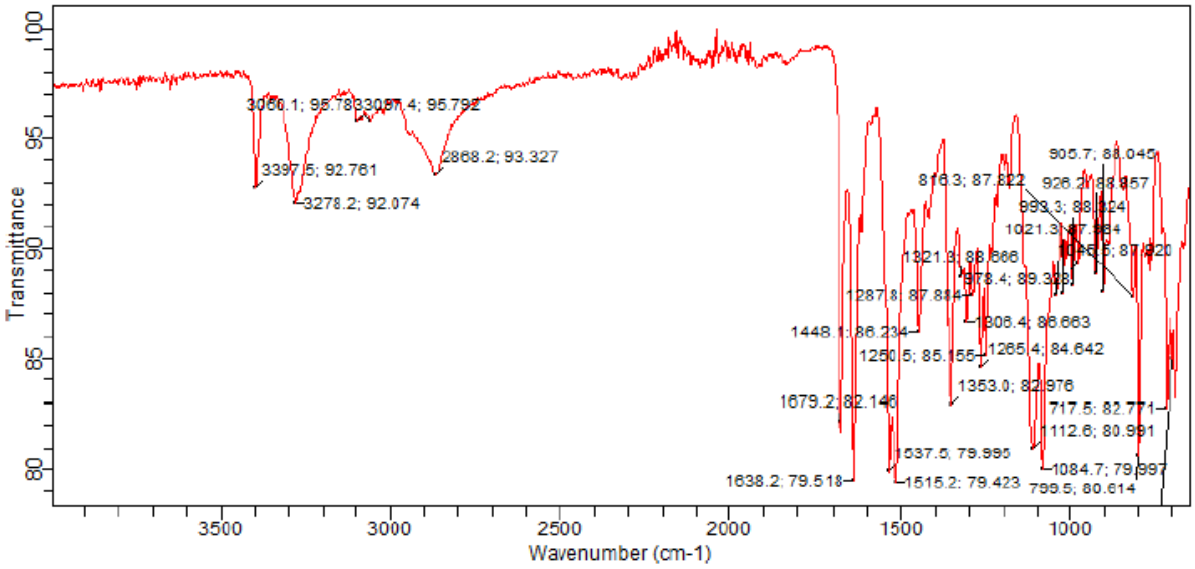


^{13}C NMR (D_6-DMSO, 100 MHz)

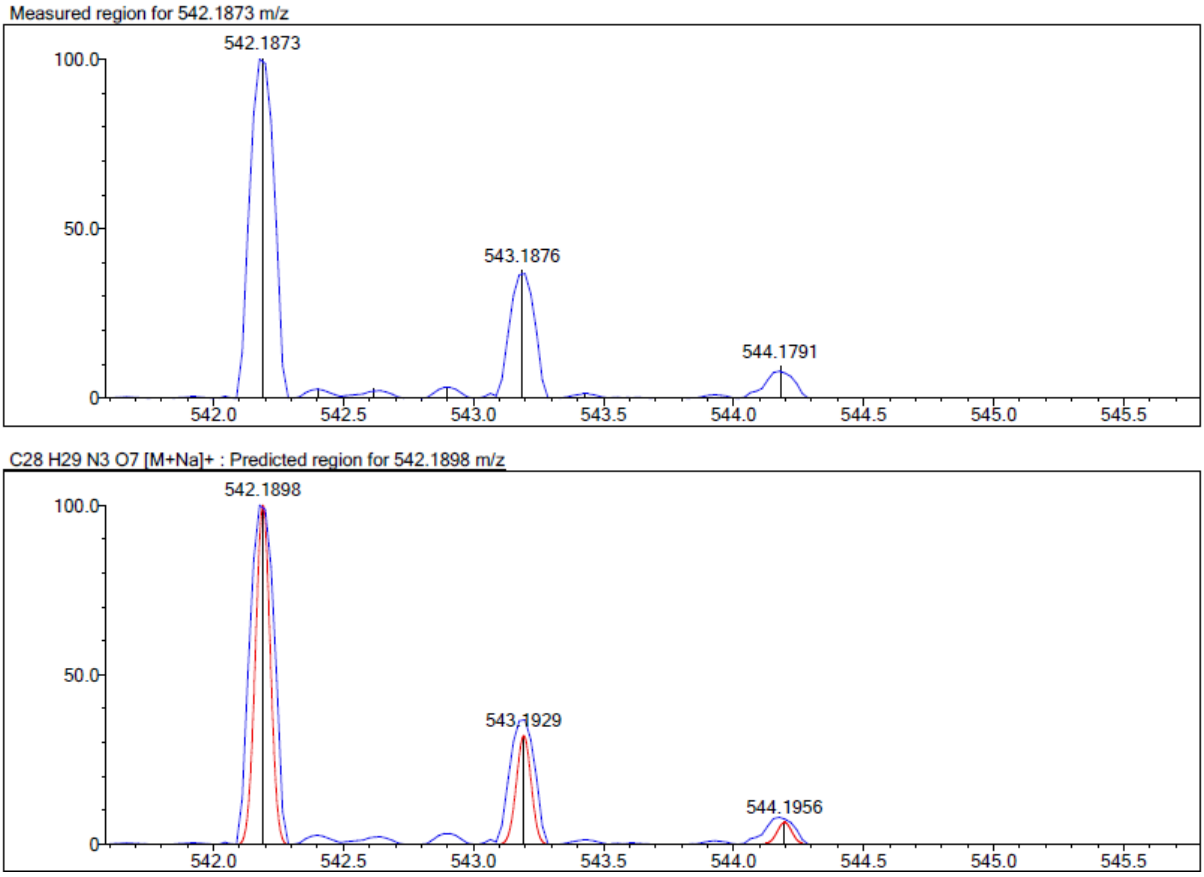


Nitro Macrocycle **ESI-4**

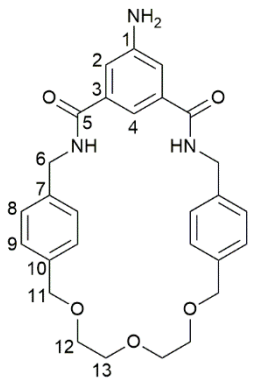
IR Spectrum (neat)



Mass Spectrum (ES +ve)



Amino Macrocycle **3**



$SnCl_2$ (690 mg, 3.64 mmol) was added to a suspension of nitro macrocycle **ESI-4** (189 mg, 0.364 mmol) in EtOH (25 mL). The reaction was heated to 60 °C for 24 h under an Ar (g) atmosphere. After cooling to r.t. the reaction mixture was basified using 10% NaOH (aq) and then this aqueous layer was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layers were dried ($MgSO_4$) and the solvent removed *in vacuo*. The resulting residue was titurated with CH_2Cl_2, the insoluble solid removed by gravity filtration and the solvent of the filtrate removed *in vacuo* to yield the product as an off-white solid (147 mg, 82%).

Mp 244 °C (dec).

ν_{max}/cm^{-1} (neat): 3340 (N–H), 2860 (C–H), 1630 (C=O), 1530, 1450, 1330, 1260, 1100.

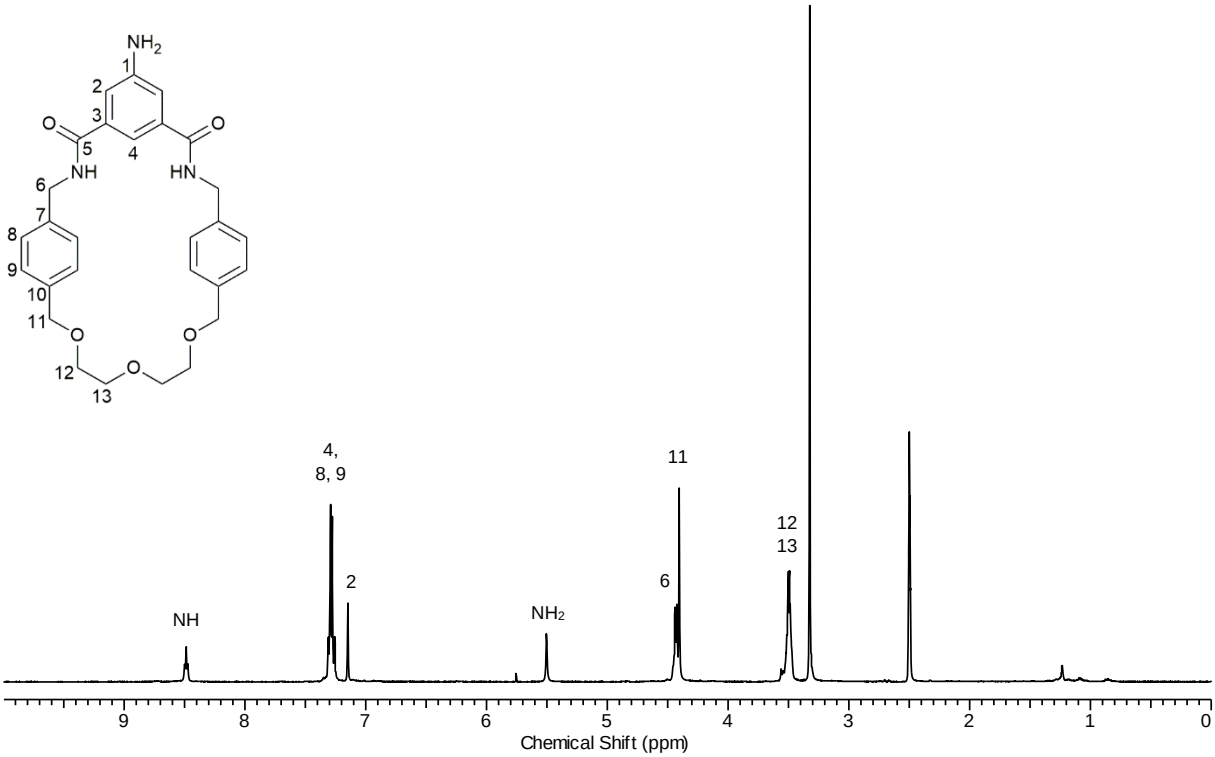
δ_H (400 MHz; D$_6$-DMSO): 8.49 (2H, t, ^3J = 5.6 Hz, C^5NH), 7.26-7.31 (9H, m, C^4, C^8 & C^9), 7.15 (2H, d, ^4J = 0.9 Hz, C^2H), 5.50 (2H, br s, C^1NH_2), 4.43 (4H, d, ^3J = 5.6 Hz, C^6H), 4.41 (4H, s, $C^{11}H$) 3.47-3.52 (8H, m, C^{12} & C^{13}).

δ_C (100 MHz; D$_6$-DMSO): 168.8 (C^5), 149.0 (C^1), 138.6 (C^7), 136.9 (C^{10}), 135.5 (C^3), 128.3 (C^9), 127.8 (C^8), 115.2 (C^2), 112.5 (C^4), 72.0 (C^{11}), 69.8 (C^{12} or C^{13}), 68.8 (C^{12} or C^{13}), 42.6 (C^6).

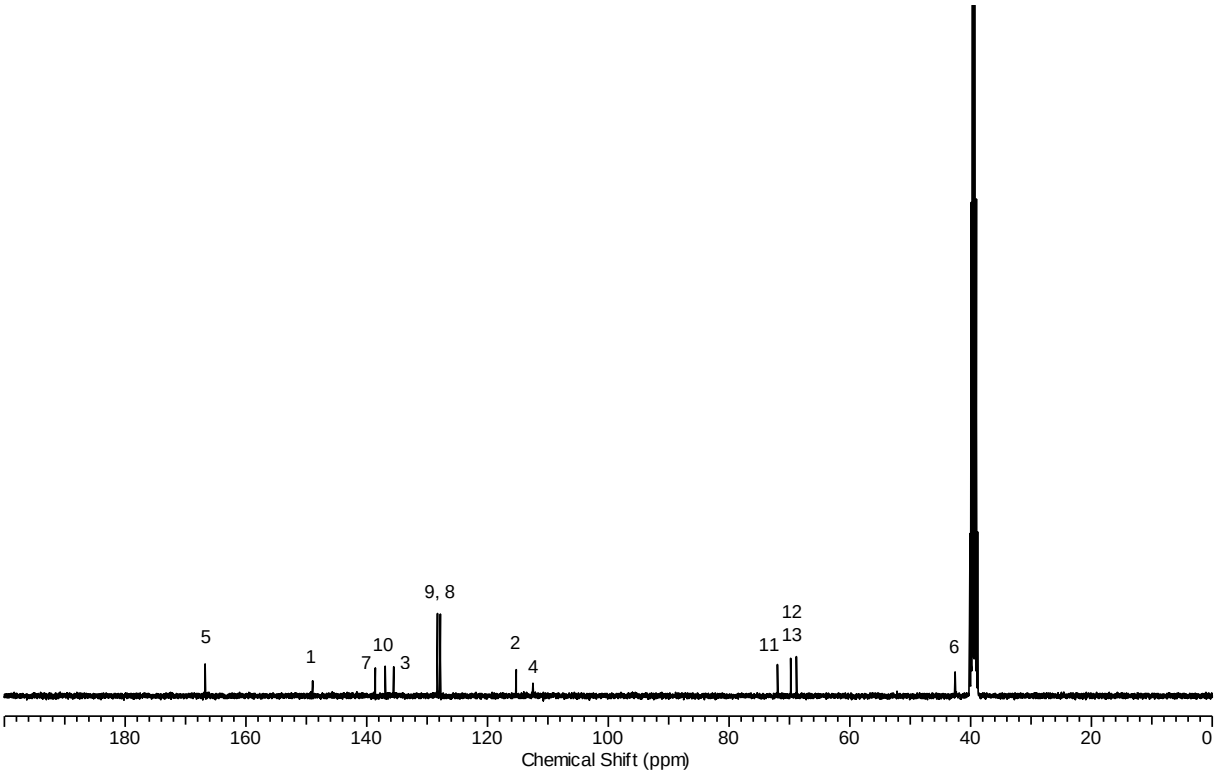
***m/z* (ES):** 490.2327 ($[M + H]^+$ $C_{28}H_{32}N_3O_7$ requires 490.2336).

Amino Macrocycle **3**

^{1}H NMR (D_6-DMSO, 400 MHz)

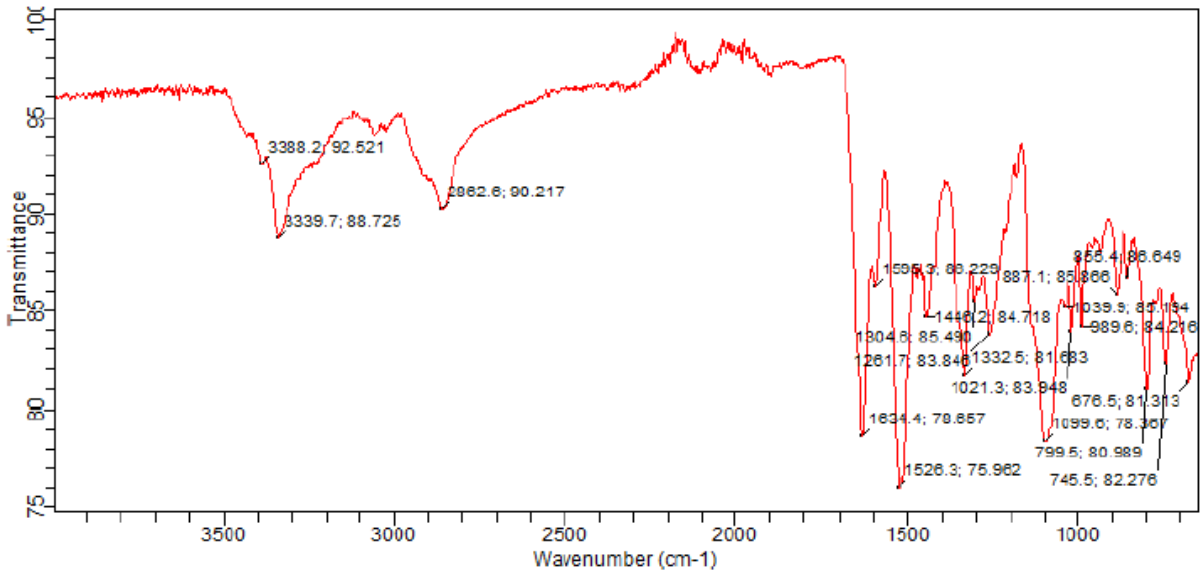


^{13}C NMR (D_6-DMSO, 100 MHz)

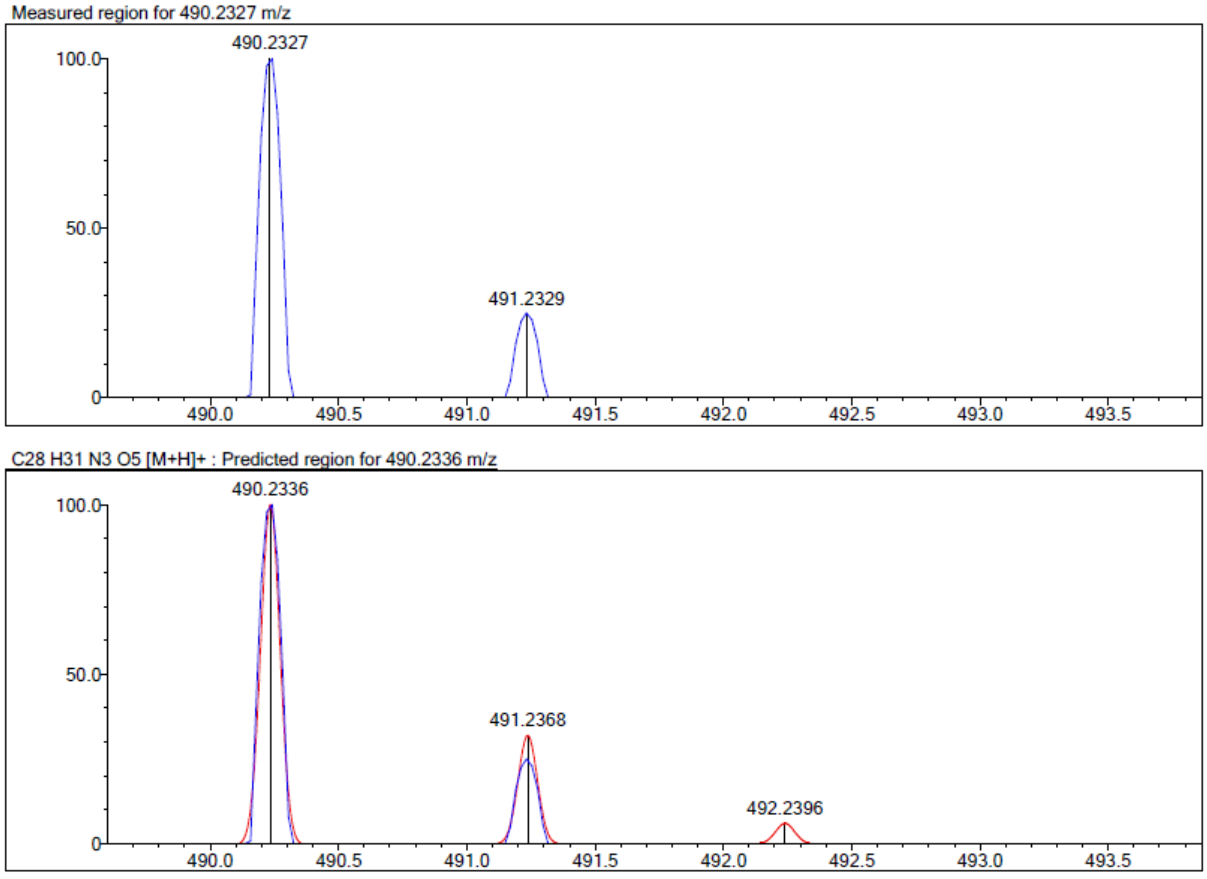


Amino Macrocycle **3**

IR Spectrum (neat)

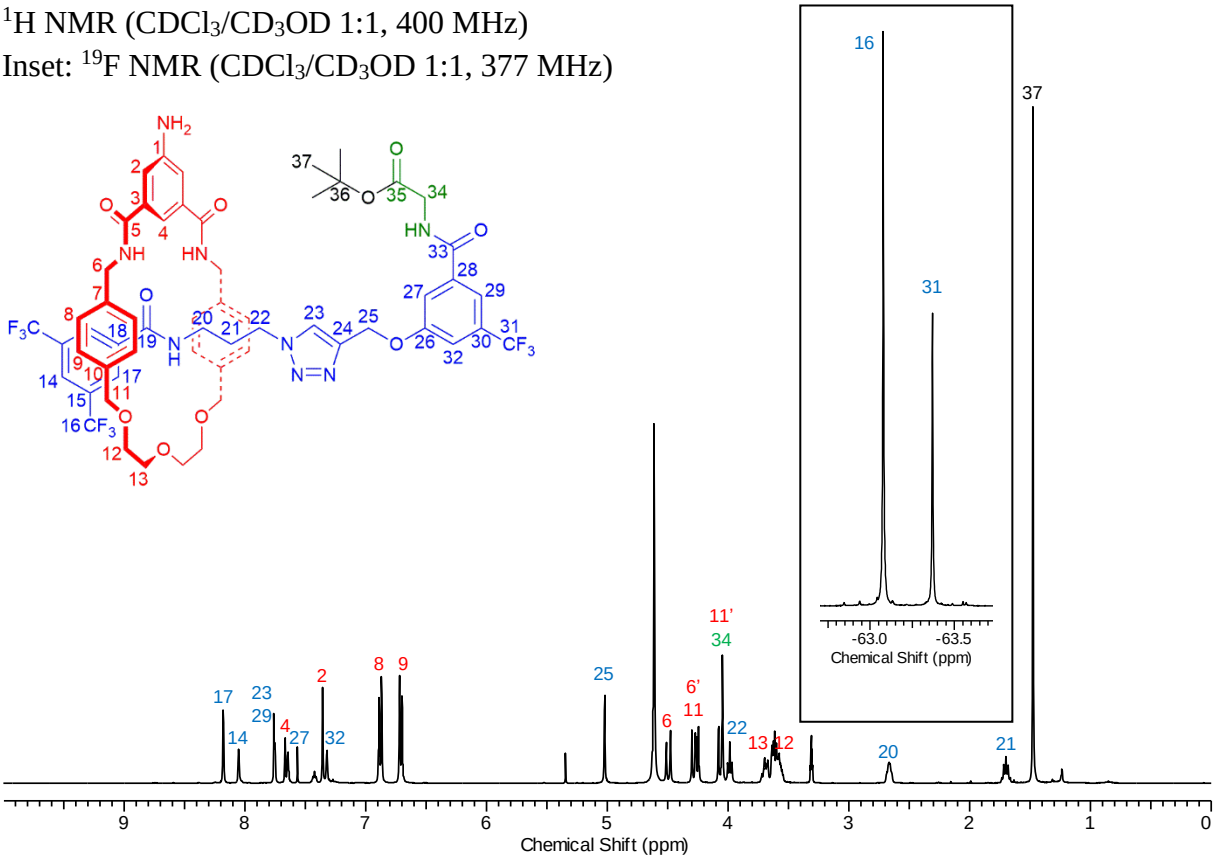


Mass Spectrum (ES +ve)

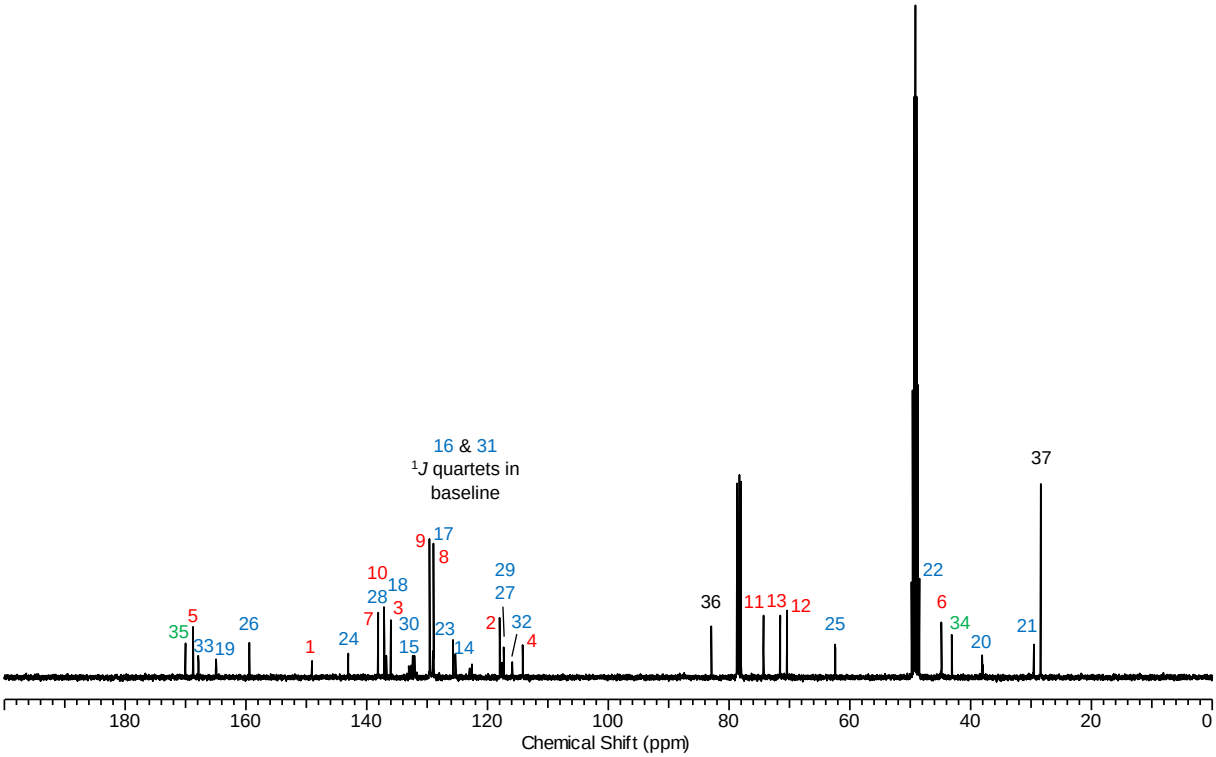


[2]Rotaxane **4**

^{1}H NMR ($CDCl_3$/CD_3OD 1:1, 400 MHz)
Inset: ^{19}F NMR ($CDCl_3$/CD_3OD 1:1, 377 MHz)

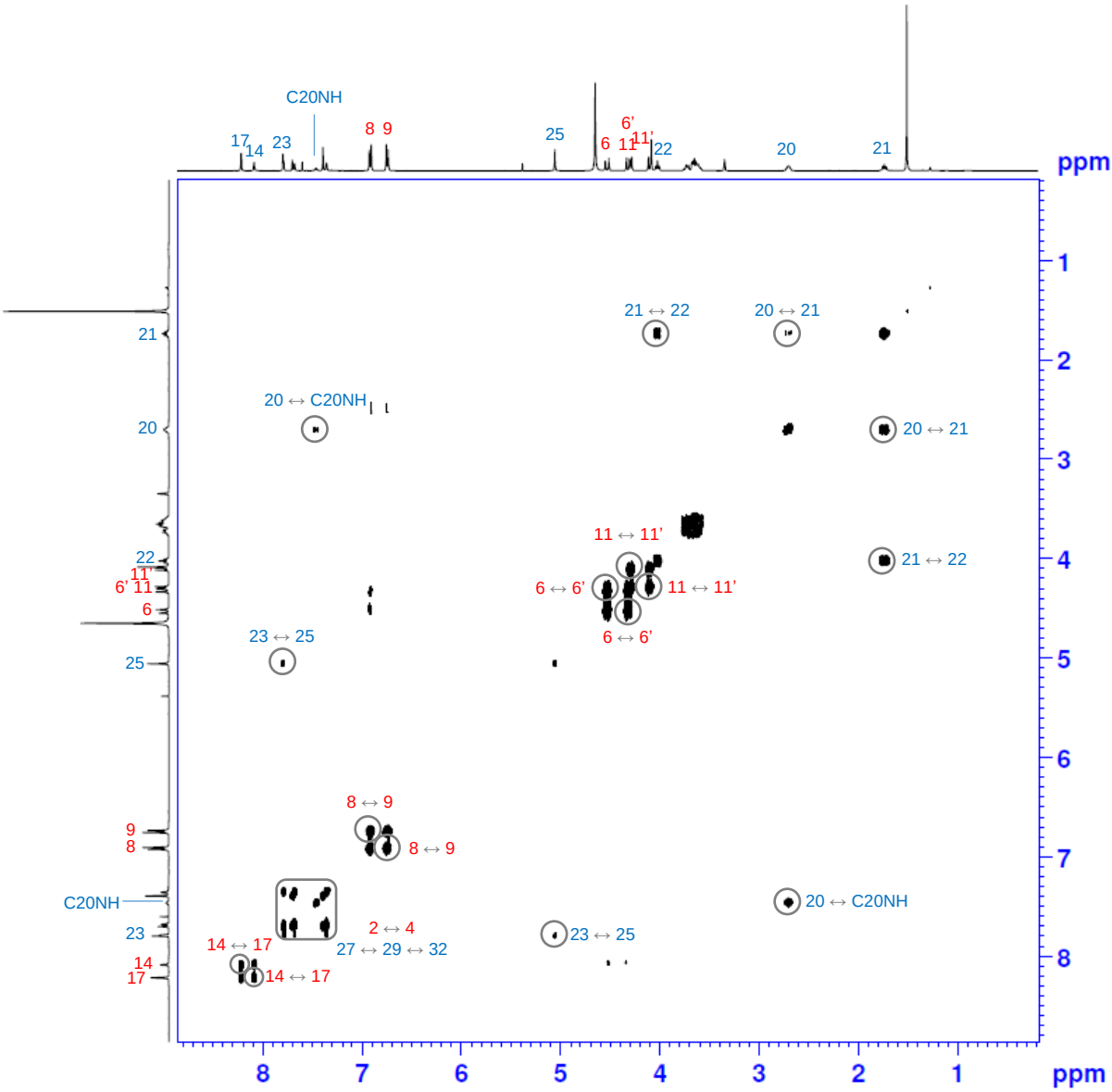


^{13}C NMR ($CDCl_3$/CD_3OD 1:1, 100 MHz)



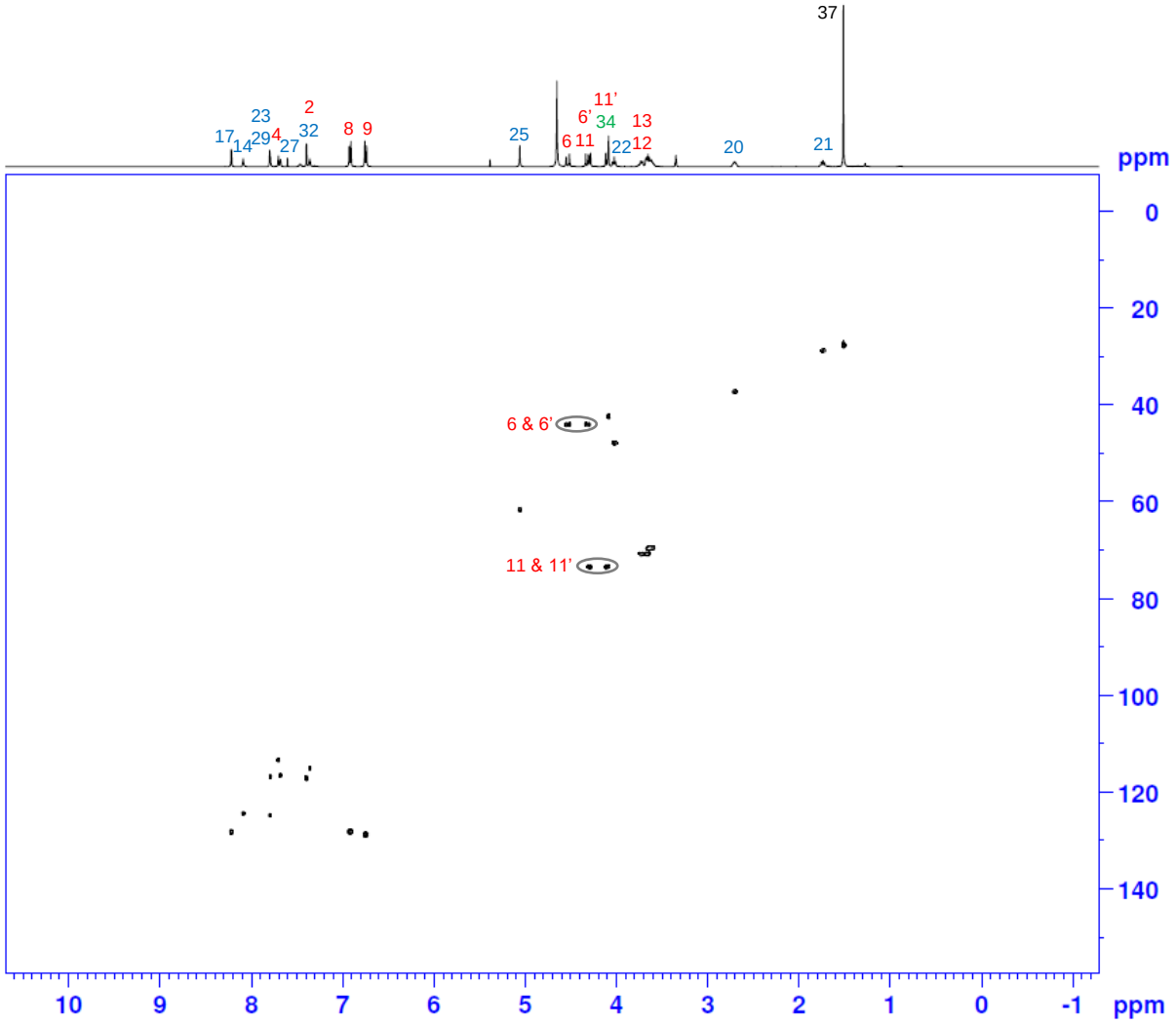
[2]Rotaxane **4**

^{1}H-^{1}H COSY NMR ($CDCl_3$/CD_3OD 1:1, 400 MHz)
Selected peaks and cross-peaks labelled



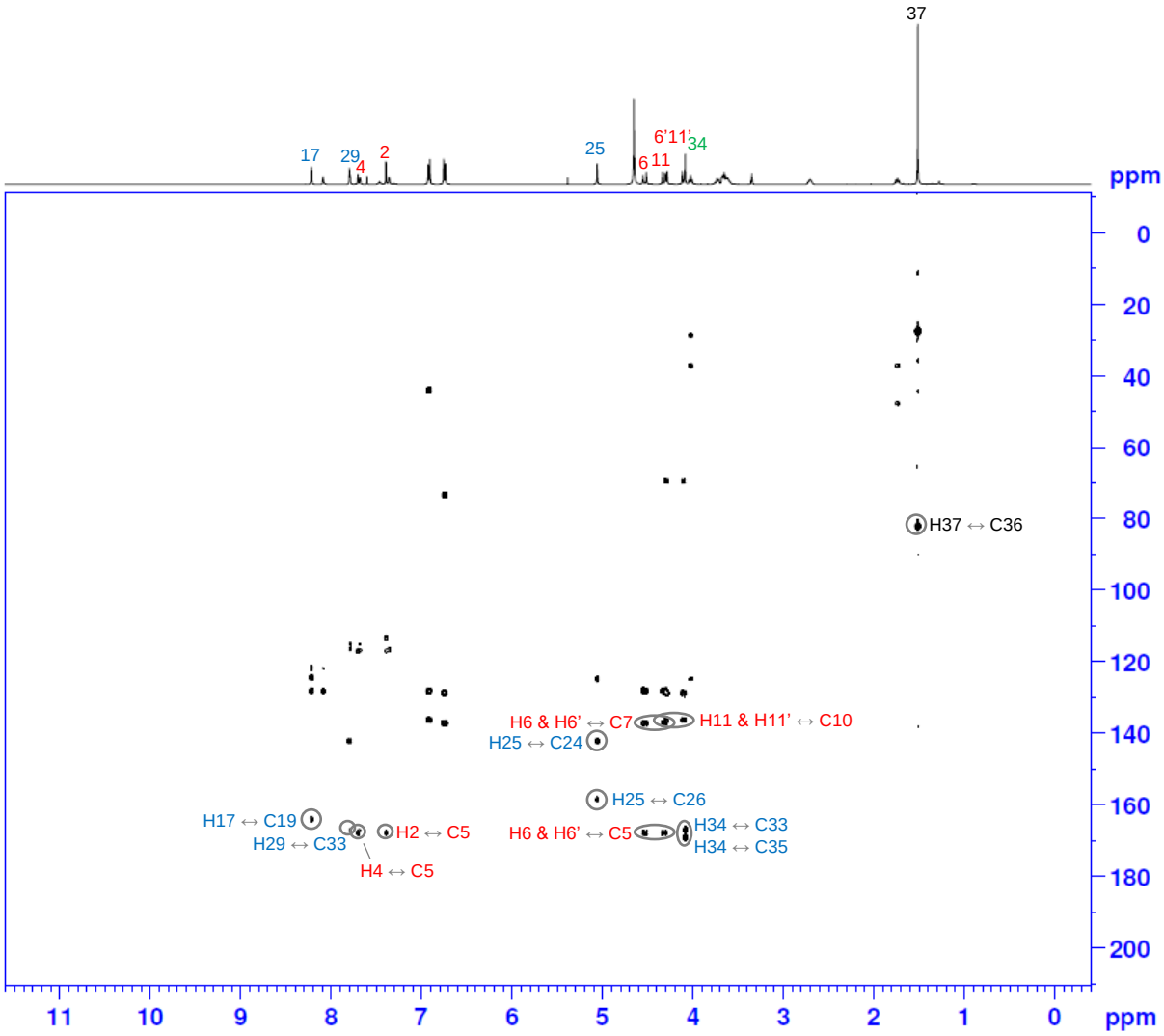
[2]Rotaxane **4**

^{1}H-^{13}C HSQC NMR ($CDCl_3$/CD_3OD 1:1, 400 MHz)
Peaks and diastereotopic correlations labelled



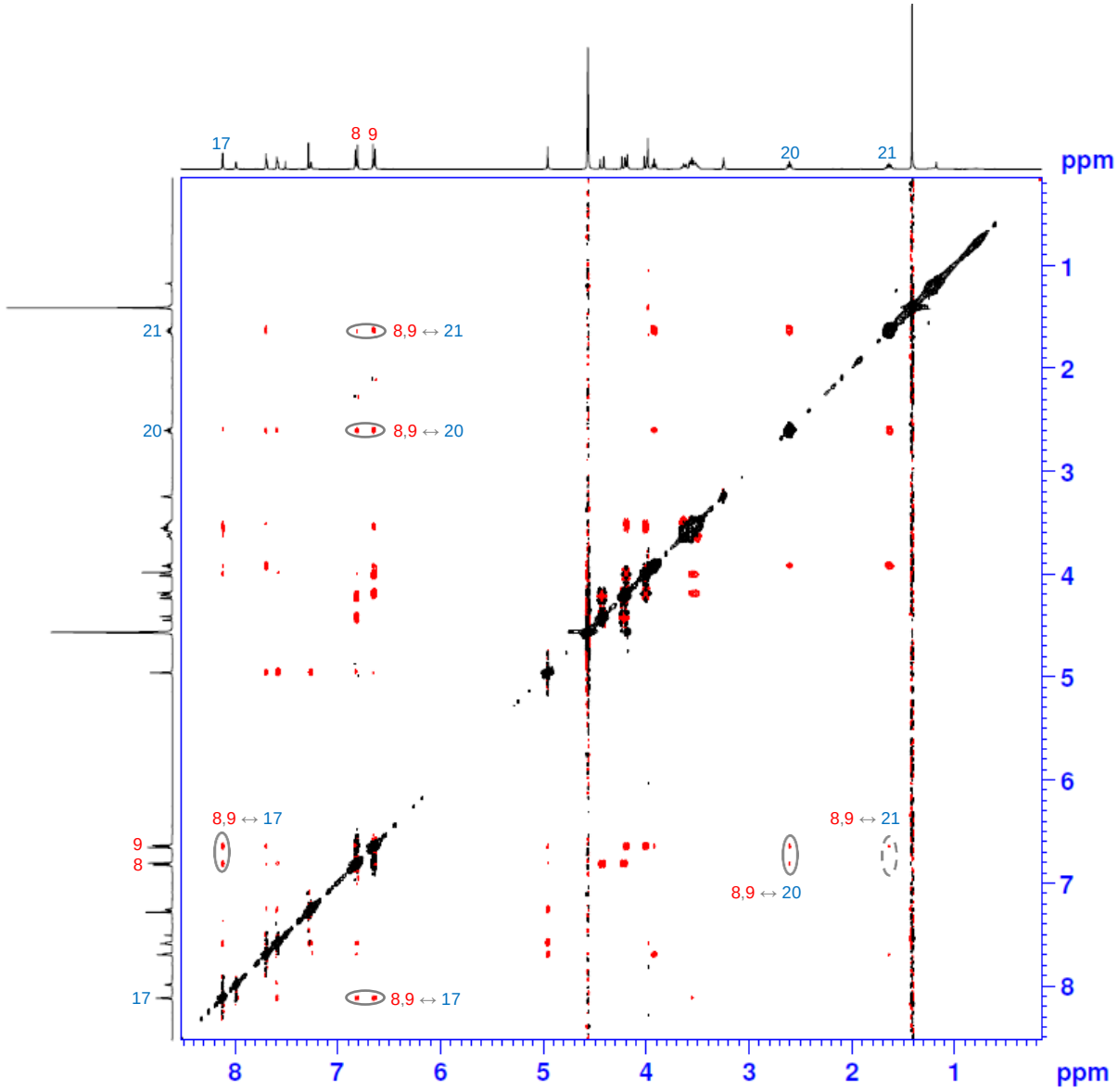
[2]Rotaxane **4**

^{1}H-^{13}C HMBC NMR ($CDCl_3$/CD_3OD 1:1, 400 MHz)
Selected peaks and quaternary correlations labelled



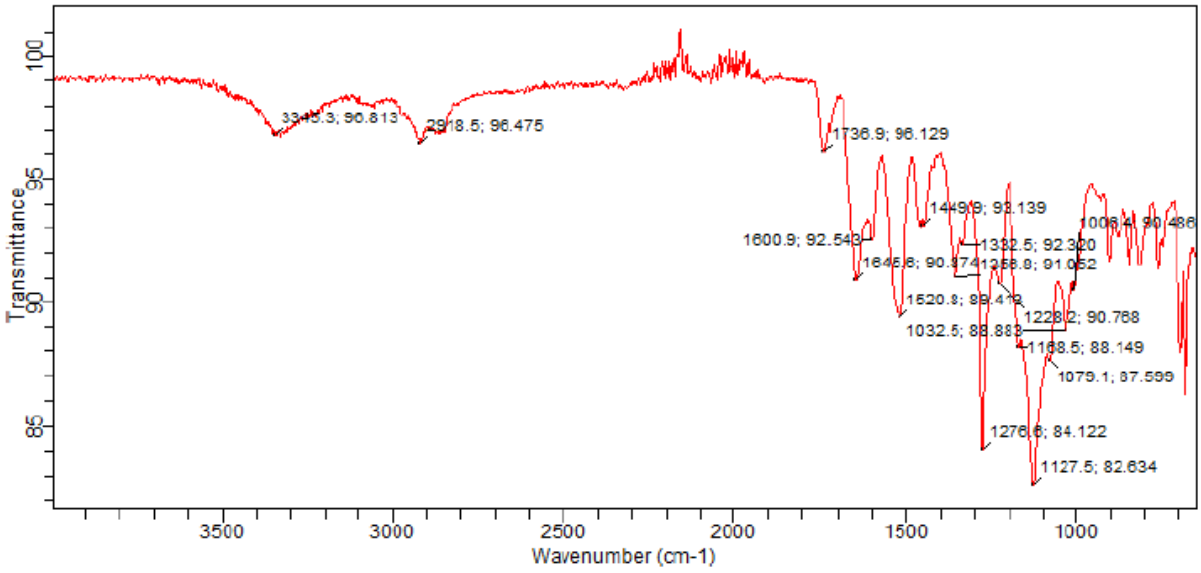
[2]Rotaxane **4**

^{1}H ROESY NMR ($CDCl_3$/CD_3OD 1:1, 400 MHz)
Unambiguous inter-component couplings labelled

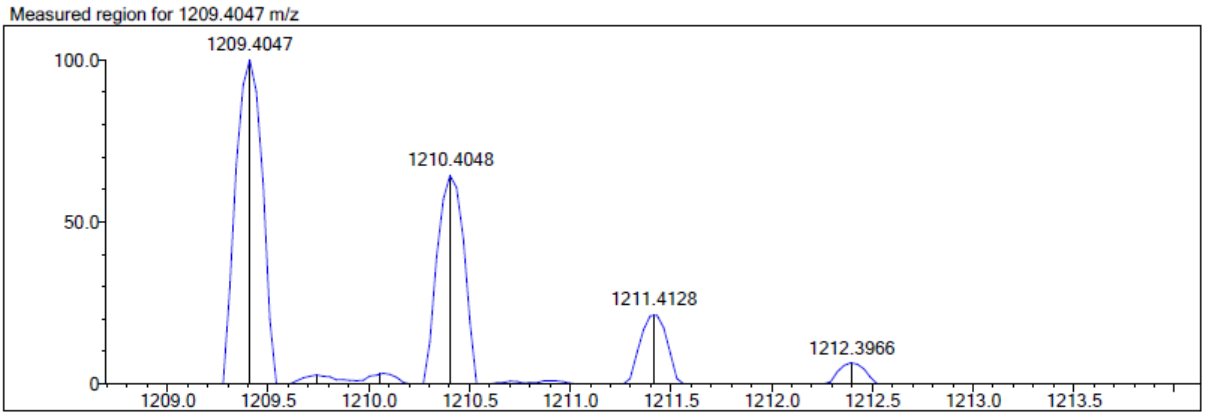


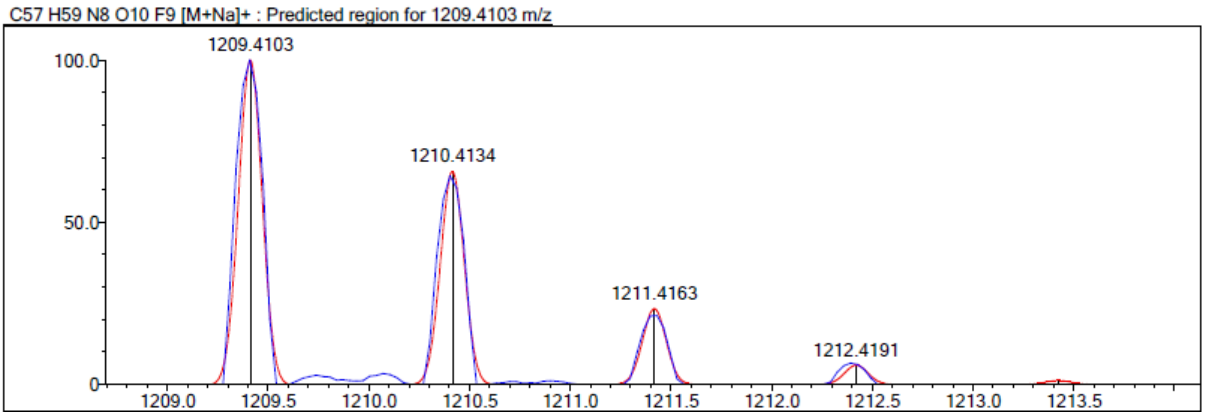
[2]Rotaxane **4**

IR Spectrum (neat)



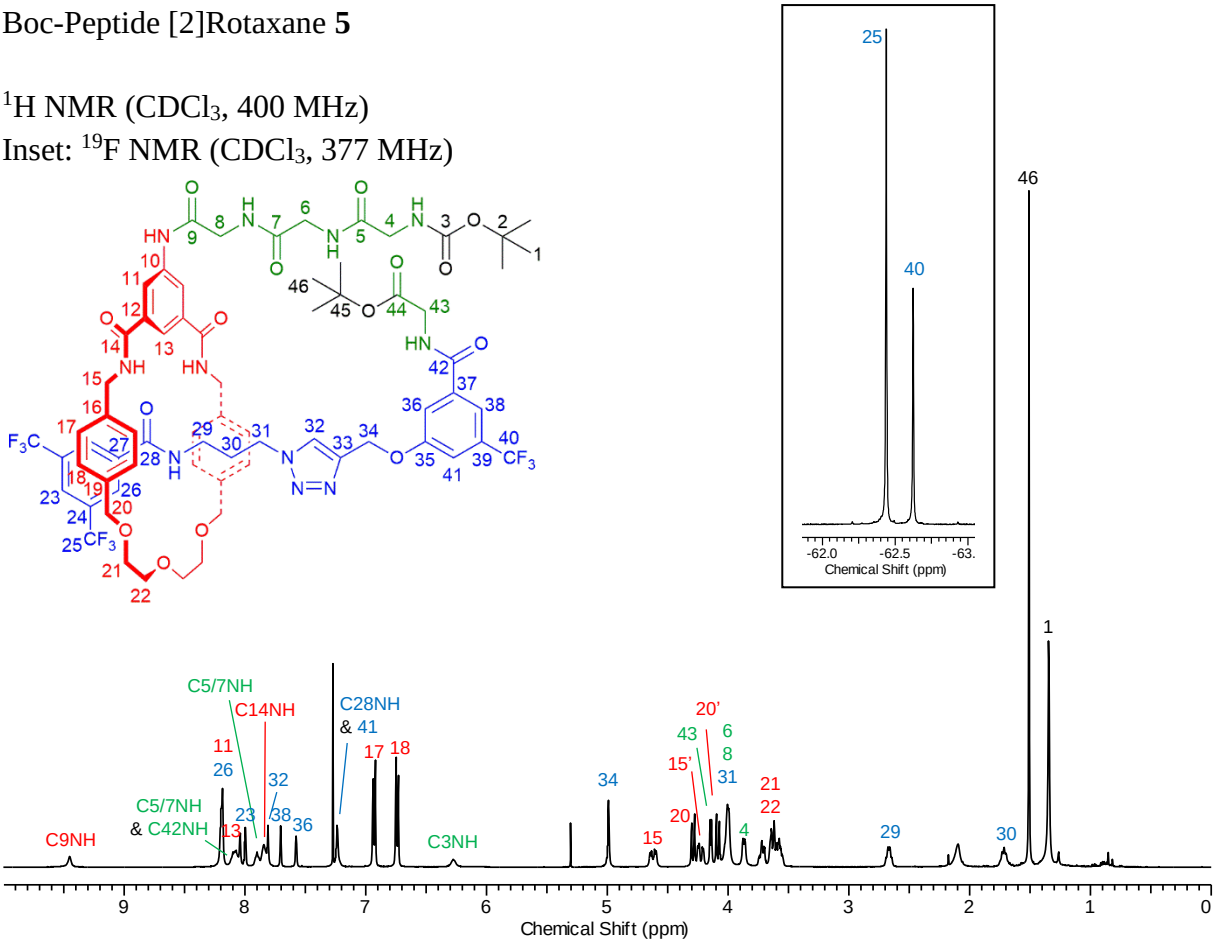
Mass Spectrum (ES +ve)



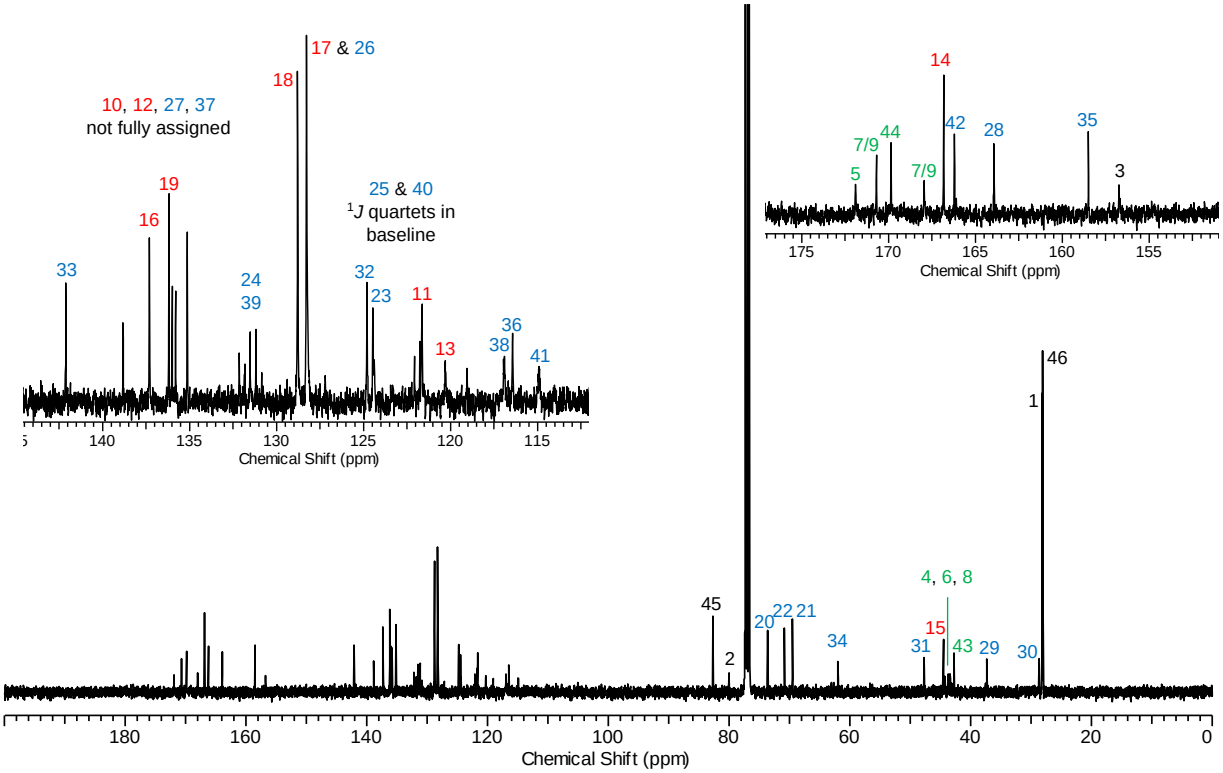


Boc-Peptide [2]Rotaxane **5**

^{1}H NMR ($CDCl_3$, 400 MHz)
Inset: ^{19}F NMR ($CDCl_3$, 377 MHz)

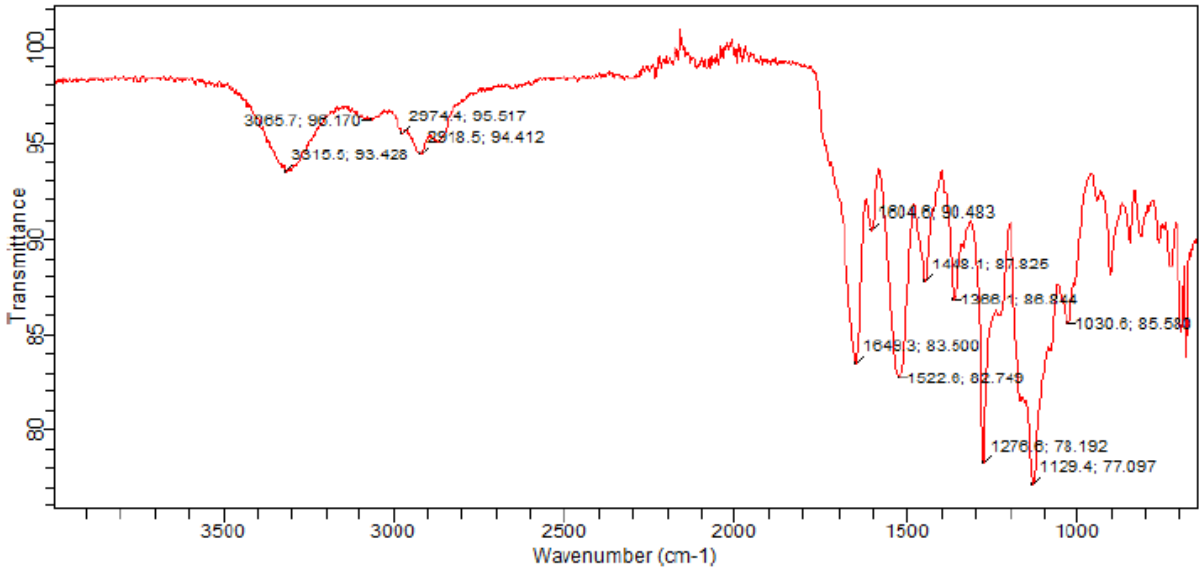


^{13}C NMR ($CDCl_3$, 100 MHz)

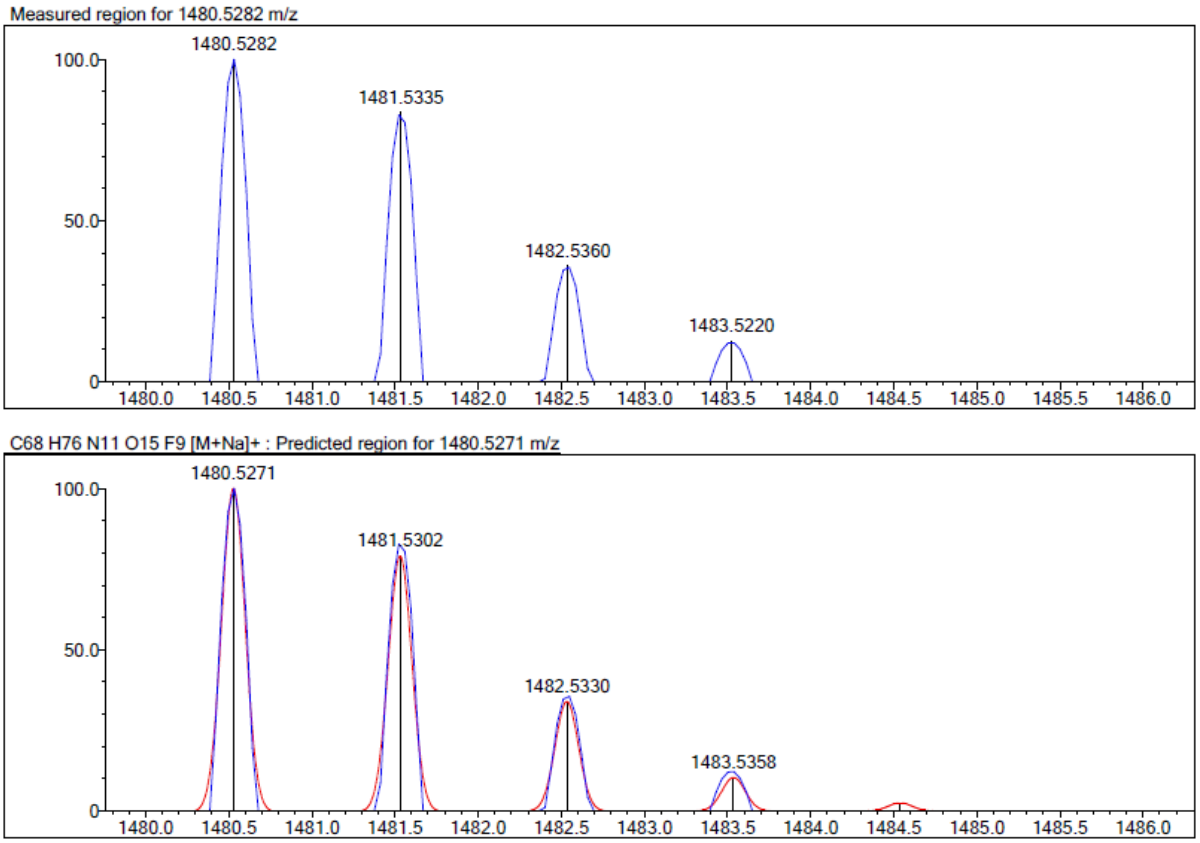


Boc-Peptide [2]Rotaxane **5**

IR Spectrum (neat)

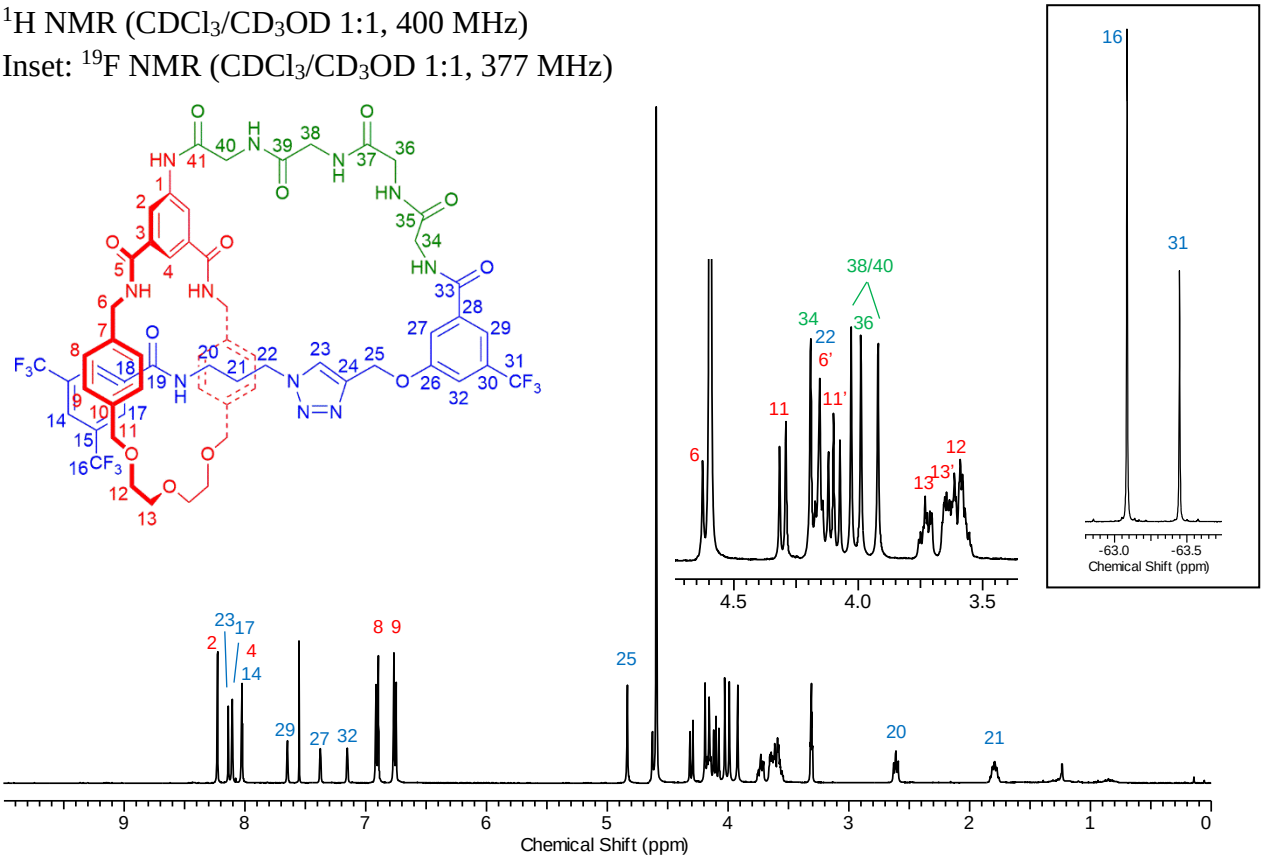


Mass Spectrum (ES +ve)

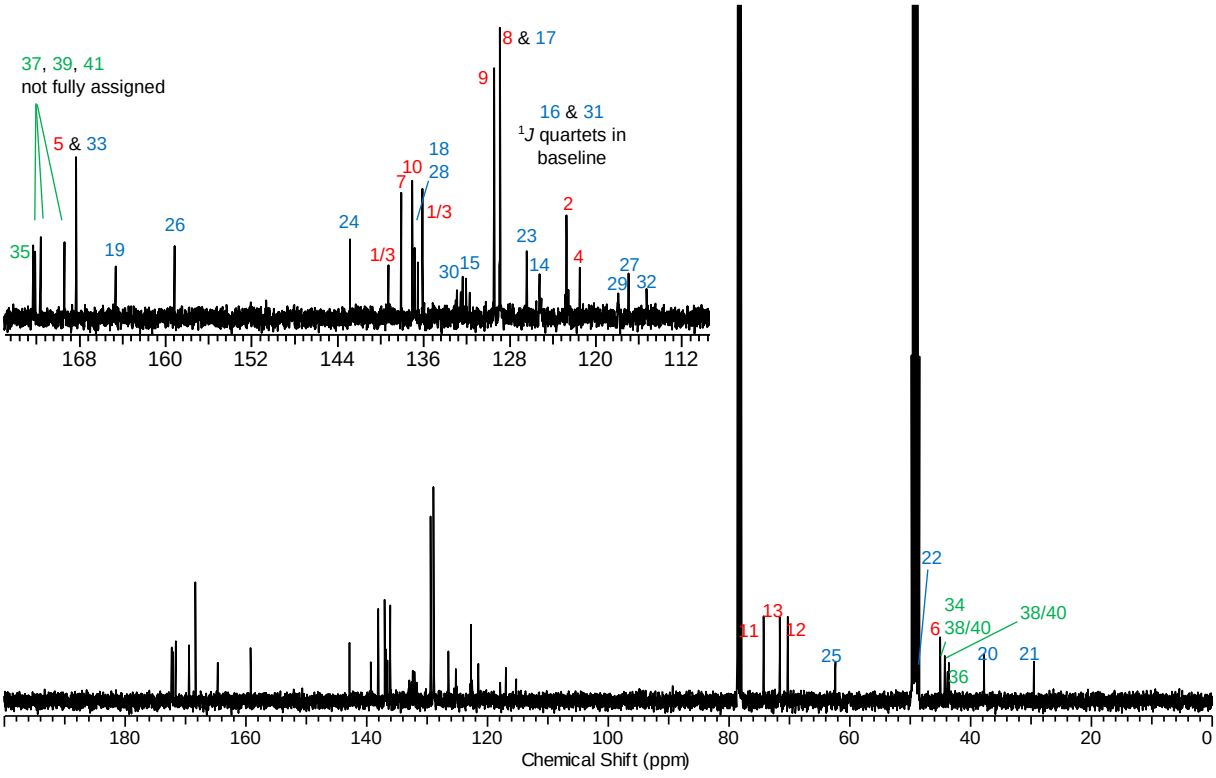


[1]Rotaxane **6** – *NMR Spectra in 1:1 $CDCl_3/CD_3OD$*

^{1}H NMR ($CDCl_3/CD_3OD$ 1:1, 400 MHz)
Inset: ^{19}F NMR ($CDCl_3/CD_3OD$ 1:1, 377 MHz)

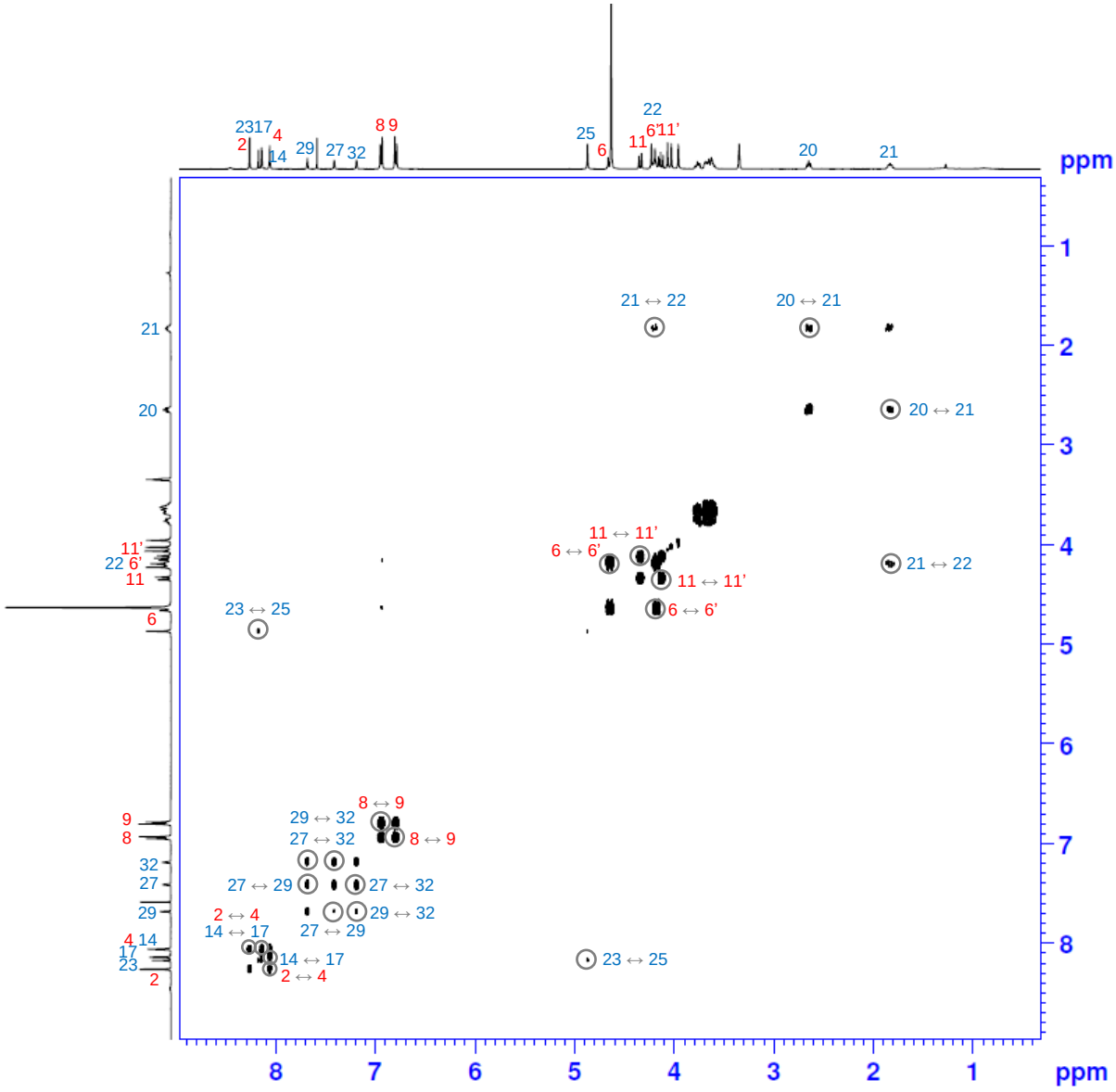


^{13}C NMR ($CDCl_3/CD_3OD$ 1:1, 100 MHz)



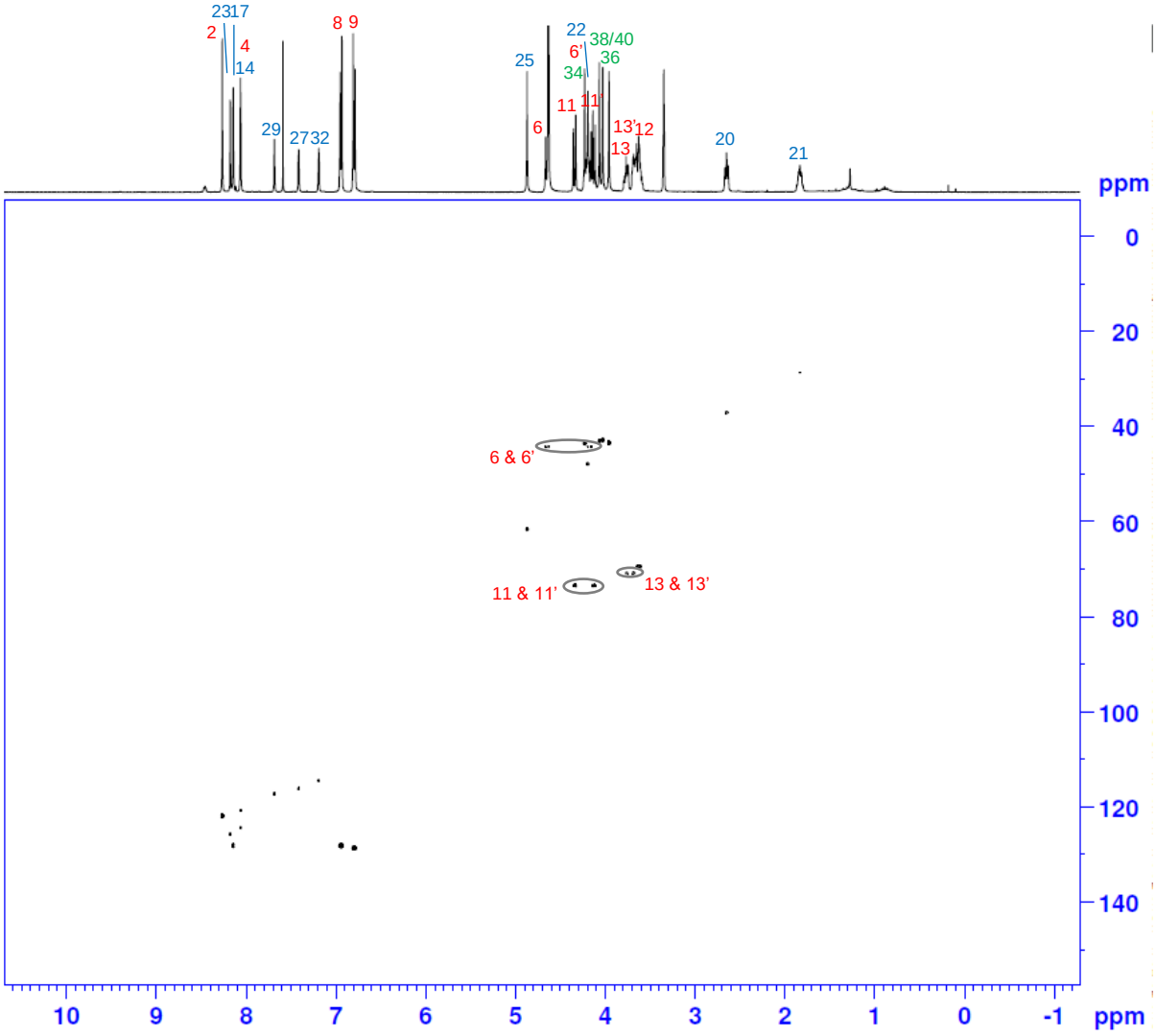
[1]Rotaxane **6** – *NMR Spectra in 1:1 $CDCl_3/CD_3OD$*

^1H-^1H COSY NMR ($CDCl_3/CD_3OD$ 1:1, 400 MHz)
Selected peaks and cross-peaks labelled



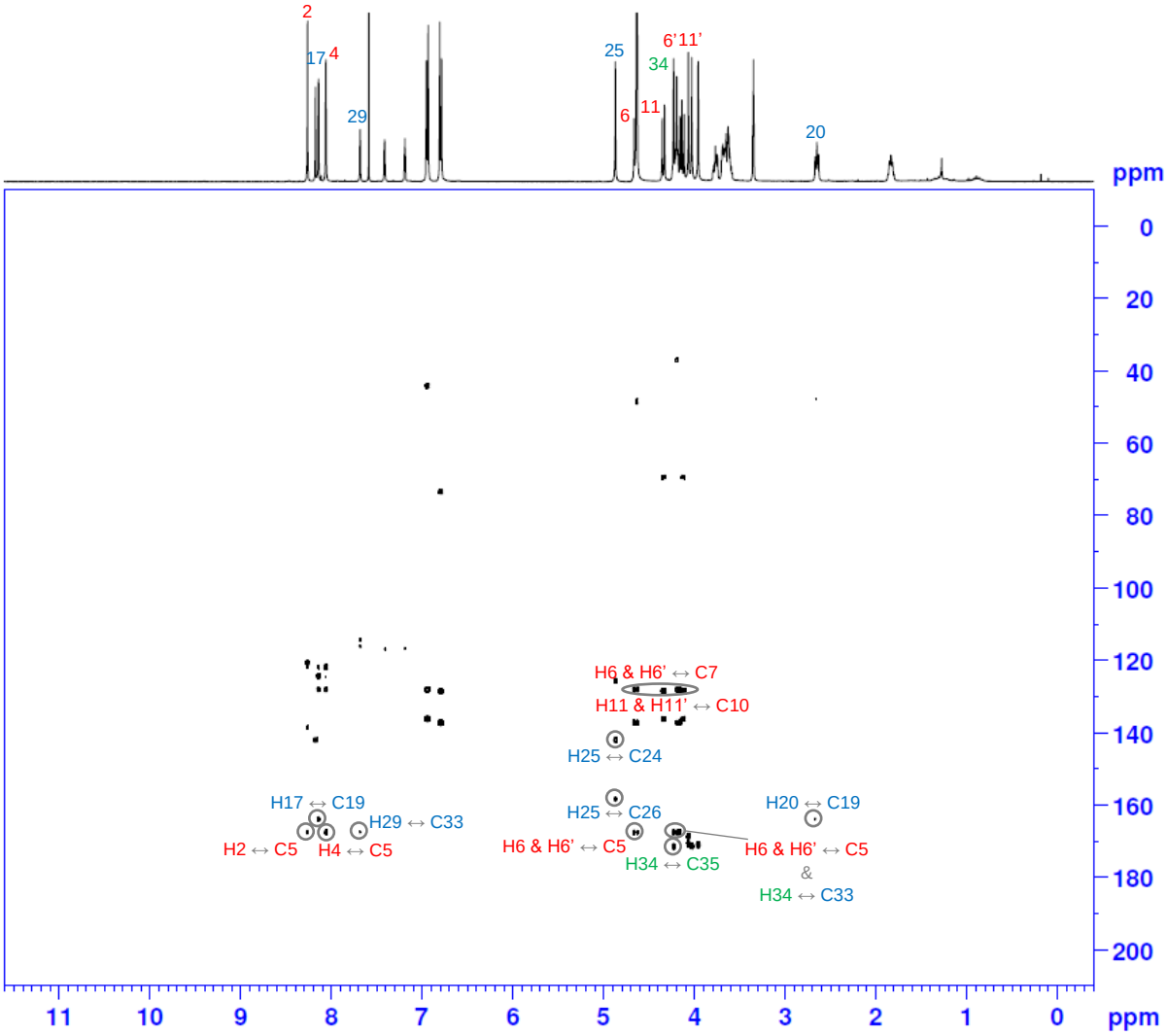
[1]Rotaxane **6** – *NMR Spectra in 1:1 $CDCl_3/CD_3OD$*

^{1}H-^{13}C HSQC NMR ($CDCl_3/CD_3OD$ 1:1, 400 MHz)
Peaks and diastereotopic correlations labelled



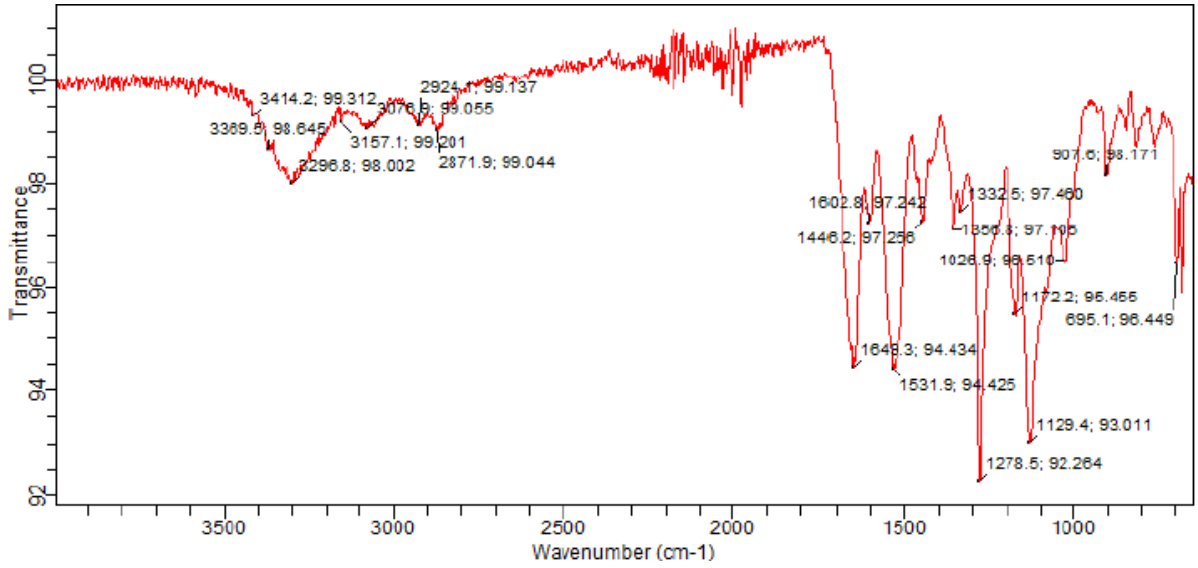
[1]Rotaxane **6** – *NMR Spectra in 1:1 $CDCl_3/CD_3OD$*

^{1}H-^{13}C HMBC NMR ($CDCl_3/CD_3OD$ 1:1, 400 MHz)
Selected peaks and quaternary correlations labelled

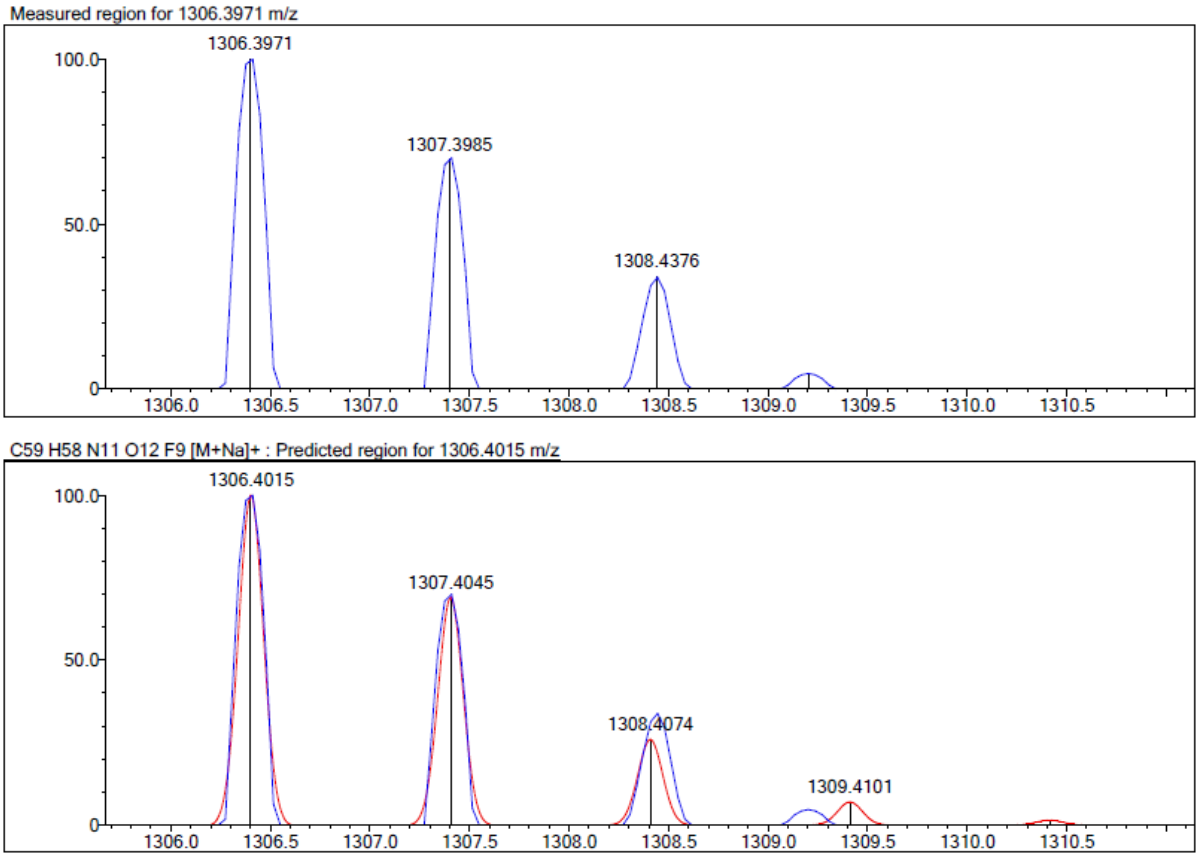


[1]Rotaxane **6**

IR Spectrum (neat)

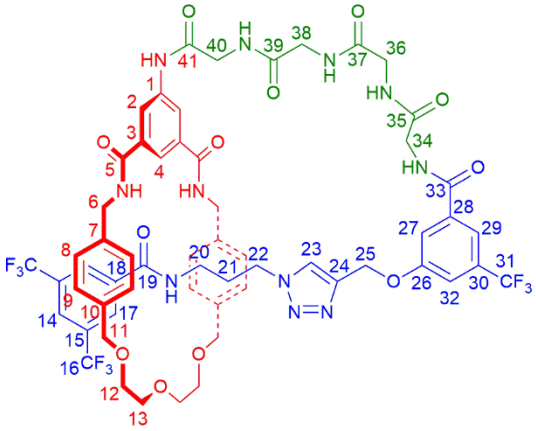


Mass Spectrum (ES +ve)



[1]Rotaxane **6** – *NMR Spectral Data in D_6-DMSO*

2.5 mg dissolved in 600 μL.
Data collected to assign ^{1}H NMR and to allow for ^{1}H DOSY NMR comparison with unthreaded isomer **8**. No 1D ^{13}C NMR spectrum recorded - ^{13}C shifts are from 2D spectra. Data re-processed to allow for signal detection due to dilute nature of sample solution.



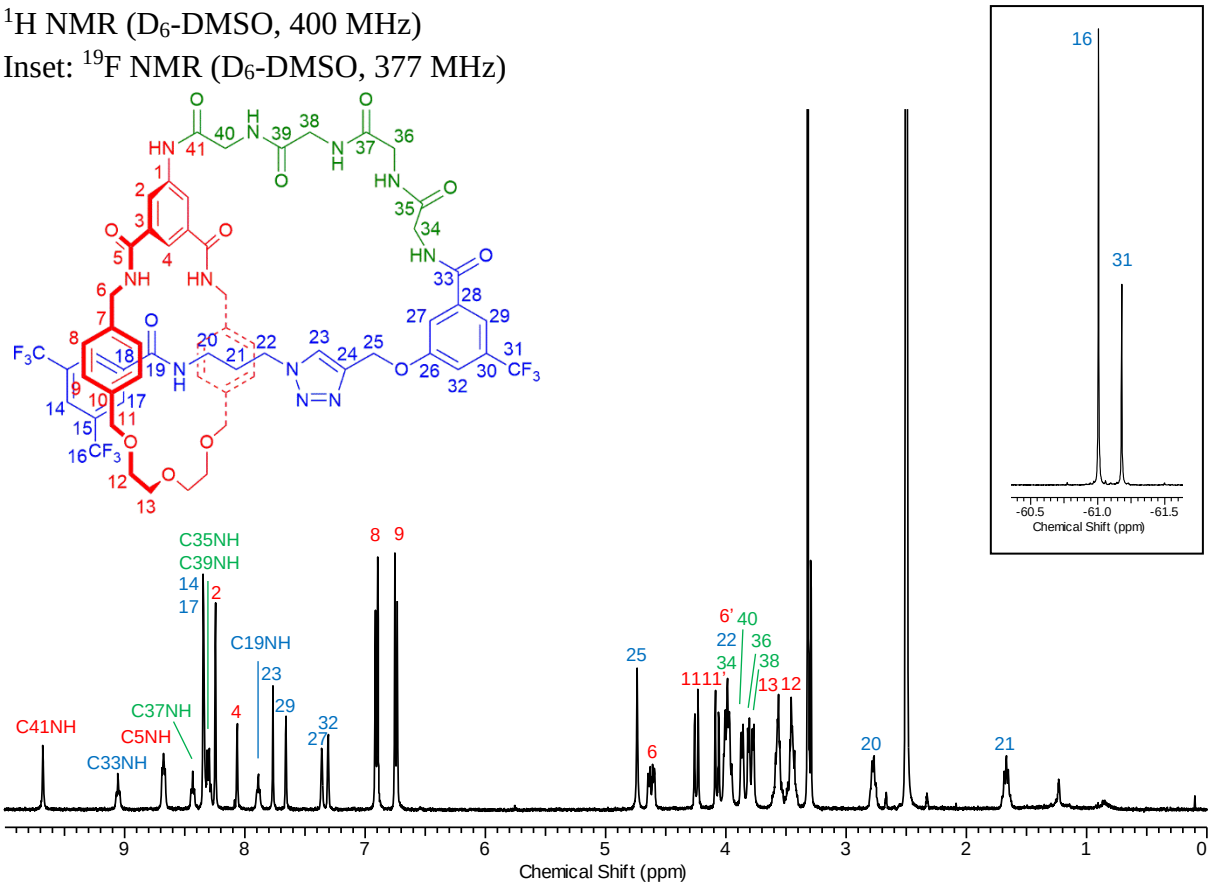
δ_H (400 MHz; D_6-DMSO): 9.68 (1H, s, C^{41}NH), 9.05 (1H, t, ^{3}J = 5.4 Hz, C^{33}NH), 8.68 (2H, t, ^{3}J = 6.4 Hz, C^{5}NH), 8.43 (1H, t, ^{3}J = 5.2 Hz, C^{37}NH), 8.35 (3H, app s, C^{14}H & C^{17}H), 8.28-8.33 (2H, m, C^{35}NH & C^{39}NH), 8.24 (2H, s, C^{2}H), 8.06 (1H, s, C^{4}H), 7.89 (1H, t, ^{3}J = 5.2 Hz, C^{19}NH), 7.77 (1H, s, C^{23}H), 7.66 (1H, s, C^{29}H), 7.36 (1H, s, C^{27}H), 7.31 (1H, s, C^{32}H), 6.90 (4H, d, ^{3}J = 7.9 Hz, C^{8}H), 6.74 (4H, d, ^{3}J = 7.9 Hz, C^{9}H), 4.74 (2H, s, C^{25}H), 4.62 (2H, dd, ^{2}J = 14 Hz, ^{3}J = 6.4 Hz, C^{6}H - diastereotopic), 4.25 (2H, d, ^{2}J = 11 Hz, C^{11}H - diastereotopic), 4.07 (2H, d, ^{2}J = 11 Hz, C^{11}H - diastereotopic), 3.95-4.02 (6H, m, C^{6}H – diastereotopic, C^{22}H & C^{34}H), 3.87 (2H, d, ^{3}J = 5.7 Hz, C^{40}H), 3.81 (2H, d, ^{3}J = 5.2 Hz, C^{36}H), 3.77 (2H, d, ^{3}J = 5.2 Hz, C^{38}H), 3.55-3.59 (4H, m, C^{13}H), 3.43-3.47 (4H, m, C^{12}H), 2.75-2.80 (2H, app q, C^{20}H), 1.63-1.70 (2H, app quin, C^{21}H).

δ_C (100 MHz; D_6-DMSO) – detected resonances only: 170.3 (C^{37}), 170.0 (C^{39}), 169.9 (C^{35}), 168.2 (C^{41}), 166.3 (C^{5}), 165.5 (C^{33}), 163.3 (C^{19}), 158.6 (C^{26}), 141.4 (C^{24}), 138.1 (C^{7}), 136.2 (C^{10}), 128.8 (C^{14} or C^{17}), 128.6 (C^{9}), 128.4 (C^{8}), 125.3 (C^{23}), 125.1 (C^{14} or C^{17}), 121.3 (C^{2}), 120.5 (C^{4}), 117.7 (C^{27}), 116.7 (C^{29}), 114.0 (C^{32}), 72.8 (C^{11}), 70.4 (C^{13}), 69.1 (C^{12}), 61.6 (C^{25}), 47.3 (C^{22}), 43.6 (C^{6} & C^{34}), 43.4 (C^{40}), 43.0 (C^{38}), 42.6 (C^{36}), 37.2 (C^{20}), 29.0 (C^{21}).

δ_F (377 MHz; D_6-DMSO): –61.1 ($C^{16}F_3$), –61.2 ($C^{31}F_3$).

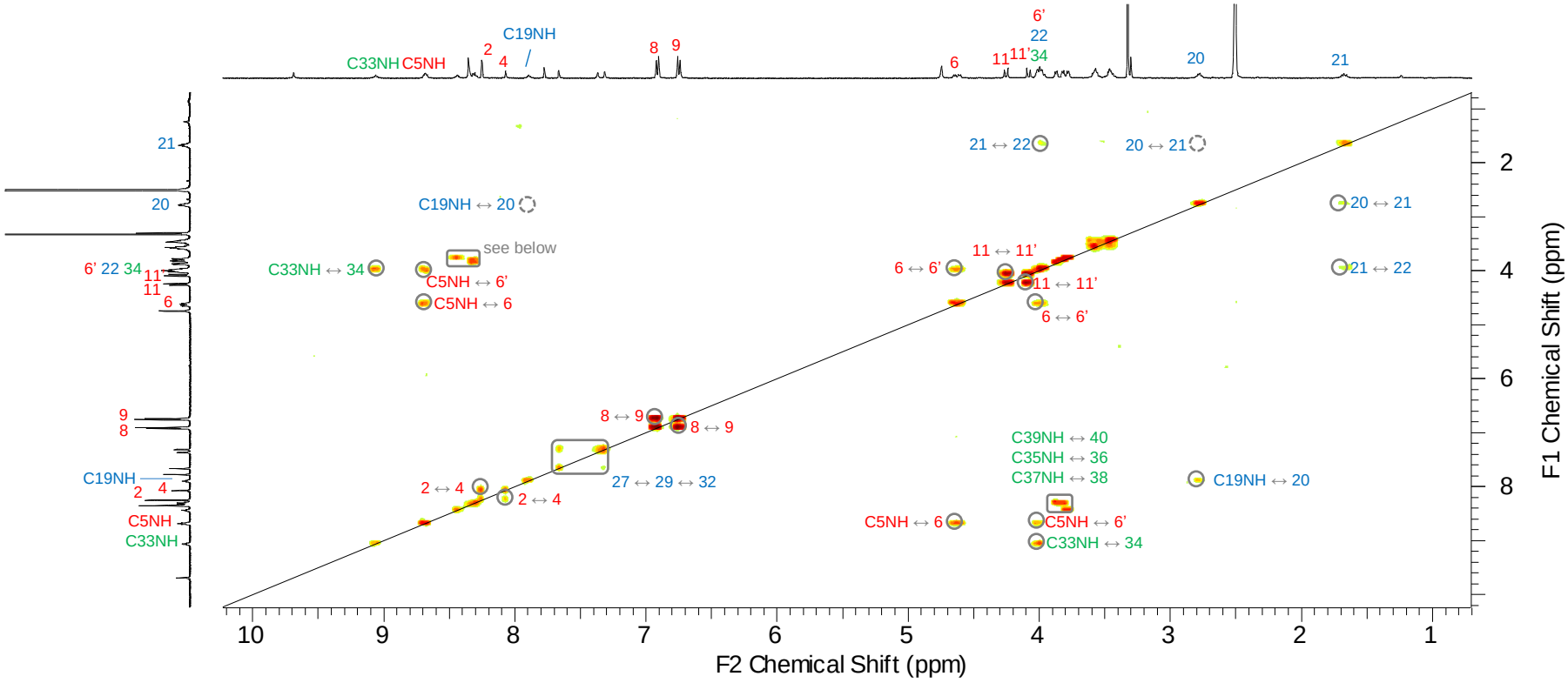
[1]Rotaxane **6** – *NMR Spectral Data in D_6-DMSO*

^{1}H NMR (D_6-DMSO, 400 MHz)
Inset: ^{19}F NMR (D_6-DMSO, 377 MHz)



[1]Rotaxane **6** – *NMR Spectral Data in D_6-DMSO*

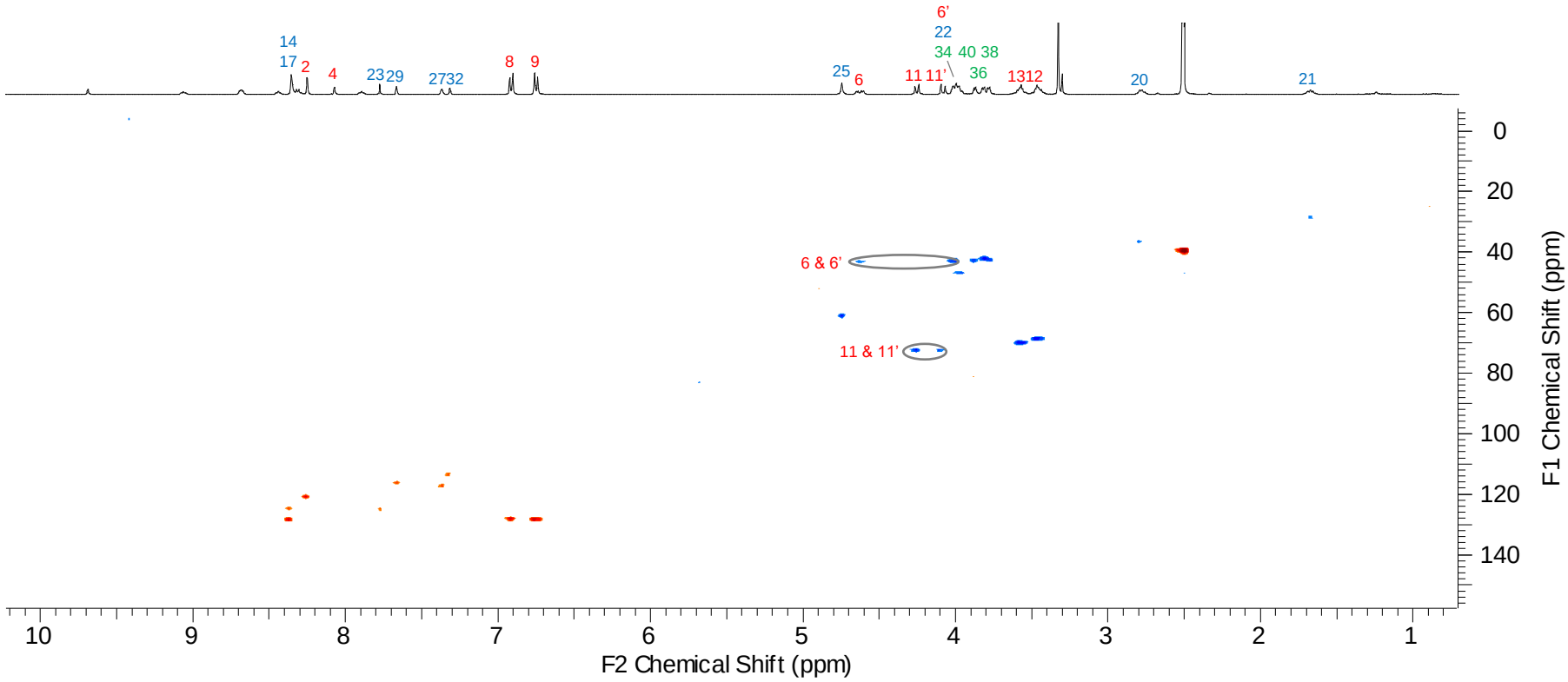
^{1}H-^{1}H COSY NMR (D_6-DMSO, 400 MHz)
Selected peaks and cross-peaks labelled



[1]Rotaxane **6** – *NMR Spectral Data in D_6-DMSO*

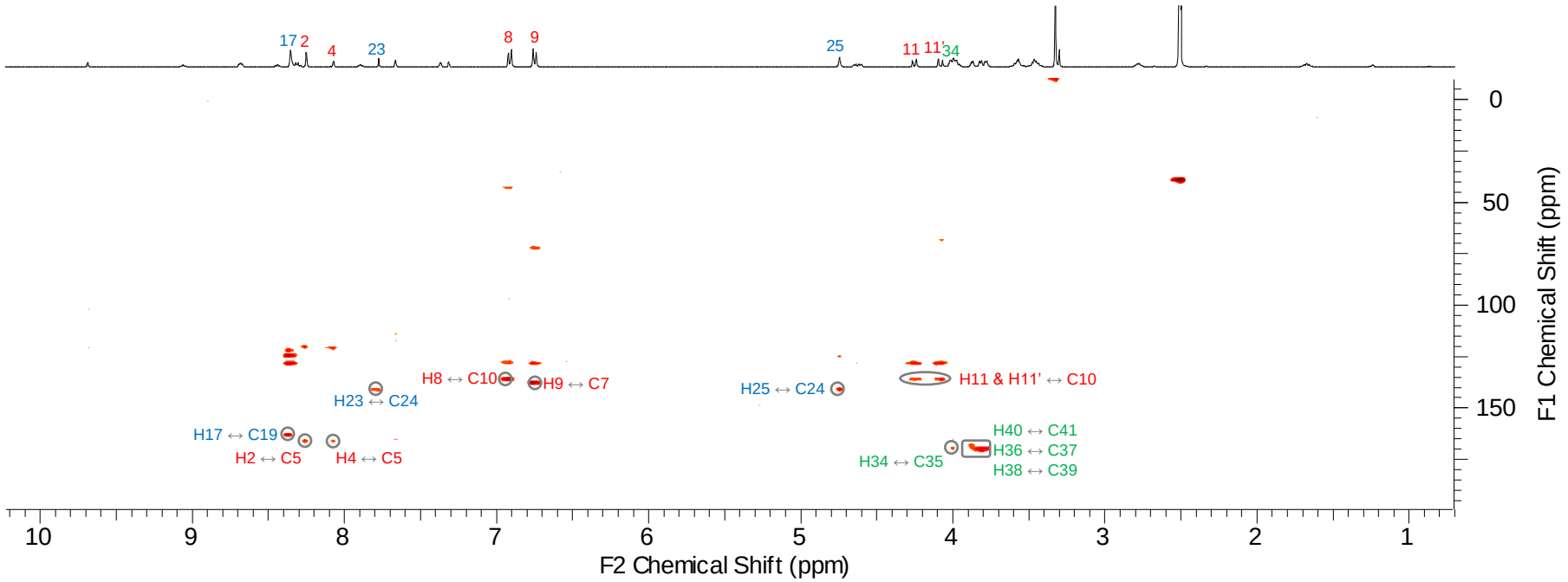
^{1}H-^{13}C HSQC NMR (D_6-DMSO, 400 MHz)

C-H peaks and diastereotopic correlations labelled

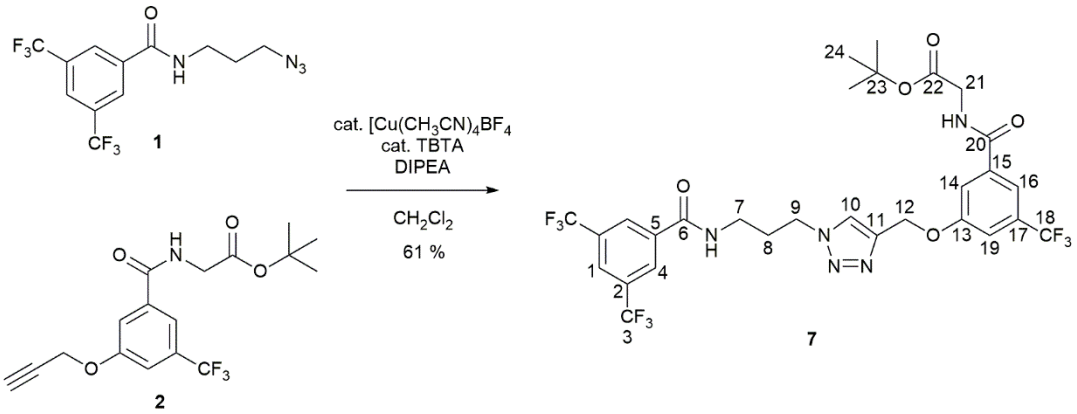


[1]Rotaxane **6** – *NMR Spectral Data in D_6-DMSO*

^{1}H-^{13}C HMBC NMR (D_6-DMSO, 400 MHz)
Selected peaks and quaternary correlations labelled



Axle **7**



*Scheme ESI-3: Preparation of axle **7***

Azide **1** (65 mg, 0.19 mmol) and alkyne **2** (68 mg, 0.19 mmol) were dissolved in dry CH_2Cl_2 (3 mL) under an Ar (g) atmosphere. Then, $Cu(CH_3CN)_4BF_4$ (6.0 mg, 0.019 mmol), TBTA (10 mg, 0.019 mmol) and DIPEA (38 μL, 27 mg, 0.21 mmol) were added. The reaction was stirred at r.t. for 24 h under an Ar (g) atmosphere. Then, the reaction was diluted to 20 mL, washed with 0.02 M EDTA in 1M NH_3 (aq) solution (2 × 20 mL) and brine (1 × 20 mL). The organic layer was dried ($MgSO_4$) and solvent removed *in vacuo*. The crude material was purified by silica gel column chromatography (CH_2Cl_2/CH_3OH 99:1 to 98:2) to yield the product as a colourless film (80 mg, 61%).

***R*f** 0.24 (CH_2Cl_2/CH_3OH 98:2).

ν_{max}/cm^{-1} (neat): 3320 (N–H), 3090 (C–H), 2980 (C–H), 2940 (C–H), 1740 (C=O ester), 1650 (C=O amide), 1600, 1540, 1450, 1370, 1280, 1230, 1120, 1040.

δ_H (400 MHz; $CDCl_3$): 8.31 (2H, s, C^4H), 7.96 (1H, s, C^1H), 7.79-7.82 (2H, m, $C^{10}H$ & C^6NH), 7.57 (1H, s, $C^{16}H$), 7.55 (1H, s, $C^{14}H$), 7.24 (1H, s, $C^{19}H$), 7.19 (1H, t, ^3J = 4.8 Hz, $C^{20}NH$), 5.18 (2H, s, $C^{12}H$), 4.49 (2H, t, ^3J = 6.1 Hz, C^9H), 4.09 (2H, d, ^3J = 4.8 Hz, $C^{21}H$), 3.48-3.50 (2H, m, C^7H), 2.25-2.28 (2H, m, C^8H), 1.48 (9H, s, $C^{24}H$).

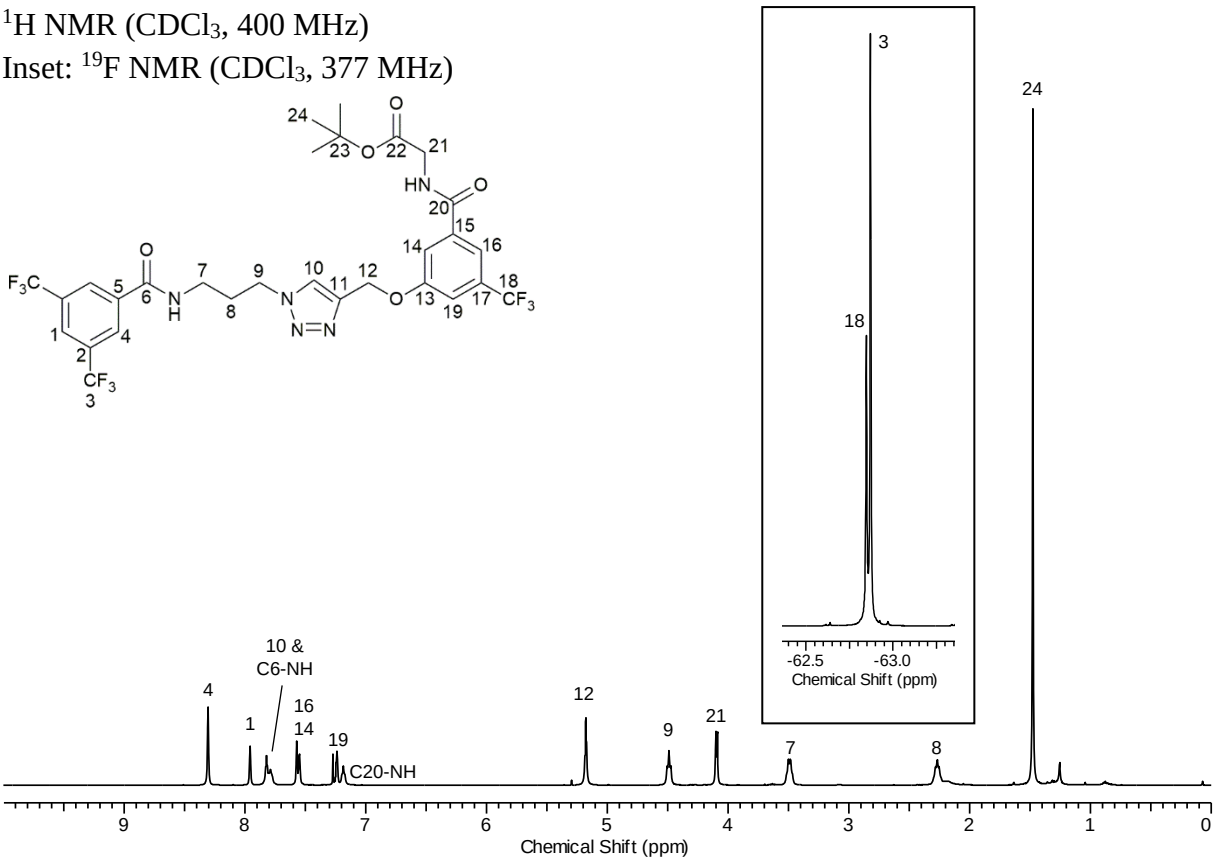
δ_C (100 MHz; $CDCl_3$): 169.1 (C^{22}), 165.9 (C^{20}), 165.1 (C^6), 158.4 (C^{13}), 143.1 (C^{11}), 136.2 (C^5 & C^{15} – coincident), 132.3 (q, ^2J = 32 Hz, C^{17}), 132.0 (q, ^2J = 34 Hz, C^2), 127.6 (q, ^3J = 2.6 Hz, C^4), 124.9 (br, C^1), 123.8 (br, C^{10}), 123.2 (q, ^1J = 271 Hz, C^{18}), 122.8 (q, ^1J = 271 Hz, C^3), 116.6 (C^{14}), 116.3 (q, ^3J = 3.7 Hz, C^{16}), 115.2 (q, ^3J = 3.7 Hz, C^{19}), 82.8 (C^{23}), 62.1 (C^{12}), 48.0 (C^9), 42.5 (C^{21}), 37.4 (C^7), 29.7 (C^8), 27.9 (C^{24}).

δ_F (377 MHz; $CDCl_3$): –62.9 ($C^{18}F_3$), –62.9 (sic, C^3F_3).

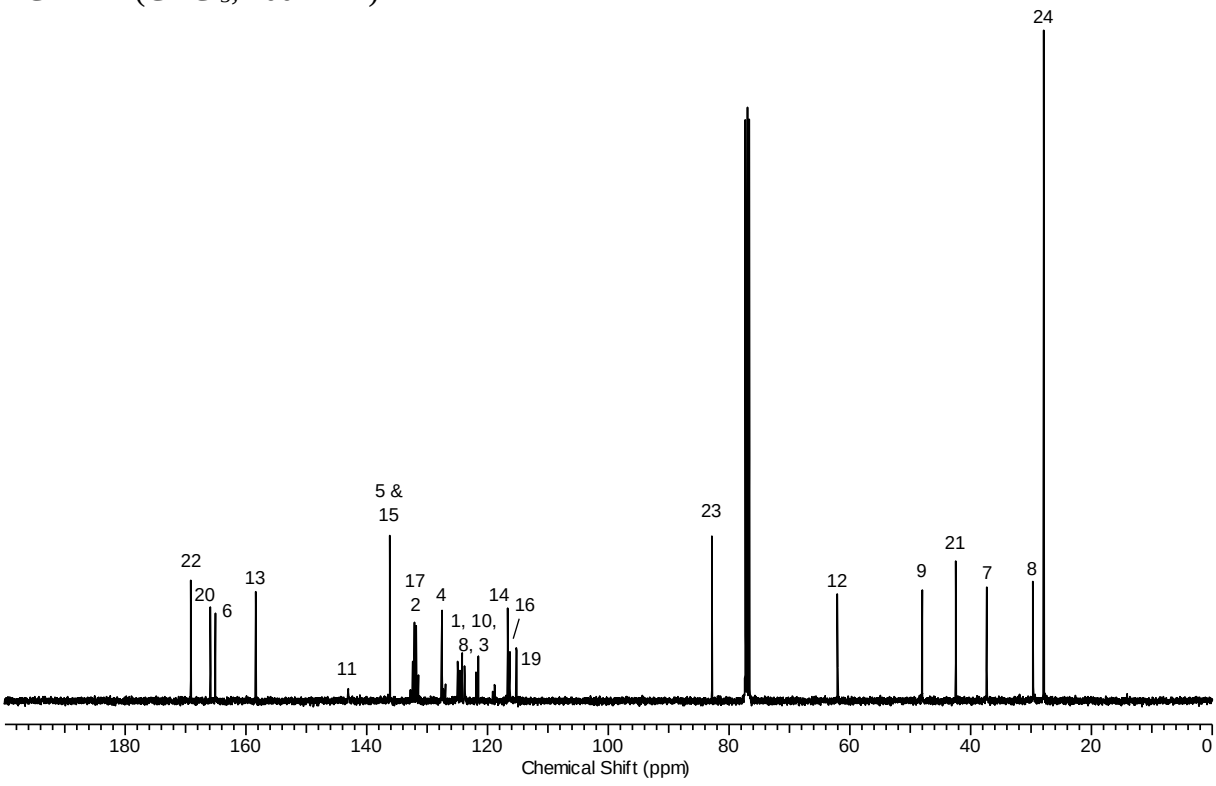
***m/z* (ES):** 720.1826 ($[M + Na]^+$ $C_{29}H_{28}F_9N_5NaO_5$ requires 720.1839).

Axle **7**

^{1}H NMR ($CDCl_3$, 400 MHz)
Inset: ^{19}F NMR ($CDCl_3$, 377 MHz)

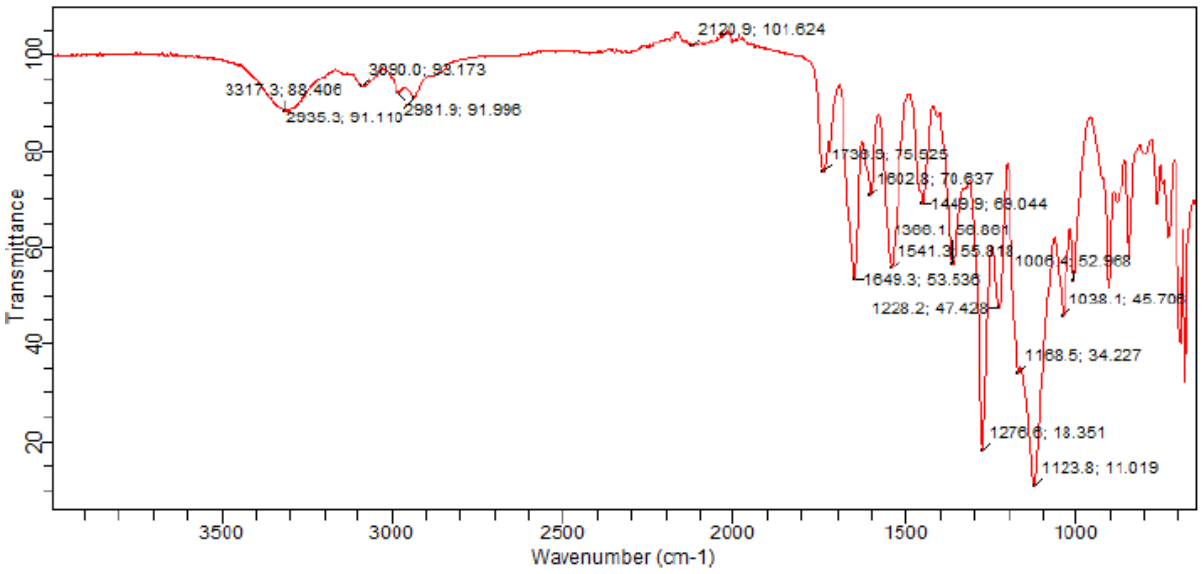


^{13}C NMR ($CDCl_3$, 100 MHz)

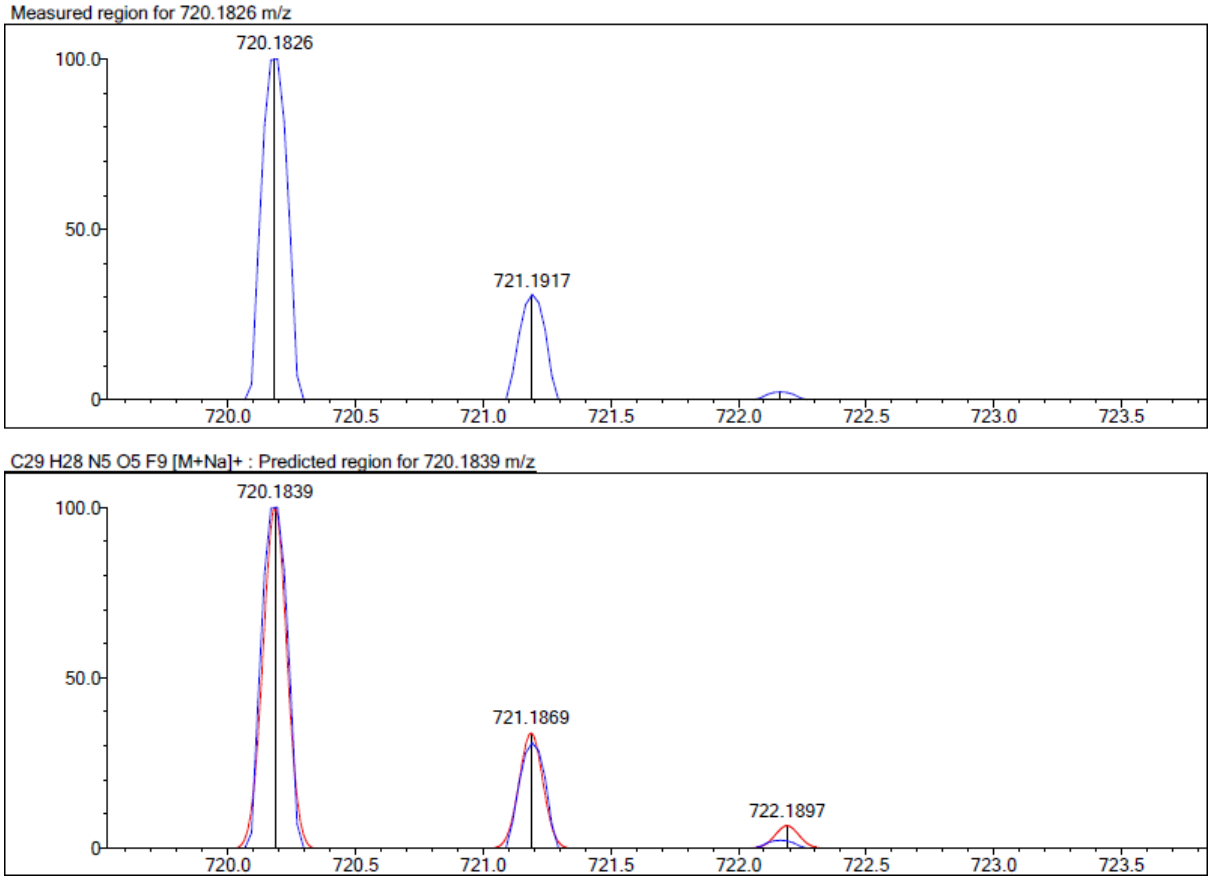


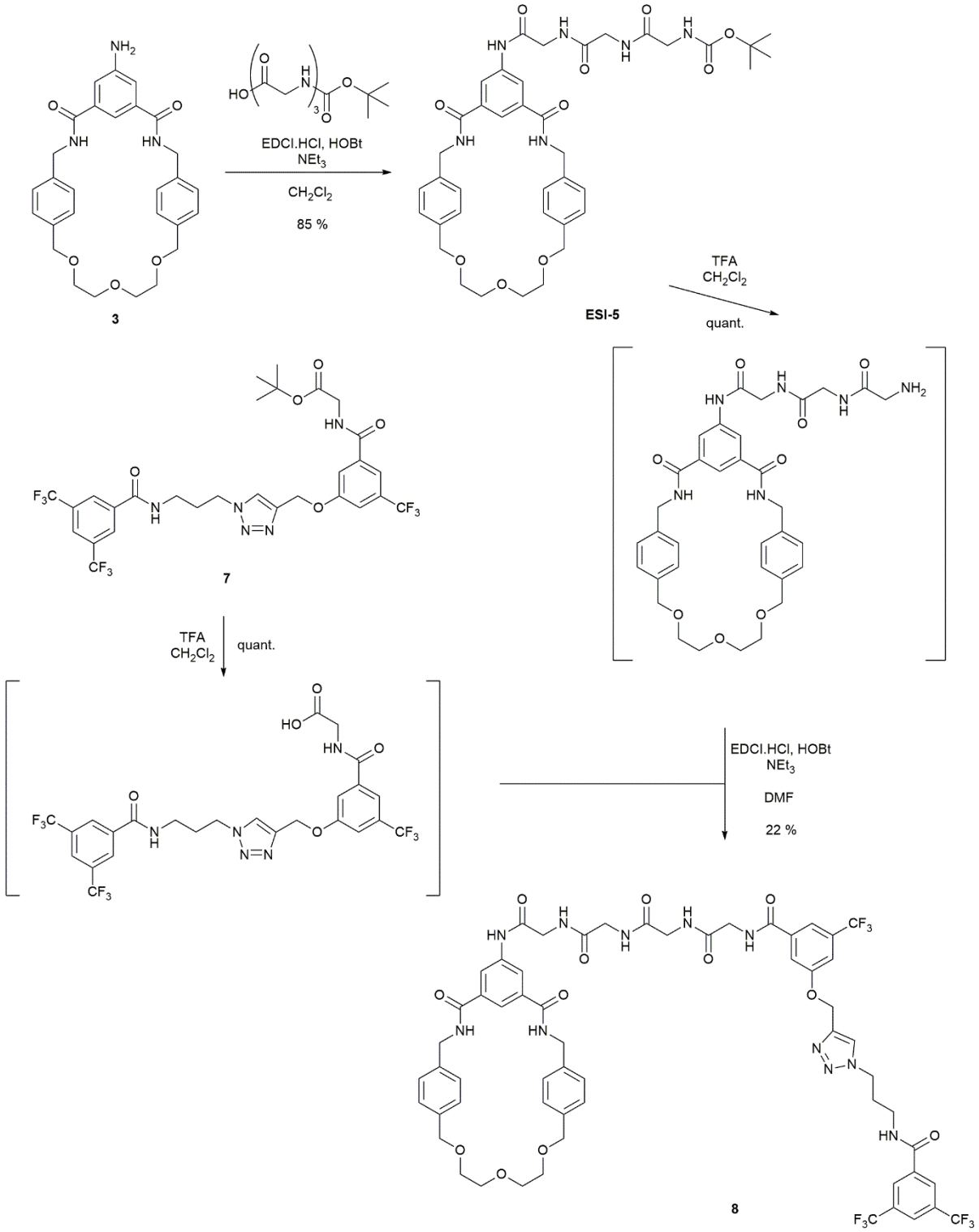
Axle **7**

IR Spectrum (neat)



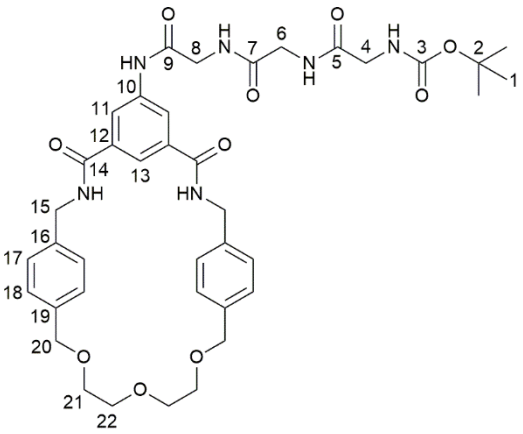
Mass Spectrum (ES +ve)





*Scheme ESI-4: Preparation of unthreaded isomer **8***

Boc-Peptide Macrocycle **ESI-5**



Macrocycle **3** (61 mg, 0.13 mmol) was dissolved in dry CH_2Cl_2 (20 mL), and cooled to 0 °C under an Ar (g) atmosphere. Then dry NEt_3 (19 mg, 26 μL, 0.19 mmol), triglycine *tert*-butyl ester (36 mg, 0.13 mmol), HOBt.hydrate (22 mg, 0.16 mmol) and EDCI.HCl (31 mg, 0.16 mmol) were added, maintaining the temperature at 0 °C. The reaction was stirred at r.t. for 6 h, at which time analytical TLC confirmed presence of **3**. The reaction flask was cooled to 0 °C and further dry NEt_3 (19 mg, 26 μL, 0.19 mmol), triglycine *tert*-butyl ester (36 mg, 0.13 mmol), HOBt.hydrate (22 mg, 0.16 mmol) and EDCI.HCl (31 mg, 0.16 mmol) were added. The reaction was stirred at r.t. for a further 16 h, at which time analytical TLC confirmed presence of **3**. The reaction flask was cooled to 0 °C and further dry NEt_3 (19 mg, 26 μL, 0.19 mmol), triglycine *tert*-butyl ester (36 mg, 0.13 mmol), HOBt.hydrate (22 mg, 0.16 mmol) and EDCI.HCl (31 mg, 0.16 mmol) were added. The reaction was stirred at r.t. for a further 24 h, then washed with H_2O (1 × 20 mL), 10% citric acid (aq) (2 × 20 mL), saturated $NaHCO_3$ (aq) (2 × 20 mL) and brine (1 × 20 mL). The organic layer was dried ($MgSO_4$), and the solvent removed *in vacuo*. The crude material was purified by silica gel chromatography (CH_2Cl_2/CH_3OH 96:4 to 90:10) to yield the product as a white solid (81 mg, 85%).

***R*f** 0.36 (CH_2Cl_2/CH_3OH 92:8).

Mp 215 °C (dec).

ν_{max}/cm^{-1} (neat): 3300 (N–H), 3080 (C–H), 2980 (C–H), 2920 (C–H), 2870 (C–H), 1640 (C=O), 1510, 1440, 1360, 1250, 1160, 1100, 1080, 1020.

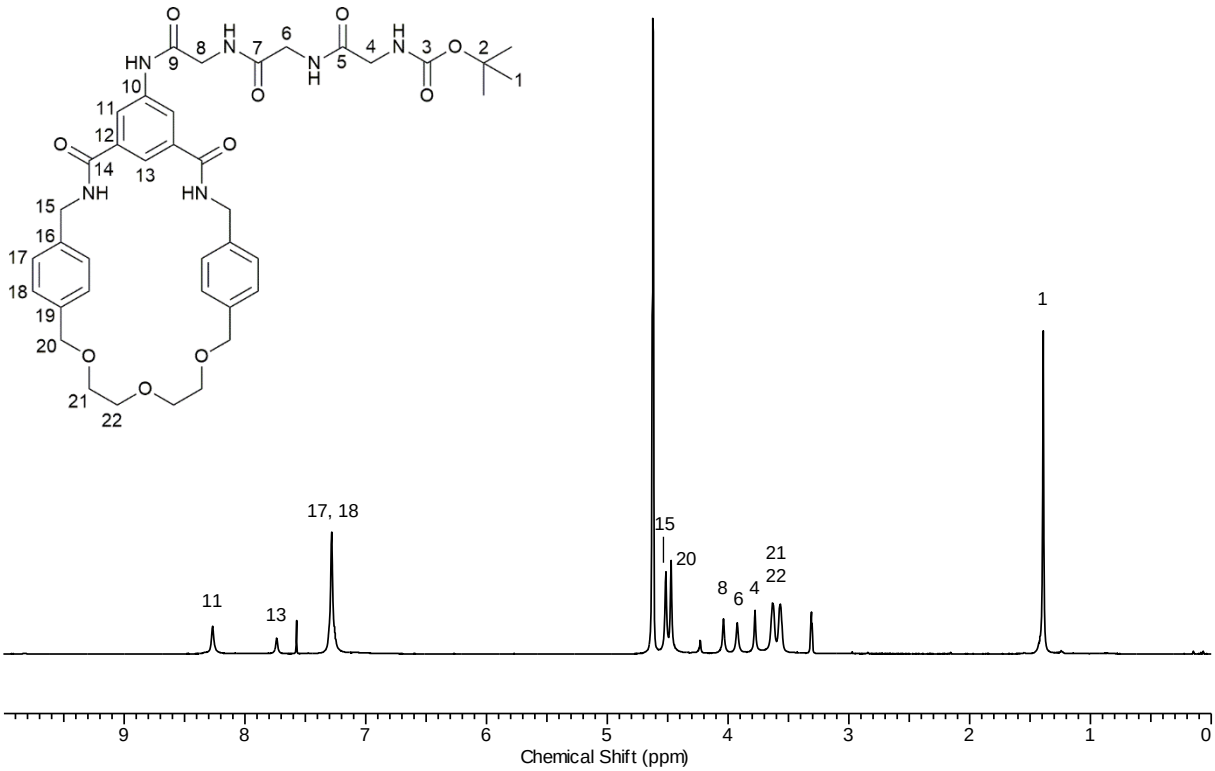
δ_H (400 MHz; $CDCl_3$/CD_3OD 1:1): 8.27 (br s, 2H, $C^{11}H$), 7.74 (br s, 1H, $C^{13}H$), 7.28 (br s, 8H, $C^{17}H$ & $C^{18}H$), 4.52 (s, 4H, $C^{15}H$), 4.47 (s, 4H, $C^{20}H$), 4.04 (s, 2H, $C^{8}H$), 3.92 (s, 2H, $C^{6}H$), 3.78 (s, 2H, $C^{4}H$), 3.57-3.63 (m, 8H, $C^{21}H$ & $C^{22}H$), 1.39 (s, 9H, $C^{1}H$).

δ_C (100 MHz; $CDCl_3$/CD_3OD 1:1): 173.1 (C^5), 171.6 (C^7), 169.3 (C^9), 168.3 (C^{14}), 158.2 (C^3), 139.7 (C^{10} or C^{12}), 138.3 (C^{16}), 138.0 (C^{19}), 136.2 (C^{10} or C^{12}), 129.3 (C^{17} or C^{18}), 129.0 (C^{17} or C^{18}), 123.2 (C^{11}), 121.1 (C^{13}), 80.9 (C^2), 73.7 (C^{20}), 71.2 (C^{21} or C^{22}), 70.0 (C^{21} or C^{22}), 44.6 (C^4 & C^{15}), 43.7 (C^6 & C^8), 28.7 (C^1).

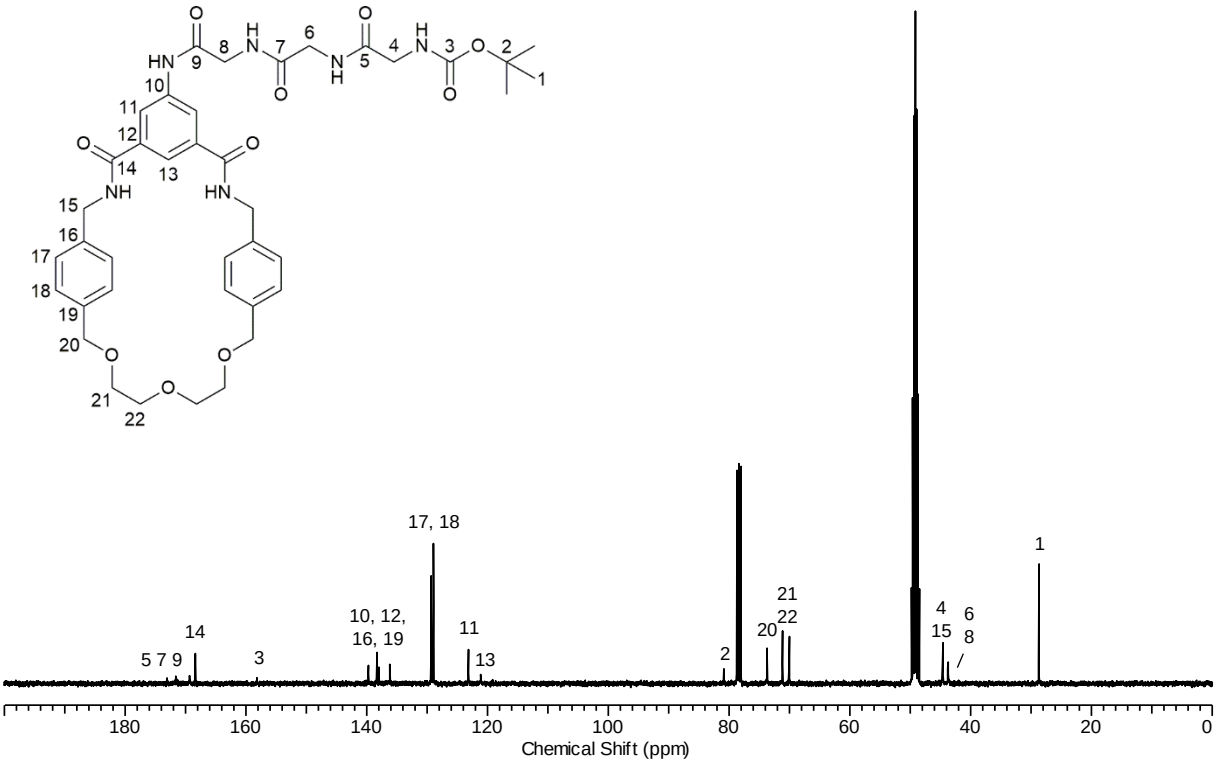
***m/z* (ES):** 783.3326 ($[M + Na]^+$ $C_{39}H_{48}N_6NaO_{10}$ requires 783.3324).

Boc-Peptide Macrocycle **ESI-5**

^{1}H NMR ($CDCl_3$/CD_3OD 1:1, 400 MHz)

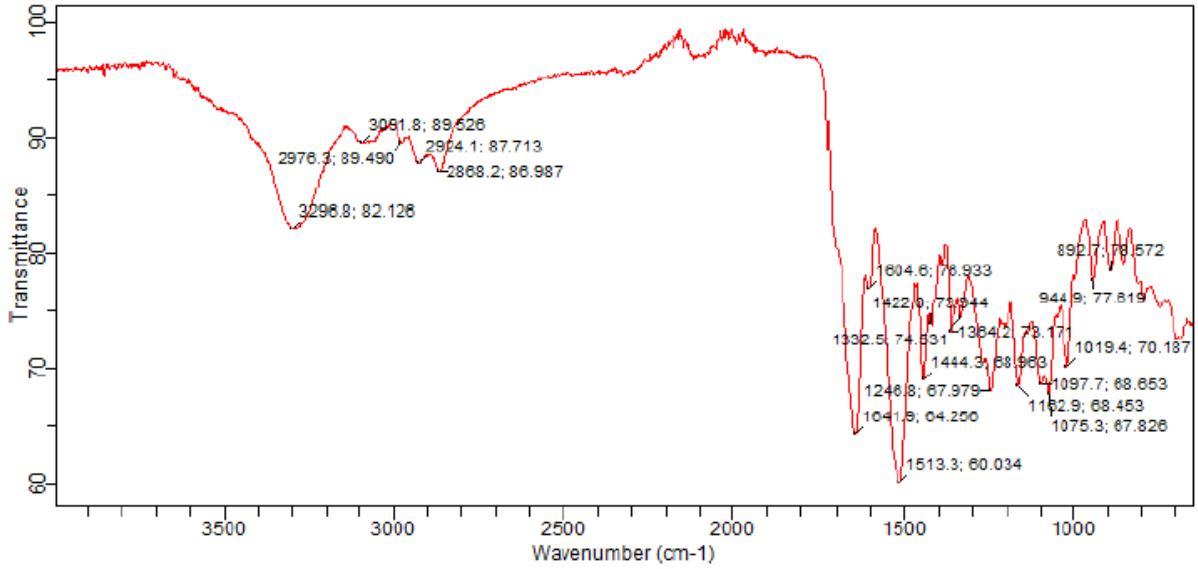


^{13}C NMR ($CDCl_3$/CD_3OD 1:1, 100 MHz)

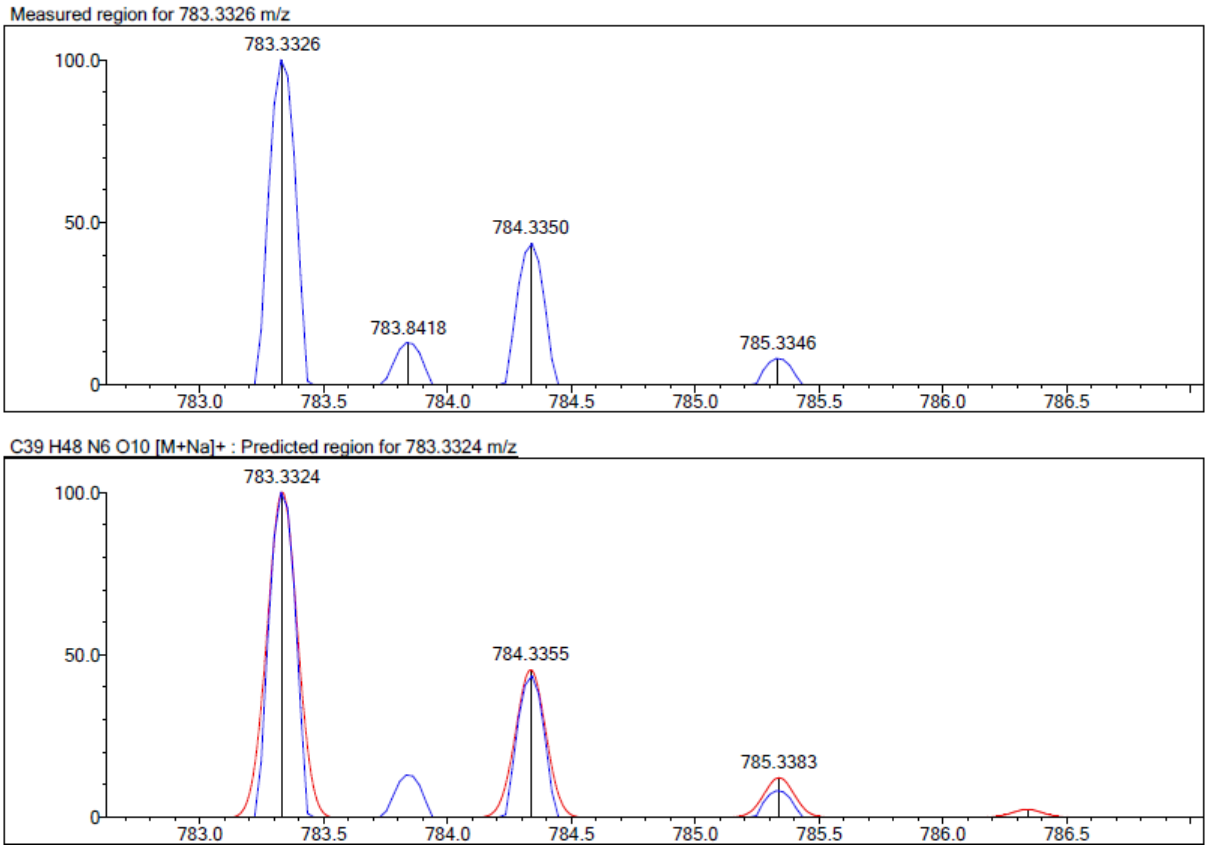


Boc-Peptide Macrocycle **ESI-5**

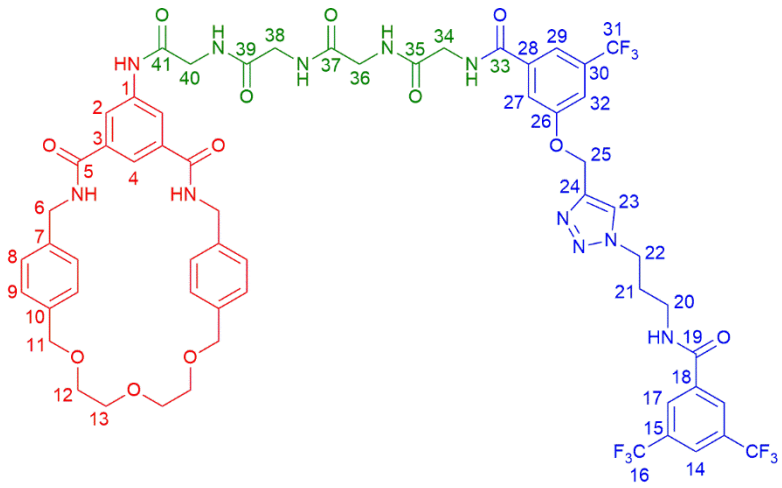
IR Spectrum (neat)



Mass Spectrum (ES +ve)



Unthreaded "[1]Rotaxane" **8**



Boc-peptide macrocycle **ESI-5** (75 mg, 0.099 mmol) was dissolved in CH_2Cl_2 (2 mL) and TFA (1 mL) added dropwise. The reaction mixture was stirred at r.t. for 4 h. Volatiles were removed *in vacuo* and and deprotection confirmed by ^{1}H NMR. Simultaneously, axle **7** (two batches of 71 mg, 0.102 mmol) was dissolved in CH_2Cl_2 (2 mL) and TFA (1 mL) added dropwise. The reaction mixture was stirred at r.t. for 4 h. Volatiles were removed *in vacuo* and deprotection confirmed by ^{1}H NMR. Deprotected macrocycle was suspended in dry DMF (5 mL) and cooled to 0 °C under an Ar (g) atmosphere. Then dry NEt_3 (103 mg, 141 μL, 1.02 mmol) was added followed by first batch of deprotected axle in dry DMF (2 mL), HOBt.hydrate (18 mg, 0.13 mmol) and EDCI.HCl (25 mg, 0.13 mmol), maintaining the temperature at 0 °C. The reaction mixture was stirred at r.t. for ~ 20 h. The reaction flask was then cooled to 0 °C and the second batch of deprotected axle in dry DMF (2 mL), HOBt.hydrate (18 mg, 0.13 mmol) and EDCI.HCl (25 mg, 0.13 mmol) were added. The reaction mixture was stirred at r.t. for 24 h. The solvent was removed *in vacuo* and an attempt to purify the crude material by silica gel chromatography (CH_2Cl_2/CH_3OH 96:4 to 85:15) undertaken. While some product (37 mg, "29%" – after washing with water) eluted this was contaminated with triethylamine hydrochloride (removed by the water wash) and other organic material. Clean product (28 mg, 22%) was obtained as a white film after mechanical recovery from the top of sand layer at the top of the column and washing with water.

***R*f** 0.26 (CH_2Cl_2/CH_3OH 90:10).

ν_{max}/cm^{-1} (neat): 3300 (N–H), 3100 (C–H), 2940 (C–H), 2870 (C–H), 1650 (C=O), 1600, 1530, 1450, 1360, 1280, 1170, 1120, 1020.

δ_H (400 MHz; D$_6$-DMSO): 10.14 (1H, s, C^{41}NH), 9.07 (1H, t, ^3J = 5.7 Hz, C^{33}NH), 9.03 (1H, t, ^3J = 5.4 Hz, C^{19}NH), 8.72 (2H, t, ^3J = 5.4 Hz, C^{5}NH), 8.49 (2H, s, C^{17}H), 8.23-8.31 (7H, m, C^{14}H, C^{23}H, C^{35}NH, C^{37}NH, C^{39}NH, C^{2}H), 7.85 (1H, s, C^{4}H), 7.83 (2H, app s, C^{27}H & C^{29}H), 7.55 (1H, s, C^{32}H), 7.32 (4H, d, ^3J = 8.0 Hz, C^{8}H), 7.27 (4H, d, ^3J = 8.0 Hz, C^{9}H), 5.31 (2H, s, C^{25}H), 4.45-4.50 (6H, m, C^{22}H & C^{6}H), 4.40 (4H, s, C^{11}), 3.96 (2H, d, ^3J = 5.7 Hz, C^{34}H), 3.92 (2H, d, ^3J = 5.7 Hz, C^{40}H), 3.80-3.82 (4H, m, C^{36}H & C^{38}H), 3.49 (8H, br, C^{12}H & C^{13}H), 3.36 (obscured, 2H, C^{20}H), 2.10-2.17 (2H, app quin, C^{21}H).

δ_C (100 MHz; D_6-DMSO): 169.4 (C^{37} or C^{39}), 169.3 (C^{37} or C^{39}), 169.2 (C^{35}), 168.0 (C^{41}), 166.0 (C^{5}), 164.9 (C^{33}), 163.4 (C^{19}), 158.4 (C^{26}), 141.9 (C^{24}), 139.1 (*), 138.3 (C^{7}), 137.0 (C^{10}), 136.5 (*), 136.5 (sic, *), 135.4 (*), 130.4 (^{2}J = 33 Hz, C^{15}), 130.4 (sic, ^{2}J = 32 Hz, C^{30}), 128.4 (C^{9}), 128.0 (C^{8} & C^{17}), 124.9 (C^{14} & C^{23}), 123.7 (q, ^{1}J = 272 Hz, C^{31}), 123.1 (q, ^{1}J = 272 Hz, C^{16}), 120.9 (C^{2}), 120.1 (C^{4}), 117.9 (C^{27}), 116.4 (br, C^{29}), 114.2 (br, C^{32}), 72.0 (C^{11}), 69.8 (C^{12} or C^{13}), 68.8 (C^{12} or C^{13}), 61.9 (C^{25}), 47.4 (C^{22}), 42.8 (C^{6}), 42.7 (C^{34} & C^{40}), 42.1 (C^{36} or C^{38}), 42.1 (sic, C^{36} or C^{38}), 36.9 (C^{20}), 29.5 (C^{21}).

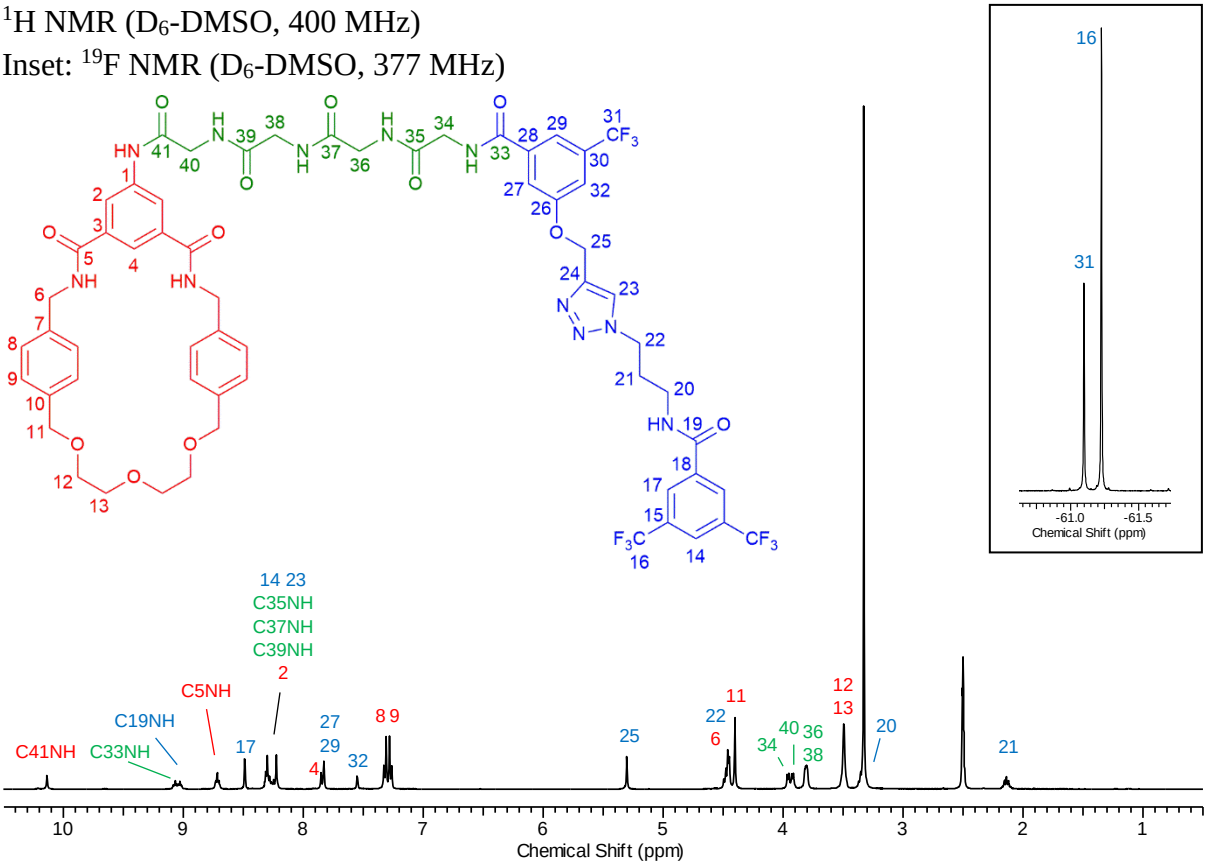
* = *C^{1} or C^{3} or C^{18} or C^{28}*.

δ_F (377 MHz; D_6-DMSO): –61.2 ($C^{31}F_3$), –61.3 ($C^{16}F_3$).

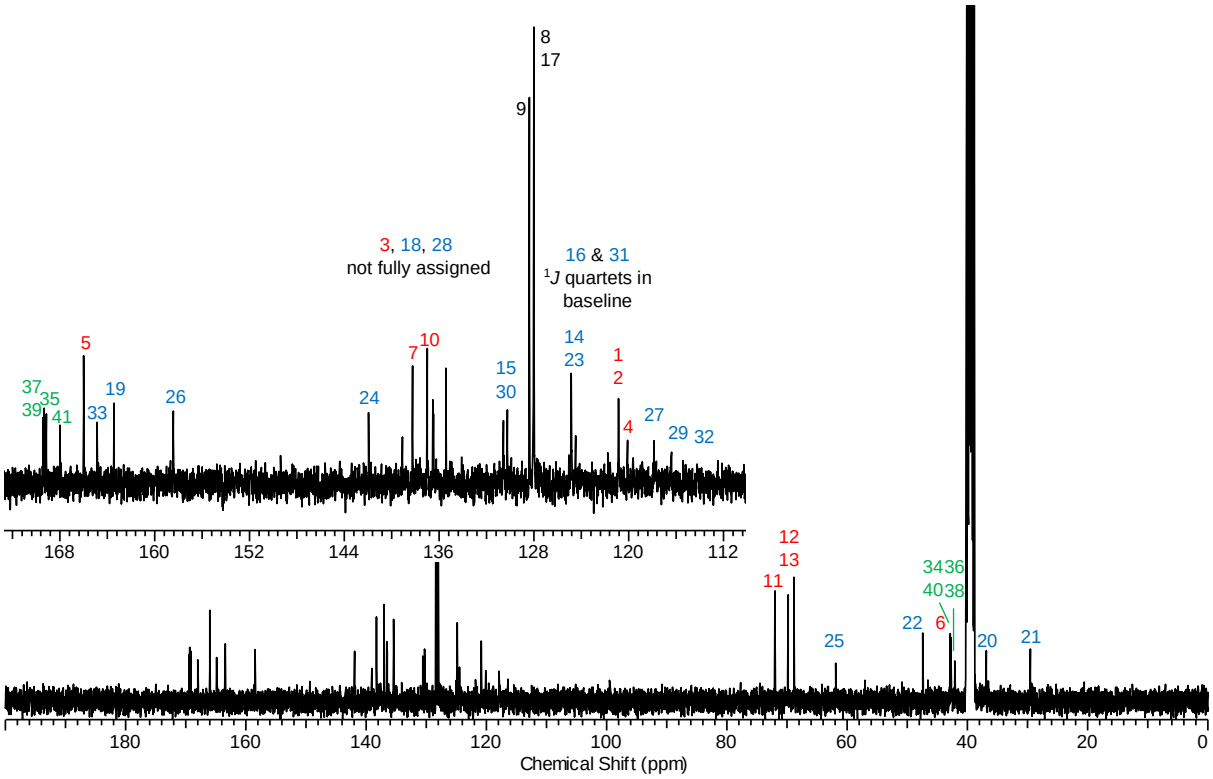
***m/z* (ES):** 1284.4160 ($[M + H]^+$ $C_{59}F_9H_{59}N_{11}O_{12}$ requires 1284.4195).

Unthreaded “[1]Rotaxane” **8**

^{1}H NMR (D_6-DMSO, 400 MHz)
Inset: ^{19}F NMR (D_6-DMSO, 377 MHz)

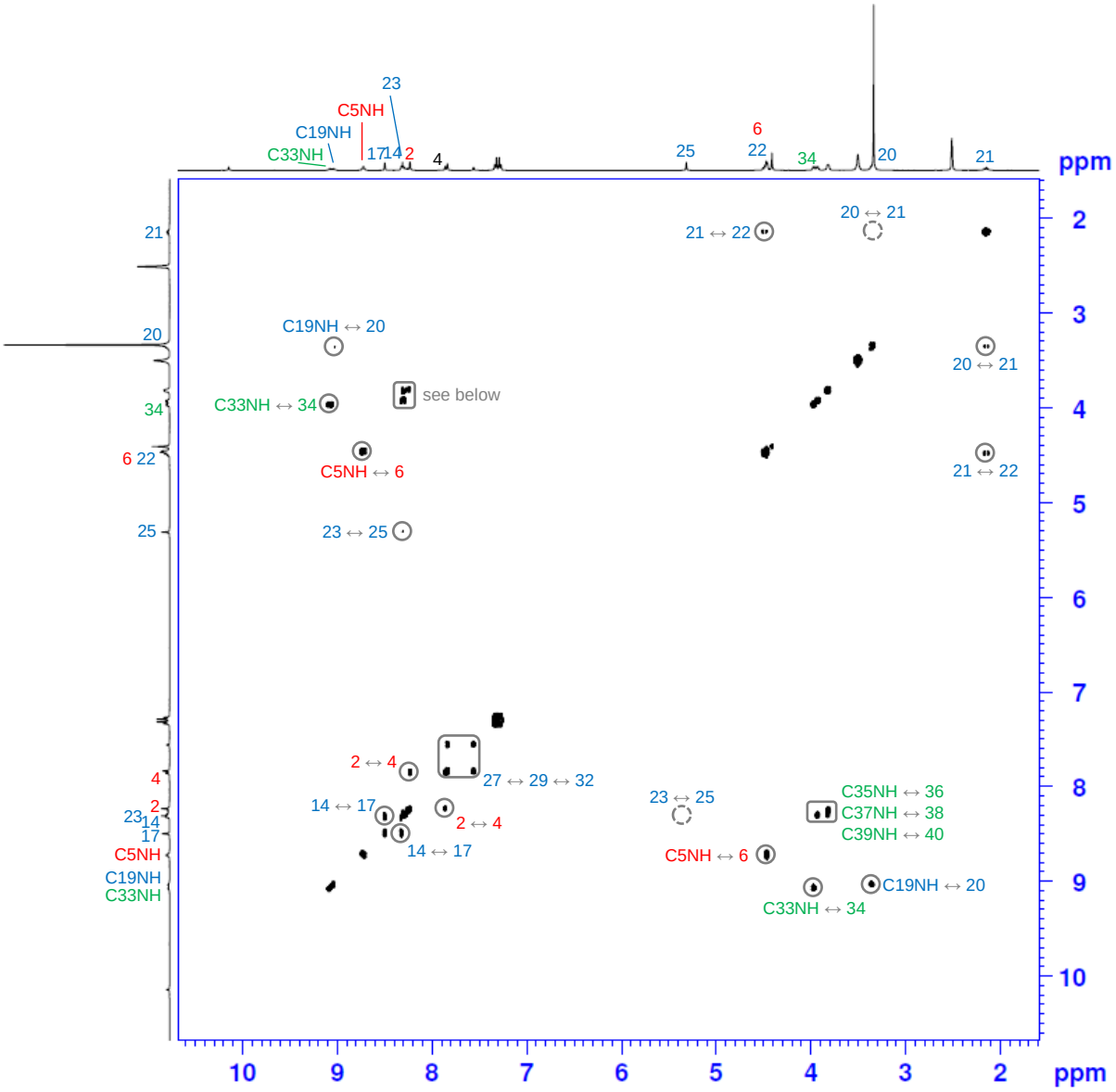


^{13}C NMR (D_6-DMSO, 100 MHz)



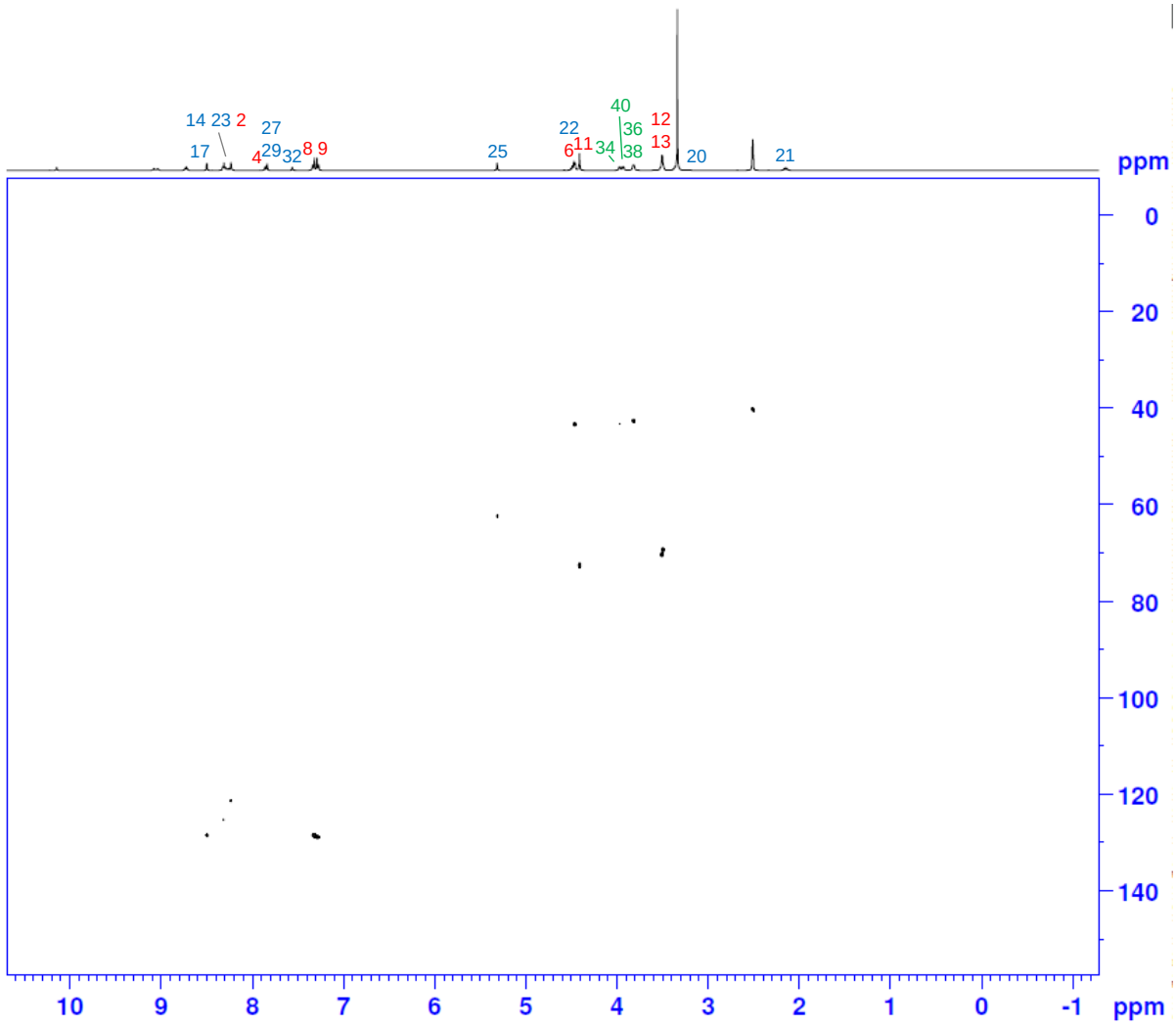
Unthreaded "[1]Rotaxane" **8**

^{1}H-^{1}H COSY NMR (D_6-DMSO, 400 MHz)
Selected peaks and cross-peaks labelled



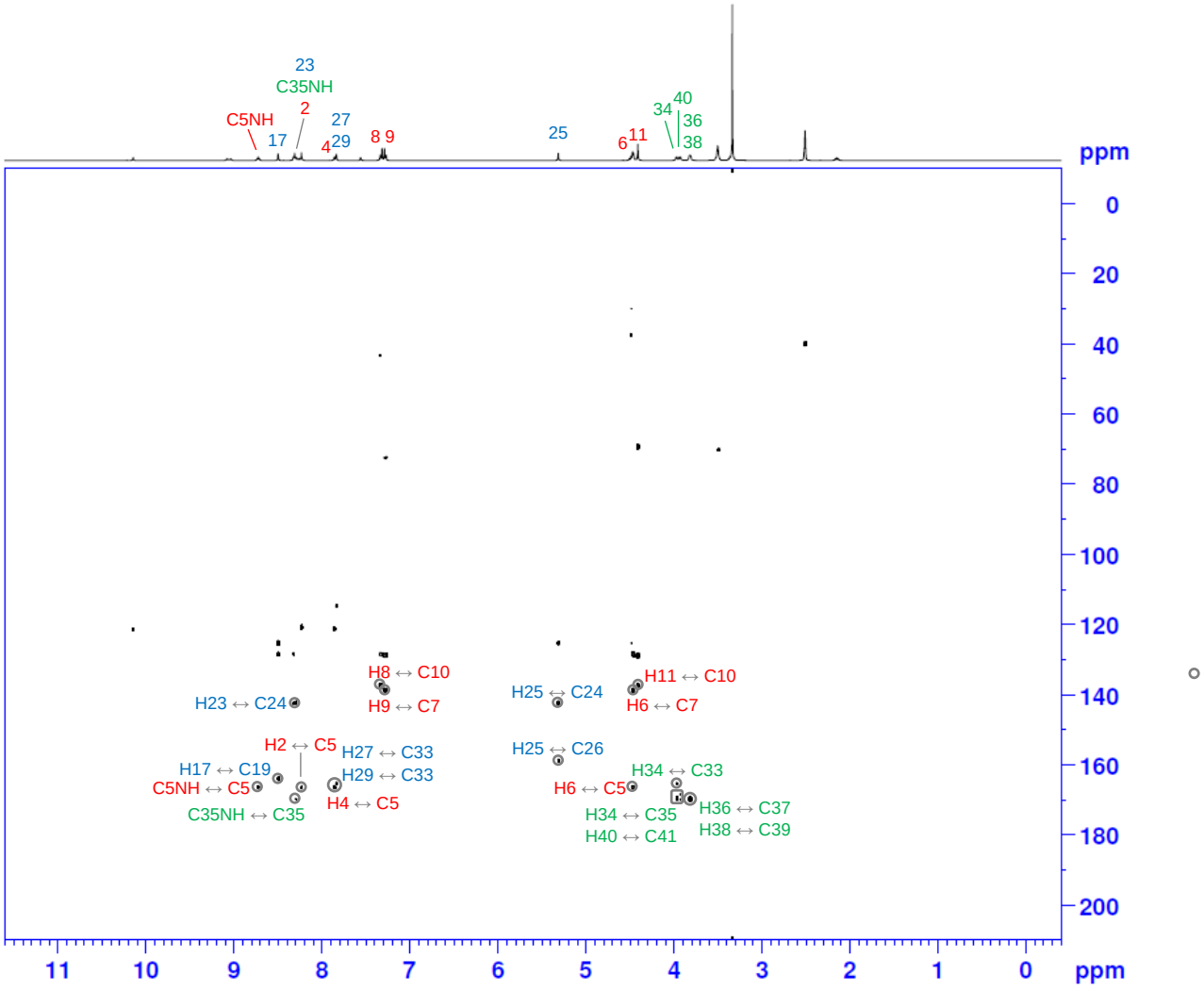
Unthreaded “[1]Rotaxane” **8**

^{1}H-^{13}C HSQC NMR (D_6-DMSO, 400 MHz)
C-H peaks labelled



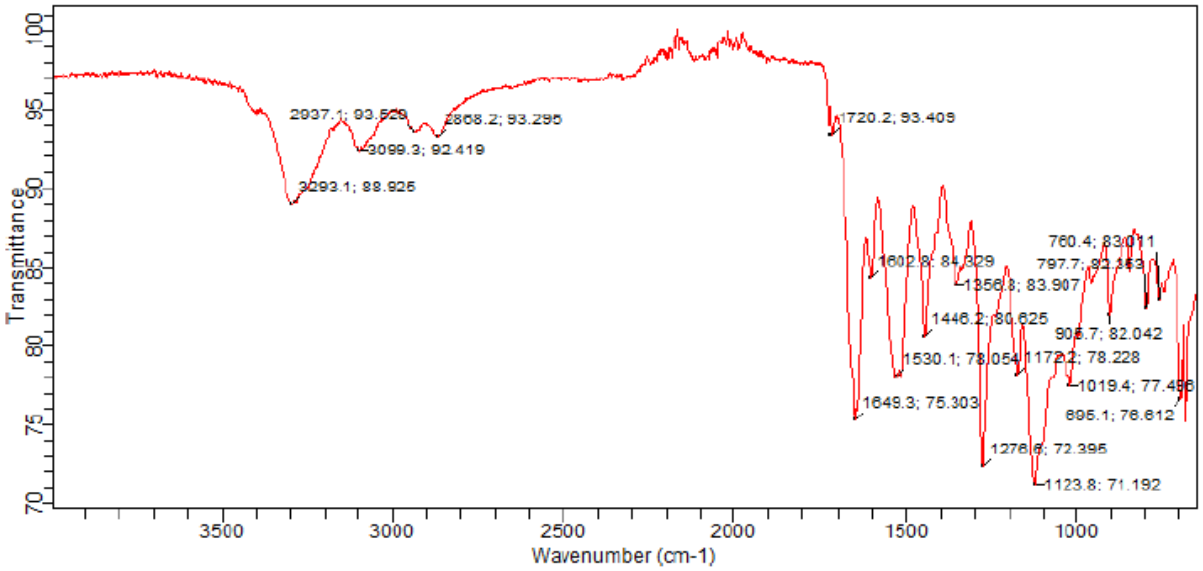
Unthreaded "[1]Rotaxane" **8**

^{1}H-^{13}C HMBC NMR (D_6-DMSO, 400 MHz)
Selected peaks and quaternary correlations labelled

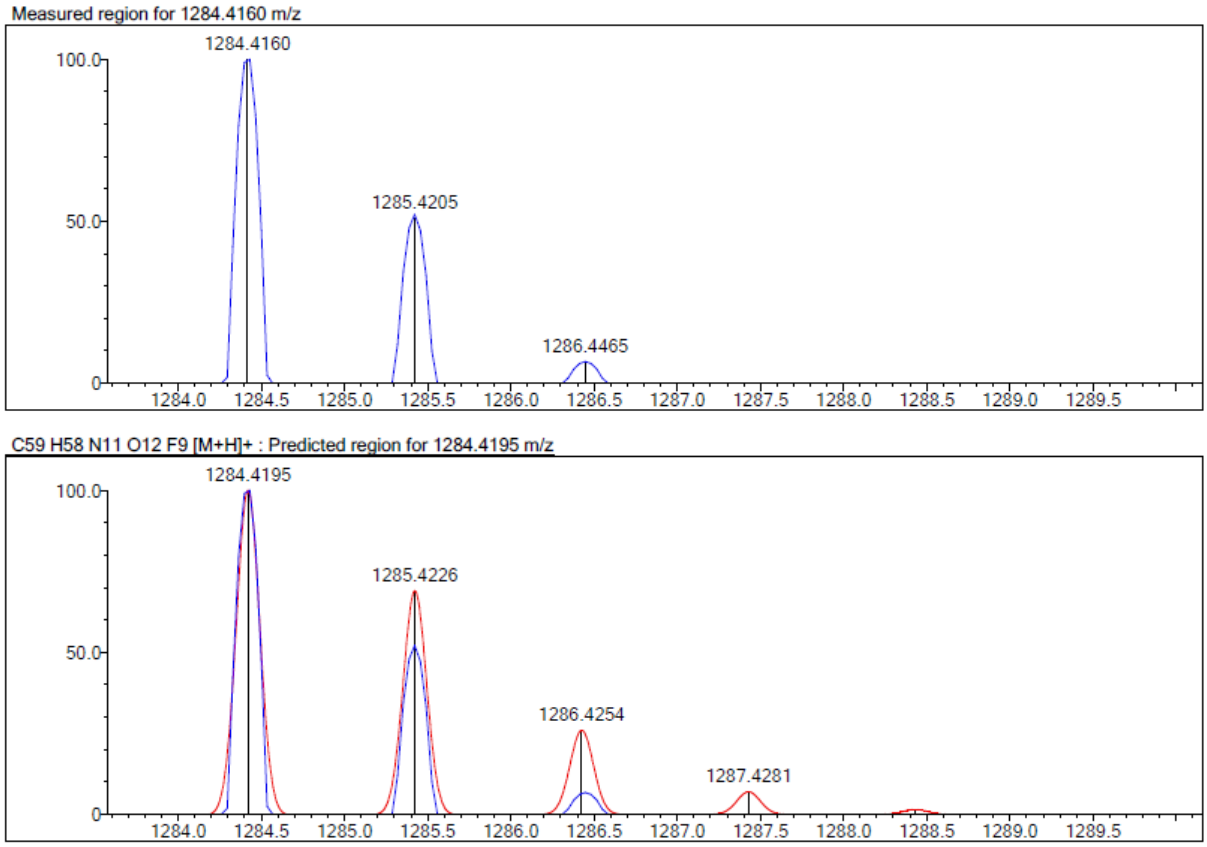


Unthreaded “[1]Rotaxane” **8**

IR Spectrum (neat)



Mass Spectrum (ES +ve)



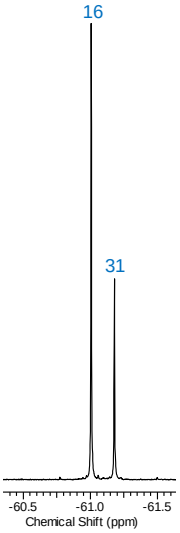
Further NMR Comparison of [1]Rotaxane **6** and Unthreaded "[1]Rotaxane" **8**

2.5 mg dissolved in 600 μL in D_6-DMSO (due to very limited solubility of **8**).

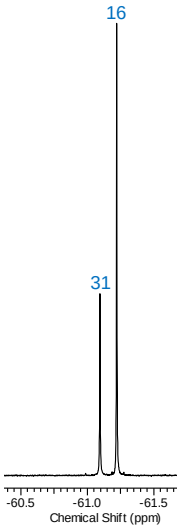
^{1}H NMR spectra depicted in Figure 3 of main article.

^{19}F NMR (D_6-DMSO, 377 MHz)

[1]Rotaxane **6**



Unthreaded "[1]Rotaxane" **8**



VT ^{1}H NMR (D_6-DMSO, 400 MHz)

The temperature dependence of amide proton shifts is commonly used in the structural determination of proteins in aqueous solution.[5] Empirically, amide chemical shifts decrease with increasing temperature. This is attributed to lengthening of hydrogen bonds (whether transiently to solvent, or persistently through intramolecular interactions), and therefore a reduction in the contribution of hydrogen bonding to the observed chemical shift.

Intramolecular hydrogen bonds are stronger and more long-lived than ones with the solvent, so are more resistant to deformation and leads to slower reductions in the chemical shift. In aqueous solution, temperature coefficients $\Delta\delta/\Delta T > -4.5$ ppb/K are attributed to intramolecular hydrogen bonds, while those that are more negative (< -4.5 ppb/K) are deemed to be freely interacting with the solvent. It is important to note that this threshold is only valid in cases where there are no changes in secondary structure.[5b] Deviations from non-linearity[5a] of shift with temperature are unambiguous indicators of this type of behaviour (this was not the case for **6** and **8** over 298-348 K, $R^2 > 0.99$), but effects of temperature on conformational equilibria can easily appear to affect the observed shifts in an apparently linear fashion, and thus give misleading information.[5b]

Since the hydrogen bonding properties of D_6-DMSO are somewhat different to water, it is reasonable to assume the appropriate threshold for hydrogen bonding is different. The limited data for rigid peptides is consistent with all coefficients being slightly less negative on average.[6]

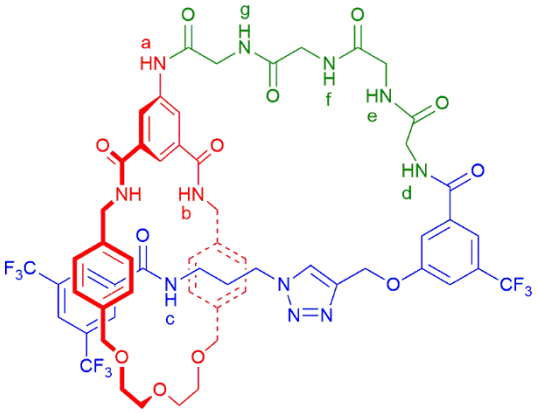
The two exceptional temperature coefficients for [1]rotaxane **6** hint at a combination of factors. While the coefficient of −1.1 ppb/K for axle amide *c* is explicable by citing inaccessibility of solvent – it would be expected that the same trend would be observed for macrocyclic protons *b*, but the recorded coefficient is −6.7 ppb/K. We note that exceptions do exist, e.g. proximity of NH to ring currents associated with aromatic amino acid residues.[5d]

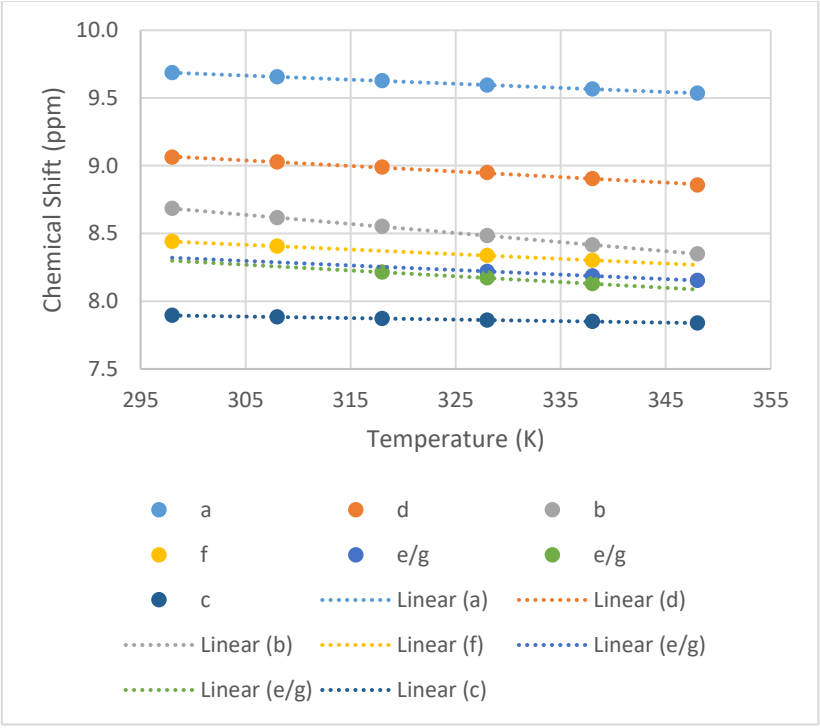
We therefore conclude that the unusual chemical environment of a threaded axle through a macrocycle leads to a range of non-covalent interactions (not just hydrogen bonding) affecting temperature coefficients.

VT ^{1}H NMR (D_6-DMSO, 400 MHz)

[1]Rotaxane **6**

Proton	Chemical Shift (ppm)						Slope (ppb/K)
	298K	308K	318K	328K	338K	348K	
a	9.687	9.656	9.626	9.595	9.565	9.536	−3.0
d	9.062	9.028	8.990	8.949	8.905	8.858	−4.1
b	8.684	8.617	8.551	8.483	8.416	8.349	−6.7
f	8.440	8.406		8.337	8.302		−3.5
e/g				8.220	8.187	8.153	−3.4
e/g			8.214	8.171	8.129		−4.3
c	7.895	7.883	7.872	7.861	7.850	7.839	−1.1

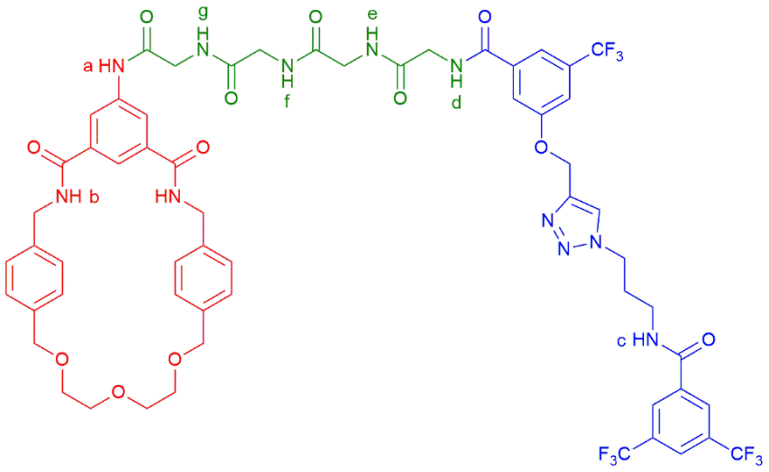


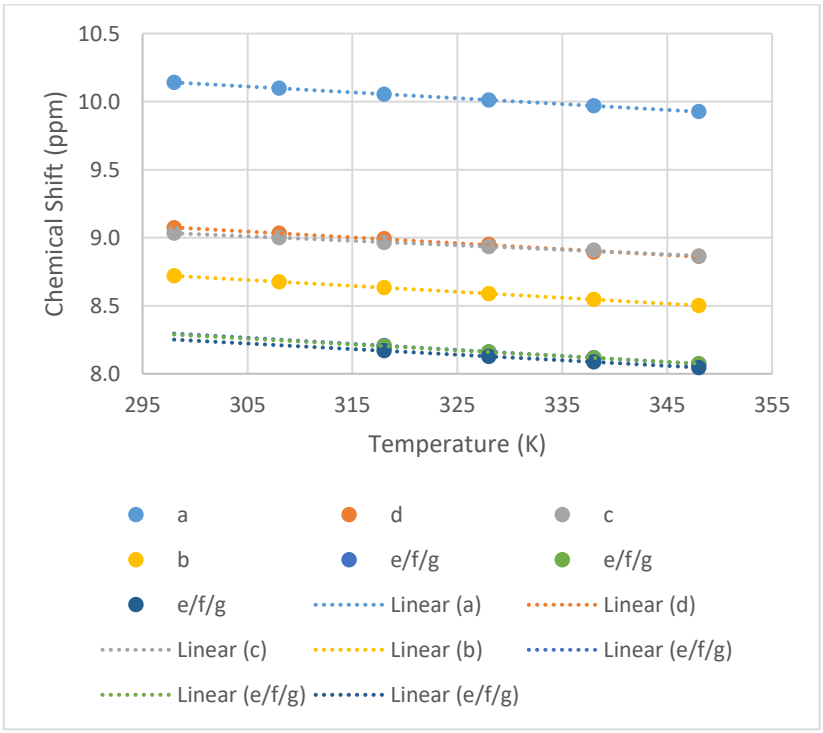


VT ^{1}H NMR (D_6-DMSO, 400 MHz)

Unthreaded "[1]Rotaxane" **8**

Proton	Chemical Shift (ppm)						Slope (ppb/K)
	298K	308K	318K	328K	338K	348K	
a	10.142	10.098	10.055	10.011	9.969	9.927	−4.3
d	9.073	9.033	8.992	8.950	8.895	8.863	−4.3
c	9.033	9.000	8.966	8.934	8.909	8.863	−3.3
b	8.720	8.676	8.633	8.589	8.546	8.502	−4.4
e/f/g			8.208	8.161	8.117	8.074	−4.5
e/f/g			8.202	8.161	8.117	8.074	−4.3
e/f/g			8.169	8.128	8.087	8.046	−4.1





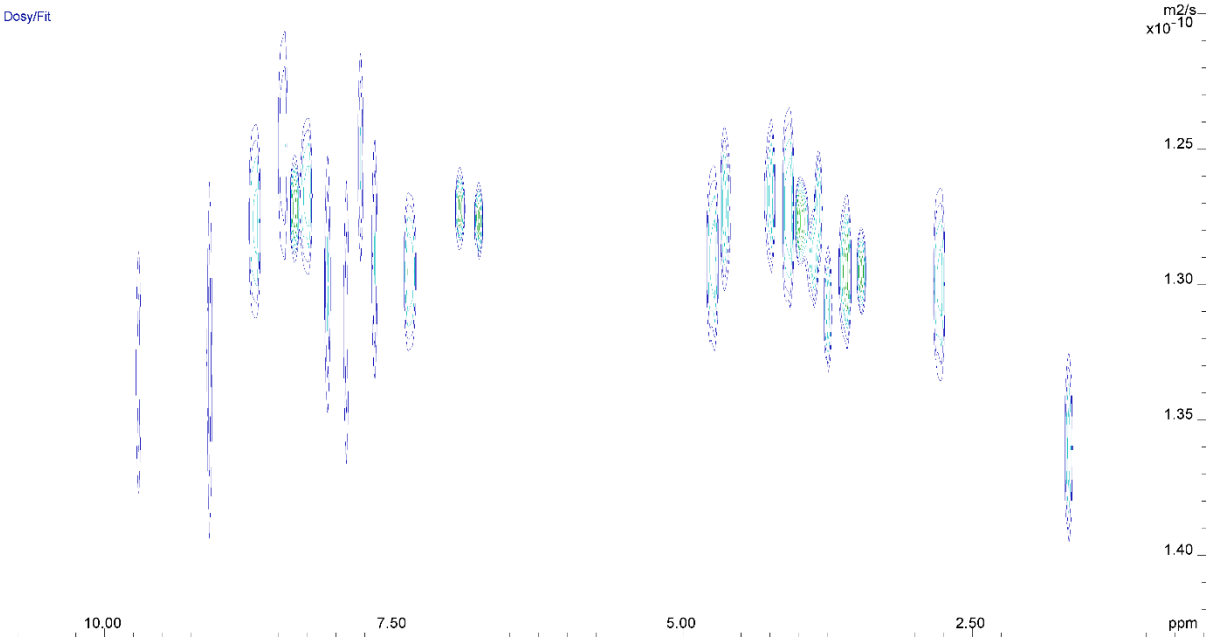
^{1}H DOSY NMR (D_6-DMSO, 400 MHz)

Diffusion NMR experiments were carried out using the Stimulated Echo technique, with bipolar diffusion-encoding gradients and longitudinal eddy current delay (bpp-LED-STE,[7] pulse program ledbpgp2s). Experiments used four dummy scans and 16 transients and a relaxation of 1 s. Gradient strength was increased from 10 to 95% of the maximum strength in 16 linear steps, with gradient pulse lengths of 1.2 ms (P30), sine-smoothed square shapes (GPNAM6 SMSQ10.100), and a diffusion period of 0.15 s. This gave diffusive attenuation on the order of 70% for unthreaded **8**. The gradient strength was calibrated as 49.7 G/cm using the standard doped water reference sample (1% H_2O in D_2O, with 0.1 mg/mL $GdCl_3$) as being 1.91e-9 m^2s^{-1},[8] at a temperature of 298.0 K calibrated using 99.8% CD_3OD.[9]

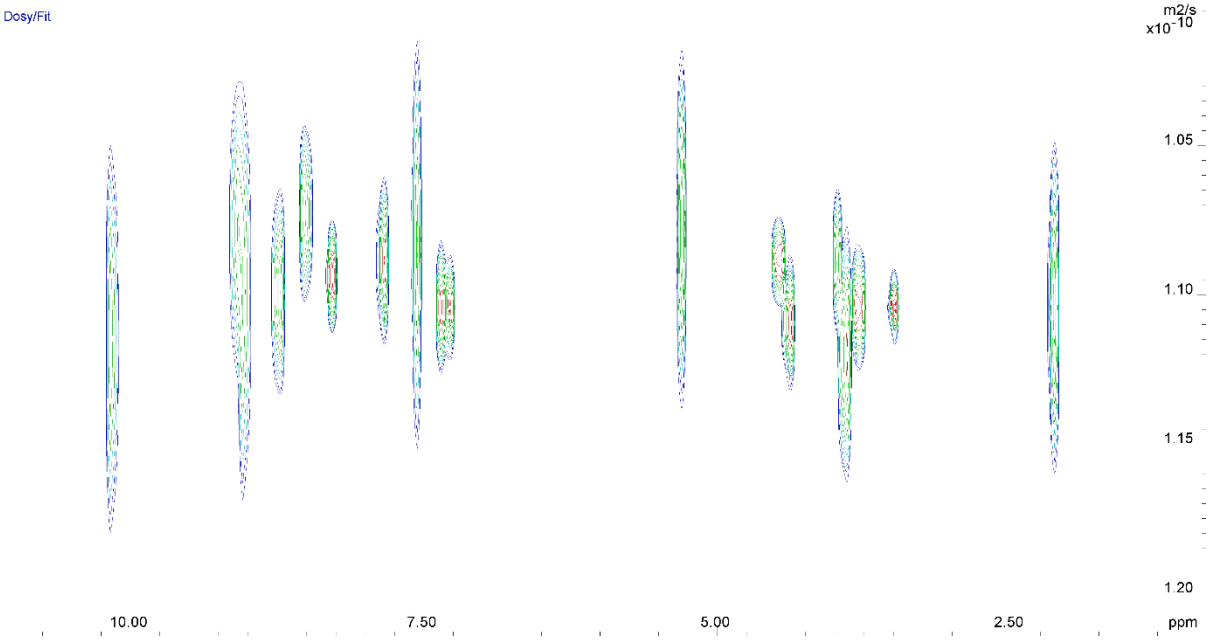
Data was processed using peak areas in Dynamics Centre 2.6. The reported values for D in the main article are the mean and standard deviation across all the peaks in the sample, with the exception of peak *20* in **8** which overlapped with adventitious water. That the D is consistent across both exchangeable and non-exchangeable peaks is evidence that exchange with water had no material impact on shift measurements made on these peaks.

^{1}H DOSY NMR (D_6-DMSO, 400 MHz)

[1]Rotaxane **6**



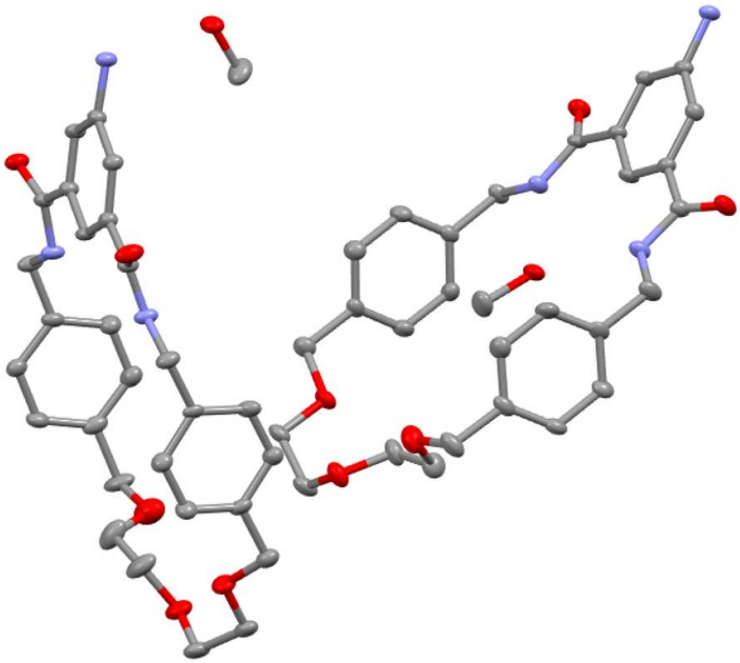
Unthreaded "[1]Rotaxane" **8**



Crystallography

Amino Macrocycle **3**

Single crystals of amino macrocycle **3** were grown by slow evaporation of a chloroform/methanol solution. A suitable crystal was selected and mounted on a MITIGEN loop using Paratone-N oil on a Supernova Dual (Cu at home) AtlasS2 diffractometer. The crystal was kept at 99.96(11) K during data collection. Using Olex2,[10] the structure was solved with the ShelXT[11] structure solution program using Intrinsic Phasing and refined with the ShelXL[12] refinement package using Least Squares minimisation.



*X-ray crystal structure of amino macrocycle **3**. Thermal ellipsoids are displayed at 50% probability.*

*Crystal data for amino macrocycle **3** (CCDC number: 1995143):*

$C_{29}H_{35}N_3O_6$ (*M* = 521.60 g/mol): monoclinic, space group $P2_1$ (no. 4), *a* = 7.9726(2) Å, *b* = 30.0112(8) Å, *c* = 11.2602(3) Å, β = 100.176(3)°, *V* = 2651.82(12) Å^3, *Z* = 4, *T* = 99.96(11) K, μ(CuKα) = 0.750 mm^{-1}, *Dcalc* = 1.306 g/cm^3, 11400 reflections measured (7.978° ≤ 2Θ ≤ 144.198°), 7672 unique (R_{int} = 0.0463, R_{sigma} = 0.0435) which were used in all calculations. The final R_1 was 0.0639 (I > 2σ(I)) and wR_2 was 0.1835 (all data).

Computation

The Gaussian 09 program was used to conduct all density functional theory calculations.[13] In each case, geometry optimisations using the B3LYP/6-31G* model chemistry were undertaken.[14-17] DMSO solvent effects were modelled using the default polarisable continuum model (and default solvent cavity parameters) as defined in Gaussian 09. To obtain reasonable starting structures, pre-optimisation was carried out using molecular mechanics and semi-empirical PM6 methods as necessary.

The log output files were converted to .xyz files using Avogadro software, before the figures presented in the main manuscript were generated using Mercury 4.0.0.

The calculated energies of the minimized structures were:
[1]Rotaxane **6** −4686.91181339 a.u.
Unthreaded "[1]Rotaxane" **8** −4686.89116433 a.u.

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