Supplementary Information:

Anion recognition in water by a rotaxane containing a secondary rim functionalised cyclodextrin stoppered axle

Michal Řezanka,[a,b] Matthew J. Langton[b] and Paul D. Beer[b]

[a] Institute for Nanomaterials, Advanced Technologies and Innovation, Technical University of Liberec, Studentská 1402/2, 461 17 Liberec, Czech Republic.

[b] Chemistry Research Laboratory, Department of Chemistry, University of Oxford, Mansfield Road, Oxford OX1 3TA, United Kingdom. Email: paul.beer@chem.ox.ac.uk

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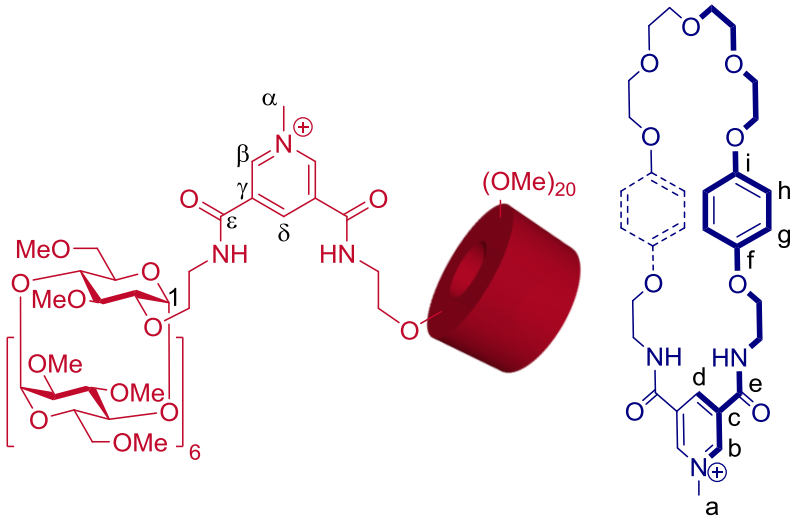
Experimental section

General information

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-1, with $CHCl_3$ as the eluent. Where mixtures of solvents were used, ratios are reported by volume. Triethylamine was distilled from and stored over potassium hydroxide. NMR spectra were recorded on a Bruker AVII 500 (with cryoprobe) and Bruker AVIII 500 spectrometers. Numbering of atoms for NMR spectra transcription was done according to SI Figure 1. Mass spectra were carried out on a Waters Micromass LCT and Bruker microTOF spectrometers.

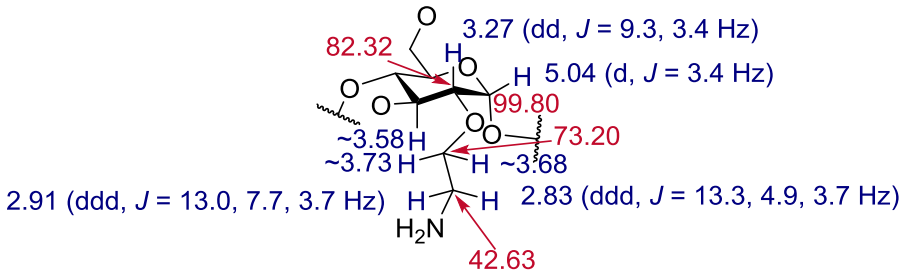


SI Figure 1. An example of atom numbering.

Experimental procedures

Compounds **1-3** were synthesized as previously described and spectral data were in agreement with those published.[1,2] Bis-amine macrocycle precursor **6** was prepared as previously described.[3]

The position of the aminoethyl substituent on cyclodextrin skeleton of compound **3** was confirmed by 2D NMR spectroscopy techniques (^{1}H–^{1}H COSY, HSQC, HMBC) (SI Figure 2).



SI Figure 2. Confirmation of substituent position in 2^A-aminoethyl-per-*O*-methyl-β-CD (**3**). NMR shifts in ppm ($CDCl_3$).

Axle precursor 4:

Dry CH_2Cl_2 (8 ml) was added to a mixture of pyridine-3,5-dicarboxylic acid (12 mg, 0.071 mmol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (55 mg, 0.285 mmol), 1-hydroxybenzotriazole hydrate (24 mg, 0.143 mmol) and 4-(dimethylamino)pyridine (catalytic amount) under nitrogen atmosphere. The reaction mixture was stirred and a solution of 2^A-*O*-aminoethyl-per-O-methyl-β-cyclodextrin (**3**, 208 mg, 0.143 mmol) and dry Et_3N (58 µl, 0.57 mmol) in dry CH_2Cl_2 (4 ml) was added dropwise. Reaction mixture was stirred at rt for 3 days. The solvent was removed *in vacuo*, and the product was purified by chromatography on silica gel column (gradient CH_2Cl_2/MeOH from 30/1 to 5/1). Workup afforded 180 mg (83 %) of the title compound as a white powder. **^{1}H NMR** (500 MHz, $CDCl_3$) δ (ppm): 9.13 (s, 2 H, *H*-β), 8.64 (s, 1 H, *H*-δ), 7.77 (s, 2 H, -N*H*-), 5.15-4.88 (m, 14 H, *H*-1), 3.93-3.03 (m, 212 H, β-CD *H*, N-C*H*$_2$-C*H*$_2$-O). **^{13}C NMR** (125 MHz, $CDCl_3$) δ (ppm): 165.12 (*C*-ε), 149.82 (aromatic *C*), 135.69 (aromatic *C*), 130.40 (aromatic *C*), 99.66-98.71 (*C*-1), 82.30-58.26 (β-CD *C*, N-*C*H$_2$-*C*H$_2$-O). **HRMS** (ESI): m/z calcd. for $C_{135}H_{231}N_3Na_2O_{72}$ $[M+2\ Na]^{2+}$ 1546.2145; found 1546.2184.

Axle 5$^{\cdot}$Cl:

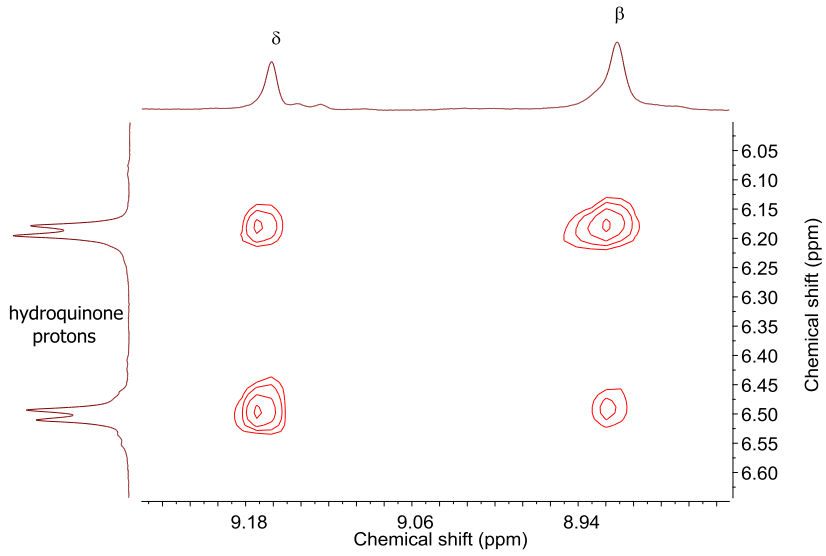
Pyridine axle precursor (**4**, 167 mg, 0.055 mmol) was dissolved in MeI (1 ml) in vial sealed with a cap. Reaction mixture was stirred at 35 °C overnight. The unreacted MeI was removed *in vacuo*, The product was dissolved in MeOH/water (1/1) and passed through an amberlite (chloride) column to give 157 mg (92 %) of chloride salt of the title compound as a white powder. **^{1}H NMR** (500 MHz, $CDCl_3$/CD_3OD = 1/1) δ (ppm): 9.50 (s, 2 H, *H*-β), 9.33 (s, 1 H, *H*-δ), 5.20-5.07 (m, 14 H, *H*-1), 4.56 (s, 3 H, *H*-α), 4.00-2.94 (m, 212 H, β-CD *H*, N-C*H*$_2$-

C*H*$_2$-O). **^{13}C NMR** (125 MHz, $CDCl_3/CD_3OD$ = 1/1) δ (ppm): 162.91 (*C*-ε), 148.18 (aromatic *C*), 142.47 (aromatic *C*), 136.04 (aromatic *C*), 99.76-99.37 (*C*-1), 83.66-58.90 (β-CD *C*, N-*C*H_2-*C*H_2-O). **HRMS** (ESI): m/z calcd. for $C_{136}H_{234}N_3NaO_{72}$ $[M+Na]^{2+}$ 1542.2314; found 1542.2346.

Rotaxane precursor 7·Cl:

Oxalyl chloride (39 µl, 0.64 mmol) and one drop of DMF was added to a suspension of pyridine-3,5-dicarboxylic acid (38 mg, 0.23 mmol) in dry CH_2Cl_2. Reaction mixture was stirred overnight and volatile compounds were removed *in vacuo*. Pyridine-3,5-dicarboxylic acid dichloride was used in next step.

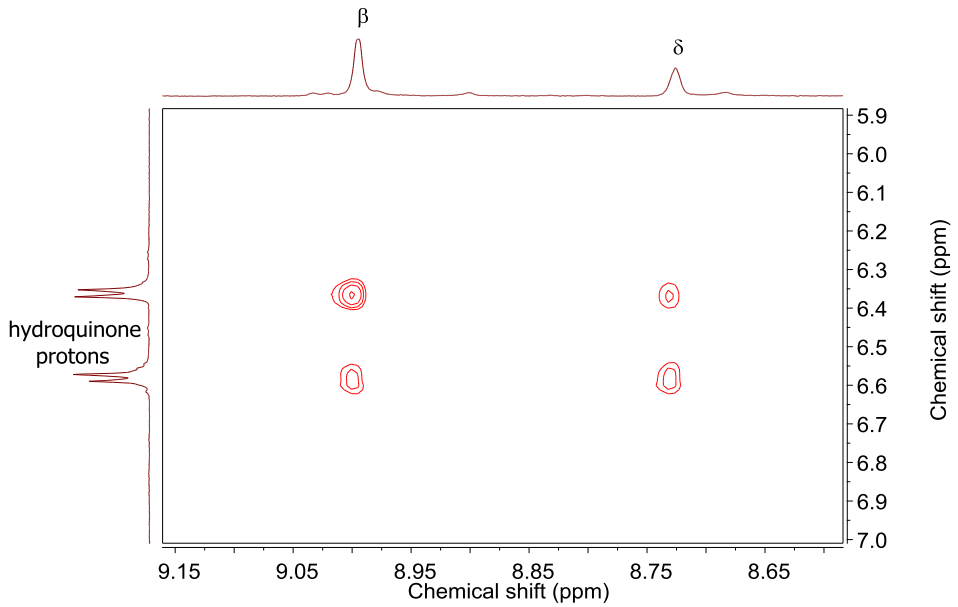
Axle **5** (71 mg, 0.023 mmol) and bis-amine **6** (106 mg, 0.23 mmol) were dissolved in dry CH_2Cl_2 (25 ml) and cooled down to 0 °C under N_2 atmosphere. Et_3N was added followed immediately by a dropwise addition of solution of pyridine-3,5-dicarboxylic acid dichloride in dry CH_2Cl_2 (8 ml). Reaction mixture was stirred overnight. The solvent was removed *in vacuo*, and the product was purified by chromatography on silica gel column (gradient CH_2Cl_2/MeOH from 40/1 to 10/1) and by size-exclusion column chromatography in $CHCl_3$. Workup afforded 36 mg (43 %) of the title compound as a white powder. **^{1}H NMR** (500 MHz, $CDCl_3/CD_3OD$ = 1/1) δ (ppm): 10.00 (s, 1 H, *H*-d), 9.36 (s, 2 H, *H*-b), 9.17 (s, 1 H, *H*-δ), 8.90 (s, 2 H, *H*-β), 6.50 (d, *J* = 8.6 Hz, 1 H, *H*-g or *H*-h), 6.18 (d, *J* = 8.4 Hz, 1 H, *H*-g or *H*-h), 5.15-5.01 (m, 14 H, *H*-1), 4.47 (s, 3 H, *H*-α), 4.22-3.11 (m, 236 H, β-CD *H*, polyether *H*, N-C*H*$_2$-C*H*$_2$-O). **^{13}C NMR** (125 MHz, $CDCl_3/CD_3OD$ = 1/1) δ (ppm): 164.45 (*C*-e), 160.86 (*C*-ε), 153.75 (*C*-f or *C*-i), 152.75 (*C*-f or *C*-i), 149.38 (*C*-b), 146.03 (*C*-β), 138.56 (*C*-δ), 137.16 (*C*-d), 133.56 (*C*-c, *C*-γ), 115.33 (*C*-g or *C*-h), 115.25 (*C*-g or *C*-h), 99.71-99.29 (*C*-1), 82.58-58.82 (β-CD *C*, polyether *C*, N-*C*H_2-*C*H_2-O), C-α overlapping with CD_3OD. **HRMS** (ESI): m/z calcd. for $C_{167}H_{271}N_6NaO_{81}$ $[M+Na]^{2+}$ 1839.8579; found 1839.8656.



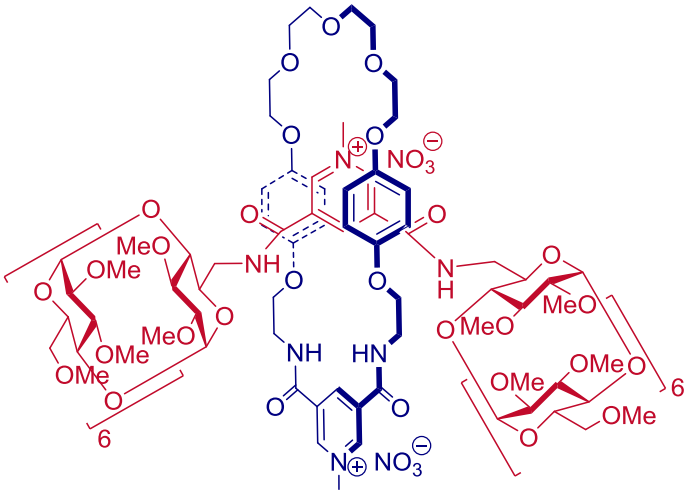
SI Figure 3. ROESY spectrum of rotaxane precursor **7 Cl**.

Rotaxane 8 $(NO_3)_2$:

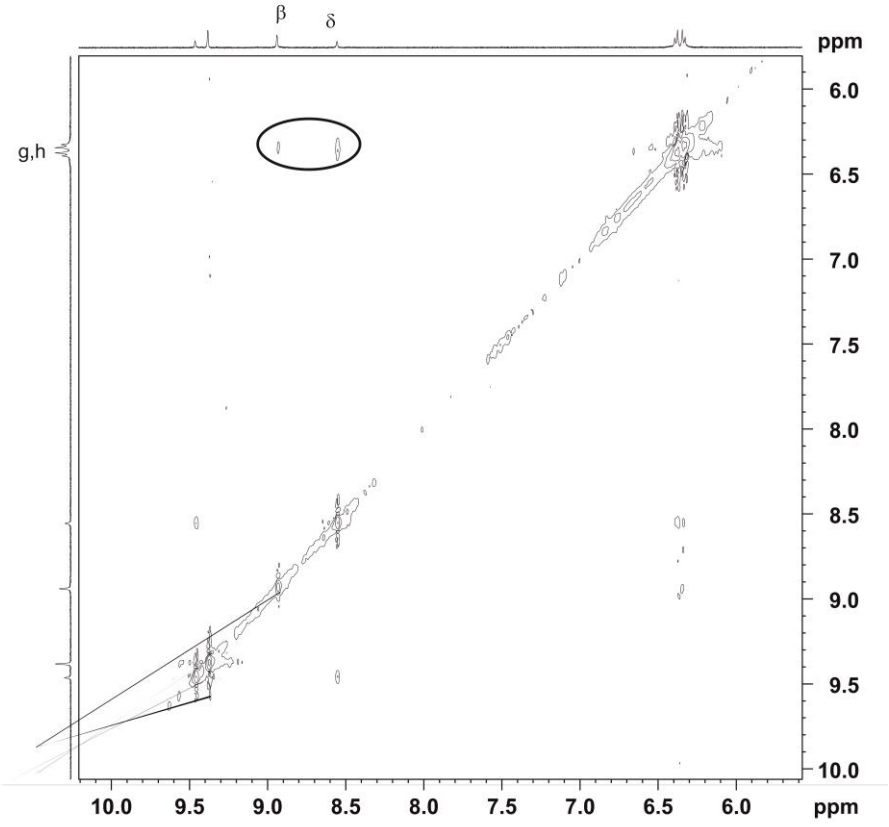
Rotaxane precursor (**7**, 36 mg, 0.01 mmol) was dissolved in MeI (1 ml) in vial sealed with a cap. Reaction mixture was stirred at 35 °C overnight. The unreacted MeI was removed *in vacuo*, The product was dissolved in MeOH/water (1/1) and passed through an amberlite (nitrate) column to give 37 mg (99 %) of di-nitrate salt of the title compound as a white powder. **^{1}H NMR** (500 MHz, $CDCl_3/CD_3OD$ = 1/1) δ (ppm): 9.82 (s, 1 H, *H*-d), 9.55 (s, 2 H, *H*-b), 8.99 (s, 2 H, *H*-β), 8.78 (s, 1 H, *H*-δ), 6.57 (d, *J* = 9.0 Hz, 1 H, *H*-g or *H*-h), 6.36 (d, *J* = 9.0 Hz, 1 H, *H*-g or *H*-h), 5.20-5.08 (m, 14 H, *H*-1), 4.60 (s, 3 H, *H*-a), 4.41 (s, 3 H, *H*-α), 4.12-3.11 (m, 236 H, β-CD *H*, polyether *H*, N-CH_2-CH_2-O). **^{13}C NMR** (125 MHz, $CDCl_3/CD_3OD$ = 1/1) δ (ppm): 163.04 (*C*-e), 161.95 (*C*-ε), 154.34 (*C*-f or *C*-i), 153.69 (*C*-f or *C*-i), 149.00 (*C*-b), 146.97 (*C*-β), 141.80 (*C*-δ), 141.70 (*C*-d), 135.37 (*C*-c), 134.44 (*C*-γ), 116.26 (*C*-g or *C*-h), 115.87 (*C*-g or *C*-h), 99.73-99.40 (*C*-1), 83.65-58.91 (β-CD *C*, polyether *C*, N-*C*H_2-*C*H_2-O), 50.42 (C-α), 49.65 (C-a). **HRMS** (ESI): m/z calcd. for $C_{168}H_{274}N_6O_{81}$ $[M]^{2+}$ 1835.8747; found 1835.8804.



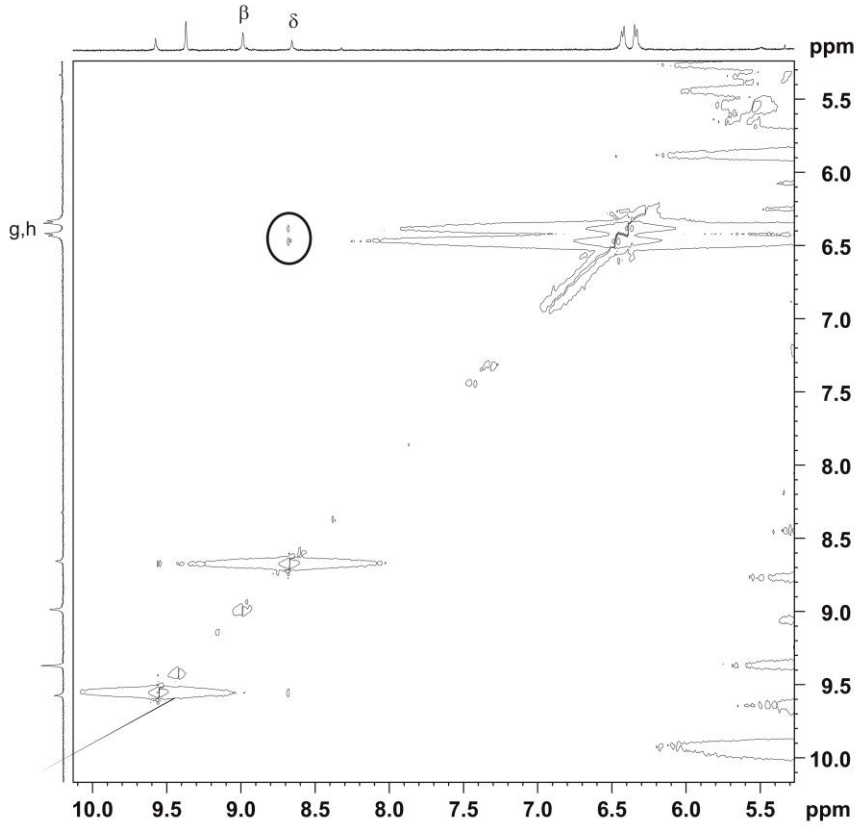
SI Figure 4. ROESY spectrum of rotaxane **8 $(NO_3)_2$**.



SI Figure 5. Rotaxane with the cyclodextrin stoppers functionalised on the primary rim.[4] (Compare with Fig. 4 in the main text.)



SI Figure 6. ROESY NMR of rotaxane **8 $(NO_3)_2$** (in D_2O)



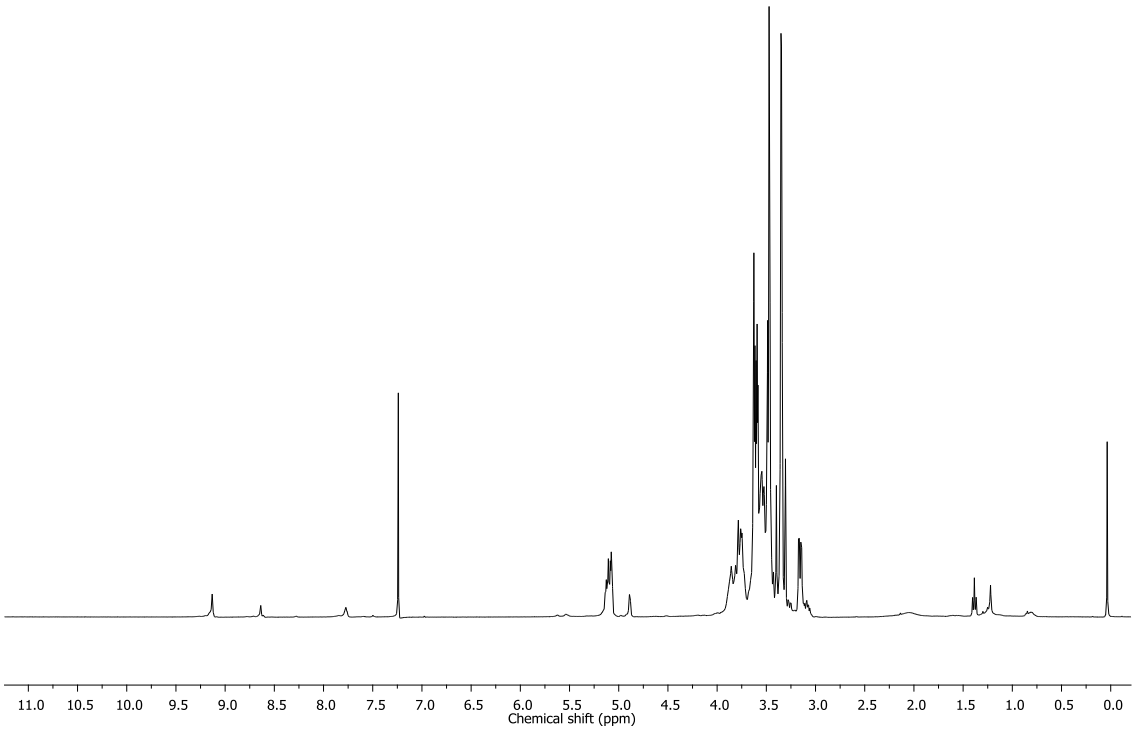
SI Figure 7. ROESY NMR of rotaxane **8 $(NO_3)_2$ +** 100 eq NaI (in D_2O)

Titration protocol

^{1}H NMR spectra were recorded on a Bruker AVIII spectrometer, at 500 MHz. Aliquots of a 0.75 M solution of the anion guest in D_2O, as the sodium salt, was added to a 1.5 mM solution of rotaxane **8 $(NO_3)_2$** in D_2O at 298 K. A trace amount of acetone (0.05%) was added as an internal reference and all spectra referenced to its resonance at 2.10 ppm. The chemical shift of proton *b* was monitored and the value of the observed chemical shift and the guest concentration were entered into winEQNMR2[5] 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 binding stoichiometry. In all experiments the association of guest and host was fast on the NMR timescale.

Copies of Spectra

^{1}H NMR of axle precursor **4**



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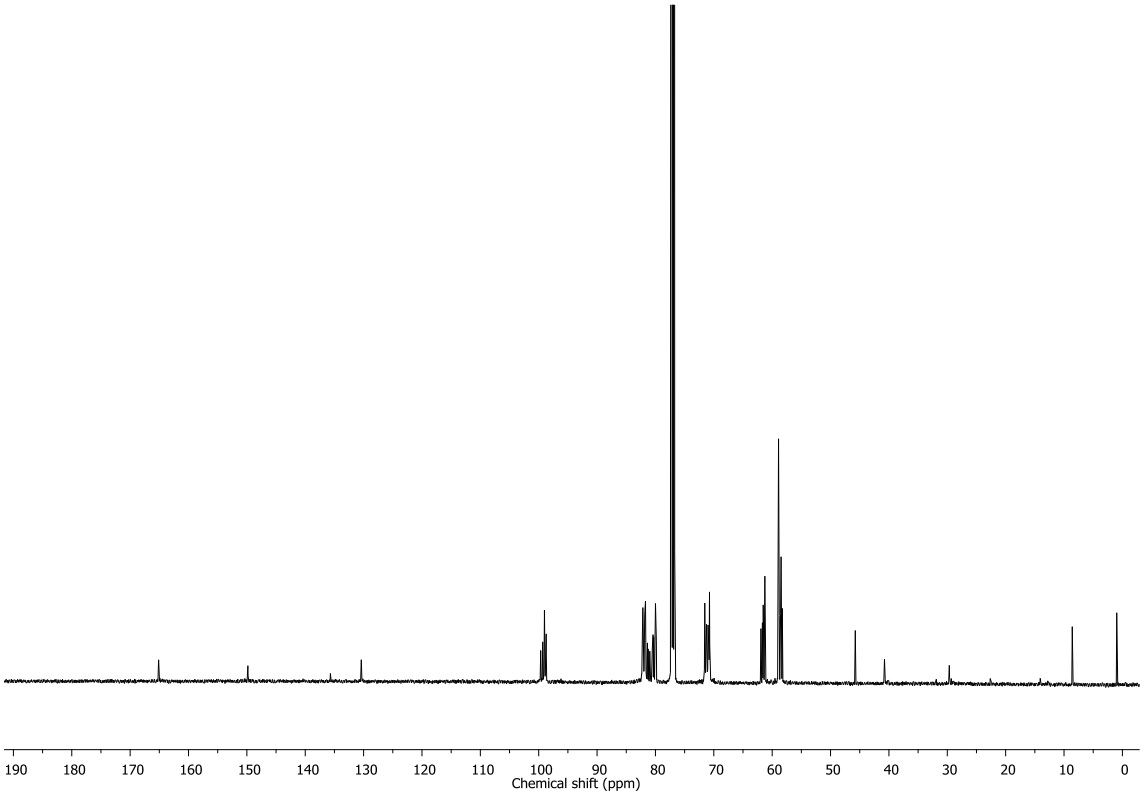
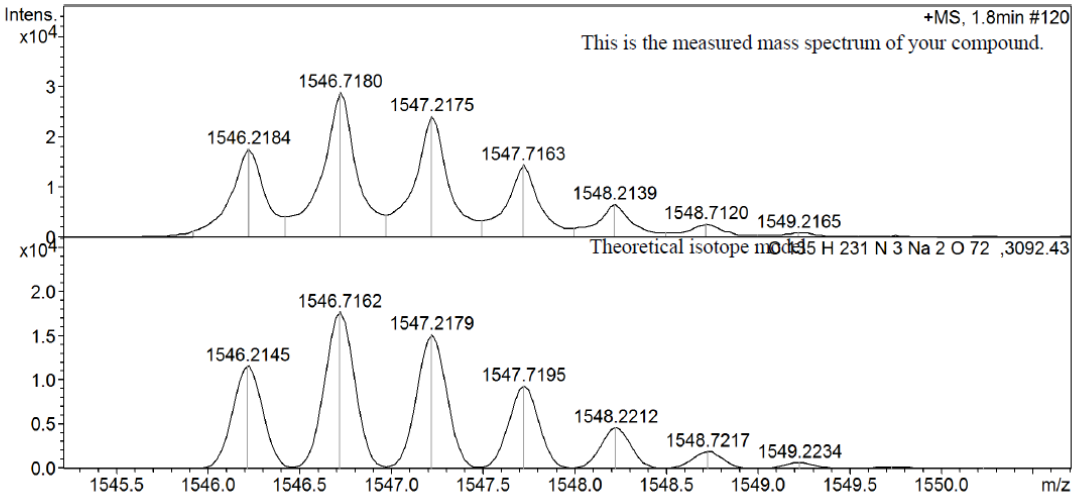


HRMS of axle precursor **4**



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	e⁻ Conf	mSigma
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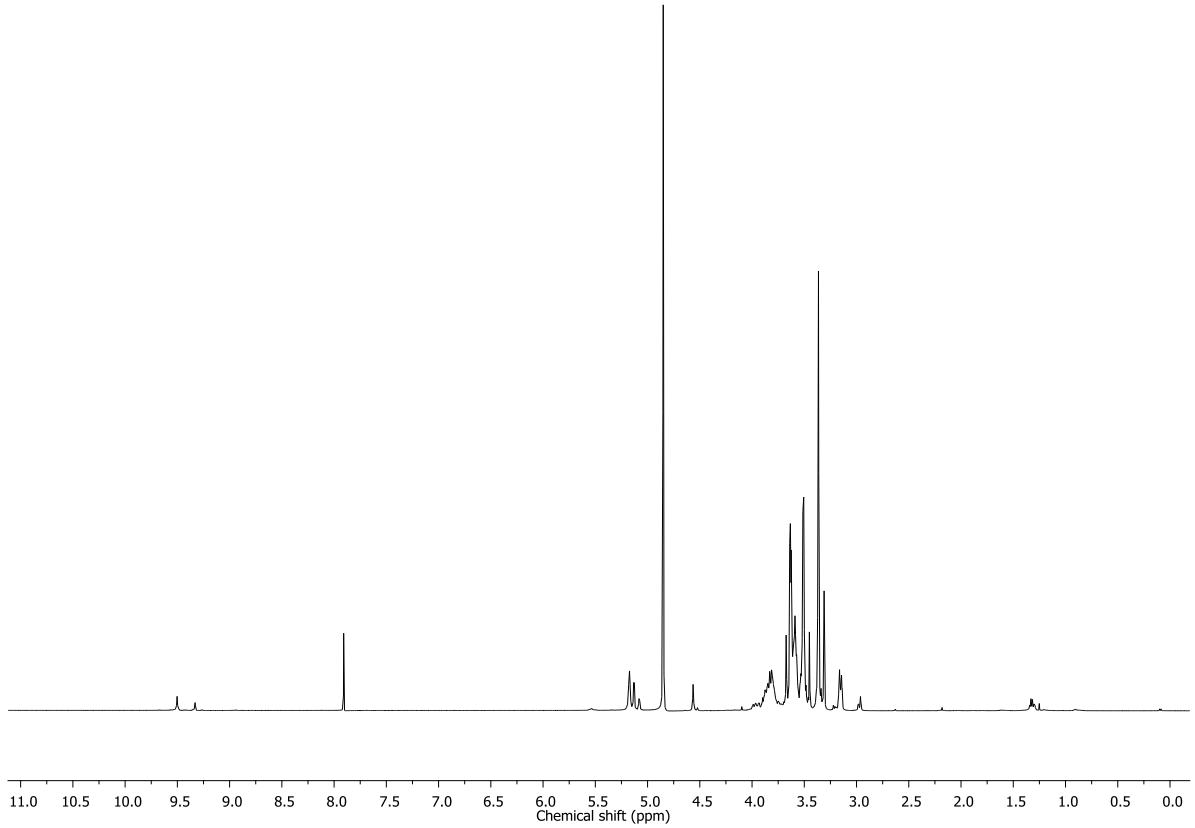
^{1}H NMR of axle **5 Cl**



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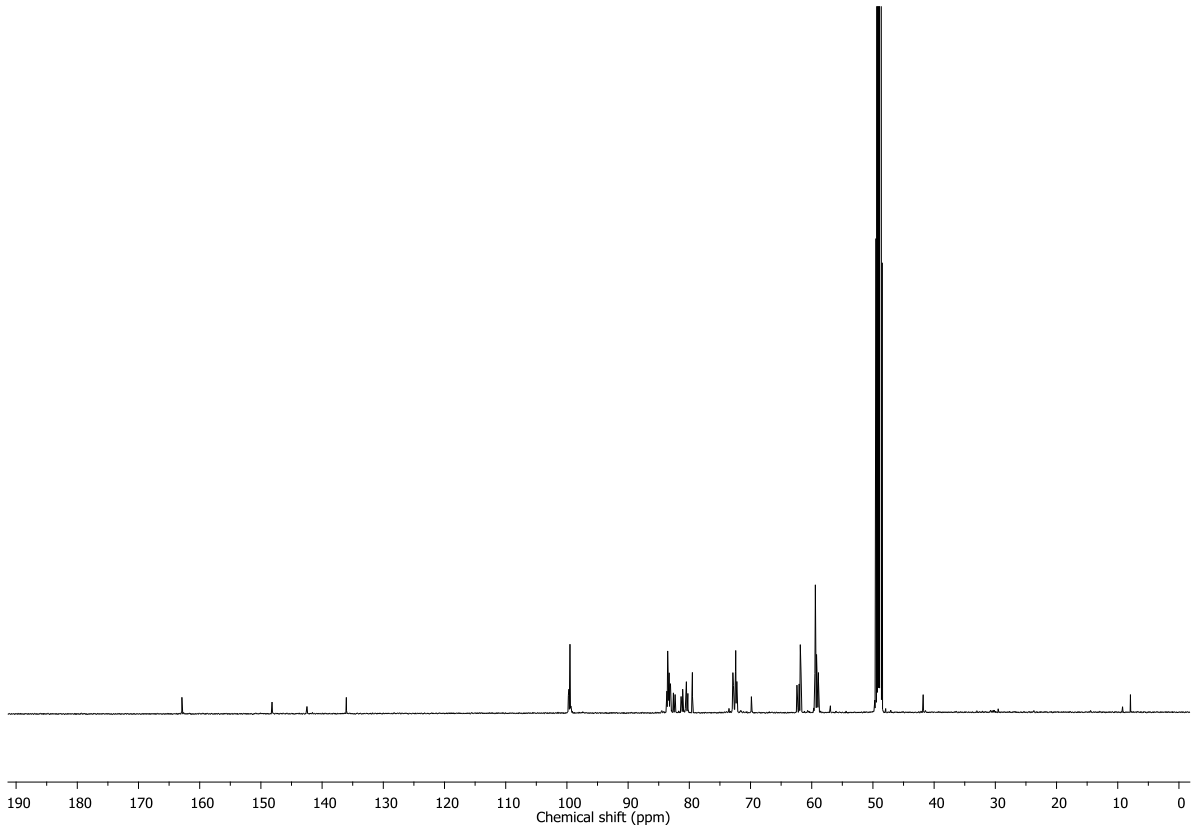
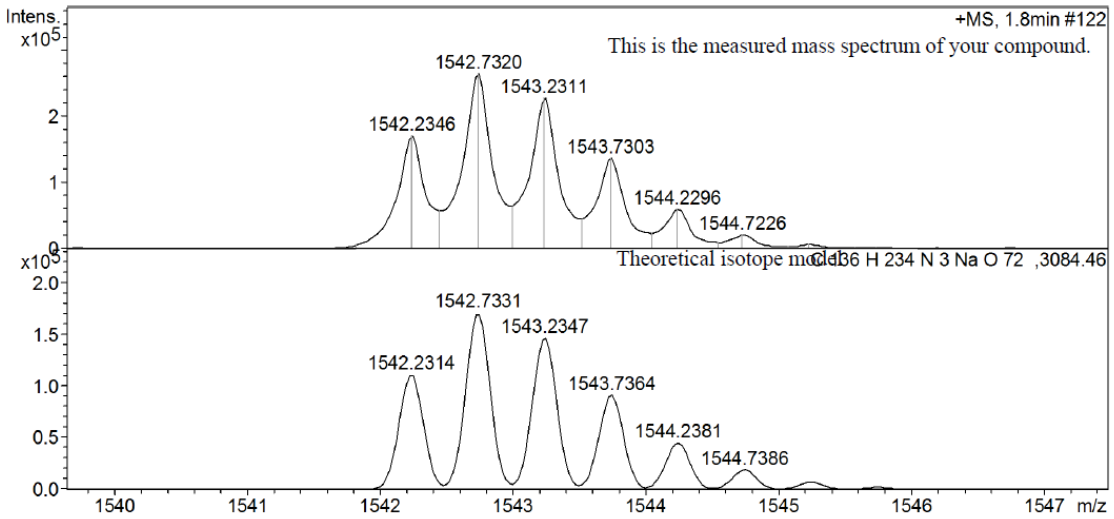


HRMS of axle **5 Cl**



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1542.2346								
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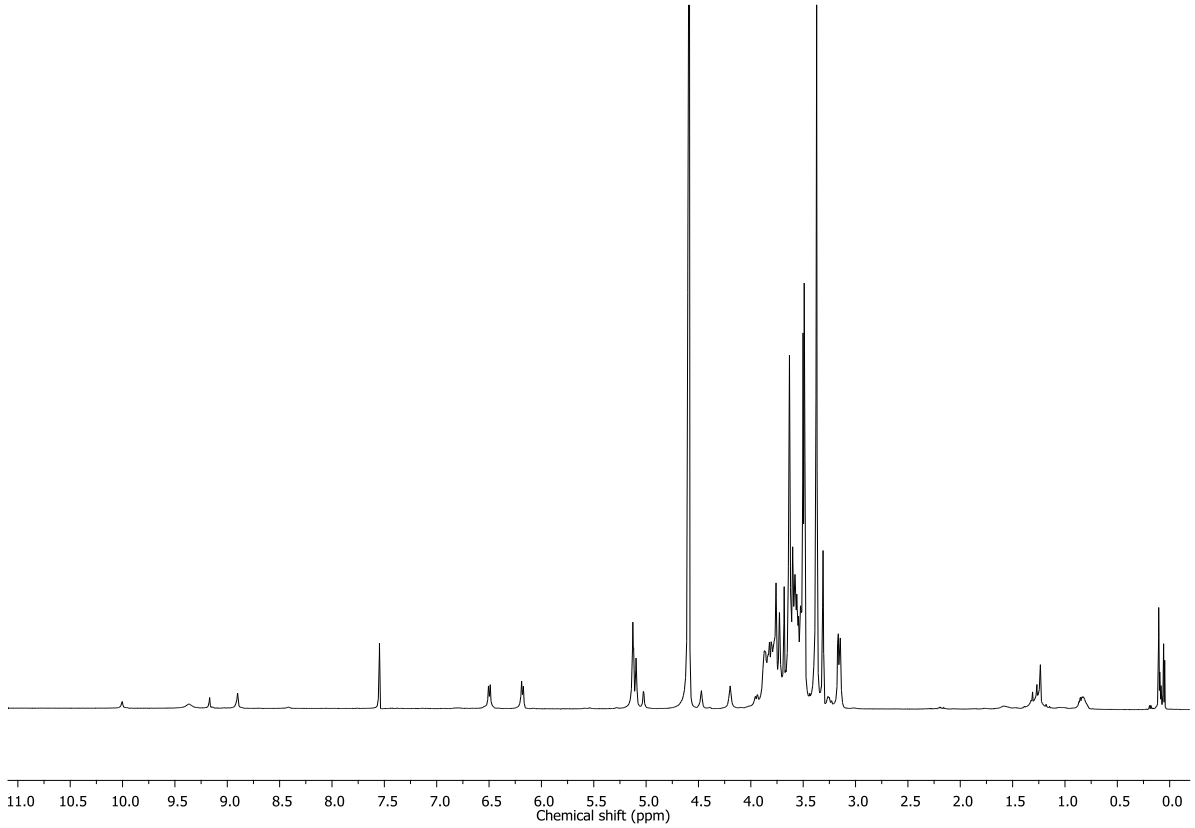
^{1}H NMR of rotaxane precursor **7·Cl**



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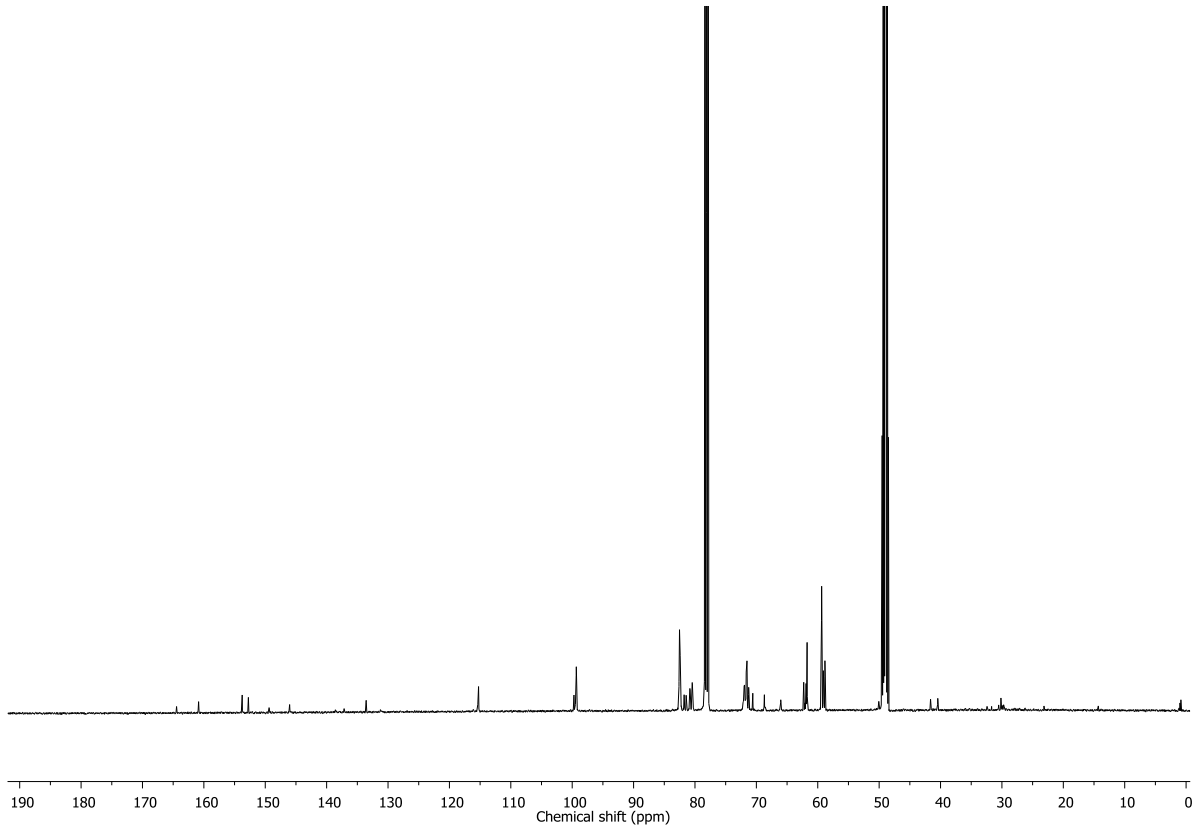

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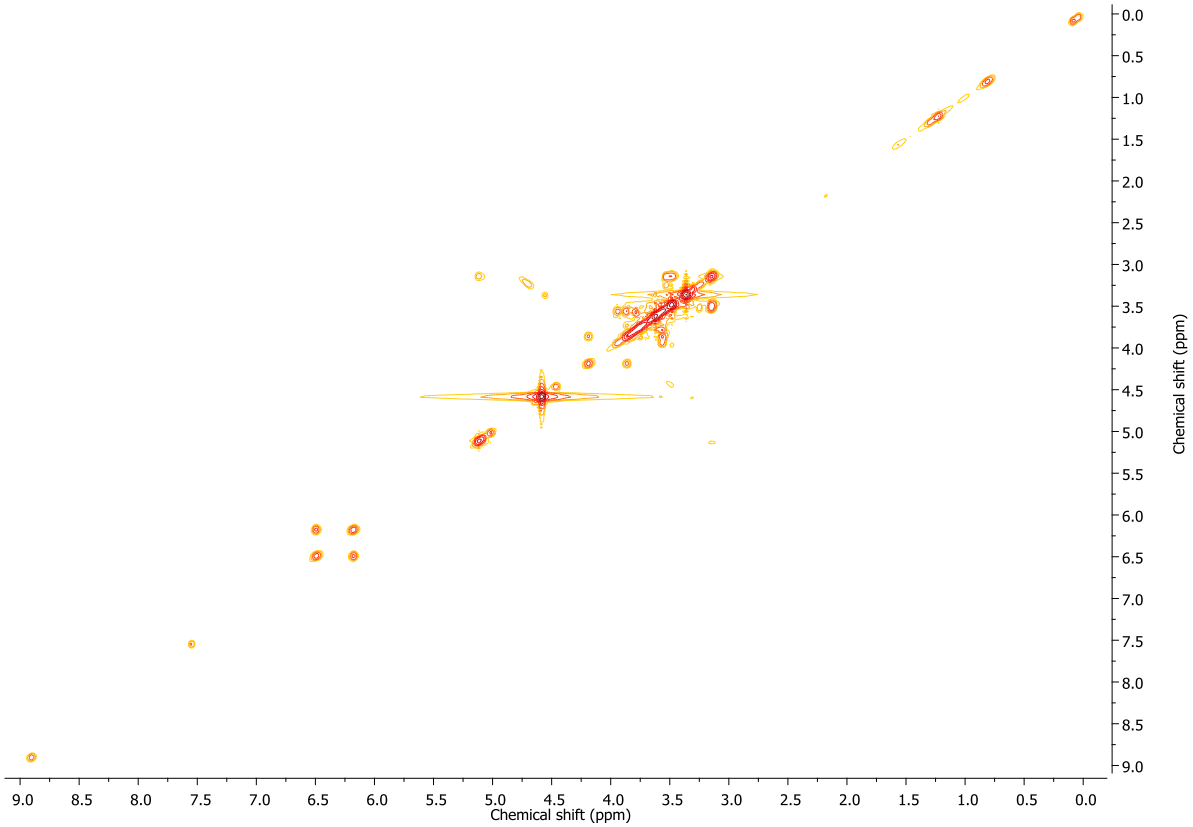
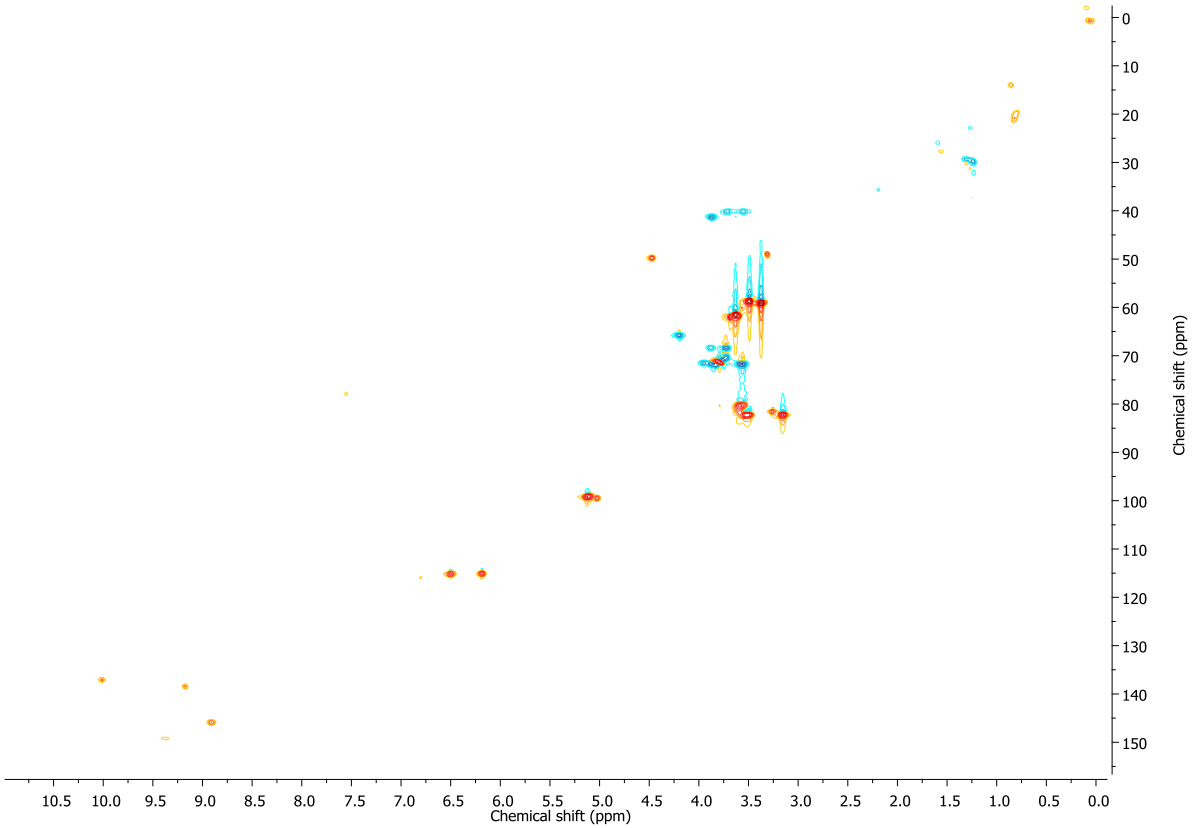


HSQC NMR of rotaxane precursor **7·Cl**



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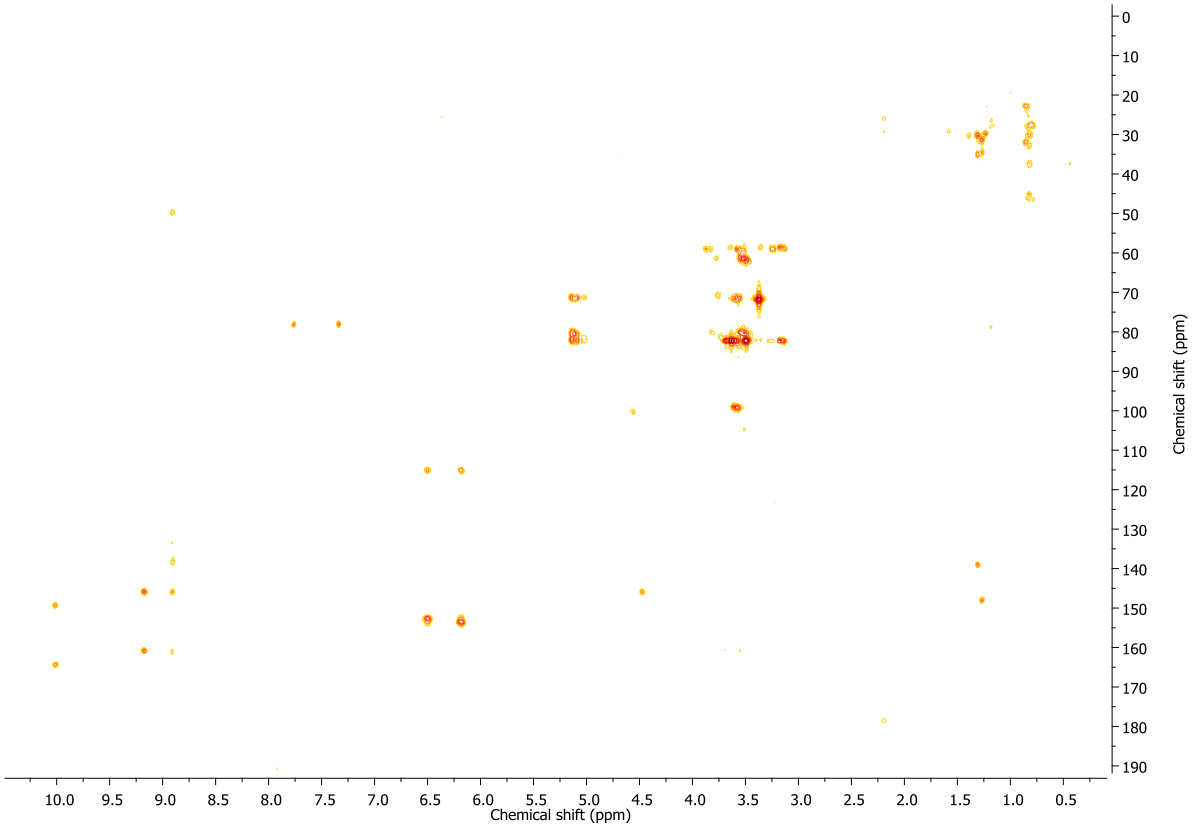
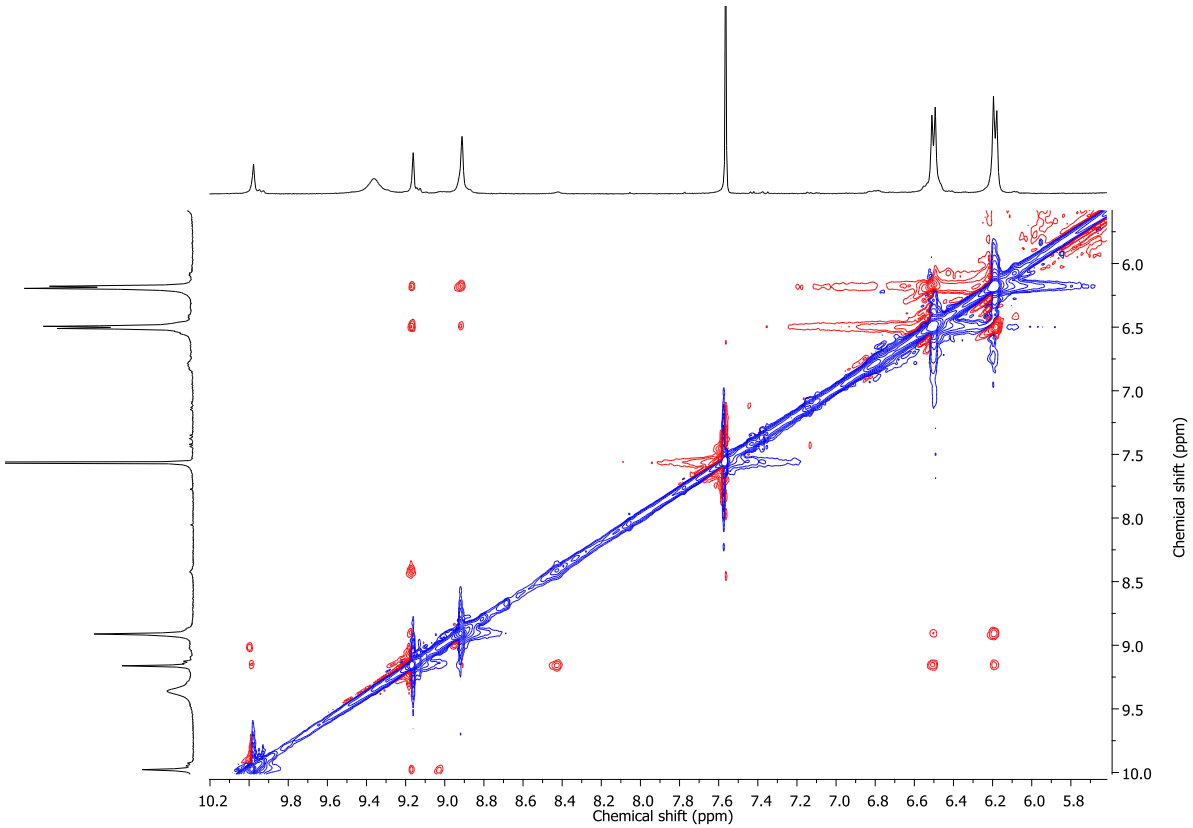
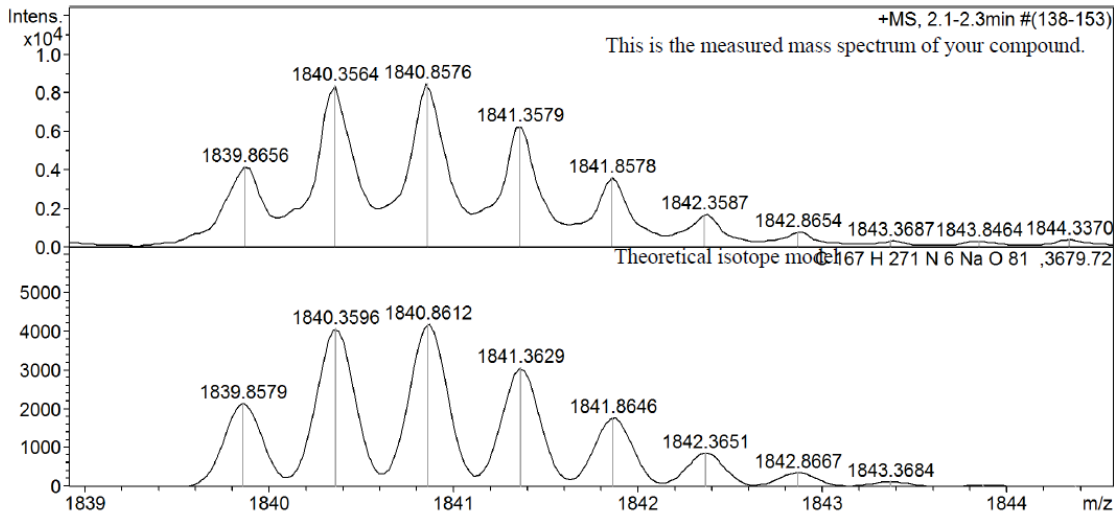


ROESY NMR of rotaxane precursor **7·Cl**



HRMS of rotaxane precursor **7·Cl**



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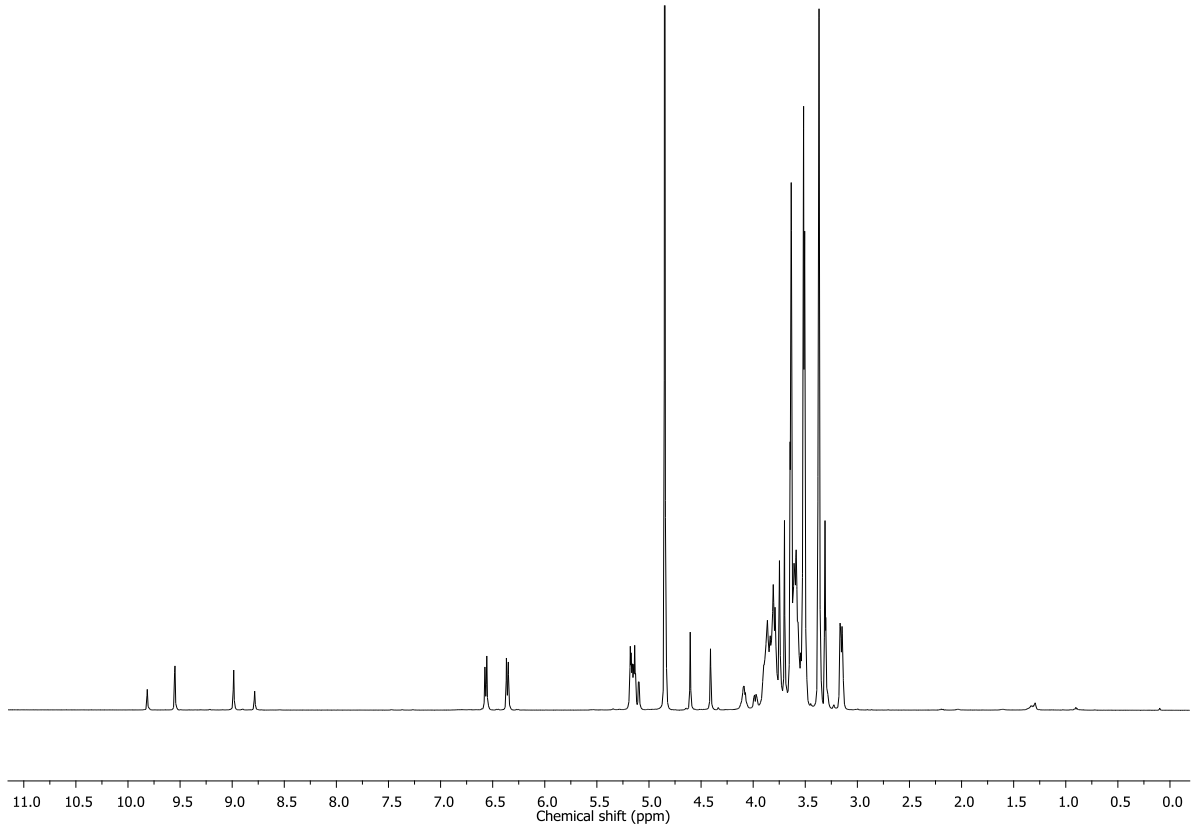
^{1}H NMR of rotaxane **8 $(NO_3)_2$**



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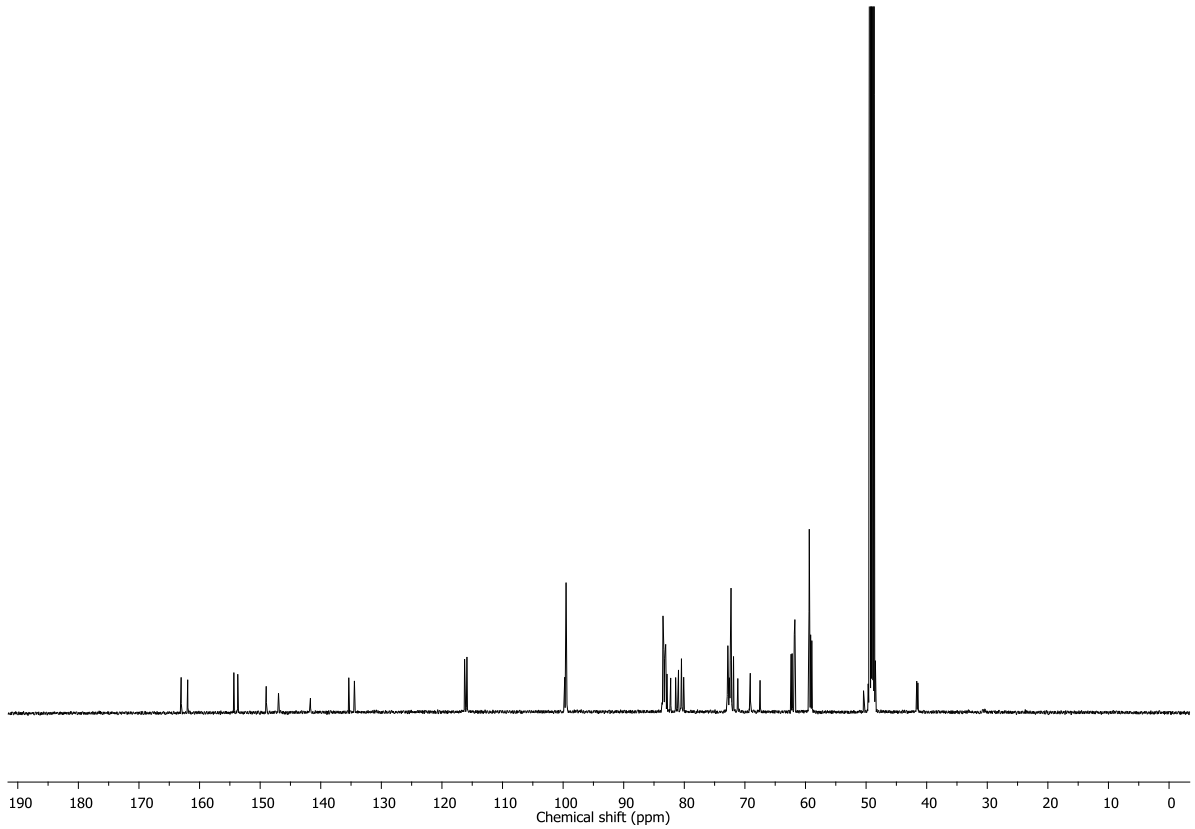
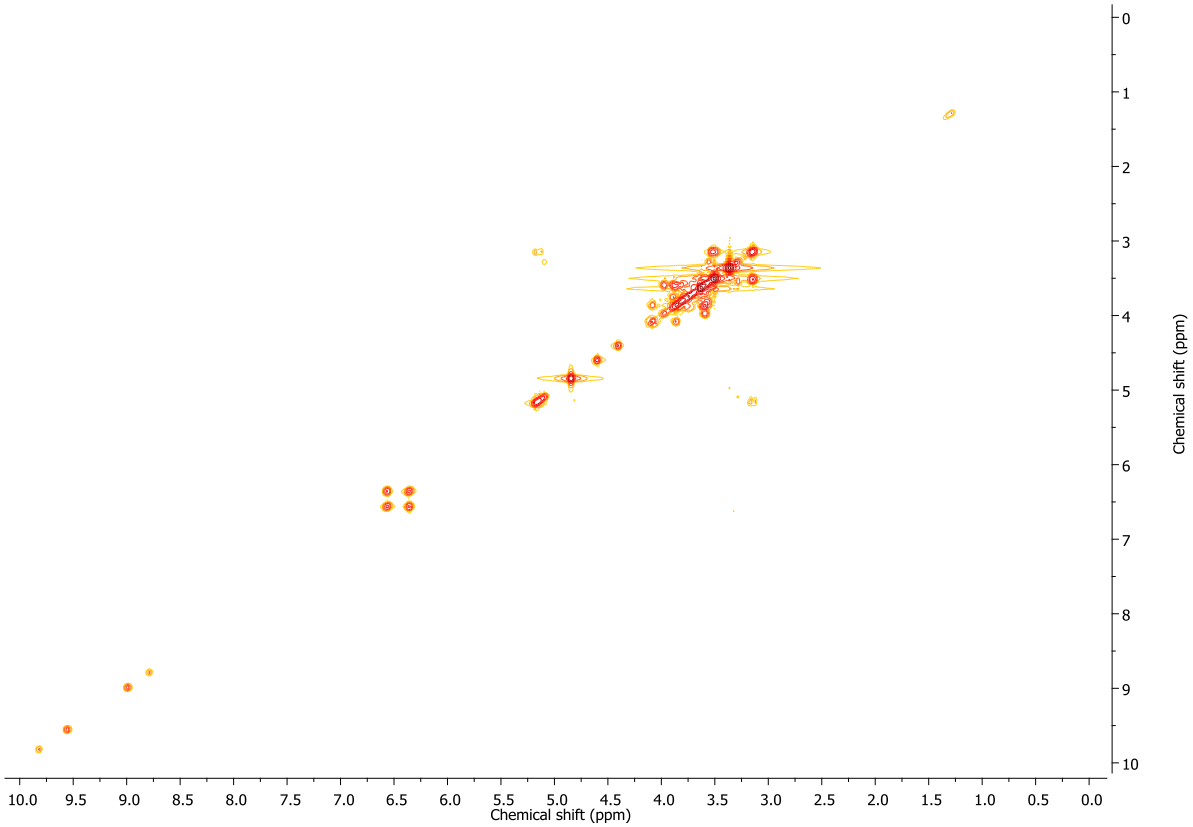
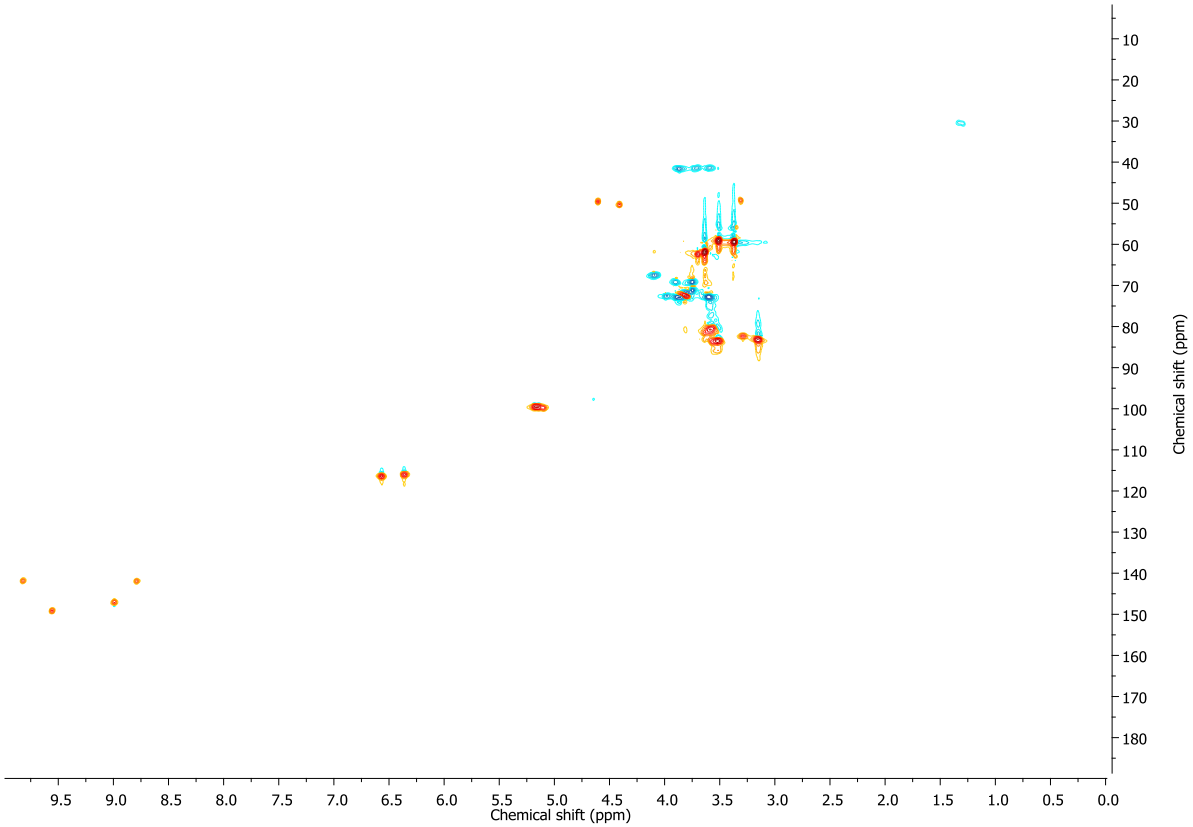


^{1}H-^{1}H COSY NMR of rotaxane **8 $(NO_3)_2$**



HSQC NMR of rotaxane **8 $(NO_3)_2$**



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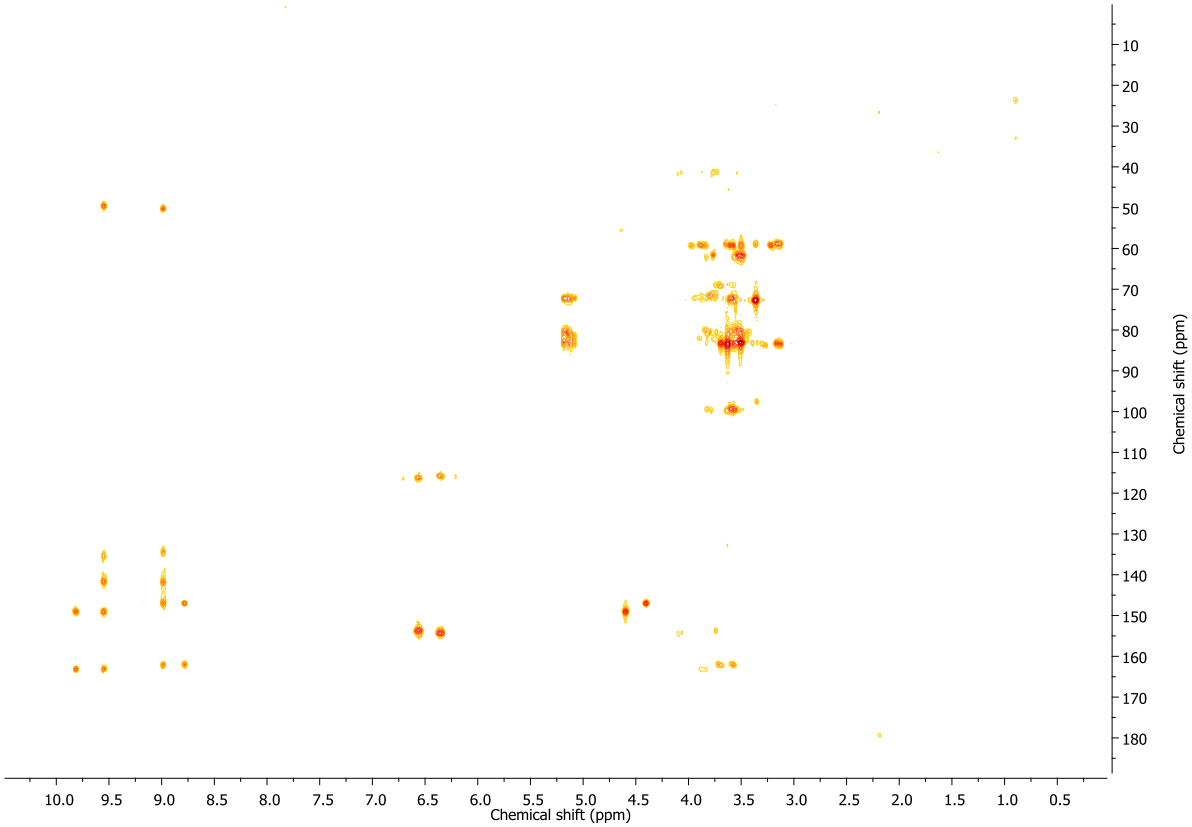
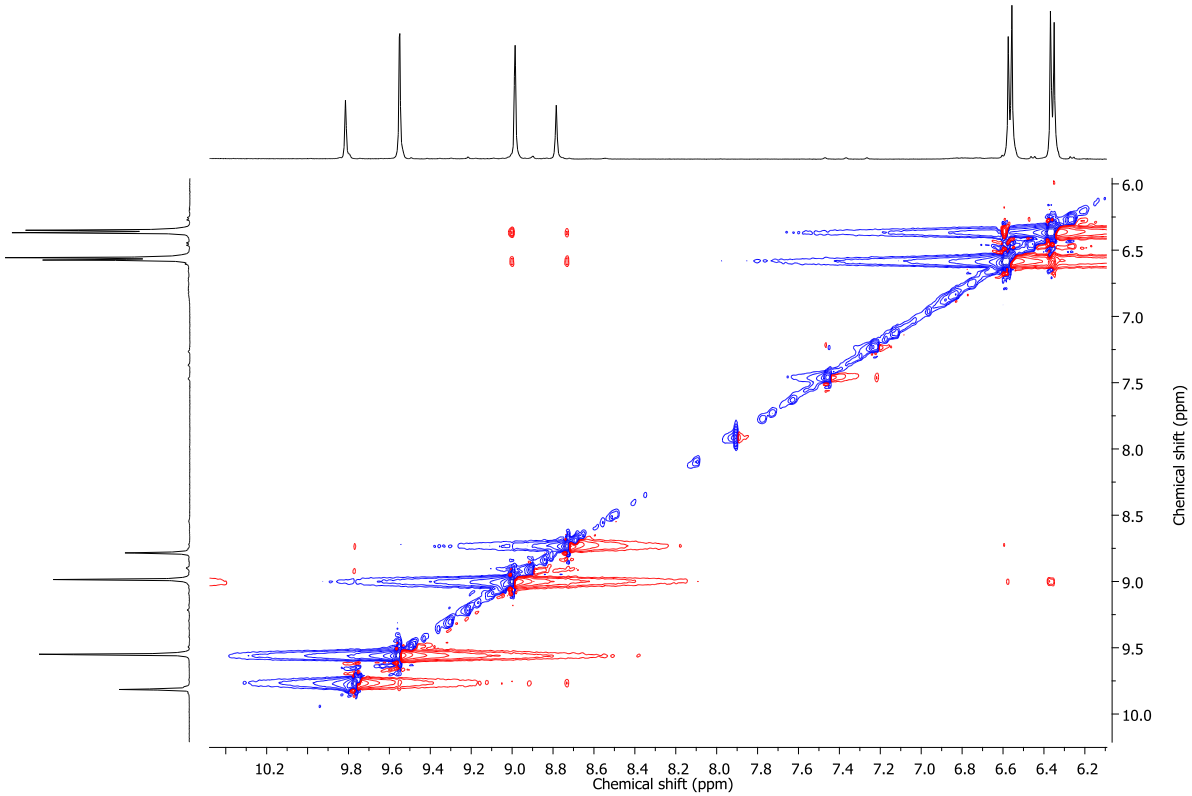
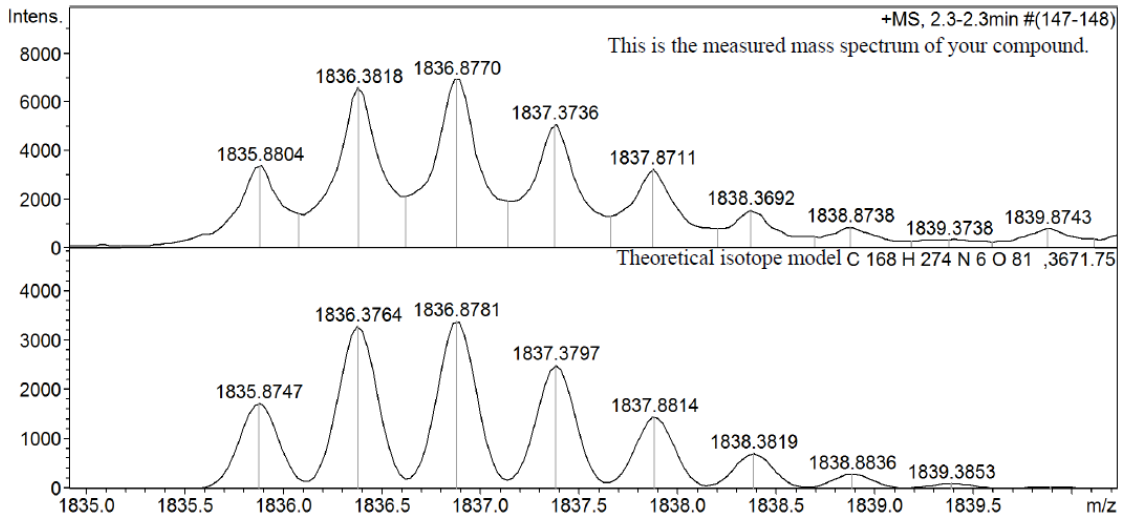


ROESY NMR of rotaxane **8 $(NO_3)_2$** (in CD_3OD)



HRMS of rotaxane **8 $(NO_3)_2$**



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	e¯ Conf	mSigma
1835.8804								
	1	C 168 H 274 N 6 O 81	1835.8747	-3.1	1.1	35.0	even	42.26

References

1 B. du Roizel, J.-P. Baltaze and P. Sinaÿ, *Tetrahedron Lett.*, 2002, **43**, 2371–2373.
2 Z. Guan, Y. Wang, Y. Chen, L. Zhang and Y. Zhang, *Tetrahedron*, 2009, **65**, 1125–1129.
3 K.-Y. Ng, V. Felix, S. M. Santos, N. H. Rees and P. D. Beer, *Chem. Commun.*, 2008, 1281–1283.
4 M. J. Langton, S. W. Robinson, I. Marques, V. Félix and P. D. Beer, *Nat. Chem.*, 2014, **6**, 1039–1043.
5 M. J. Hynes, *J. Chem. Soc. Dalton Trans.*, 1993, **2**, 311–312.