SUPPORTING INFORMATION

Modulating the Shuttling Motion of [2]Rotaxanes Built of *p*-Xylylenediamine Units through Permethylation at the Benzylic Positions of the Ring

Julio Puigcerver,[a] Mateo Alajarin,[a] Alberto Martinez-Cuezva*[a] and Jose Berna*[a]

[a] Departamento de Química Orgánica, Facultad de Química, Regional Campus of International Excellence "Campus Mare Nostrum", Universidad de Murcia, E-30100 Murcia, Spain.

E-mail: amcuezva@um.es; ppberna@um.es

Table of Contents S2
1. General experimental section.. S3
2. Synthesis of methylated diamine 3 .... S4
3. Synthesis of fumaramide thread with one binding site (6) S5
4. Synthesis of fumaramide thread with two binding sites (8a) S5
5. Synthesis of succinamide thread with two binding sites (8b) S7
6. Synthesis of glycylglycine thread with two binding sites (8c) S8
7. General procedure for the preparation of non-methylated rotaxanes (1).............. S10
8. General procedure for the preparation of methylated rotaxanes (2 and 7).............. S13
9. Calculation of the pKa of the deprotonation of model amides S17
10. VT-NMR experiment of [2]rotaxane 7 .... S18
11. VT-NMR experiments of degenerate [2]rotaxanes.. S20
12. Crystal data and structure refinement for rotaxane 7 .... S34
13. ^{1}H and ^{13}C NMR spectra of synthesized compounds S37
14. References S72

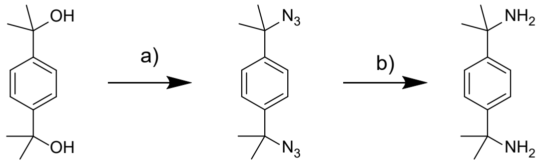
1. General Experimental Section

Unless stated otherwise, all reagents were purchased from Aldrich Chemicals and used without further purification. HPLC grade solvents (Scharlab) were nitrogen saturated and were dried and deoxygenated using an Innovative Technology Inc. Pure-Solv 400 Solvent Purification System. Column chromatography was carried out using silica gel (60 Å, 70-200 µm, SDS) as stationary phase, and TLC was performed on precoated silica gel on aluminium cards (0.25 mm thick, with fluorescent indicator 254 nm, Fluka) and observed under UV light. Size exclusion chromatography (SEC) was carried out using TOYOPEARL® size exclusion media (HW-40S, 20 – 40 µm). All melting points were determined on a Kofler hot-plate melting point apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance 300, 400 and 600 MHz instruments. ^{1}H NMR chemical shifts are reported relative to Me_4Si and were referenced via residual proton resonances of the corresponding deuterated solvent, whereas ^{13}C NMR spectra are reported relative to Me_4Si using the carbon signals of the deuterated solvent. Signals in the ^{1}H and ^{13}C NMR spectra of the synthesized compounds were assigned with the aid of DEPT, APT, or two-dimensional NMR experiments (COSY, HMQC and HMBC). Abbreviations of coupling patterns are as follows: br, broad; s, singlet; d, doublet; t, triplet; q, quadruplet; qui, quintuplet; m, multiplet. The deuterated solvent $CDCl_3$ was dried over $CaCl_2$ and stored with molecular sieves prior to use. Coupling constants (*J*) are expressed in Hz. High-resolution mass spectra (HRMS) were obtained using a time-of-flight (TOF) instrument equipped with electrospray ionization (ESI).

Abbreviation list:

EDCI·HCl: *N*-(3-dimethylaminopropyl)-*N*’-ethylcarbodiimide hydrochloride
DIPEA: *N,N*-diisopropylethylamine
HOBt: hydroxybenzotriazole
TFA: trifluoroacetic acid
DCM: dichloromethane
TLC: thin layer chromatography
THF: tetrahydrofurane

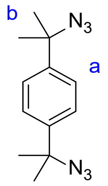
2. Synthesis of methylated diamine (3)



4 **5** **3**

Scheme S1. *Reactions conditions*: a) NaN_3, TFA, $CHCl_3$, 0 ºC to 25 °C, 6 h; b) H_2, Pd/C 10%, MeOH, 25 °C, overnight.

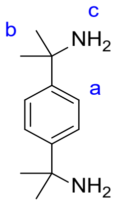
Synthesis of 1,4-*bis*(2-azidopropan-2-yl)benzene (5)



5

To a cooled at 0 ºC solution of commercial 2,2’-(1,4-phenylene)*bis*(propan-2-ol) (**4**) (8.0 g, 41.2 mmol) in $CHCl_3$ (100 mL) was added NaN_3 (12.1 g, 185.3 mmol). Then, a solution of TFA (25.4 mL, 329.4 mmol) in $CHCl_3$ (75 mL) was added dropwise. The mixture was stirred at room temperature for 6 hours. After this time, the medium was made basic by adding a solution of NH_3 30% (100 mL). The two phases were separated, and the aqueous phase was extracted with $CHCl_3$ (2 x 30 mL). The organic fractions were combined and washed with brine (2 x 40 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated to dryness. A white solid was obtained without further purifications steps (8.9 g, 89 %); mp 38-40 ºC; **^{1}H-NMR (400 MHz, $CDCl_3$, 298 K) δ:** 7.46 (s, 4H, H_a), 1.66 (s, 12H, H_b) ppm; **^{13}C-NMR (100 MHz, $CDCl_3$, 298 K) δ:** 143.9 (C), 125.4 (CH), 63.5 (C), 28.4 (CH_3) ppm; **HRMS (ESI)** calcd. for $C_{12}H_{15}$ $[M + H - H_2N_6]^+$ 159.1168, found 159.1177; **IR (neat) ν:** 2974.7, 2931.3, 2359.5, 2097.2, 1255.4, 831.2, 576.6 cm^{-1}.

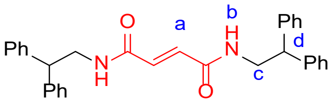
Synthesis of 2,2’-(1,4-phenylene)*bis*(propan-2-amine) (3)



3

To a solution of compound **5** (2.5 g, 10.2 mmol) in MeOH (50 mL) was added Pd/C 10% (1.1 g, 1.0 mmol of Pd). Then, the atmosphere was saturated by H_2 by coupling a balloon and the mixture was stirred at room temperature for overnight. After this time, the black suspension was filtered into a Celite® pad to remove the catalyst. The solution was concentrated to dryness to yield the title product as a white solid, which was subjected to the next reaction step without further purification (2.0 g, 99 %); mp < 330 ºC; **^{1}H-NMR (400 MHz, $CDCl_3$, 298 K) δ:** 7.45 (s, 4H, H_a), 1.67 (s, 4H, H_c), 1.48 (s, 12H, H_b) ppm; **^{13}C-NMR (100 MHz, $CDCl_3$, 298 K) δ:** 148.2 (C), 124.6 (CH), 52.2 (C), 32.9 (CH_3) ppm; **IR (neat) ν:** 2960.2, 2924.5, 2862.8, 2361.4, 2341.2, 827.3, 583.4 cm^{-1}.

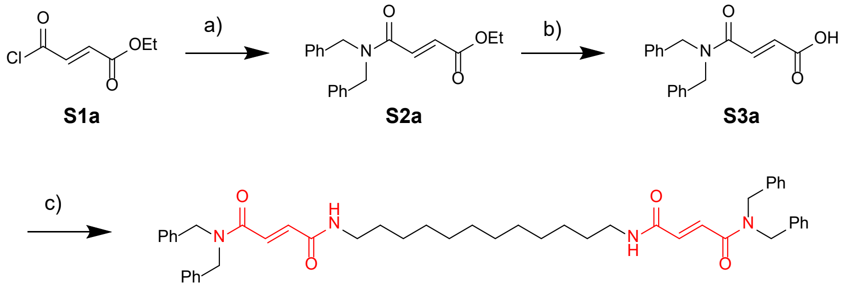
3. Synthesis of fumaramide thread with one binding site (6)



6

To a stirred solution of 2,2-diphenylethylamine (2.8 g, 14.5 mmol) in anhydrous DCM (60 mL) was added Et_3N (2.0 mL, 14.5 mmol). The solution was kept under N_2 atmosphere and cooled to 0 ºC. Then, a solution of fumaroyl chloride (1.0 g, 6.6 mmol) in DCM (5.0 mL) was added dropwise. The mixture was stirred at room temperature for overnight. After this time, the orange suspension was washed with water (3 x 25 mL), HCl 1M (3 x 25 mL), a saturated solution of $NaHCO_3$ (3 x 25 mL) and brine (3 x 25 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated to dryness. The resulting solid was washed with a little volume of Et_2O and pentane to obtain the title product as a white solid (2.7 g, 87 %); mp 257-259 ºC; **^{1}H-NMR (400 MHz, $CDCl_3$, 298 K) δ:** 7.31-7.21 (m, 20H, H_{Ph}), 6.66 (s, 2H, H_a), 5.67 (t, *J* = 6.1 Hz, 2H, H_b), 4.18 (t, *J* = 8.0 Hz, 2H, H_d), 3.97 (dd, *J* =8.0 Hz, 6.1 Hz, 4H, H_c) ppm; **^{13}C-NMR (100 MHz, DMSO, 298 K) δ:** 163.7 (CO), 142.7 (C), 132.5 (CH), 128.5 (CH), 127.8 (CH), 126.4 (CH), 50.0 (CH), 43.3 (CH_2) ppm; **HRMS (ESI)** calcd. for $C_{32}H_{31}N_2O_2$ $[M + H]^+$ 475.2380, found 475.2385; **IR (neat) ν:** 3263.0, 3076.9, 2922.6, 1631.5, 1550.5, 1327.8, 695.2 cm^{-1}.

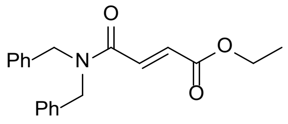
4. Synthesis of fumaramide thread with two binding sites (8a)



8a

Scheme S2. *Reactions conditions*: a) Dibenzylamine, CH_2Cl_2, 0°C to 25°C, overnight; b) NaOH, EtOH, 25°C, overnight; c) 1,12-dodecanediamine, HOBt, EDCI·HCl, DiPEA, CH_2Cl_2, 0°C to 25°C, 24 h.

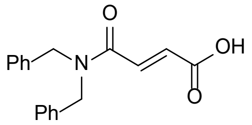
Synthesis of compound S2a



S2a

Fragment **S2a** was synthesized following a described procedure reported in N. S. Simpkins, D. F. Weske, L. Male, S. J. Coles and M. B. Pitak, *Chem. Commun.*, **2013**, 49, 5010-5012 and showed identical spectroscopic data as those reported therein.

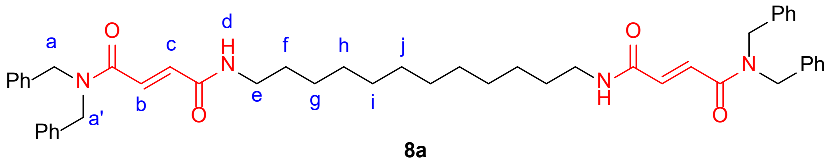
Synthesis of compound S3a



S3a

Fragment **S3a** was synthesized following a described procedure reported in N. S. Simpkins, D. F. Weske, L. Male, S. J. Coles and M. B. Pitak, *Chem. Commun.*, **2013**, 49, 5010-5012 and showed identical spectroscopic data as those reported therein.

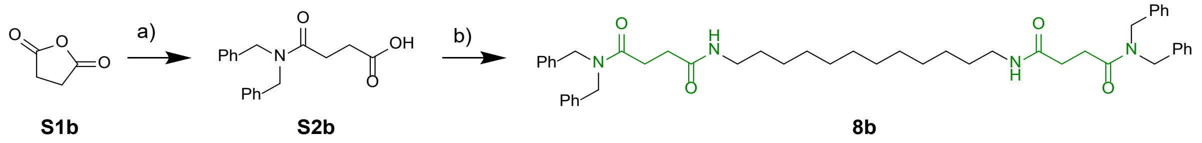
Synthesis of fumaramide thread with two binding sites (8a)



8a

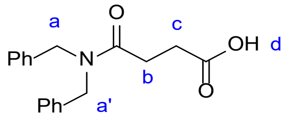
To a cooled at 0 °C solution of carboxylic acid **S3a** (1.3 g, 4.4 mmol) in DCM (50 mL) were added HOBt (0.6 g, 4.4 mmol), DiPEA (0.8 mL, 4.4 mmol) and EDCI·HCl (0.7 g, 4.4 mmol). The mixture was stirred at room temperature for 30 min. After this time, 1,12-dodecanediamine (0.4 g, 2.0 mmol) was added and the reaction was stirred at room temperature for overnight. Then, the mixture was washed with HCl 1M (2 x 40 mL), NaOH 1M (2 x 40 mL) and brine (2 x 40 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated to dryness. The resulting crude was subjected to column chromatography on silica gel using $CHCl_3$/acetone (3:1) as eluent to give the title product as a white solid (1.0 g, 66%); mp 161-163 °C; **^{1}H-NMR (400 MHz, $CDCl_3$, 298 K) δ**: 7.46 (d, J = 14.7 Hz, 2H, H_b), 7.37-7.28 (m, 12H, H_{Ph}), 7.23-7.09 (m, 10H, H_{Ph+c}), 6.40-6.31 (m, 2H, NH_d), 4.65 (s, 4H, H_a), 4.56 (s, 4H, $H_{a'}$), 3.30-3.25 (m, 4H, H_e), 1.50-1.46 (m, 4H, H_f), 1.28-1.24 (m, 16H, $H_{g+h+i+j}$) ppm; **^{13}C-NMR (100 MHz, $CDCl_3$, 298 K) δ**: 166.1 (CO), 164.2 (CO), 136.7 (C), 136.1 (CH), 136.0 (C), 129.8 (CH), 129.1 (CH), 128.9 (CH), 128.3 (CH), 128.1 (CH), 127.8 (CH), 126.9 (CH), 50.3 (CH_2), 48.6 (CH_2), 40.0 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 27.0 (CH_2) ppm; **HRMS (ESI)**: calcd for $C_{48}H_{59}N_4O_4$ $[M+H]^+$ 755.4531, found 755.4554; **IR (neat) ν:** 3323.7, 2924.5, 2852.2, 1636.3, 1604.5, 1557.2, 1441.5, 1189.9, 699.1 cm^{-1}.

5. Synthesis of succinamide thread with two binding sites (8b)



Scheme S3. *Reactions conditions*: a) Dibenzylamine, THF, 25°C, overnight; b) 1,12-dodecanediamine, HOBt, EDCI·HCl, DiPEA, CH_2Cl_2, 0°C to 25°C, 24 h.

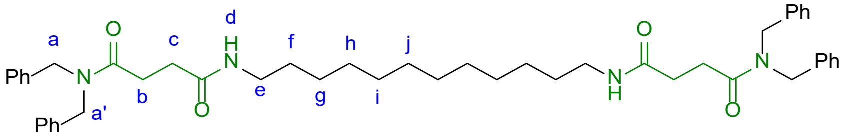
Synthesis of compound S2b



S2b

To a stirred suspension of succinic anhydride (**S1b**) (6.9 g, 69.4 mmol) in anhydrous THF (60 mL) was added dibenzylamine (16.0 mL, 83.2 mmol). The mixture was stirred under N_2 atmosphere, at room temperature for overnight. After this time, the medium was acidified with HCl 1M (50 mL) and the resulting suspension was filtered to obtain the title product as a white solid without further purification (17.5 g, 85%); mp 115-117 °C; **^{1}H-NMR (400 MHz, $CDCl_3$, 298 K) δ**: 11.19 (s, 1H, H_d), 7.42 – 7.19 (m, 10H, H_{Ph}), 4.64 (s, 2H, H_a), 4.51 (s, 2H, $H_{a'}$), 2.84 – 2.73 (m, 4H, H_{b+c}) ppm; **^{13}C-NMR (100 MHz, $CDCl_3$, 298 K) δ**: 177.7 (CO), 172.3 (CO), 136.9 (C), 136.0 (C), 129.0 (CH), 128.6 (CH), 128.2 (CH), 127.7 (CH), 127.4 (CH), 126.4 (CH), 49.9 (CH_2), 48.5 (CH_2), 29.5 (CH_2), 28.0 (CH_2) ppm; **HRMS (ESI)**: calcd for $C_{18}H_{20}NO_3$ $[M+H]^+$ 298.1438, found 298.1438; **IR (neat) ν:** 3031.6, 2929.3, 1701.9, 1636.3, 730.9, 695.2 cm^{-1}.

Synthesis of succinamide thread with two binding sites (8b)

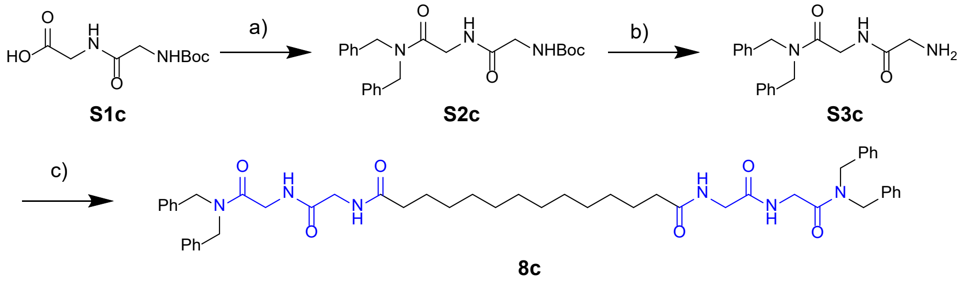


8b

To a cooled at 0 °C solution of carboxylic acid **S2b** (2.5 g, 8.41 mmol) in DCM (150 mL) were added HOBt (1.14 g, 8.41 mmol), DiPEA (1.47 mL, 8.41 mmol) and EDCI·HCl (1.31 g, 8.41 mmol). The mixture was stirred at room temperature for 30 min. After this time, 1,12-dodecanediamine (0.77 g, 3.82 mmol) was added and the reaction was stirred at room temperature for overnight. Then, the mixture was washed with HCl 1M (2 x 40 mL), NaOH 1M (2 x 40 mL) and brine (2 x 40 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated to dryness. The resulting crude was subjected to column chromatography on silica gel using $CHCl_3$/acetone (5:1) as eluent to give the title product as a white solid (1.72 g, 59%); mp 129-131 °C; **^{1}H-NMR (300 MHz, $CDCl_3$, 298 K) δ**: 7.39-7.27 (m, 12H, H_{Ph}), 7.20-7.14 (m, 8H, H_{Ph}), 6.21 (s, 2H, NH_d), 4.60 (s, 4H, H_a), 4.48 (s, 4H, $H_{a'}$), 3.22 (dd, J = 13.3 Hz,

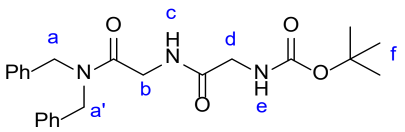
$J = 6.7$ Hz, 4H, H_e), 2.78 (t, $J = 6.4$ Hz, 4H, H_b), 2.58 (t, $J = 6.4$ Hz, 4H, H_c), 1.50-1.44 (m, 4H, H_f), 1.33-1.25 (m, 16H, $H_{g+h+i+j}$) ppm; **^{13}C-NMR (75 MHz, $CDCl_3$, 298 K) δ**: 172.9 (CO), 172.4 (CO), 137.2 (C), 136.3 (C), 129.1 (CH), 128.8 (CH), 128.2 (CH), 127.8 (CH), 127.6 (CH), 126.6 (CH), 50.1 (CH_2), 48.6 (CH_2), 39.8 (CH_2), 31.8 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 27.0 (CH_2) ppm; **HRMS (ESI)**: calcd for $C_{48}H_{59}N_4O_4$ $[M+H]^+$ 759.4731, found 759.4757; **IR (neat) ν:** 3317.0, 2931.3, 2850.3, 2359.5, 1635.3, 1538.9, 1428.0, 1193.7, 693.3 cm^{-1}.

6. Synthesis of glycylglycine thread with two binding sites (8c)



Scheme S4. *Reactions conditions*: a) Dibenzylamine, HOBt, DiPEA, EDCI·HCl, CH_2Cl_2, 0°C to 25°C, overnight; b) TFA, $CHCl_3$, 0°C to 25°C, overnight; c) Tetradecanedioyl dichloride, Et_3N, THF, 0°C to 25°C, overnight.

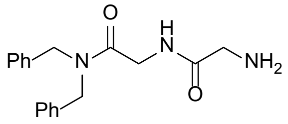
Synthesis of compound S2c



S2c

To a stirred solution of N-Boc-glycylglycine (**S1c**) (6.0 g, 25.8 mmol) in DCM (100 mL) were added dibenzylamine (5.0 mL, 25.8 mmol), HOBt (5.2 g, 38.7 mmol) and DiPEA (2.3 mL, 12.9 mmol). The solution was cooled at 0 °C and EDCI·HCl (6.0 g, 38.7 mmol) was added. The mixture was stirred at that temperature for 30 min and at room temperature for overnight. After this time, the mixture was washed with HCl 0.1M (2 x 50 mL), NaOH 0.1M (2 x 50 mL) and brine (2 x 50 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated to dryness. The resulting crude was subjected to column chromatography on silica gel using hexane/AcOEt (1:1) as eluent to give the title product as a white solid (3.3 g, 31 %); mp 33-35 °C; **^{1}H-NMR (400 MHz, $CDCl_3$, 298 K) δ:** 7.39 – 7.21 (m, 7H, H_{Ph+c}), 7.19 – 7.07 (m, 4H, H_{Ph}), 5.59 (s, 1H, H_e), 4.57 (s, 2H, H_a), 4.38 (s, 2H, $H_{a'}$), 4.19 (d, $J = 4.3$ Hz, 2H, H_b), 3.87 (d, $J = 4.8$ Hz, 2H, H_d), 1.43 (s, 9H, H_f) ppm; **^{13}C-NMR (100 MHz, $CDCl_3$, 298 K) δ:** 169.7 (CO), 168.5 (CO), 156.0 (CO), 136.3 (C), 135.2 (C), 129.0 (CH), 128.7 (CH), 128.2 (CH), 127.9 (CH), 127.6 (CH), 126.5 (CH), 79.9 (C), 49.0 (CH_2), 48.5 (CH_2), 44.0 (CH_2), 41.3 (CH_2), 28.3 (CH_3) ppm; **HRMS (ESI)** calcd for $C_{23}H_{29}N_3NaO_4$ $[M+Na]^+$ 434.2050, found 434.2059; **IR (neat) ν:** 3313.1, 2974.7, 2929.3, 1638.2, 1161.9, 697.1 cm^{-1}.

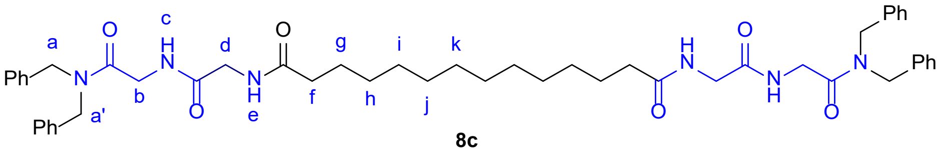
Synthesis of compound S3c



S3c

To a cooled at 0 ºC solution of compound **S2c** (3.0 g, 7.3 mmol) in $CHCl_3$ (25 mL) was added dropwise TFA (5.6 mL, 73.0 mmol). The mixture was stirred at room temperature for overnight. After this time, the solvent was removed in vacuo and the resulting residue was treated for 2 hours with Amberlyst® basic resin in $CHCl_3$/MeOH (2:1) (20 mL). Then the resin was removed by filtration and the solvent removed under reduced pressure to obtain the title product **S3c** as a white solid, that was employed in the next synthesis step without further purification (2.2 g, 99 %).

Synthesis of glycylglycine thread with two binding sites (8c)



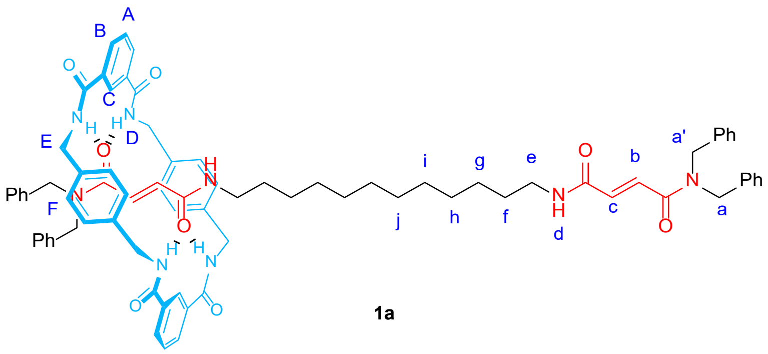
8c

To a cooled at 0 °C solution of 2-(2-aminoacetamido)-*N,N*-dibenzylacetamide (**S3c**) (0.5 g, 1.5 mmol) and triethylamine (0.32 mL, 2.3 mmol) in anhydrous THF (30 mL) was added dropwise tetradecanedioyl dichloride (0.23 g, 0.77 mmol). The mixture was stirred at room temperature for overnight. Then, the solvent was removed under reduced pressure and the crude redissolved in DCM (30 mL). The solution was washed with HCl 1 M (2 x 10 mL), saturated $NaHCO_3$ (2 x 10 mL) and brine (2 x 10 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated to dryness. The resulting crude was subjected to column chromatography on silica gel using $CHCl_3$/MeOH (10:1) as eluent to give the title product as a white solid (0.44 g, 43%); mp 64 - 66 °C; **^{1}H-NMR (400 MHz, $CDCl_3$, 298 K) δ**: 7.39 – 7.27 (m, 12H, H_{Ph+c}), 7.19 – 7.10 (m, 10H, H_{Ph}), 6.42 (m, 2H, H_e), 4.60 (s, 4H, H_a), 4.39 (s, 4H, $H_{a'}$), 4.19 (d, J = 4.2 Hz, 4H, H_b), 4.01 (d, J = 5.2 Hz, 4H, H_d), 2.24 (t, J = 7.6 Hz, 4H, H_f), 1.68 – 1.59 (m, 4H, H_g), 1.29 – 1.23 (m, 16H, $H_{k+j+i+h}$) ppm; **^{13}C-NMR (100 MHz, $CDCl_3$, 298 K) δ**: 173.8 (CO), 169.0 (CO), 168.4 (CO), 136.4 (C), 135.2 (C), 129.3 (CH), 128.9 (CH), 128.4 (CH), 128.1 (CH), 127.9 (CH), 126.6 (CH), 49.1 (CH_2), 48.7 (CH_2), 43.0 (CH_2), 41.6 (CH_2), 36.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 25.7 (CH_2) ppm; **HRMS (ESI)**: calcd for $C_{50}H_{65}N_6O_6$ $[M+H]^+$ 845.4960, found 845.4965. **IR (neat) ν:** 3313.1, 2920.7, 2852.2, 1634.4, 1452.1, 1425.1, 1220.7, 695.2 cm^{-1}.

7. General procedure for the preparation of the non-methylated rotaxanes (1)

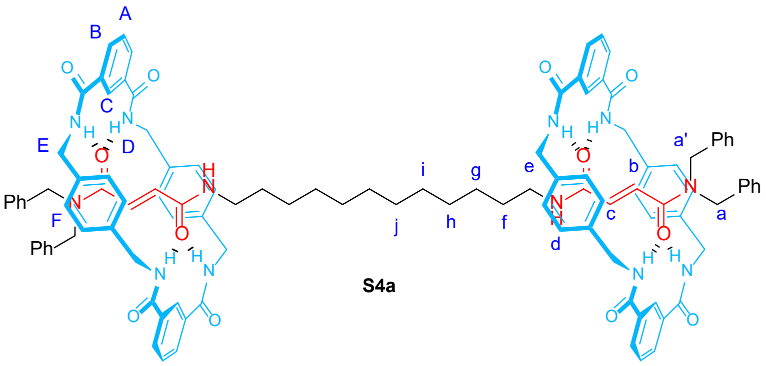
Thread (1 equiv.) and Et_3N (24 equiv.) in anhydrous $CHCl_3$ (300 mL) were stirred vigorously whilst solutions of *p*-xylylenediamine (4 equiv.) in anhydrous $CHCl_3$ (20 mL) and isophthaloyl dichloride (4 equiv.) in anhydrous $CHCl_3$ (20 mL) were simultaneously added for 4 h using motor-driven syringe pumps. After a further 4 h, the resulting suspension was filtered through a Celite® pad, washed with water (2 x 50 mL), an aqueous solution of HCl 1N (2 x 50 mL), a saturated solution of $NaHCO_3$ (2 x 50 mL) and brine (2 x 50 mL). The organic phase was dried over $MgSO_4$ and the solvent removed under reduced pressure. The resulting solid was subjected to column chromatography (silica gel) and/or size exclusion chromatography to yield unconsumed thread, the [2]rotaxane and, in some cases, the [3]rotaxane.

Synthesis of fumaramide non-methylated [2]rotaxane (1a)



1a

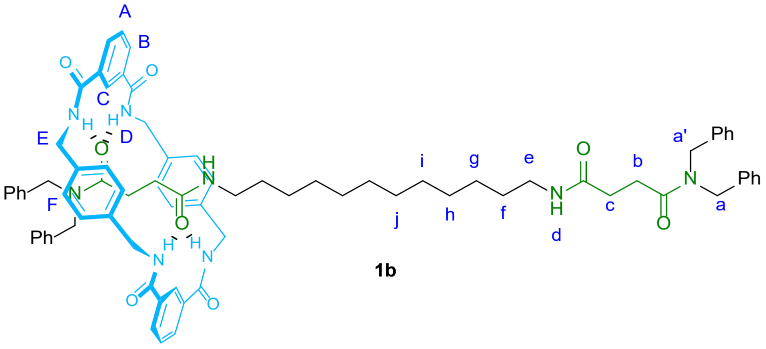
[2]Rotaxane **1a** was obtained following the described method from fumaramide thread **8a** (0.25 g, 0.33 mmol), *p*-xylylendiamine (0.18 g, 1.32 mmol) and isophthaloyl dichloride (0.27 g, 1.32 mmol). The resulting crude was purified by column chromatography (silica gel) using $CHCl_3$/Acetone (2:1) as eluent to give the title product as a white solid (0.11 g, 23% isolated, 29% conversion by crude ^{1}H-NMR); mp 79 - 81 °C; **^{1}H-NMR (400 MHz, $CDCl_3$, 298 K) δ**: 8.39 (s, 2H, H_C), 8.14 (dd, *J* = 7.8, 1.5 Hz, 4H, H_B), 7.59 (t, *J* = 5.2 Hz, 4H, H_D), 7.54 (t, *J* = 7.8 Hz, 2H, H_A), 7.35 – 7.10 (m, 20H, H_{Ph}), 7.05 – 6.77 (m, 4H, H_{b+c}), 6.93 (s, 8H, H_F), 4.61 – 4.24 (m, 16H, $H_{E+a+a'}$), 3.26 (q, *J* = 6.7 Hz, 4H, H_e), 1.62 – 1.47 (m, 4H, H_f), 1.35 – 1.15 (m, 16H, $H_{g+h+i+j}$) ppm; **^{13}C-NMR (100 MHz, $CDCl_3$, 298 K) δ**: 166.3 (CO), 166.1 (CO), 164.9 (CO), 137.4 (C), 136.3 (C), 135.3 (C), 133.5 (C), 131.7 (CH), 129.3 (CH), 129.1 (CH), 128.9 (CH), 128.2 (CH), 126.3 (CH), 123.8 (CH), 50.9 (CH_2), 44.0 (CH_2), 40.2 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 29.3 (CH_2), 27.1 (CH_2) ppm; **HRMS (ESI)**: calcd for $C_{80}H_{87}N_8O_8$ $[M+H]^+$ 1287.6641, found 1287.6636. **IR (neat) ν:** 3288.0, 2921.6, 2854.1, 1619.9, 1530.2, 696.2, 603.6 cm^{-1}.



S4a

The corresponding byproduct [3]rotaxane **S4a** was also obtained as a white solid (3.9 mg, 0.6% isolated, 2.3% conversion by crude ^{1}H-NMR); mp 126 - 128 °C; **^{1}H-NMR (400 MHz, CDCl$_3$, 298 K) δ**: δ 8.38 (s, 4H, H_C), 8.09 (dd, J = 7.7, 1.5 Hz, 8H, H_B), 8.02 (bs, 2H, H_d), 7.59 (t, J = 5.3 Hz, 8H, H_D), 7.47 (t, J = 7.7 Hz, 4H, H_A), 7.36 – 7.27 (m, 4H, H_{Ph}), 7.18 – 7.02 (m, 12H, H_{Ph}), 6.95 (s, 16H, H_F), 6.67 (d, J = 7.5 Hz, 4H, H_{Ph}), 6.04 – 5.93 (m, 4H, H_{b+c}), 4.43 – 4.24 (m, 24H, $H_{E+a+a'}$), 3.25 (q, J = 6.8 Hz, 4H, H_e), 1.57 (q, J = 6.8 Hz, 4H, H_f), 1.29 – 1.22 (m, 16H, $H_{g+h+i+j}$) ppm; **^{13}C-NMR (100 MHz, CDCl$_3$, 298 K) δ**: 166.3 (CO), 165.9 (CO), 165.6 (CO), 137.4 (C), 136.0 (C), 134.7 (C), 133.7 (CH), 133.6 (C), 131.6 (CH), 129.5 (CH), 129.2 (CH), 129.2 (CH), 129.1 (CH), 128.9 (CH), 128.6 (CH), 128.3 (CH), 126.2 (CH), 125.8 (CH), 123.8 (CH), 51.5 (CH$_2$), 51.1 (CH$_2$), 44.1 (CH$_2$), 40.5 (CH$_2$), 29.8 (CH$_2$), 29.4 (CH$_2$), 29.3 (CH$_2$), 29.2 (CH$_2$), 27.1(CH$_2$) ppm; **HRMS (ESI)**: calcd for $C_{112}H_{115}N_{12}O_{12}$ [M+H]$^+$ 1819.8779, found 1819.8723; **IR (neat) ν:** 3290.0, 2922.6, 2850.3, 1649.8, 1529.3, 1300.8, 1268.9, 697.1, 601.7 cm^{-1}.

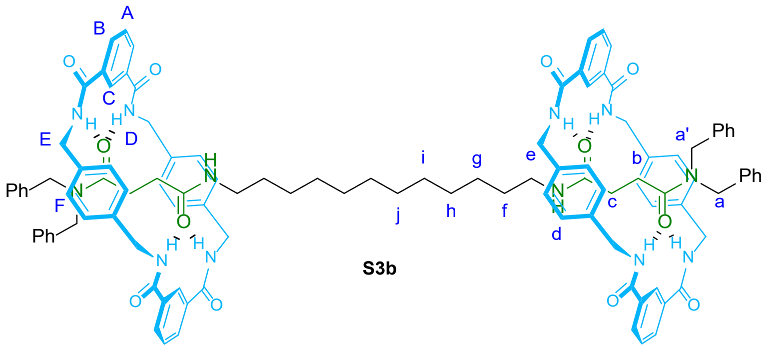
Synthesis of succinamide non-methylated [2]rotaxane (1b)



1b

[2]Rotaxane **1b** was obtained following the described method from succinamide thread **8b** (0.25 g, 0.33 mmol), *p*-xylylendiamine (0.18 g, 1.32 mmol) and isophthaloyl dichloride (0.27 g, 1.32 mmol). The resulting crude was purified by column chromatography (silica gel) using CHCl$_3$/Acetone (3:1) as eluent to give the title product as a white solid (0.075 g, 17% isolated, 34% conversion by crude ^{1}H-NMR); mp 81 - 83 °C; **^{1}H-NMR (400 MHz, CDCl$_3$, 298 K) δ**: 8.48 (s, 2H, H_C), 8.24 (d, J = 7.8 Hz, 4H, H_B), 7.60 (t, J = 7.8 Hz, 2H, H_A), 7.49 (t, J = 5.2 Hz, 4H, H_D), 7.34 – 7.15 (m, 16H, H_{Ph}), 6.95 – 6.92 (m, 12H, H_{Ph+F}), 6.23 – 6.19 (m, 2H, H_d), 4.51 (s, 4H, H_a), 4.41 (d, J = 4.9 Hz, 8H, H_E), 4.31 (s, 4H, $H_{a'}$), 3.15 (q, J = 6.5 Hz, 4H, H_e), 2.07 – 1.39 (m, 8H, H_{b+c}), 1.49 – 1.44 (m, 4H, H_f), 1.28 – 1.22 (m, 16H, $H_{g+h+i+j}$) ppm; **^{13}C-NMR (100 MHz, CDCl$_3$, 298 K) δ**: 173.1 (CO), 172.8 (CO), 166.0 (CO), 138.0 (C), 136.9 (C), 135.7

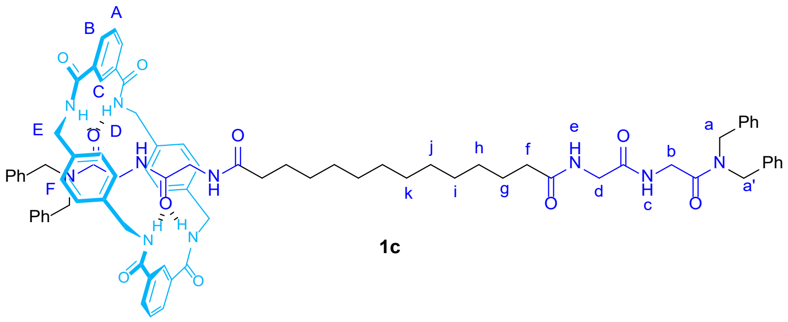
(C), 133.6 (C), 131.8 (CH), 129.2 (CH), 129.2 (CH), 129.0 (CH), 129.0 (CH), 128.8 (CH), 128.5 (CH), 127.9 (CH), 126.1 (CH), 123.4 (CH), 50.8 (CH_2), 49.9 (CH_2), 43.7 (CH_2), 39.8 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 28.5 (CH_2), 27.0 (CH_2) ppm; **HRMS (ESI)**: calcd for $C_{80}H_{91}N_8O_8$ $[M+H]^+$ 1291.6954, found 1291.6919; **IR (neat) ν:** 3303.5, 2922.6, 2852.2, 1643.1, 1627.6, 1532.2, 697.1 cm^{-1}.



S3b

The corresponding byproduct [3]rotaxane **S3b** was also obtained as a white solid (9.0 mg, 1.5% isolated, 2.5% conversion by crude ^{1}H-NMR); mp 153 - 155 °C; **^{1}H-NMR (400 MHz, $CDCl_3$, 298 K) δ**: 8.49 (s, 4H, H_C), 8.22 (dd, J = 7.7, 1.6 Hz, 8H, H_B), 7.57 (t, J = 7.7 Hz, 4H, H_A), 7.48 (t, J = 5.5 Hz, 8H, H_D), 7.39 – 7.30 (m, 6H, H_{Ph}), 7.24 – 7.18 (m, 6H, H_{Ph}), 7.08 (t, J = 7.6 Hz, 4H, H_{Ph}), 6.96 (s, 16H, H_F), 6.74 (d, J = 7.6 Hz, 4H, H_{Ph}), 6.11 (t, J = 5.6 Hz, 2H, H_d), 4.50 (s, 4H, H_a), 4.48 – 4.34 (m, 16H, H_E), 4.18 (s, 4H, $H_{a'}$), 3.09 (q, J = 6.6 Hz, 4H, H_e), 1.47 – 1.41 (m, 4H, H_f), 1.27 – 1.22 (m, 16H, $H_{g+h+i+j}$), 1.12 – 0.94 (m, 8H, H_{b+c}) ppm; **^{13}C-NMR (100 MHz, $CDCl_3$, 298 K) δ**: 173.6 (CO), 173.3 (CO), 166.0 (CO), 138.1 (C), 136.8 (C), 135.1 (C), 133.6 (C), 132.0 (CH), 129.4 (CH), 129.2 (CH), 129.2 (CH), 129.1 (CH), 129.0 (CH), 128.5 (CH), 128.2 (CH), 125.8 (CH), 123.4 (CH), 53.9 (CH_2), 51.8 (CH_2), 51.6 (CH_2), 43.7 (CH_2), 40.0 (CH_2), 31.9, 29.7, 29.4, 29.3, 29.3 (CH_2), 29.1 (CH_2), 28.1 (CH_2), 26.9 (CH_2) ppm; **HRMS (ESI)**: calcd for $C_{112}H_{118}N_{12}NaO_{12}$ $[M+Na]^+$ 1845.8884, found 1845.8778; **IR (neat) ν:** 3308.3, 2922.6, 1649.8, 1627.6, 1532.2, 697.1, 608.4 cm^{-1}.

Synthesis of glycylglycine non-methylated [2]rotaxane (1c)



1c

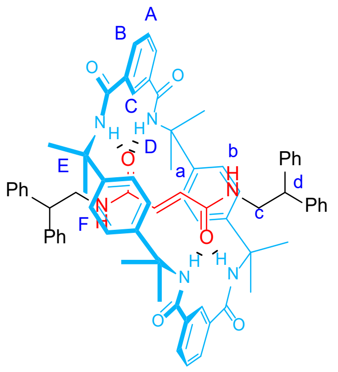
[2]Rotaxane **1c** was obtained following the described method from glycylglycine thread **8c** (0.25 g, 0.30 mmol), *p*-xylylendiamine (0.16 g, 1.18 mmol) and isophthaloyl dichloride (0.24 g, 1.18 mmol). The resulting crude was purified by column chromatography (silica gel) using $CHCl_3$/MeOH (20:1) as eluent to give a fraction that contains the unconsumed thread and the desired [2]rotaxane. Then, portions of 100

mg of this fraction were subjected to a SEC column using $CHCl_3$/MeOH (1:1) as eluent to yield the title product as a white solid (0.01 g, 2% isolated, 25% conversion by crude ^{1}H-NMR); mp 93 - 95 °C; **^{1}H-NMR (400 MHz, $CDCl_3$, 298 K) δ**: 8.51 (s, 2H, H_C), 8.24 (dd, J = 7.8, 1.5 Hz, 4H, H_B), 7.64 – 7.56 (m, 6H, H_{A+D}), 7.33 – 7.24 (m, 12H, H_{Ph}), 7.15 – 7.11 (m, 4H, H_{Ph}), 7.05 – 6.99 (m, 10H, H_{F+c}), 6.95 – 6.91 (m, 4H, H_{Ph}), 6.45 (bs, 2H, H_e), 4.53 (s, 4H, H_a), 4.44 (d, J = 5.1 Hz, 8H, H_E), 4.27 (s, 4H, $H_{a'}$), 3.63 (bs, 8H, H_{b+d}), 2.06 (t, J = 7.7 Hz, 4H, H_f), 1.43 – 1.39 (m, 4H, H_g), 1.17 – 1.13 (m, 16H, $H_{h+i+j+k}$) ppm; **^{13}C-NMR (100 MHz, $CDCl_3$, 298 K) δ**: 174.5 (CO), 169.8 (CO), 168.2 (CO), 166.4 (CO), 137.7 (C), 136.2 (C), 134.9 (C), 133.8 (C), 132.0 (CH), 129.4 (CH), 129.3 (CH), 129.1 (CH), 129.0 (CH), 128.6 (CH), 128.3 (CH), 128.2 (CH), 126.2 (CH), 124.0 (CH), 50.2 (CH_2), 50.0 (CH_2), 44.1 (CH_2), 43.4 (CH_2), 41.7 (CH_2), 36.2 (CH_2), 29.2 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 25.3 (CH_2) ppm; **HRMS (ESI)**: calcd for $C_{82}H_{93}N_{10}O_{10}$ $[M+H]^+$ 1377.7071, found 1377.7036. **IR (neat) ν:** 3294.8, 2922.6, 2852.2, 1634.4, 1525.4, 697.1 cm^{-1}.

8. General procedure for the preparation of the methylated rotaxanes (2 and 7)

Thread (1 equiv.) and Et_3N (24 equiv.) in anhydrous $CHCl_3$ (300 mL) were stirred vigorously whilst solutions of 2,2’-(1,4-phenylene)*bis*(propan-2-amine) (**3**) (8 equiv.) in anhydrous $CHCl_3$ (20 mL) and isophthaloyl dichloride (8 equiv.) in anhydrous $CHCl_3$ (20 mL) were simultaneously added for 4 h using motor-driven syringe pumps. After a further 4 h, the resulting suspension was filtered through a Celite® pad, washed with water (2 x 50 mL), an aqueous solution of HCl 1N (2 x 50 mL), a saturated solution of $NaHCO_3$ (2 x 50 mL) and brine (2 x 50 mL). The organic phase was dried over $MgSO_4$ and the solvent removed under reduced pressure. The resulting solid was subjected to column chromatography (silica gel) and/or size exclusion chromatography (SEC) to yield unconsumed thread, the methylated macrocycle, the [2]rotaxane and, in some cases, the [3]rotaxane.

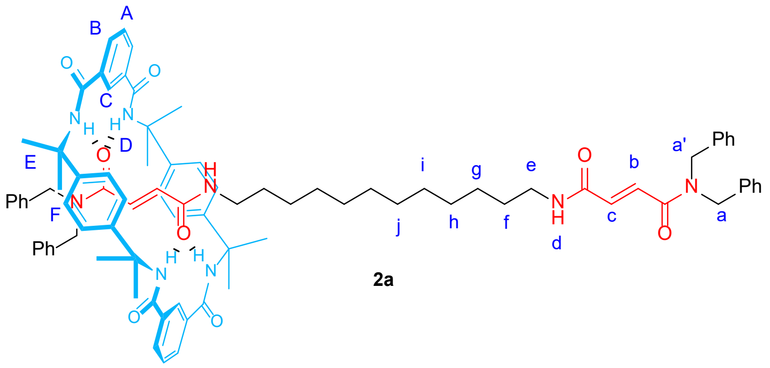
Synthesis of fumaramide methylated [2]rotaxane with one binding site (7)



7

[2]Rotaxane **7** was obtained following the described method from fumaramide thread **6** (0.59 g, 1.25 mmol), 2,2'-(1,4-phenylene)*bis*(propan-2-amine) (**3**) (1.93 g, 10.0 mmol) and isophthaloyl dichloride (2.03 g, 10.0 mmol). The resulting crude was purified by column chromatography (silica gel) using $CHCl_3$/MeOH (20:1) as eluent to give a fraction that contains the unconsumed thread and the desired [2]rotaxane. Then, portions of 100 mg of this fraction were subjected to a SEC column using $CHCl_3$/MeOH (1:1) as eluent to yield the title product as a white solid (0.15 g, 11% isolated); mp > 300 °C; **^{1}H-NMR (400 MHz, $CDCl_3$, 298 K) δ**: 8.14 (s, 2H, H_C), 7.93 (dd, J = 7.7, 1.4 Hz, 4H, H_B), 7.52 (t, J = 7.7 Hz, 2H, H_A), 7.28-7.00 (m, 32H, H_{Ph+D+F}), 6.87 (t, J = 5.3 Hz, 2H, H_b), 5.84 (s, 2H, H_a), 4.02 (t, J = 7.9 Hz, 2H, H_d), 3.38 (dd, J = 7.9, 5.3 Hz, 4H, H_c), 1.78 (s, 24H, H_E) ppm; **^{13}C-NMR (100 MHz, $CDCl_3$, 298 K) δ**: 168.0 (CO), 164.9 (CO), 145.8 (C), 142.2 (C), 136.1 (C), 132.4 (CH), 130.3 (CH), 128.7 (CH), 128.1 (CH), 126.9 (CH), 126.5 (CH), 125.4 (CH), 56.7 (C), 50.0 (CH), 44.7 (CH_2), 28.6 (CH_3) ppm; **HRMS (ESI)**: calcd for $C_{72}H_{75}N_6O_6$ $[M+H]^+$ 1119.5743, found 1119.5779; **IR (neat) ν:** 3264.9, 1634.4, 1529.3, 1297.9, 690.4, 590.1 cm^{-1}.

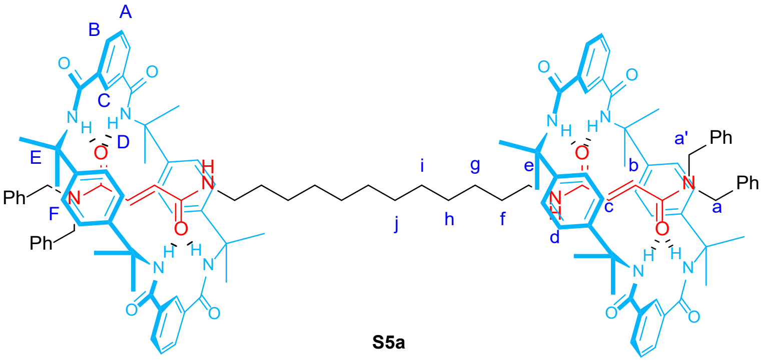
Synthesis of fumaramide methylated [2]rotaxane (2a)



2a

[2]Rotaxane **2a** was obtained following the described method from fumaramide thread **8a** (0.65 g, 0.86 mmol), 2,2'-(1,4-phenylene)*bis*(propan-2-amine) (**3**) (1.32 g, 6.89 mmol) and isophthaloyl dichloride (1.40 g, 6.89 mmol). The resulting crude was purified by column chromatography (silica gel) using $CHCl_3$/Acetone (3:1) as eluent to give a fraction that contains the unconsumed thread, the desired [2]rotaxane and the corresponding [3]rotaxane. Then, portions of 100 mg of this fraction were subjected to a SEC column using $CHCl_3$/MeOH (1:1) as eluent to yield the title product as a white solid (0.13 g, 11% isolated, 31% conversion by crude ^{1}H-NMR); mp 109 - 111 °C; **^{1}H-NMR (400 MHz, $CDCl_3$, 298 K) δ**: 8.51 (s, 2H, H_C), 7.98 (dd, J = 7.8 Hz, 1.5 Hz, 4H, H_B), 7.44 (t, J = 7.8 Hz, 2H, H_A), 7.34-7.21 (m, 20H, H_{Ph+D}), 7.20 (s, 8H, H_F), 7.05-7.03 (m, 4H, H_{Ph}), 6.84 (bs, 4H, H_{b+c}), 6.66 (bs, 2H, H_d), 4.44 (s, 4H, H_a), 4.10 (bs, 4H, $H_{a'}$), 3.01 (bs, 4H, H_e), 1.75 (s, 24H, H_E), 1.46-1.42 (m, 4H, H_f), 1.23 (m, 16H, $H_{g+h+i+j}$) ppm; **^{13}C-NMR (100 MHz, $CDCl_3$, 298 K) δ**: 166.5 (CO), 166.0 (CO), 164.7 (CO), 146.3 (CH), 136.4 (C), 136.1 (CH), 135.9 (C), 135.5 (C), 130.6 (CH), 128.9 (CH), 128.8 (CH), 128.0 (CH), 127.9 (CH), 127.7 (CH), 126.5 (CH), 125.7 (CH), 125.2 (CH), 56.7 (C), 49.9 (CH_2), 48.4 (CH_2), 40.2 (CH_2), 29.6

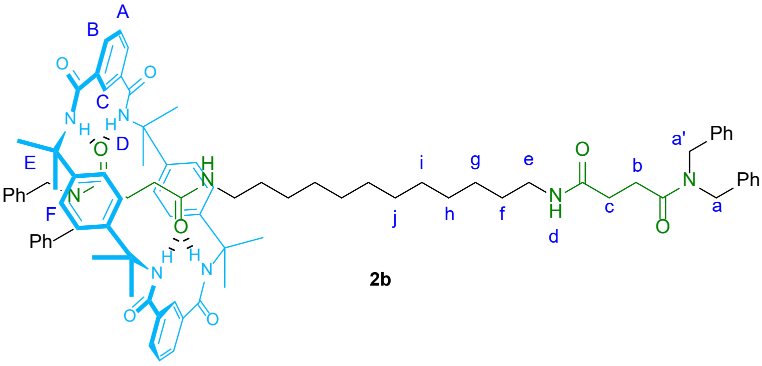
(CH_2), 29.5 (CH_2), 29.3 (CH_2), 29.0 (CH_2), 28.6 (CH_3), 27.0 (CH_2) ppm; **HRMS (ESI)**: calcd for $C_{88}H_{103}N_8O_8$ $[M+H]^+$ 1399.7893, found 1399.7917; **IR (neat) ν:** 3279.4, 2926.5, 2852.2, 1622.8, 1527.4, 1188.9, 694.2 cm^{-1}.



S5a

The corresponding byproduct [3]rotaxane **S5a** was also obtained as white solid (25 mg, 1.4% isolated, 4.2% conversion by crude ^{1}H-NMR); mp 140 - 142 °C; **^{1}H-NMR (400 MHz, $CDCl_3$, 298 K) δ**: 8.51 (s, 4H, H_C), 7.98 (d, $J = 7.8$ Hz, 8H, H_B), 7.49 – 7.45 (m, 6H, H_{A+d}), 7.35 – 7.27 (m, 14H, H_{Ph}), 7.21 (s, 16H, H_F), 7.19 – 7.16 (m, 6H, H_{Ph+D}), 6.89 (d, $J = 7.6$ Hz, 4H, H_{Ph}), 6.57 – 6.51 (m, 4H, H_D), 6.22 – 6.09 (m, 4H, H_{b+c}), 4.26 (s, 4H, H_a), 3.67 (s, 4H, $H_{a'}$), 2.76 (q, $J = 6.9$ Hz, 4H, H_e), 1.84 (s, 24H, H_E), 1.67 (s, 24H, $H_{E'}$), 1.47 – 1.39 (m, 4H, H_f), 1.27 (bs, 16H, $H_{g+h+i+j}$) ppm; **^{13}C-NMR (100 MHz, $CDCl_3$, 298 K) δ**: 166.5 (CO), 165.7 (CO), 165.0 (CO), 146.3 (C), 136.3 (C), 136.0 (CH), 135.6 (C), 130.6 (CH), 128.9 (CH), 128.8 (CH), 128.8 (CH), 127.9 (CH), 127.7 (CH), 127.0 (CH), 126.0 (CH), 125.7 (CH), 125.2 (CH), 56.7 (C), 49.5 (CH_2), 48.3 (CH_2), 40.5 (CH_2), 29.8 (CH_2), 29.8 (CH_2), 29.6 (CH_3), 29.5 (CH_2), 29.0 (CH_2), 27.7 (CH_3), 27.2 (CH_2) ppm; **HRMS (ESI)**: calcd for $C_{128}H_{147}N_{12}O_{12}$ $[M+H]^+$ 2044.1256, found 2044.1231; **IR (neat) ν:** 3283.2, 2924.5, 2852.2, 1626.7, 1527.4, 1188.9, 728.0, 698.1, 592.0 cm^{-1}.

Synthesis of succinamide methylated [2]rotaxane (2b)

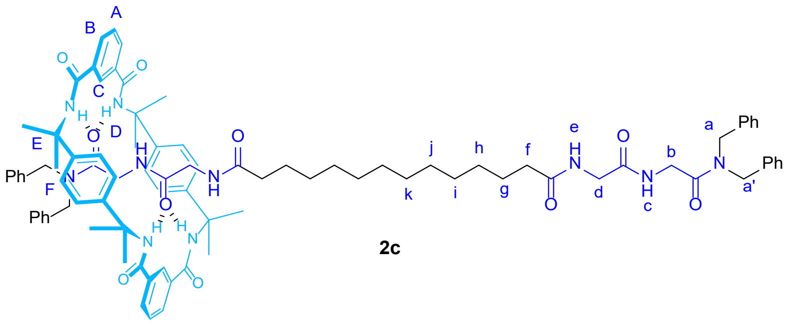


2b

[2]Rotaxane **2b** was obtained following the described method from succinamide thread **8b** (0.30 g, 0.40 mmol), 2,2'-(1,4-phenylene)*bis*(propan-2-amine) (**3**) (0.61 g, 3.16 mmol) and isophthaloyl dichloride (0.64 g, 3.16 mmol). The resulting crude was purified by column chromatography (silica gel) using $CHCl_3$/Acetone (3:1) as eluent to give a fraction that contains the unconsumed thread and the desired [2]rotaxane. Then, portions of 100 mg of this fraction were subjected to a SEC column using

$CHCl_3$/MeOH (1:1) as eluent to yield the title product as a white solid (17 mg, 3% isolated, 7% conversion by crude ^{1}H-NMR); mp 110 - 112 °C; **^{1}H-NMR (400 MHz, $CDCl_3$, 298 K) δ**: 8.40 (t, J = 1.8 Hz, 2H, H_C), 8.03 (dd, J = 7.7, 1.8 Hz, 4H, H_B), 7.51 (t, J = 7.7 Hz, 2H, H_A), 7.35 – 7.26 (m, 10H, H_{Ph+D}), 7.25 – 7.22 (m, 6H, H_{Ph}), 7.21 (s, 8H, H_F), 7.03 (d, J = 7.1 Hz, 4H, H_{Ph}), 6.86 (d, J = 7.4 Hz, 4H, H_{Ph}), 6.50 (t, J = 5.2 Hz, 2H, H_d), 4.43 (s, 4H, H_a), 4.01 (s, 4H, $H_{a'}$), 2.96 (q, J = 6.6 Hz, 4H, H_e), 2.21 – 2.16 (m, 4H, H_b), 2.04 – 1.99 (m, 4H, H_c), 1.76 (s, 24H, H_E), 1.47 – 1.40 (m, 4H, H_f), 1.31 – 1.24 (m, 16H, $H_{g+h+i+j}$) ppm; **^{13}C-NMR (100 MHz, $CDCl_3$, 298 K) δ**: 172.5 (CO), 172.3 (CO), 166.4 (CO), 146.5 (C), 137.1 (C), 136.4 (C), 135.7 (C), 130.7 (CH), 129.0 (CH), 128.8 (CH), 128.8 (CH), 127.7 (CH), 127.5 (CH), 126.1 (CH), 125.7 (CH), 125.2 (CH), 56.6 (C), 49.7 (CH_2), 48.6 (CH_2), 39.9 (CH_2), 31.3 (CH_2), 29.8 (CH_2), 29.7 (CH_2), 29.7 (CH_2), 29.4 (CH_2), 29.4 (CH_2), 28.5 (CH_3), 27.1 (CH_2) ppm; **HRMS (ESI)**: calcd for $C_{88}H_{107}N_8O_8$ $[M+H]^+$ 1403.8206, found 1403.8206; **IR (neat) ν:** 3301.5, 2924.5, 1631.5, 1529.3, 730.9, 695.2, 590.1 cm^{-1}.

Synthesis of glycylglycine methylated [2]rotaxane (2c)

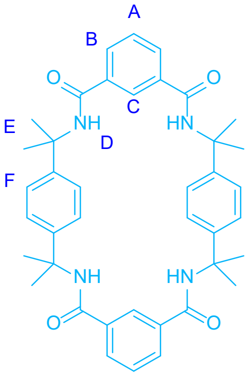


2c

[2]Rotaxane **2c** was obtained following the described method from glycylglycine thread **8c** (0.30 g, 0.35 mmol), 2,2'-(1,4-phenylene)*bis*(propan-2-amine) (**3**) (0.55 g, 2.84 mmol) and isophthaloyl dichloride (0.58 g, 2.84 mmol). The resulting crude was purified by column chromatography (silica gel) using $CHCl_3$/Acetone (2:1) as eluent to give a fraction that contains the unconsumed thread and the desired [2]rotaxane. Then, portions of 100 mg of this fraction were subjected to a SEC column using $CHCl_3$/MeOH (1:1) as eluent to yield the title product as a white solid (0.025 g, 5% isolated, 10% conversion by crude ^{1}H-NMR); mp 99 - 101 °C; **^{1}H-NMR (400 MHz, $CDCl_3$, 298 K) δ**: 8.44 (t, J = 1.8 Hz, 2H, H_C), 7.95 (dd, J = 7.7, 1.8 Hz, 4H, H_B), 7.47 (t, J = 7.7 Hz, 2H, H_A), 7.39 – 7.23 (m, 24H, H_{Ph+D+F}), 7.10 – 7.06 (m, 4H, H_{Ph}), 7.02 – 6.97 (m, 6H, H_{Ph+c}), 6.58 (t, J = 5.0 Hz, 2H, H_e), 4.49 (s, 4H, H_a), 4.23 (s, 4H, $H_{a'}$), 3.77 (s, 4H, H_b), 3.56 (s, 4H, H_d), 2.05 (t, J = 7.7 Hz, 4H, H_f), 1.79 (s, 24H, H_E), 1.52 – 1.45 (m, 4H, H_g), 1.17 (bs, 16H, $H_{h+i+j+k}$) ppm; **^{13}C-NMR (100 MHz, $CDCl_3$, 298 K) δ**: 173.7 (CO), 169.4 (CO), 168.3 (CO), 166.6 (CO), 145.8 (C), 136.5 (C), 135.5 (C), 135.4 (C), 130.2 (CH), 129.2 (CH), 128.8 (CH), 128.6 (CH), 128.3 (CH), 128.1 (CH), 127.8 (CH), 126.6 (CH), 126.3 (CH), 125.3 (CH), 56.4 (C), 49.1 (CH_2), 49.0 (CH_2), 42.6 (CH_2), 41.4 (CH_2), 36.3 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 28.4

(CH_3), 25.6 (CH_2) ppm; **HRMS (ESI)**: calcd for $C_{90}H_{108}N_{10}NaO_{10}$ $[M+Na]^+$ 1511.8142, found 1511.8163; **IR (neat) ν:** 3290.0, 2924.5, 2852.2, 1638.2, 1525.4, 1452.1, 699.1 cm^{-1}.

Isolation of the methylated macrocycle (S1)

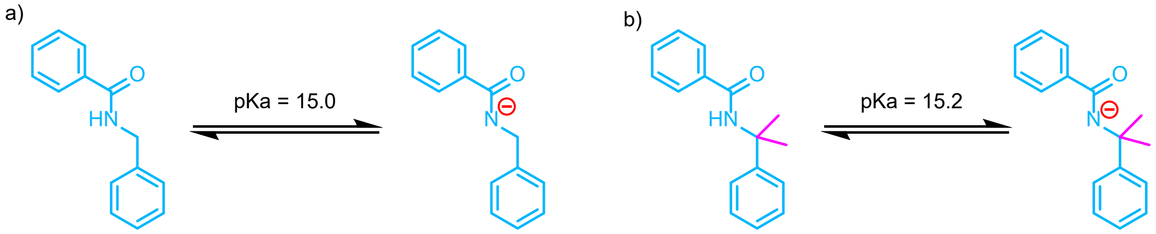


S1

In each reaction of formation of rotaxanes in which the 2,2'-(1,4-phenylene)*bis*(propan-2-amine) (**3**) was employed, a considerably amount of methylated macrocycle **S1** was isolated. This compound shows greater solubility in halogenated solvents than his non-methylated analogous; mp 230 - 232 °C; **^{1}H-NMR (400 MHz, $CDCl_3$, 298 K) δ**: 8.08 (s, 2H, H_C), 7.81 (d, J = 7.7 Hz, 4H, H_B), 7.43 - 7.39 (m, 10H, H_{A+F}), 6.59 (s, 4H, H_D), 1.79 (s, 24H, H_E) ppm; **^{13}C-NMR (100 MHz, $CDCl_3$, 298 K) δ**: 165.7 (CO), 145.1 (C), 135.7 (C), 129.9 (CH), 129.0 (CH), 125.3 (CH), 125.2 (CH), 56.5 (C), 29.2 (CH_3) ppm; **HRMS (ESI)**: calcd for $C_{40}H_{45}N_4O_4$ $[M+H]^+$ 645.3435, found 645.3446; **IR (neat) ν:** 3286.1, 2973.7, 1645.0, 1523.5, 1507.1, 585.3 cm^{-1}.

9. Estimation of the pKa of the deprotonation of model amides

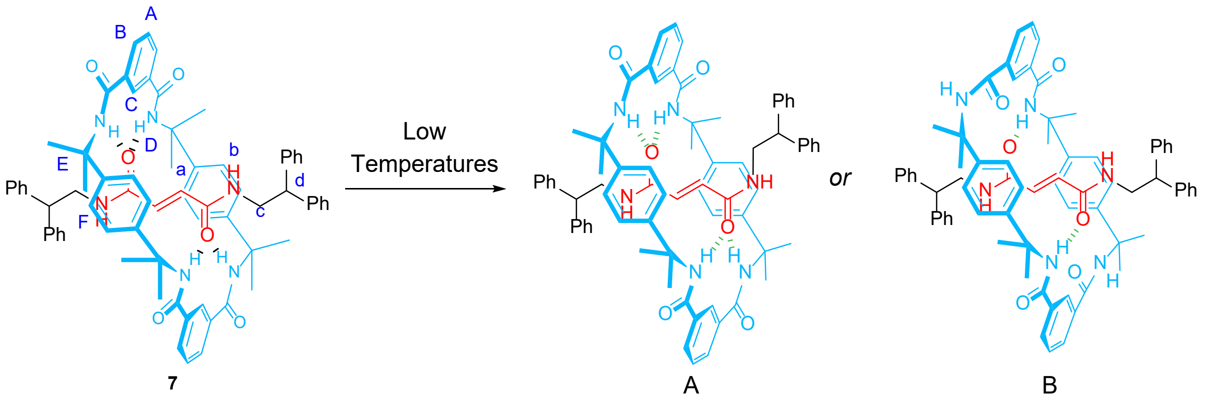
The pKa of the NH amide groups has been computationally estimated for two model molecules: a) *N*-benzylbenzamide and b) *N*-(2-phenylpropan-2-yl)benzamide, which mimic the amides present at the macrocycle in rotaxanes **2** and **7** (Scheme S5). The estimation was carried out using the software package Marvin Suite version 5.11.5 and chemicalize.org, both developed by ChemAxom. The obtained data indicates the less acidic character of the NH group in the dimethylated analogue compared to that of the *N*-benzylbenzamide.



Scheme S5. Computational estimation of the pKa (error: ± 0.2) for the deprotonation of: a) *N*-benzylbenzamide and b) *N*-(2-phenylpropan-2-yl)benzamide.

10. VT-NMR experiment of [2]rotaxane 7

Variation temperature ^{1}H NMR experiments were performed for the methylated rotaxane **7** in order to get valuable information about the rotational motion of the polyamide macrocycle. The temperature dependence ^{1}H NMR spectrum of the systems was studied in CD_2Cl_2 (from 293 K to 193 K). As the temperature decreases, the signal corresponding to the eight methyl groups at 1.78 ppm broadens, until it splits into two other signals around 234 K, at 2.15 and 1.63 ppm, which could tentatively be assigned to methyl groups in *pseudoaxial* and *pseudoequatorial* positions. However, as the temperature drops to 213 K, the splitting of each of these signals into two more is observed, due to other conformational processes, possibly associated with *cis-trans* isomerism of any amide bond. Moreover, the splitting of other signals is also observed at low temperatures, related to both thread (H_c) and macrocycle (H_A, H_B and H_C). If these processes occur at the amides of the thread, the observed conformation would correspond to a conformer of **7** in which the O=C-N-H groups of the fumaramide fragment would have a different geometry (conformation A). Alternatively, if these *cis-trans* isomerization processes occur in the amide groups of the macrocycle, the conformation observed at low temperature could correspond to a conformation similar to that observed in the solid state, where two of the four carbonyl groups point alternately into the cavity (conformation B). Different conformations cannot be excluded from this analysis.



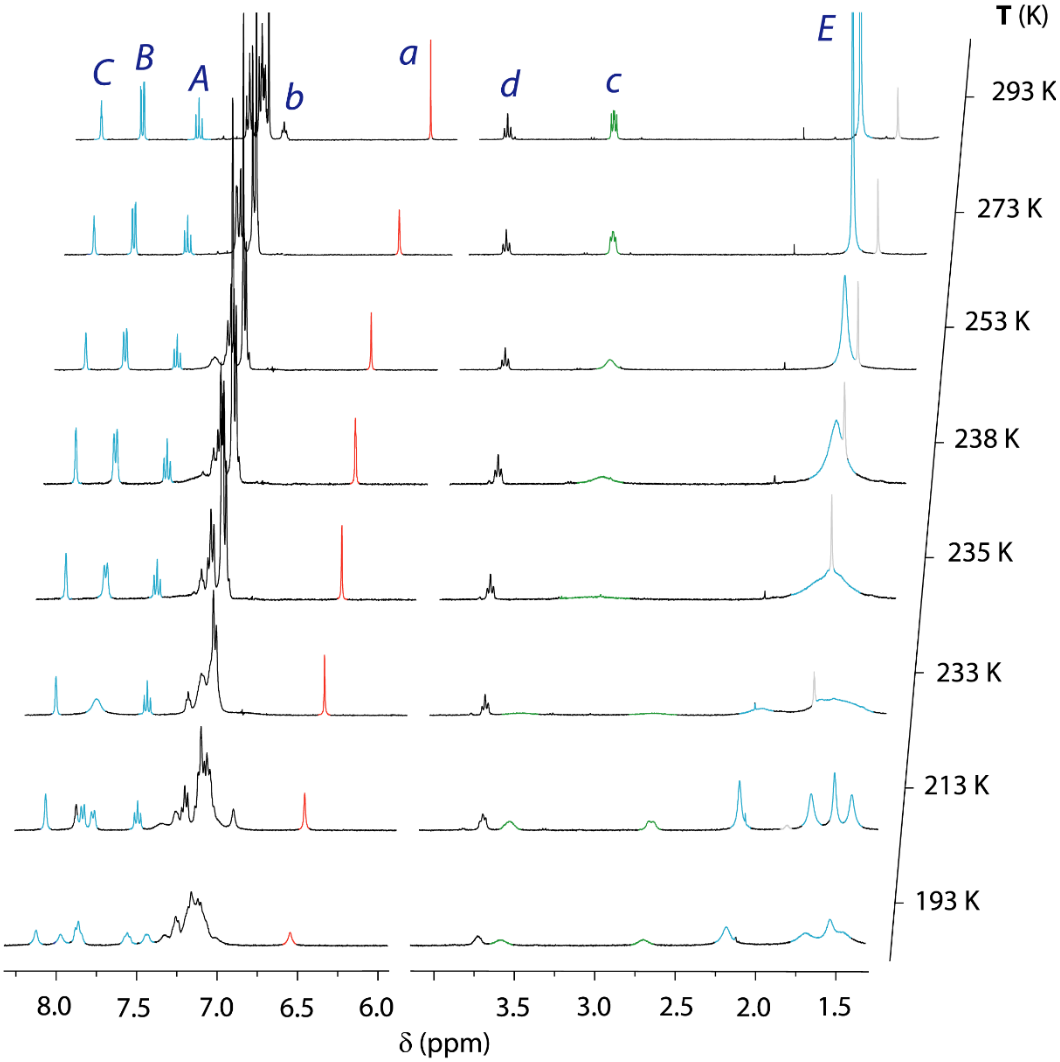


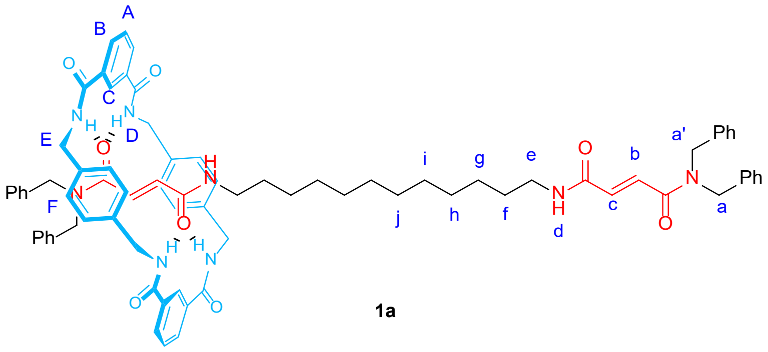
Figure S1. Partial VT-^{1}H NMR spectra (400 MHz, CD_2Cl_2) of [2]rotaxane **7**.

11. VT-NMR experiments of degenerate [2]rotaxanes

Variation temperature ^{1}H NMR experiments were performed for degenerate [2]rotaxanes (both methylated and non-methylated) in order to get valuable information about the translational motion of the polyamide macrocycle along the two binding-sites thread, estimating the relative strength of the intercomponent interactions and the shuttling rates of different systems. The free energies of activation for the translation of the macrocycle were calculated using the Eyring equation, $\Delta G_c^{\neq} = -RT_c{\cdot}ln(k_c h/k_b T_c)$, where $k_c = \pi\sqrt{(\Delta\upsilon^2 + 6J^2)}/\sqrt{2}$, T_c is the coalescence temperature and R, h and k_b are the gas, Planck and Boltzmann constants, respectively. The temperature dependence ^{1}H NMR spectrum of the systems was studied in $CDCl_3$ (from 308 K to 218 K).

Non-methylated fumaramide [2]rotaxane 1a

We followed the coalescence of the signals related to the fumaramide function (H_{b+c}).



1a

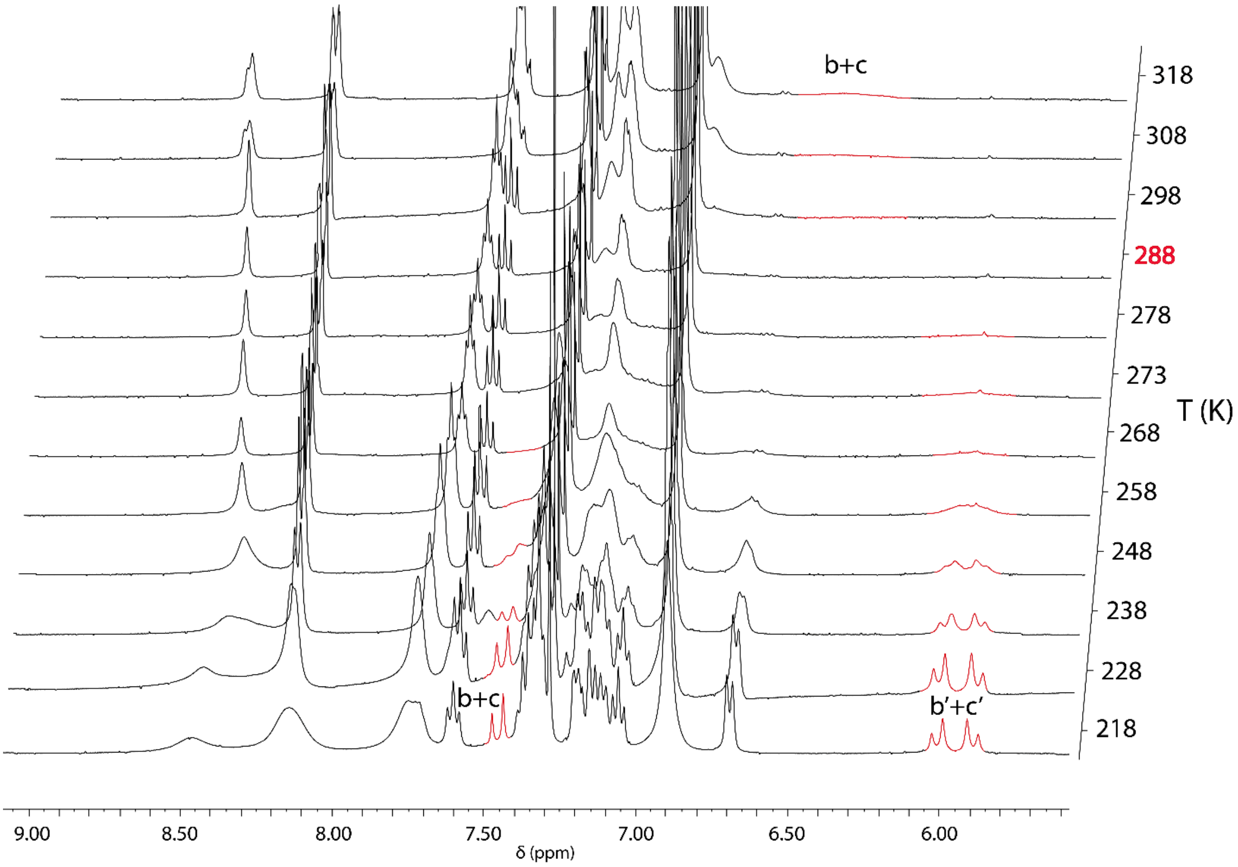
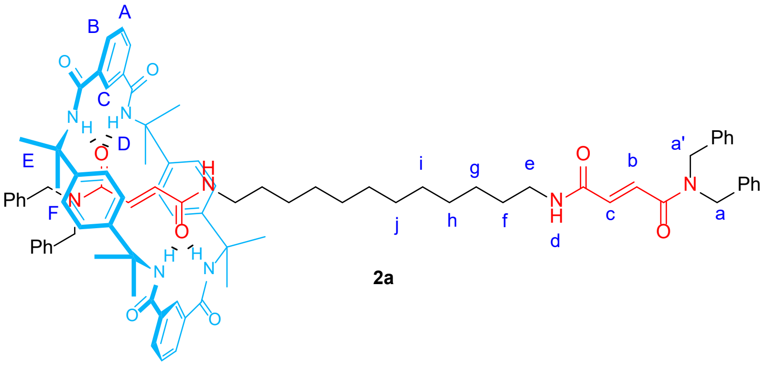


Figure S2. Partial VT-^{1}H NMR spectra (400 MHz, $CDCl_3$) of [2]rotaxane **1a**.

Methylated fumaramide [2]rotaxane 2a

We followed the coalescence of the signals related to the fumaramide function (H_{b+c}).



2a

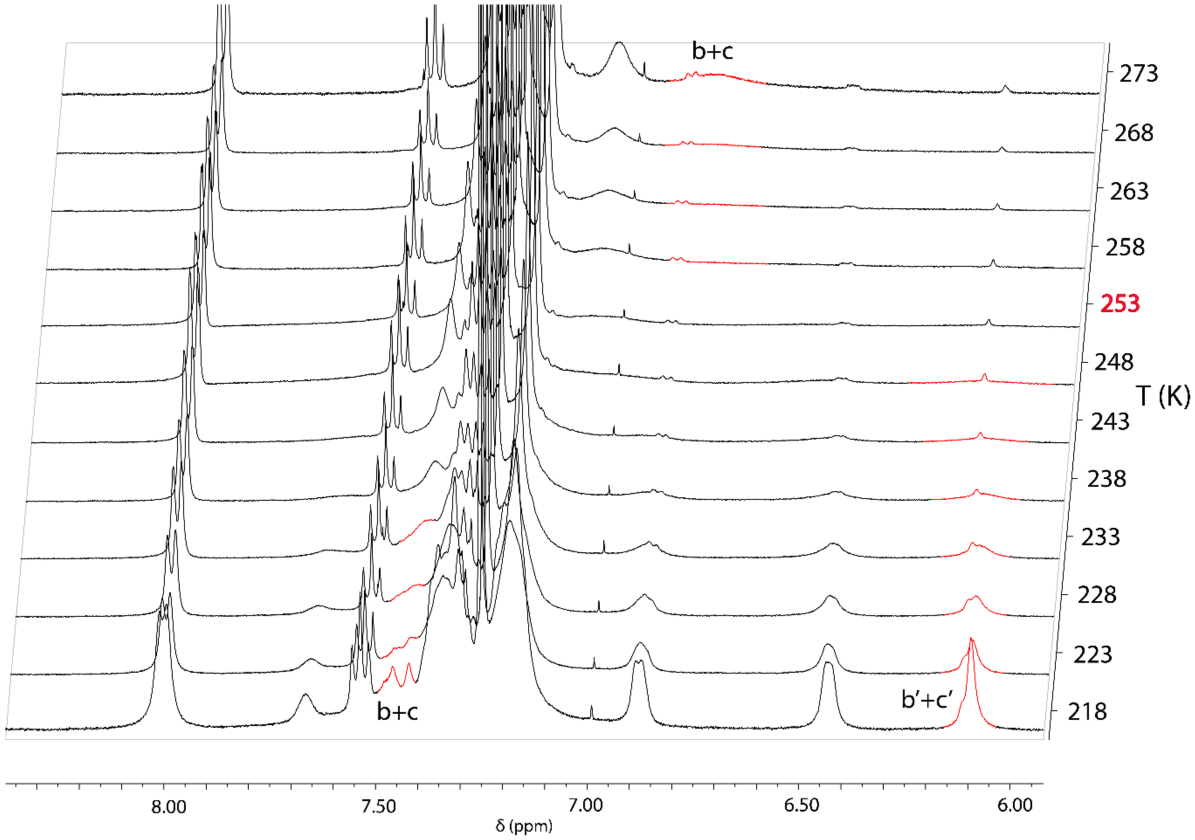
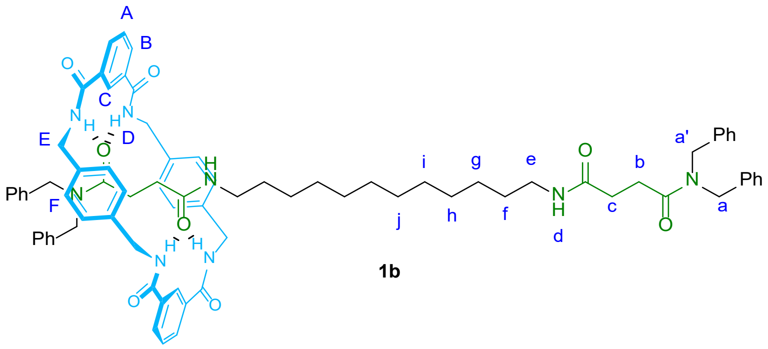


Figure S3. Partial VT-^{1}H NMR spectra (400 MHz, $CDCl_3$) of [2]rotaxane **2a**.

Non-methylated succinamide [2]rotaxane 1b

We followed the coalescence of the signals related to the methylene linked to the amide group in the aliphatic chain of the thread (H_e).



1b

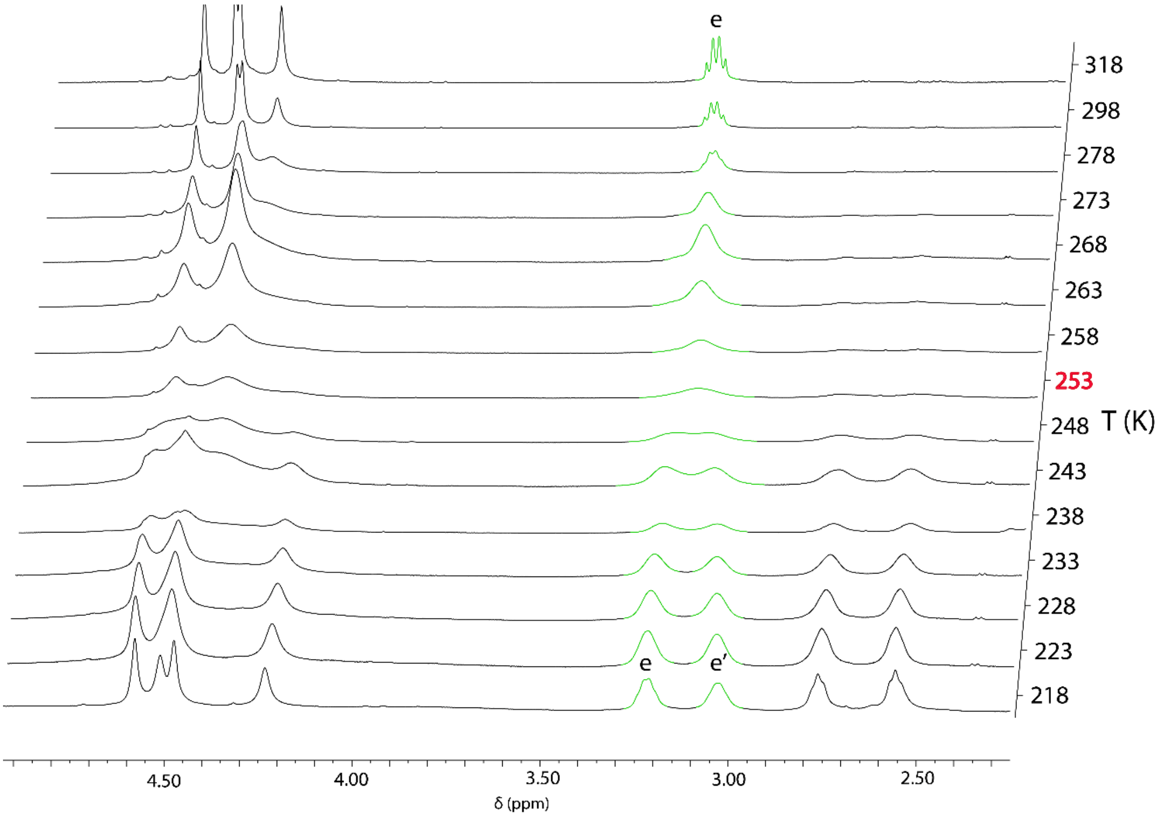
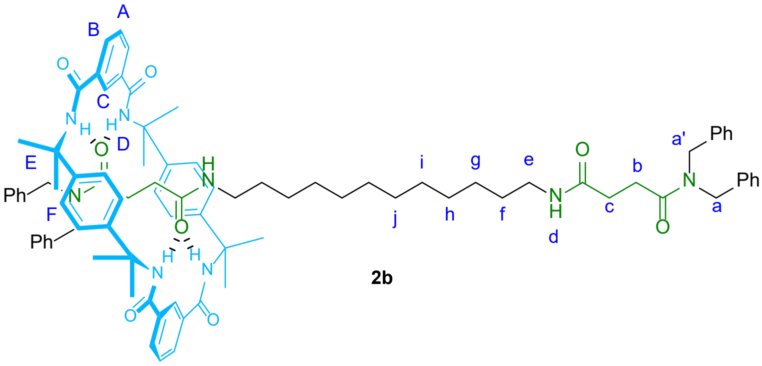


Figure S4. Partial VT-^{1}H NMR spectra (400 MHz, $CDCl_3$) of [2]rotaxane **1b**.

Methylated succinamide [2]rotaxane 2b

We followed the coalescence of the signals related to the methylene linked to the amide group in the aliphatic chain of the thread (H_e).



2b

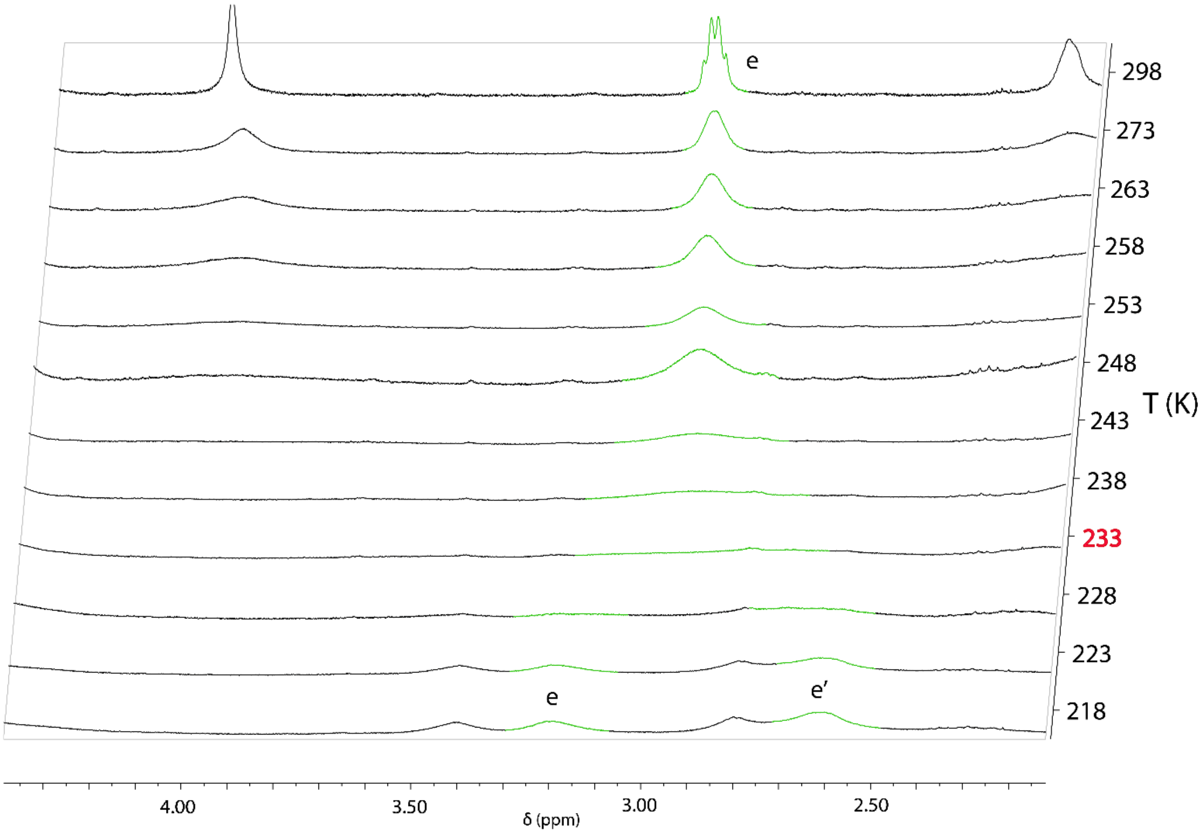
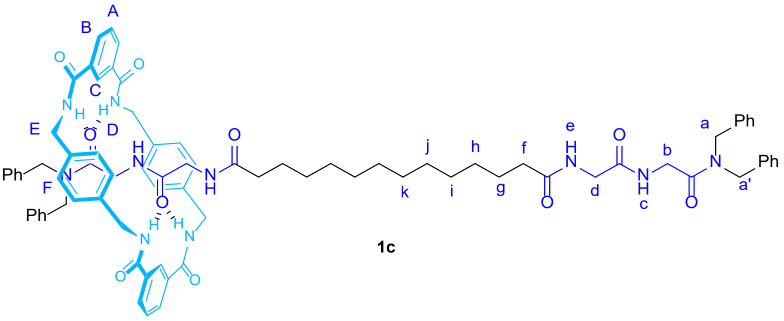


Figure S5. Partial VT-^{1}H NMR spectra (400 MHz, $CDCl_3$) of [2]rotaxane **2b**. The identity of the signals was confirmed by COSY NMR experiments.

Non-methylated glycylglycine [2]rotaxane 1c

We followed the coalescence of the signals related to the methylene linked to the amide group in the aliphatic chain of the thread (H_f).



1c

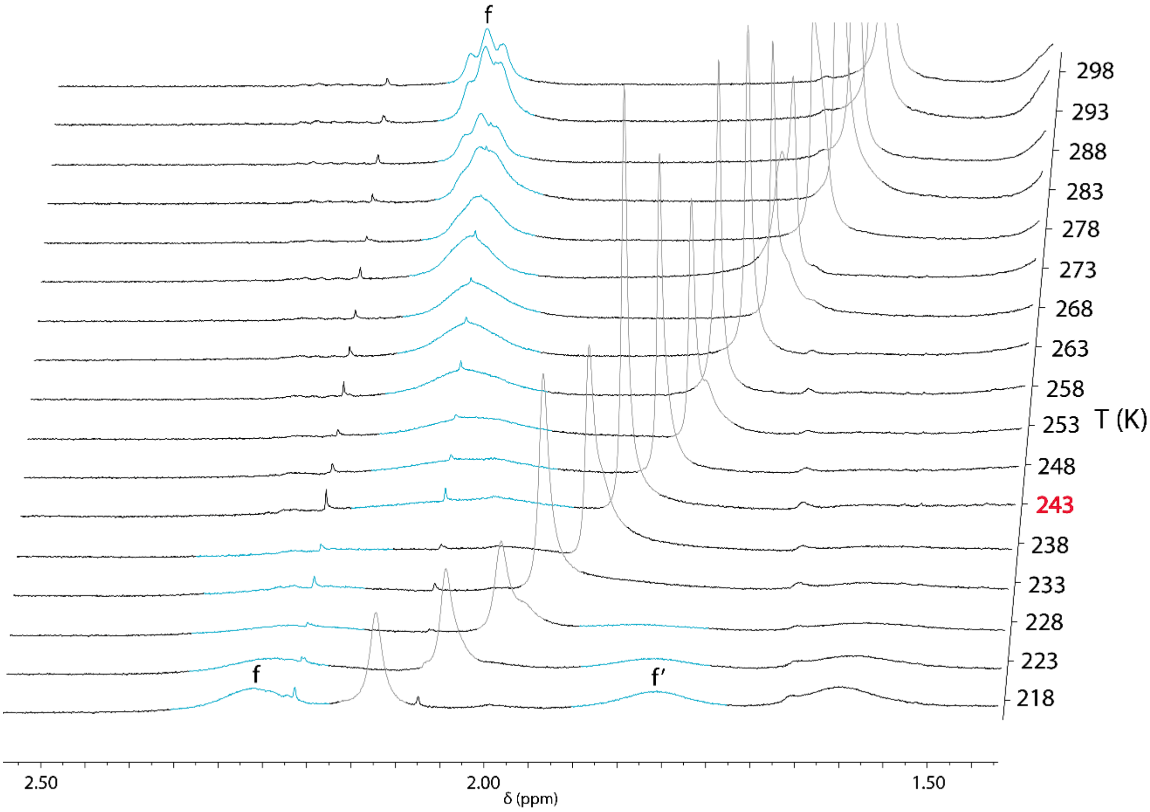
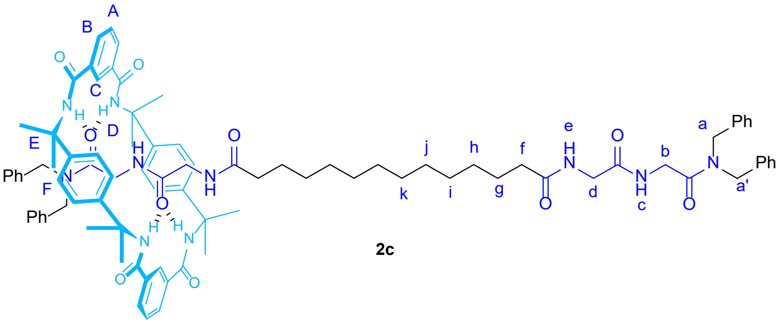


Figure S6. Partial VT-^{1}H NMR spectra (400 MHz, $CDCl_3$) of [2]rotaxane **1c**. In grey: water.

Methylated glycylglycine [2]rotaxane 2c

We followed the coalescence of the signals related to the methylene linked to the amide group in the aliphatic chain of the thread (H_f).



2c

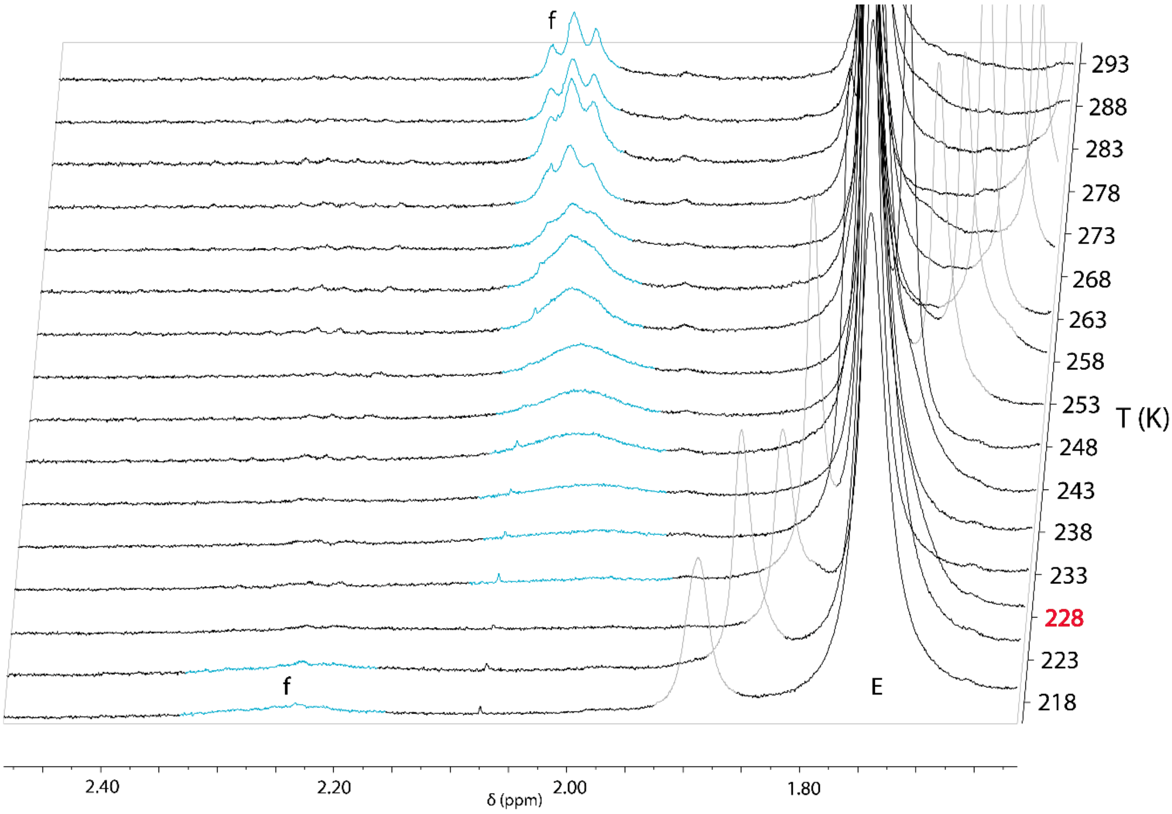


Figure S7. Partial VT-^{1}H NMR spectra (400 MHz, $CDCl_3$) of [2]rotaxane **2c**. The other H_f signal at low temperature is overlapped with the benzylic methyl signal H_E. In grey: water.

Table S1. Kinetic and thermodynamic parameters for macrocycle shuttling obtained from VT-^{1}H NMR spectra of the degenerate [2]rotaxanes at the respective coalescence temperatures.

Entry		**T_c (K)**[a]	**Δν (Hz)**	**k_c (s^{-1})**	**$\Delta G_c^‡$ (kcal·mol^{-1})**[b]
1	**Non-methylated fumaramide [2]rotaxane 1a**	288	580	1288.4	12.7
2	**Methylated fumaramide [2]rotaxane 2a**	253	535	1188.5	11.2
3	**Non-methylated succinamide [2]rotaxane 1b**	253	76	168.8	12.1
4	**Methylated succinamide [2]rotaxane 2b**	233	230	510.9	10.6
5	**Non-methylated glycyclglycine [2]rotaxane 1c**	243	180	399.9	11.2
6	**Methylated glycylglycine [2]rotaxane 2c**	228	160	355.4	10.6

[a] NMR temperature calibration was performed using a pure methanol sample. [b] Calculated value ± 0.2

To ensure a comprehensive comparison of the shuttling process, we calculated the kinetic constants for each rotaxane at the same temperature (298 K). When the exchange is fast ($T > T_c$), the data treatment becomes straightforward since the widths of the NMR lines involved in the exchange are influenced by the uncertainty principle.[1]

We considered a simple (uncoupled) two-site exchange A ⇄ B between the protons with equal populations. A single Lorentzian line is observed at T>Tc, with a line width given by the following equation:

$$\Delta\nu_{1/2} = \frac{1}{2}\pi(\nu_A - \nu_B)^2 k^{-1}$$

where: $\Delta\nu_{1/2}$ is the peak width at half-height at each temperature, and ($\nu_A - \nu_B$) is the maximum separation of the two peaks at low temperature.[1]

We obtained both parameters ($\Delta\nu_{1/2}$ and $\nu_A - \nu_B$) at different temperatures from experimental ^{1}H NMR spectra. $\Delta\nu_{1/2}$ was easily extracted by using "Line fitting" tools in MestReNova software. With these values we calculated the kinetic constants at different temperatures and plotted the *ln(k/T)* versus *1/T* to obtain a linear relationship. Finally, we calculated the kinetic constants at 298 K by extrapolation.

Non-methylated fumaramide [2]rotaxane 1a

In [2]rotaxane **1a** we evaluated the signals related with the second methylene in the aliphatic chain of the thread (H_f):

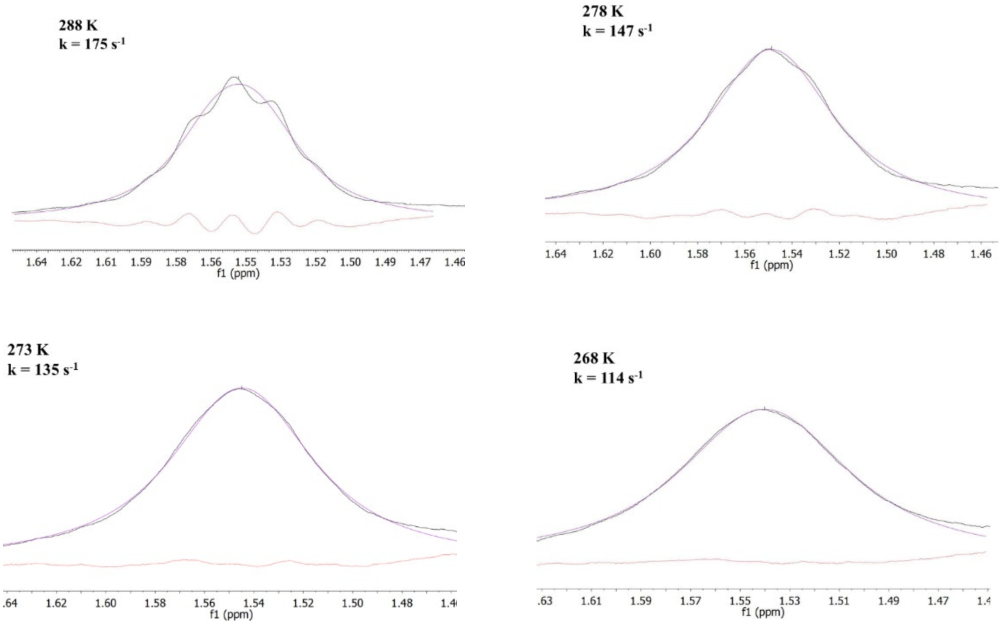


Figure S8. Fittings to Lorentzian peaks of the ^1H NMR signal H_f of rotaxane **1a** at different temperatures (400 MHz, $CDCl_3$) and the corresponding calculated exchange rate constants ***k***. Black line: experimental data; Purple line: Fittings; Orange line: fitting error.

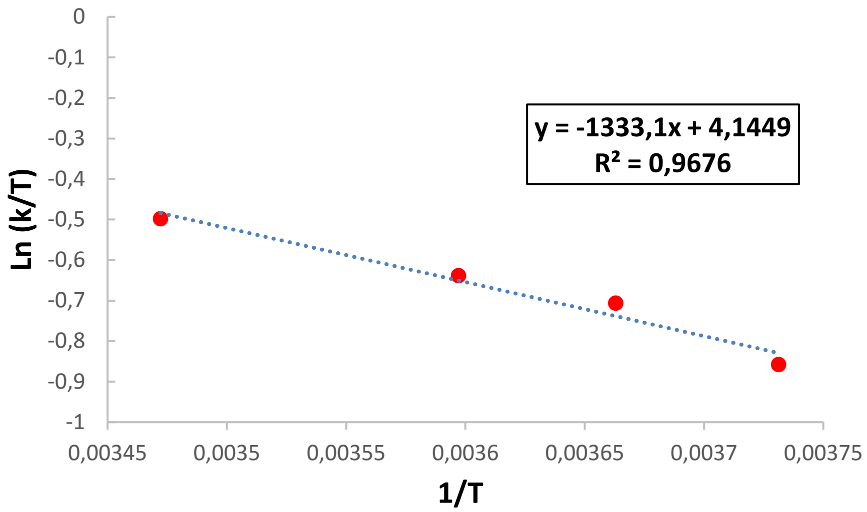


Figure S9. Eyring plot of the exchange rate constants ***k*** for rotaxane **1a**.

Methylated fumaramide [2]rotaxane 2a

In [2]rotaxane **2a** we evaluated the signals related with the first methylene in the aliphatic chain of the thread (H$_e$):

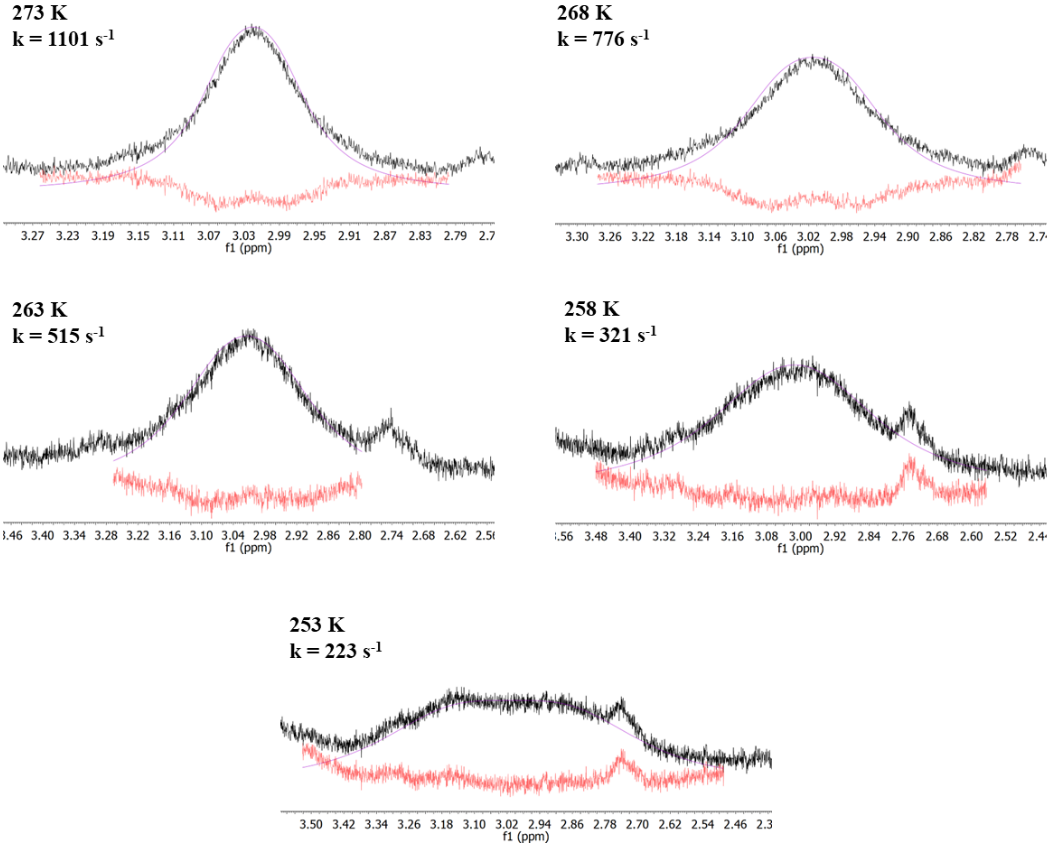


Figure S10. Fittings to Lorentzian peaks of the ^{1}H NMR signal H$_f$ of rotaxane **2a** at different temperatures (400 MHz, CDCl$_3$) and the corresponding calculated exchange rate constants ***k***. Black line: experimental data; Purple line: Fittings; Orange line: fitting error.

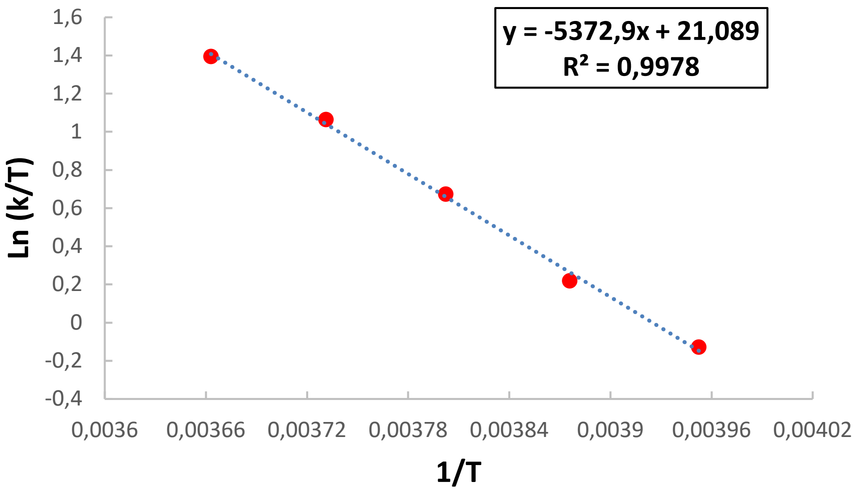


Figure S11. Eyring plot of the exchange rate constants ***k*** for rotaxane **2a**

Non-methylated succinamide [2]rotaxane 1b

In [2]rotaxane **1b** we evaluated the signals related with the first methylene in the aliphatic chain of the thread (H_e):

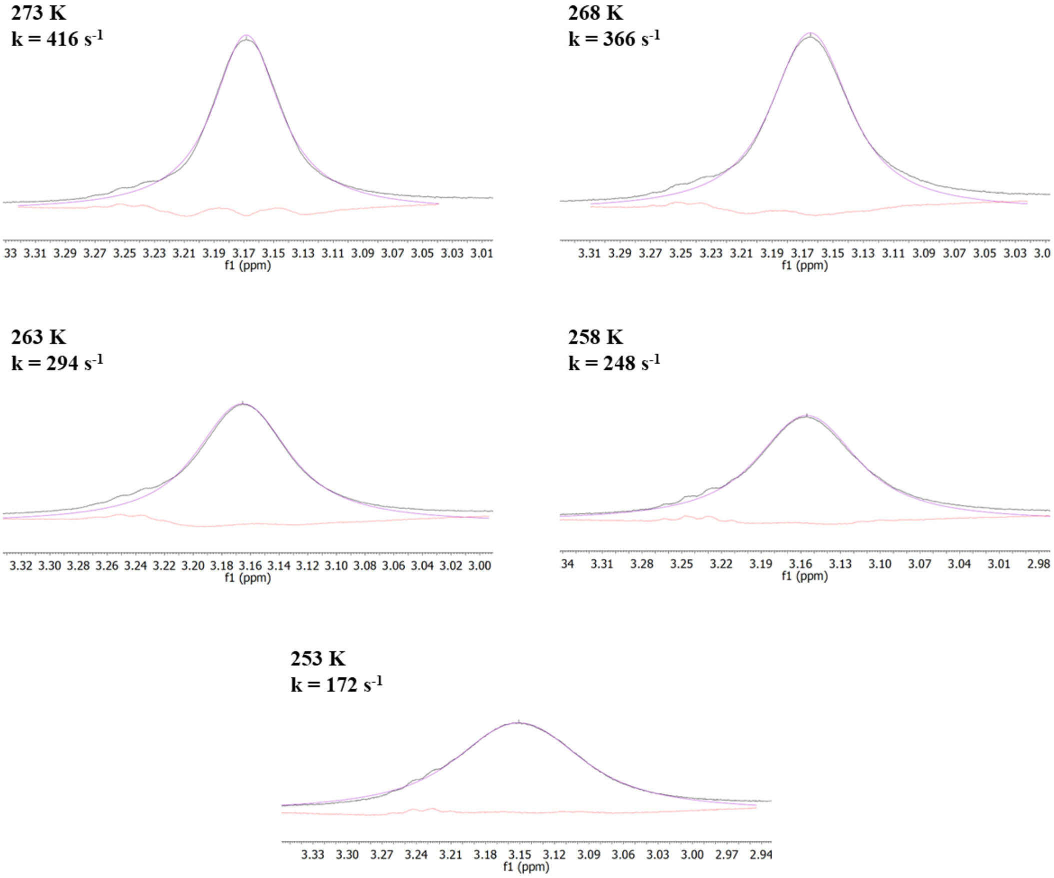


Figure S12. Fittings to Lorentzian peaks of the ^{1}H NMR signal H_f of rotaxane **1b** at different temperatures (400 MHz, $CDCl_3$) and the corresponding calculated exchange rate constants ***k***. Black line: experimental data; Purple line: Fittings; Orange line: fitting error.

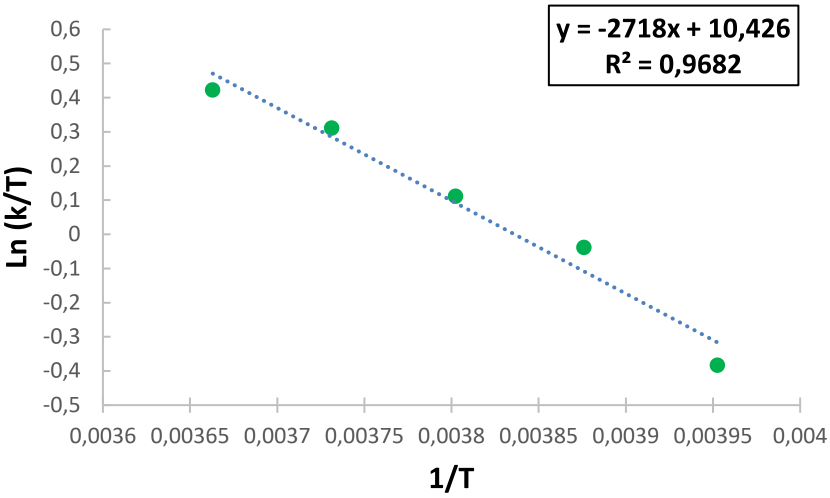


Figure S13. Eyring plot of the exchange rate constants ***k*** for rotaxane **1b**.

Methylated succinamide [2]rotaxane 2b

In [2]rotaxane **2b** we evaluated the signals related with the first methylene in the aliphatic chain of the thread (H_e):

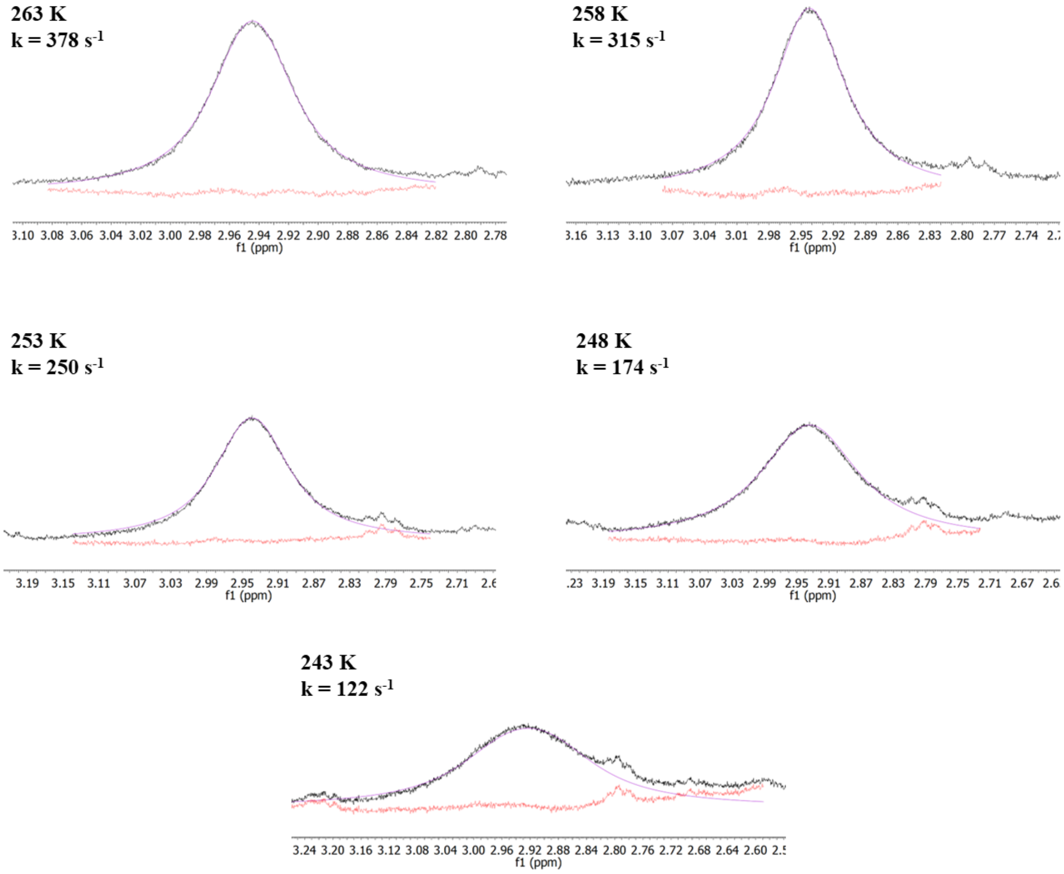


Figure S14. Fittings to Lorentzian peaks of the ^{1}H NMR signal H_f of rotaxane **2b** at different temperatures (400 MHz, $CDCl_3$) and the corresponding calculated exchange rate constants ***k***. Black line: experimental data; Purple line: Fittings; Orange line: fitting error.

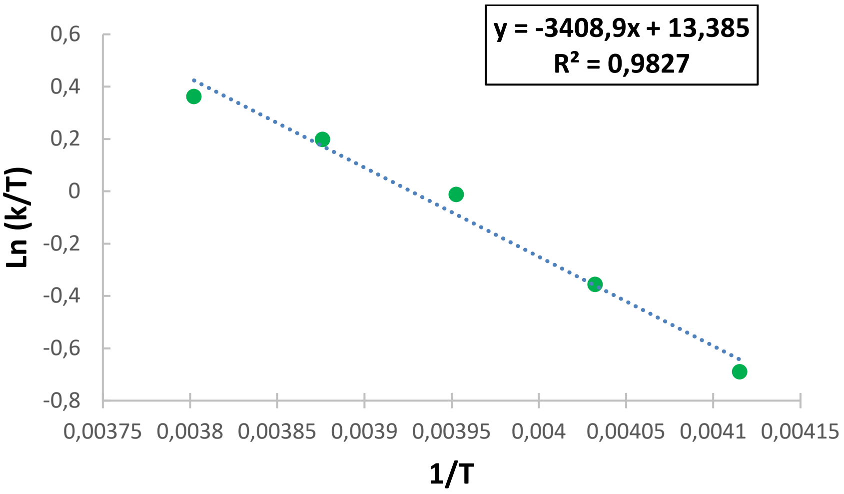


Figure S15. Eyring plot of the exchange rate constants ***k*** for rotaxane **2b**.

Non-methylated glycylglycine [2]rotaxane 1c

In [2]rotaxane **1c** we evaluated the signals related with the first methylene in the aliphatic chain of the thread (H_f):

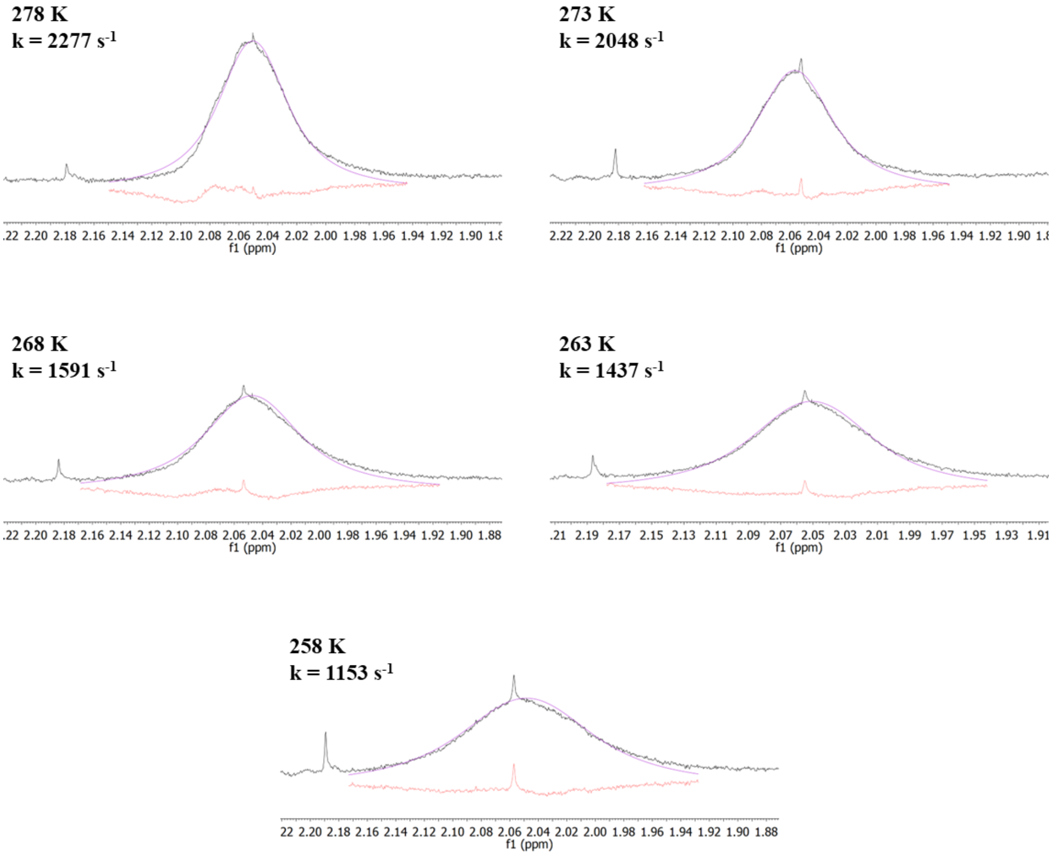


Figure S16. Fittings to Lorentzian peaks of the ^{1}H NMR signal H_f of rotaxane **1c** at different temperatures (400 MHz, $CDCl_3$) and the corresponding calculated exchange rate constants ***k***. Black line: experimental data; Purple line: Fittings; Orange line: fitting error.

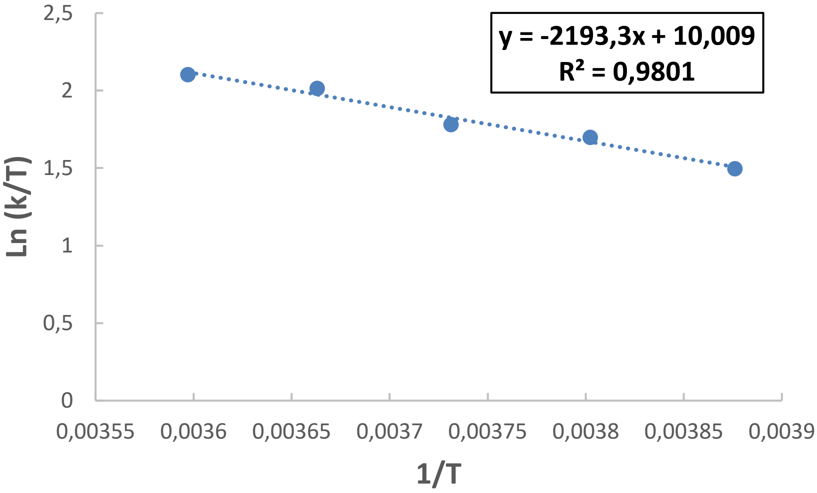


Figure S17. Eyring plot of the exchange rate constants ***k*** for rotaxane **1c**.

Methylated glycylglycine [2]rotaxane 2c

In [2]rotaxane **2c** we evaluated the signals related with the first methylene in the aliphatic chain of the thread (H_f):

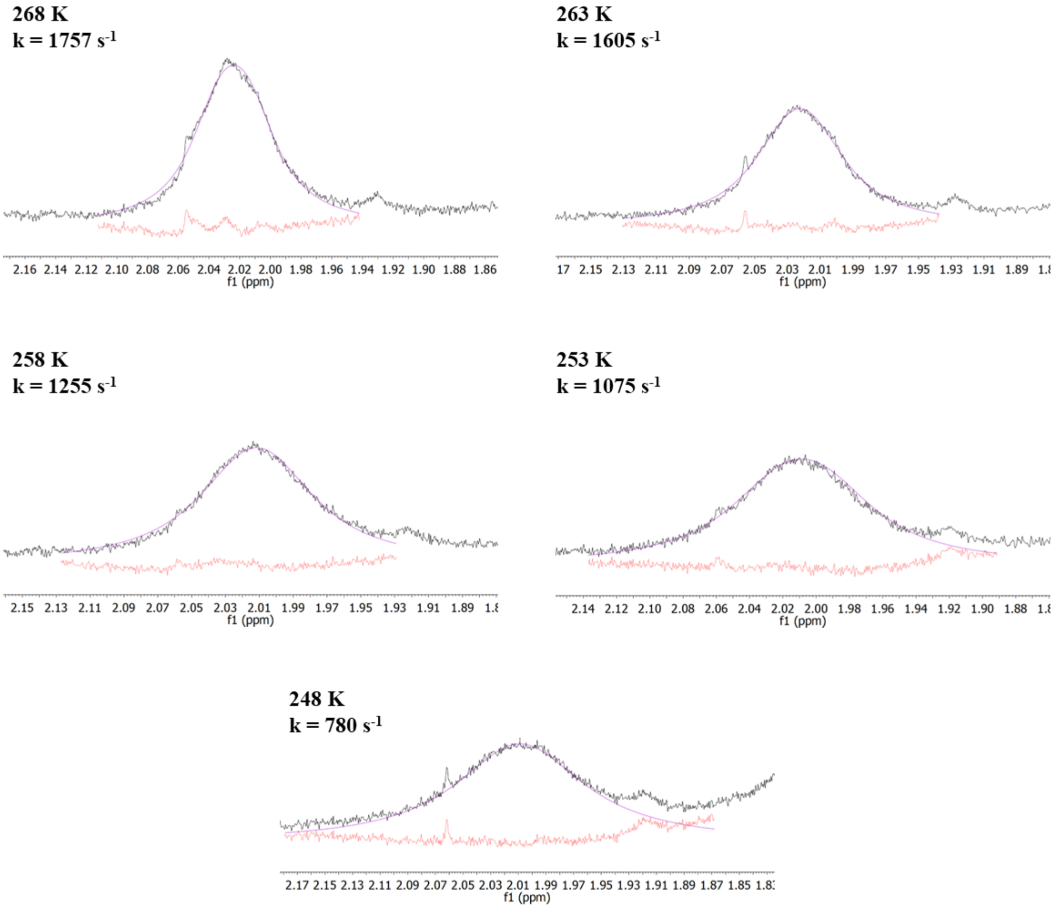


Figure S18. Fittings to Lorentzian peaks of the ^{1}H NMR signal H_f of rotaxane **2c** at different temperatures (400 MHz, $CDCl_3$) and the corresponding calculated exchange rate constants ***k***. Black line: experimental data; Purple line: Fittings; Orange line: fitting error.

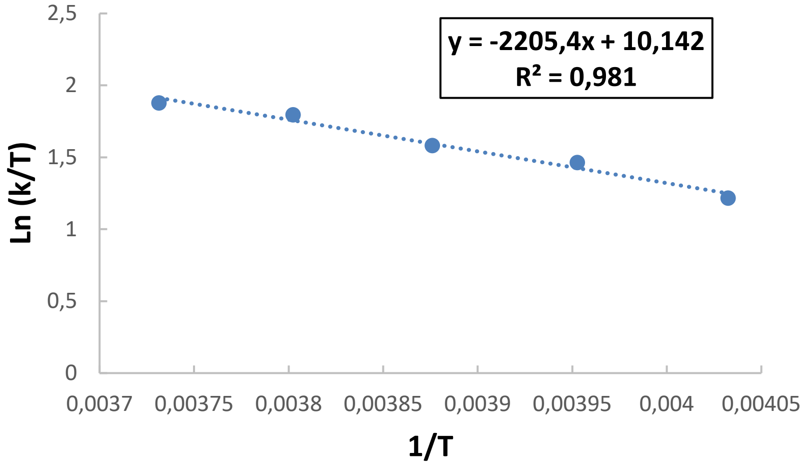


Figure S19. Eyring plot of the exchange rate constants ***k*** for rotaxane **2c**.

Table S2. Calculated exchange rate constants ***k*** of degenerate [2]rotaxanes at 298K.

Entry	**Compound**	***k*** **(s^{-1})**
1	**Non-methylated fumaramide [2]rotaxane 1a**	235
2	**Methylated fumaramide [2]rotaxane 2a**	6350
3	**Non-methylated succinamide [2]rotaxane 1b**	1099
4	**Methylated succinamide [2]rotaxane 2b**	2085
5	**Non-methylated glycylglycine [2]rotaxane 1c**	4213
6	**Methylated glycylglycine [2]rotaxane 2c**	4621

12. Crystal data and structure refinement for rotaxane 7

Single crystals of $C_{76}H_{82}Cl_8N_6O_6$ [JPA_2_0msp_a] were obtained by slow diffusion of pentane in a solution of CH_2Cl_2. Intensities were registered at low temperature (100.0 K) on a Bruker D8 QUEST system equipped with a multilayer monochromator and a Mo K/a Incoatec microfocus sealed tube (λ = 0.71073 Å). Absorption corrections were based on multi-scans (program SADABS). Using Olex2,[2] the structure was solved with the SHELXT[3] structure solution program using Intrinsic Phasing and refined with the SHELXL[4] refinement package using Least Squares minimisation. Hydrogen atoms were included using a riding model. Four molecules of dichloromethane are included in the unit cell. The disorder in one part of the rotaxane and in solvent molecules has been modelled. The structure was deposited with CSD (deposition number CCDC 2279619).

Table S3. Crystal data and structure refinement for **7**.

Empirical formula	$C_{76}H_{82}Cl_8N_6O_6$	
Formula weight	1459.07	
Temperature	100(2) K	
Wavelength	1.54178 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 14.7489(8) Å	a= 85.290(2)°.
	b = 15.2963(9) Å	b= 82.032(2)°.
	c = 16.4862(9) Å	g = 87.045(2)°.
Volume	3668.0(4) Å^3	
Z	2	
Density (calculated)	1.321 Mg/m^3	
Absorption coefficient	3.253 mm^{-1}	
F(000)	1528	
Crystal size	0.220 x 0.120 x 0.070 mm^3	
Theta range for data collection	5.428 to 136.848°.	
Index ranges	-17<=h<=17, -18<=k<=18, -19<=l<=19	
Reflections collected	120345	
Independent reflections	13442 [R(int) = 0.0466]	
Completeness to theta = 67.679°	99.9 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7531 and 0.6096	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	13442 /116/ 982	
Goodness-of-fit on F^2	1.046	
Final R indices [I>2sigma(I)]	R1 = 0.0471, wR2 = 0.1156	

R indices (all data)	R1 = 0.0544, wR2 = 0.1211
Largest diff. peak and hole	0.85 and -0.89 e.Å^{-3}

Table S4. Hydrogens bonds for **7** [Å and (°)]

D-H...A	**d(D-H)**	**d(H...A)**	**d(D...A)**	**<(DHA)**
N(1)-H(1)...O(2)#1	0.88	2.02	2.900(2)	178.0
N(2)-H(2)...O(3')#2	0.88	2.15	2.952(2)	151.2
N(2’)-H(2’)...O(3)#3	0.88	2.05	2.930(2)	178.4
N(3’)-H(3’)...O(1’)#3	0.88	2.12	2.987(2)	166.5

Symmetry transformations used to generate equivalent atoms:
#1 +x,+y,-1+z #2 1-x,1-y,-z #3 1+x,+y,-1+z #4 2-x,1-y,-z

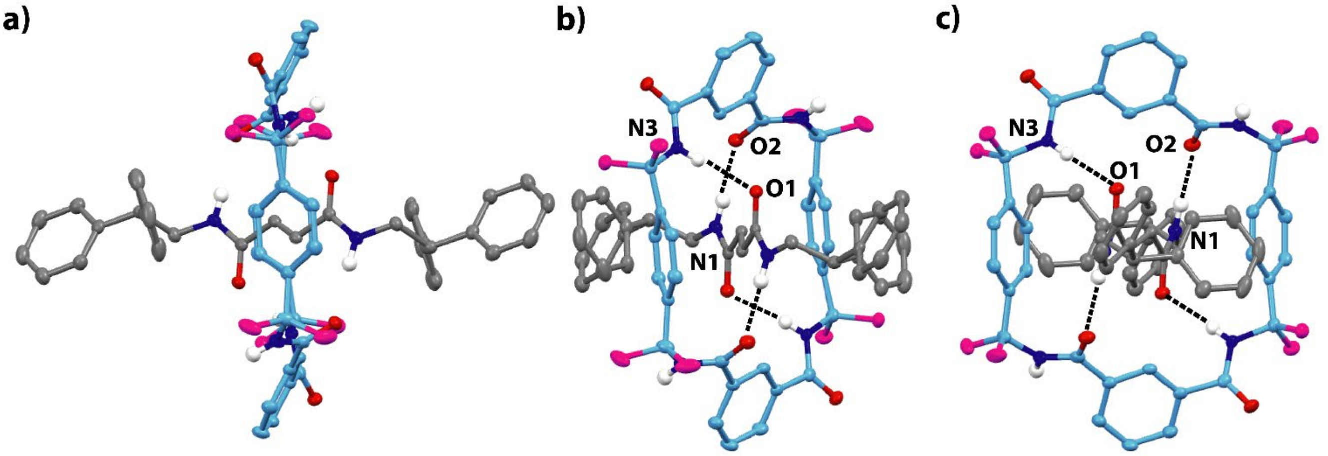


Figure S20. Molecular structure of **7** (molecule 1) with thermal ellipsoids drawn at 50% probability: a) lateral view; b) tilted view; c) front view. For clarity, selected hydrogens atoms and solvent molecules have been deleted. Methyl groups at the macrocycle are highlighted in magenta.

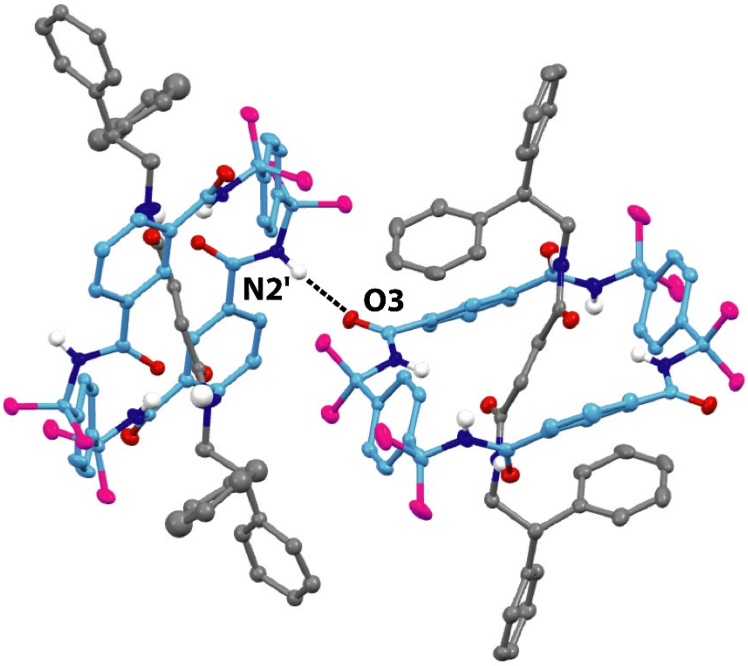
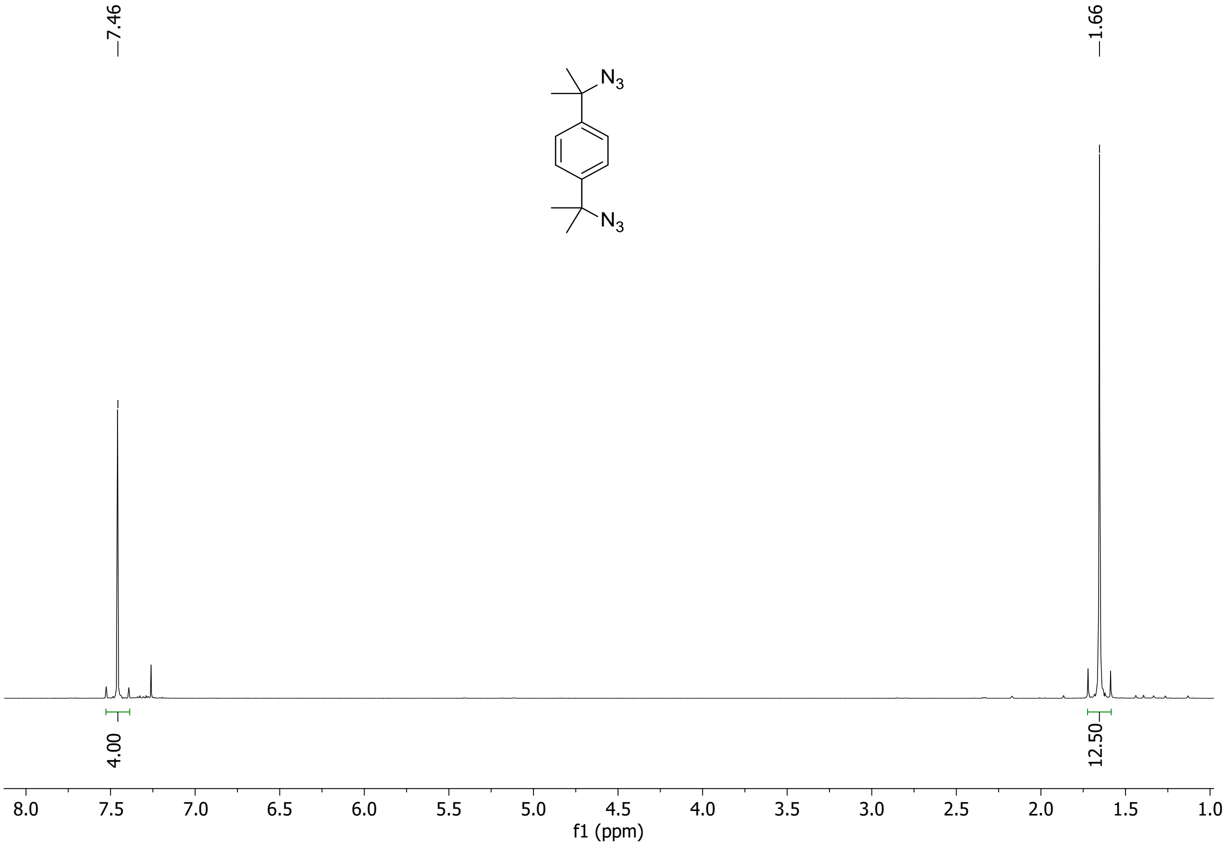


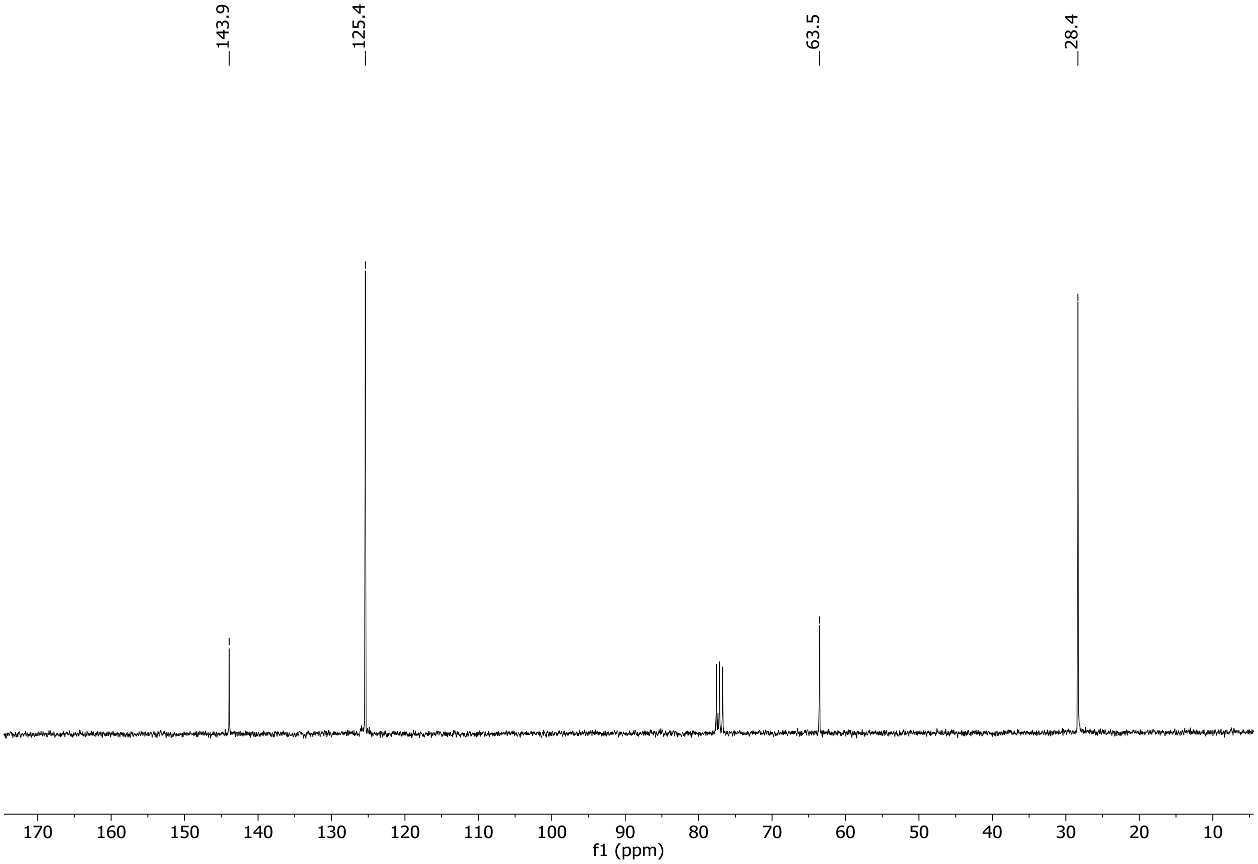
Figure S21. Hydrogen bond between two molecules of rotaxane **7** in the unit cell. For clarity, selected hydrogens atoms and solvent molecules have been deleted. Methyl groups at the macrocycle are highlighted in magenta.

13. ^{1}H and ^{13}C NMR Spectra of synthesized compounds

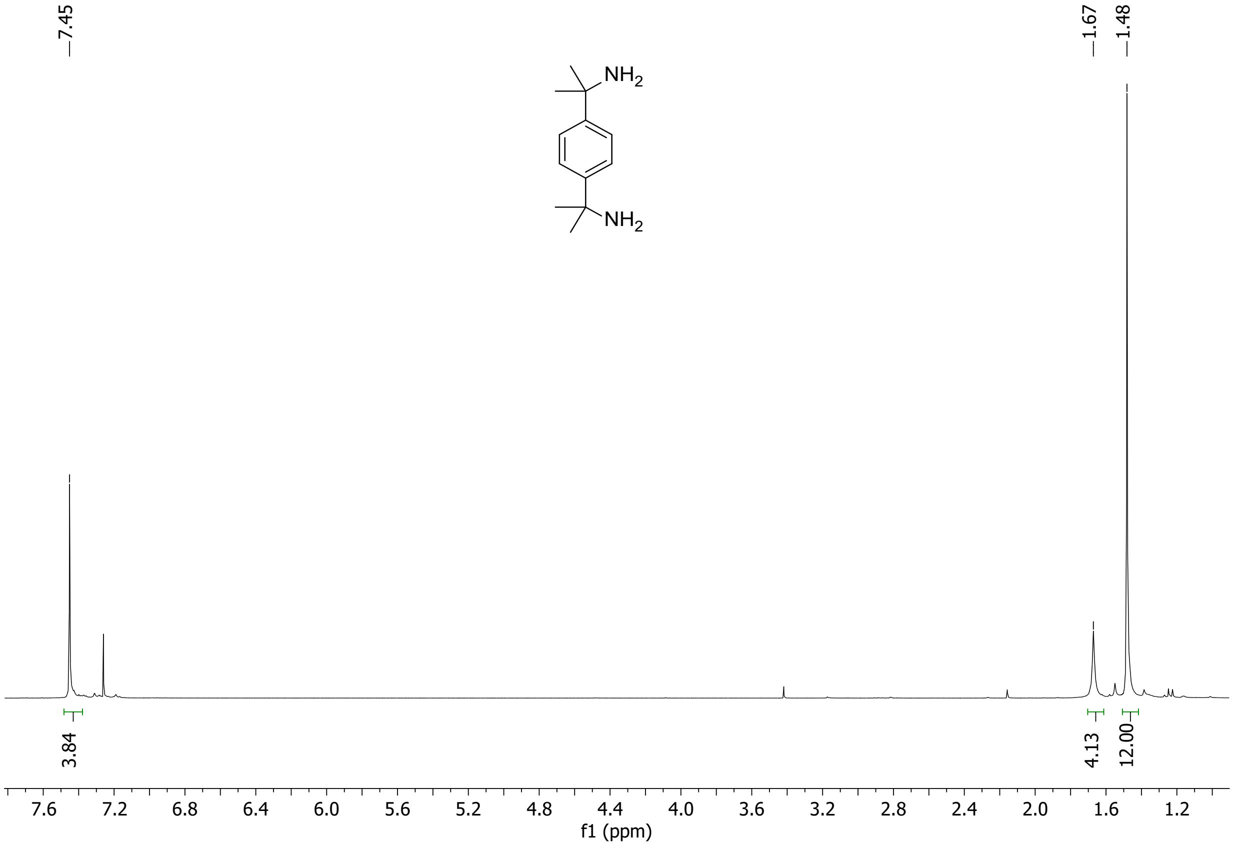
5 (^{1}H NMR, 400 MHz, $CDCl_3$, 298 K)



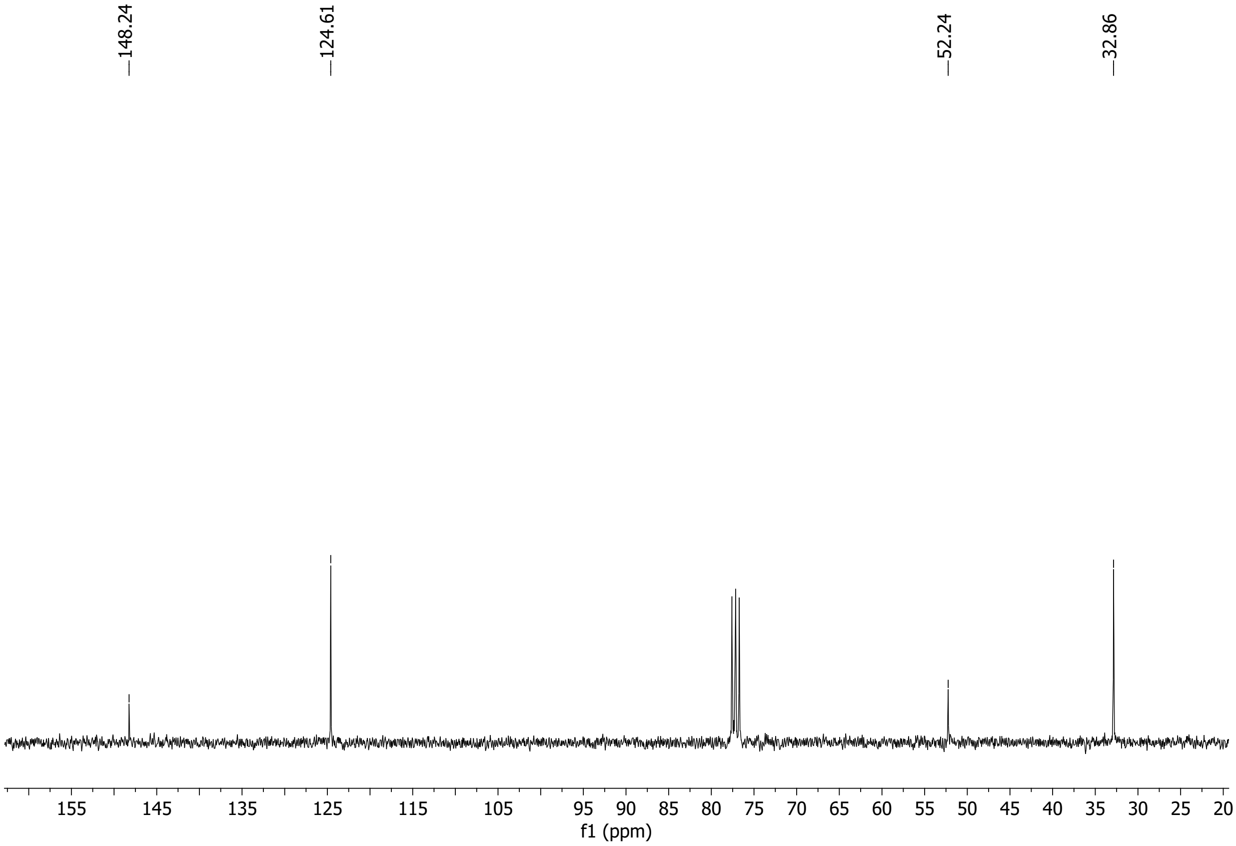
5 (^{13}C NMR, 100 MHz, $CDCl_3$, 298 K)



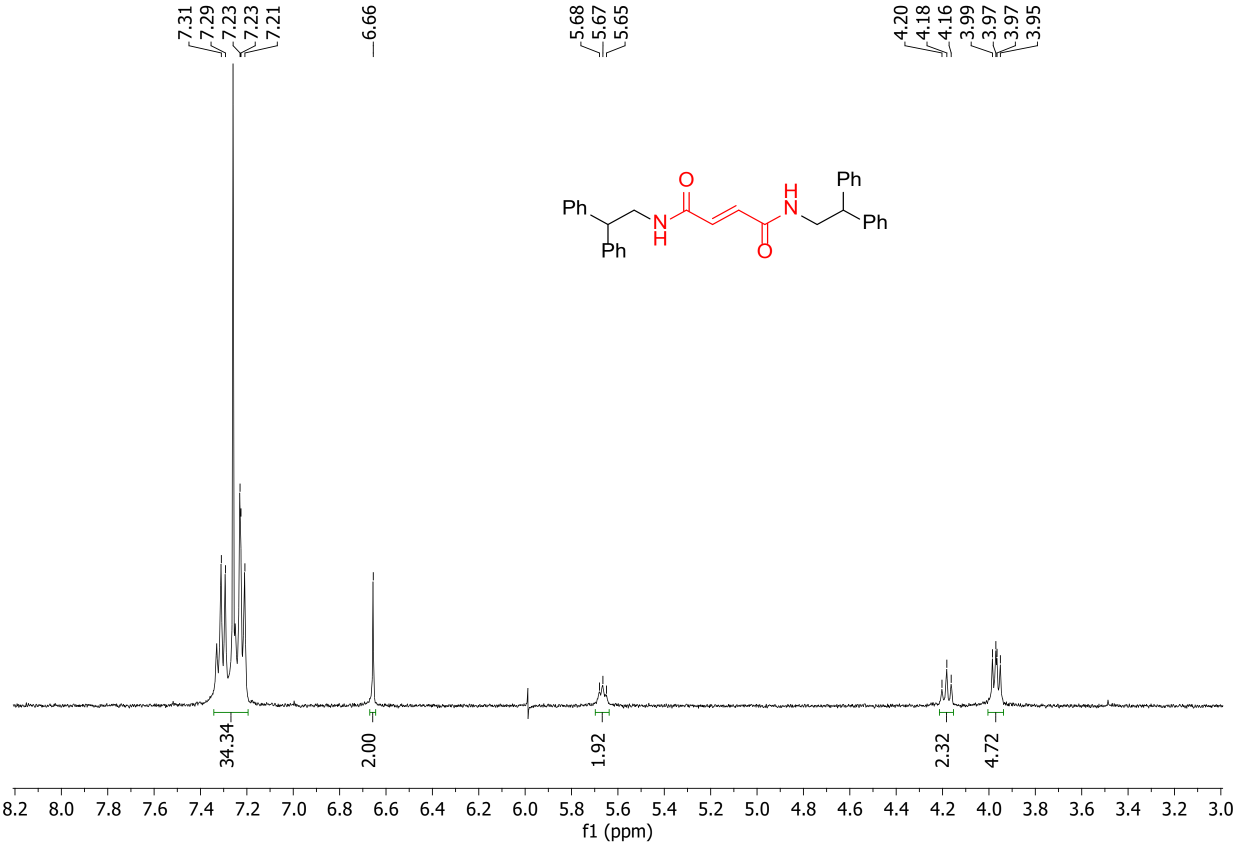
3 (^{1}H NMR, 400 MHz, CDCl$_3$, 298 K)



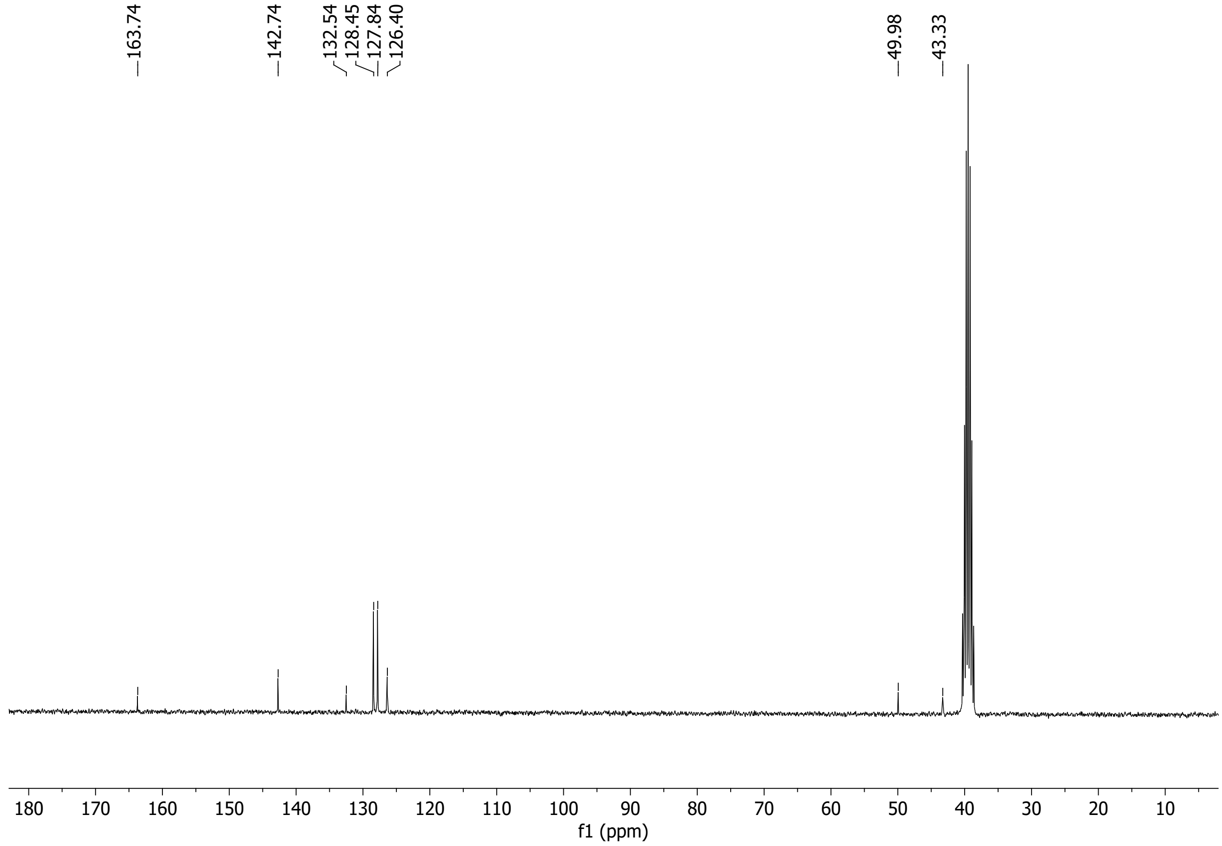
3 (^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



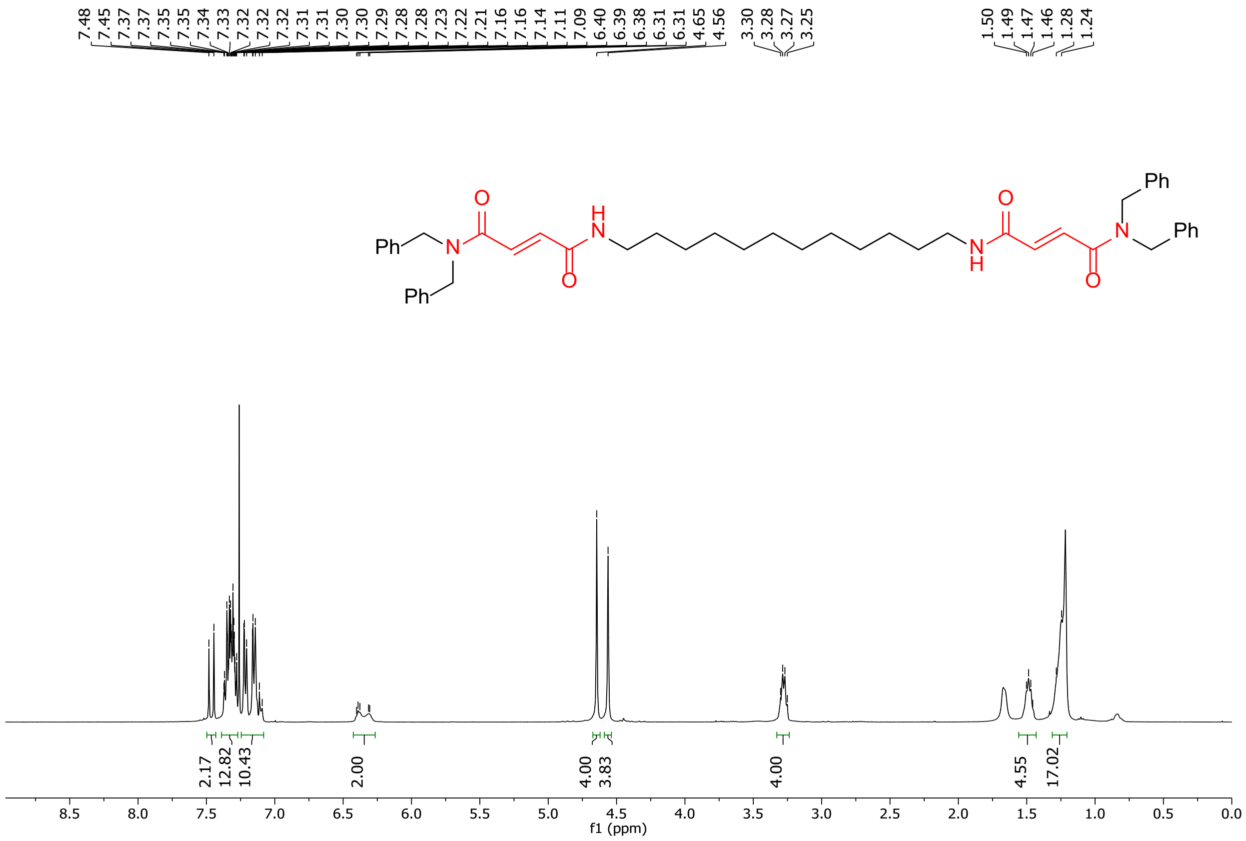
6 (^{1}H NMR, 400 MHz, CDCl$_3$, 298 K)



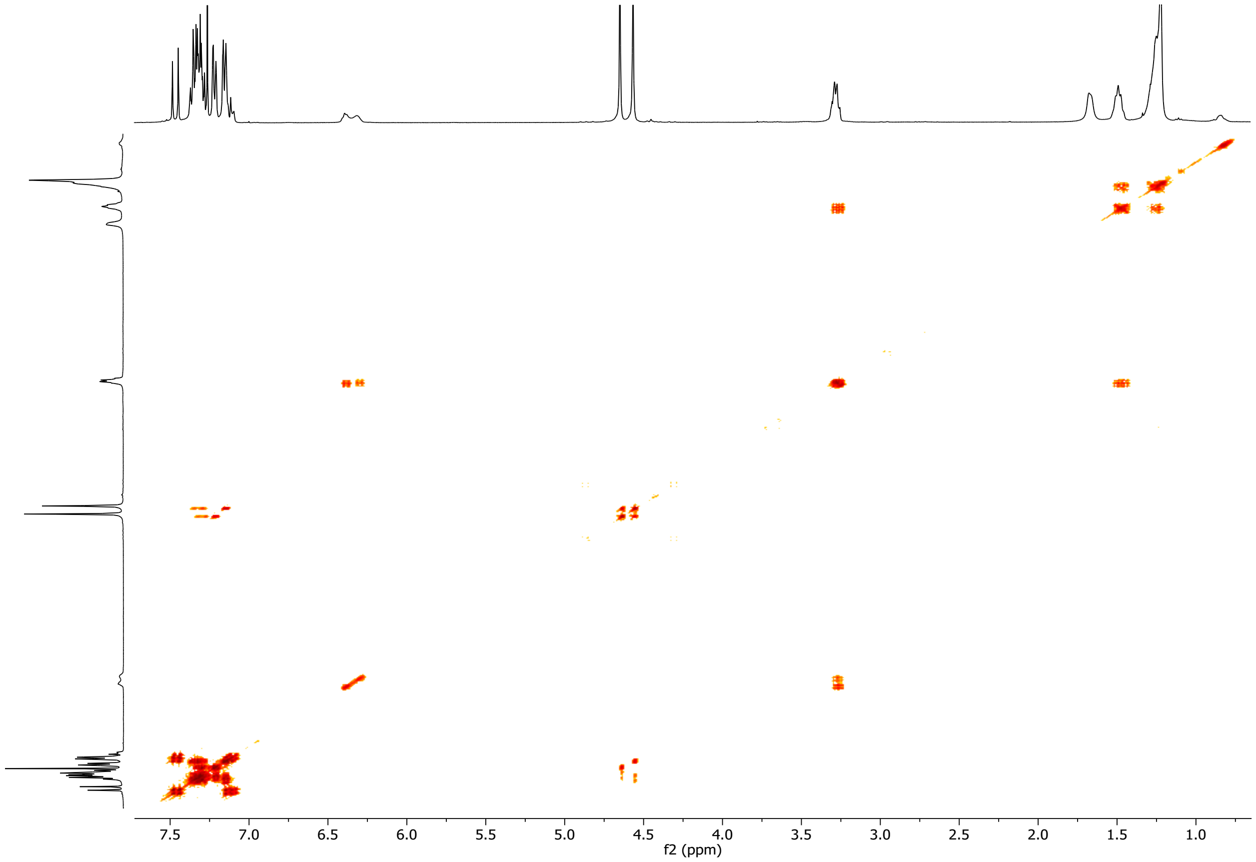
6 (^{13}C NMR, 100 MHz, DMSO, 298 K)



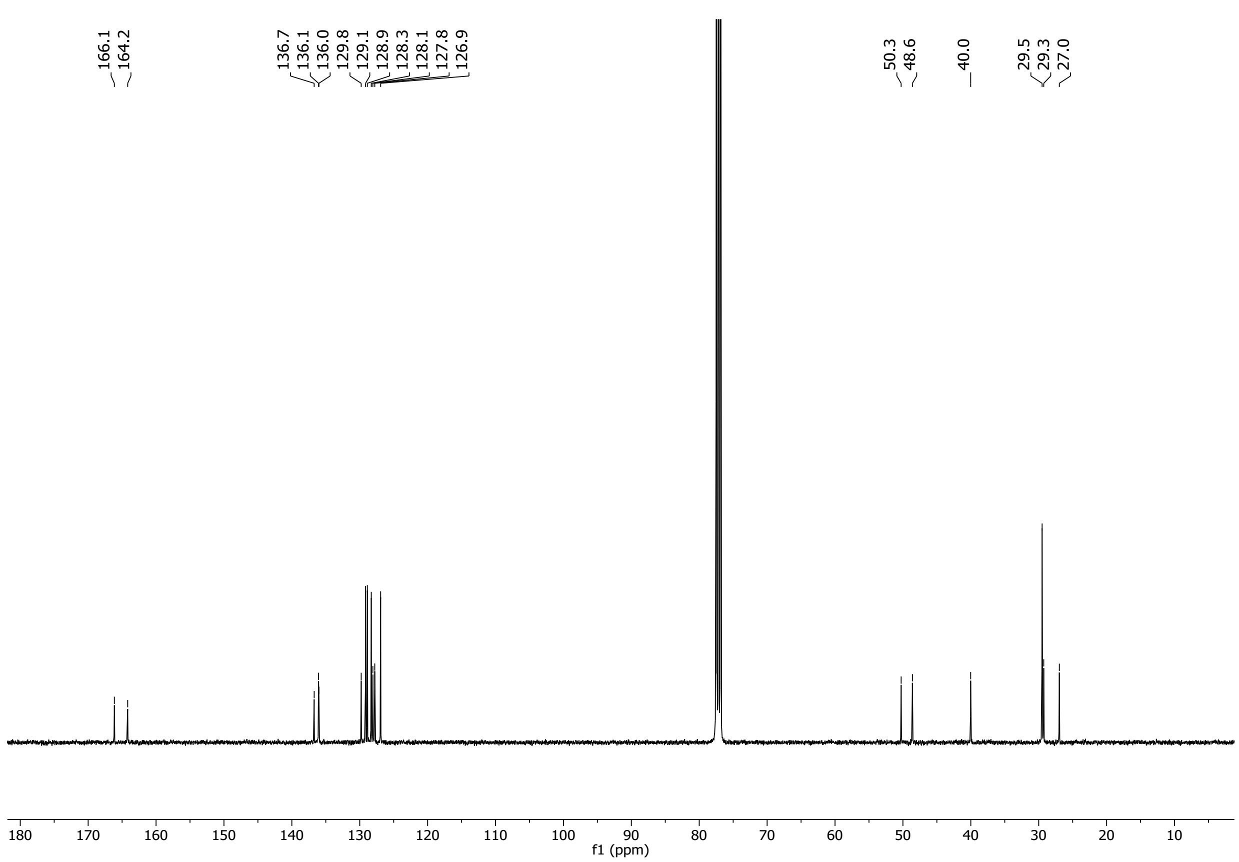
8a (^{1}H NMR, 400 MHz, CDCl$_3$, 298 K)



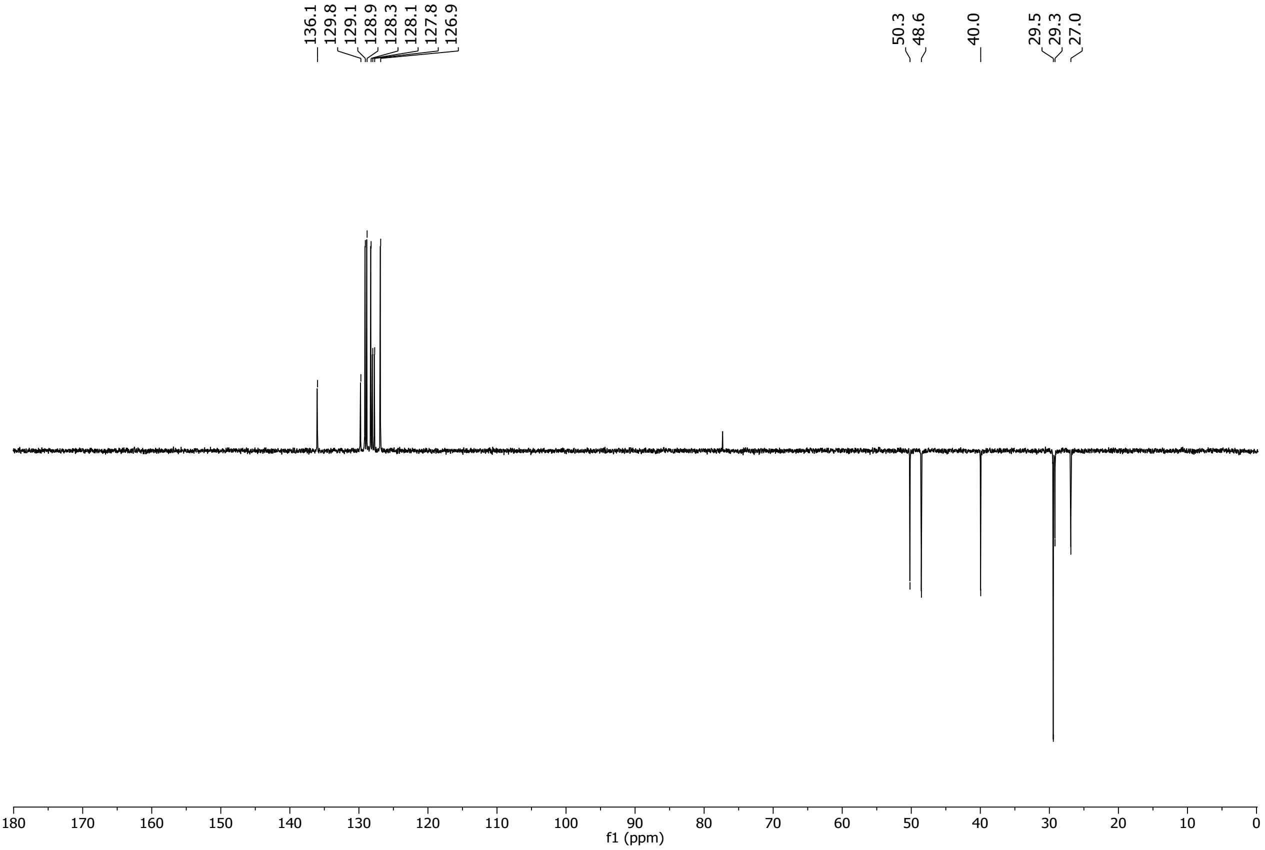
8a (COSY ^{1}H-^{1}H NMR, 400 MHz, CDCl$_3$, 298 K)



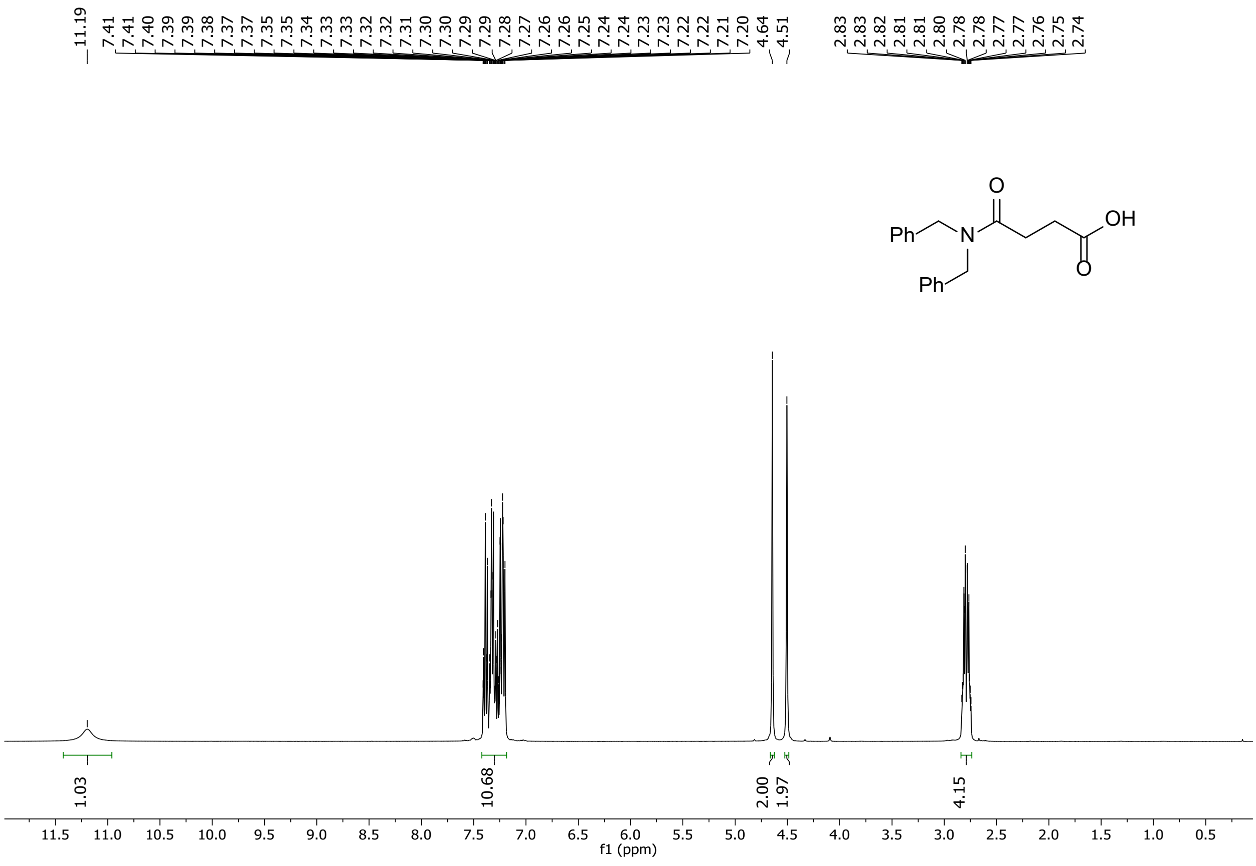
8a (^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



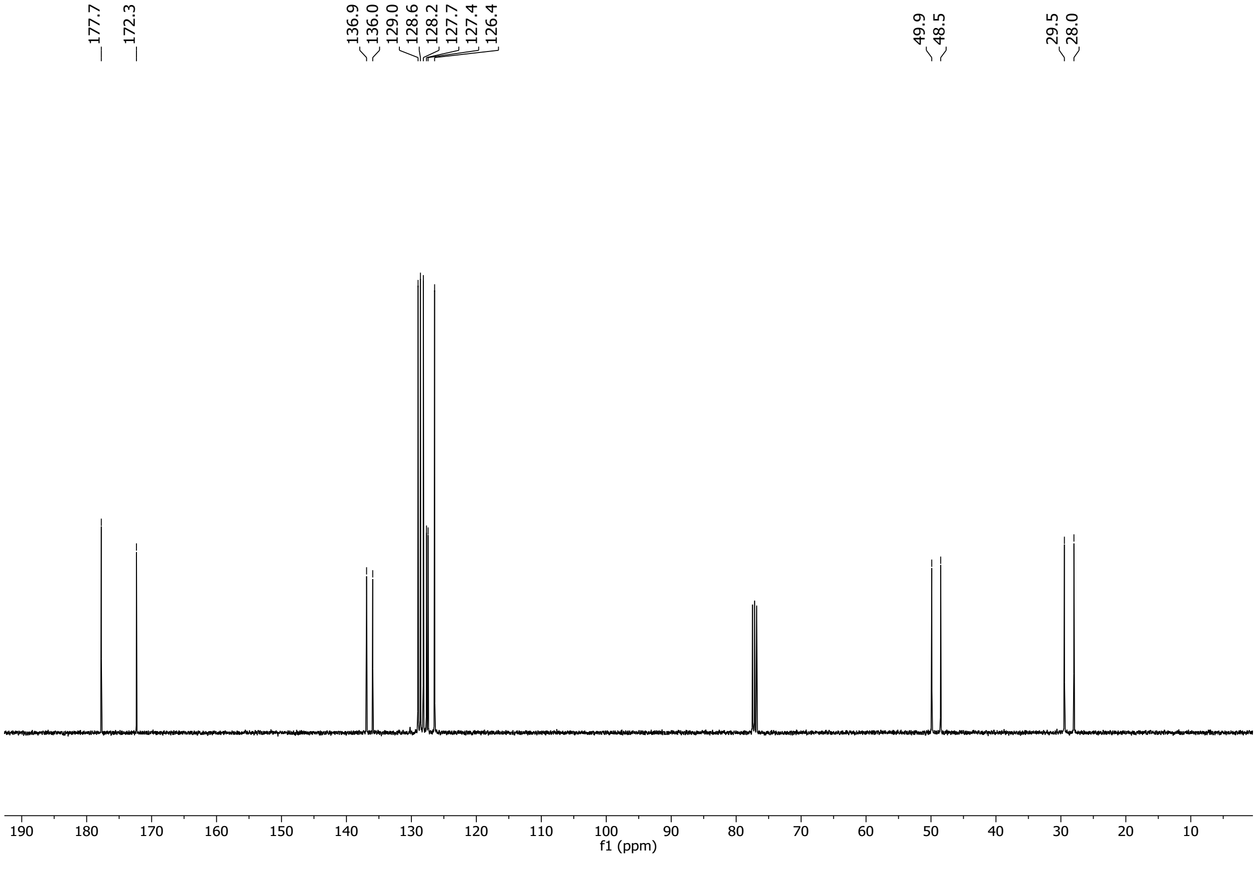
8a (DEPT ^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



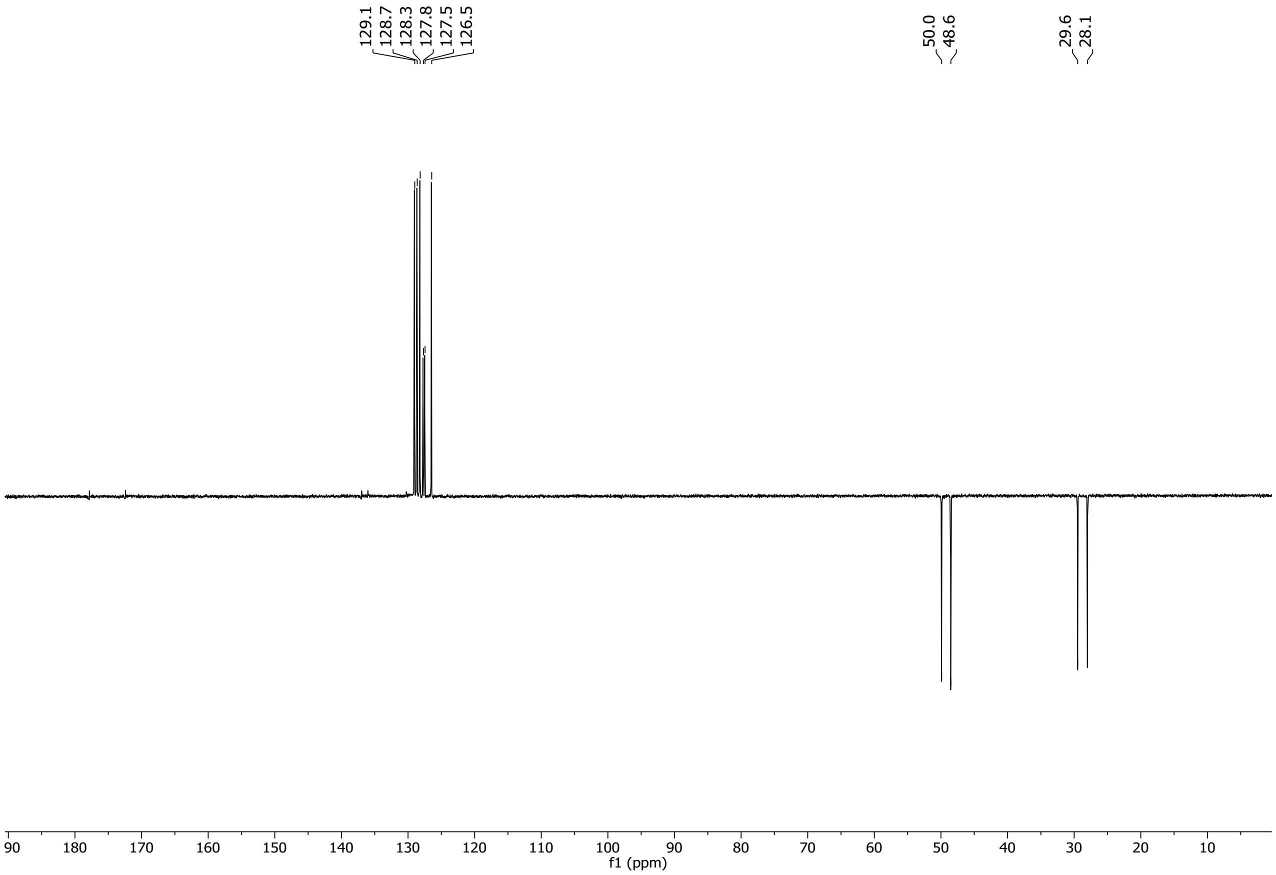
S2b (^{1}H NMR, 400 MHz, CDCl$_3$, 298 K)



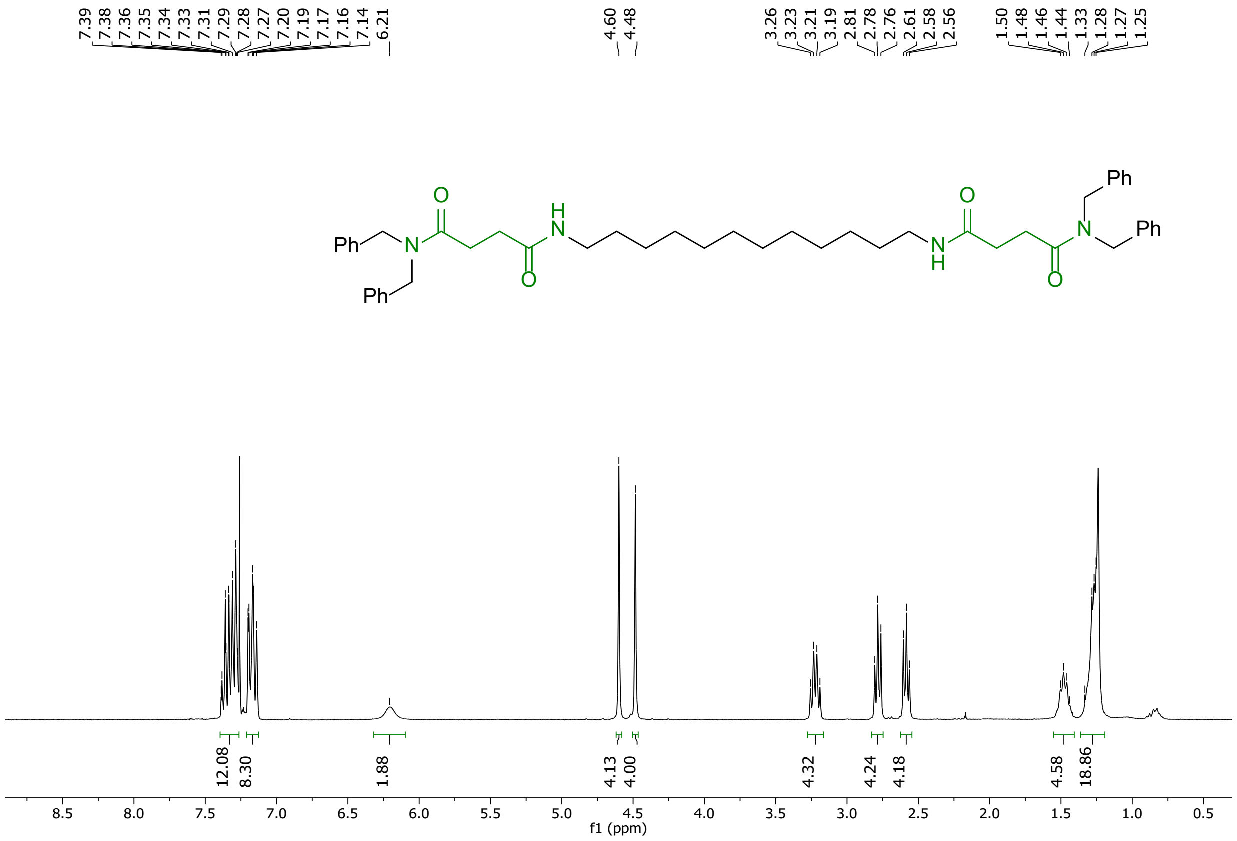
S2b (^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



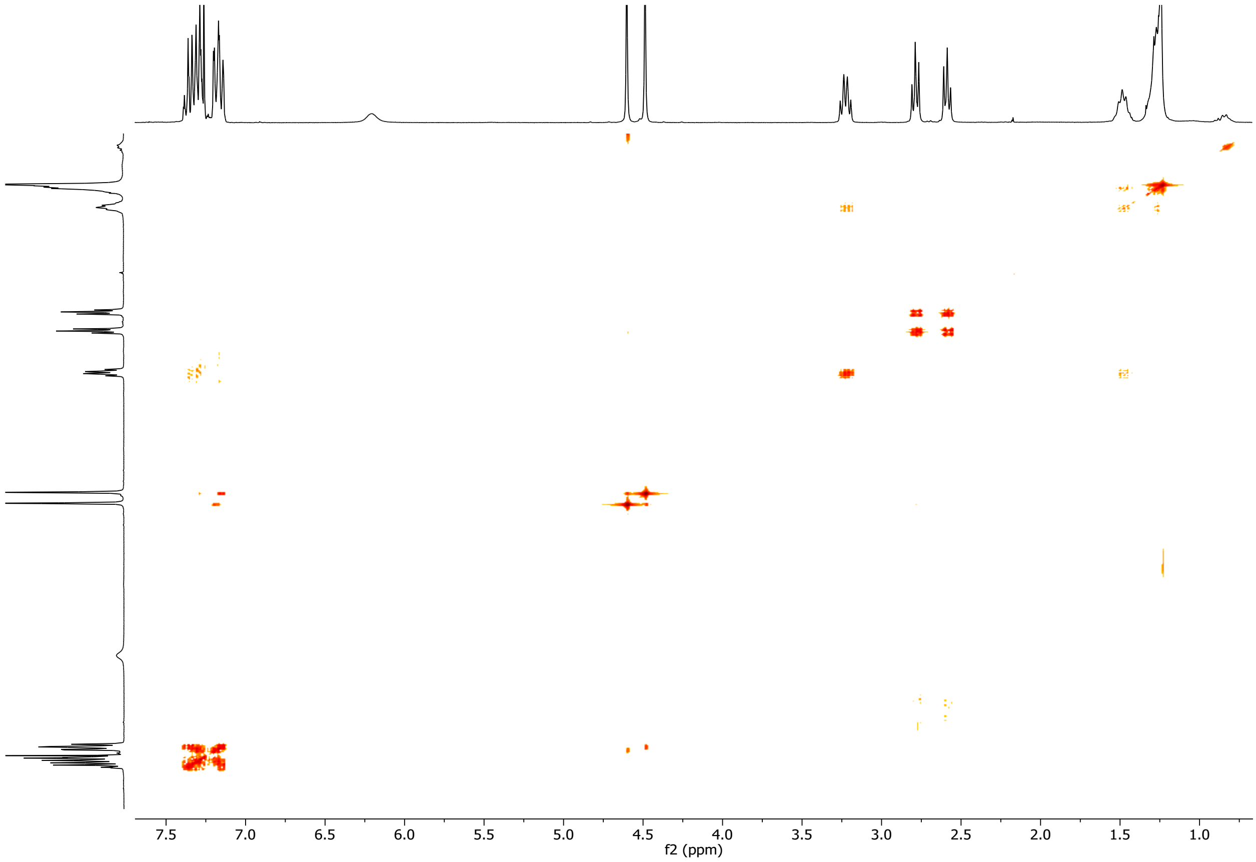
S2b (DEPT ^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



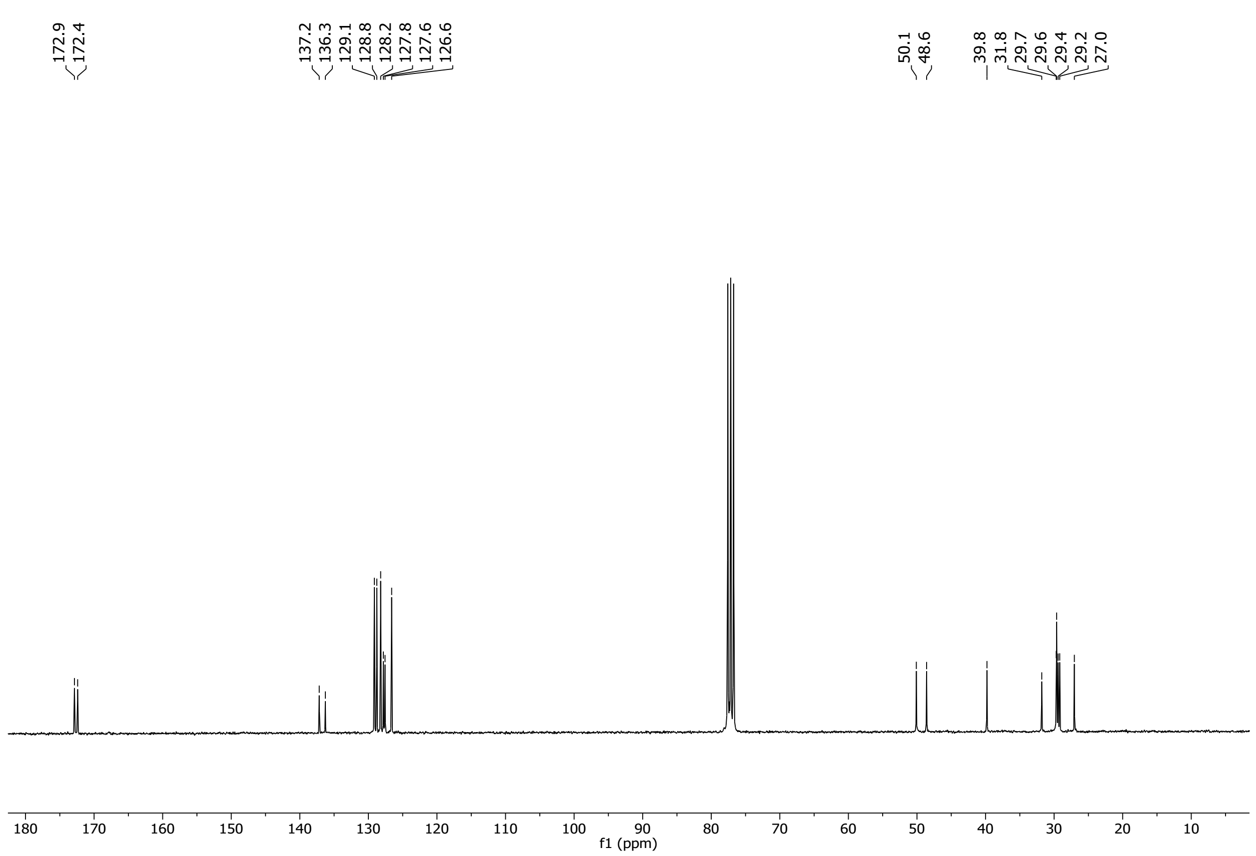
8b (^{1}H NMR, 300 MHz, CDCl$_3$, 298 K)



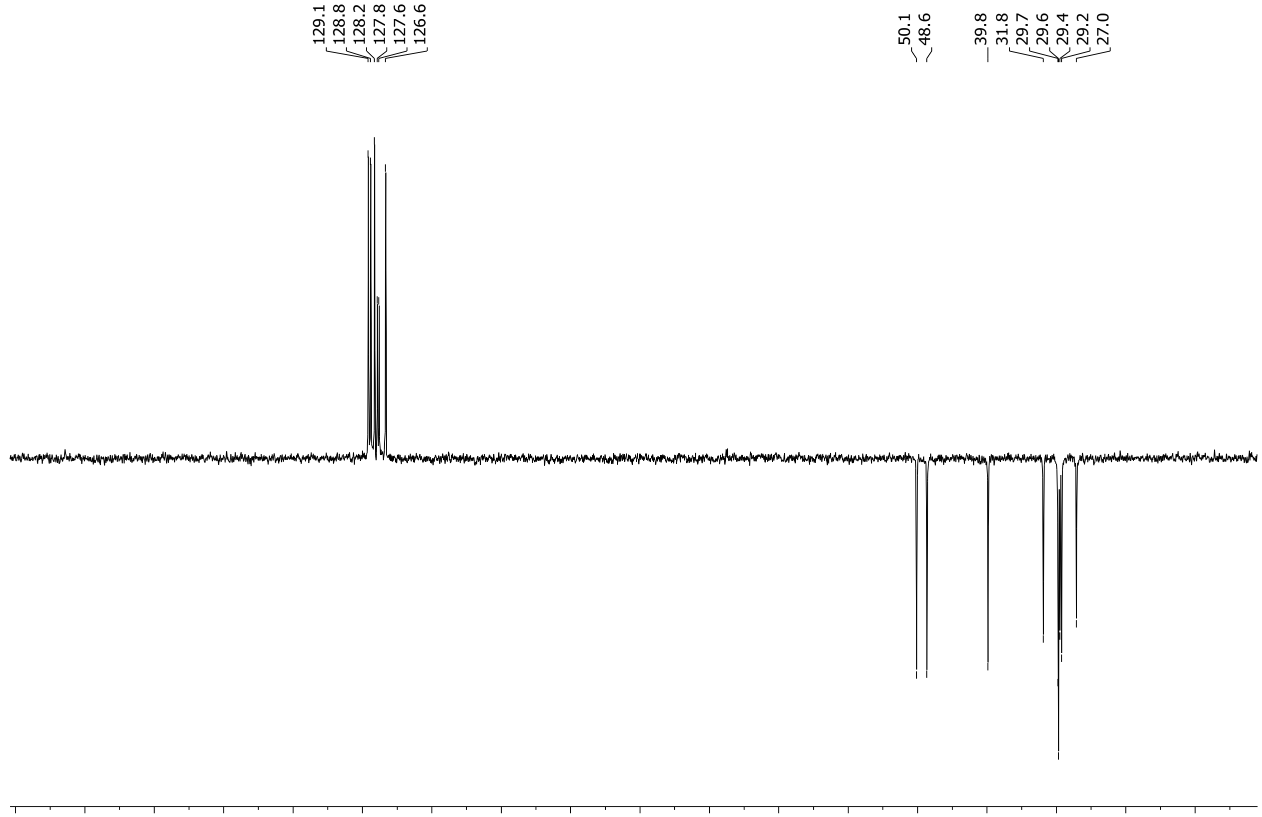
8b (COSY ^{1}H-^{1}H NMR, 300 MHz, CDCl$_3$, 298 K)



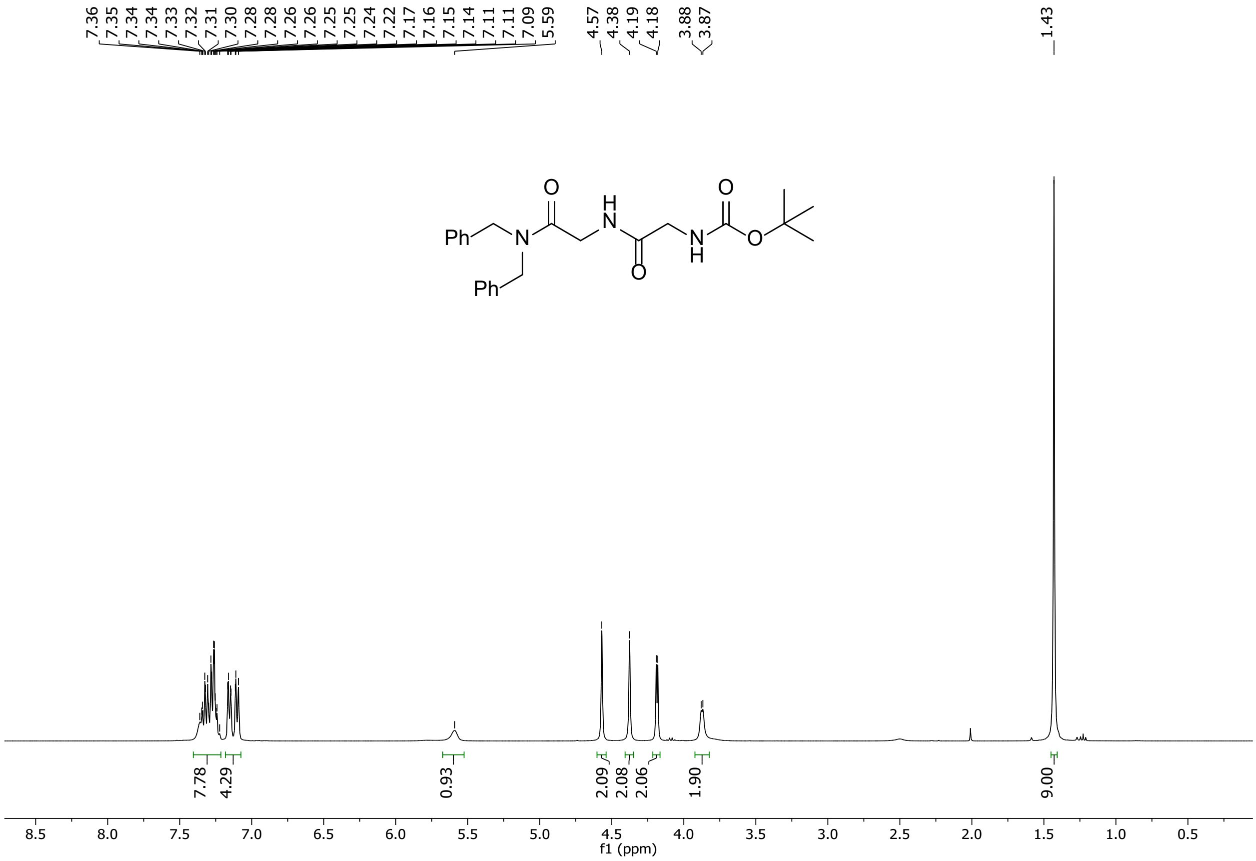
8b (^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



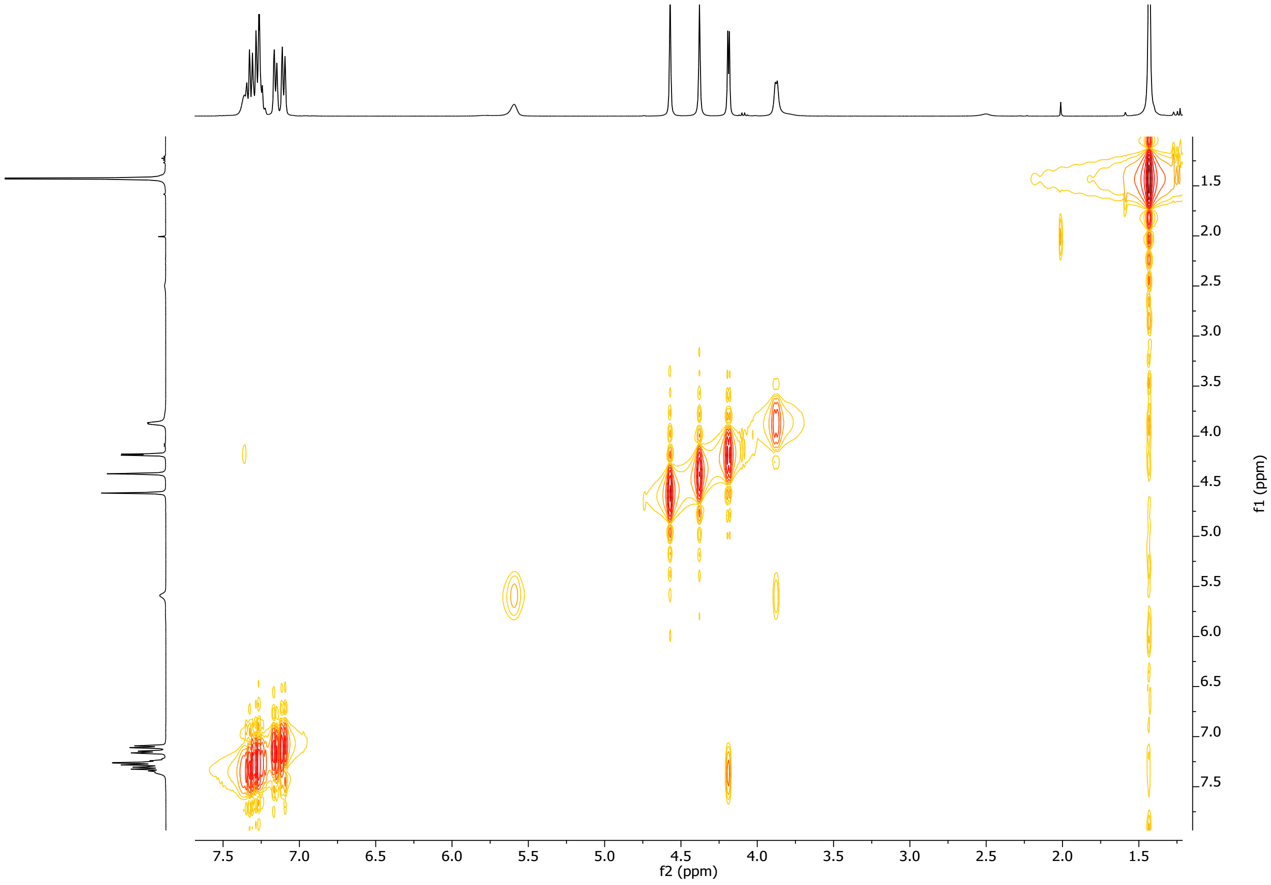
8b (DEPT ^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



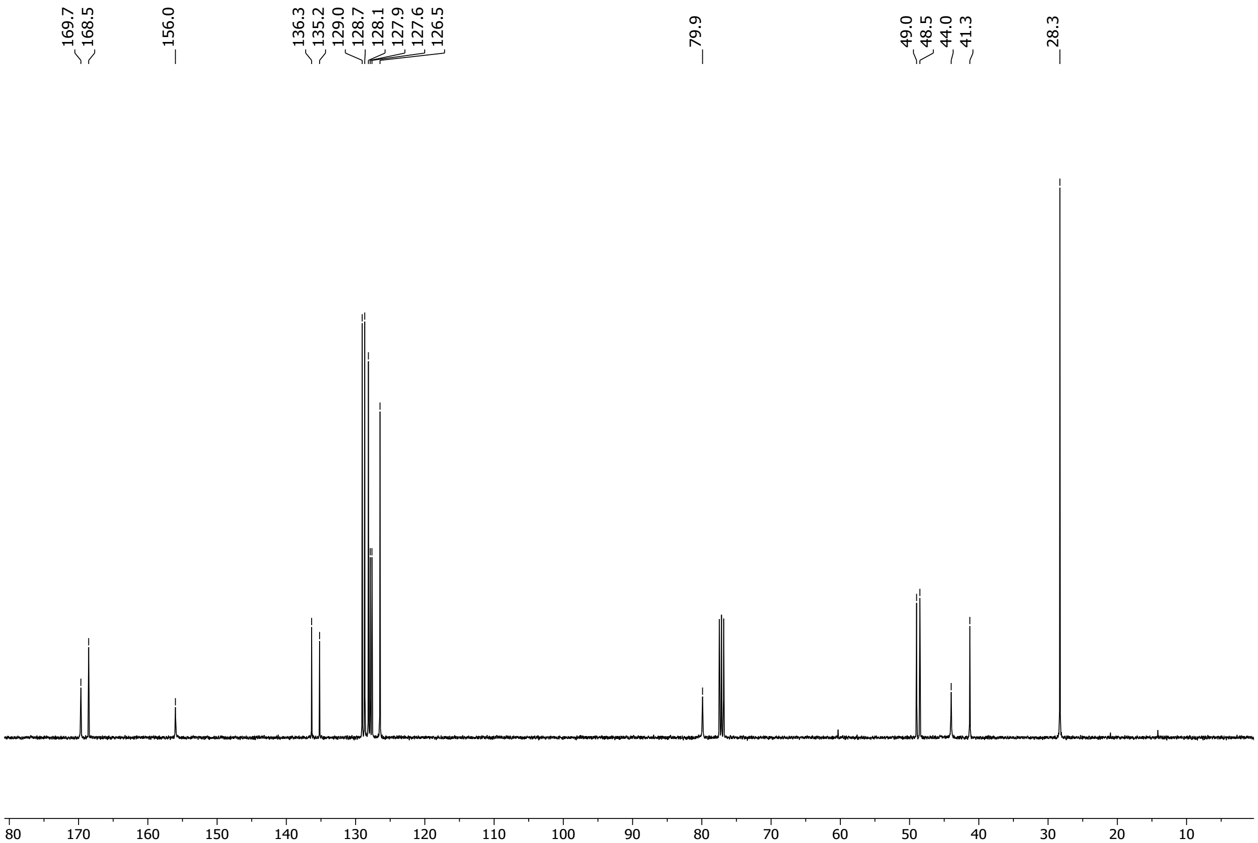
S2c (^{1}H NMR, 400 MHz, CDCl$_3$, 298 K)



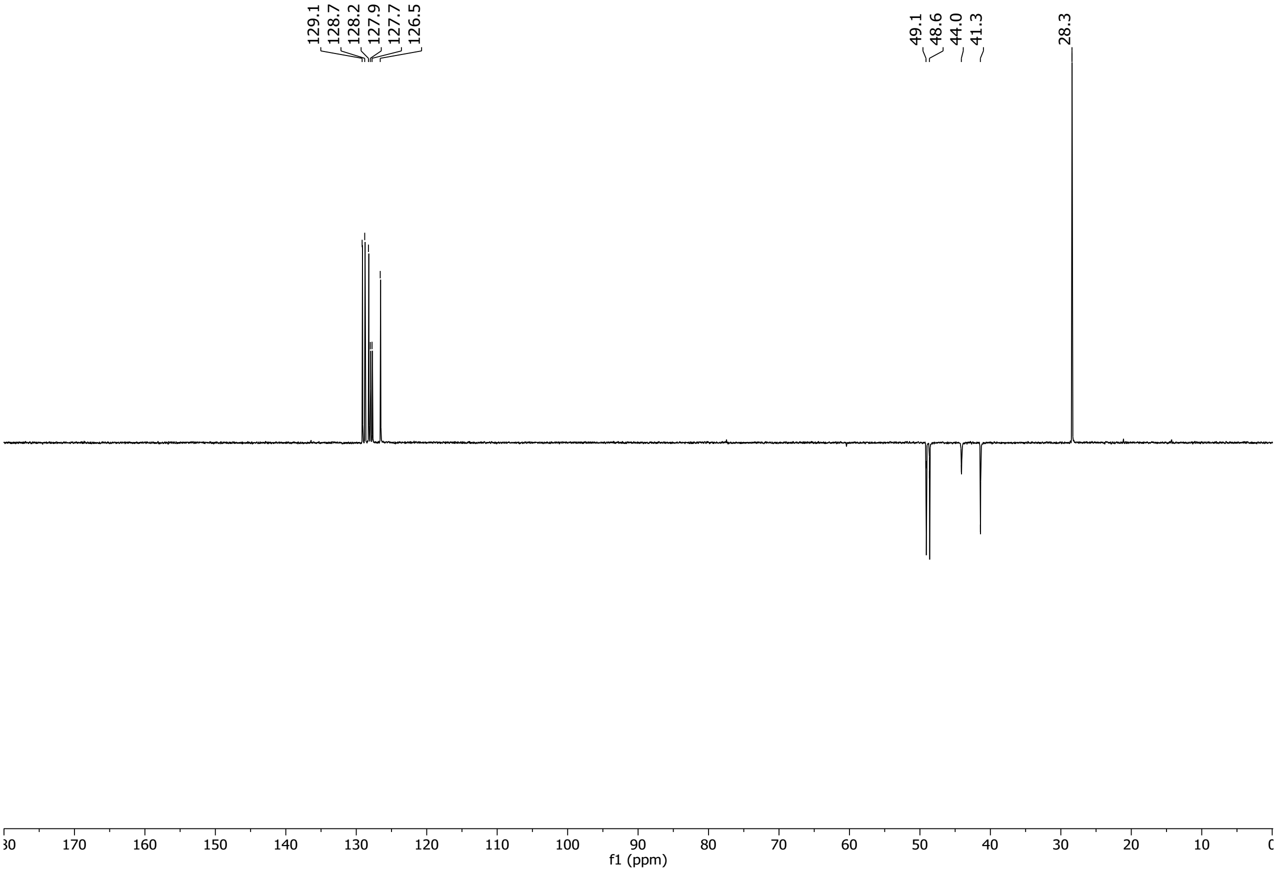
S2c (COSY ^{1}H-^{1}H NMR, 400 MHz, CDCl$_3$, 298 K)



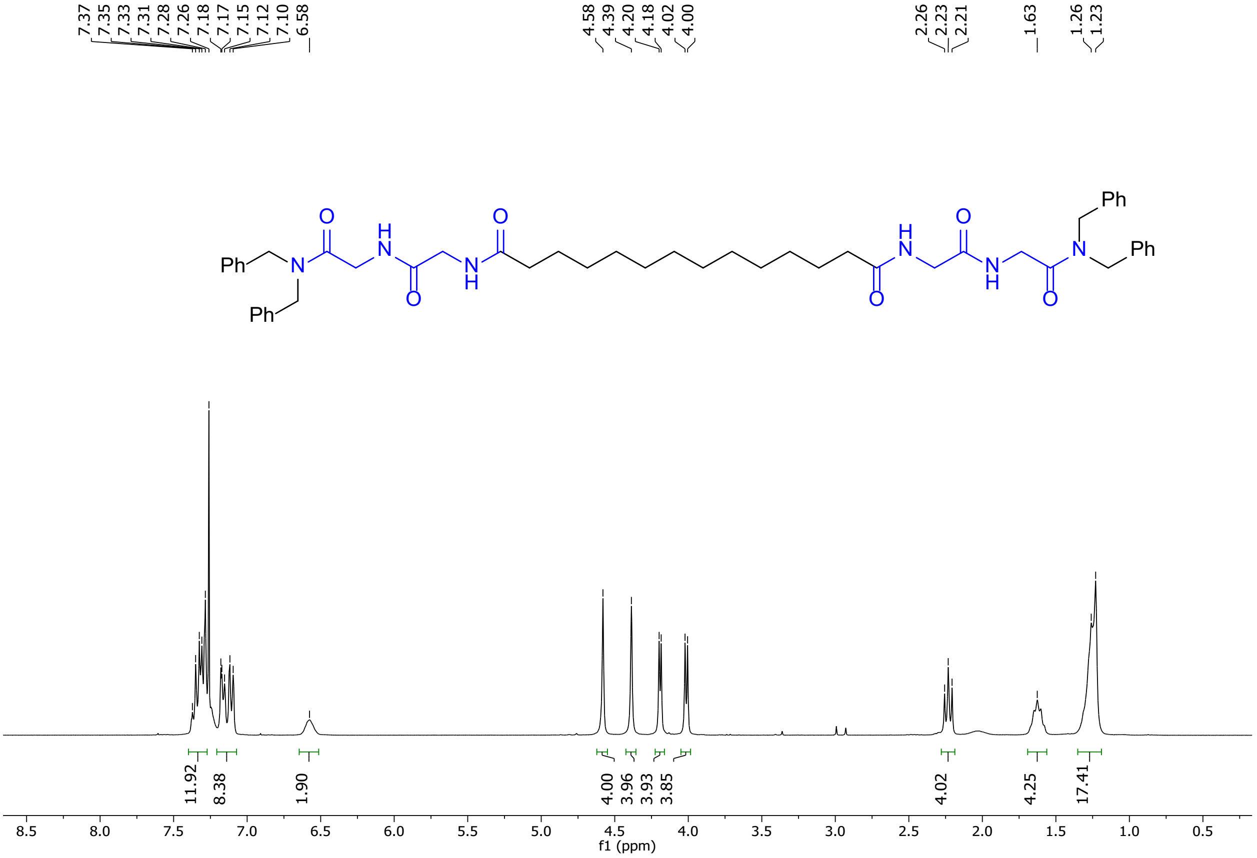
S2c (^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



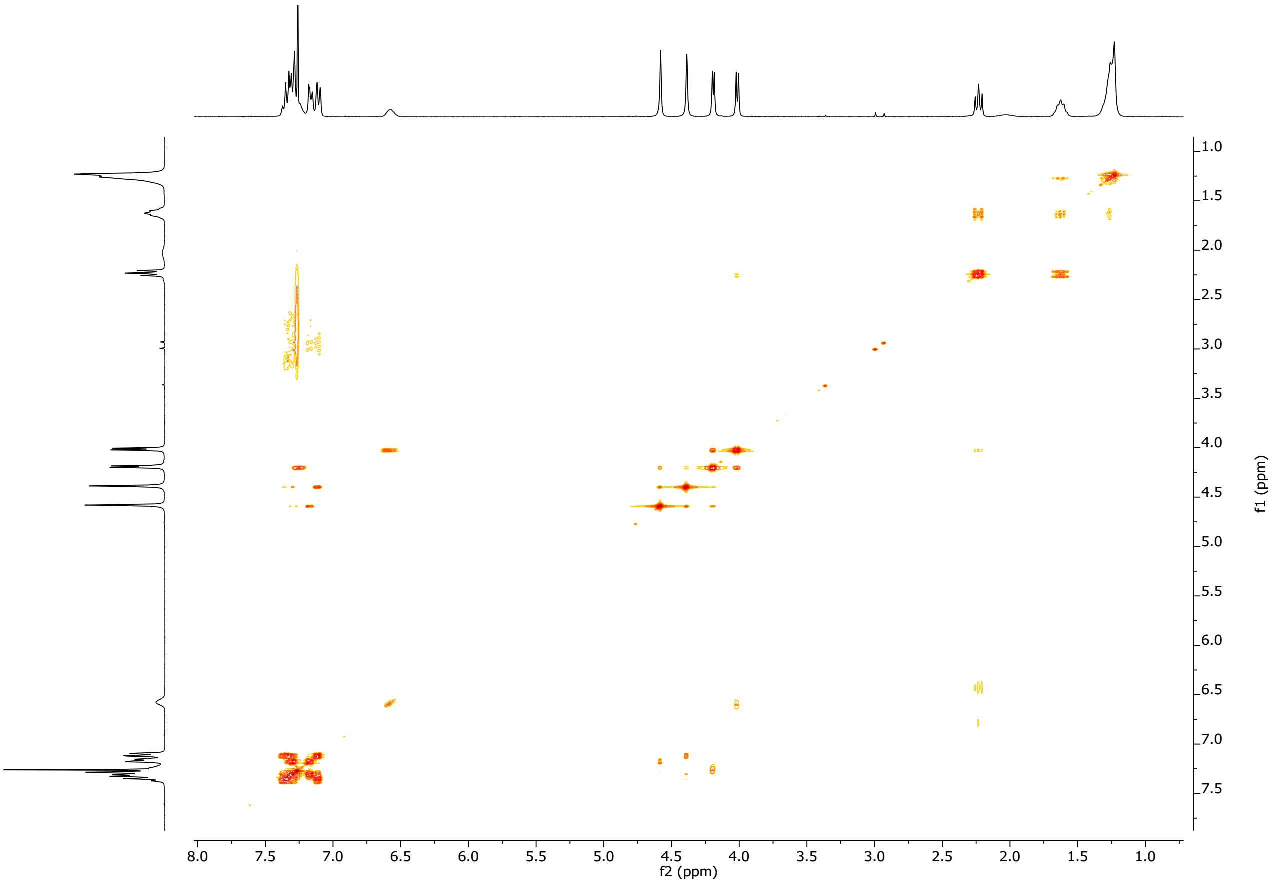
S2c (DEPT ^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



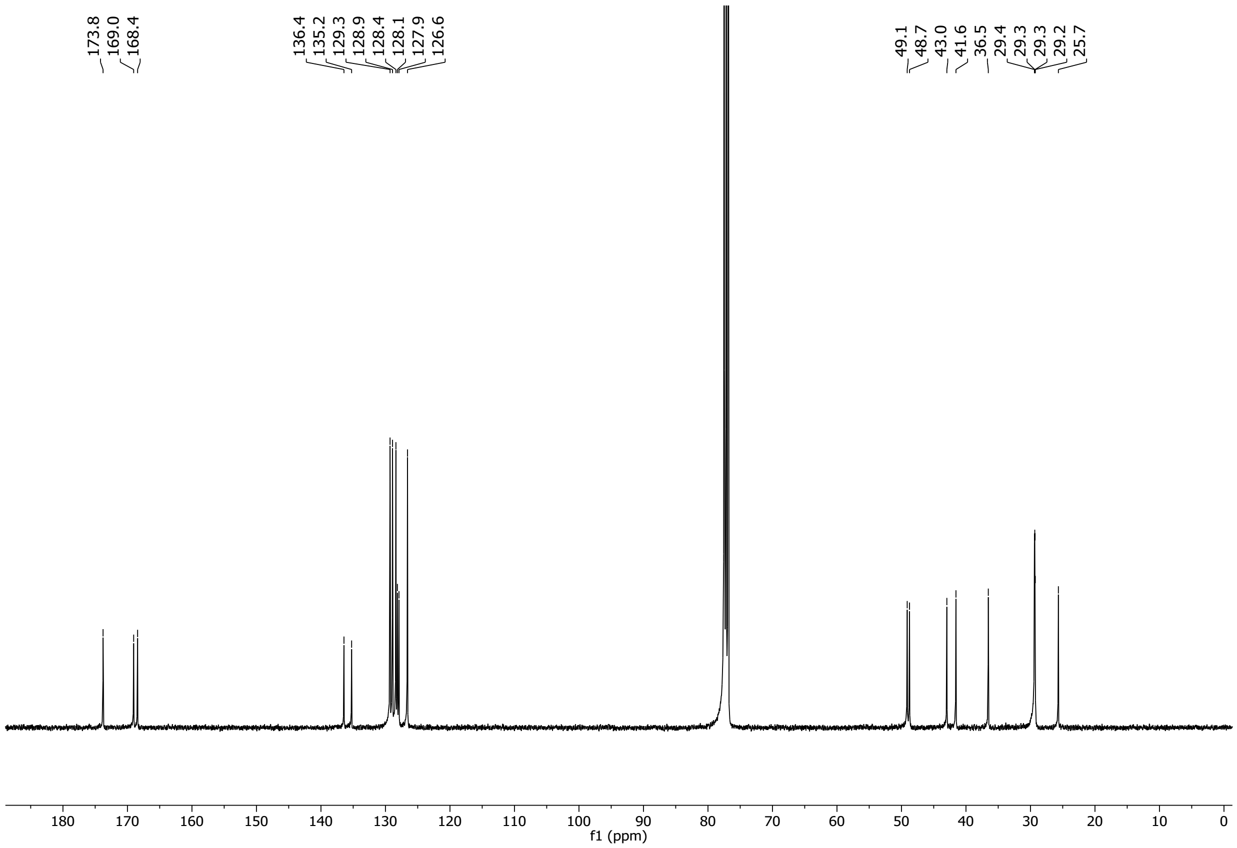
8c (^{1}H NMR, 400 MHz, CDCl$_3$, 298 K)



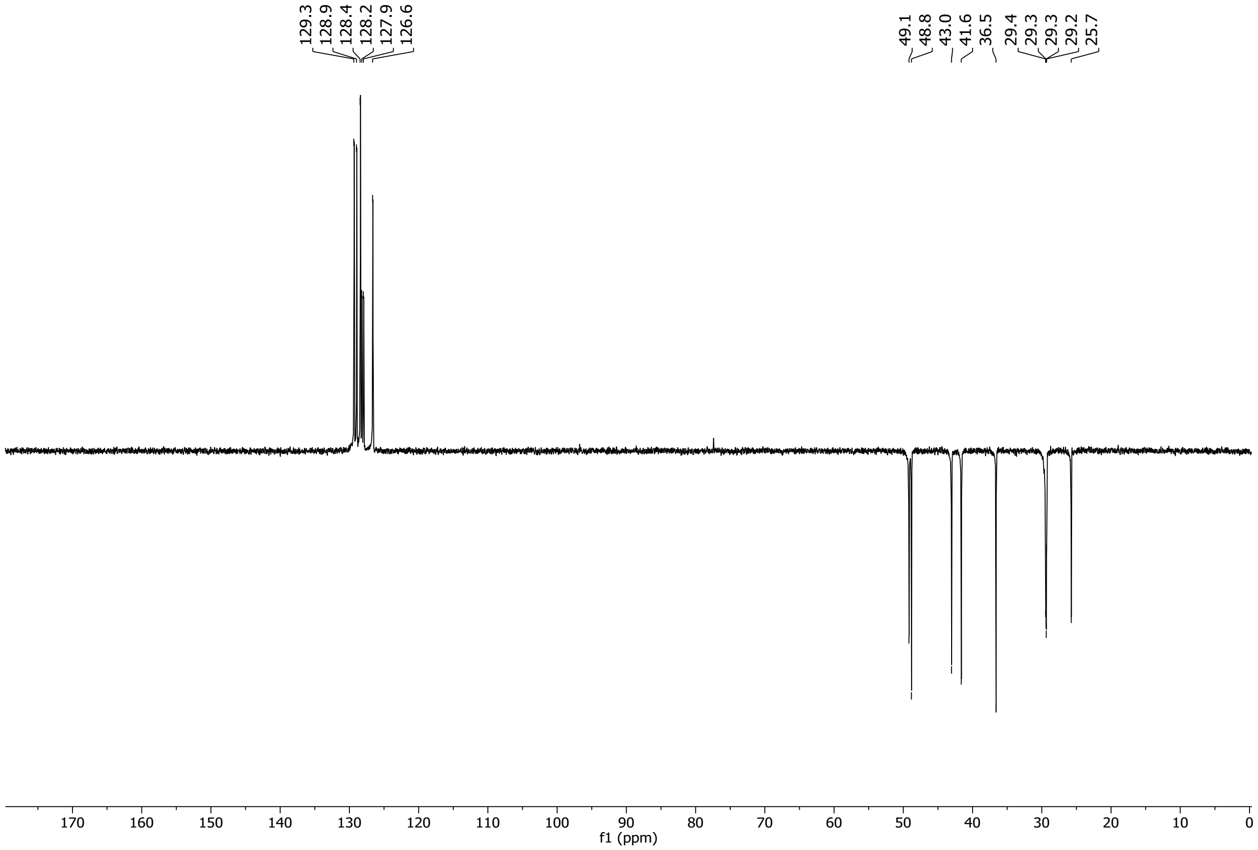
8c (COSY ^{1}H-^{1}H NMR, 400 MHz, CDCl$_3$, 298 K)



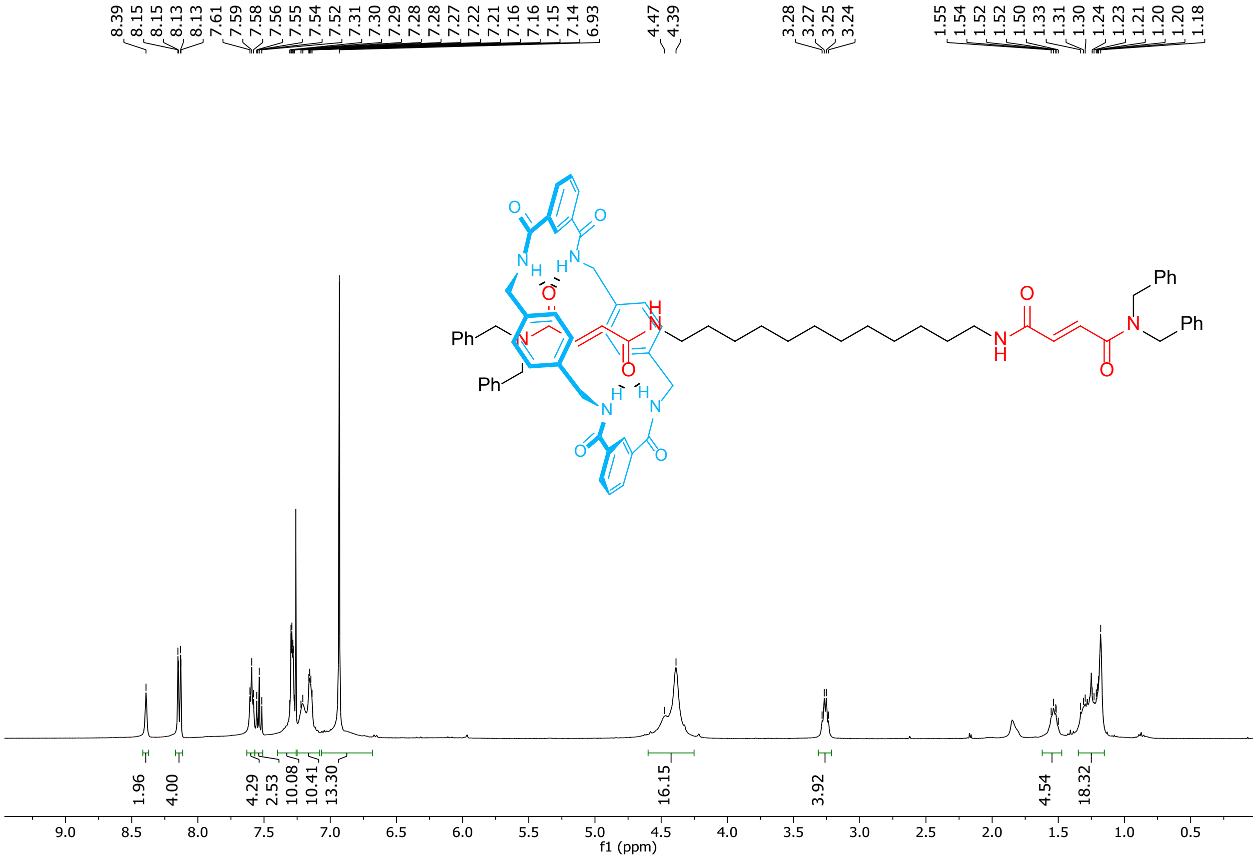
8c (^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



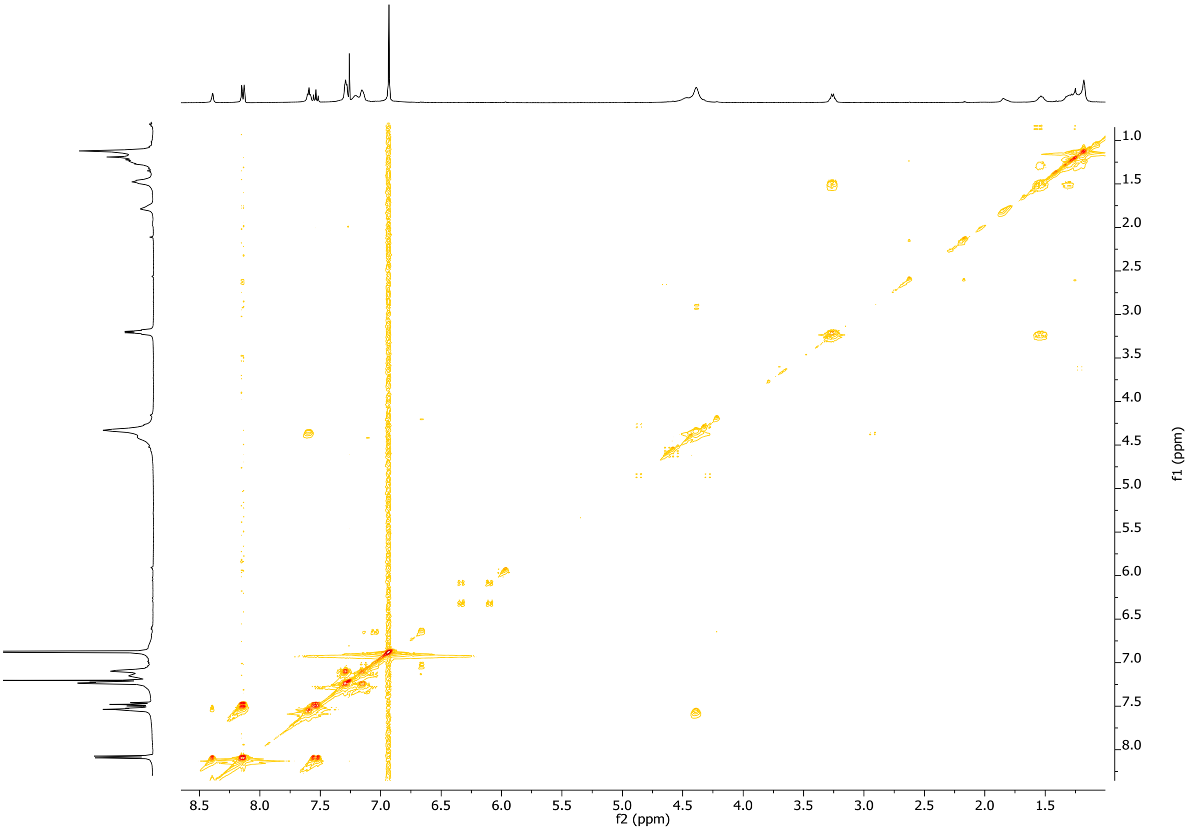
8c (DEPT ^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



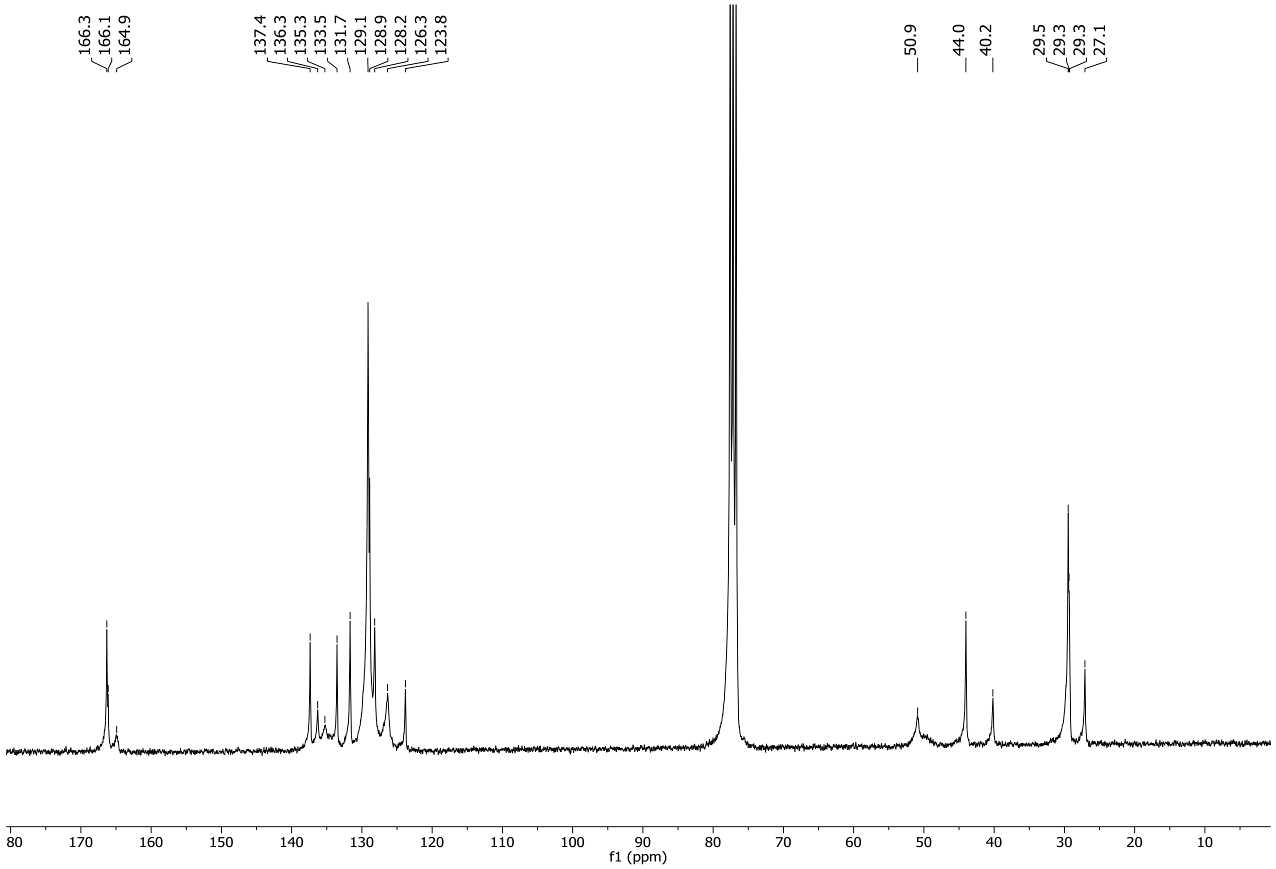
1a (^{1}H NMR, 400 MHz, CDCl$_3$, 298 K)



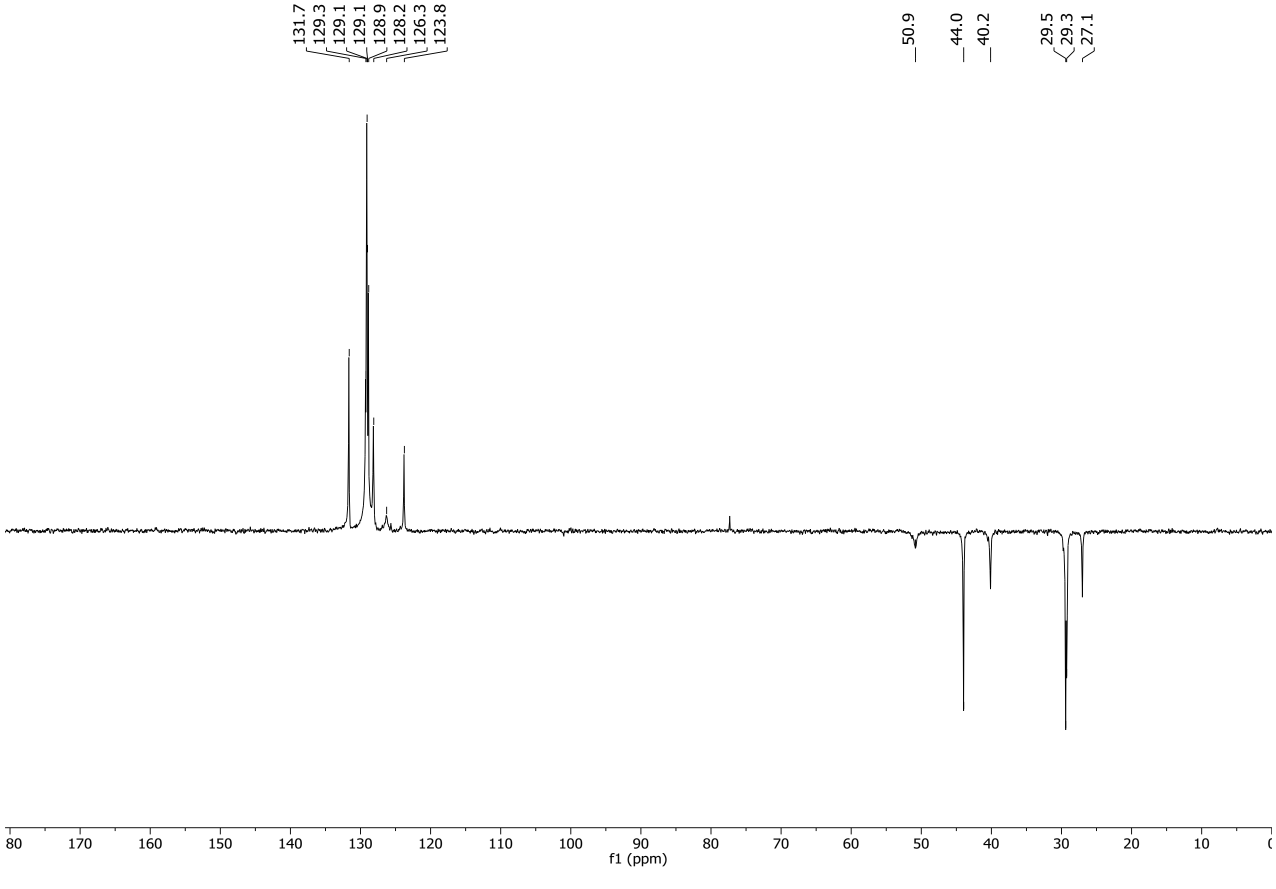
1a (COSY ^{1}H-^{1}H NMR, 400 MHz, CDCl$_3$, 298 K)



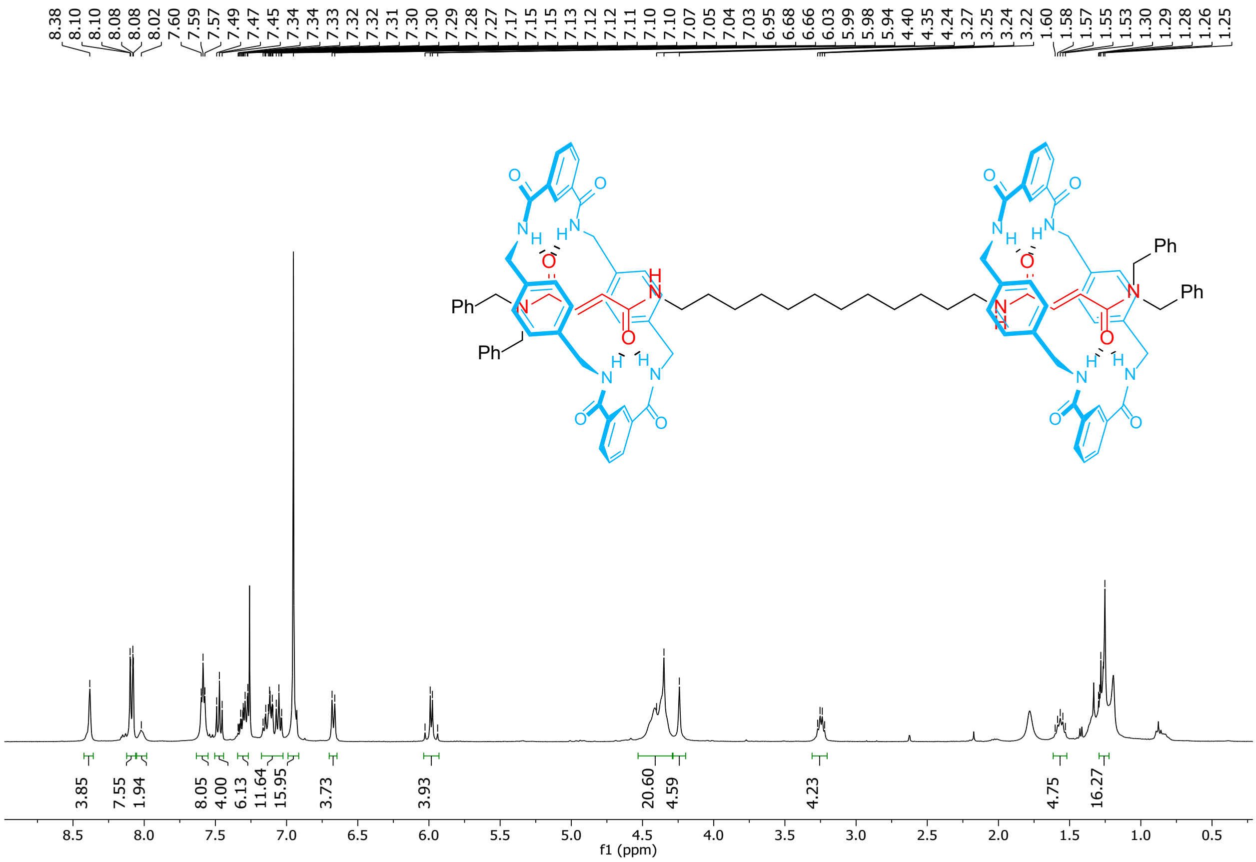
1a (^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



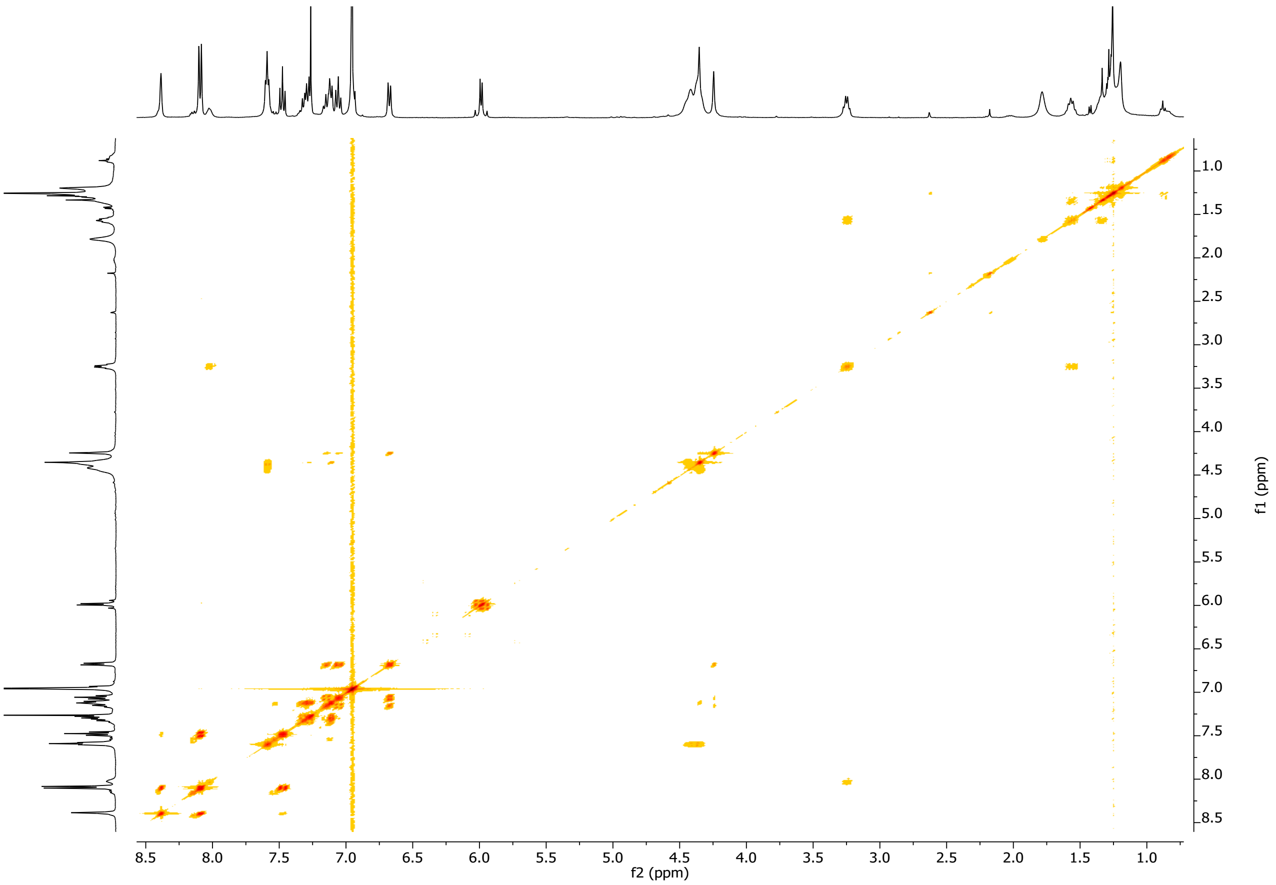
1a (DEPT ^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



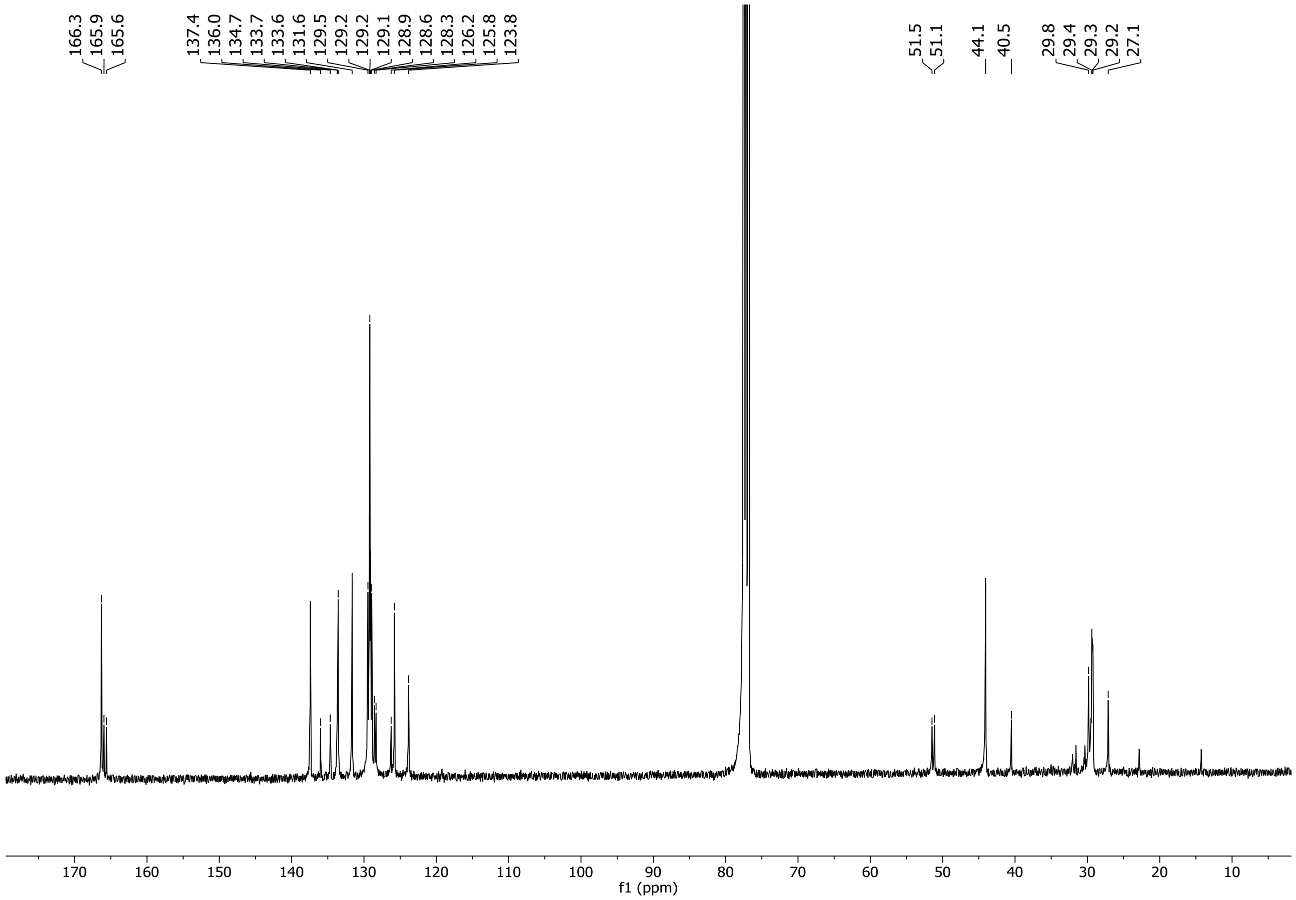
S4a (^{1}H NMR, 400 MHz, CDCl$_3$, 298 K)



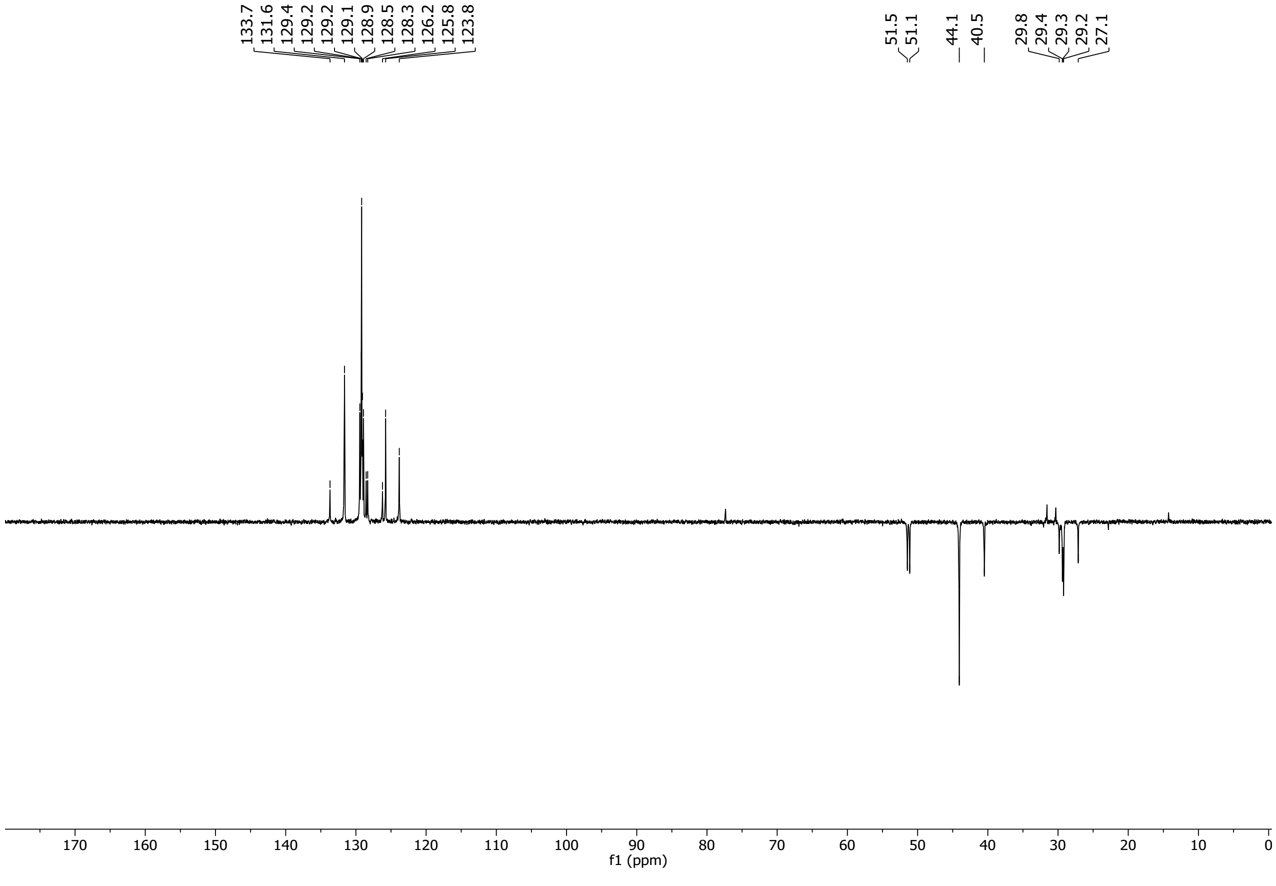
S4a (COSY ^{1}H-^{1}H NMR, 400 MHz, CDCl$_3$, 298 K)



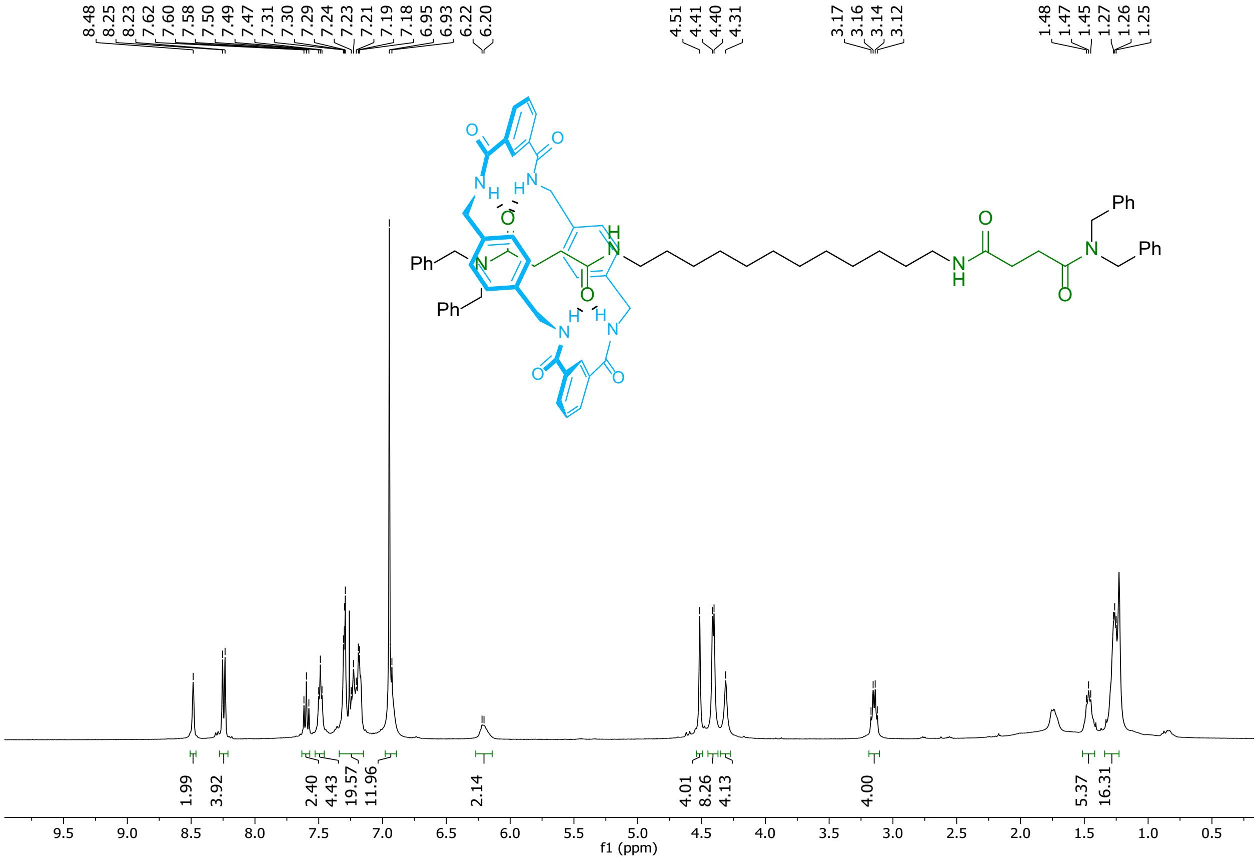
S4a (^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



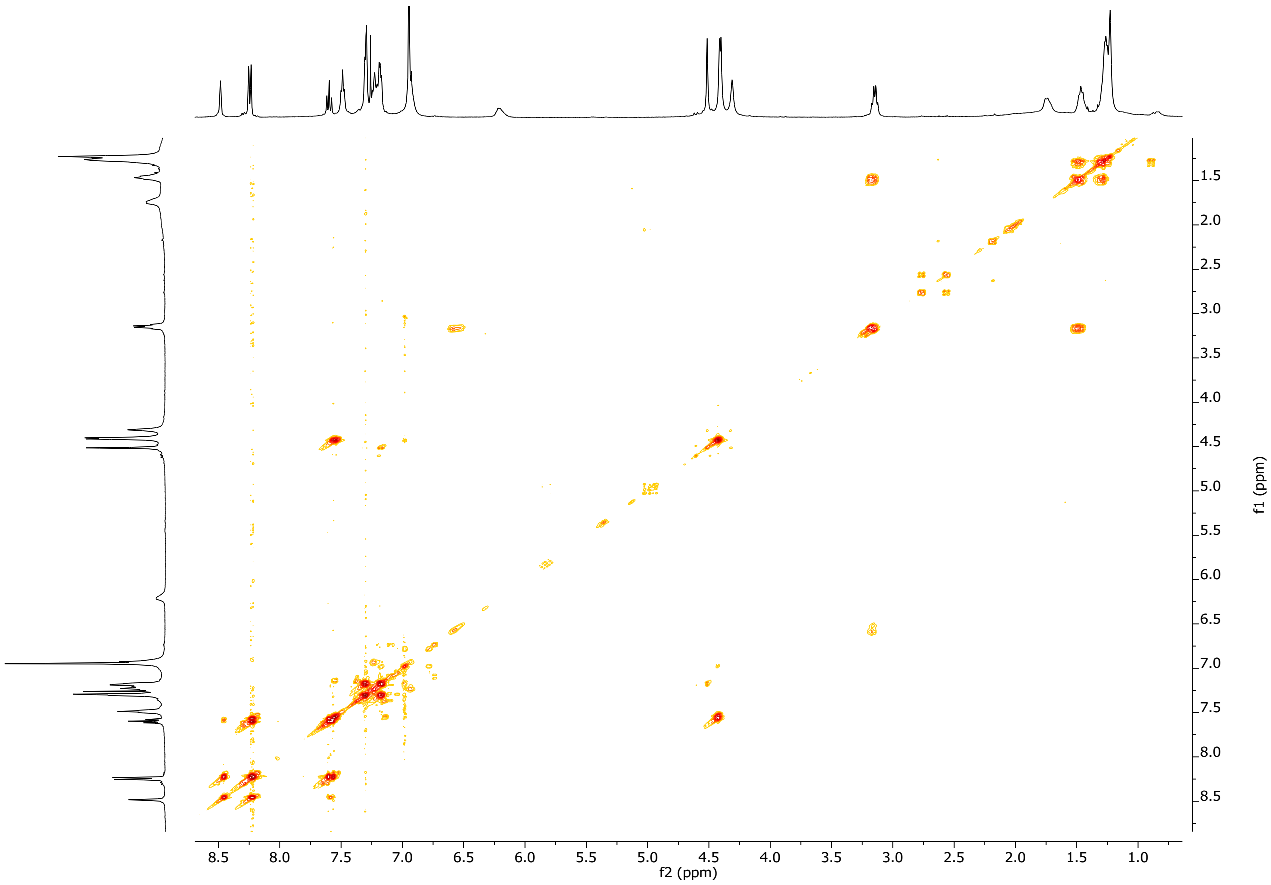
S4a (^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



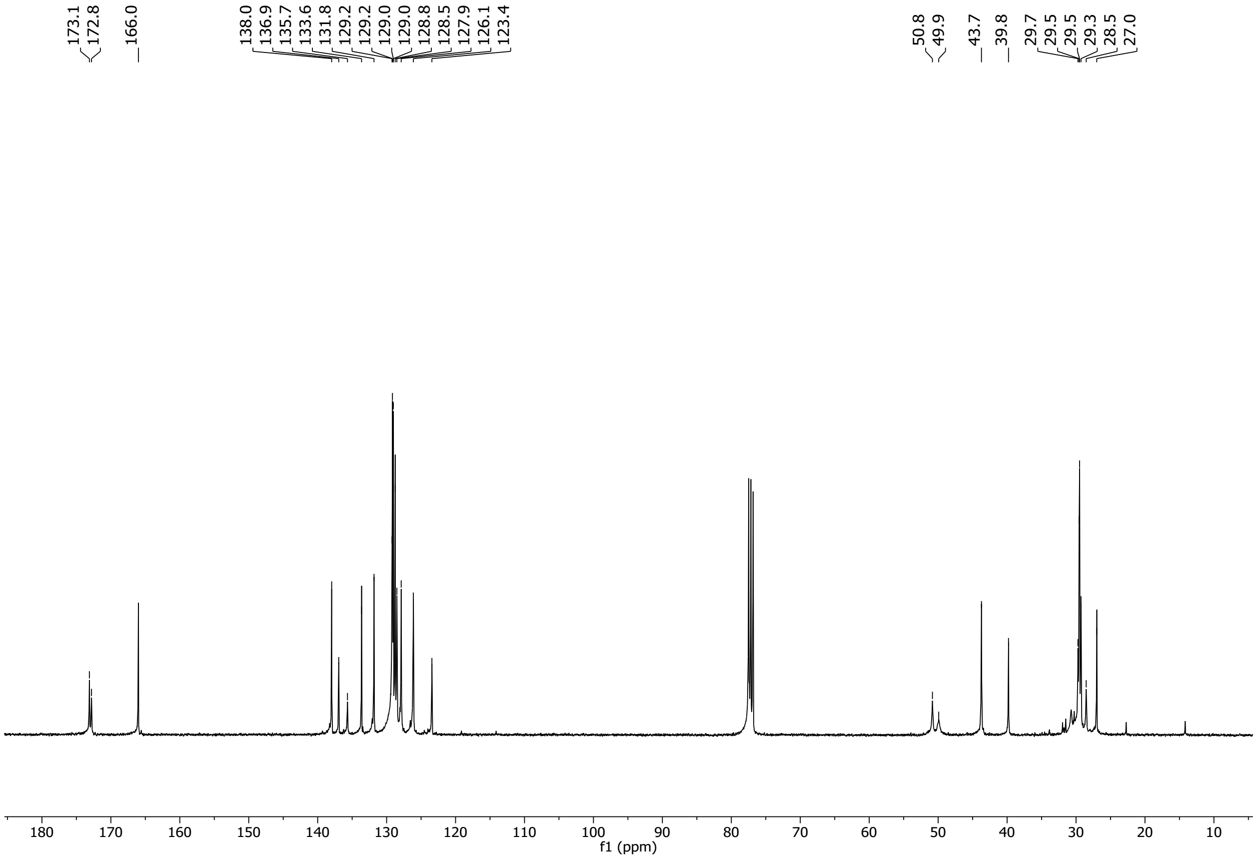
1b (^{1}H NMR, 400 MHz, CDCl$_3$, 298 K)



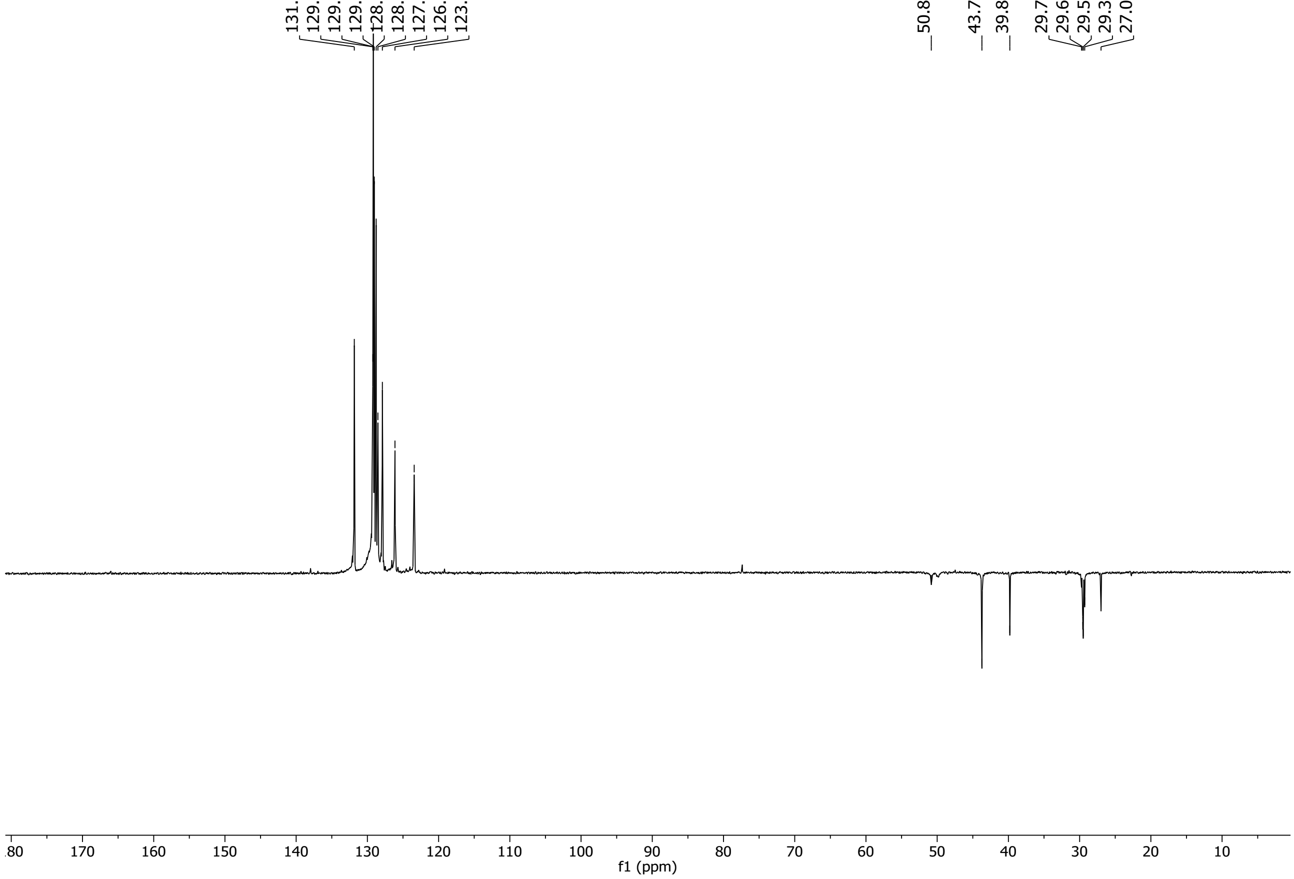
1b (COSY ^{1}H-^{1}H NMR, 400 MHz, CDCl$_3$, 298 K)



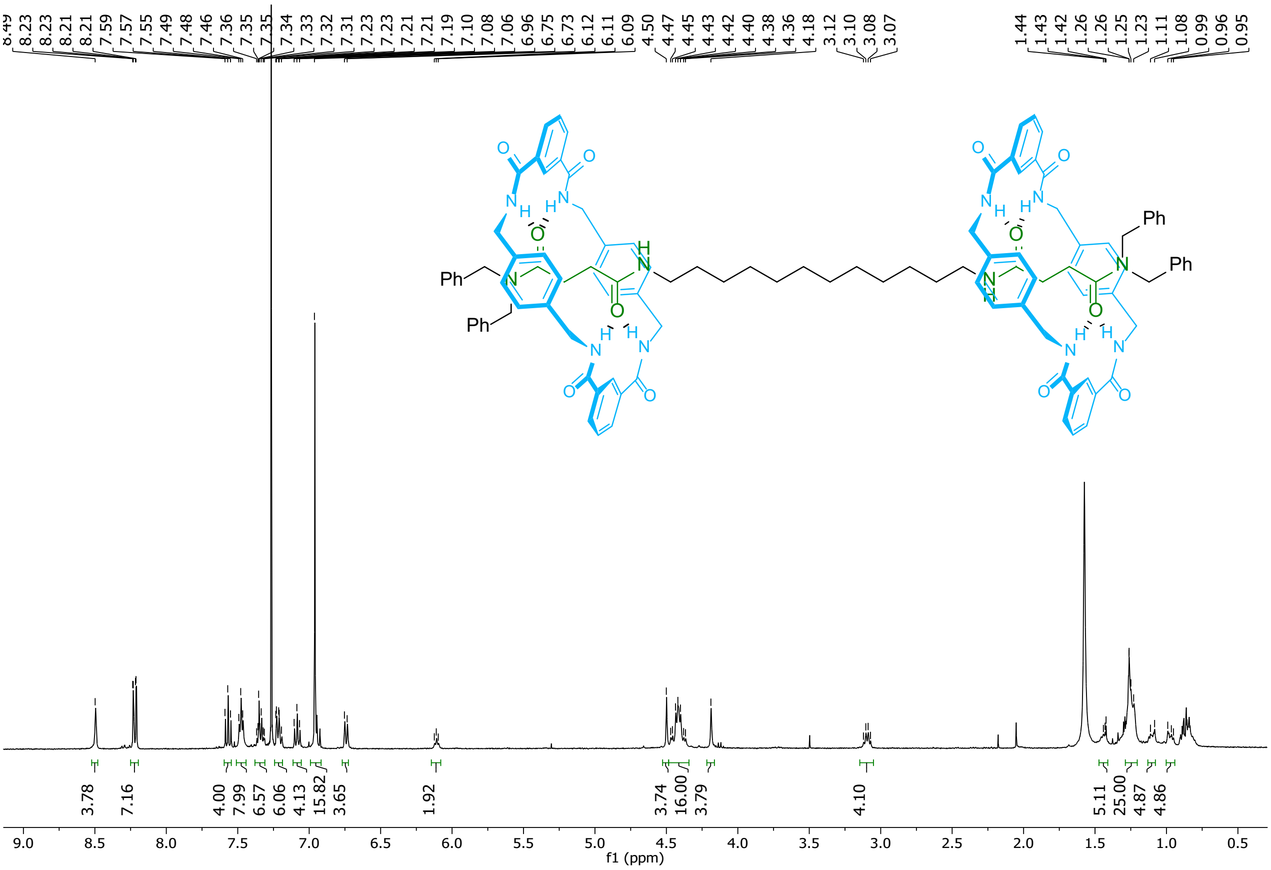
1b (^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



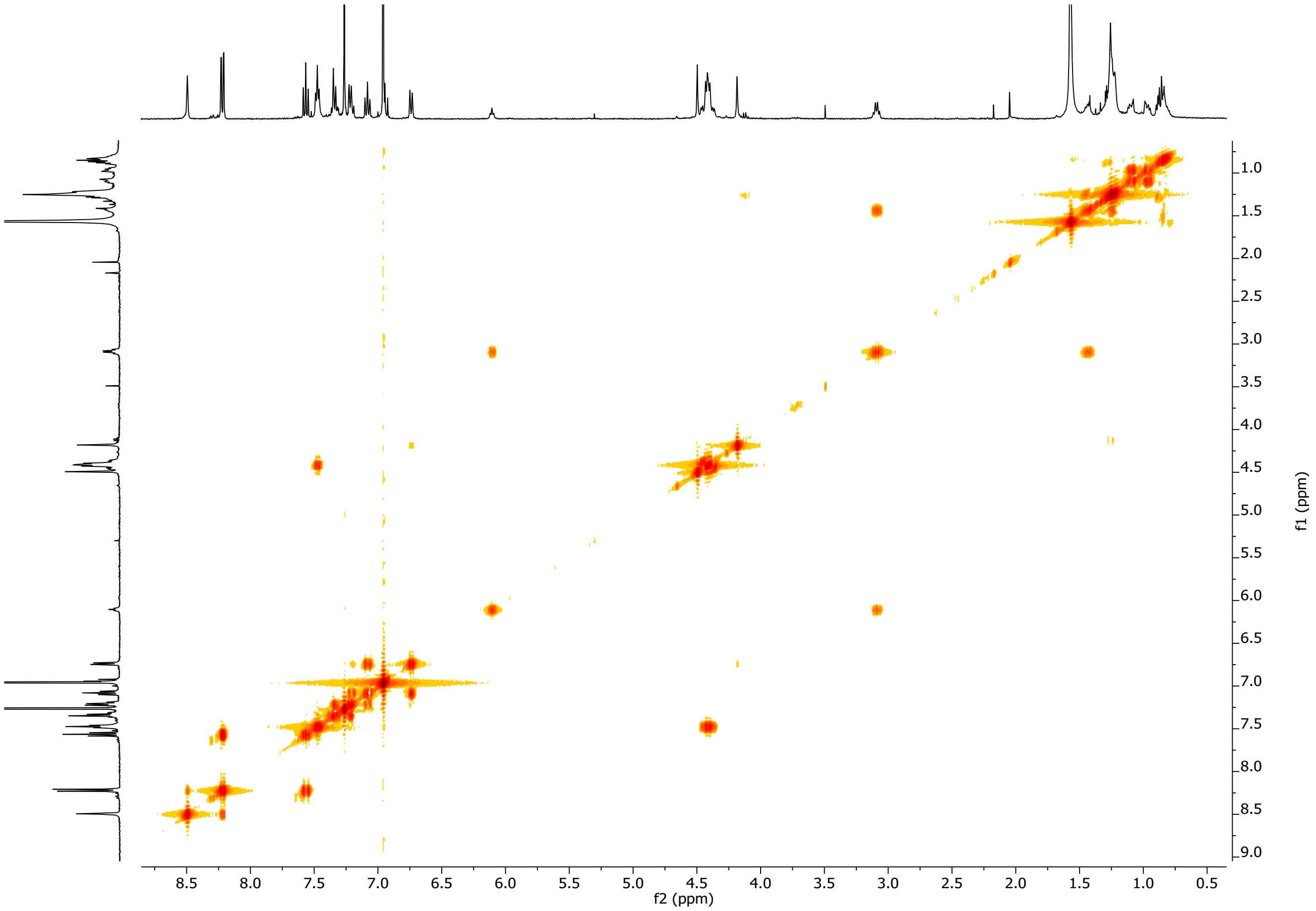
1b (DEPT ^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



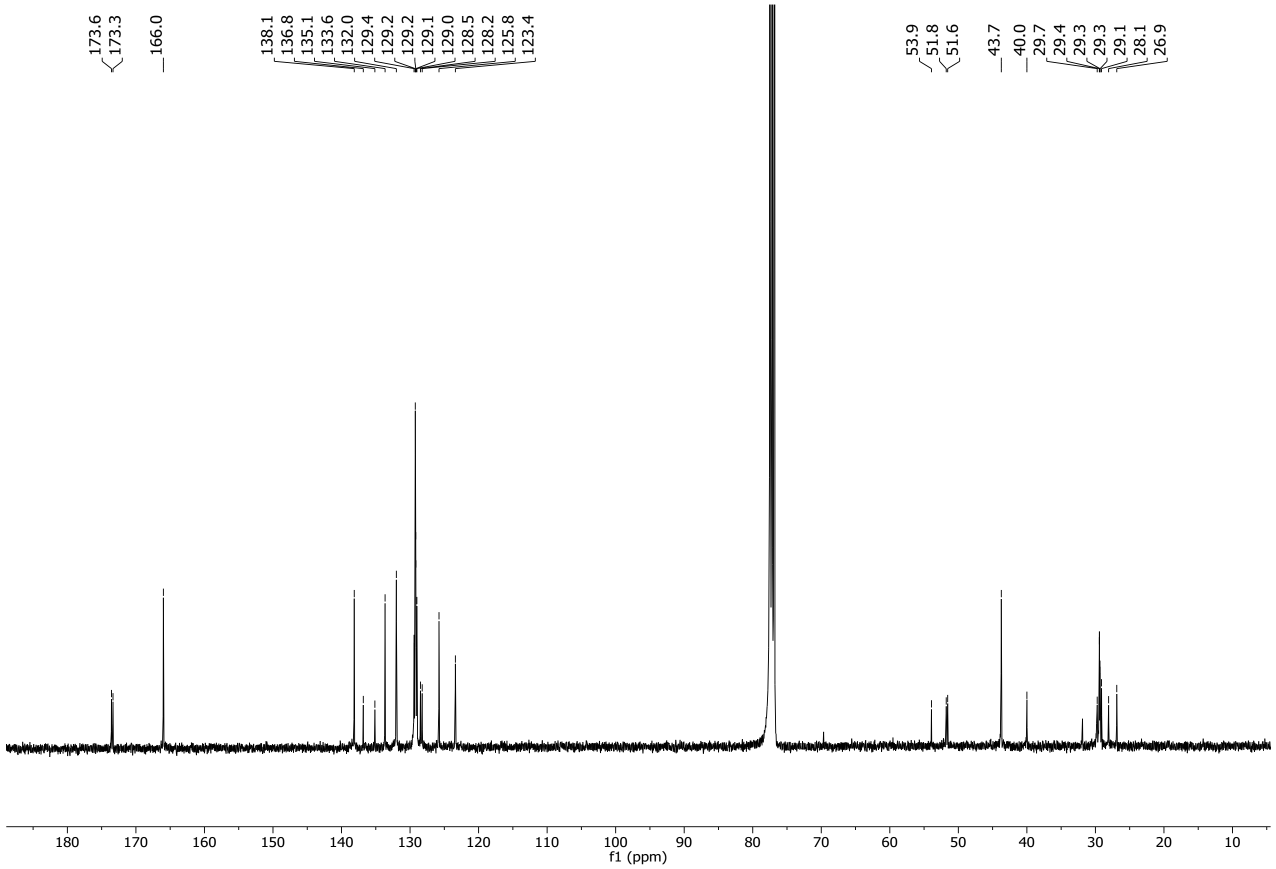
S3b (^{1}H NMR, 400 MHz, $CDCl_3$, 298 K)



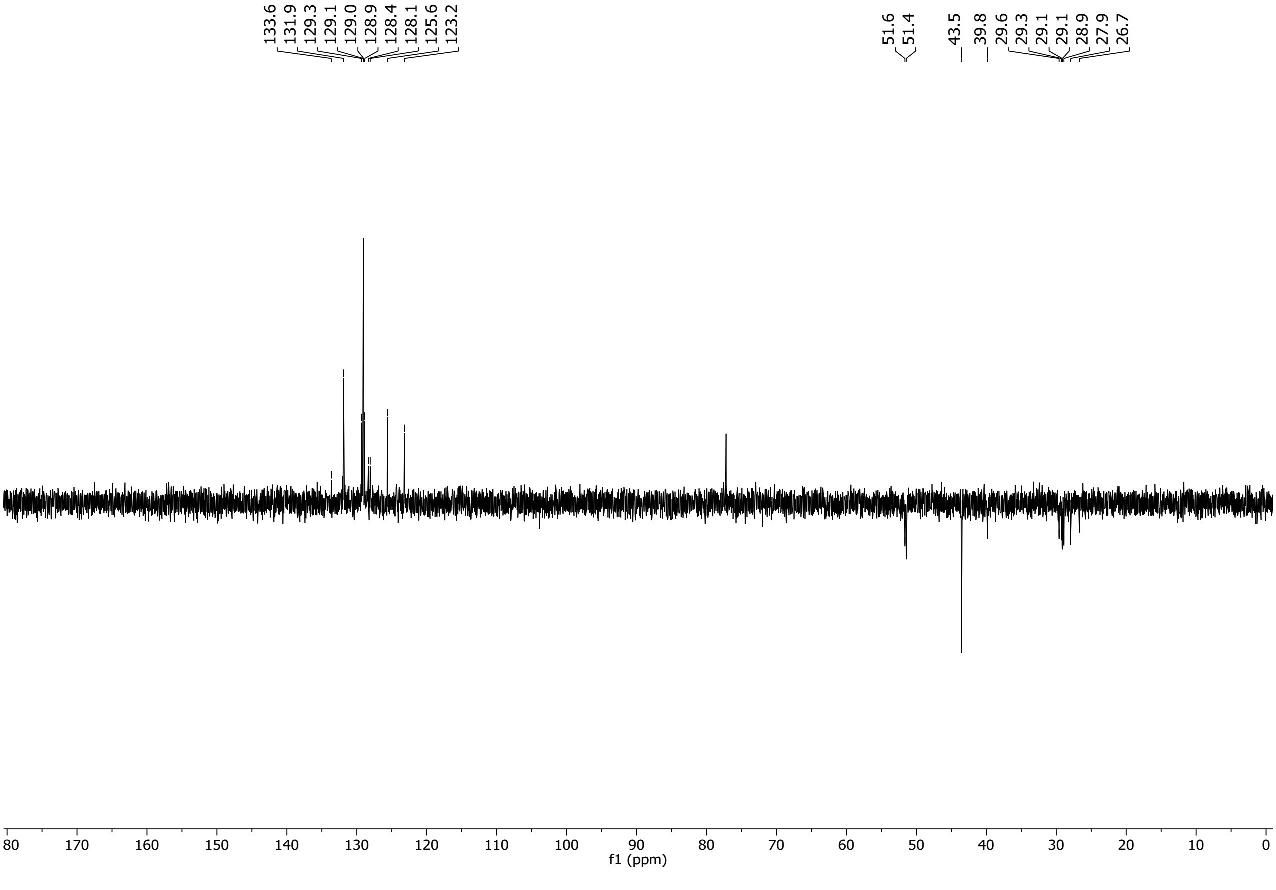
S3b (COSY ^{1}H-^{1}H NMR, 400 MHz, $CDCl_3$, 298 K)



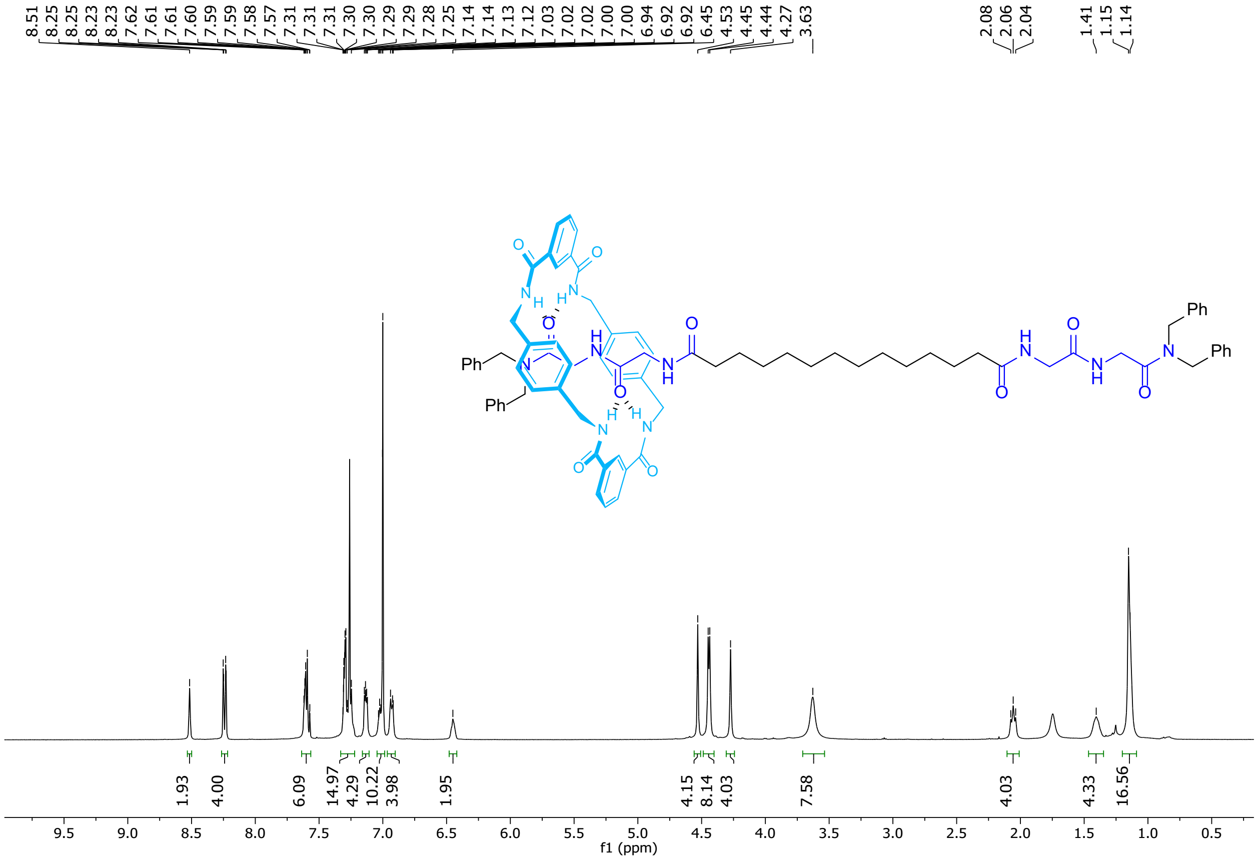
S3b (^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



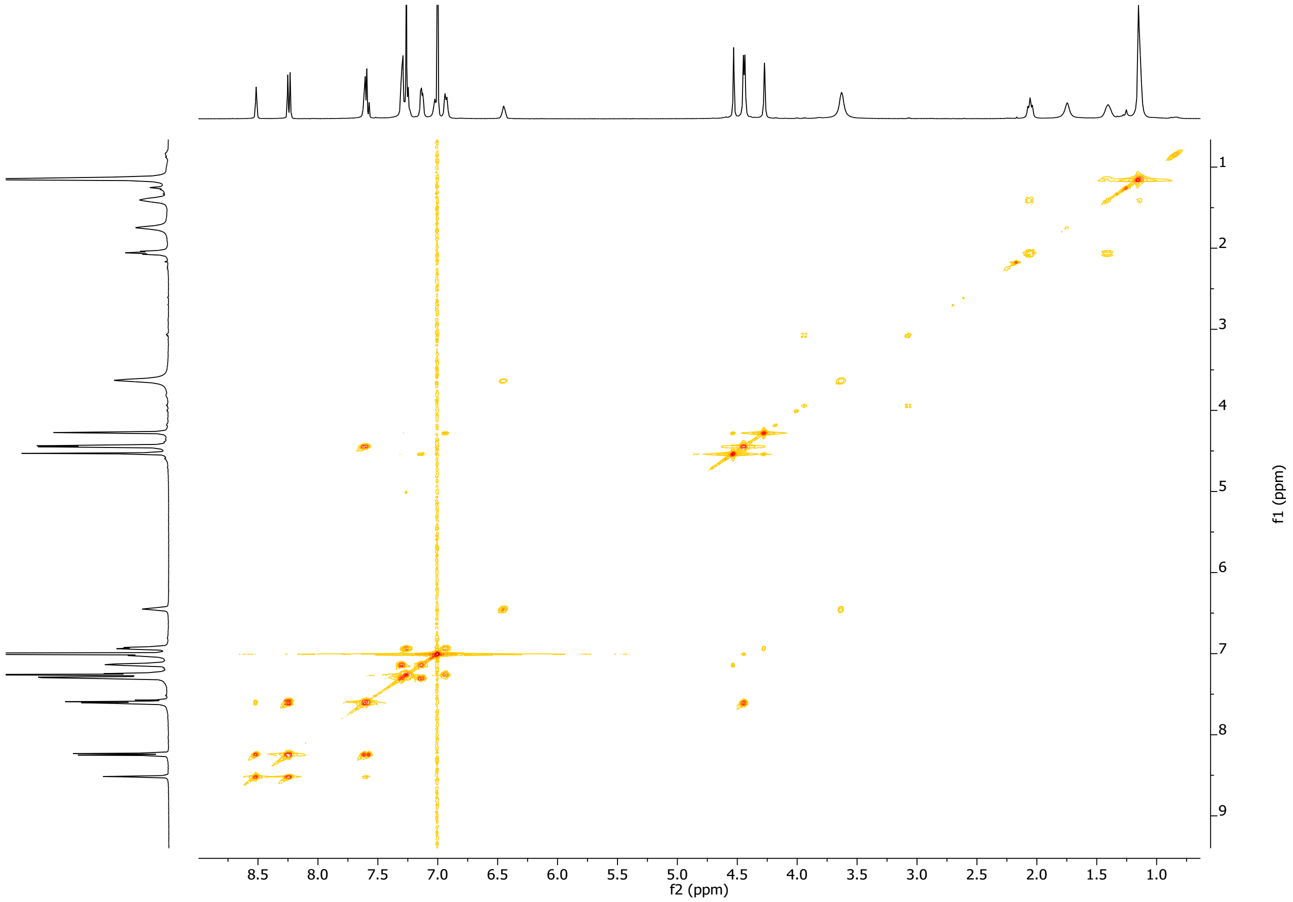
S3b (DEPT ^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



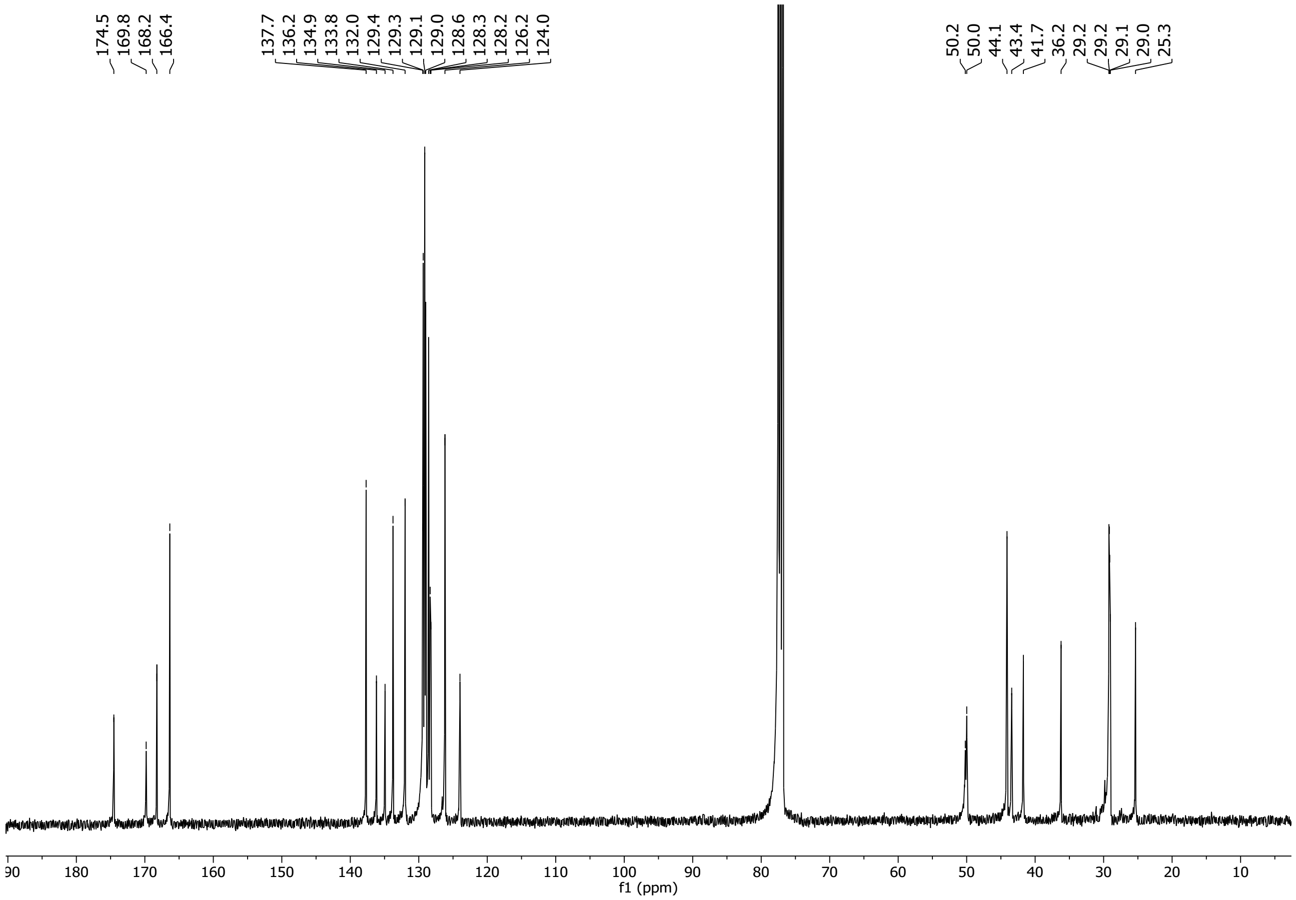
1c (^{1}H NMR, 400 MHz, $CDCl_3$, 298 K)



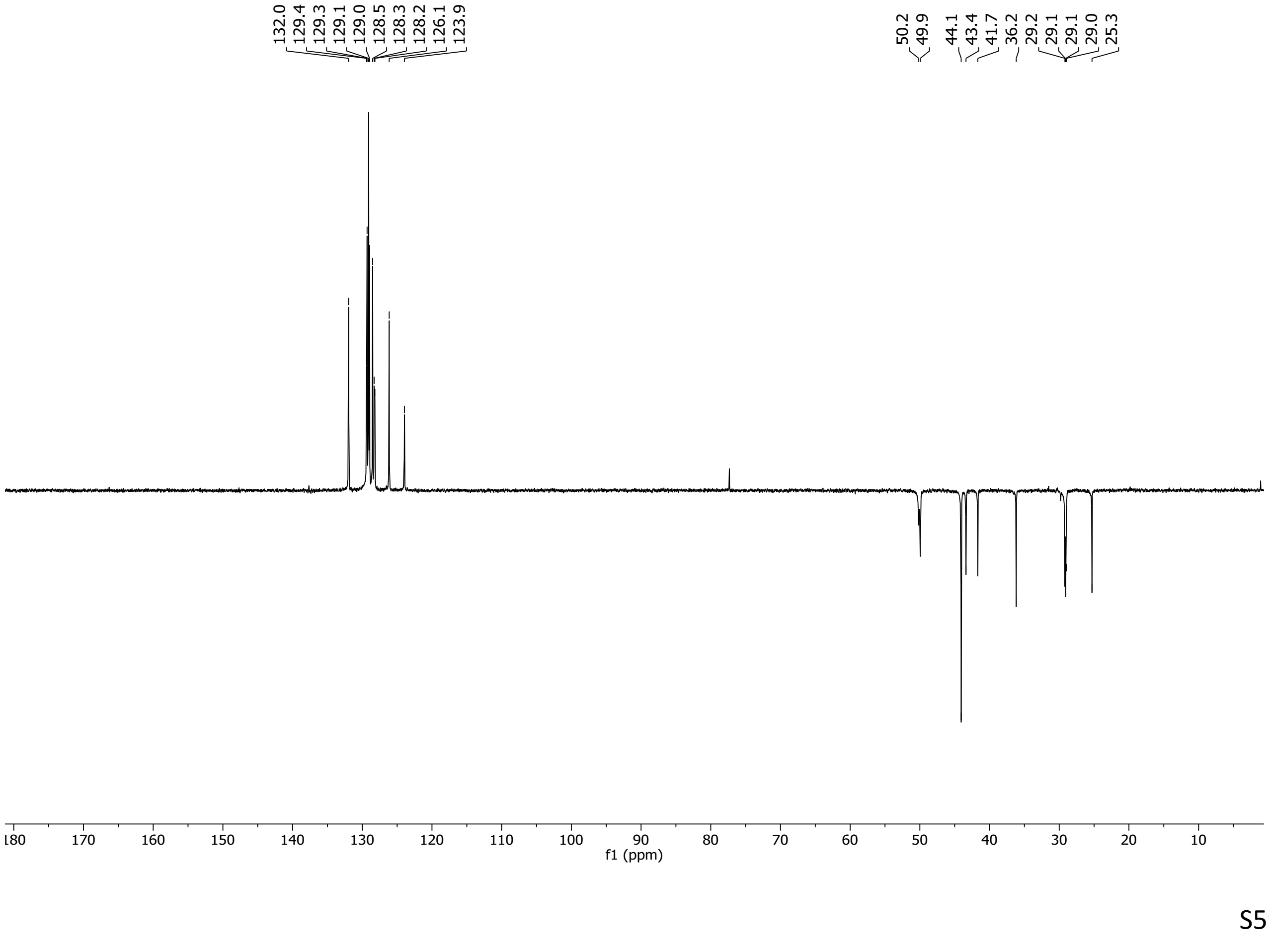
1c (COSY ^{1}H-^{1}H NMR, 400 MHz, $CDCl_3$, 298 K)



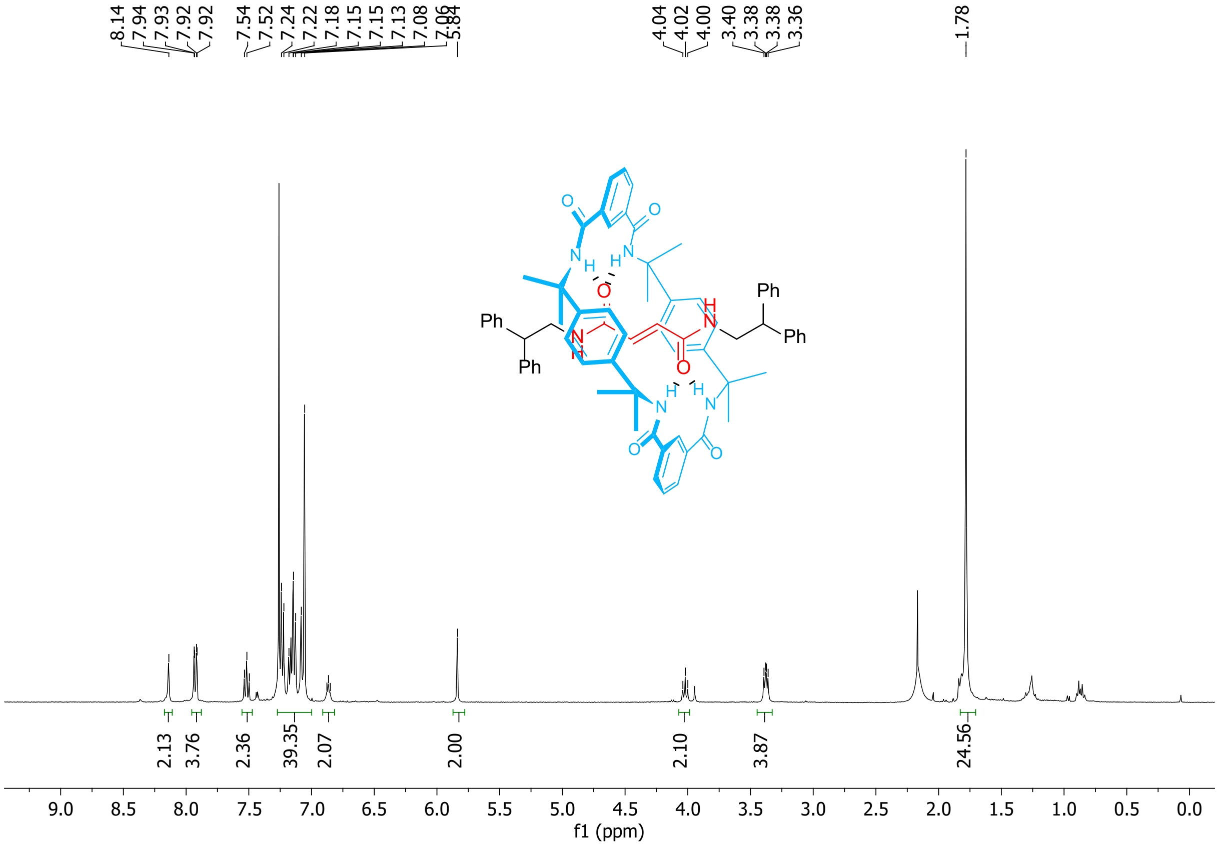
1c (^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



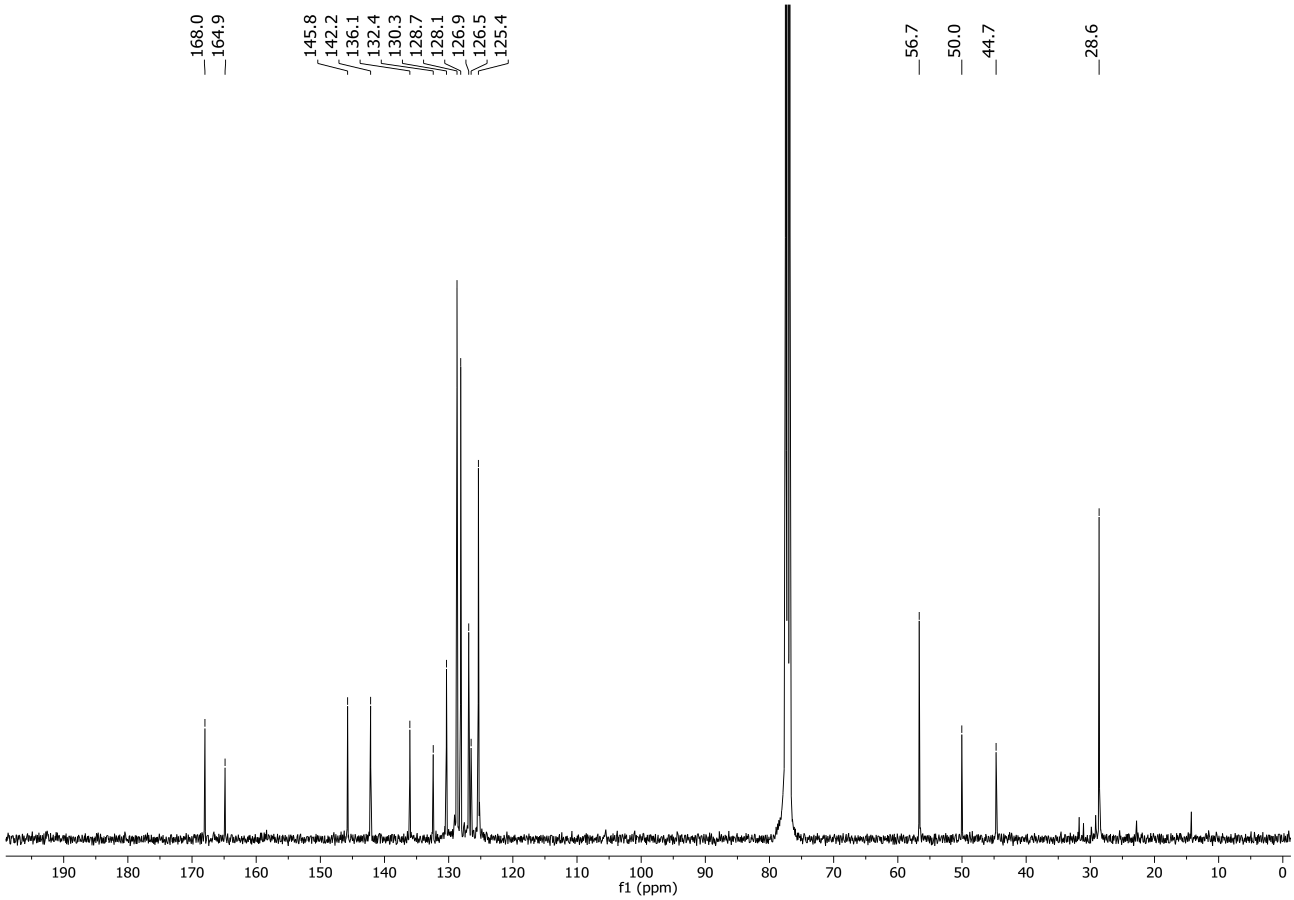
1c (DEPT ^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



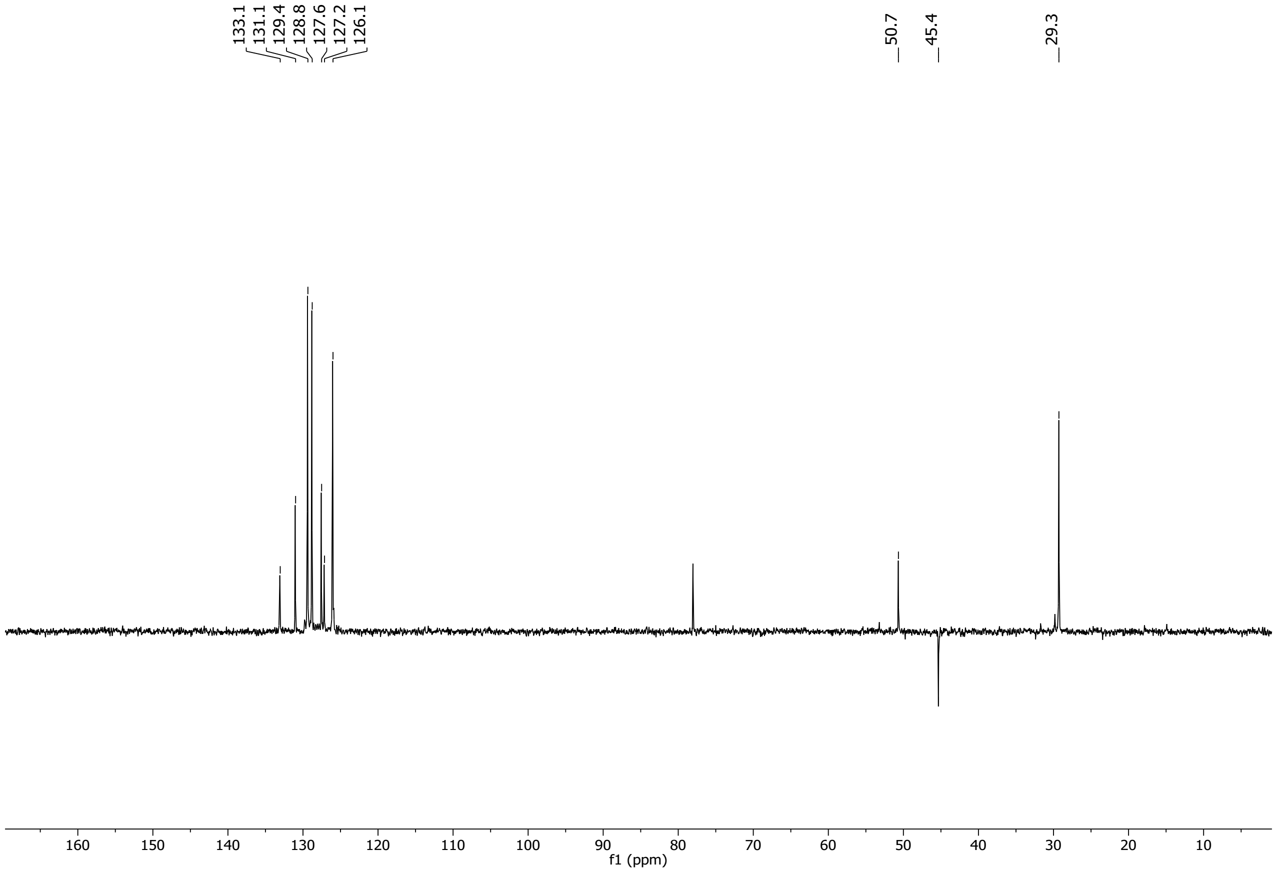
7 (^{1}H NMR, 400 MHz, CDCl$_3$, 298 K)



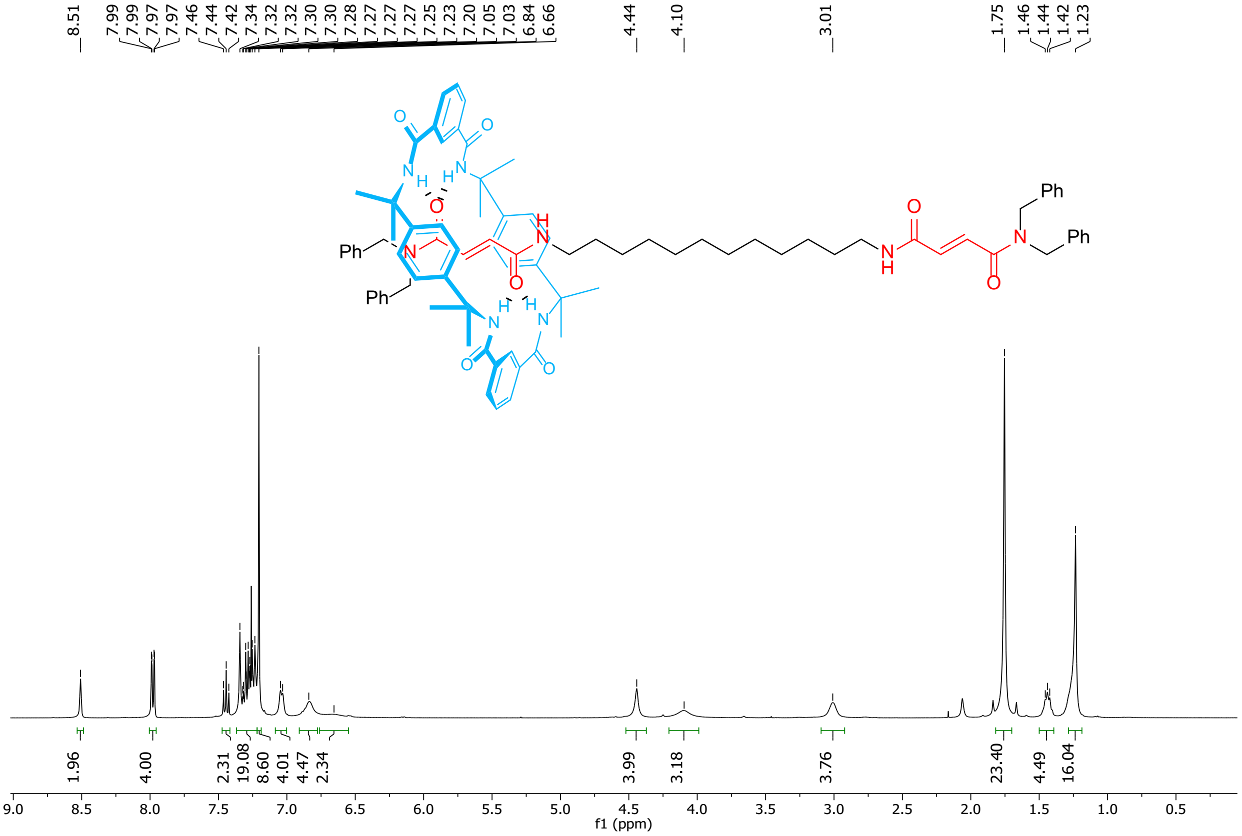
7 (^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



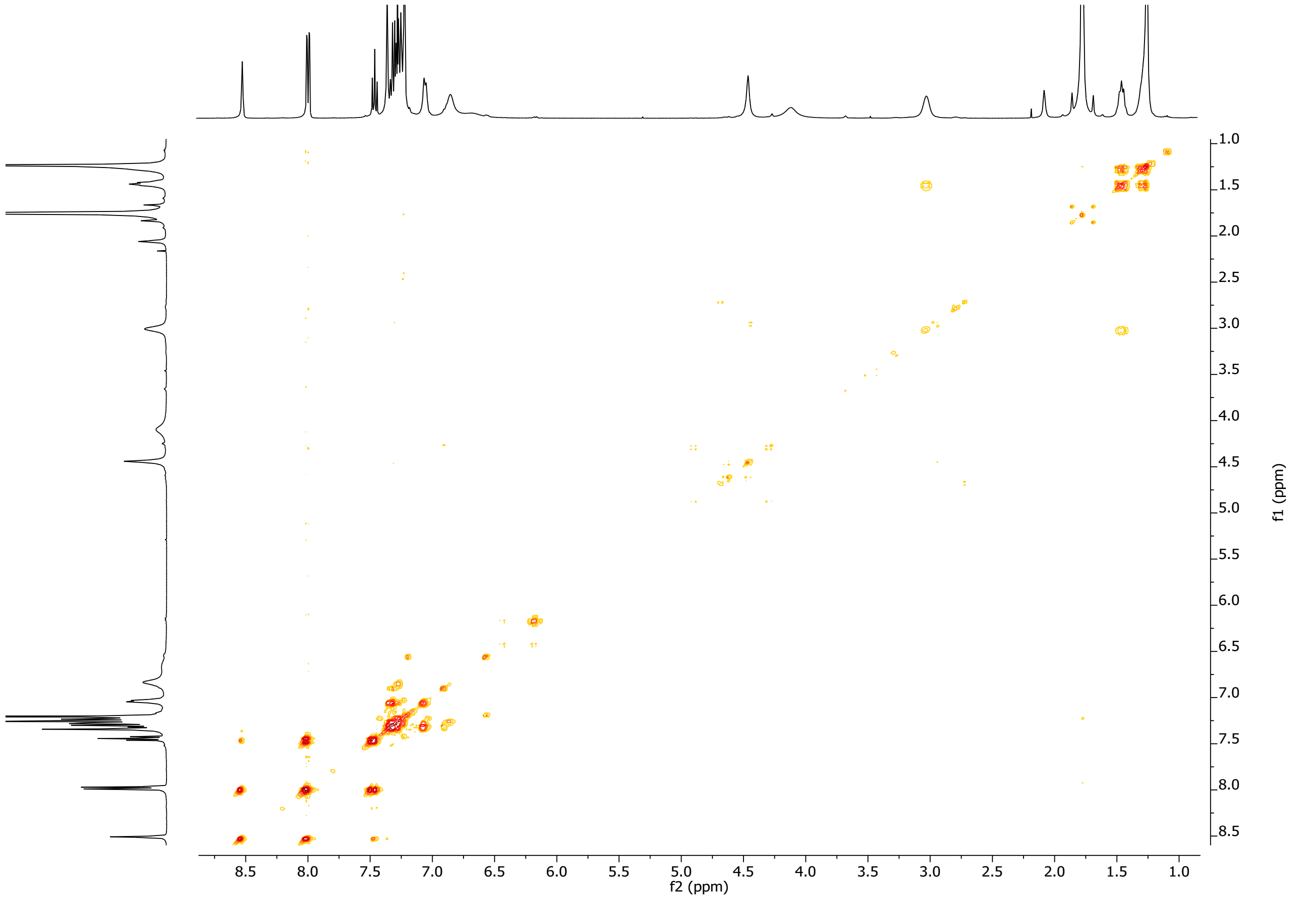
7 (DEPT ^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



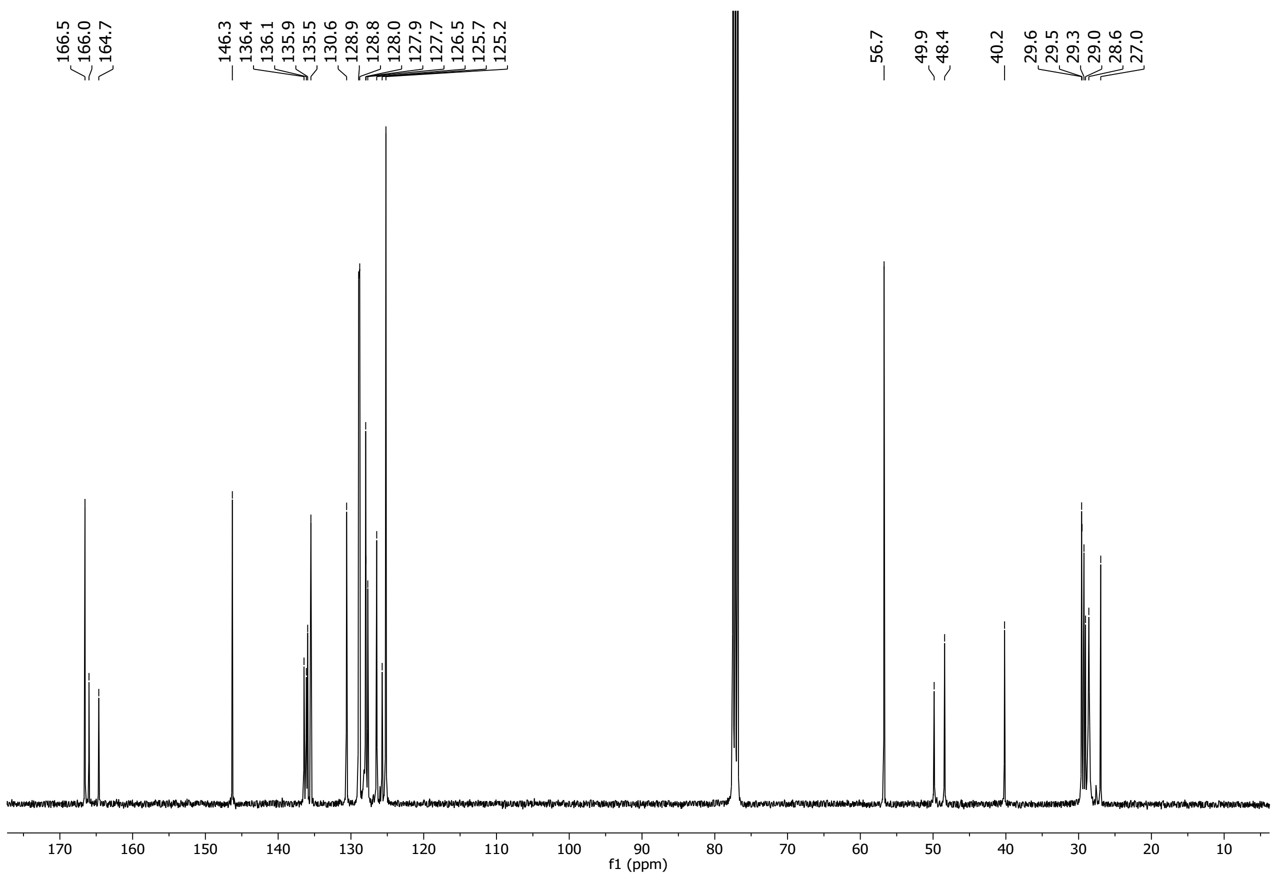
2a (^{1}H NMR, 400 MHz, CDCl$_3$, 298 K)



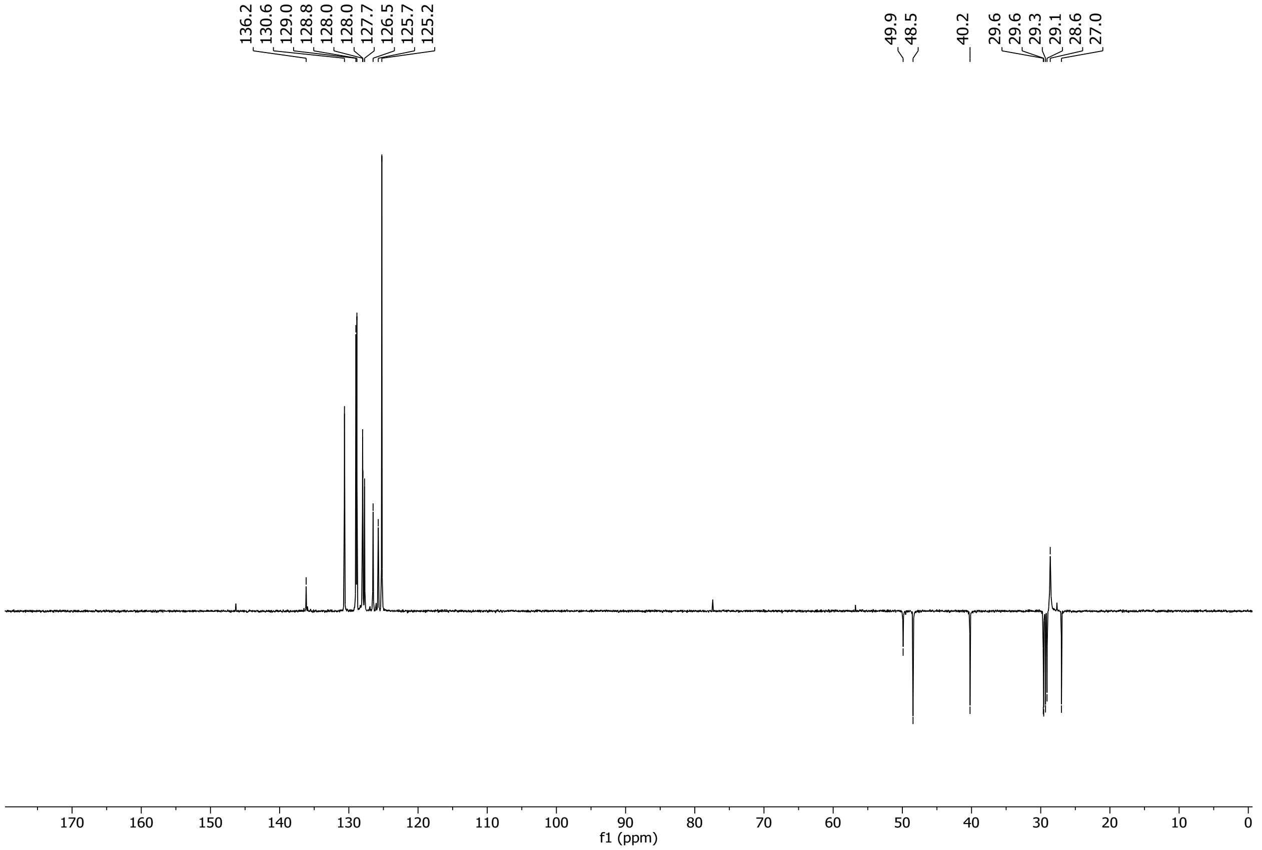
2a (COSY ^{1}H-^{1}H NMR, 400 MHz, CDCl$_3$, 298 K)



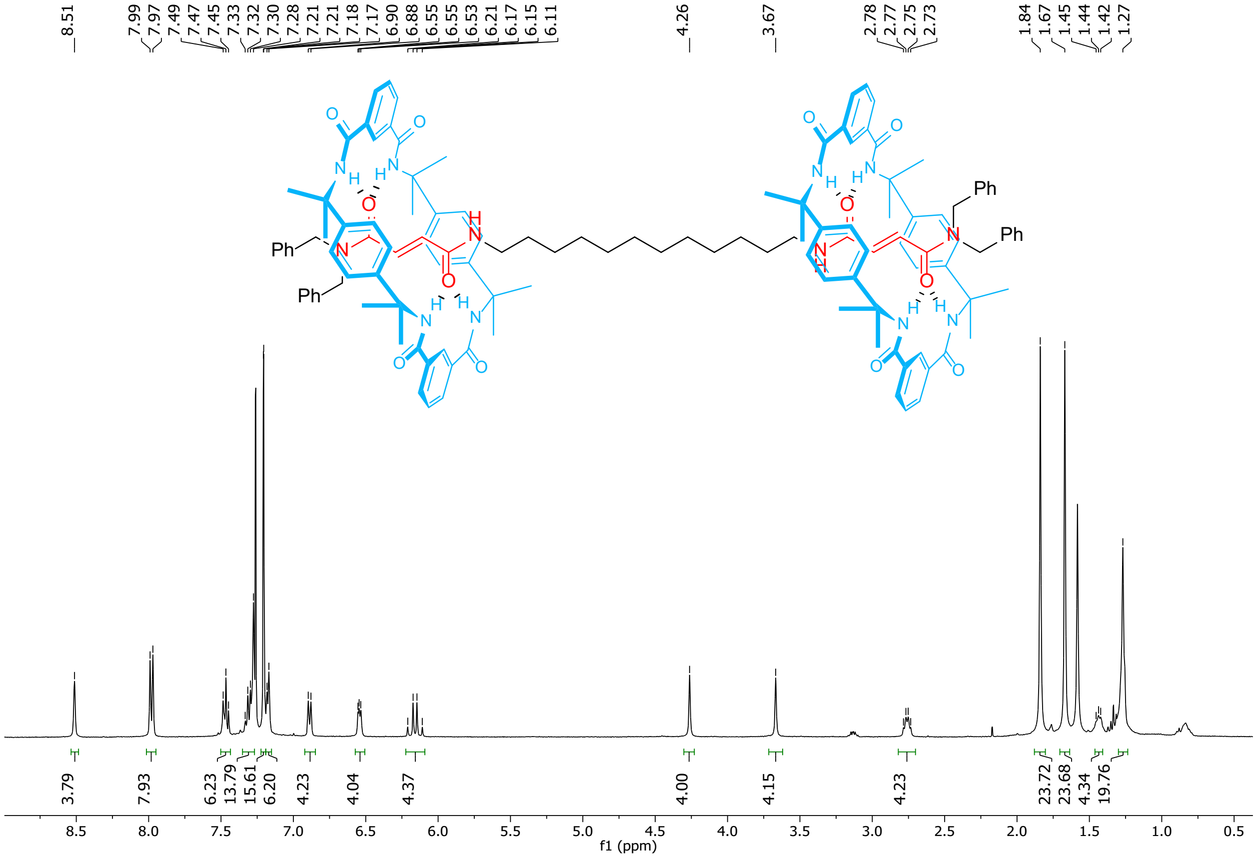
2a (^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



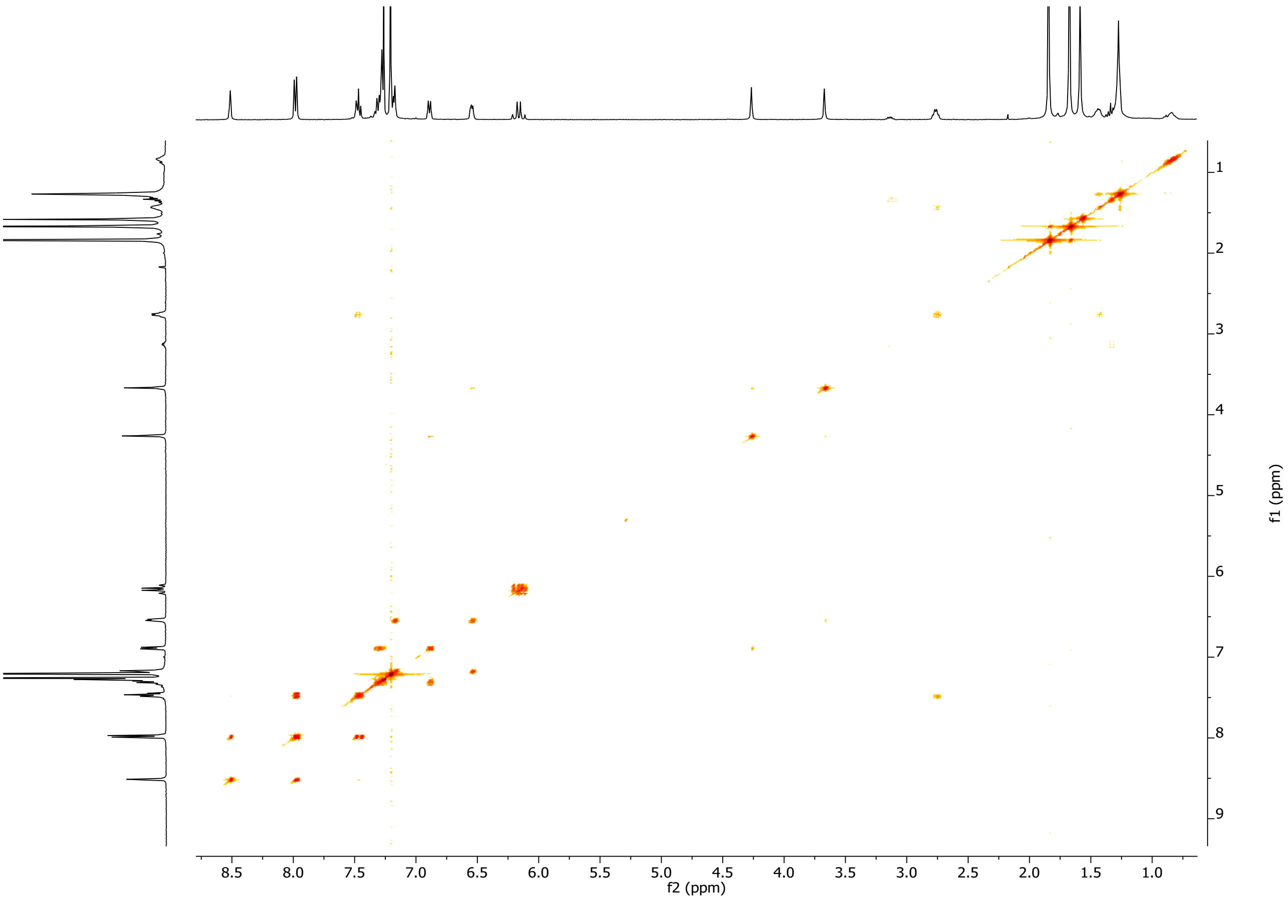
2a (DEPT ^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



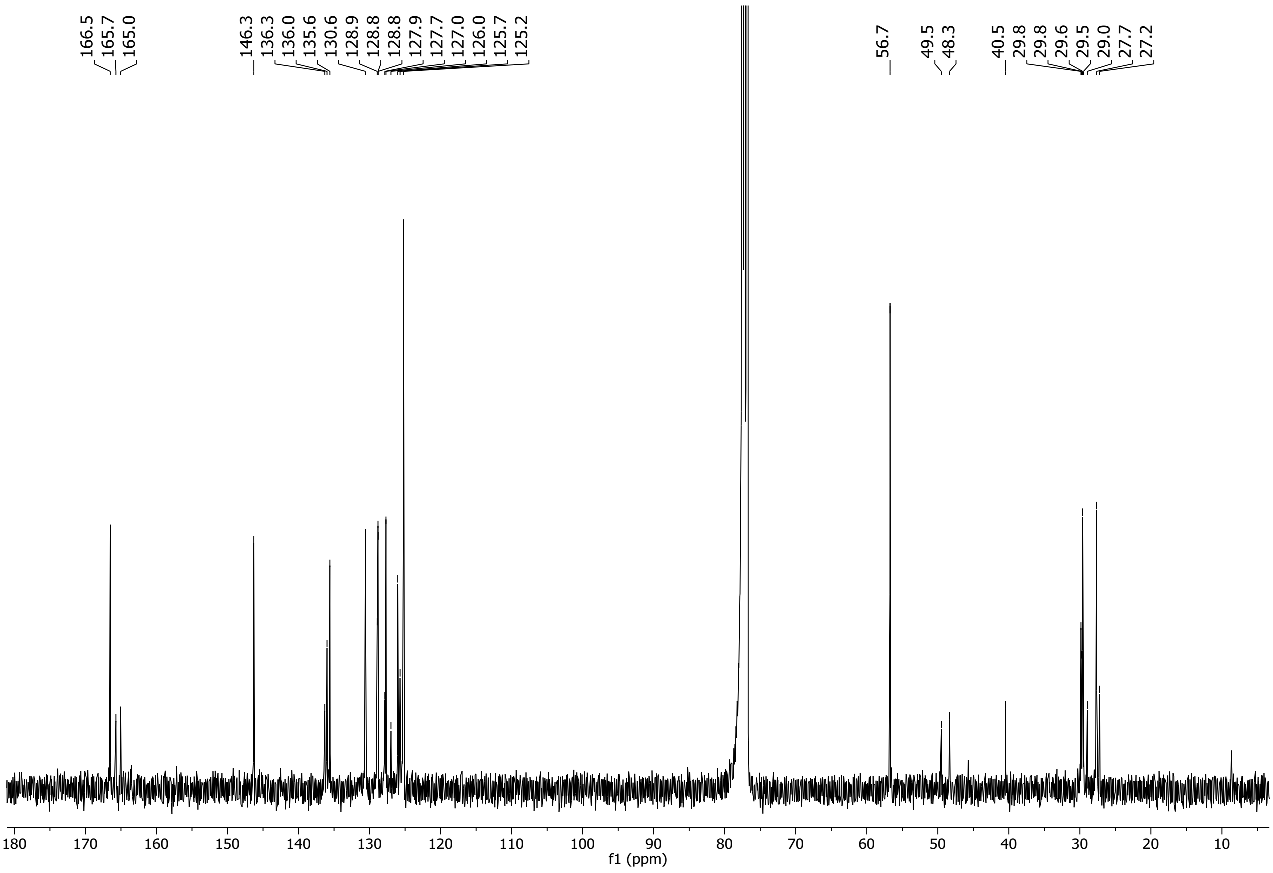
S5a (^{1}H NMR, 400 MHz, CDCl$_3$, 298 K)



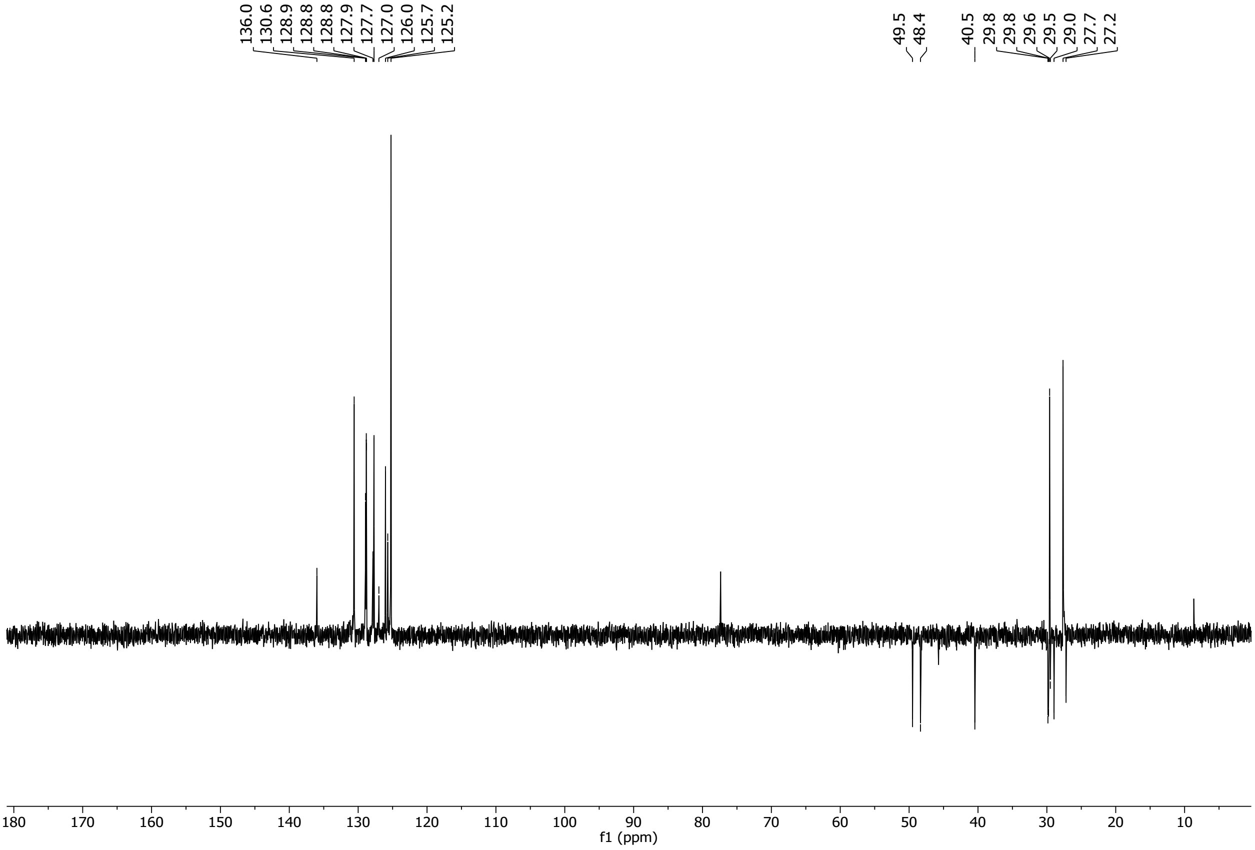
S5a (COSY ^{1}H-^{1}H NMR, 400 MHz, CDCl$_3$, 298 K)



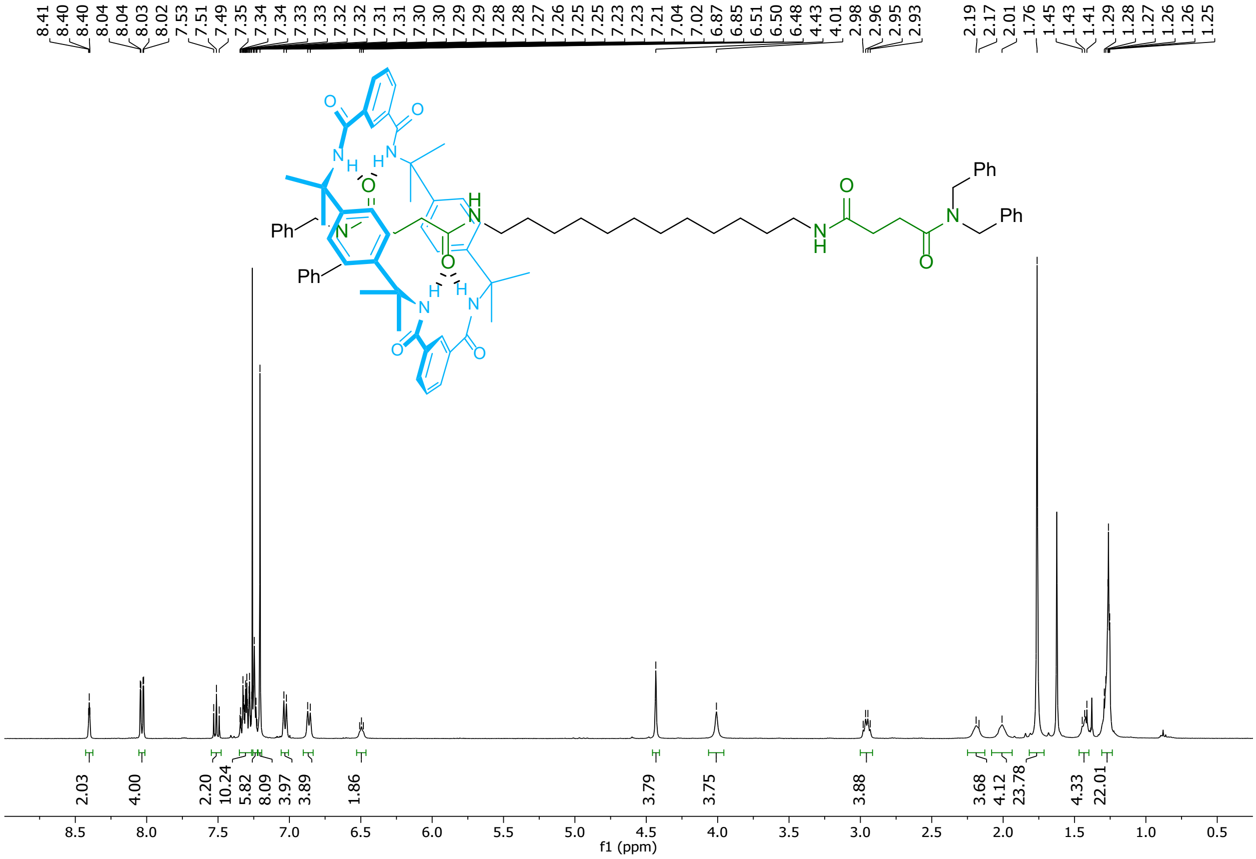
S5a (^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



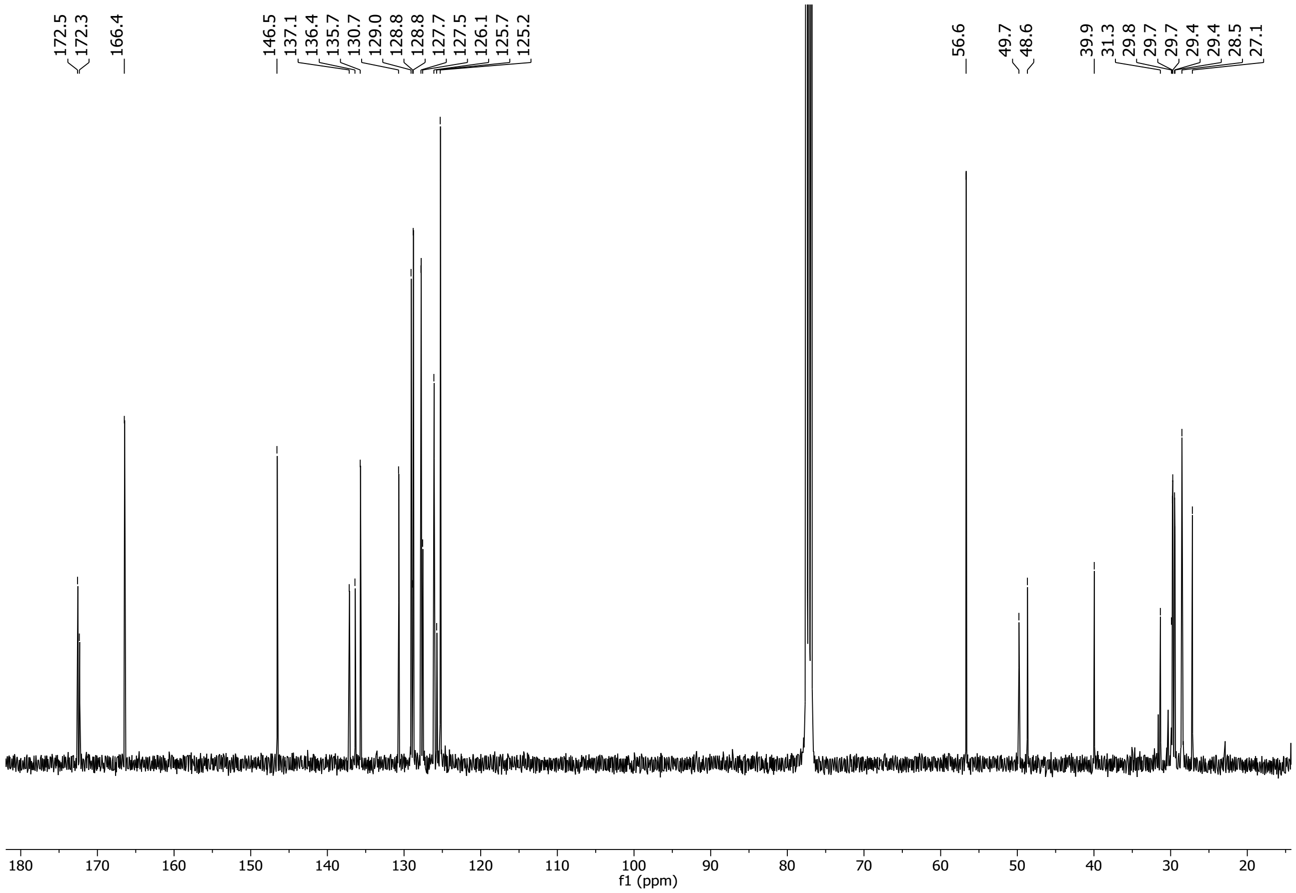
S5a (DEPT ^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



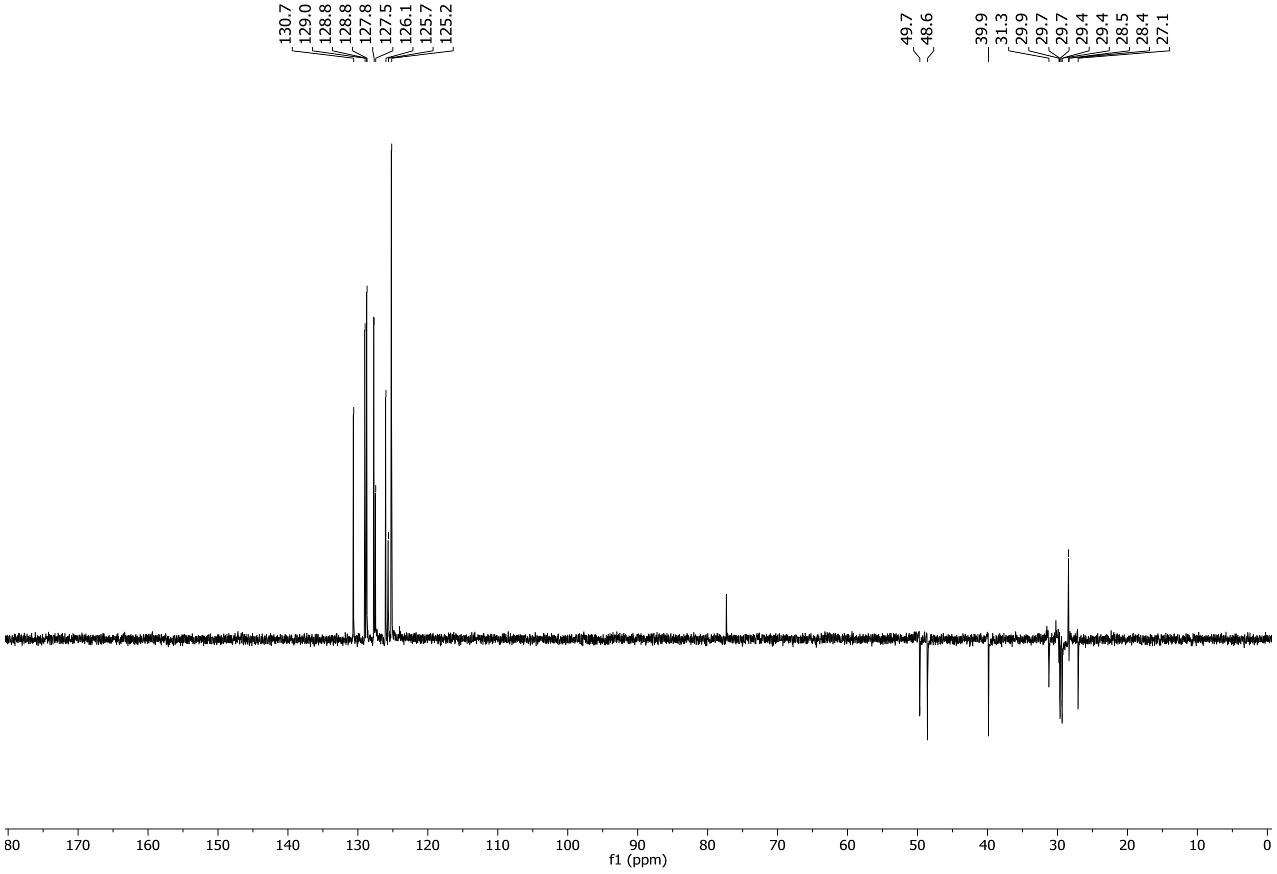
2b (^{1}H NMR, 400 MHz, CDCl$_3$, 298 K)



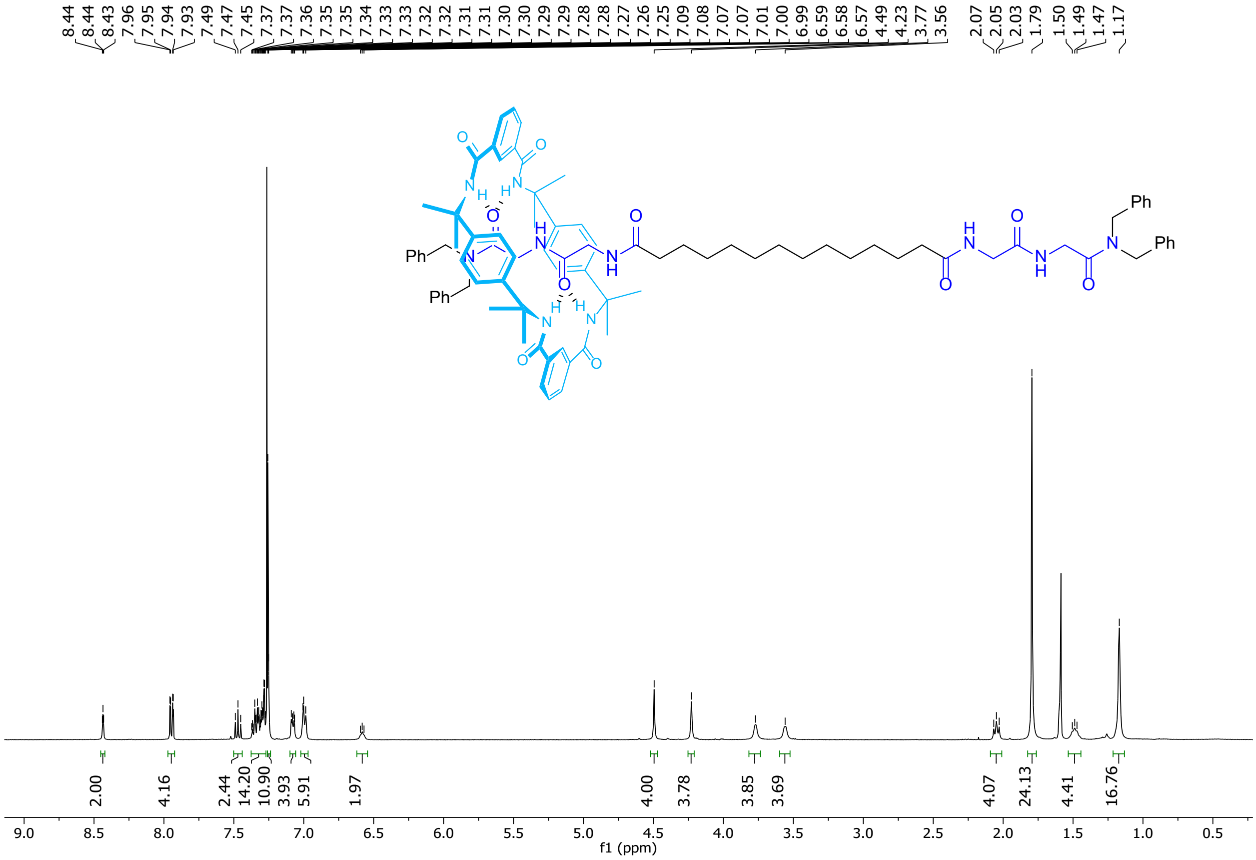
2b (^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



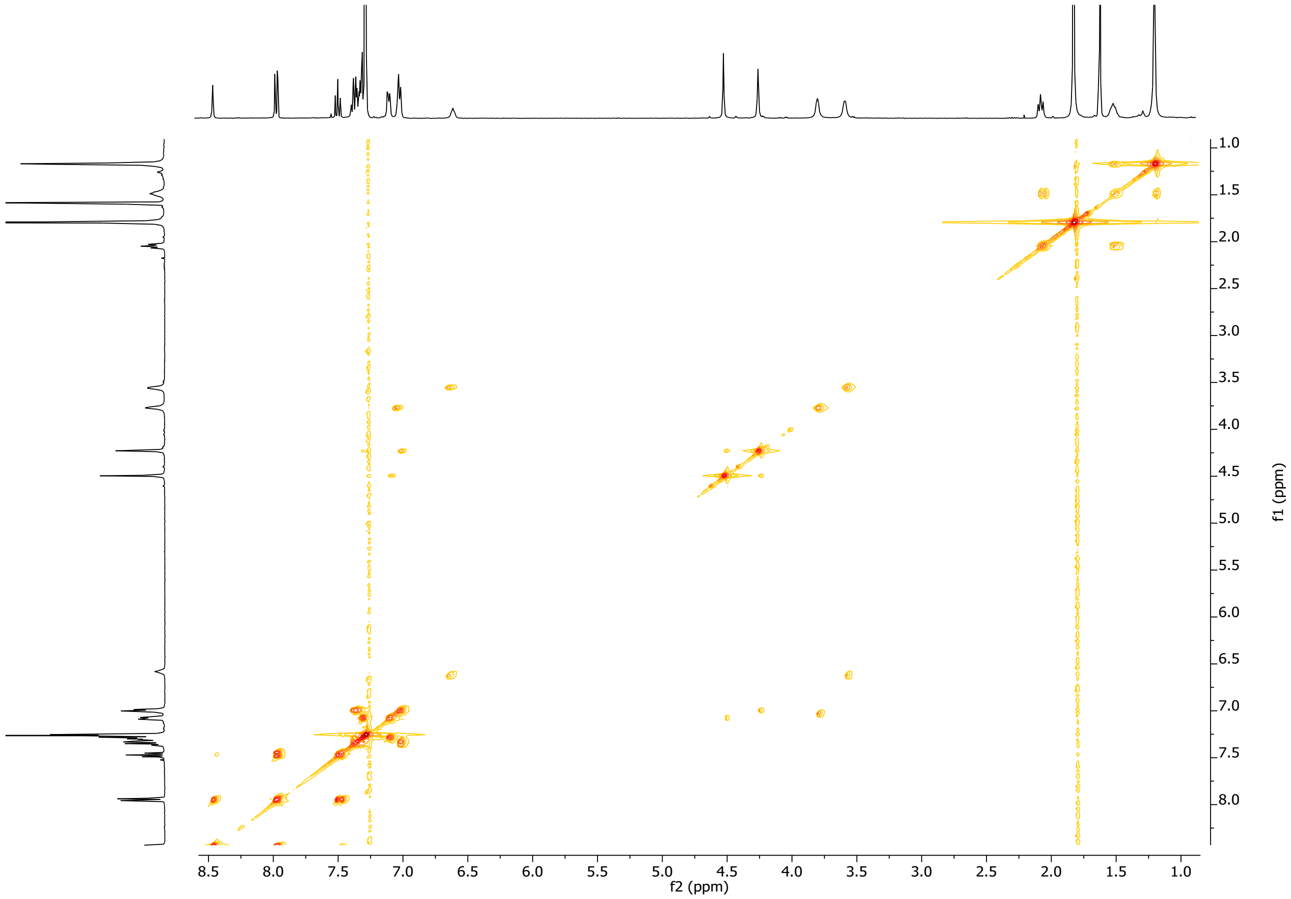
2b (DEPT ^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



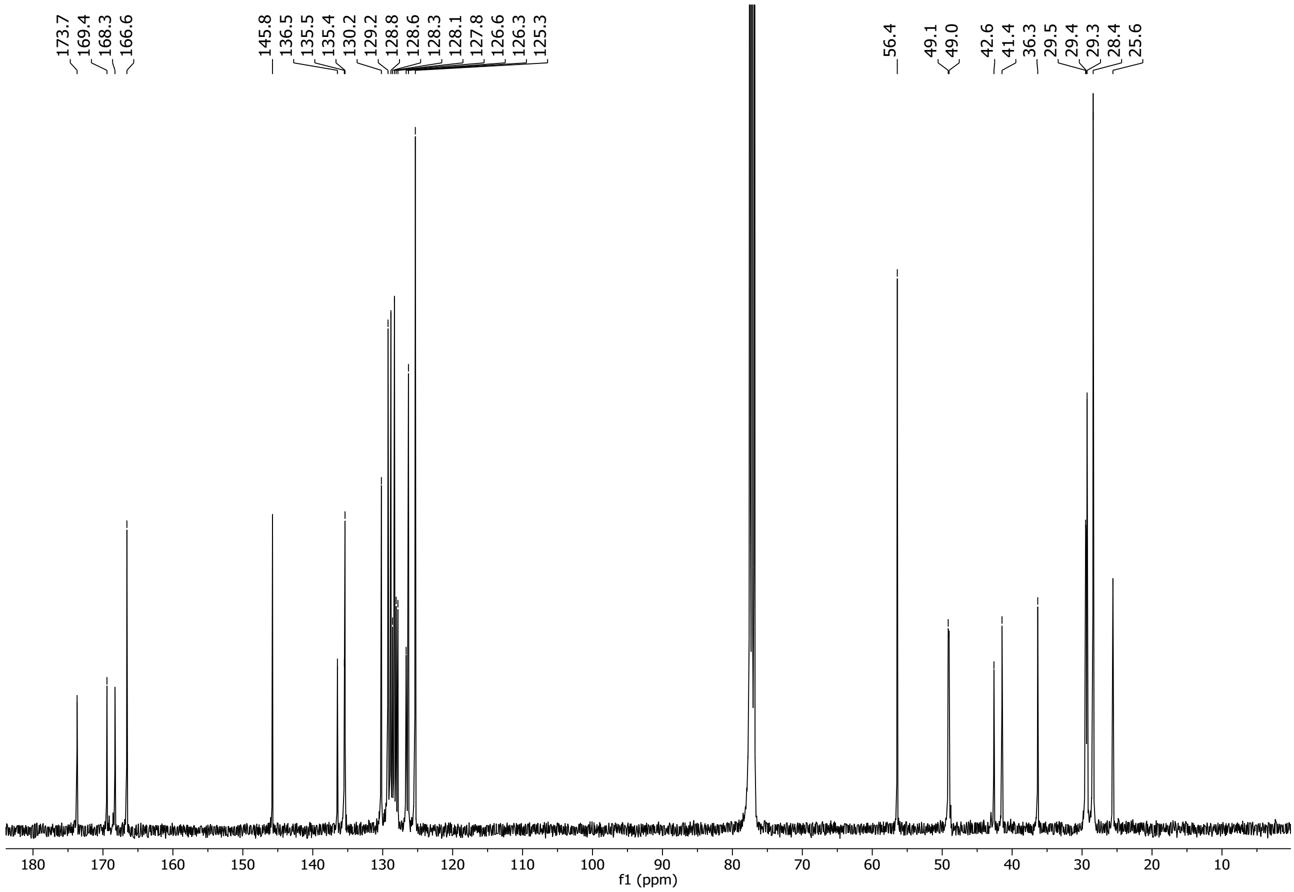
2c (^{1}H NMR, 400 MHz, CDCl$_3$, 298 K)



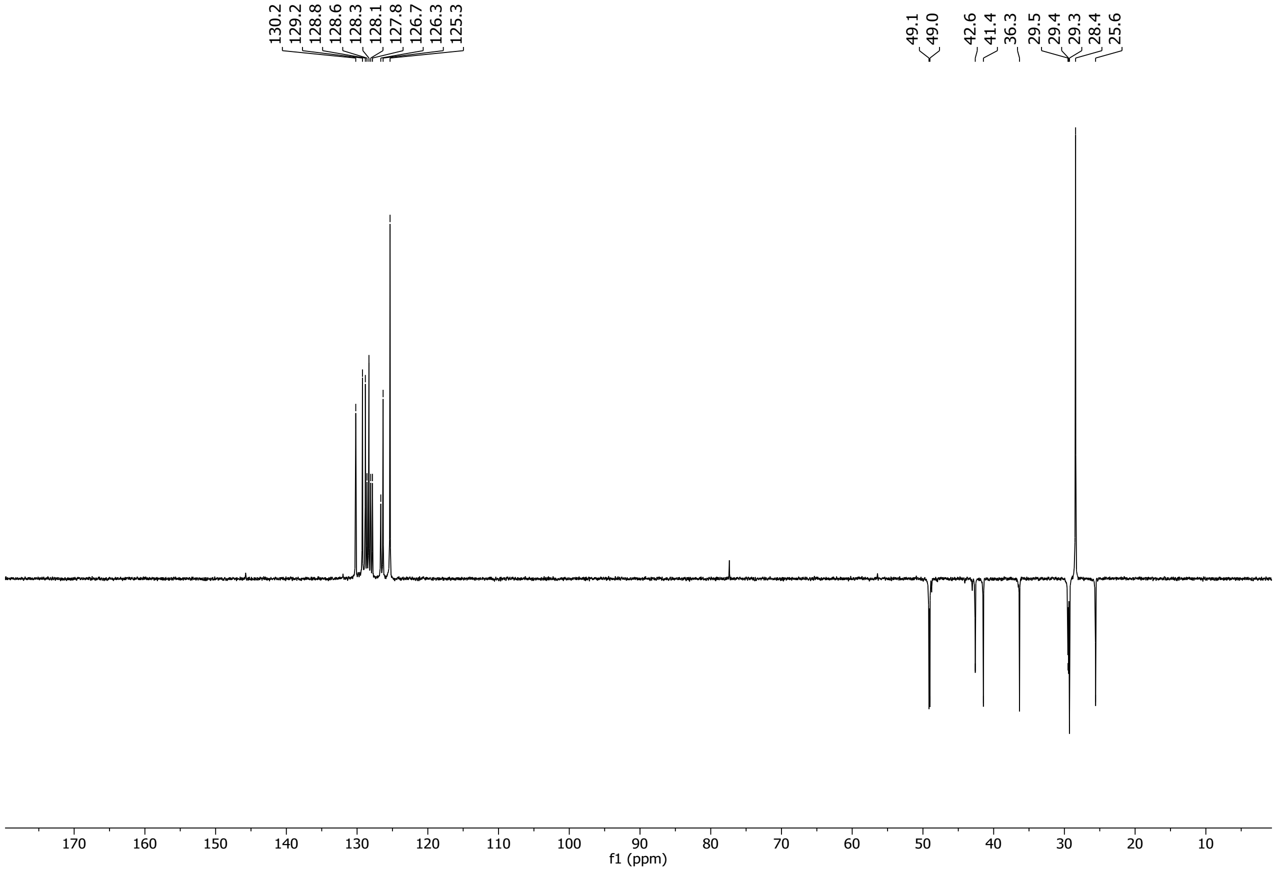
2c (COSY ^{1}H-^{1}H NMR, 400 MHz, CDCl$_3$, 298 K)



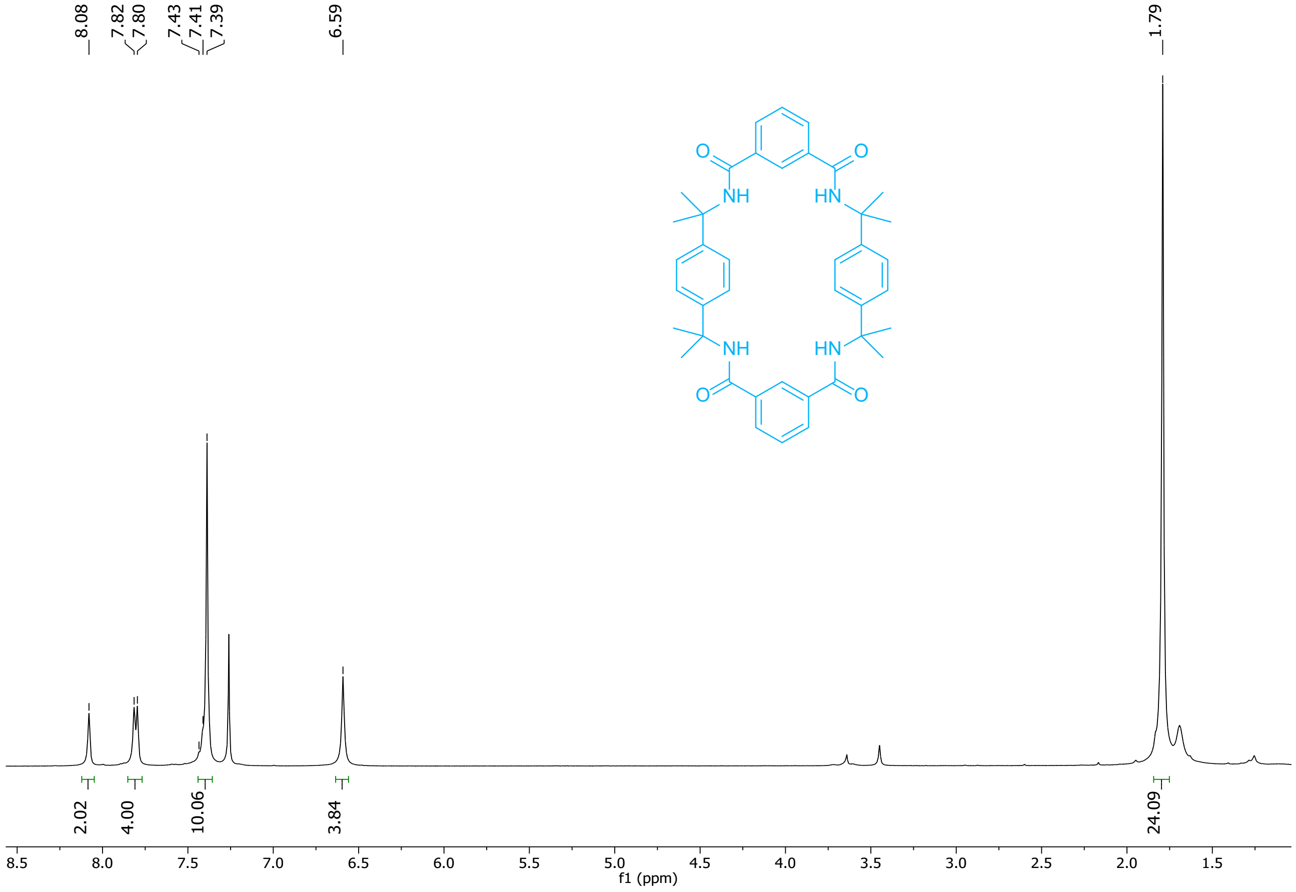
2c (^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



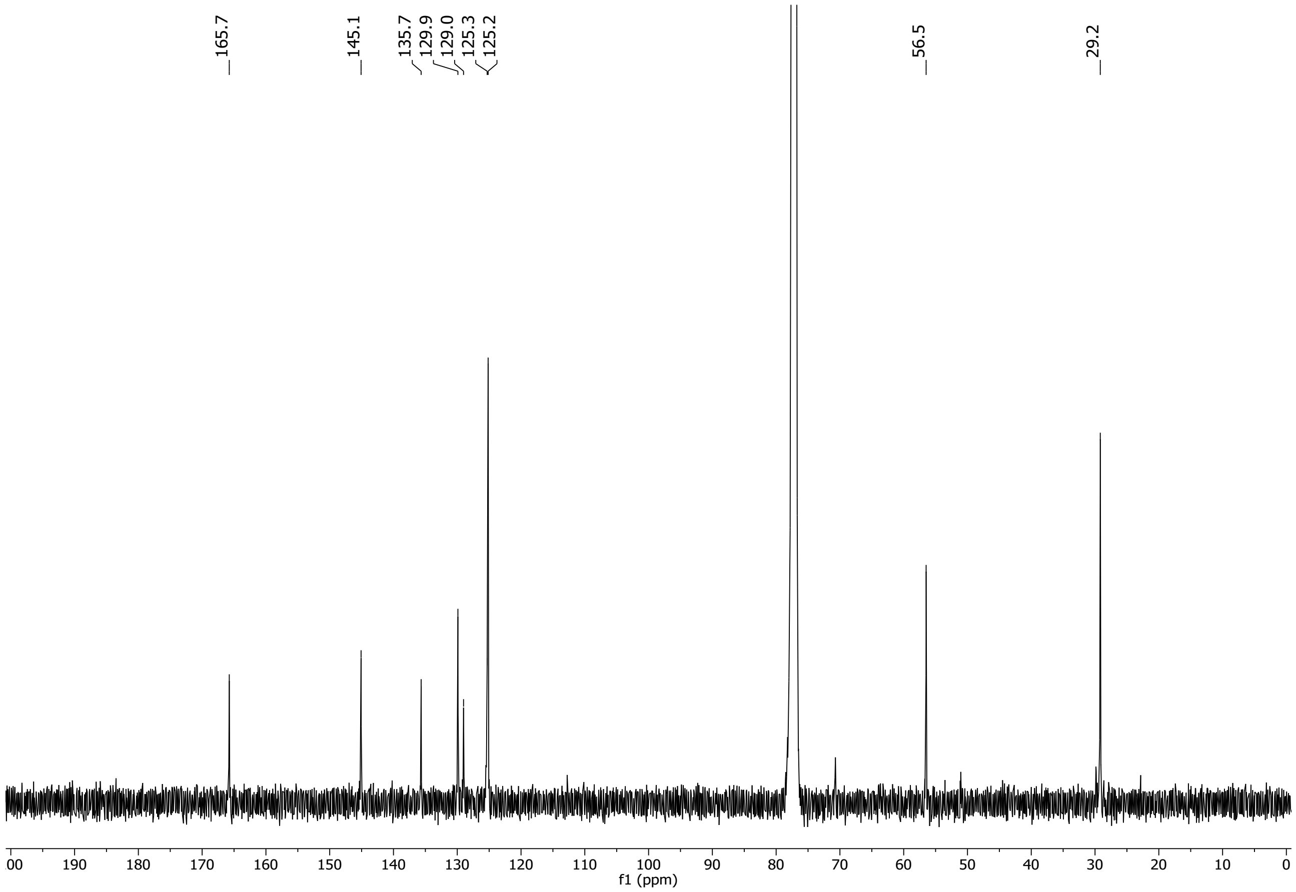
2c (DEPT ^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



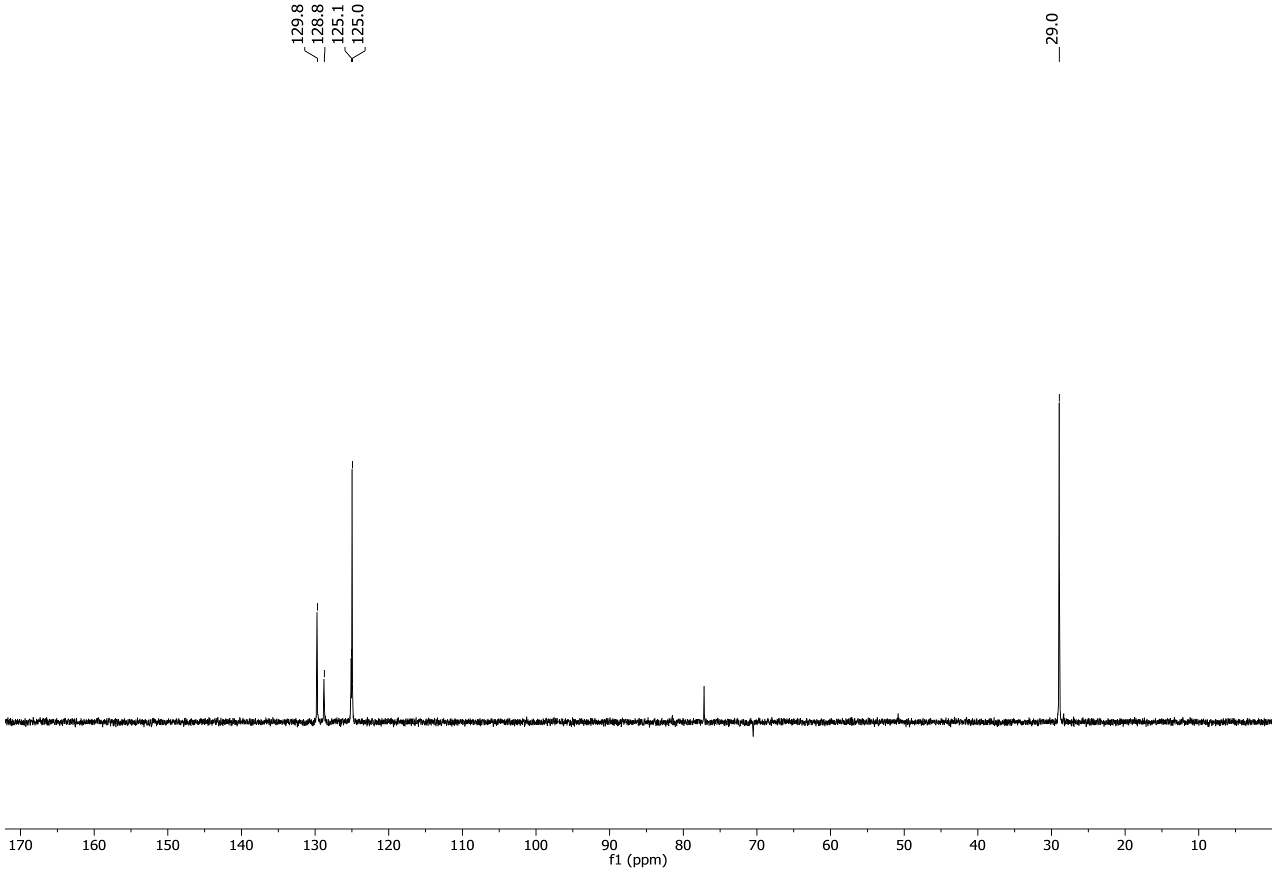
S1 (^{1}H NMR, 400 MHz, CDCl$_3$, 298 K)



S1 (^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



S1 (DEPT ^{13}C NMR, 100 MHz, CDCl$_3$, 298 K)



14. References

[1] Harris, R. K. *Nuclear Magnetic Resonance Spectroscopy*; Longman Scientific & Technical: Essex, U.K., **1986**.

[2] O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard, H. Puschmann, *J. Appl. Cryst.* **2009**, *42*, 339-341.

[3] A. Altomare, G. Cascarano, C. Giacovazzo, A. Guagliardi, *J. Appl. Crystallogr.***1993**, *26*, 343.

[4] a) G. M. Sheldrick, F2 SHELXL-2014/7: Program for the Solution of Crystal Structures; University of Göttingen: Göttingen, Germany, **2014**. b) G. M. Sheldrick, Crystal structure refinement with SHELXL, *Acta Cryst.* **2015**, C71, 3-8.