Supporting Information

Second Generation Specific-Enzyme-Activated Rotaxane Propeptides

Antony Fernandes,[a,b] Aurélien Viterisi,[a] Vincent Aucagne,*[c] David A. Leigh*[a] and Sébastien Papot*[b]

[a] School of Chemistry, University of Edinburgh, The King's Buildings, West Mains Road, Edinburgh, EH9 3JJ. Fax: (+44) 131-650-6453, http://www.catenane.net ; E-mail: david.leigh@ed.ac.uk

[b] Université de Poitiers, UMR 6514 Laboratoire de Synthèse et Réactivité des Substances Naturelles, 40 Avenue du Recteur Pineau, 86022 Poitiers (France) ; E-mail: sebastien.papot@univ-poitiers.fr

[c] Centre de Biophysique Moléculaire, UPR 4301 CNRS, Rue Charles Sadron, 45071 Orléans, Cedex 2 (France) ; E-mail: aucagne@cnrs-orleans.fr

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1. General Experimental Section

Unless otherwise stated, all reactions were run under an atmosphere of N_2. Prior to use, isophthaloyl dichloride was purified by recrystallization from hexane; *p*-xylylenediamine was purified by distillation under reduced pressure. Dry acetonitrile, chloroform, dichloromethane, *N*,*N*-dimethylformamide, methanol, tetrahydrofuran and toluene were obtained by passing these solvents through activated alumina columns on a PureSolv^{TM} solvent purification system (Innovative Technologies, Inc., MA). Unless otherwise stated, all other reagents were purchased from commercial sources and used without further purification. Flash column chromatography was carried out using Kiesegel C60 (Fisher Scientific) as the stationary phase. Analytical TLC was performed on aluminium-backed sheets pre-coated with silica 60 F254 adsorbent (0.25 mm thick, Merck, Germany) and visualized under UV light. Preparative TLC was carried out using precoated silica gel plates (2000 μm thick, Silica gel GF, Uniplate, Germany). Size exclusion chromatography was performed using Toyopearl HW-405 (Tosoh, Japan) with methanol/chloroform in a 1:1 v/v ratio as the eluent. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV 400 instrument. Chemical shifts (δ) are reported in parts per million from low to high field and referenced to residual solvent. Coupling constants (*J*) are reported in hertz (Hz). Standard abbreviations indicating multiplicity are used as follows: b = broad, s = singulet, d = doublet, m = multiplet, q = quartet, quint. = quintet, t = triplet. All melting points were determined using a Sanyo Gallenkamp apparatus and are uncorrected. Analytical RP-HPLC was carried out on a Gilson instrument composed of 306 pumps, 811C dynamic mixer (100 μL), 806 manometric module and an Applied Biosciences 759A UV detector with a Phenomenex C18 (2) Luna column (2 x 250 mm, 5 μm, 100 Å). Chromatograms were recorded at 220 nm unless stated otherwise. H_2O and CH_3CN were used as mobile phase, buffered with TFA (0.1% v/v, pH ≈ 2). Preparative RP-HPLC was carried out on a Gilson instrument composed of 306 pumps, 811C dynamic mixer (1.5 mL), 806 manometric module and a 118 UV detector with a Spherisorb ODS2 column (21.2 x 250 mm, 5 μm, 100 Å) , using H_2O and CH_3CN as mobile phase, buffered with HCOOH (6.6 mmol/L, pH ≈ 3). LCMS was carried out on a Finnigan Mat system composed of an LCQ mass spectrometer, P4000 pumps, and a UV2000 UV detector with a Phenomenex C18 (2) Luna column (2 x 250 mm, 5 μm, 100 Å). Chromatograms were recorded at 220 nm unless stated

otherwise. Low-resolution ESI spectra were recorded either on a Micromass ZMD or on a Finnigan Mat LCQ spectrometer. MALDI and high resolution ESI spectrometry were carried out by the EPSRC national mass spectrometry service centre (Swansea, UK). FAB mass spectrometry was carried out by the mass spectrometry service at the University of Edinburgh. Aldehyde **11**[1] and alkyne **17**[2] and were synthesized according to literature procedures.

Enzymatic Hydrolysis

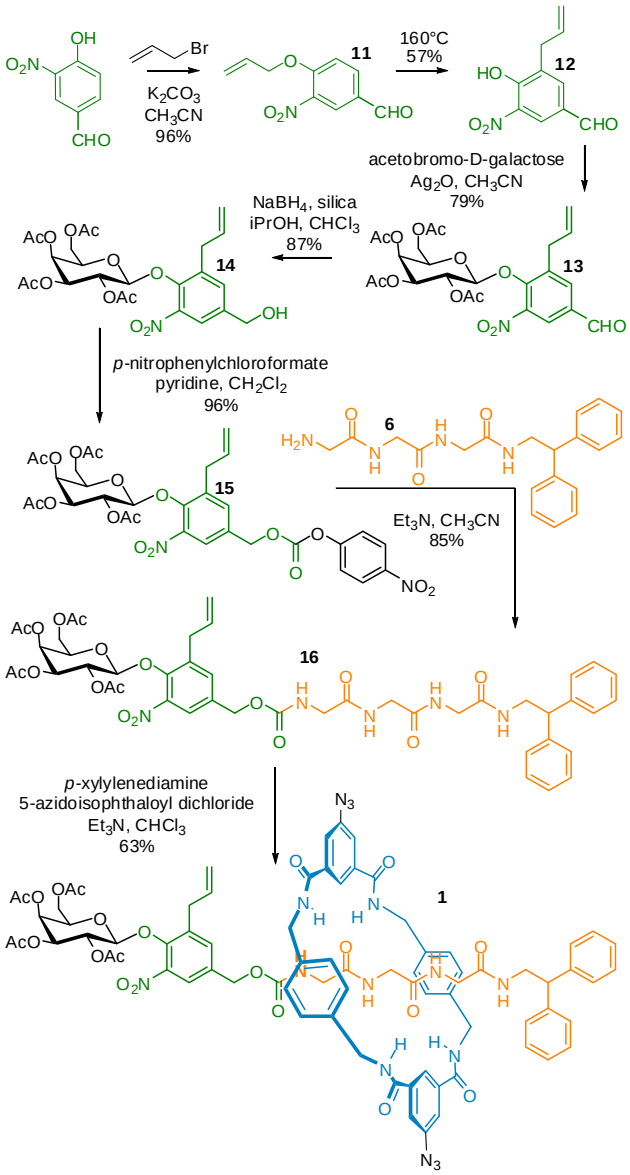
Enzymatic hydrolysis was carried out with commercial β-galactosidase from *Escherichia coli* E.C. 3.2.1.23 (1000 units/mg protein (biuret), aqueous glycerol suspension (1:1), 10 mM Tris buffer salts and 10 mM magnesium chloride, pH 7.3). Prodrugs were incubated with the enzyme in phosphate buffer (0.02 M, pH 7.0) at 37°C. Aliquots (20 μL) were periodically withdrawn from the medium and diluted into a solution of TFA (0.1%) in H_2O (40 μL). Rate of hydrolysis was monitored by analytical HPLC and LCMS.

Solubility of Propeptides

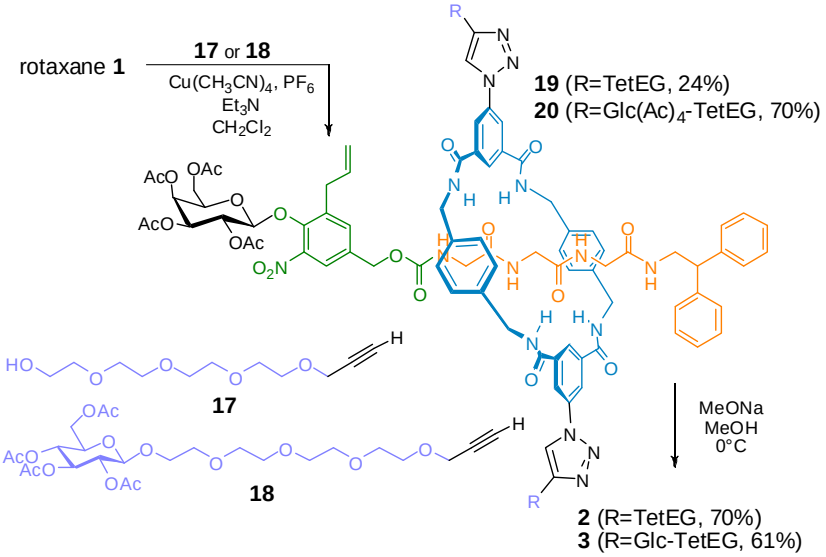
A known quantity of each compound was dissolved in a known volume of DMSO/X/H_2O (X= MeOH, EtOH or CH_3CN depending on the solubility of each compound) and the solution was analyzed by HPLC. The absolute area of the peak of interest was then compared with the area of the peak produced by a saturated solution of the same compound in pure water at 20°C. Care was taken to record the chromatograms in the linearity regime of the UV detector (Manufacturer specifications: linearity: 1% when value is within sensitivity range of 0.001 to 2.0 A.U.) and final solubility are given with a maximum error of 10%.

2. Synthetic Routes

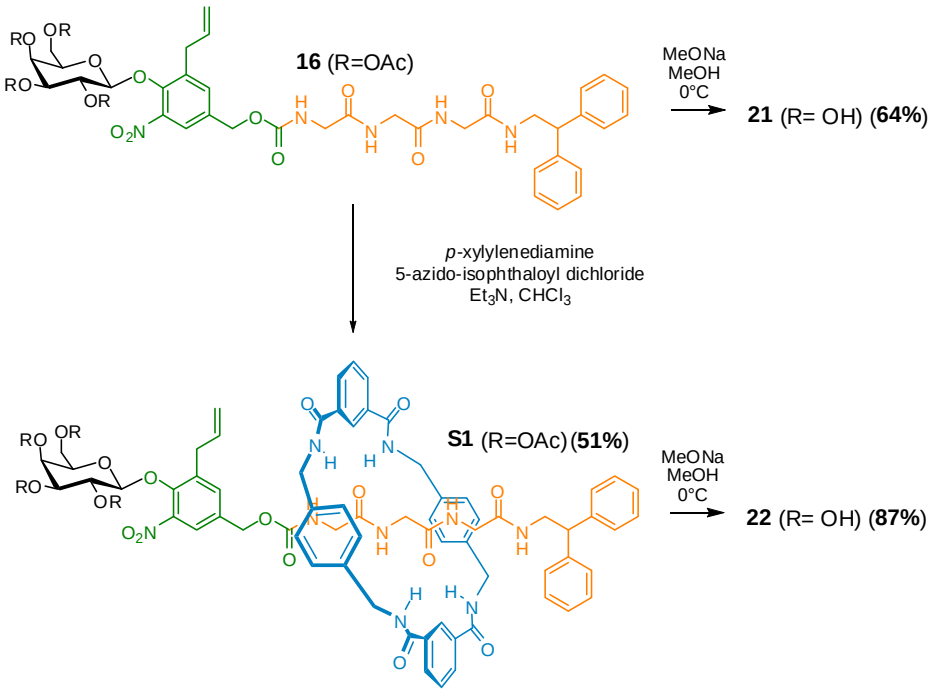
Scheme S1 - *Synthesis of bis-Azido Rotaxane* ***1***



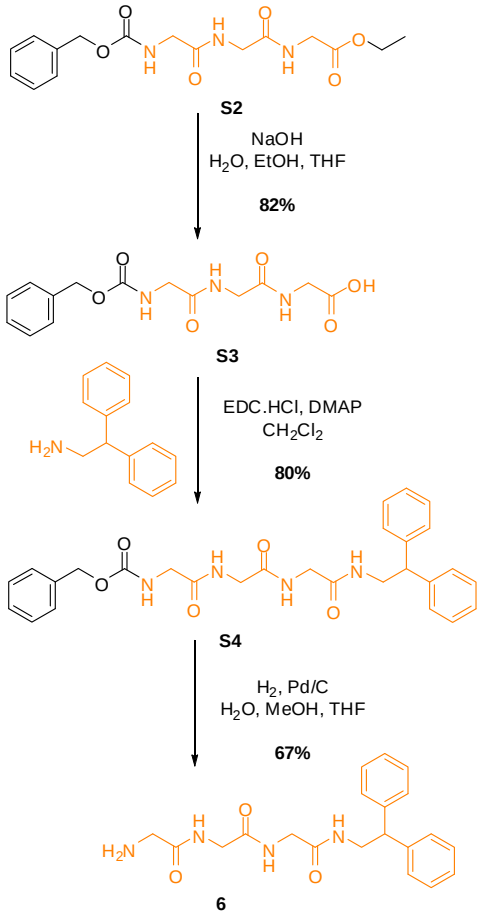
Scheme S2 - *Synthesis of Functionalized Rotaxane Propeptides* ***2*** *and* ***3***



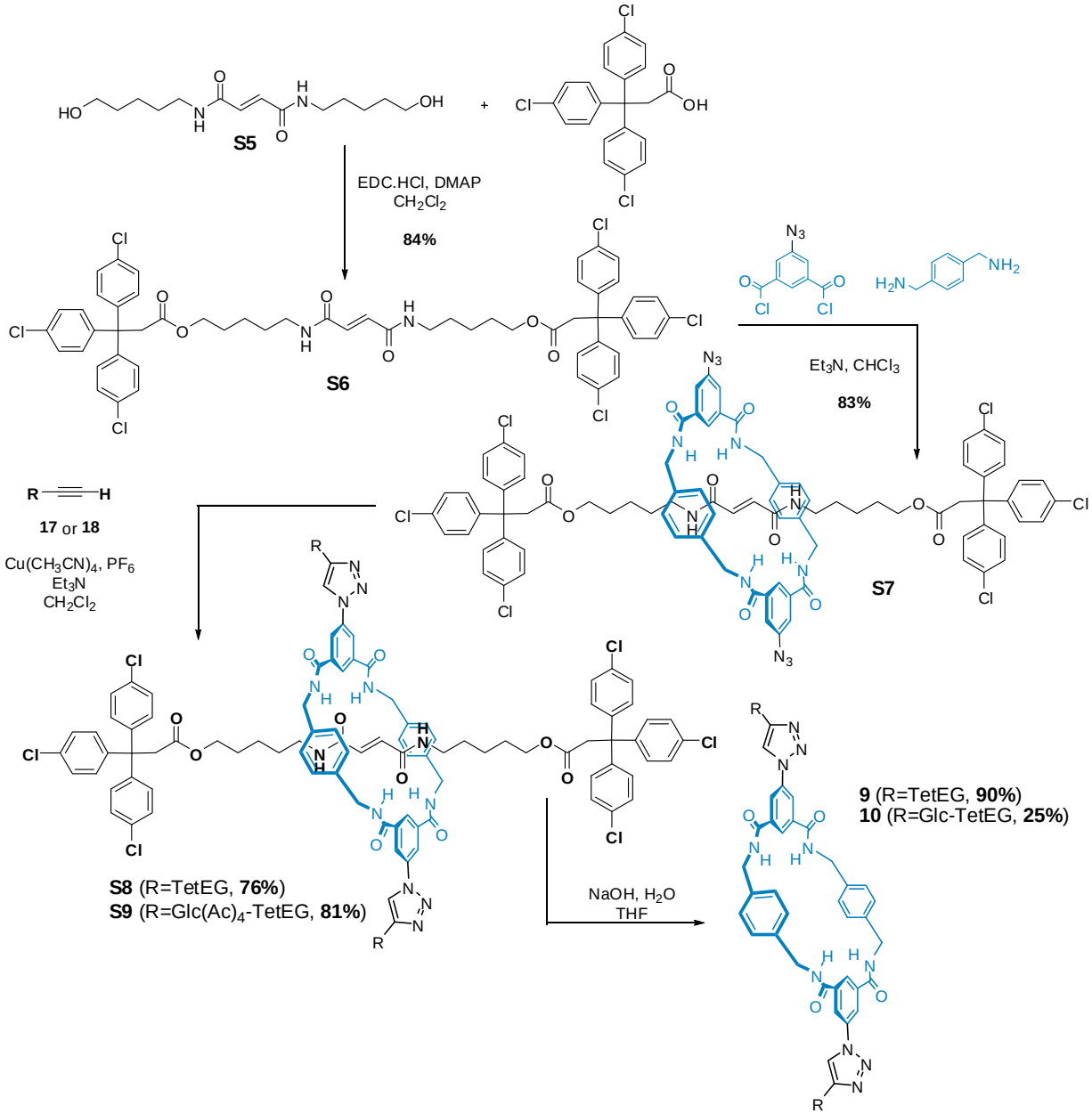
Scheme S3 - *Synthesis of Propeptide* ***21*** *and Unfunctionalised Rotaxane* ***22***



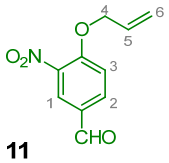
Scheme S4 *- Synthesis of Peptide* ***6***



Scheme S5 *- Synthesis of Macrocycles* ***9*** *and* ***10***



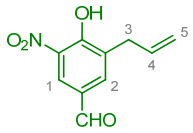
3. Experimental Procedures



11

CAS Registry Number: 125872-98-2

To a solution of 4-hydroxy-3-nitrobenzaldehyde (5.00 g, 29.94 mmol) in CH_3CN (200 mL) were added allyl bromide (10.40 mL, 4.0 equiv.) and K_2CO_3 (12.40 g, 3.0 equiv.). After being stirred at 60°C for 72 hours, the mixture was diluted with EtOAc and washed with water and brine. The combined organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by column chromatography over silica gel (petroleum ether/ethyl acetate: 7/3 then 6/4) afforded **11** (5.70 g, 27.54 mmol, 92%) as a pale yellow powder. The compound showed identical spectroscopic data to that reported in the literature.[2] ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.91 (s, 1H, C*H*O), 8.32 (d, 1H, J = 2.0 Hz, H_1), 8.04 (dd, 1H, J = 8.8 Hz, J = 2.0 Hz, H_2), 7.21 (d, 1H, J = 8.8 Hz, H_3), 6.07 to 5.98 (m, 1H, H_5), 5.50 (dd, 1H, J = 17.6 Hz, J = 1.2 Hz, H_6), 5.37 (dd, 1H, J = 7.8 Hz, J = 1.2 Hz, H_6), 4.78 (dt, 2H, J = 4.8 Hz, J = 1.6 Hz, H_4).

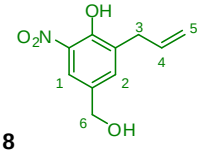


12

CAS Registry Number: 914769-85-0

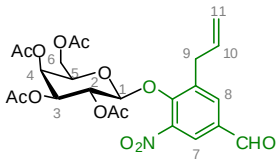
3-Nitro-4-(2-propenyloxy)benzaldehyde **11** (5.60 g, 27.05 mmol) was added in a 10 mL round-bottom flask equipped with a condenser and then heated at 160-165°C over a period of 17 hours. After allowing the dark brown mixture to reach room temperature, it was poured into 1M HCl and extracted with dichloromethane. The combined organic layers were dried with $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography over silica gel (petroleum ether/ethyl acetate: 8/2 to 6/4) afforded **12** (3.20 g, 15.46 mmol, 57%) as a yellow powder and the remaining starting material **11**. The latter was put in a second run in the same conditions described before to afford 680 mg of **12** (3.29 mmol, 12%) resulting in a total yield of 69%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.41 (bs, 1H, O*H*), 9.91 (s, 1H,

C*H*O), 8.51 (d, 1H, J = 2.0 Hz, H_1), 8.01 (d, 1H, J = 2.0 Hz, H_2), 6.03 to 5.93 (m, 1H, H_4), 5.20 to 5.18 (m, 1H, H_5), 5.16 (dq, 1H, J = 11.2 Hz, J = 1.6 Hz, H_5), 3.53 (d, 2H, J = 6.8 Hz, H_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 189.0, 157.7, 135.8, 134.1, 133.5, 133.3, 128.4, 126.9, 118.0, 33.7; LRFAB-MS (3-NOBA matrix): *m/z* 208 $[M+H]^+$; m.p. 82-84°C.



8

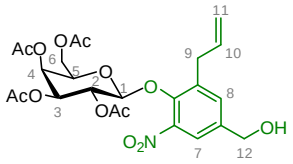
To a solution of **12** (100 mg, 0.48 mmol) in $CHCl_3$/*i*PrOH (2.40 mL/1.60 mL) was added silica (90 mg). The mixture was cooled in an ice-water bath and $NaBH_4$ (320 mg, 12.0 equiv.) was added portion-wise. After 1 hour at 0°C, the solution was allowed to reach room temperature and filtered on Celite®, the pad being washed with CH_2Cl_2. Evaporation and purification by column chromatography over silica gel (petroleum ether/ethyl acetate: 9/1 to 8/2) afforded **8** as a yellow solid (40 mg, 0.19 mmol, 40%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.92 (bs, 1H, O*H*), 7.98 (d, 1H, J = 2.0 Hz, H_1), 7.48 (d, 1H, J = 2.0 Hz, H_2), 6.03 to 5.93 (m, 1H, H_4), 5.15 (t, 1H, J = 1.2 Hz, H_5), 5.12 (dq, 1H, J = 6.4 Hz, J = 1.2 Hz, H_5), 4.65 (s, 2H, H_6), 3.48 (d, 2H, J = 6.4 Hz, H_3), 1.91 (bs, 1H, CH_2-O*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.8, 136.4, 135.1, 133.3, 132.5, 131.9, 121.2, 117.2, 64.0, 33.9; LRESI-MS (negative): *m/z* 208 $[M-H]^-$; m.p. 40-42°C.



13

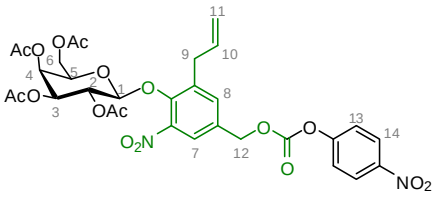
To a solution of **12** (570 mg, 2.75 mmol, 1.1 equiv.) in CH_3CN (30 mL) cooled in an ice-water bath was added α-D-galactopyranosyl bromide-2,3,4,6-tetraacetate (1.03 g, 2.51 mmol) and Ag_2O (872 mg, 1.5 equiv.). After overnight stirring at room temperature, the solution was filtered through silica, eluted with ethyl acetate and concentrated under reduced pressure. Purification by column chromatography over silica gel (petroleum ether/ethyl acetate: 85/15 then 6/4) afforded **13** as a pale yellow powder (1.16 g, 2.16 mmol, 79%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.97 (s, 1H, C*H*O),

8.11 (d, 1H, J = 2.0 Hz, H_7), 7.96 (d, 1H, J = 2.0 Hz, H_8), 5.96 to 5.86 (m, 1H, H_{10}), 5.48 (dd, 1H, J = 10.4 Hz, J = 8.0 Hz, H_2), 5.37 (d, 1H, J = 2.8 Hz, H_4), 5.22 to 5.14 (m, 2H, H_{11}), 5.05 (dd, 1H, J = 10.4 Hz, J = 2.8, H_3), 4.95 (d, 1H, J = 8.0 Hz, H_1), 4.02 (d, 2H, J = 6.8 Hz, H_6), 3.81 (dt, 1H, J = 6.8 Hz, J = 0.8 Hz, H_5), 3.58 (dd, 2H, J = 6.4 Hz, J = 1.2 Hz, H_9), 2.18 (s, 3H, *H*-Ac), 2.16 (s, 3H, *H*-Ac), 1.99 (s, 3H, *H*-Ac), 1.96 (s, 3H, *H*-Ac); ^{13}C NMR (100 MHz, $CDCl_3$): δ 189.1, 170.3, 170.2, 170.1, 169.6, 149.6, 145.5, 139.2, 134.7, 134.6, 133.1, 124.1, 118.3, 102.3, 71.5, 70.6, 68.7, 66.6, 60.8, 34.1, 20.9, 20.8, 20.7, 20.6; LRFAB-MS (3-NOBA matrix): *m/z* 538 $[M+H]^+$, 560 $[M+Na]^+$; HRFAB-MS (3-NOBA matrix): *m/z* 538.1552 (calcd. for $C_{24}H_{28}NO_{13}$ 538.1561 $[M+H]^+$); m.p. 78-80°C.



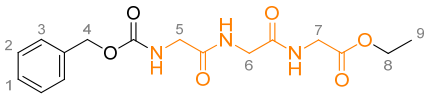
14

To a solution of **13** (700 mg, 1.30 mmol) in $CHCl_3$/*i*PrOH (20 mL/14 mL) was added silica (1.0 g). The mixture was cooled in an ice-water bath and $NaBH_4$ (594 mg, 12.0 equiv.) was added portion-wise. After 2 hours at 0°C, the solution was allowed to reach room temperature and filtered on Celite®, the pad being washed with CH_2Cl_2. Evaporation and purification by column chromatography over silica gel (petroleum ether/ethyl acetate: 6/4 to 3/7) afforded **14** as a pale yellow powder (608 mg, 1.13 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (d, 1H, J = 2.0 Hz, H_9), 7.40 (d, 1H, J = 1.6 Hz, H_{11}), 5.93 to 5.83 (m, 1H, H_{14}), 5.45 (dd, 1H, J = 10.4 Hz, J = 8.0 Hz, H_2), 5.35 (d, 1H, J = 2.8 Hz, H_4), 5.14 (bs, 1H, H_{15}), 5.11 (dd, 1H, J = 7.2 Hz, J = 1.2 Hz, H_{15}), 5.02 (dd, 1H, J = 10.4 Hz, J = 3.6 Hz, H_3), 4.82 (d, 1H, J = 8.0 Hz, H_1), 4.69 (bs, 2H, H_{16}), 4.03 (dd, 2H, J = 6.8 Hz, J = 3.6 Hz, H_6), 3.77 (t, 1H, J = 6.4 Hz, H_5), 3.50 (t, 2H, J = 4.8 Hz, H_{13}), 2.37 (bs, 1H, CH_2-O*H*), 2.18 (s, 3H, *H*-Ac), 2.15 (s, 3H, *H*-Ac), 1.98 (s, 3H, *H*-Ac), 1.96 (s, 3H, *H*-Ac); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.4, 170.2, 169.7, 145.3, 144.4, 139.0, 137.5, 135.6, 132.2, 121.0, 117.4, 102.7, 71.2, 70.7, 68.7, 66.7, 63.5, 60.8, 34.1, 21.1, 20.9, 20.7, 20.6 LRFAB-MS (3-NOBA matrix): *m/z* 538 $[M+H]^+$, 562 $[M+Na]^+$; HRFAB-MS (3-NOBA matrix): *m/z* 562.1528 (calcd. for $C_{24}H_{29}NO_{13}Na$ 562.1537 $[M+Na]^+$); m.p. 110-112°C.



15

To a solution of **14** (433 mg, 0.80 mmol) in CH_2Cl_2 (15 mL) was added *para*-nitrophenyl chloroformate (356 mg, 2.2 equiv.) and pyridine (160 µL, 2.5 equiv.). After one day at room temperature, the solution was diluted with water and extracted with CH_2Cl_2. The combined organic layers were washed with water, dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography over silica gel (petroleum ether/ethyl acetate: 6/4) afforded **15** (540 mg, 0.77 mmol, 96%) as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.29 (d, 2H, J = 9.2 Hz, H_{14}), 7.71 (d, 1H, J = 2.0 Hz, H_7), 7.50 (d, 1H, J = 2.0 Hz, H_8), 7.39 (d, 2H, J = 9.2 Hz, H_{13}), 5.96 to 5.85 (m, 1H, H_{10}), 5.48 (dd, 1H, J = 10.8 Hz, J = 8.0 Hz, H_2), 5.38 (d, 1H, J = 2.4 Hz, H_4), 5.27 (bs, 2H, H_{12}), 5.20 to 5.14 (m, 2H, H_{11}), 5.05 (dd, 1H, J = 10.8 Hz, J = 3.6 Hz, H_3), 4.88 (d, 1H, J = 8.0 Hz, H_1), 4.06 (t, 2H, J = 6.8 Hz, H_6), 3.80 (t, 1H, J = 7.6 Hz, H_5), 3.56 (t, 2H, J = 6.0 Hz, H_9), 2.20 (s, 3H, *H*-Ac), 2.17 (s, 3H, *H*-Ac), 2.01 (s, 3H, *H*-Ac), 1.97 (s, 3H, *H*-Ac); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 170.3, 170.2, 169.7, 155.4, 152.4, 145.7, 145.4, 138.5, 135.3, 134.1, 132.1, 125.5, 122.9, 121.8, 118.0, 102.7, 71.3, 70.7, 69.1, 68.7, 66.7, 60.8, 34.1, 20.9, 20.8, 20.7, 20.6; LRFAB-MS (3-NOBA matrix): *m/z* 705 $[M+H]^+$, 727 $[M+Na]^+$; m.p. 66-68°C.

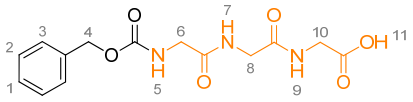


S2

CAS Registry Number: 2503-35-7

To a solution of Cbz-glycine (3.62 g, 17.3 mmol), EDCI.HCl (3.31 g, 1.0 equiv.) and DMAP (2.11 g, 1.0 equiv.) in CH_2Cl_2 (180 mL) at room temperature, was added glycylglycine ethylester hydrochloride (3.74 g, 1.1 equiv.) and the reaction mixture was stirred overnight. An aqueous solution of HCl (1 M) (100 mL) was added, the layers were separated and the aqueous layer was extracted with $CHCl_3$/iPrOH (3:1) (3×50 mL). The combined organic fractions were dried ($MgSO_4$) and concentrated under reduced pressure. To the crude solid was added Et_2O (50 mL). The resulting white suspension was sonicated for 5 minutes and filtered by gravity to give **S2** as a

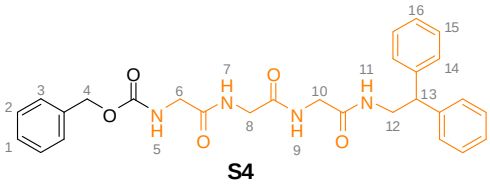
colourless solid (5.42 g, 15.4 mmol, 90%). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ (1:1)): δ 7.38-7.20 (m, 5H, H_{1+2+3}), 5.06 (s, 2H, H_4), 4.15 (q, 2H, J = 7.1 Hz, H_8), 3.99-3.74 (m, 6H, H_{5+6+7}), 3.30 (t, 3H, J = 7.1 Hz, H_8); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$ (3:1)): δ 170.8, 170.3, 170.2, 136.2, 128.6, 128.3, 128.1, 107.0, 67.3, 61.7, 44.3, 42.4, 41.1, 14.0; LRESI-MS: *m/z* 374 $[M+Na]^+$; HRESI-MS: *m/z* 374.1321 $[M+Na]^+$ (calcd. for $C_{16}H_{21}O_6N_3Na_1$ 374.1323 $[M+Na]^+$); m.p. 133-135°C.



S3

CAS Registry Number: 2566-20-3

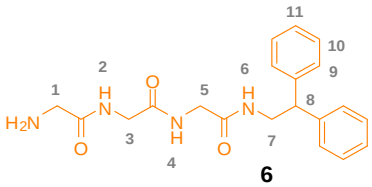
To a solution of **S2** (2.24 g, 6.37 mmol) in a 2:3:3 mixture H_2O/EtOH/THF (320 mL) at 0ºC was added crushed NaOH (395 mg, 1.5 equiv.) in one portion and the reaction mixture was stirred at that temperature for 20 min. An aqueous solution (1 M) of HCl (40 mL) was added and solvents were evaporated under reduced pressure. To the resulting solid was added H_2O (30 mL), the suspension was sonicated for 5 minutes, filtered under suction and washed with H_2O (50 mL). The solid was re-suspended in a 1:6 mixture of MeOH/Et_2O (50 mL), sonicated for 5 minutes, filtered by gravity and washed with Et_2O (20 mL) to afford **S3** as a colourless solid (1.70 g, 5.25 mmol, 82%). ^{1}H NMR (400 MHz, DMSO d_6): δ 12.58 (s, 1H, H_{11}), 8.16 (dt, J = 5.6 Hz and J = 5.6 Hz, 2H, H_{5+4}), 7.49 (t, J = 6.0 Hz, 1H, H_9), 7.14-7.25 (m, 5H, H_{1+2+3}), 5.03 (s, 2H, H_4), 3.75 (dd, J = 5.5 Hz and J = 5.5 Hz, 4H, H_{6+8}), 3.67 (d, J = 6.0 Hz, 2H, H_{10}); ^{13}C NMR (100 MHz, DMSO d_6): δ 171.0, 169.3, 169.0, 156.4, 136.9, 128.2, 127.7, 127.6, 65.4, 43.4, 41.6, 40.4; LRESI-MS: *m/z* 346 $[M+Na]^+$; HRESI-MS: *m/z* 324.1196 $[M+H]^+$ (calcd. for $C_{14}H_{18}O_6N_3$ 324.1190 $[M+H]^+$); m.p. 182-184°C.



S4

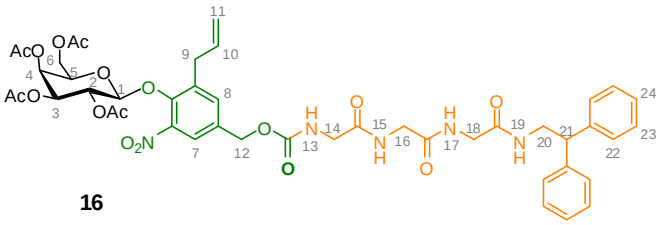
To a solution of **S3** (1.52 g, 4.7 mmol), EDCI.HCl (991 mg, 1.1 equiv.) and DMAP (631 mg, 1.1 equiv.) in CH_2Cl_2 (100 mL) at room temperature, was added 2,2-diphenylethanamine (878 mg, 4.4 mmol, 0.95 equiv.) and the reaction mixture was stirred overnight. An aqueous solution (1 M) of HCl (50 mL) was added, the layers

were separated and the aqueous layer was extracted with CH_2Cl_2 (3×50 mL). The combined organic fractions were washed with H_2O (40 mL), dried ($MgSO_4$) and concentrated under reduced pressure to afford **S4** as a colourless solid (1.87 g, 3.72 mmol, 80%). ^{1}H NMR (400 MHz, DMSO d_6): δ 8.13, 8.05, 7.49 (3t, J = 5.6 Hz, J = 5.8 Hz and J = 6.0 Hz, 3H, H_{5+7+9}), 7.86 (t, J = 5.5 Hz, 1H, H_{11}), 7.38-7.09 (m, 15H, $H_{1+2+3+14+15+16}$), 5.03 (s, 2H, H_4), 4.18 (t, J = 7.7 Hz, 1H, H_{13}), 3.75-3.67 (m, 4H, H_{6+11}), 3.66, 3.58 (2d, J = 6.1 Hz and J = 5.7 Hz, 4H, H_{8+10}); ^{13}C NMR (100 MHz, DMSO d_6): δ 169.4, 168.9, 168.6, 156.4, 142.7, 136.9, 128.3, 128.2, 127.8, 127.7, 127.6, 126.3, 65.4, 49.9, 43.4, 43.0, 41.9, 41.7; LRESI-MS: *m/z* 525 $[M+Na]^+$; HRESI-MS: *m/z* 503.2286 $[M+H]^+$ (calcd. for $C_{28}H_{30}O_5N_4$ 503.2289 $[M+H]^+$); m.p. 210-212°C.



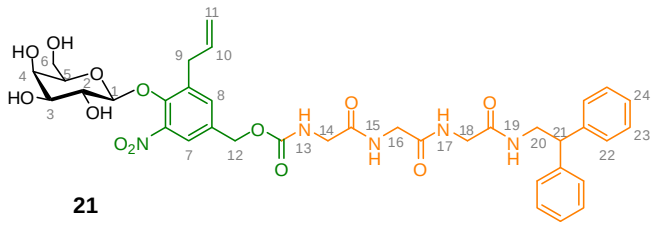
6

A solution of **S4** (2.65 g, 5.27 mmol) in a 3:4:13 mixture H_2O/MeOH/THF (500 mL) was heated up to 50°C until it had fully dissolved (5 min). The solution was then allowed to cool to room temperature and palladium (10%) on carbon (561 mg) was added in one portion. The reaction vessel was purged with H_2 by means of three vacuum/H_2 cycles and the mixture stirred at room temperature for 15 hours. The solid was filtered off with Celite®, MeOH and THF were evaporated under reduced pressure and the remaining aqueous layer was extracted with $CHCl_3$/iPrOH (3/1) (3×50 mL). The combined organic fractions were dried ($MgSO_4$) and concentrated under reduced pressure. The resulting yellow oil was re-dissolved in CH_2Cl_2 (10 mL) and Et_2O (50 mL) was added. The resulting precipitate was sonicated for 5 minutes and filtered by gravity to afford **6** as a colourless solid (1.30 g, 3.52 mmol, 67%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07-7.81 (m, 1H, H_6), 7.42-7.25, 6.75-6.58 (2m, 2H, H_{2+4}), 7.25-6.95 (m, 10H, $H_{9+10+11}$), 4.10 (t, 1H, J = 7.8 Hz, H_8), 3.75-3.67 (m, 4H, H_{6+11}), 3.75, 3.64, 3.28 (3s, 4H, H_{3+5+7}); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.7 (×2), 169.0, 141.7, 128.6, 128.0, 126.7, 50.2, 43.8 (×2), 42.8, 42.6 LRESI-MS: *m/z* 391 $[M+Na]^+$; HRESI-MS: *m/z* 369.1924 (calcd. for $C_{20}H_{25}O_3N_4$ 369.1921 $[M+H]^+$); m.p. 50-52°C.



16

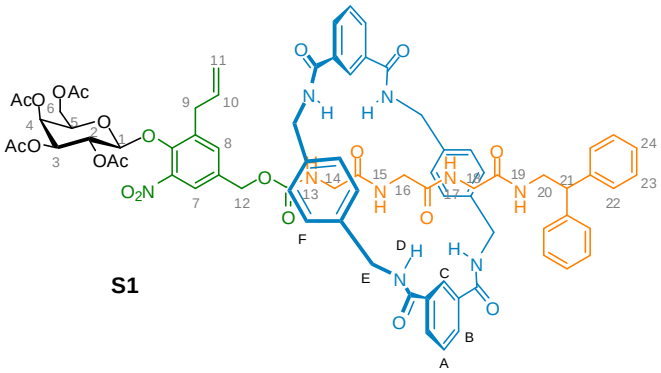
To a solution of **15** (529 mg, 0.75 mmol) in CH_3CN (35 mL) was added the peptide **6** (332 mg, 1.2 equiv.) and Et_3N (260 µL, 2.5 equiv.). Stirring was continued at room temperature during one day and the solution was diluted with water and extracted with ethyl acetate. The combined extracts were washed with water, dried over $MgSO_4$ and concentrated. Purification by column chromatography over silica gel (petroleum ether/ethyl acetate: 4/6 to 2/8 then CH_2Cl_2/MeOH: 9/1) yielded **16** as a pale brown powder (599 mg, 0.64 mmol, 85%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57 (d, 1H, J = 2.0 Hz, H_7), 7.39 (m, 2H, NH_{15+17}), 7.37 (d, 1H, J = 2.0 Hz, H_8), 7.27 to 7.16 (m, 10H, $H_{22+23+24}$), 6.65 (t, 1H, J = 5.2 Hz, NH_{19}), 6.08 (t, 1H, J = 4.8 Hz, NH_{13}), 5.93 to 5.82 (m, 1H, H_{10}), 5.46 (dd, 1H, J = 10.4 Hz, J = 8.0 Hz, H_2), 5.37 (d, 1H, J = 2.8 Hz, H_4), 5.15 (d, 1H, J = 1.2 Hz, H_{11}), 5.12 (dd, 1H, J = 9.2 Hz, J = 1.2 Hz, H_{11}), 5.06 (dd, 1H, J = 10.4 Hz, J = 2.8 Hz, H_3), 5.00 (s, 2H, H_{12}), 4.85 (d, 1H, J = 8.0 Hz, H_1), 4.17 (t, 1H, J = 8.0 Hz, H_{21}), 4.03 (d, 2H, J = 6.4 Hz, H_6), 3.91 to 3.76 (m, 9H, $H_{5+14+16+18+20}$), 3.50 (m, 2H, H_9), 2.19 (s, 3H, *H*-Ac), 2.15 (s, 3H, *H*-Ac), 2.00 (s, 3H, *H*-Ac), 1.95 (s, 3H, *H*-Ac); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.4, 170.2, 169.8, 169.7, 169.3, 168.9, 156.4, 145.4, 145.0, 141.9, 137.9, 135.4, 134.4, 133.5, 128.8, 128.1, 126.9, 122.2, 117.7, 102.7, 71.1, 70.7, 68.8, 66.7, 65.3, 60.7, 50.5, 44.4, 44.0, 43.1, 42.9, 34.0, 29.8, 20.9, 20.8, 20.7, 20.6; LRESI-MS: *m/z* 957 $[M+Na]^+$; LRFAB-MS (3-NOBA matrix): *m/z* 935 $[M+H]^+$; HRFAB-MS (3-NOBA matrix): *m/z* 934.3384 (calcd. for $C_{45}H_{52}N_5O_{17}$ 934.3359 $[M+H]^+$); m.p. 126-128°C.



21

To a solution of **16** (100 mg, 0.11 mmol) in MeOH (10 mL) cooled in an ice-water bath was added dropwise a 0°C solution of MeONa (58 mg, 10.0 equiv.) in MeOH (5 mL). Stirring was continued for one hour at 0°C and the solution was neutralised with

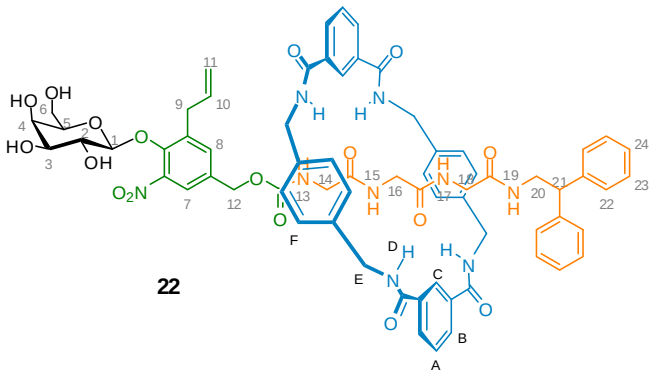
Amberlite IRC-50 and filtered. MeOH was then evaporated and the resulting mixture was purified by column chromatography over silica gel (CH_2Cl_2/MeOH: 5 to 20% MeOH) to afford **21** (57 mg, 0.07 mmol, 64%) as a pale brown powder. ^{1}H NMR (400 MHz, CD_3OD): δ 7.57 (d, 1H, J = 2.0 Hz, H_7), 7.45 (d, 1H, J = 2.0 Hz, H_8), 7.24 to 7.19 (m, 8H, H_{22+23}), 7.14 to 7.10 (m, 2H, H_{24}), 5.99 to 5.89 (m, 1H, H_{10}), 5.11 to 5.04 (m, 4H, H_{11+12}), 4.47 (d, 1H, J = 8.0 Hz, H_1), 4.24 (t, 1H, J = 8.0 Hz, H_{21}), 3.80 to 3.73 (m, 8H, $H_{14+16+18+20}$), 3.70 (m, 2H, H_{4+5}), 3.66 to 3.54 (m, 5H, H_{2+6+9}), 3.44 (dd, 1H, J = 10.0 Hz, J = 3.6 Hz, H_3); ^{13}C NMR (100 MHz, CD_3OD): δ 173.1, 172.1, 171.5, 159.0, 147.9, 147.0, 143.8, 138.5, 137.5, 135.5, 134.7, 129.6, 129.2, 127.7, 122.8, 117.4, 107.1, 77.1, 74.7, 72.5, 69.7, 66.5, 61.6, 51.6, 49.9, 45.1, 43.8, 43.3, 34.5; LRES$^+$-MS: *m/z* 766 $[M+H]^+$, 788-789-790 $[M+Na]^+$; LRFAB-MS (3-NOBA matrix): *m/z* 766 $[M+H]^+$; HRFAB-MS (3-NOBA matrix): *m/z* 766.2928 (calcd. for $C_{37}H_{44}N_5O_{13}$ 766.2936 $[M+H]^+$); m.p. 145-147°C.



S1

Thread **16** (200 mg, 0.21 mmol) and Et_3N (1.0 mL, 35.0 equiv.) were dissolved in anhydrous chloroform (40 mL) and stirred vigorously whilst solutions of *p*-xylylene diamine (466 mg, 16.0 equiv.) in anhydrous chloroform (40 mL) and isophthaloyl dichloride (696 mg, 16.0 equiv.) in anhydrous chloroform (40 mL) were simultaneously added over a period of 3 hours using motor-driven syringe pumps. After overnight stirring, 1 mL of MeOH was added and the resulting suspension filtered through Celite®. The pad was washed with $CHCl_3$/MeOH 2% (3 x 100 mL) and the combined filtrates were concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using CH_2Cl_2/MeOH (1 to 15% MeOH) as eluent to give a mixture of thread **16** and rotaxane **S1**. This mixture was resolved by running a size exclusion chromatography using $CHCl_3$/MeOH (50/50) as eluent to give rotaxane **S1** as a pale brown powder (161 mg, 0.11 mmol, 51%).^{1}H

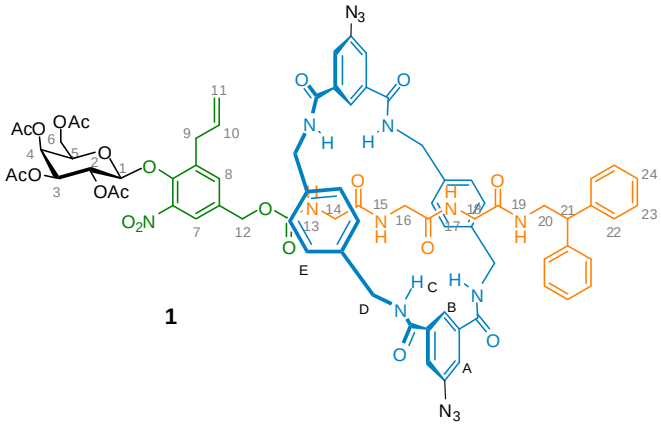
NMR (400 MHz, $CDCl_3$): δ 8.19 (s, 2H, H_C), 8.00 (d, 4H, J = 7.6 Hz, H_B), 7.81 (bs, 4H, H_D), 7.47 (t, 2H, J = 7.6 Hz, H_A), 7.35 (s, 1H, H_7), 7.23 (s, 1H, H_8), 7.21 to 7.09 (m, 18H, $H_{22+23+24+F}$), 6.98 (bs, 3H, N$H_{15+17+19}$), 5.89 to 5.79 (m, 1H, H_{10}), 5.73 (bs, 1H, NH_{13}), 5.46 (t, 1H, J = 8.0 Hz, H_2), 5.37 (bs, 1H, H_4), 5.14 to 5.05 (m, 3H, H_{3+11}), 4.84 (d, 1H, J = 8.0 Hz, H_1), 4.74 (bs, 2H, H_{12}), 4.48 to 4.39 (m, 8H, H_E), 4.05 (t, 1H, J = 8.0 Hz, H_{21}), 4.02 (d, 2H, J = 6.0 Hz, H_6), 3.80 (t, 1H, J = 6.0 Hz, H_5), 3.70 (bs, 2H, H_{20}), 3.46 to 3.42 (m, 4H, H_{9+14}), 3.30 (bs, 2H, H_{18}), 2.93 (bs, 2H, H_{16}), 2.17 (s, 3H, *H*-Ac), 2.16 (s, 3H, *H*-Ac), 2.01 (s, 3H, *H*-Ac), 1.94 (s, 3H, *H*-Ac); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.3, 170.2, 170.1, 169.7, 169.2, 168.2, 167.2, 156.3, 145.3, 145.0, 141.7, 137.7, 137.4, 135.3, 134.3, 133.9, 133.7, 131.0, 129.1, 129.0, 128.8, 128.0, 127.0, 125.4, 122.2, 117.7, 102.6, 71.1, 70.7, 68.8, 66.7, 65.3, 60.7, 50.5, 44.5, 44.2, 42.7, 42.1, 33.9, 20.9, 20.8, 20.7, 20.6; LRFAB-MS (3-NOBA matrix): *m/z* 1467 $[M+H]^+$; HRFAB-MS (3-NOBA matrix): *m/z* 1466.5485 (calcd. for $C_{77}H_{80}N_9O_{21}$ 1466.5469 $[M+H]^+$); m.p. 160-162°C.



22

To a solution of **S1** (98 mg, 0.067 mmol) in MeOH (10 mL) cooled in an ice-water bath was added dropwise a 0°C solution of MeONa (36 mg, 10.0 equiv.) in MeOH (5 mL). Stirring was continued for one hour at 0°C and the solution was neutralised with Amberlite IRC-50 resin and filtered. MeOH was then evaporated and the resulting mixture was purified by column chromatography over silica gel (CH_2Cl_2/MeOH: 2 to 10% MeOH) to afford **22** (75 mg, 0.058 mmol, 87%) as a pale brown powder. ^{1}H NMR (400 MHz, CD_3OD): δ 8.34 (bs, 2H, H_C), 8.05 to 8.03 (m, 4H, H_B), 7.59 (t, 2H, J = 7.6 Hz, H_A), 7.23 to 7.01 (m, 18H, $H_{22+23+24+F}$), 5.97 to 5.87 (m, 1H, H_{10}), 5.09 (dd, 1H, J = 8.8 Hz, J = 1.6 Hz, H_{10}), 5.05 (bs, 1H, H_{11}), 4.57 (bs, 2H, H_{12}), 4.48 to 4.39 (m, 9H), 3.93 (t, 1H, J = 8.0 Hz, H_{21}), 3.82 (t, 1H, J = 3.2 Hz), 3.76 (dd, 1H, J = 9.6 Hz, J = 7.6 Hz), 3.65 (dd, 1H, J = 11.2 Hz, J = 6.8 Hz), 3.57 to 3.44 (m, 10H,

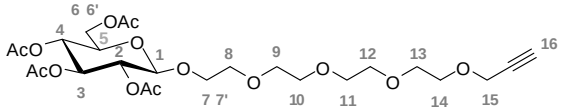
$H_{14+16+18+20}$); ^{13}C NMR (100 MHz, CD_3OD): δ 172.4, 171.2, 170.5, 169.0, 158.1, 147.7, 147.0, 143.6, 138.8, 138.3, 137.5, 135.7, 134.9, 134.7, 131.7, 130.1, 130.0, 129.9, 129.8, 129.6, 129.4, 129.0, 127.8, 127.7, 122.8, 117.4, 107.1, 77.1, 74.7, 72.5, 69.7, 66.2, 61.7, 51.7, 49.9, 45.2, 45.0, 44.9, 43.2, 42.6, 34.4; LRFAB-MS (3-NOBA matrix): *m/z* 1299 $[M+H]^+$; HRFAB-MS (3-NOBA matrix): *m/z* 1299.5104 (calcd. for $C_{69}H_{73}N_9O_{17}$ calcd. 1299.5124 $[M+H]^+$); m.p. 178-180°C.



1

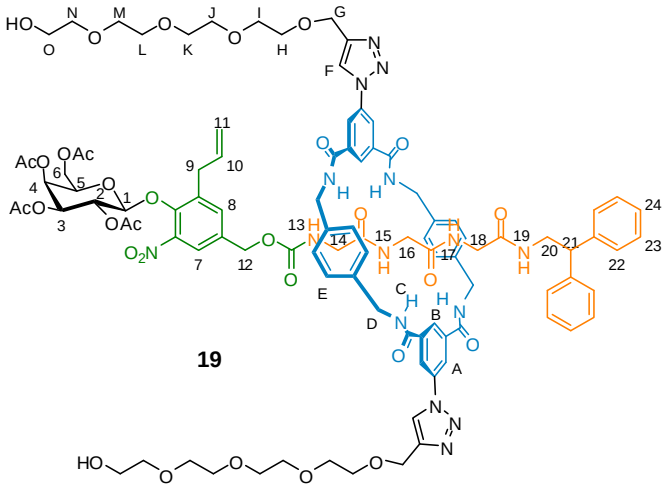
Thread **16** (400 mg, 0.43 mmol) and Et_3N (2.1 mL, 35.0 equiv.) were dissolved in anhydrous chloroform (80 mL) and stirred vigorously whilst solutions of *p*-xylylene diamine (933 mg, 16.0 equiv.) in anhydrous chloroform (60 mL) and 5-azido-isophthaloyl dichloride (1.67 g, 16.0 equiv.) in anhydrous chloroform (60 mL) were simultaneously added over a period of 5 hours using motor-driven syringe pumps. After overnight stirring, 2 mL of MeOH were added and the resulting suspension filtered through Celite®. The pad was washed with $CHCl_3$/MeOH 2% (2x200 mL) and the combined filtrates were concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using $CHCl_3$/acetone (10 to 60% acetone) as eluent to give rotaxane **1** as a pale yellow powder (421 mg, 0.27 mmol, 63%). ^1H NMR (400 MHz, $CDCl_3$): δ 7.88 (bs, 2H, H_B), 7.62 (bs, 4H, H_C), 7.54 (bs, 4H, H_A), 7.34 (s, 1H, H_7), 7.18 to 7.17 (m, 1H, N*H*), 7.14 (d, 4H, *J* = 7.6 Hz, H_E), 7.10 (s, 1H, H_8), 7.07 (bs, 10H, $H_{22+23+24}$), 7.01 (d, 4H, *J* = 7.2 Hz, H_E), 6.86 (bs, 2H, N*H*), 5.83 to 5.73 (m, 1H, H_{10}), 5.63 (bs, 1H, NH_{13}), 5.39 (dd, 1H, *J* = 10.4 Hz, *J* = 8.0 Hz, H_2), 5.30 (d, 1H, *J* = 3.6 Hz, H_4), 5.08 to 4.97 (m, 3H, H_{3+11}), 4.77 to 4.76 (m, 3H, H_{1+12}), 4.50 to 4.46 (m, 4H, H_D), 4.31 to 4.27 (m, 4H, H_D), 4.00 to 3.91 (m, 3H, H_{6+21}), 3.74 (t, 1H, *J* = 7.2 Hz, H_5), 3.66 (bs, 2H, H_{20}), 3.45 (bs, 2H, H_{14}), 3.41 (d, 2H, *J* = 6.4 Hz, H_9), 3.25 (bs, 2H, $H_{16/18}$), 2.97 (bs, 2H, $H_{16/18}$), 2.10 (s, 3H, *H*-Ac), 2.08 (s,

3H, *H*-Ac), 1.93 (s, 3H, *H*-Ac), 1.88 (s, 3H, *H*-Ac); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.4, 170.2, 169.7, 169.4, 168.1, 166.0, 156.5, 145.3, 145.1, 141.6, 141.5, 137.8, 137.2, 135.7, 135.3, 133.8, 133.5, 129.3, 128.9, 127.9, 127.2, 122.1, 121.6, 121.0, 117.8, 102.7, 71.1, 70.7, 68.7, 66.7, 65.4, 60.7, 53.9, 50.6, 44.5, 44.4, 42.9, 42.4, 34.0, 31.9, 29.4, 21.0, 20.8, 20.7 (x2); LRESI-MS: *m/z* 1550 $[M+H]^+$; LRFAB-MS (3-NOBA matrix): *m/z* 1551 $[M+H]^+$; m.p. degradation.



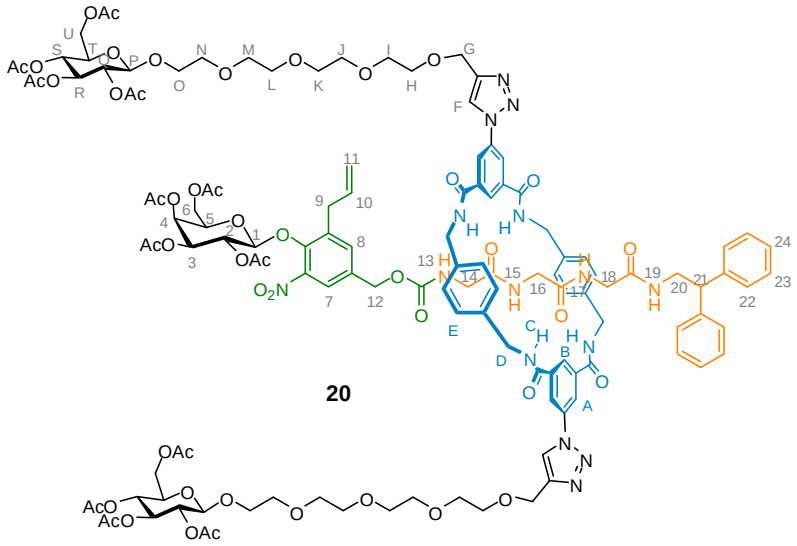
18

To a solution of peracetylated β-D-glucose (317 mg, 0.81 mmol) and alkyne **17** (283 mg, 1.5 equiv.) in anhydrous CH_2Cl_2 (2 mL) under N_2 at 0ºC, was added $BF_3.Et_2O$ (0.52 mL, 5.0 equiv.) dropwise. The reaction mixture was allowed to warm up to room temperature and stirred overnight. An aqueous saturated solution of $NaHCO_3$ (10 mL) was added, the layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3×15 mL). The combined organic fractions were dried (Na_2SO_4) and concentrated under reduced pressure. The resulting brown residue was purified by flash column chromatography on silica gel with CH_2Cl_2/acetone (93/7) as eluent to give pure **18** as a colourless oil (378 mg, 0.67 mmol, 83%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.13 (t, 1H, J = 9.6 Hz, H_3), 5.01 (t, 1H, J = 9.6 Hz, H_4), 4.91 (dd, 1H, J = 8.0 Hz, J = 9.6 Hz, H_2), 4.55 (d, 1H, J = 8.0 Hz, H_1), 4.19 (dd, 1H, J = 4.7 Hz, J = 12.3 Hz, H_6), 4.14 (d, 2H, J = 2.4 Hz, H_{15}), 4.06 (dd, 1H, J = 2.3 Hz, J = 12.3 Hz, $H_{6'}$), 3.87 (dt, 1H, J = 4.0 Hz, J = 11.0 Hz, H_7), 3.71 to 3.52 (m, 16H, $H_{5+7'+8+9+10+11+12+13+14}$), 2.40 (t, 1H, J = 2.3 Hz, H_{16}), 2.01, 1.97, 1.95, 1.93 (4s, 12H, *H*-Ac); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 170.0, 169.2, 169.1, 100.6, 79.5, 74.4, 72.6, 71.5, 71.0, 70.5, 70.4 (×2), 70.3, 70.1, 70.0, 68.8 (×2), 68.2, 61.7, 58.1, 20.6, 20.5, 20.4, 20.3; LRESI-MS: *m/z* 585 $[M+Na]^+$; HRESI-MS: *m/z* 580.2600 (calcd. for $C_{25}H_{42}O_{14}N$ 580.2600 $[M+ NH_4]^+$).



19

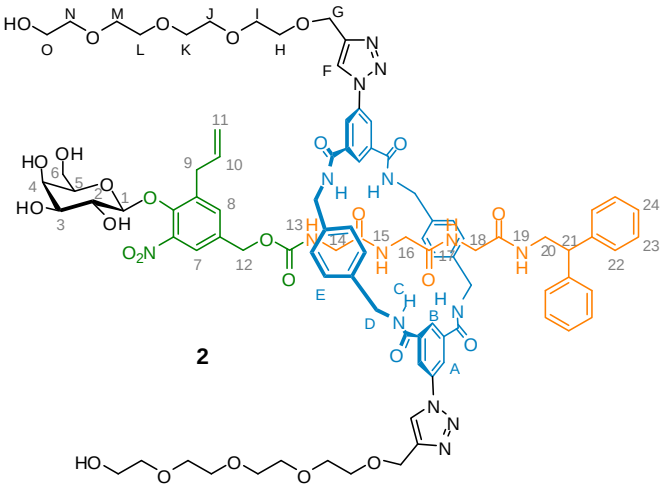
To a solution of rotaxane **1** (100 mg, 64.60 μmol) and alkyne **17** (30 mg, 2.0 equiv.) in CH_2Cl_2 (10 mL) was added $Cu(CH_3CN)_4PF_6$ (12 mg, 0.5 equiv.) and Et_3N (20 μL, 2.2 equiv.) and the solution stirred overnight at room temperature. A saturated NH_4Cl solution was then added to the reaction mixture and air was bubbled for 30 min. The organic layer was separated and washed with 1M EDTA, dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The resulting solid was purified by column chromatography on silica gel using $CHCl_3$/acetone (30 to 55% acetone) to give rotaxane **19** as a pale yellow powder (31 mg, 15.40 μmol, 24%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.31 (bs, 6H, H_{A+F}), 8.22 (bs, 2H, H_B), 8.18 (bs, 4H, H_C), 7.35 (s, 1H, H_7), 7.18 (s, 1H, H_8), 7.09 to 6.99 (m, 21H, $H_{E+22+23+24+15+17+19}$), 5.96 (bs, 1H, H_{13}), 5.79 to 5.69 (m, 1H, H_{10}), 5.36 (dd, 1H, J = 10.4 Hz, J = 8.0 Hz, H_2), 5.30 (d, 1H, J = 3.2 Hz, H_4), 5.03 to 4.98 (m, 3H, H_{3+11}), 4.77 to 4.74 (m, 3H, H_{1+12}), 4.68 (bs, 4H, H_G), 4.39 to 4.35 (m, 8H, H_D), 3.97 to 3.94 (m, 3H, H_{6+21}), 3.77 (t, 1H, J = 6.8 Hz, H_5), 3.69 to 3.47 (m, 34H, $H_{20+H+I+J+K+L+M+N+O}$), 3.37 to 3.35 (m, 2H, H_9), 2.96 to 2.79 (m, 7H, $H_{14+16+18}$), 2.11 (s, 3H, *H*-Ac), 2.06 (s, 3H, *H*-Ac), 1.93 (s, 3H, *H*-Ac), 1.87 (s, 3H, *H*-Ac); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.4, 170.2, 170.1, 170.0, 169.7, 169.2, 168.6, 166.0 (x2), 156.3, 146.2, 145.3, 145.0, 141.9, 137.7, 137.3, 137.2, 136.3, 136.2, 135.4, 134.2, 133.4, 129.1, 128.7, 128.0, 126.9, 126.2, 122.0, 121.4, 117.7, 102.6, 72.7, 71.1, 70.7, 70.5 (x2), 70.2, 70.1, 68.8, 66.7, 65.2, 64.6, 61.5, 60.7, 50.5, 44.7, 44.2, 42.4, 41.8, 34.0, 29.8, 20.9, 20.8, 20.7 (x2); LRFAB-MS (3-NOBA matrix): *m/z* 2014 $[M+H]^+$, 2036 $[M+Na]^+$; HRFAB-MS (3-NOBA matrix): *m/z* 2013.8153 (calcd. for $C_{98}H_{118}{}^{13}CN_{15}O_{31}$ 2013.8152 $[M+H]^+$).



20

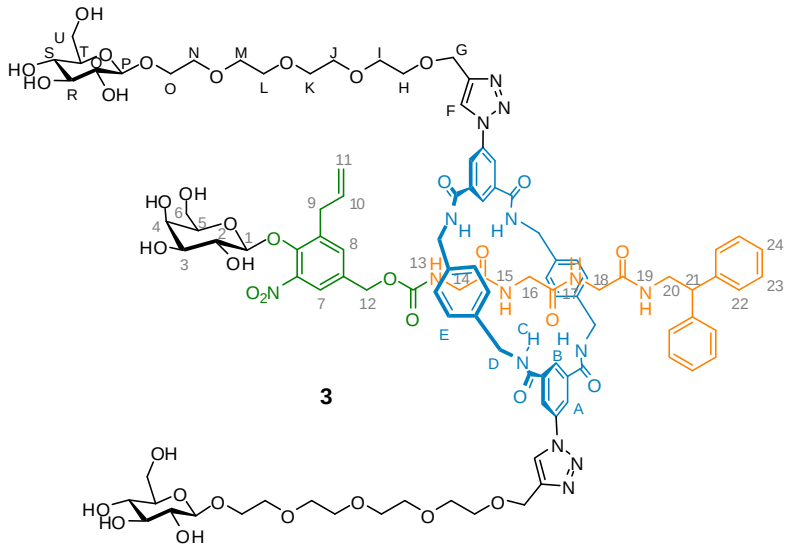
To a solution of rotaxane **1** (70 mg, 45.20 µmol) and alkyne **18** (51 mg, 2.0 equiv.) in CH_2Cl_2 (7 mL) was added $Cu(CH_3CN)_4PF_6$ (8 mg, 0.5 equiv.) and Et_3N (14 µL, 2.2 equiv.) and the solution was stirred overnight at room temperature. A saturated NH_4Cl solution was then added to the reaction mixture and air was bubbled for 30 min. The organic layer was separated and washed with 1M EDTA, dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The resulting solid was purified by preparative TLC on silica eluted 4 times with $CHCl_3$/acetone (1:1) as eluent to give rotaxane **20** as a pale yellow powder (84 mg, 31.6 µmol, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.38 (bs, 2H, H_B), 8.34 (bs, 2H, H_F), 8.25 to 8.19 (m, 8H, H_{A+C}), 7.40 (s, 1H, H_7), 7.22 (s, 1H, H_8), 7.18 to 6.95 (m, 21H, $H_{15+17+19+22+23+24+E}$), 5.85 to 5.75 (m, 2H, H_{10+13}), 5.43 (dd, 1H, J = 10.4 Hz, J = 8.0 Hz, H_2), 5.37 (d, 1H, J = 3.6 Hz, H_4), 5.20 (t, 2H, J = 9.6 Hz, H_R), 5.10 to 5.04 (m, 5H, H_{3+11+S}), 5.97 (dd, 2H, J = 9.6 Hz, J = 8.0 Hz, H_Q), 4.82 (m, 3H, H_{1+12}), 4.76 (bs, 4H, H_G), 4.58 (d, 2H, J = 8.0 Hz, H_P), 4.55 to 4.43 (m, 8H, H_D), 4.24 (dd, 2H, J = 12.0 Hz, J = 4.4 Hz, H_U), 4.11 (dd, 2H, J = 12.0 Hz, J = 2.0 Hz, H_U), 4.07 to 3.98 (m, 3H, H_{6+21}), 3.77 to 3.76 (m, 3H, H_{5+T}), 3.72 to 3.56 (m, 36H, $H_{14+20+H+I+J+K+L+M+N+O}$), 3.42 (d, 2H, J = 6.4 Hz, H_9), 3.34 to 3.25 (m, 2H, H_{18}), 2.84 (bs, 2H, H_{16}), 2.17 (s, 3H, *H*-Ac), 2.13 (s, 3H, *H*-Ac), 2.06 (s, 6H, *H*-Ac), 2.03 (s, 6H, *H*-Ac), 2.01 (s, 6H, *H*-Ac), 2.00 (s, 3H, *H*-Ac), 1.99 (s, 6H, *H*-Ac), 1.95 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.9, 170.4 (x2), 170.2, 169.7, 169.6, 165.8, 146.5, 145.0, 141.8, 137.7, 137.4, 137.3, 136.4, 136.3, 135.3, 134.1, 133.3, 129.9, 129.1, 128.8, 128.0, 126.9, 126.1, 121.9 (x2), 121.2, 117.7, 102.6, 101.0 (x2), 72.9, 71.9, 71.4, 71.0, 70.7, 70.6, 70.3, 70.1, 69.3, 68.8, 68.5, 66.8, 65.2, 64.6, 62.1, 60.7, 50.5, 44.7, 44.3, 44.1, 42.4, 42.3, 41.8, 33.9, 29.8, 29.4, 21.0, 20.9 (x2),

20.8, 20.7; LRFAB-MS (3-NOBA matrix): *m/z* 2675 $[M+H]^+$, 2699 $[M+Na]^+$; HRFAB-MS (3-NOBA matrix): *m/z* 2695.9802 (calcd. for $C_{126}{}^{13}CH_{153}N_{15}O_{49}Na$ calcd. 2695.9873 $[M+Na]^+$); m.p. 133-135°C.



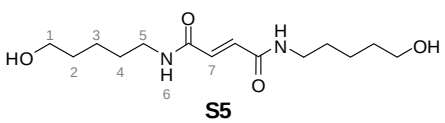
2

To a solution of **19** (27 mg, 13.40 µmol) in MeOH (4 mL) cooled in an ice-water bath was added dropwise at 0°C solution of MeONa (7 mg, 10.0 equiv.) in MeOH (2 mL). Stirring was continued for one hour at 0°C and the solution was neutralised with Amberlite IR-120 resin and filtered. MeOH was then evaporated and the resulting mixture was purified by preparative RP-HPLC using a linear gradient (37 to 55% in 20 min) of CH_3CN (containing 6.6 mM of HCOOH) in H_2O (containing 6.6 mM of HCOOH) at a flow rate of 10 ml/min to give rotaxane **2** as a pale yellow solid (17 mg, 9.40 µmol, 70%). Purity (HPLC): >94%. ^{1}H NMR (400 MHz, CD_3OD): δ 8.57 (s, 2H, H_B), 8.36 (s, 4H, H_A), 8.29 (s, 2H, H_F), 7.16 to 6.89 (m, 20H, $H_{7+8+22+23+24+E}$), 5.78 to 5.68 (m, 1H, H_{10}), 4.95 to 4.90 (m, 2H, H_{11}), 4.45 to 4.34 (m, 12H, H_{D+G}), 4.21 (d, 1H, *J* = 8.0 Hz, H_1), 3.88 (t, 1H, *J* = 8.0 Hz, H_{21}), 3.69 to 3.28 (m, 52H, $H_{2+3+4+5+6+9+12+14+16+18+20+H+I+J+K+L+M+N+O}$); ^{13}C NMR (100 MHz, CD_3OD): δ 171.2, 167.5, 167.4, 158.1, 147.4, 143.6, 138.7 (x2), 137.4, 134.4, 130.0, 129.6, 129.0, 127.8, 127.7, 127.3, 123.5, 123.1, 123.0, 122.6, 117.4, 106.9, 77.1, 74.6, 73.6, 72.4, 71.6, 71.5, 71.4, 71.0, 69.7, 66.1, 65.0, 62.2, 61.8, 51.7, 45.2, 45.0, 43.1, 42.8, 34.3, 30.8; m.p. 102-104°C.



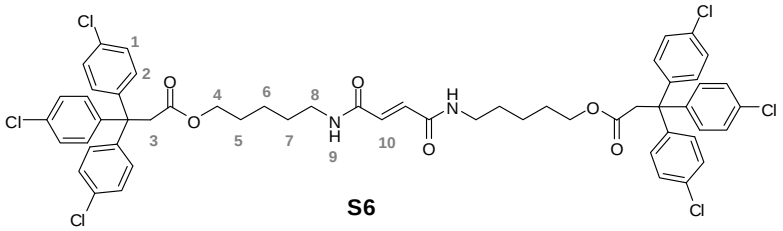
3

To a solution of **20** (24 mg, 9.00 μmol) in MeOH (4 mL) cooled in an ice-water bath was added dropwise at 0°C solution of MeONa (16 mg, 30.0 equiv.) in MeOH (3 mL). Stirring was continued for one hour at 0°C and the solution was neutralised with Amberlite IRC-50 resin and filtered. MeOH was then evaporated and the resulting mixture was purified by preparative RP-HPLC using a linear gradient (33 to 45 % in 15 min) of MeCN (containing 6.6 mM of HCOOH) in H_2O (containing 6.6 mM of HCOOH) at a flow rate of 10 mL/min to give rotaxane **3** as a pink solid (12 mg, 5.50 μmol, 61%). Purity (HPLC): >94%. ^{1}H NMR (400 MHz, CD_3OD): δ 8.69 (s, 2H, H_B), 8.47 (s, 4H, H_A), 8.39 (s, 2H, H_F), 7.41-6.92 (m, 20H, $H_{7+8+22+23+24+E}$), 5.92-5.77 (m, 1H, H_{10}), 5.07-4.98 (m, 2H, H_{11}), 4.78 (s, 4H, H_G), 4.56-4.38 (m, 8H, H_D), 4.32 (d, J = 7.7 Hz, 2H, H_1), 4.29 (d, J = 7.8 Hz, 2H, H_P), 4.04-3.93 (m, 3H, H_{O+21}), 3.90-3.10 (m, 52H, $H_{H+I+J+K+L+M+N+O'+U+2+3+4+5+6+9+12+14+16+18+20}$); ^{13}C NMR (100 MHz, CD_3OD): δ 172.5, 171.2, 170.6, 167.5, 167.4, 158.1, 147.4, 146.8, 143.6, 138.8, 138.7, 138.2, 137.4, 134.7, 134.5, 130.0 (×2), 129.6, 129.5, 129.0 (×2), 127.9, 127.7, 123.6, 123.2, 123.1, 122.6, 117.4, 106.9, 104.4, 78.0, 77.9, 77.1, 75.1, 74.6, 72.4, 71.7, 71.6, 71.6, 71.5, 71.4, 71.0, 69.7, 69.6, 66.1, 65.0, 62.8, 61.8, 51.7, 45.2, 45.0, 43.1, 42.8, 34.3; LRESI-MS: *m/z* 1085.4 $[M+2H]^{2+}$; isotopic distribution matches that calculated for $C_{103}H_{131}N_{15}O_{37}$; m.p. 129-131°C (decomposition).



S5

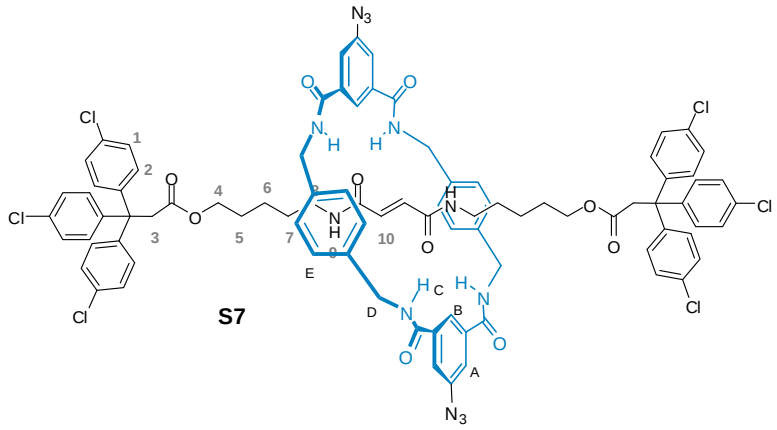
To a solution of 5-amino-pentan-1-ol (3.0 g, 29.10 mmol) in dry DMF (40 mL) at 0 ^{o}C was added a solution of fumaroyl dichloride (0.90 g, 2.0 equiv.) in DMF (15 mL) over a period of 2 hours using motor-driven syringe pumps. The reaction mixture was stirred for a further 2 hours, concentrated under reduced pressure. The residue was re-dissolved in CH_2Cl_2 (300 mL) and a solution of HCl (3 M) in diethyl ether was added until all of the unreacted 5-aminopentan-1-ol had precipitated. The solid was filtered through a plug of Celite®, the plug was washed with warm DMF (2×15 mL) and solvents were removed under reduced pressure to afford **S5** as a pale yellow solid. (1.16 g, 4.05 mmol, 70%). ^{1}H NMR (400 MHz, DMSO *d6*): δ 8.36 (t, 2H, J = 5.6 Hz, H_6), 6.80 (s, 2H, H_7), 3.36 (t, 4H, J = 6.4 Hz, H_1), 3.12 (q, 4H, J = 6.0 Hz, H_5), 1.47-1.34 (m, 8H, $H_{2+}H_4$), 1.32-1.21 (m, 4H, H_3); ^{13}C NMR (100 MHz, DMSO *d6*): δ 163.6, 132,6, 60.6, 32.2, 28.8, 28.6, 23.0; FAB-MS: *m/z* 286 $[M]^+$; HRESI-MS: *m/z* 287.1967 $[M+H]^+$ (calcd. for $C_{14}H_{27}N_2O_4$ 287.1965 $[M+H]^+$); m.p. = 219-222^{o}C.



S6

To a solution of 3,3,3-tris-(4-chloro-phenyl)-propionic acid (1.53 g, 2.0 equiv.), EDC.HCl (0.98 g, 3.0 equiv.) and DMAP (0.63 g, 3.0 equiv.) in CH_2Cl_2 (30 mL) at room temperature, was added bis-alcohol **S5** (0.49 g, 1.71 mmol). The reaction mixture was stirred overnight. CH_2Cl_2 (250 mL) and an aqueous solution (1 M) of HCl (50 mL) were added. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2×25 mL). The combined organic fractions were washed with an aqueous saturated solution of $NaHCO_3$, dried ($MgSO_4$) and concentrated under reduced pressure. Purification by flash column chromatography on silica using CH_2Cl_2/MeOH (98/2) as eluent gave **S6** as a colourless solid (1.50 g, 1.41 mmol, 84%). ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD: 9/1): δ 8.01 (t, 2H, J = 5.6 Hz, H_9), 7.23 to 7.16 (m, 12H, H_1), 7.12 to 7.04 (m, 12H, H_2), 6.71 (s, 2H, H_{10}), 3.75 (t, 4H, J = 6.5 Hz, H_4), 3.59 (s, 4H, H_3), 3.23 to 3.17 (m, 4H, H_8), 1.51-1.26 (m, 8H, H_{5+7}), 1.19 to 1.05 (m, 4H, H_6); ^{13}C NMR (400 MHz, $CDCl_3$/CD_3OD: 9/1): δ 170.4, 164.2, 144.2, 133.0, 132.6, 130.3, 128.2, 64.3, 54.6, 46.0, 39.6, 29.0, 28.0, 23.2; LRFAB-MS: *m/z*

1061 $[M+H]^+$; HRFAB-MS (glycerol matrix): *m/z* 1061.2029 (calcd. for $C_{56}{}^{13}CH_{52}D^{35}Cl_6N_2O_6$ 1061.2005 $[M+H]^+$); m.p. 132-136°C.

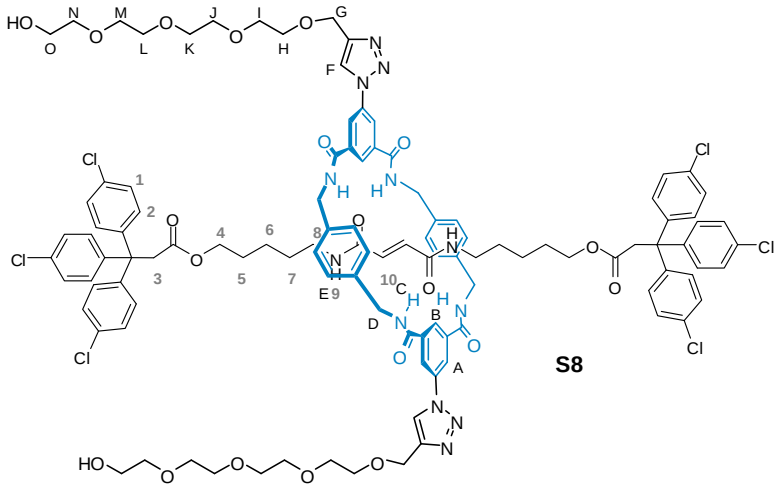


To a vigorously stirred solution of thread **S6** (0.53 g, 4.94 mmol) and Et_3N (2 mL, 30.0 equiv.) in 1 L of dry $CHCl_3$ under nitrogen, was simultaneously added a solution of *p*-xylylenediamine (1.00 g, 15.0 equiv.) in $CHCl_3$ (50 mL) and a solution of 5-azido-isophthaloyl dichloride (1.81 g, 15.0 equiv.) in $CHCl_3$ (50 mL) over a period of 3 hours using motor-driven syringe pumps. The reaction mixture was stirred for another 2 hours, filtered over a Celite® pad and the filtrate was concentrated under reduced pressure. The resulting orange solid was purified by flash column chromatography on silica with acetone/CH_2Cl_2 (10/90) as eluent to give rotaxane **S7** (0.69 g, 4.12 mmol, 83%). ^1H NMR (400 MHz, $CDCl_3$): δ 8.03 (m, 2H, H_C), 7.81-7.67 (m, 6H, H_{10+D}), 7.66 (s, 4H, H_B), 7.21-7.12 (m, 20H, H_{1+F}), 7.09-7.01 (m, 12H, H_2), 5.74 (s, 2H, H_{10}), 4.45 (bs, 8H, H_E), 3.72 (t, 4H, J = 6.5 Hz, H_4), 3.14 (m, 2H, H_8), 1.45 (bquint, 4H, J = 7.2 Hz, H_5), 1.37 to 1.21 (m, 4H, H_7), 1.17 to 1.05 (m, 4H, H_6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 165.9, 165.4, 143.8, 141.6, 136.6, 135.3, 132.3, 130.0, 129.5, 128.7, 127.9, 121.4, 120.8, 64.0, 54.4, 45.7, 43.8, 39.5, 28.4, 27.7, 23.0; LRESI-MS: *m/z* 1697 $[M+Na]^+$; HRESI-MS: *m/z* 1694.4408 (calcd. for $C_{88}H_{82}C_{l6}N_{13}O_{10}$ 1694,4439 $[M+NH_4]^+$); isotopic distribution matches that calculated for $C_{88}H_{82}Cl_6N_{13}O_{10}$; m.p. 174-176°C (decomposition)

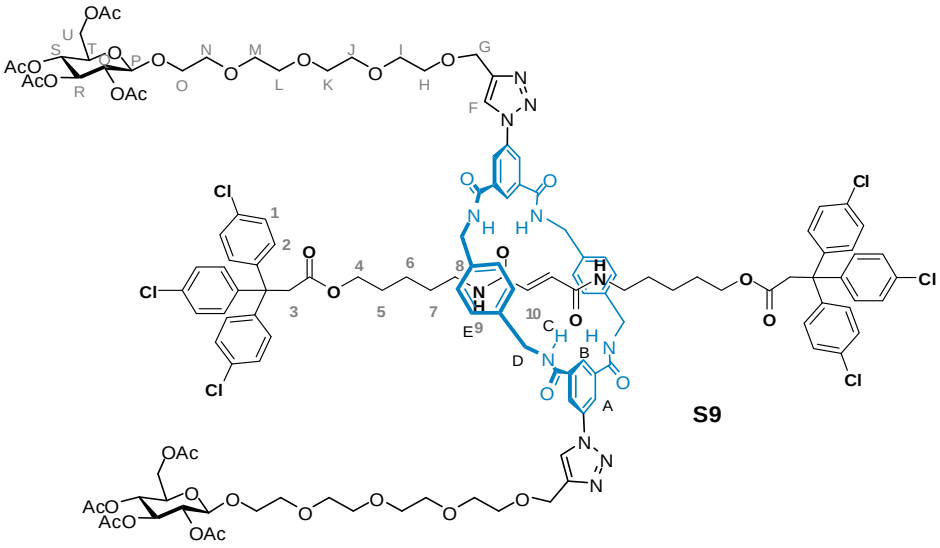
General method to access functionalised macrocycle 9 and 10

To a solution of rotaxane **S7**, alkyne (2.0 equiv.), and $Cu(CH_3CN)_4PF_6$ (0.5 equiv.) in dry CH_2Cl_2 (5 mL) and under N_2 at room temperature was added Et_3N (2.2 equiv.) and the mixture was stirred overnight. Air was bubbled through the mixture for 15 min.

then a saturated solution of NH_4Cl was added (15 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (10 mL). The combined organic fractions were washed with a 1 M solution of EDTA/K_2CO_3 (2x10 mL) and brine (10 mL), then dried ($MgSO_4$) and concentrated under reduced pressure.

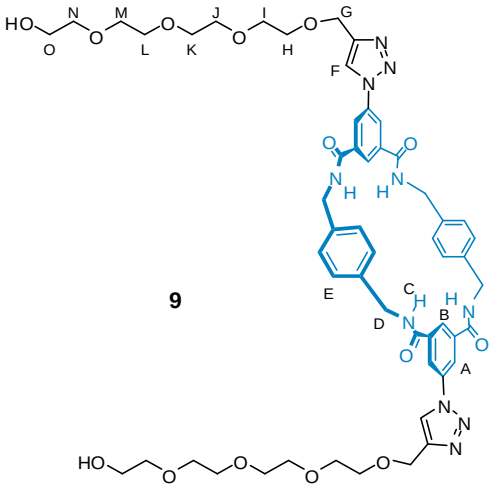


Using the general method, rotaxane **S7** (97 mg, 0.060 mmol), alkyne **17** (27 mg, 2.0 equiv.), $Cu(CH_3CN)_4PF_6$ (11 mg, 0.5 equiv.) and Et_3N (16 μL, 2.2 equiv.) in CH_2Cl_2 (15 mL) at room temperature overnight gave **S8** as a pale yellow solid (115 mg, 0.054 mmol, 93%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.50 to 8.23 (m, 8H, H_A, H_{B+F}), 8.23 to 8.07 (m, 2H, H_9), 7.95 to 7.74 (m, 4H, H_C), 7.21 to 6.92 (m, 32H, H_1, H_{2+E}), 5.86 (s, 2H, H_{10}), 4.80 (s, 4H, H_G), 4.46 to 4.09 (m, 8H, H_D), 3.84 to 3.43 (m, 40H, $H_{3+4+H+I+J+K+L+M+N+O}$), 3.21 to 3.03 (m, 4H, H_8), 1.53 to 1.19 (m, 8H, H_{5+7}), 1.18 to 1.00 (m, 4H, H_6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2, 165.6, 165.0, 146.1, 144.0, 137.4, 136.6, 135.5, 132.4, 130.2, 129.1 (x 2), 129.0, 128.1, 124.5, 122.2, 121.8, 72.5, 70.5, 70.4, 70.3, 70.0, 69.9, 64.5, 64.1, 61.4, 54.5, 45.8, 44.1, 39.7, 29.6, 27.9, 23.2; LRMALDI-MS: *m/z* 2164 $[M+Na]^+$; isotopic distribution matches that calculated for $C_{110}H_{118}Cl_6N_{12}O_{20}Na$; m.p. 100-102°C.



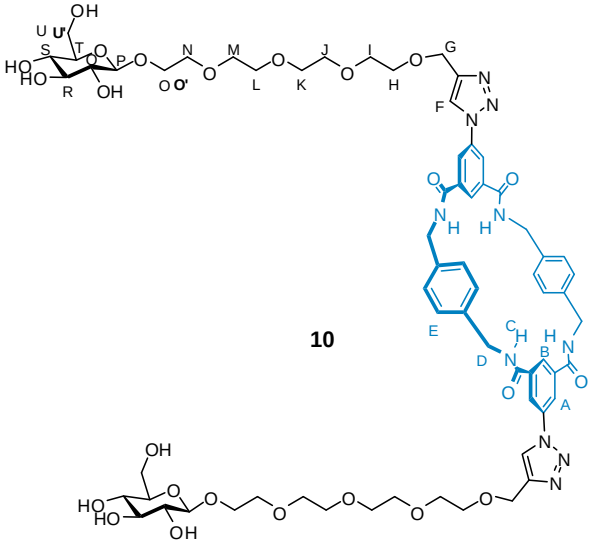
S9

Using the general method, rotaxane **S7** (250 mg, 0.150 mmol), alkyne **18** (168 mg, 2.0 equiv.), $Cu(CH_3CN)_4PF_6$ (28 mg, 0.5 equiv.) and Et_3N (455 μL, 2.2 equiv.) in CH_2Cl_2 (15 mL) at room temperature overnight gave **S9** as a pale yellow solid (296 mg, 0.105 mmol, 78%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.41 (s, 2H, H_B), 8.36 (s, 4H, H_A), 8.26 (s, 2H, H_F), 7.98-7.77 (m, 4H, H_C), 7.74-7.56 (m, 2H, H_9), 7.22-7.12 (m, 12H, H_1), 7.12-6.97 (m, 20H, H_2 and H_E), 5.75 (s, 2H, H_{10}) 5.21 (t, 2H, J = 9.5 Hz, H_R), 5.08 (t, 2H, J = 9.6 Hz, H_S), 4.98 (t, 2H, J = 8.8 Hz, H_Q), 4.80 (s, 4H, H_G), 4.60 (d, 2H, J = 7.9 Hz, H_P), 4.47-4.30 (m, 8H, H_D), 4.25 (dd, 2H, J = 4.4 Hz and J = 12.2 Hz, H_U), 4.13 (d, 2H, J = 11.5Hz, $H_{U'}$), 3.93 (dt, 2H, J = 4.3Hz and J = 11.1 Hz, H_H), 3.81-3.45 (m, 40H, $H_{3+4+H'+I-O}$), 3.20-297 (m, 4H, H_8), 2.07, 2.04, 2.01, 1.99 (4s, 24H, *H*-Ac), 1.51-1.17 (m, 8H, H_5 and H_7), 1.17-1.00 (m, 4H, H_6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 170.2, 169.4, 165.6, 165.5, 165.1, 144.0, 132.5, 130.2, 128.9, 128.1, 122.3, 100.8, 72.8, 71.7, 71.2, 70.6, 70.5, 70.5, 70.2, 70.0, 69.0, 68.3, 64.5, 64.1, 61.9, 55.4, 54.5, 45.9, 44.2, 44.2, 39.6, 29.6, 28.7, 27.8, 20.7, 20.6, 20.6, 20.5; LR-MALDI-MS: m/z 2824 $[M+Na]^+$; isotopic distribution matches that calculated for $C_{138}H_{154}Cl_6N_{12}O_{38}Na$; m.p. 96-98°C.



9

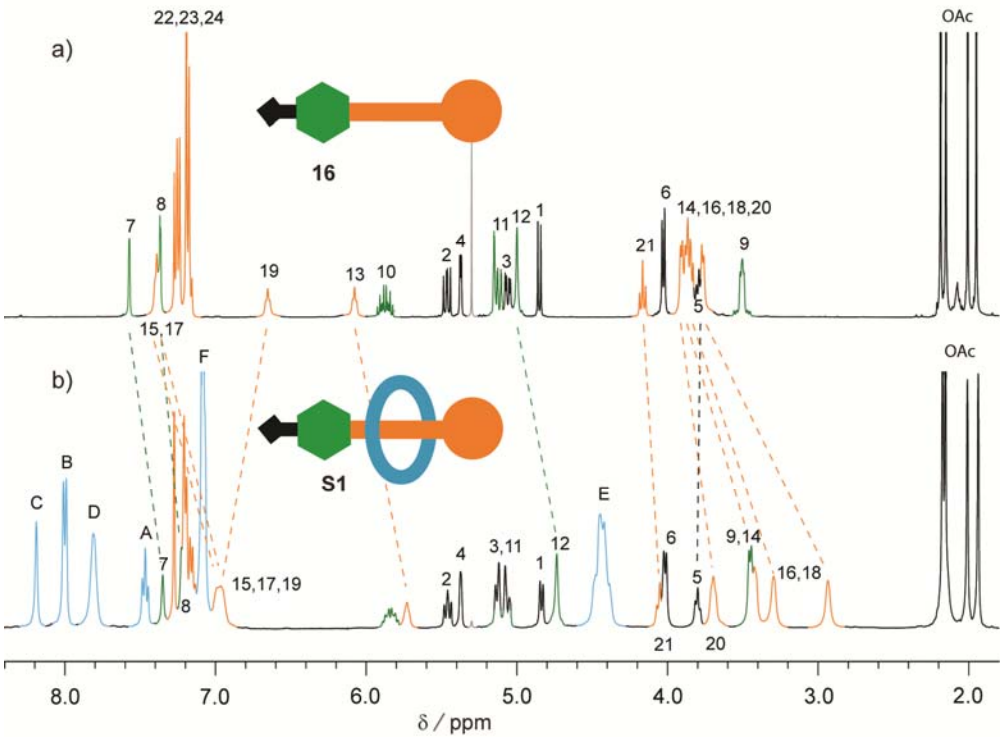
Rotaxane **S8** (40 mg, 0.018 mmol) was stirred for 4 days at room temperature in a 1 M solution of NaOH in THF/H_2O/EtOH (3/2/5) (10 mL). A 1 M aqueous solution of HCl was added drop wise until pH equalled 7. The whole solution was loaded on a preparative silica TLC plate that was eluted with CH_2Cl_2/MeOH (15:85) to give macrocycle **9** as a waxy solid (18 mg, 0.016 mmol, 90%). ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$: 1/1): δ 8.41 (2s, 4H, H_A), 8.35 (bs, 2H, H_B), 8.11 (t, 2H, J = 1.4 Hz, H_F), 7.28 (s, 8H, H_E), 4.53 (s, 8H, H_D), 4.70 (s, 2H, H_G), 3.72-3.49 (m, 32H, $H_{H+I+J+K+L+M+N+O}$); ^{13}C NMR (100 MHz, $CD_3OD/CDCl_3$: 1/1): δ 166.4, 137.8, 137.7, 136.9, 128.9, 125.3, 122.7, 122.1, 72.8, 70.8, 70.7, 70.6, 70.5, 70.3, 70.1, 64.4, 61.4, 44.3 (x2); LRESI-MS: *m/z* 1101 $[M+Na]^+$; HRESI-MS: *m/z* 1101.4651 (calcd. for $C_{54}H_{66}N_{10}O_{14}Na$ 1101.4652 $[M+Na]^+$).



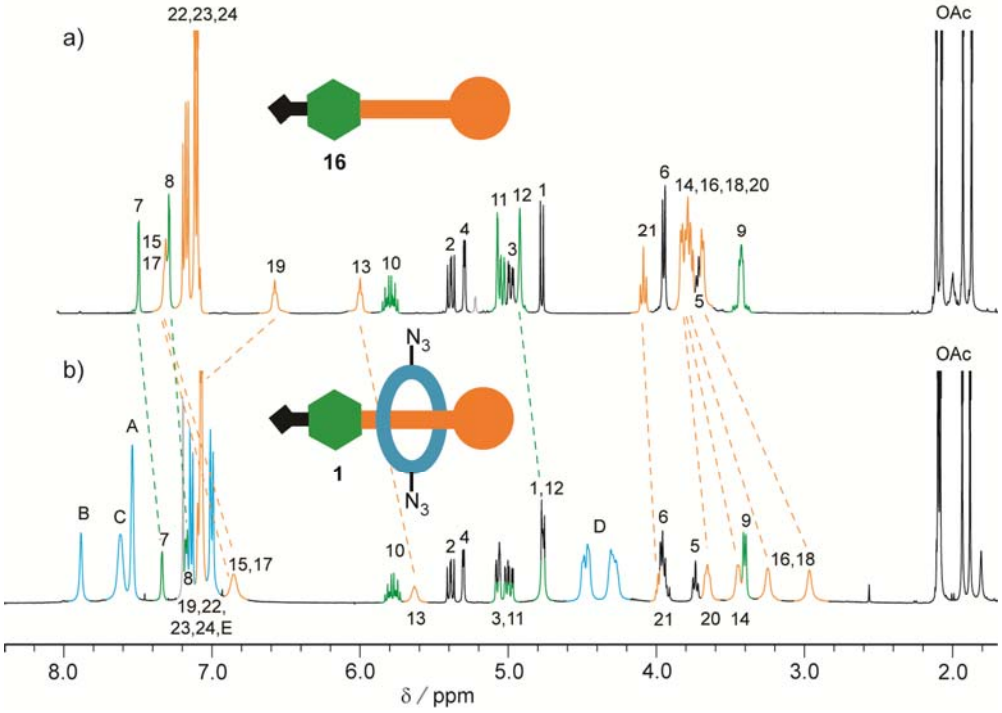
10

Rotaxane **S9** (122 mg, 0.050 mmol) was stirred for 48 hours at room temperature in a 10 M solution of NaOH in THF/H_2O/EtOH (1/1/1) (10 mL). A 1M aqueous solution of HCl was added drop wise until pH equalled 7. Solvents were removed under vacuum. The resulting solid was re-dissolved in a 1/1 mixture of CH_3CN/H_2O (10 mL), filtered by gravity and the filtrate used for purification by preparative RP-HPLC using a linear gradient of CH_3CN (25 to 40% in 20 min) in H_2O to give macrocycle **10** as a colourless solid (18 mg, 0.012 mmol, 25%). ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$: 1/1): δ 8.41, 8.40 (2s, 6H, H_{A+B}), 8.09 (s, 2H, H_F), 7.23 (s, 8H, H_E), 4.70 (s, 4H, H_G), 4.55-4.42 (m, 8H, H_D), 4.16 (d, J = 7.8 Hz, 2H, H_P), 3.95 to 3.83 (m, 2H, H_O), 3.74 (dd, 2H, J = 2.7 Hz, J = 12.1 Hz, H_U) 3.73-3.49 (m, 32H, $H_{H+I+J+K+L+M+N+O'+U'}$) 3.26 to 3.00 (m, 8H, $H_{Q+R+S+T}$); ^{13}C NMR (100 MHz, $CD_3OD/CDCl_3$: 1/1): δ 166.5, 146.3, 137.8, 137.7, 136.8, 128.9, 125.4, 122.7, 122.2, 103.1, 76.6, 76.5, 73.8, 70.8 (x 2), 70.7, 70.6, 70.5, 70.4, 70.3, 70.0, 68.7, 64.4, 61.9, 44.3; LRESI-MS: *m/z* 1425 $[M+H]^+$; HRESI-MS: *m/z* 1425.5709 (calcd. for $C_{66}H_{87}N_{10}O_{24}$ 1425.5709 $[M+H]^+$); mp. 205-207°C.

4. Representative ^{1}H NMR Stack Plots

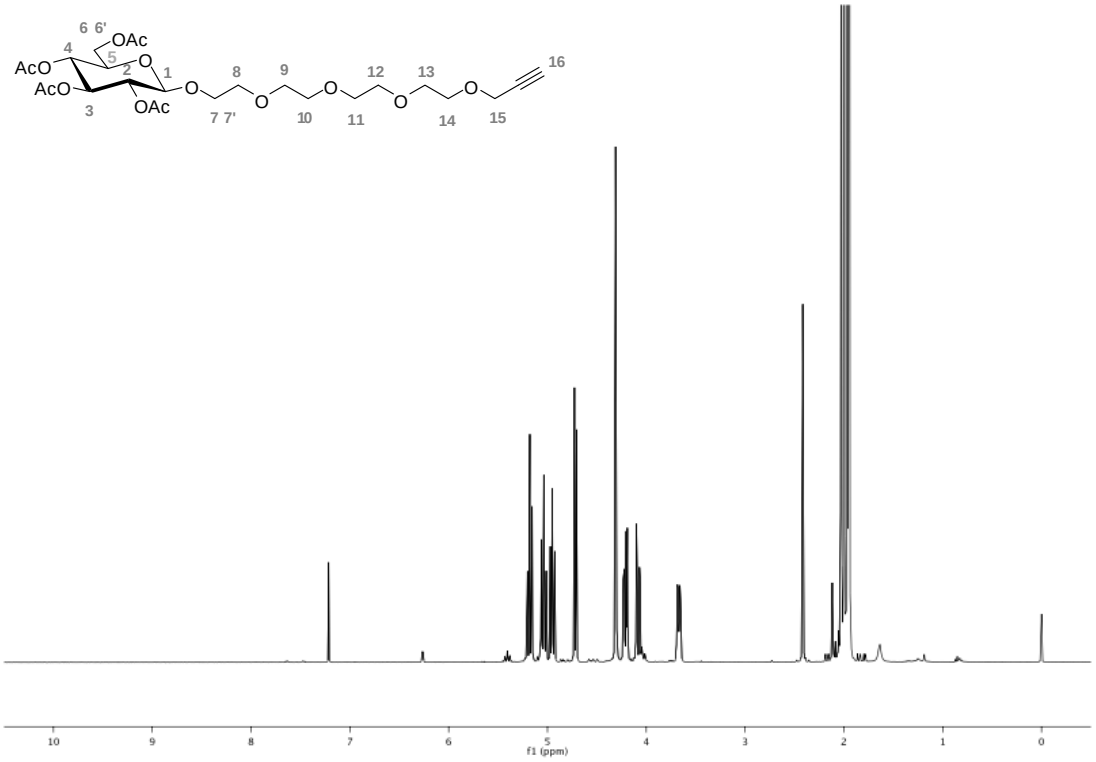


^{1}H NMR spectra of a) thread **16** and b) rotaxane **S1**, (400 MHz, 298 K, $CDCl_3$)

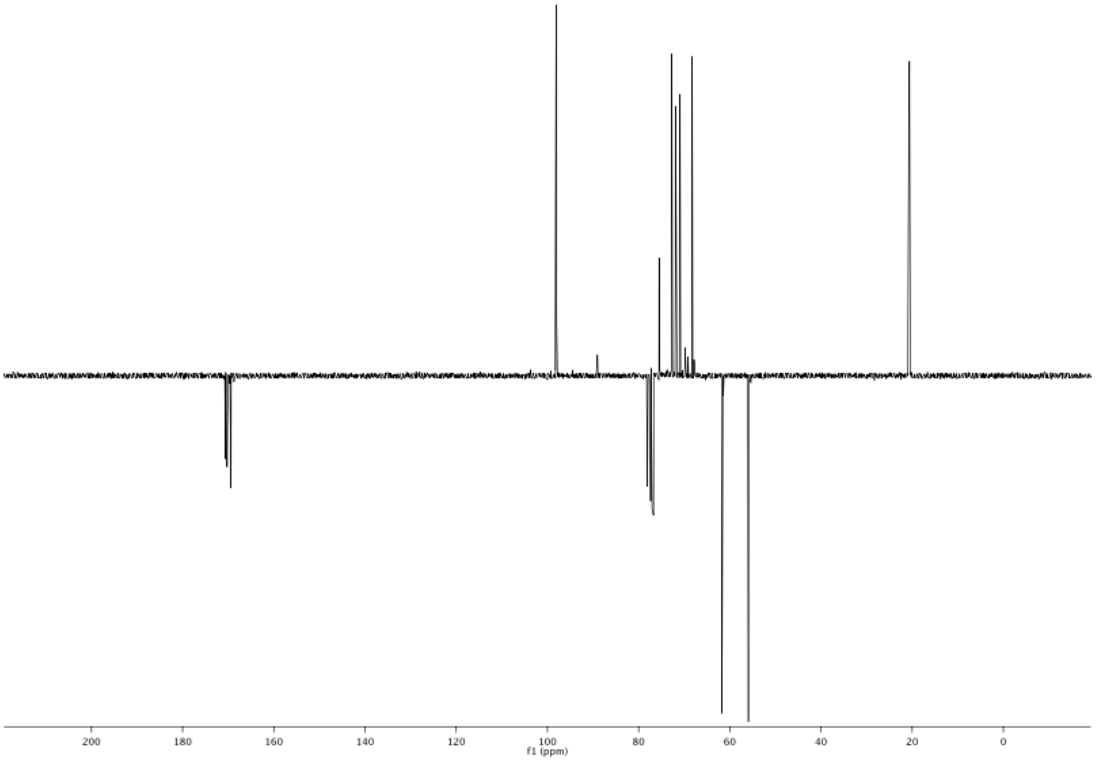


^{1}H NMR spectra of a) thread **16** and b) rotaxane **1**, (400 MHz, 298 K, CDCl3)

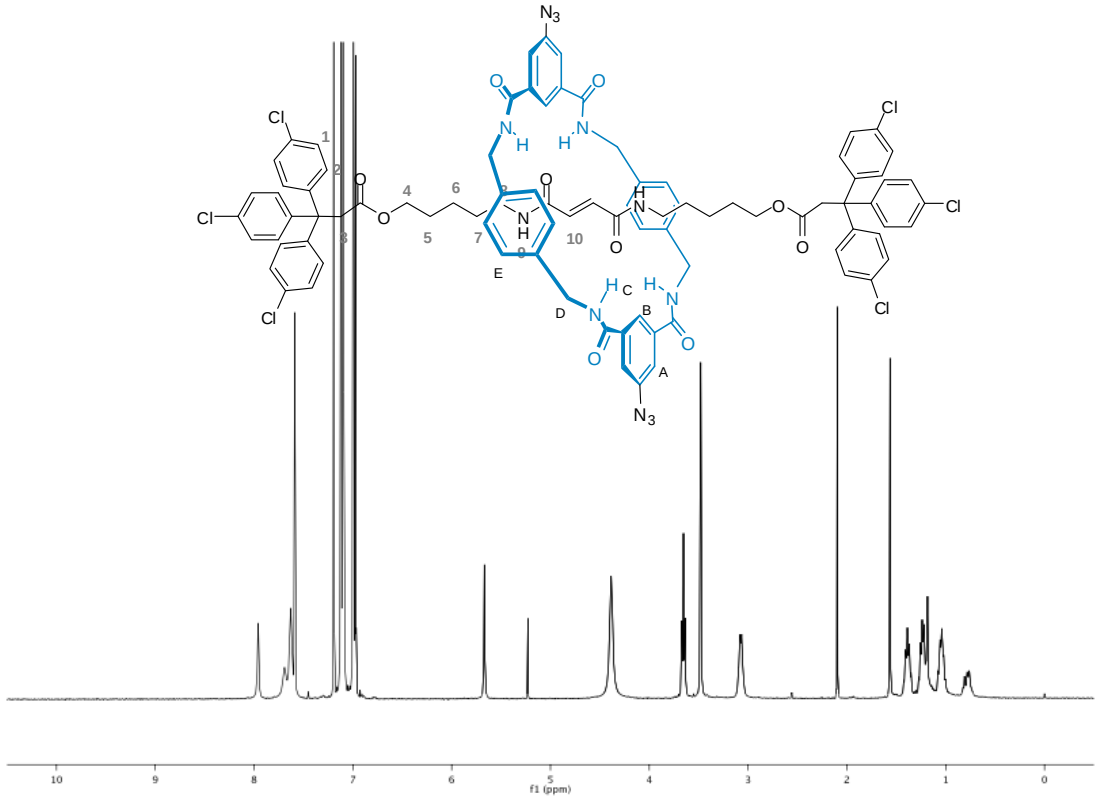
5. Selected ^{1}H and ^{13}C NMR Spectra



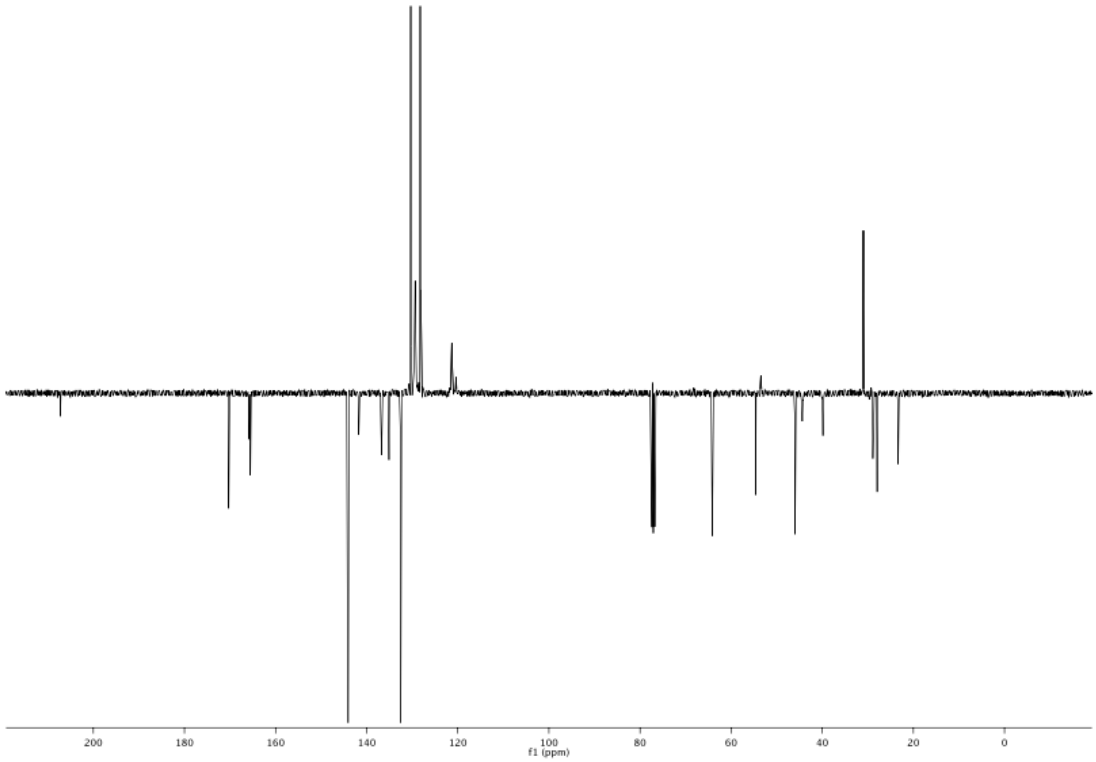
^{1}H NMR of **18**, 400 MHz, 298 K, $CDCl_3$



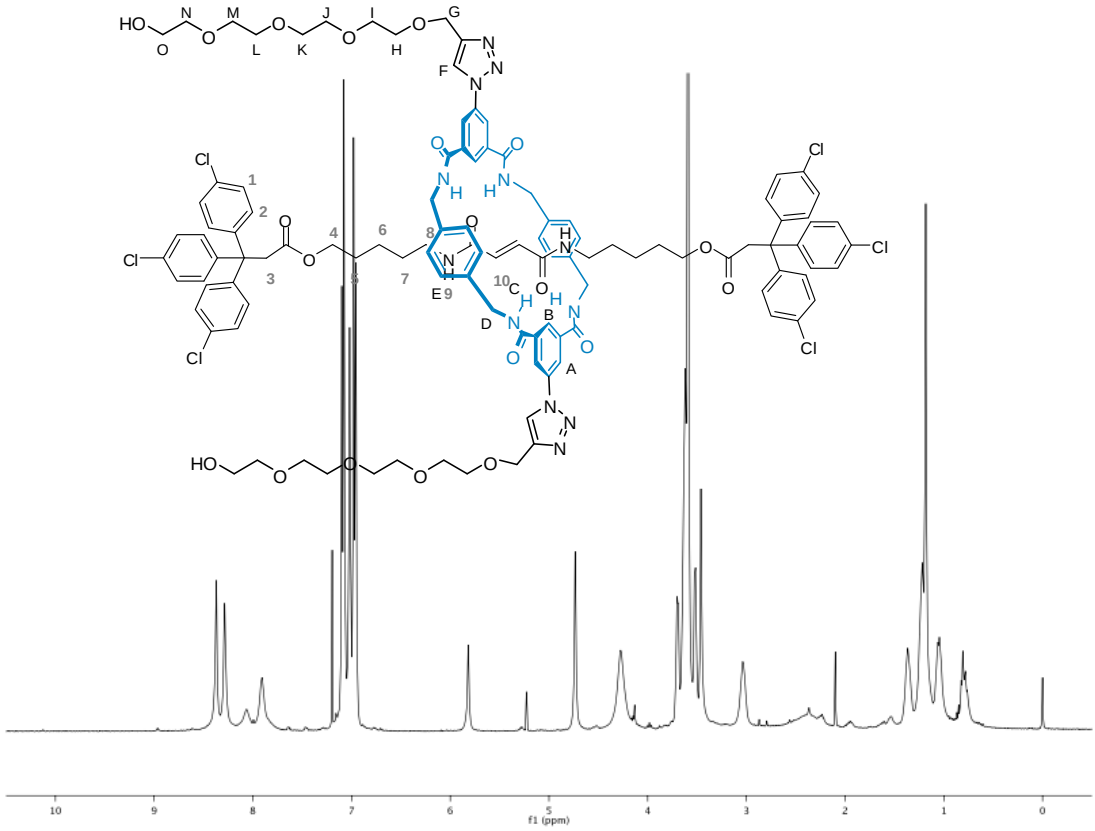
^{13}C NMR (pendant) of **18**, 100 MHz, 298 K, $CDCl_3$



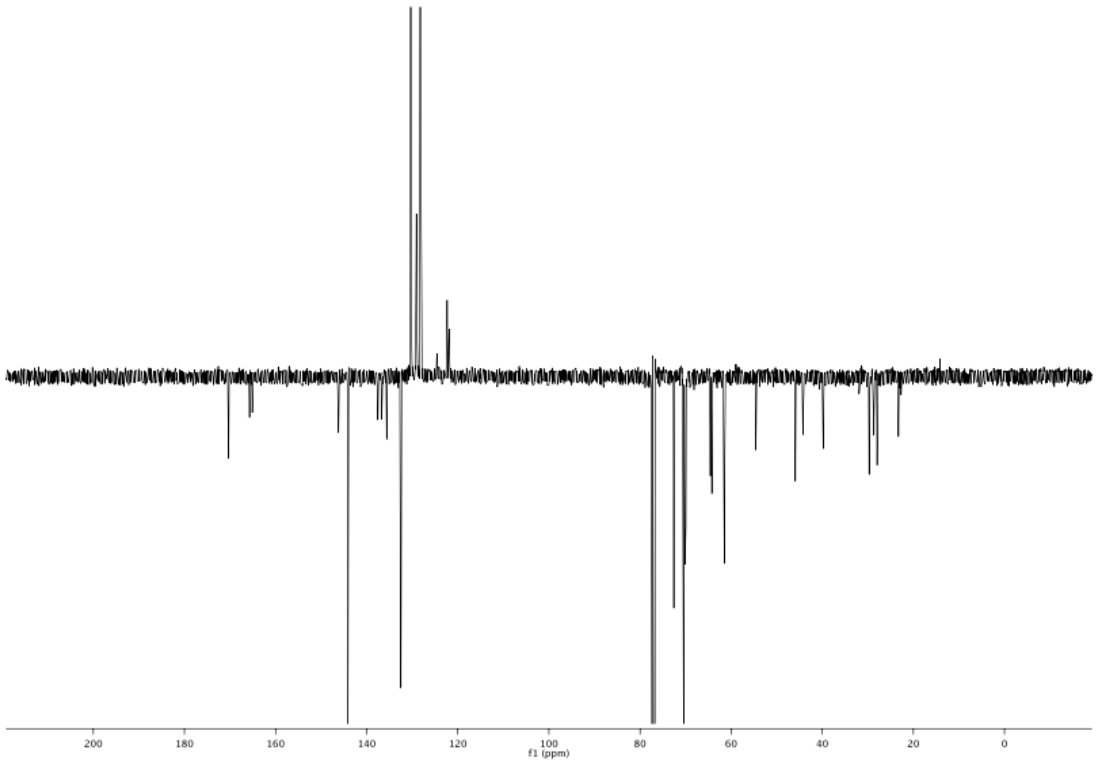
^{1}H NMR of **S7**, 400 MHz, 298 K, $CDCl_3$



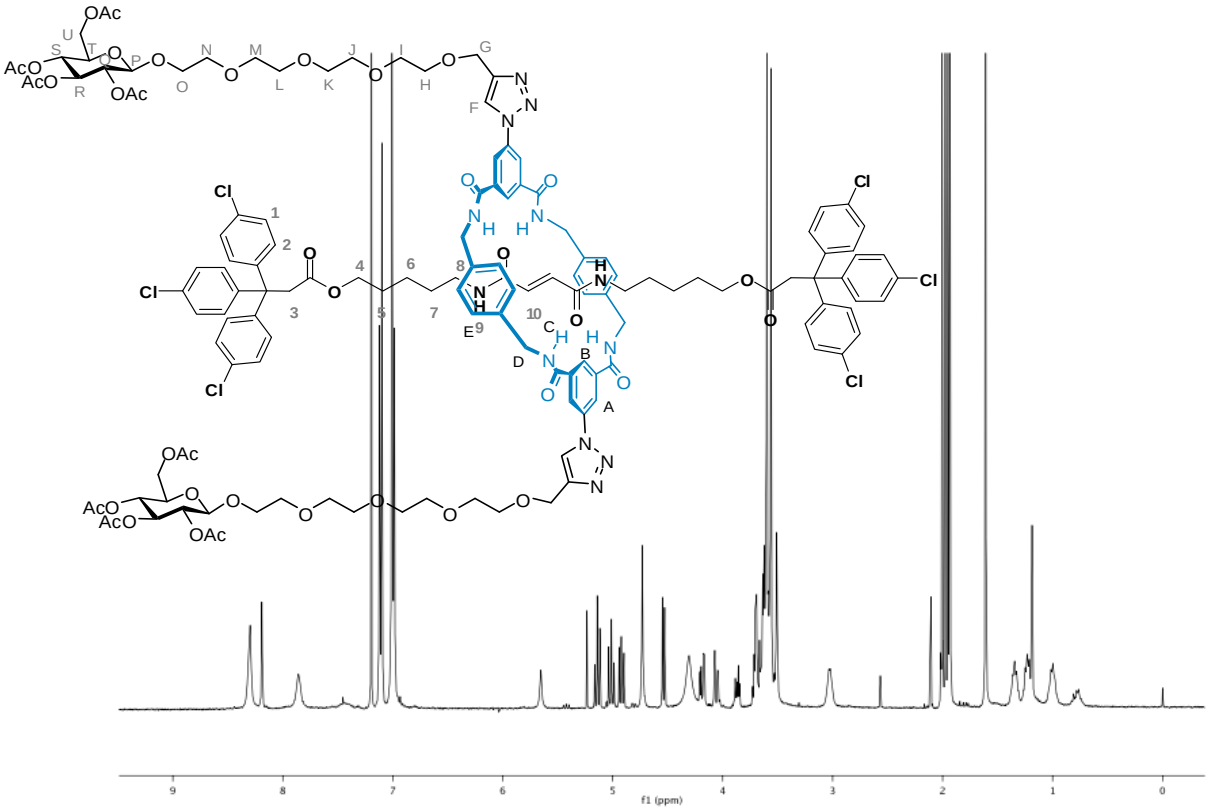
^{13}C NMR (pendant) of **S7**, 100 MHz, 298 K, $CDCl_3$



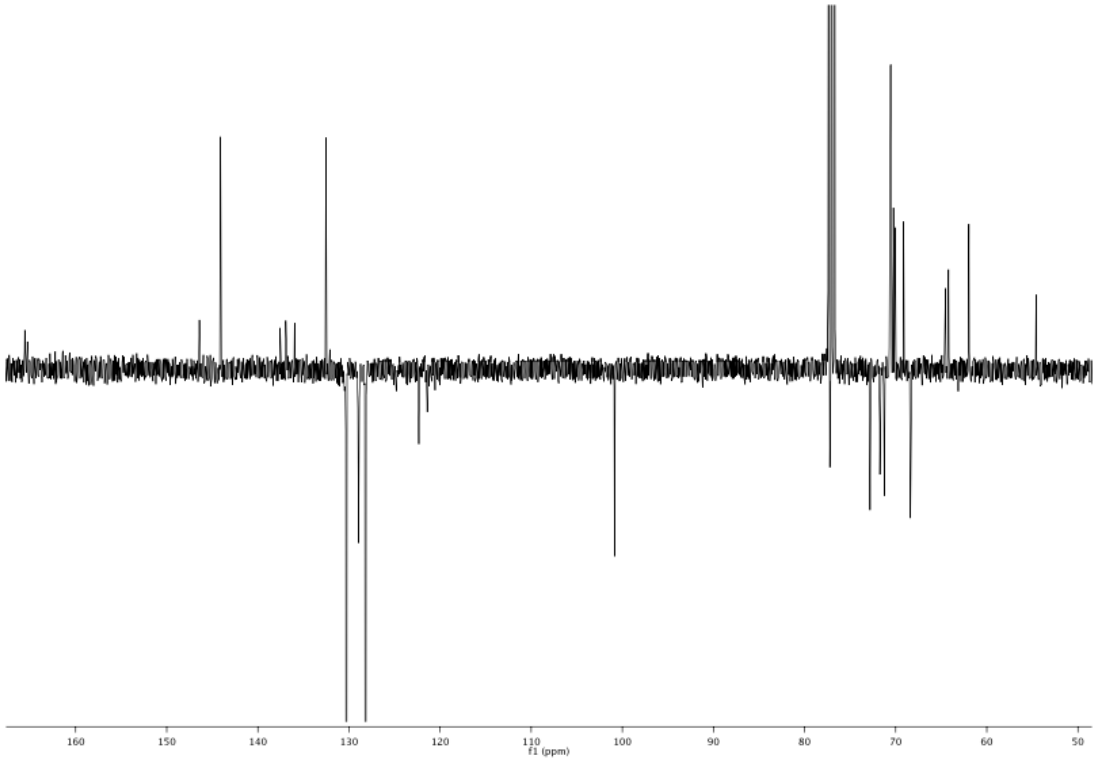
^{1}H NMR of **S8**, 400 MHz, 298 K, $CDCl_3$



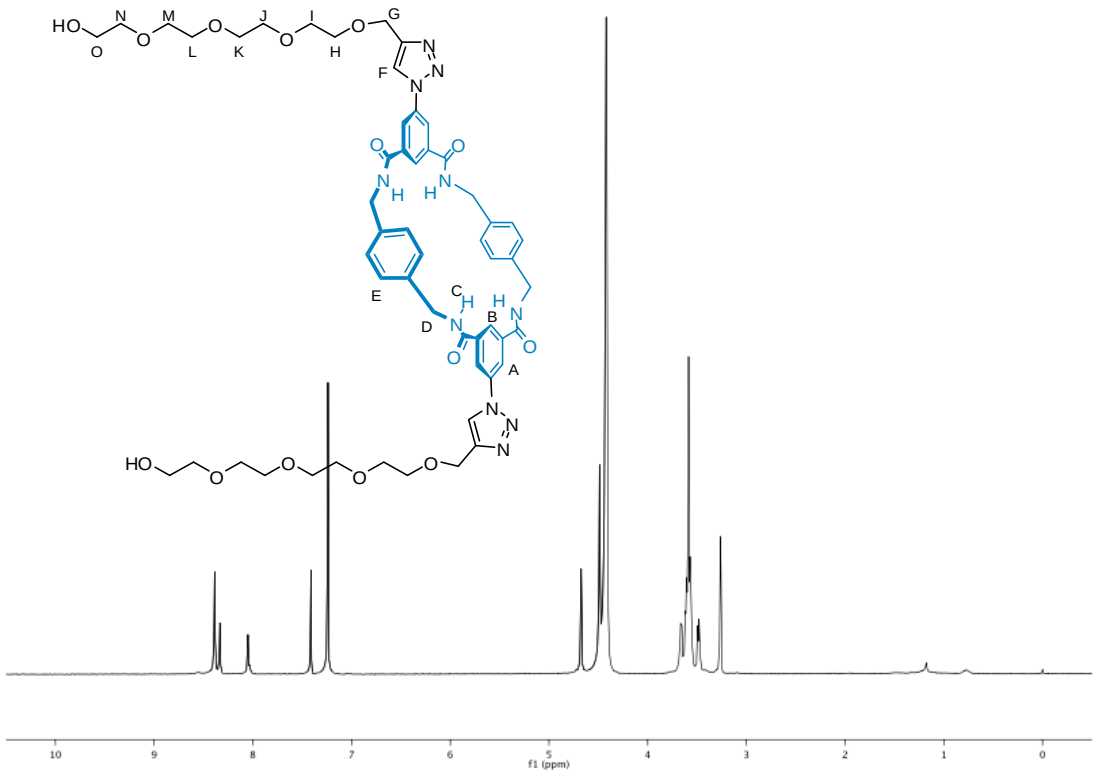
^{13}C NMR (pendant) of **S8**, 100 MHz, 298 K, $CDCl_3$



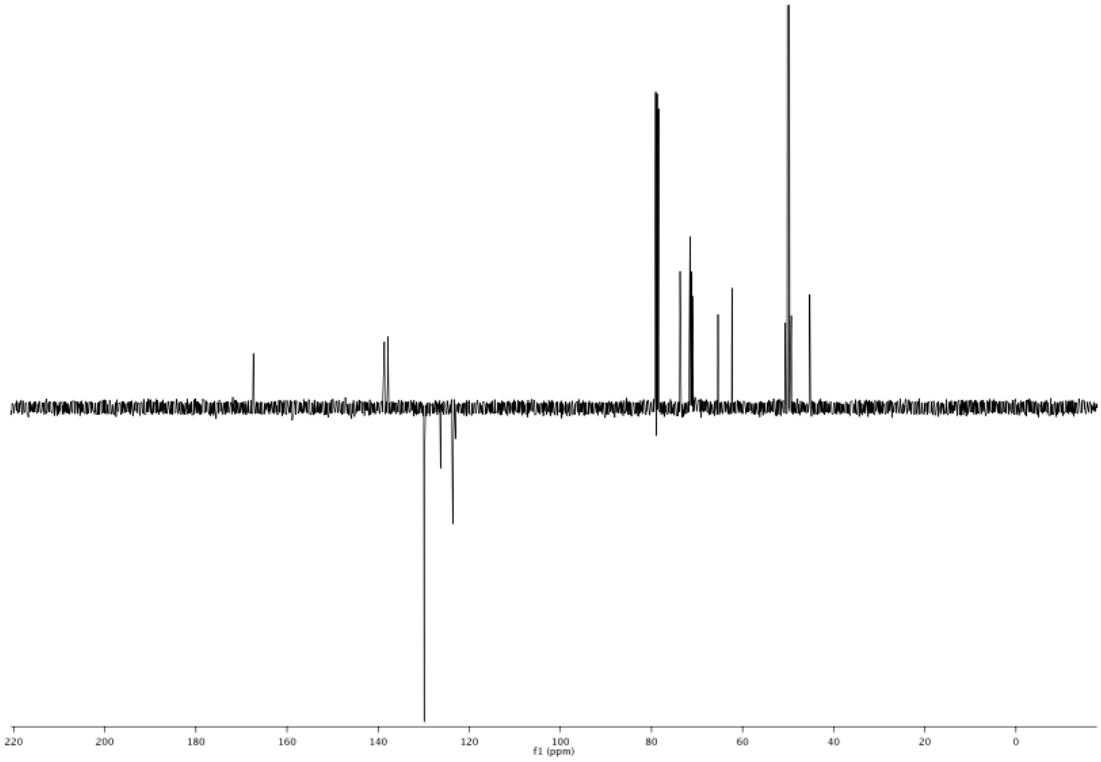
^{1}H NMR of **S9**, 400 MHz, 298 K, $CDCl_3$



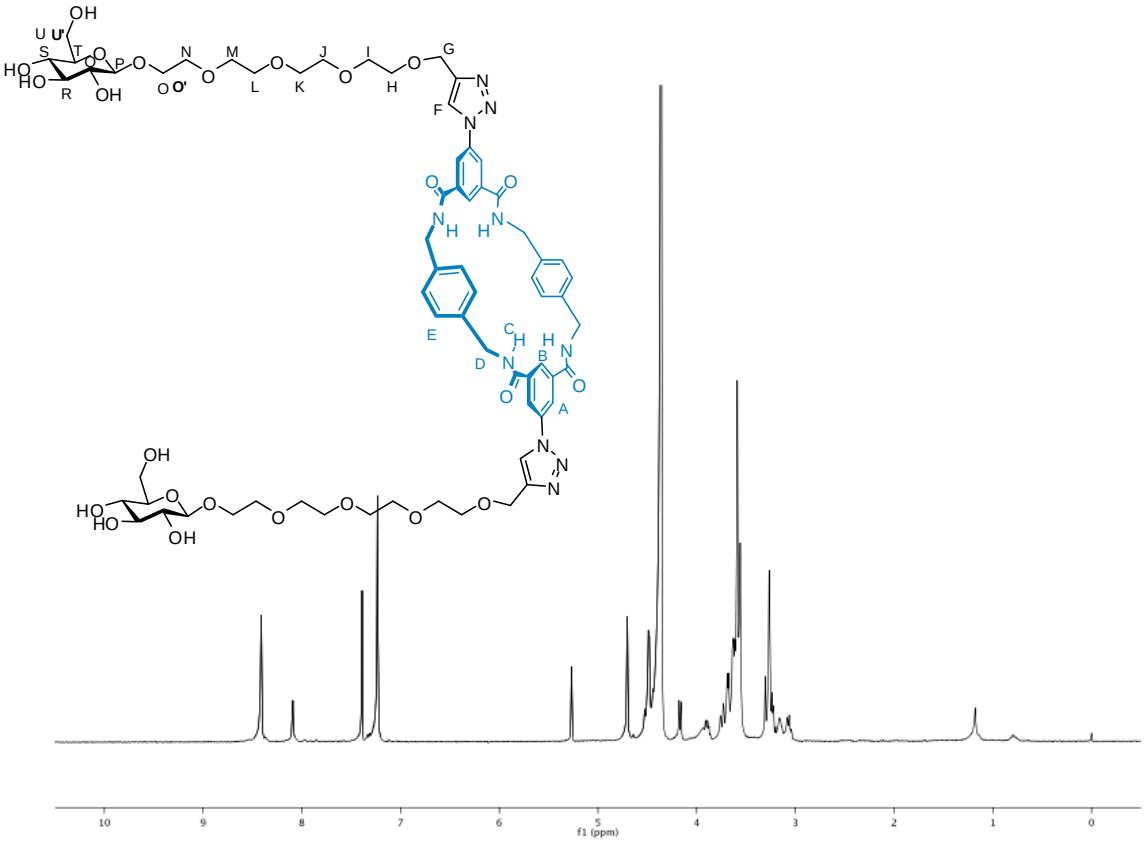
^{13}C NMR (pendant) of **S9**, 100 MHz, 298 K, $CDCl_3$



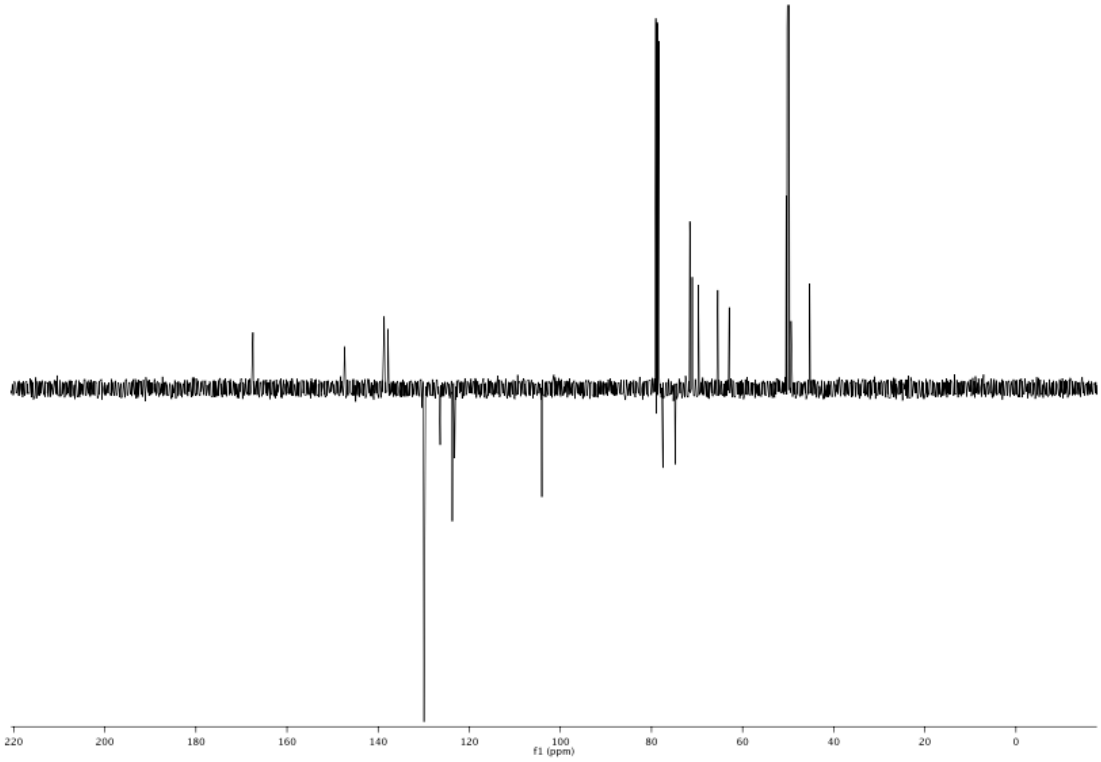
^{1}H NMR of **9**, 400 MHz, 298 K, $CD_3OD/CDCl_3$: 1/1



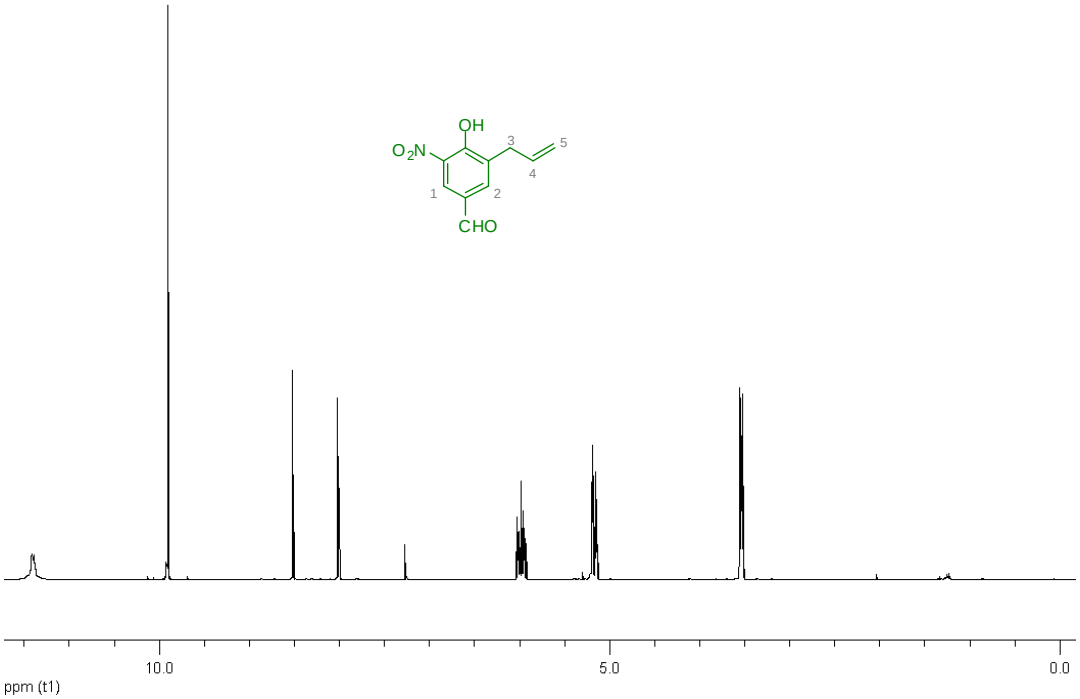
^{13}C NMR (pendant) of **9**, 100 MHz, 298 K, $CD_3OD/CDCl_3$: 1/1



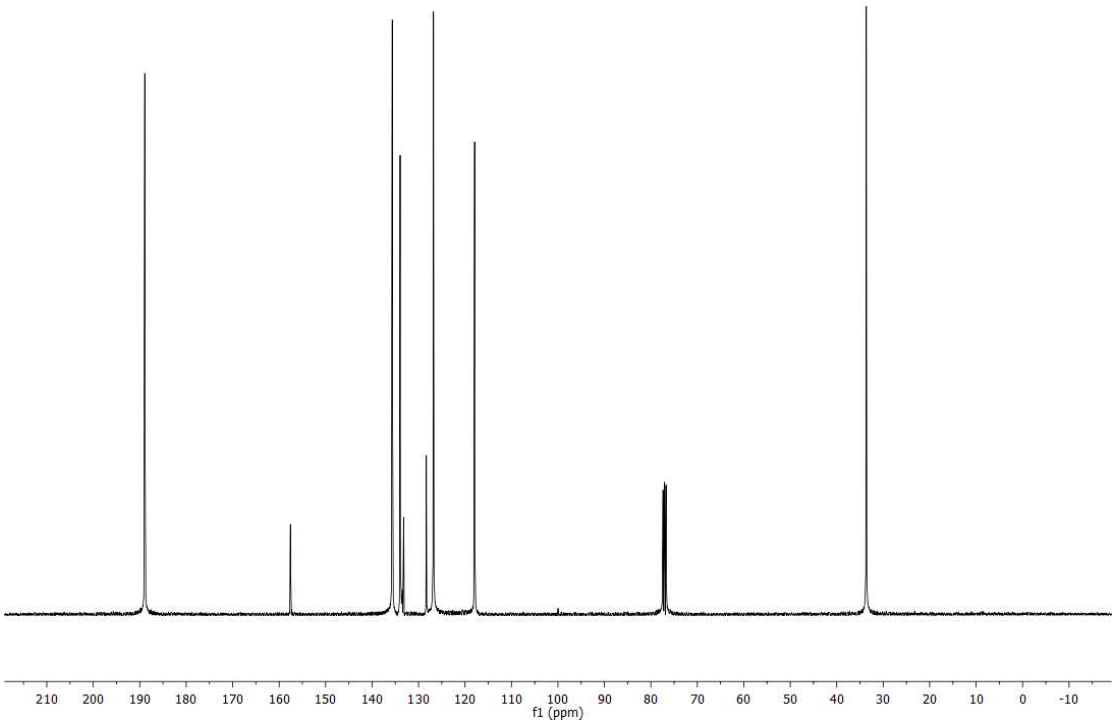
^{1}H NMR of **10**, 400 MHz, 298 K, $CD_3OD/CDCl_3$: 1/1



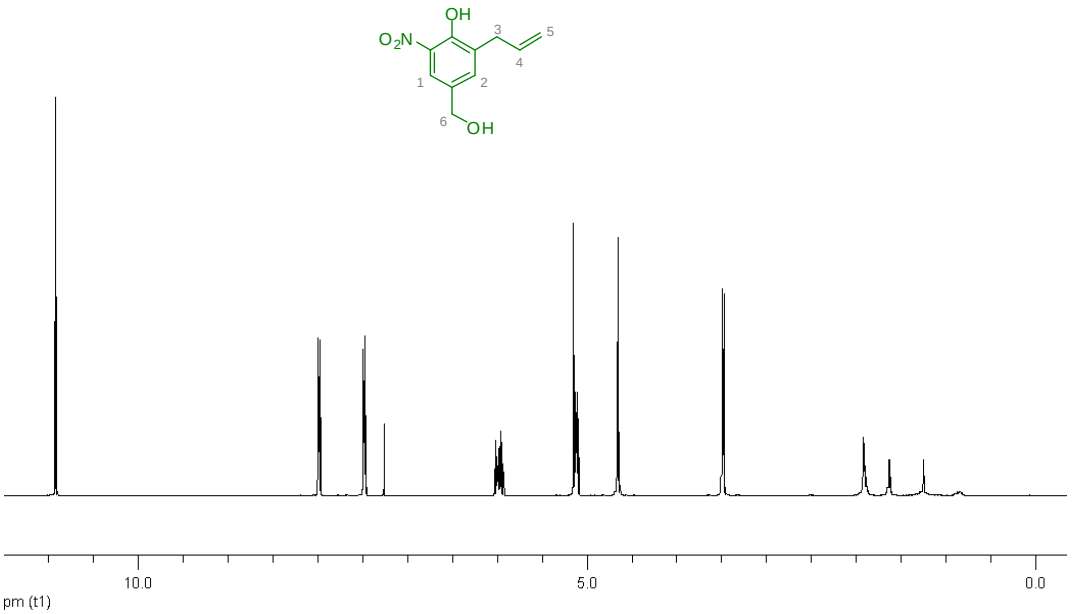
^{13}C NMR (pendant) of **10**, 100 MHz, 298 K, $CD_3OD/CDCl_3$: 1/1



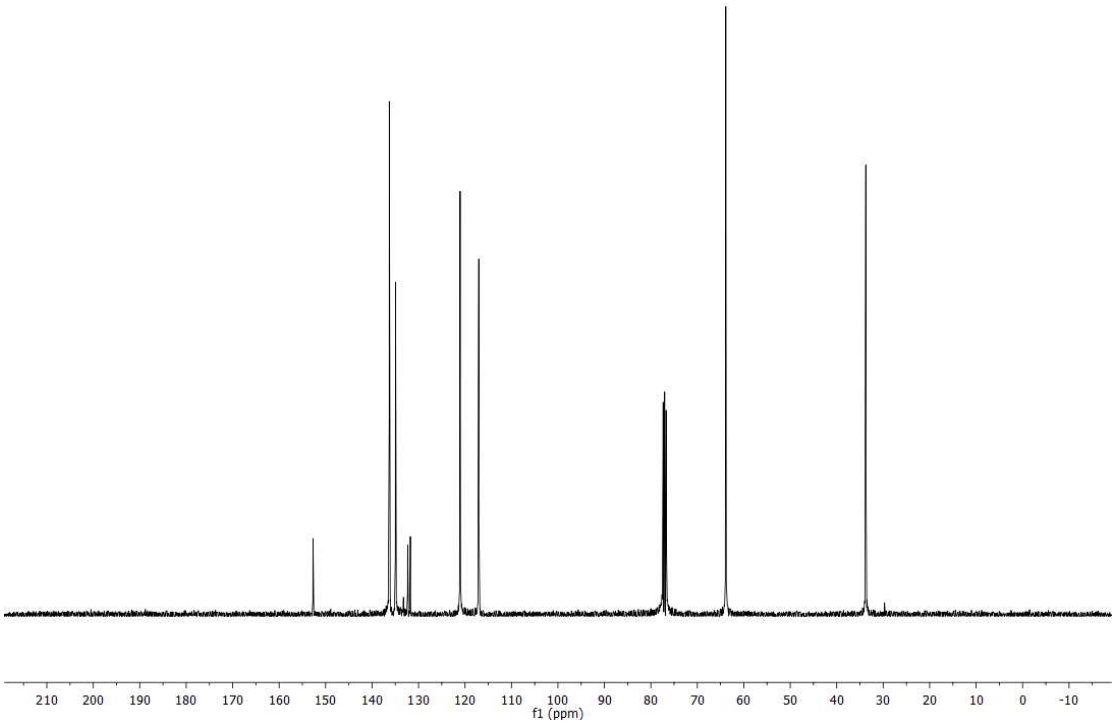
^{1}H NMR of **12**, 400 MHz, 298 K, $CDCl_3$



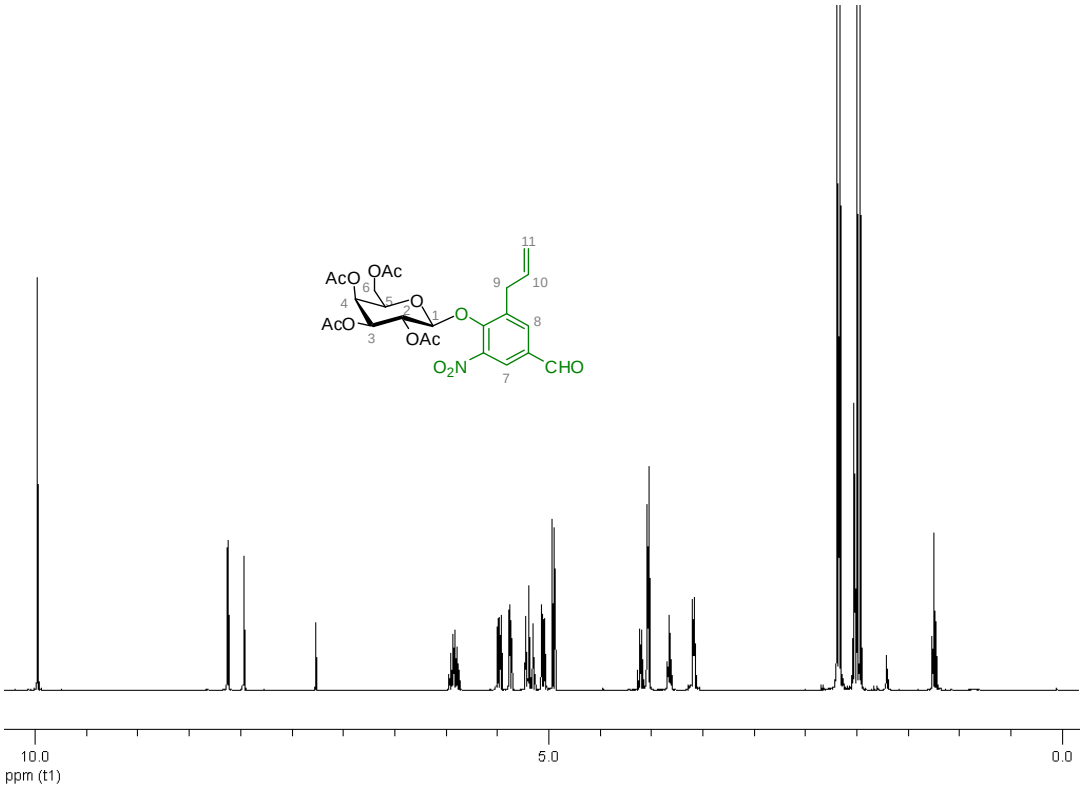
^{13}C NMR of **12**, 100 MHz, 298 K, $CDCl_3$



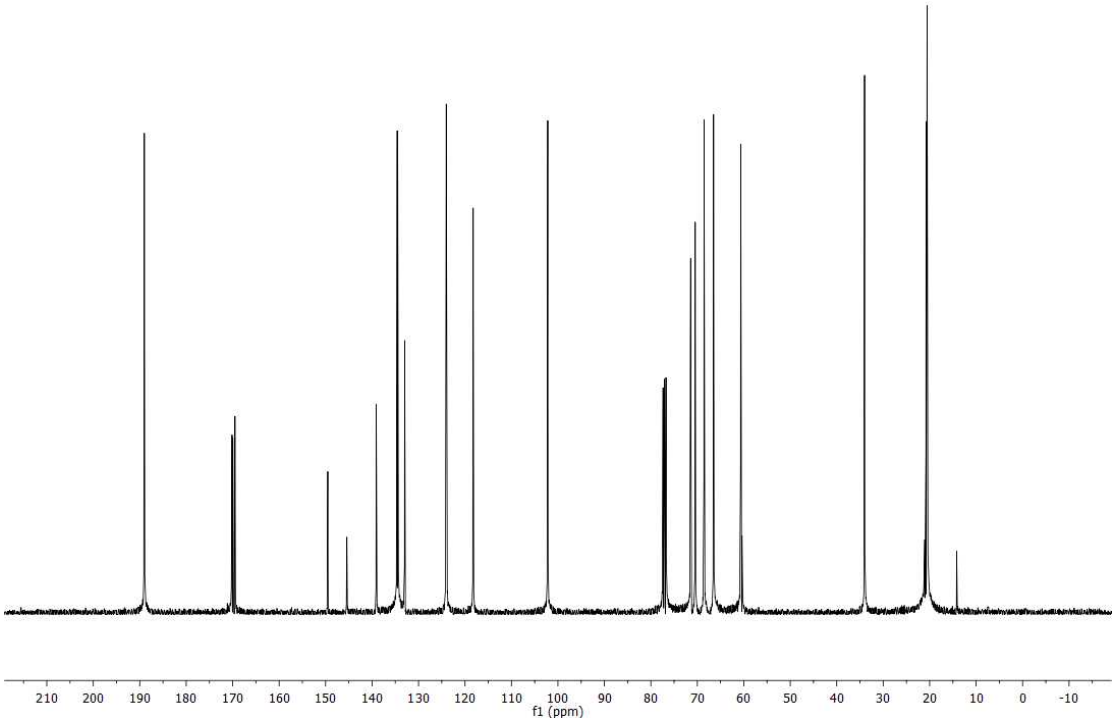
^{1}H NMR of **8**, 400 MHz, 298 K, $CDCl_3$



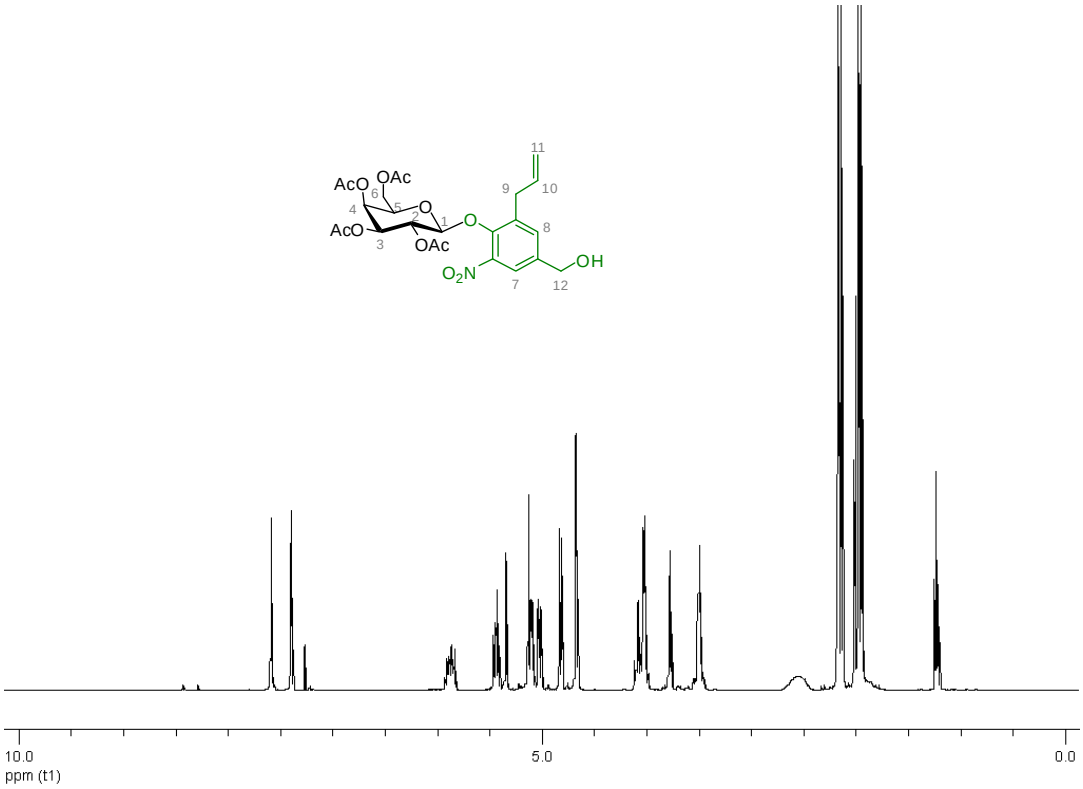
^{13}C NMR of **8**, 100 MHz, 298 K, $CDCl_3$



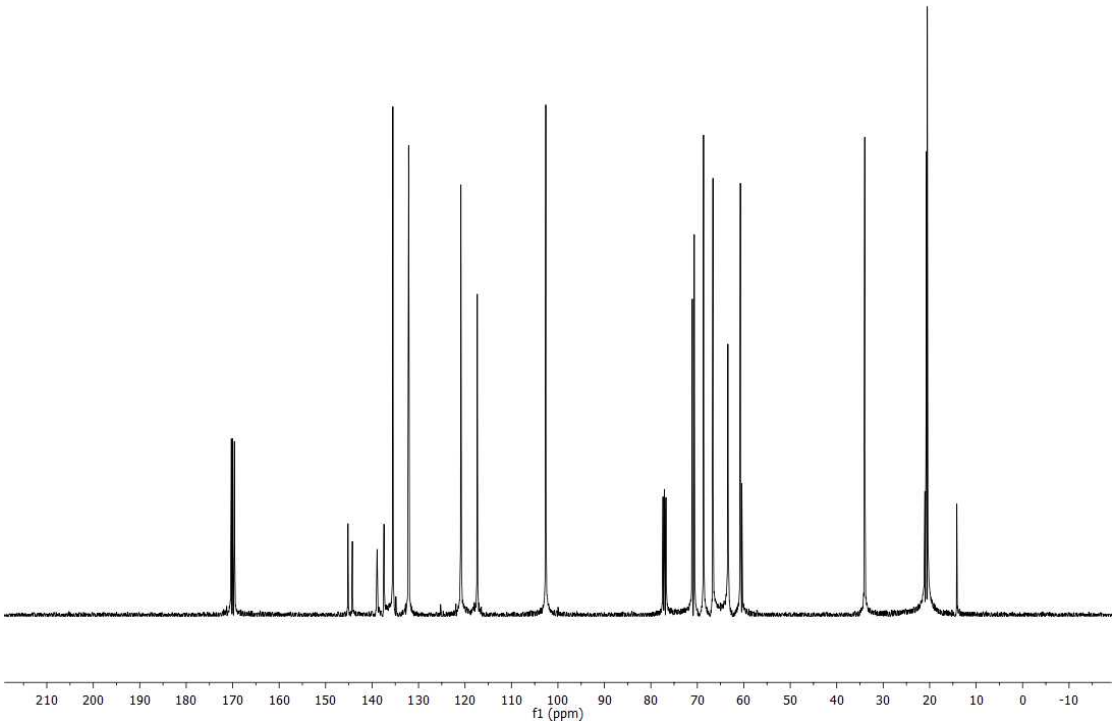
^{1}H NMR of **13**, 400 MHz, 298 K, $CDCl_3$



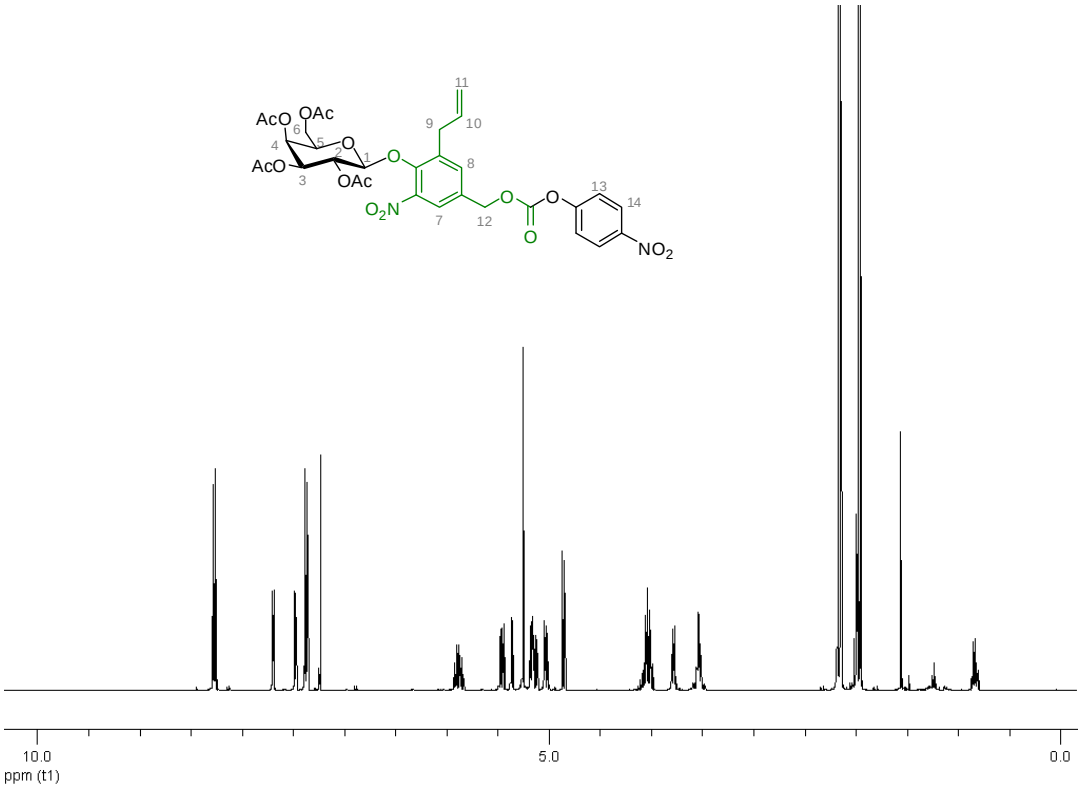
^{13}C NMR of **13**, 100 MHz, 298 K, $CDCl_3$



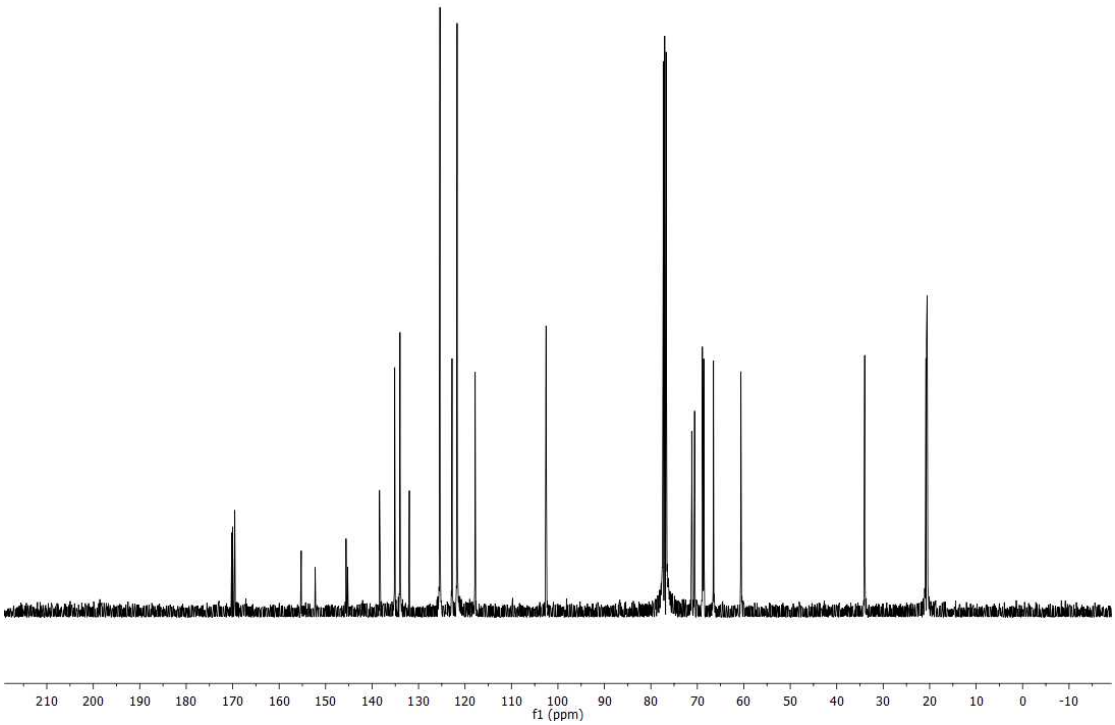
^{1}H NMR of **14**, 400 MHz, 298 K, $CDCl_3$



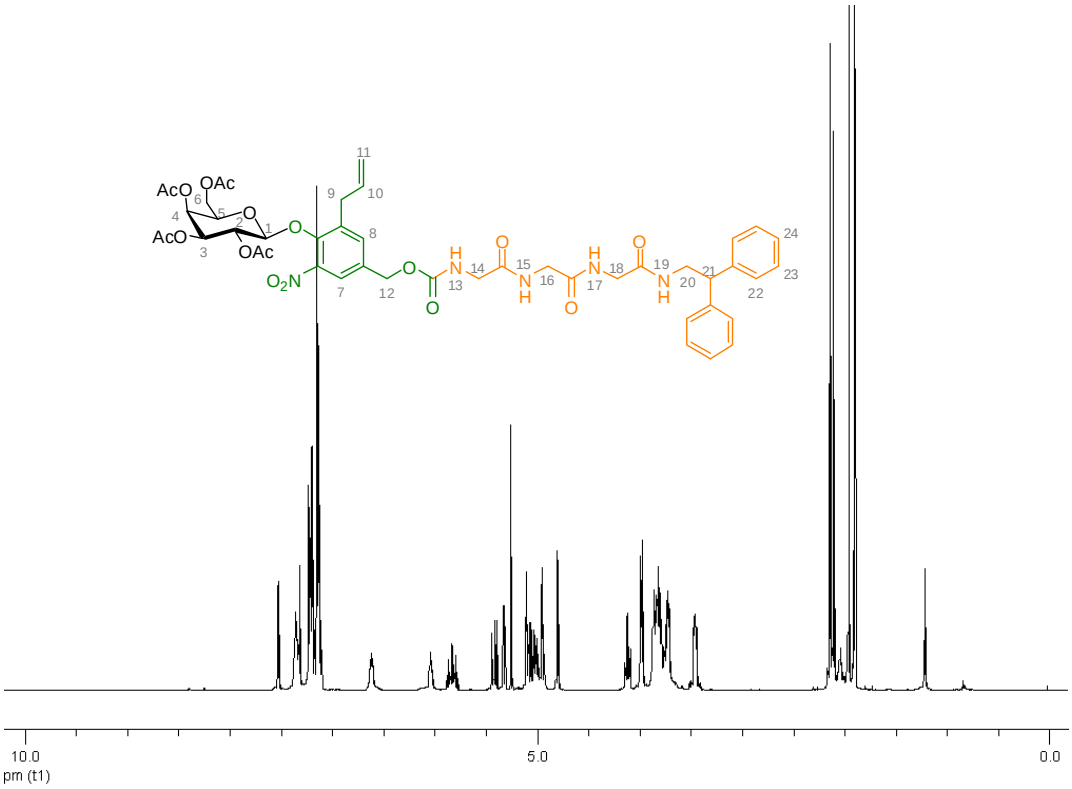
^{13}C NMR of **14**, 100 MHz, 298 K, $CDCl_3$



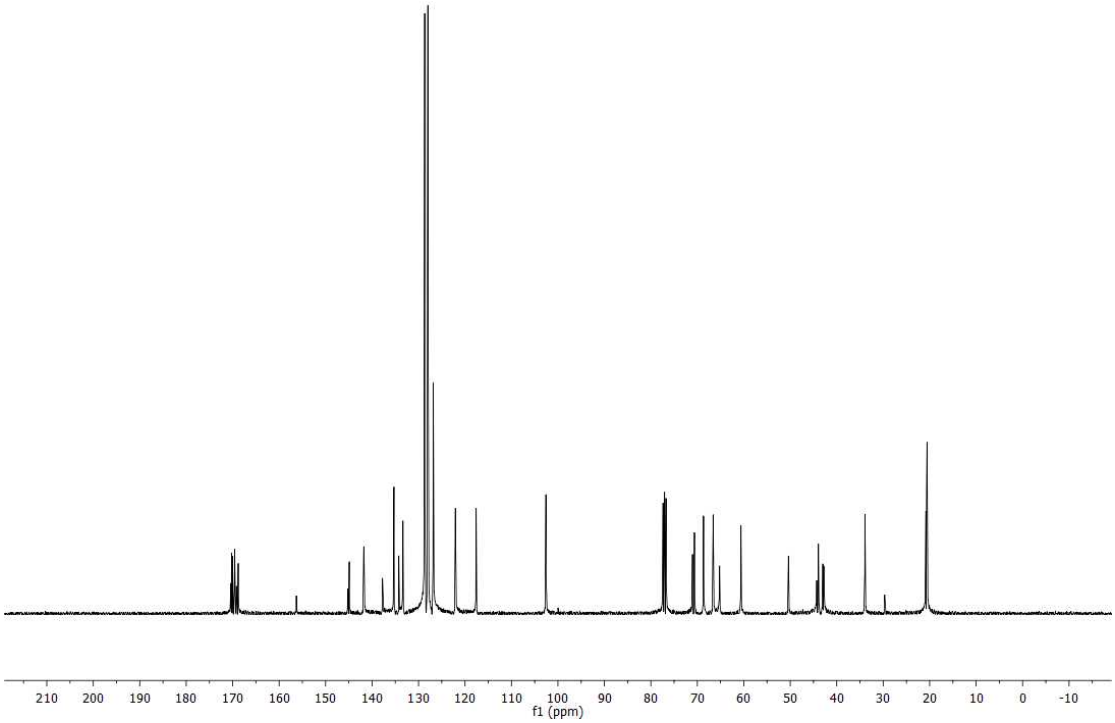
^{1}H NMR of **15**, 400 MHz, 298 K, $CDCl_3$



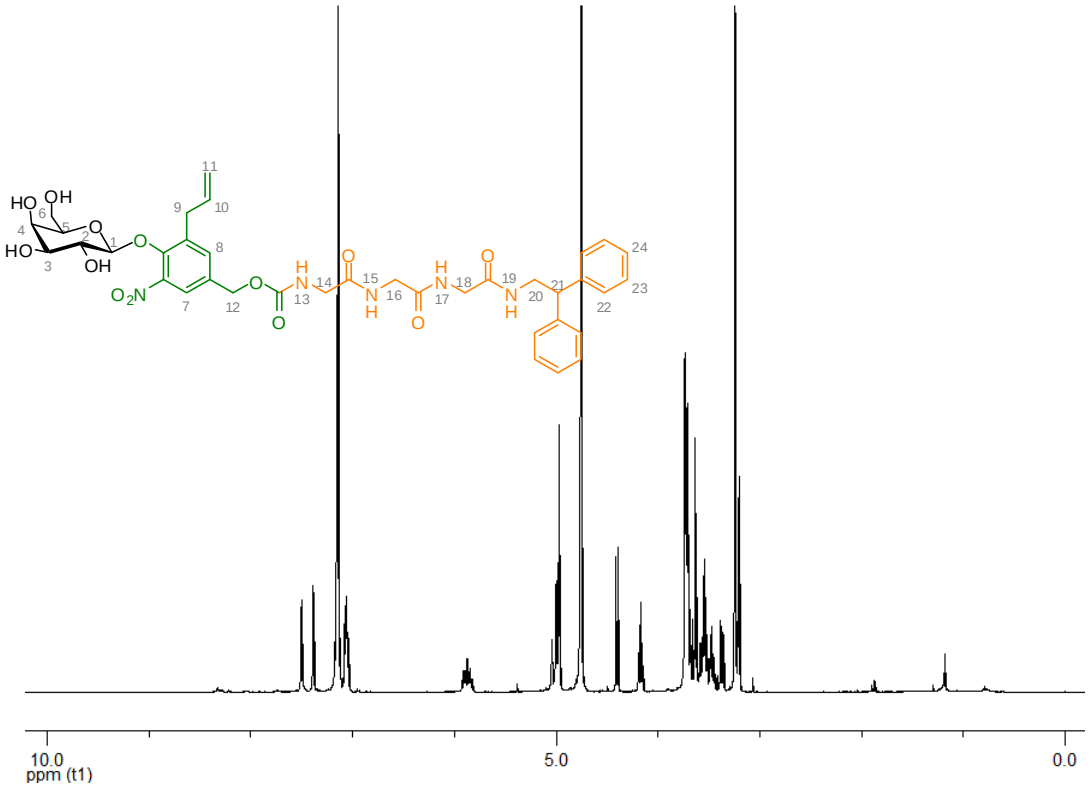
^{13}C NMR of **15**, 100 MHz, 298 K, $CDCl_3$



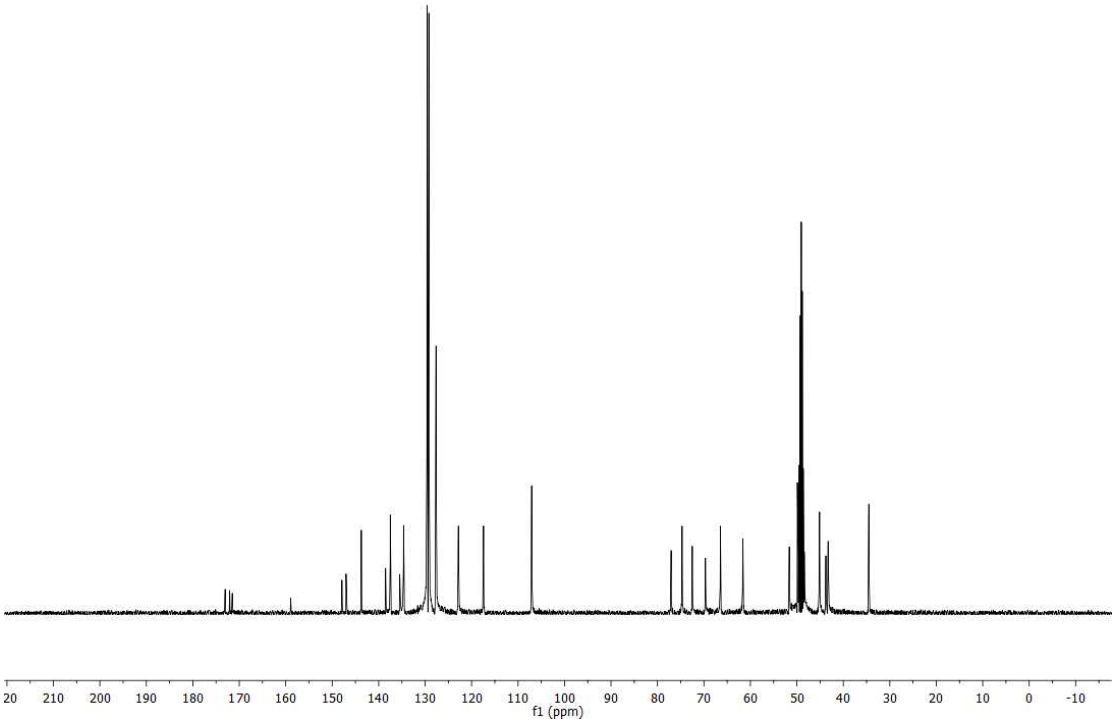
^{1}H NMR of **16**, 400 MHz, 298 K, $CDCl_3$



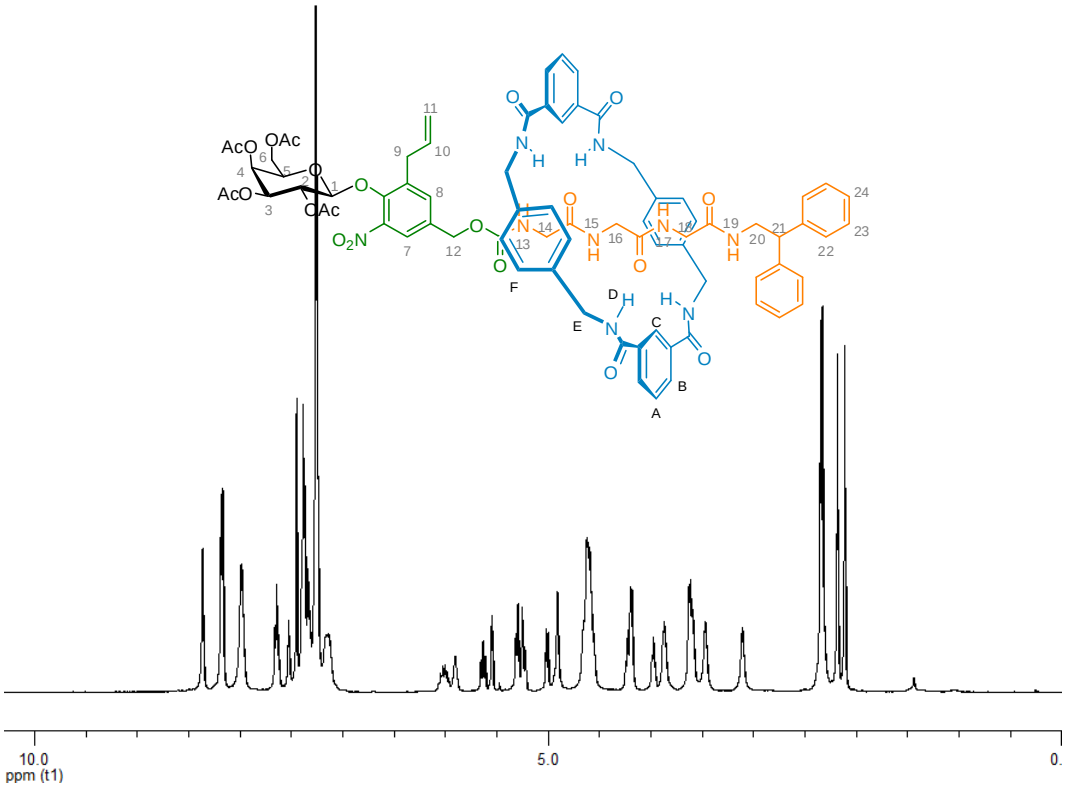
^{13}C NMR of **16**, 100 MHz, 298 K, $CDCl_3$



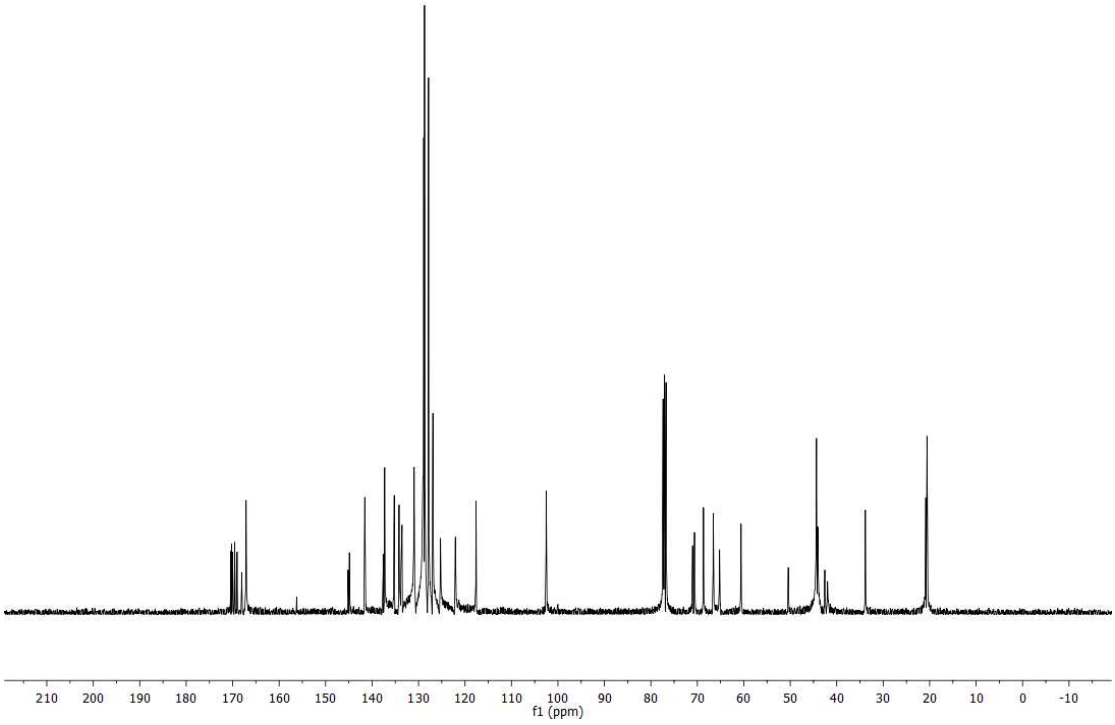
^{1}H NMR of **21**, 400 MHz, 298 K, CD_3OD



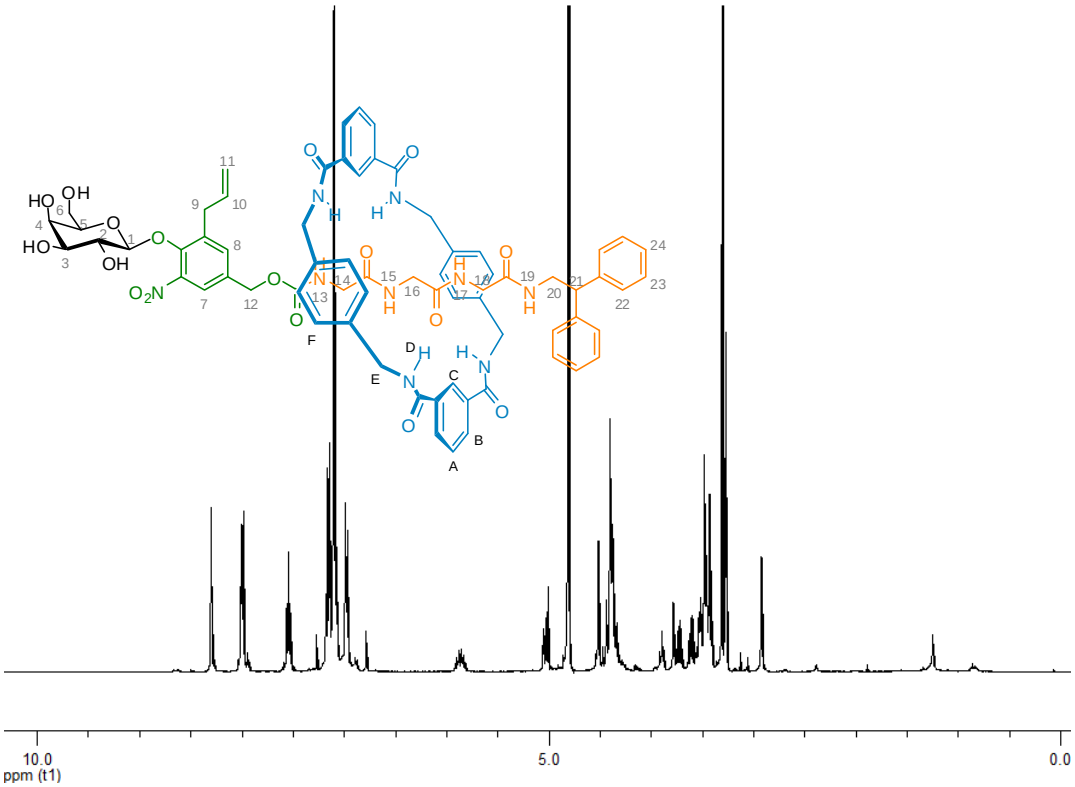
^{13}C NMR of **21**, 100 MHz, 298 K, CD_3OD



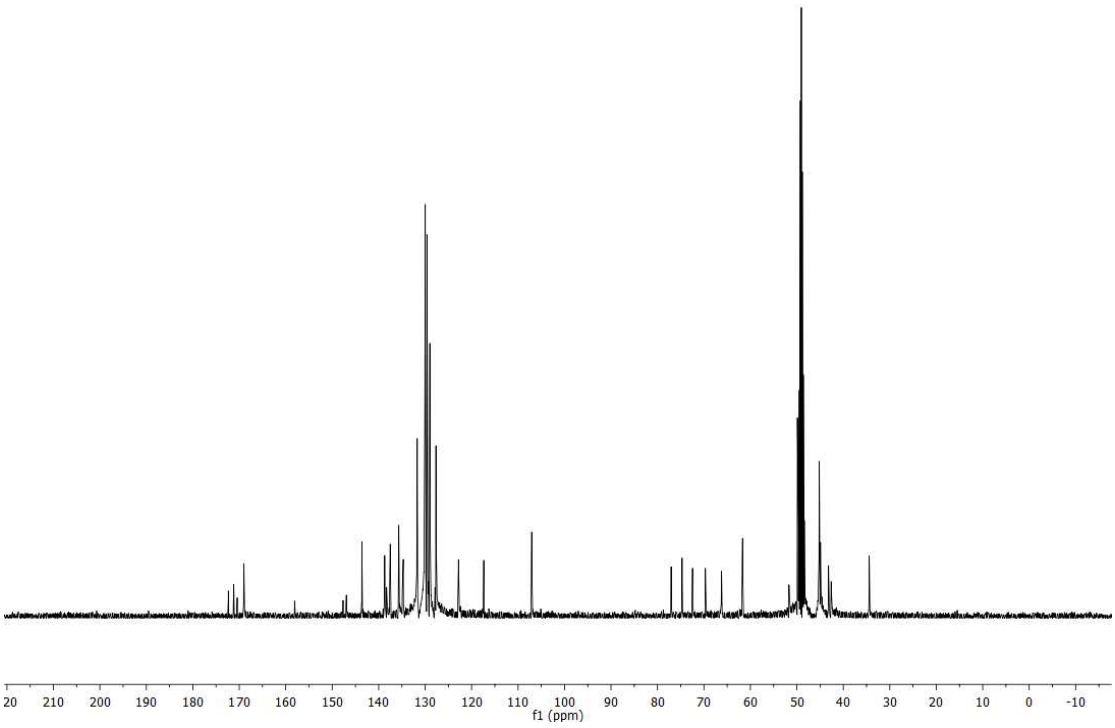
^{1}H NMR of **S1**, 400 MHz, 298 K, $CDCl_3$



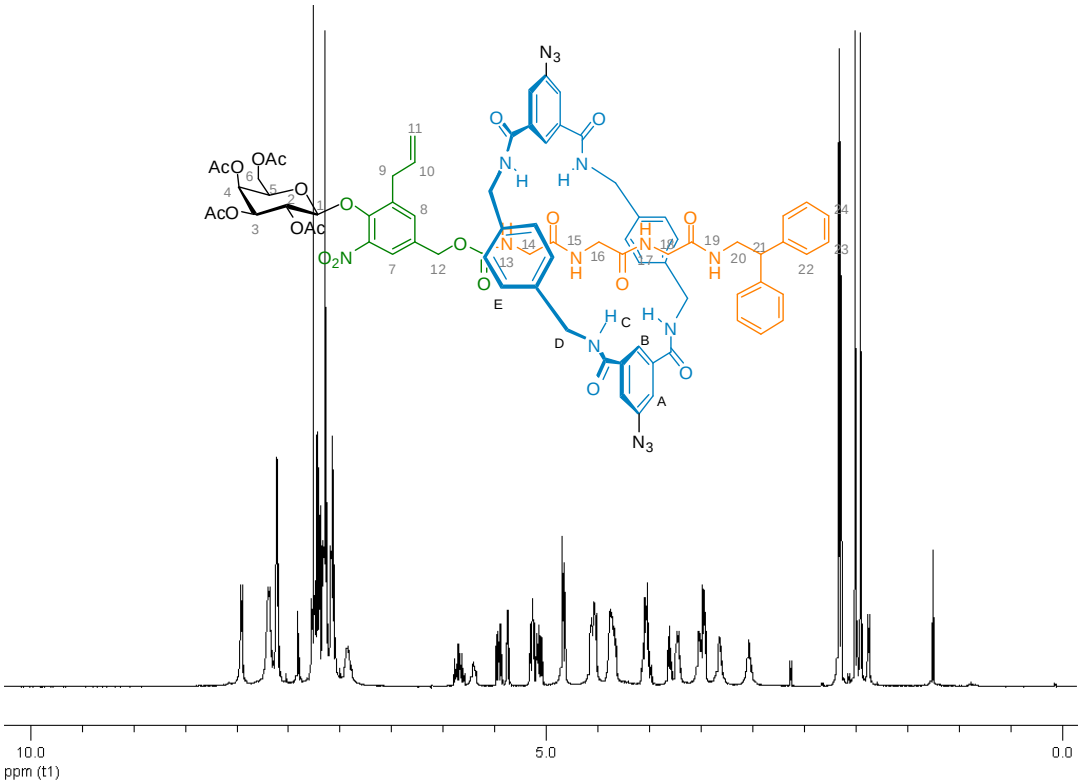
^{13}C NMR of **S1**, 100 MHz, 298 K, $CDCl_3$



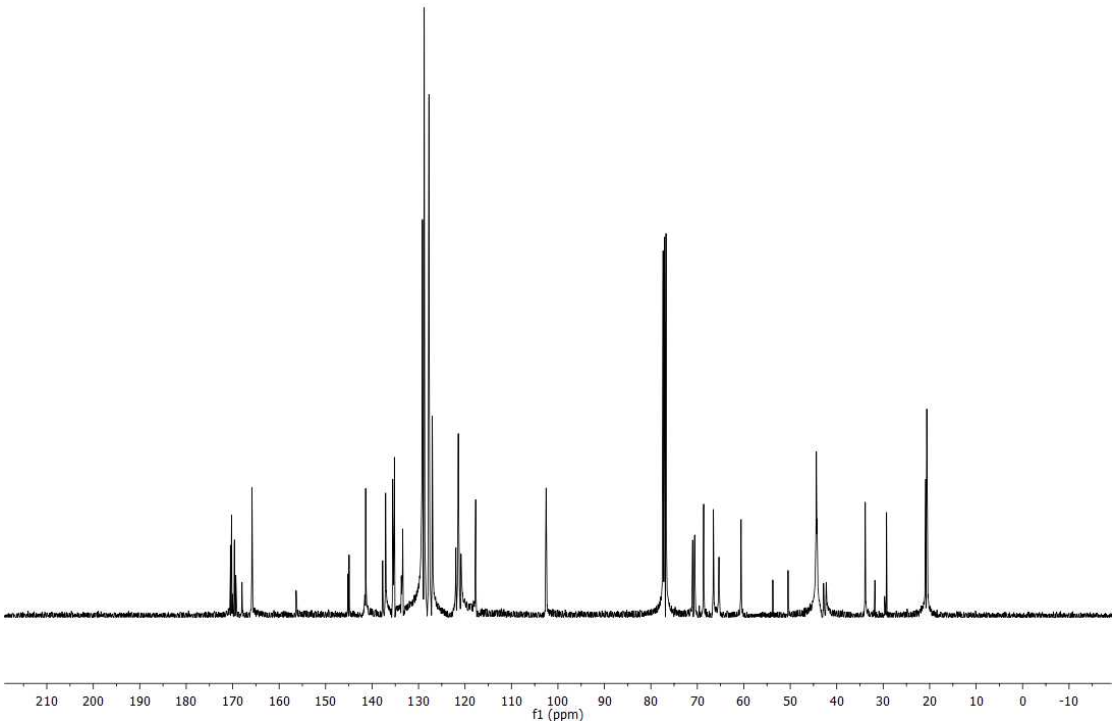
^{1}H NMR of **22**, 400 MHz, 298 K, CD_3OD



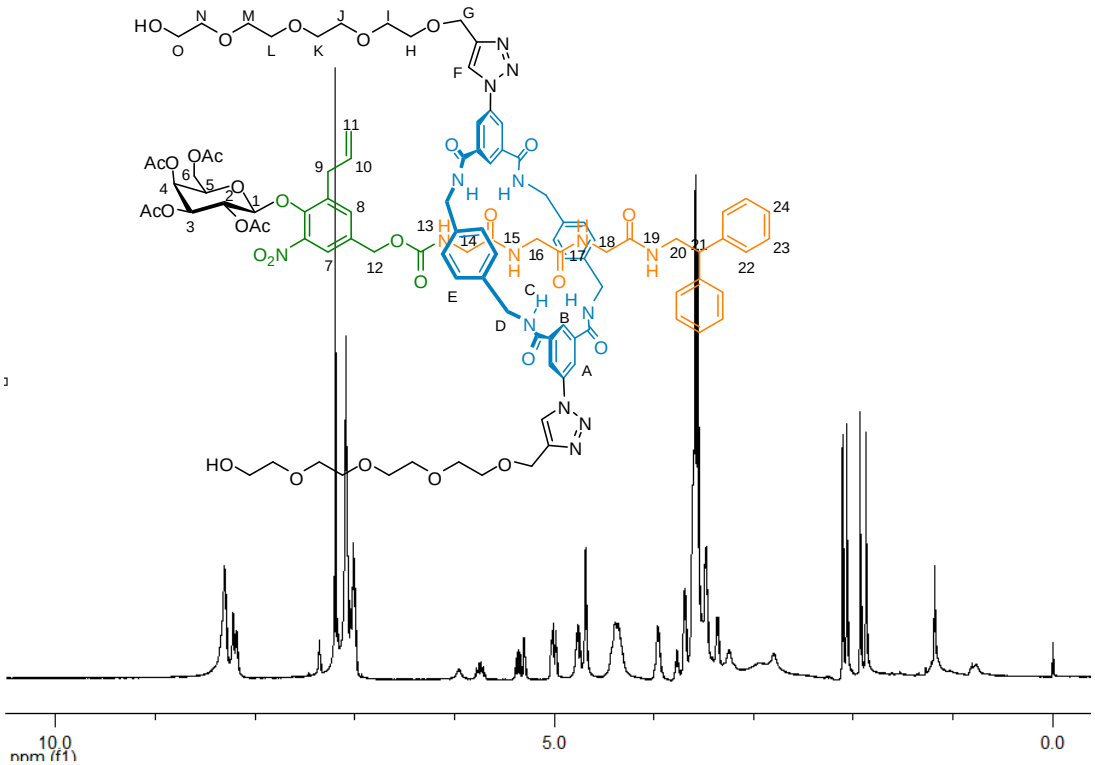
^{13}C NMR of **22**, 100 MHz, 298 K, CD_3OD



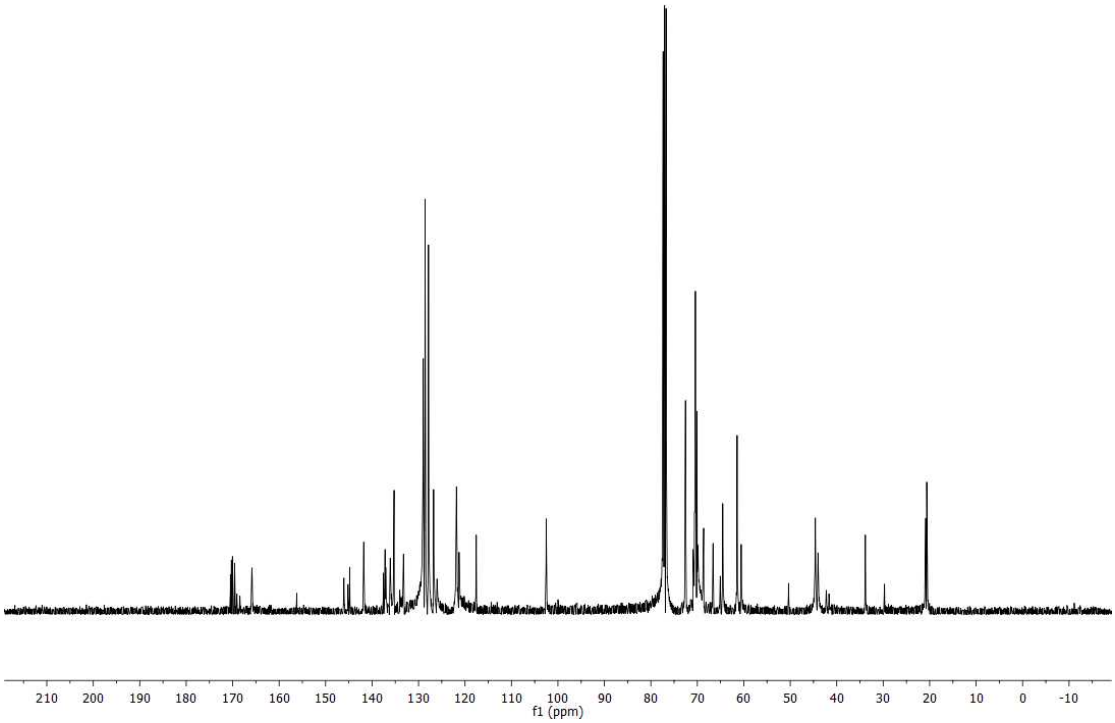
^{1}H NMR of **1**, 400 MHz, 298 K, $CDCl_3$



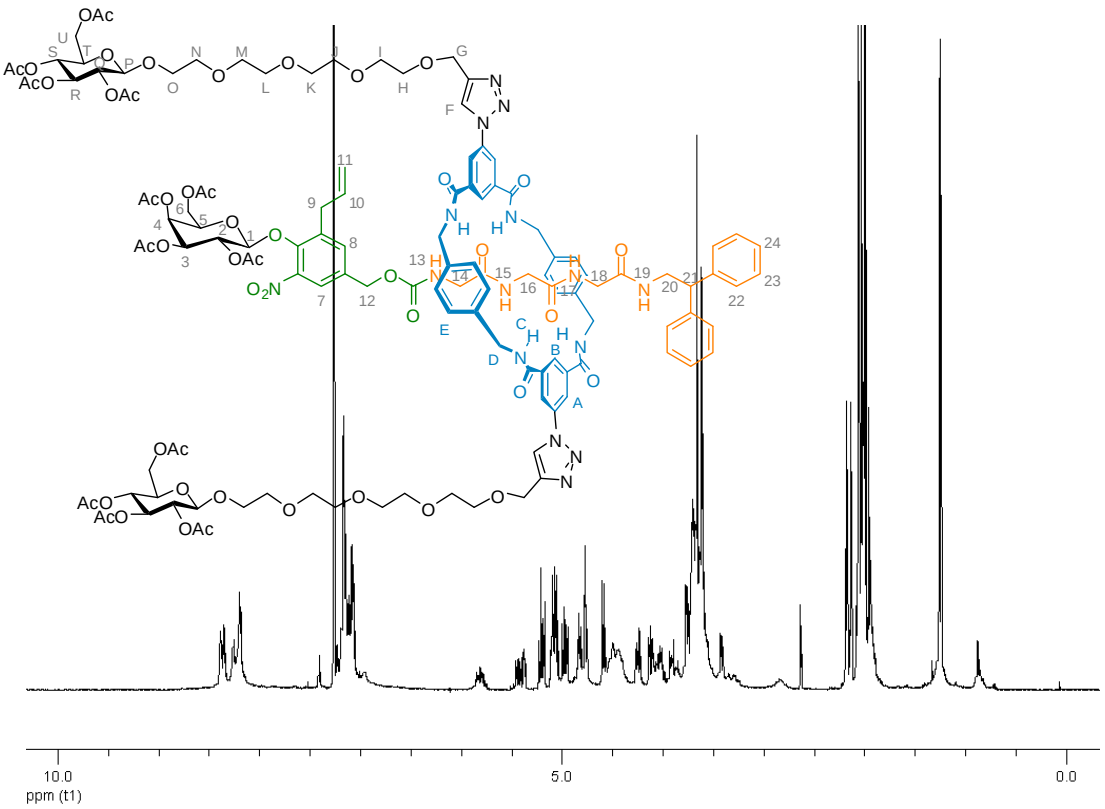
^{13}C NMR of **1**, 100 MHz, 298 K, $CDCl_3$



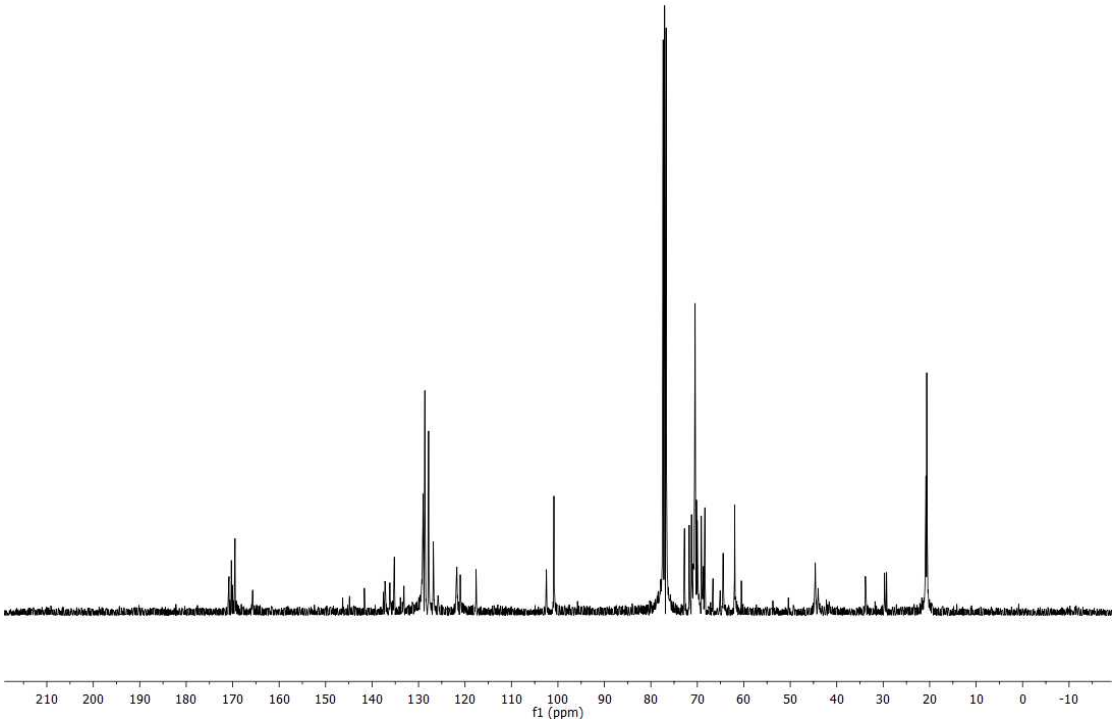
^{1}H NMR of **19**, 400 MHz, 298 K, $CDCl_3$



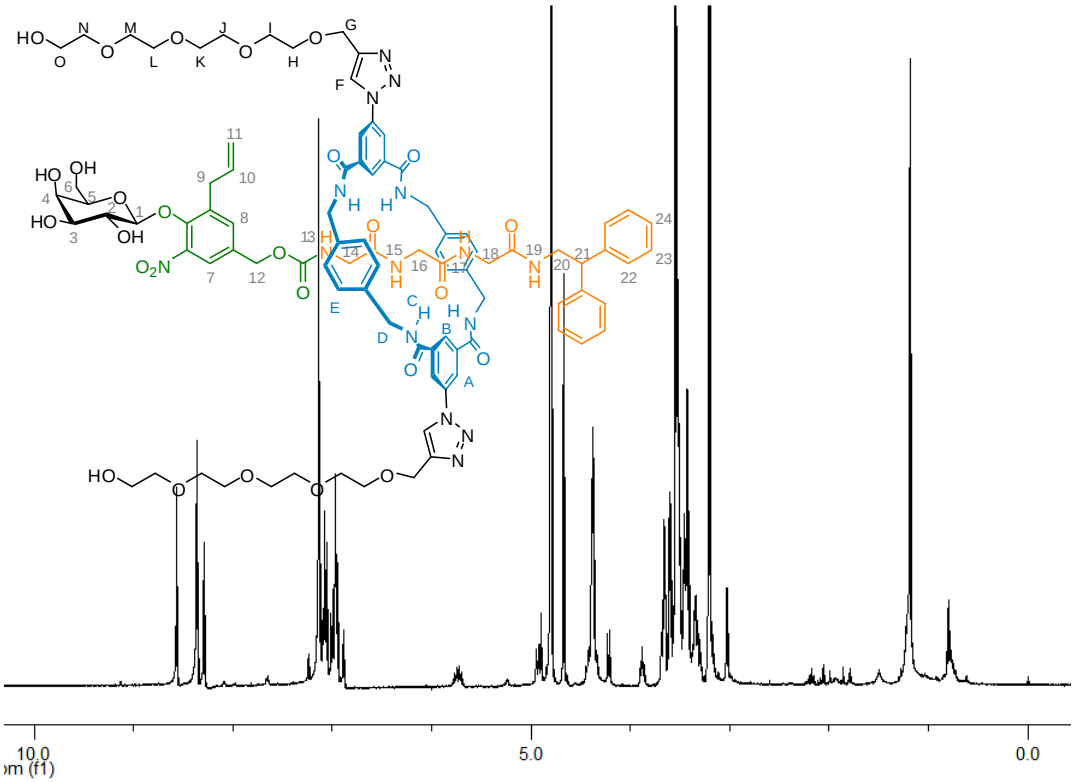
^{13}C NMR of **19**, 100 MHz, 298 K, $CDCl_3$



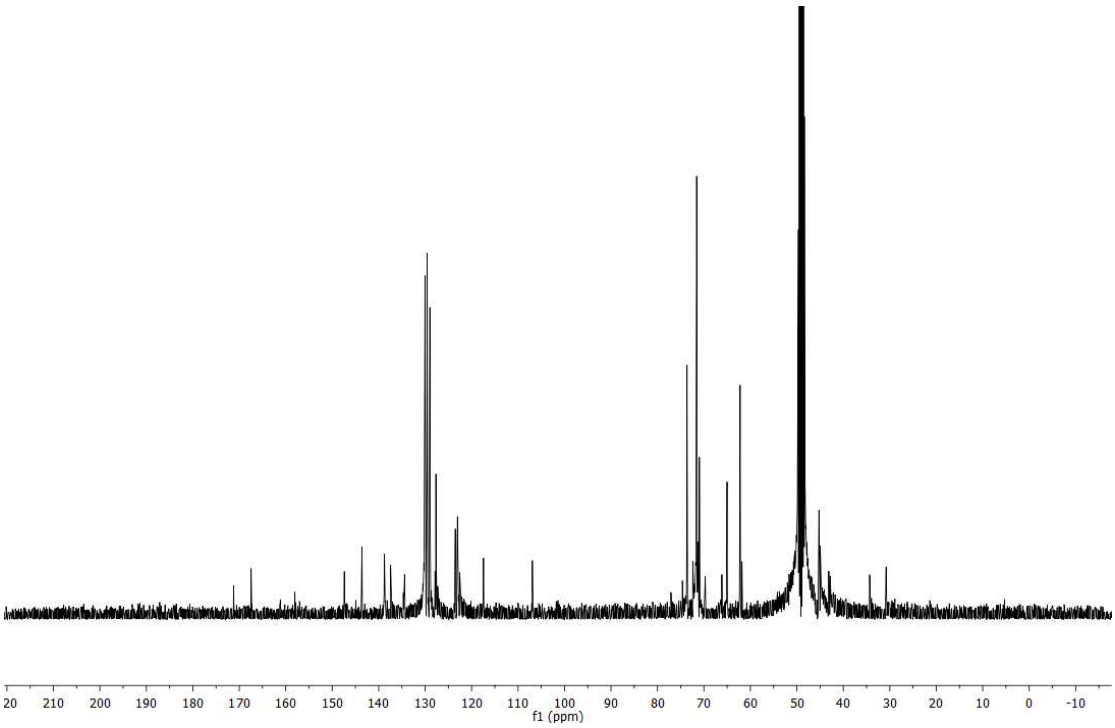
^{1}H NMR of **20**, 400 MHz, 298 K, $CDCl_3$



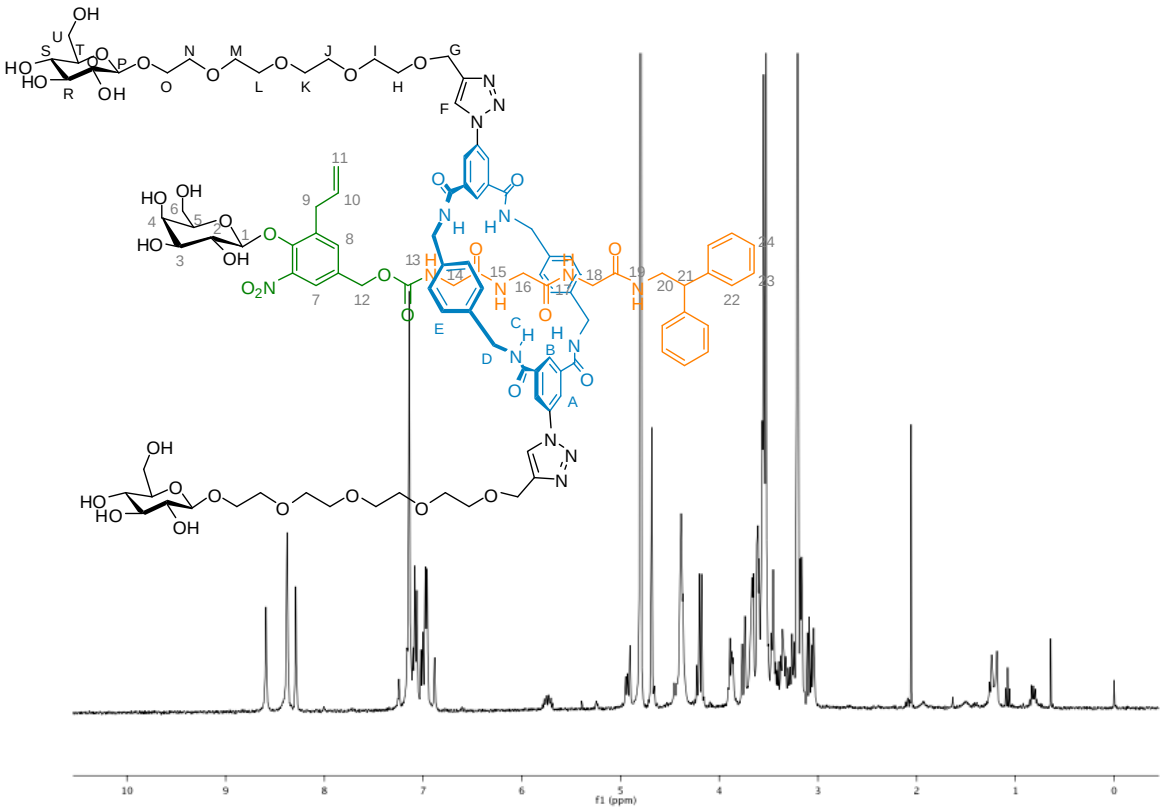
^{13}C NMR of **20**, 100 MHz, 298 K, $CDCl_3$



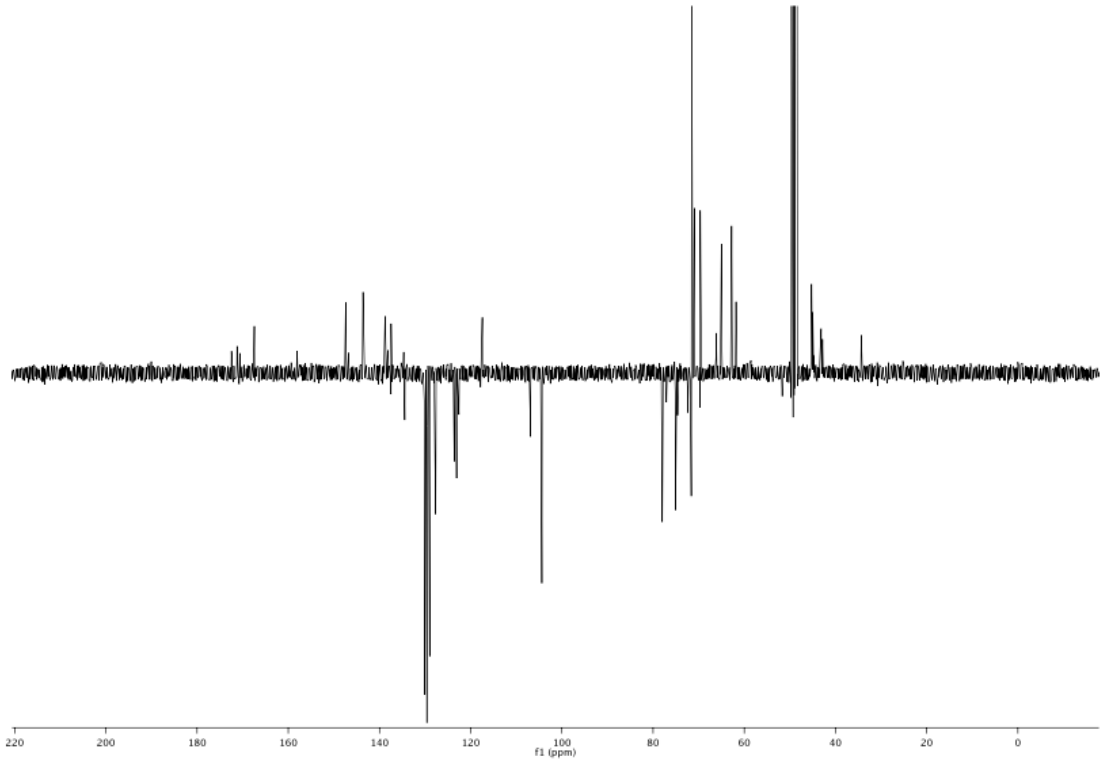
^{1}H NMR of **2**, 400 MHz, 298 K, CD_3OD



^{13}C NMR of **2**, 100 MHz, 298 K, CD_3OD



^{1}H NMR of **3**, 400 MHz, 298 K, CD_3OD



^{13}C NMR (pendant) of **3**, 100 MHz, 298 K, CD_3OD

6. References

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