Supporting Information

Second Generation Specific-Enzyme-Activated Rotaxane Propeptides

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1. General Experimental Section

Unless otherwise stated, all reactions were run under an atmosphere of N₂. Prior to use, isophthaloyl dichloride was purified by recrystallization from hexane; p-xylenelediamine 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™ 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. ¹H and ¹³C 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. H₂O and CH₃CN 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 H₂O and CH₃CN 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 $\beta$-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$_2$O (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$_2$O (X= MeOH, EtOH or CH$_3$CN 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

- Acetobromo-D-galactose with NaBH₄ and silica to give 13 in 79% yield.
- Reaction of 13 with p-nitrophenylchloroformate in pyridine and CH₂Cl₂ to yield 15 in 96% yield.
- Reaction of 15 with p-xylylenediamine and 5-azidoisophthaloyl dichloride in Et₃N and CHCl₃ to give 16 in 63% yield.
- Completion of the synthesis of 1 by reaction of 16 with p-nitrophenylchloroformate in pyridine and CH₂Cl₂ at 160°C for 57% efficiency.
**Scheme S2 - Synthesis of Functionalized Rotaxane Propeptides 2 and 3**

rotaxane 1

\[ \text{Cu(CH}_3\text{CN)}_4PF_6 \]

\[ \text{Et}_3\text{N} \]

\[ \text{CH}_2\text{Cl}_2 \]

17 or 18

\[ \text{17} \text{ or } 18 \]

19 (R=TetEG, 24%)

20 (R=Glc(Ac)=TetEG, 70%)

2 (R=TetEG, 70%)

3 (R=Glc-TetEG, 61%)

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

16 (R=OAc)

MeONa

MeOH

0°C

21 (R=OH) (64%)

p-xylylenediamine

5-azido-isophthaloyl dichloride

Et\_3\text{N}, CHCl\_3

S1 (R=OAc)(51%)

MeONa

MeOH

0°C

22 (R=OH) (87%)
**Scheme S4 - Synthesis of Peptide 6**

1. **S2**
   - Reagents: NaOH, H₂O, EtOH, THF
   - Yield: 82%

2. **S3**
   - Reagents: EDC·HCl, DMAP, CH₂Cl₂
   - Yield: 80%

3. **S4**
   - Reagents: H₂, Pd/C, H₂O, MeOH, THF
   - Yield: 67%
**Scheme S5 - Synthesis of Macrocycles 9 and 10**

1. \(\text{HO-C-(NH}_2\text{)-COOH} + \text{Cl}_2\text{CH-Cl}_2\text{OH} \rightarrow \text{S6} \quad \text{EDC.HCl, DMAP, CH}_2\text{Cl}_2 \quad 84\%\)

2. \(\text{S6} \rightarrow \text{S7} \quad \text{Et}_3\text{N, CHCl}_3 \quad 83\%\)

3. \(\text{R} = \text{H, 17 or 18} \quad \text{Cu(CH}_3\text{CN)}_4\text{PF}_6, \text{Et}_3\text{N, CH}_2\text{Cl}_2\)

4. \(\text{S7} \rightarrow \text{S8} (\text{R}=\text{TetEG, 76\%}) \quad \text{S9} (\text{R}=\text{Glc(Ac)}_2\text{-TetEG, 81\%}) \quad \text{S8} \rightarrow \text{S9} \quad \text{NaOH, H}_2\text{O, THF} \quad 84\%\)

5. \(\text{S7} \rightarrow \text{S8} (\text{R}=\text{TetEG, 76\%}) \quad \text{S9} (\text{R}=\text{Glc-TetEG, 81\%}) \quad \text{S8} \rightarrow \text{S9} \quad \text{NaOH, H}_2\text{O, THF} \quad 9\%\)

6. \(\text{S8} (\text{R}=\text{TetEG, 76\%}) \quad \text{S9} (\text{R}=\text{Glc(Ac)}_2\text{-TetEG, 81\%}) \quad \text{NaOH, H}_2\text{O, THF} \quad 9\%\)

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3. Experimental Procedures

To a solution of 4-hydroxy-3-nitrobenzaldehyde (5.00 g, 29.94 mmol) in CH₃CN (200 mL) were added allyl bromide (10.40 mL, 4.0 equiv.) and K₂CO₃ (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 (MgSO₄), 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.¹ ¹H NMR (400 MHz, CDCl₃): δ 9.91 (s, 1H, CHO), 8.32 (d, 1H, J = 2.0 Hz, H₁), 8.04 (dd, 1H, J = 8.8 Hz, J = 2.0 Hz, H₂), 7.21 (d, 1H, J = 8.8 Hz, H₃), 6.07 to 5.98 (m, 1H, H₅), 5.50 (dd, 1H, J = 17.6 Hz, J = 1.2 Hz, H₆), 5.37 (dd, 1H, J = 7.8 Hz, J = 1.2 Hz, H₆), 4.78 (dt, 2H, J = 4.8 Hz, J = 1.6 Hz, H₄).

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 MgSO₄, 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%. ¹H NMR (400 MHz, CDCl₃): δ 11.41 (bs, 1H, OOH), 9.91 (s, 1H, CHO).
(CHO), 8.51 (d, 1H, J = 2.0 Hz, H1), 8.01 (d, 1H, J = 2.0 Hz, H2), 6.03 to 5.93 (m, 1H, H4), 5.20 to 5.18 (m, 1H, H5), 5.16 (dq, 1H, J = 11.2 Hz, J = 1.6 Hz, H5), 3.53 (d, 2H, J = 6.8 Hz, H3); 13C NMR (100 MHz, CDCl3): 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.

To a solution of 12 (100 mg, 0.48 mmol) in CHCl3/iPrOH (2.40 mL/1.60 mL) was added silica (90 mg). The mixture was cooled in an ice-water bath and NaBH4 (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 CH2Cl2. 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%). 1H NMR (400 MHz, CDCl3): δ 10.92 (bs, 1H, OH), 7.98 (d, 1H, J = 2.0 Hz, H1), 7.48 (d, 1H, J = 2.0 Hz, H2), 6.03 to 5.93 (m, 1H, H4), 5.15 (t, 1H, J = 1.2 Hz, H5), 5.12 (dq, 1H, J = 6.4 Hz, J = 1.2 Hz, H5), 4.65 (s, 2H, H6), 3.48 (d, 2H, J = 6.4 Hz, H3), 1.91 (bs, 1H, CH2-OH); 13C NMR (100 MHz, CDCl3): 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.

To a solution of 12 (570 mg, 2.75 mmol, 1.1 equiv.) in CH3CN (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 Ag2O (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%). 1H NMR (400 MHz, CDCl3): δ 9.97 (s, 1H, CHO),
8.11 (d, 1H, J = 2.0 Hz, H7), 7.96 (d, 1H, J = 2.0 Hz, H8), 5.96 to 5.86 (m, 1H, H10), 5.48 (dd, 1H, J = 10.4 Hz, J = 8.0 Hz, H2), 5.37 (d, 1H, J = 8.0 Hz, H4), 5.22 to 5.14 (m, 2H, H11), 5.05 (dd, 1H, J = 10.4 Hz, J = 8.0 Hz, H3), 4.95 (d, 1H, J = 8.0 Hz, H1), 4.02 (d, 2H, J = 6.8 Hz, H6), 3.81 (dt, 1H, J = 6.8 Hz, J = 0.8 Hz, H5), 3.58 (dd, 2H, J = 6.4 Hz, J = 1.2 Hz, H14), 2.18 (s, 3H, H-Ac), 2.16 (s, 3H, H-Ac), 1.99 (s, 3H, H-Ac), 1.96 (s, 3H, H-Ac); 13C NMR (100 MHz, CDCl3): 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 C24H28NO13 538.1561 [M+H]+); m.p. 78-80°C.

To a solution of 13 (700 mg, 1.30 mmol) in CHCl3/iPrOH (20 mL/14 mL) was added silica (1.0 g). The mixture was cooled in an ice-water bath and NaBH4 (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 CH2Cl2. 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%). 1H NMR (400 MHz, CDCl3): δ 7.60 (d, 1H, J = 2.0 Hz, H9), 7.40 (d, 1H, J = 1.6 Hz, H11), 5.93 to 5.83 (m, 1H, H14), 5.45 (dd, 1H, J = 10.4 Hz, J = 8.0 Hz, H2), 5.35 (d, 1H, J = 2.8 Hz, H4), 5.14 (bs, 1H, H15), 5.11 (dd, 1H, J = 7.2 Hz, J = 1.2 Hz, H15), 5.02 (dd, 1H, J = 10.4 Hz, J = 3.6 Hz, H1), 4.82 (d, 1H, J = 8.0 Hz, H1), 4.69 (bs, 2H, H16), 4.03 (dd, 2H, J = 6.8 Hz, J = 3.6 Hz, H6), 3.77 (t, 1H, J = 6.4 Hz, H3), 3.50 (t, 2H, J = 4.8 Hz, H13), 2.37 (bs, 1H, CH2-OH), 2.18 (s, 3H, H-Ac), 2.15 (s, 3H, H-Ac), 1.98 (s, 3H, H-Ac), 1.96 (s, 3H, H-Ac); 13C NMR (100 MHz, CDCl3): 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 C24H29NO13Na 562.1537 [M+Na]+); m.p. 110-112°C.
To a solution of 14 (433 mg, 0.80 mmol) in CH₂Cl₂ (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 CH₂Cl₂. The combined organic layers were washed with water, dried over MgSO₄ 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. ¹H NMR (400 MHz, CDCl₃): δ 8.29 (d, 2H, J = 9.2 Hz, H₁₄), 7.71 (d, 1H, J = 2.0 Hz, H₇), 7.50 (d, 1H, J = 2.0 Hz, H₈), 7.39 (d, 2H, J = 9.2 Hz, H₁₃), 5.96 to 5.85 (m, 1H, H₁₀), 5.48 (dd, 1H, J = 10.8 Hz, J = 8.0 Hz, H₂), 5.38 (d, 1H, J = 2.4 Hz, H₄), 5.27 (bs, 2H, H₁₂), 5.20 to 5.14 (m, 2H, H₁₁), 5.05 (dd, 1H, J = 10.8 Hz, J = 3.6 Hz, H₃), 4.88 (d, 1H, J = 8.0 Hz, H₁), 4.06 (t, 2H, J = 6.8 Hz, H₆), 3.80 (t, 1H, H₂), 2.20 (s, 3H, H₁₀), 2.17 (s, 3H, H₁₁), 2.01 (s, 3H, H₁₂), 1.97 (s, 3H, H₁₃); ¹³C NMR (100 MHz, CDCl₃): 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, 68.6, 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.

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 CH₂Cl₂ (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 CHCl₃/iPrOH (3:1) (3×50 mL). The combined organic fractions were dried (MgSO₄) and concentrated under reduced pressure. To the crude solid was added Et₂O (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$_3$OD (1:1)): $\delta$ 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$_3$); $^{13}$C NMR (100 MHz, CDCl$_3$/CD$_3$OD (3:1)): $\delta$ 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$_6$N$_3$Na$_1$ 374.1323 [M+Na]+$^+$); m.p. 133-135°C.

To a solution of S$_2$ (2.24 g, 6.37 mmol) in a 2:3:3 mixture H$_2$O/EtOH/THF (320 mL) at 0°C 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$_2$O (30 mL), the suspension was sonicated for 5 minutes, filtered under suction and washed with H$_2$O (50 mL). The solid was re-suspended in a 1:6 mixture of MeOH/Et$_2$O (50 mL), sonicated for 5 minutes, filtered by gravity and washed with Et$_2$O (20 mL) to afford S$_3$ as a colourless solid (1.70 g, 5.25 mmol, 82%). $^1$H NMR (400 MHz, DMSO $d_6$): $\delta$ 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 $d_6$): $\delta$ 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$_6$N$_3$ 324.1190 [M+H]+$^+$); m.p. 182-184°C.

To a solution of S$_3$ (1.52 g, 4.7 mmol), EDCI.HCl (991 mg, 1.1 equiv.) and DMAP (631 mg, 1.1 equiv.) in CH$_2$Cl$_2$ (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 CH₂Cl₂ (3×50 mL). The combined organic fractions were washed with H₂O (40 mL), dried (MgSO₄) and concentrated under reduced pressure to afford S₄ as a colourless solid (1.87 g, 3.72 mmol, 80%). ¹H NMR (400 MHz, DMSO d₆): δ 8.13, 8.05, 7.49 (3t, J = 5.6 Hz, J = 5.8 Hz and J = 6.0 Hz, 3H, H₅+7+9), 7.86 (t, J = 5.5 Hz, 1H, H₁₁), 7.38-7.09 (m, 15H, H₁+2+3+14+15+16), 5.03 (s, 2H, H₄), 4.18 (t, J = 7.7 Hz, 1H, H₁₃), 3.75-3.67 (m, 4H, H₆+₁₁), 3.66, 3.58 (2d, J = 6.1 Hz and J = 5.7 Hz, 4H, H₈+₁₀); ¹³C NMR (100 MHz, DMSO d₆): 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 C₂₈H₃₀O₅N₄ 503.2289 [M+H]⁺); m.p. 210-212°C.

A solution of S₄ (2.65 g, 5.27 mmol) in a 3:4:13 mixture H₂O/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 H₂ by means of three vacuum/H₂ 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 CHCl₃/iPrOH (3/1) (3×50 mL). The combined organic fractions were dried (MgSO₄) and concentrated under reduced pressure. The resulting yellow oil was re-dissolved in CH₂Cl₂ (10 mL) and Et₂O (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%). ¹H NMR (400 MHz, CDCl₃): δ 8.07-7.81 (m, 1H, H₆), 7.42-7.25, 6.75-6.58 (2m, 2H, H₁₂+₁₄), 7.25-6.95 (m, 10H, H₉+₁₀+₁₁), 4.10 (t, 1H, J = 7.8 Hz, H₁₃), 3.75-3.67 (m, 4H, H₁₆+₁₁), 3.75, 3.64, 3.28 (3s, 4H, H₃+₅+₇); ¹³C NMR (100 MHz, CDCl₃): 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 C₂₀H₂₅O₃N₄ 369.1921 [M+H]⁺); m.p. 50-52°C.
To a solution of 15 (529 mg, 0.75 mmol) in CH$_3$CN (35 mL) was added the peptide 6 (332 mg, 1.2 equiv.) and Et$_3$N (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$_2$Cl$_2$/MeOH: 9/1) yielded 16 as a pale brown powder (599 mg, 0.64 mmol, 85%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 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$_3$): $\delta$ 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$_5$O$_{17}$ 934.3359 [M+H]$^+$); m.p. 126-128°C.

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 (CH₂Cl₂/MeOH: 5 to 20% MeOH) to afford 21 (57 mg, 0.07 mmol, 64%) as a pale brown powder. ¹H NMR (400 MHz, CD₃OD): δ 7.57 (d, 1H, J = 2.0 Hz, H₇), 7.45 (d, 1H, J = 2.0 Hz, H₈), 7.24 to 7.19 (m, 8H, H₂₂+2₃), 7.14 to 7.10 (m, 2H, H₂₄), 5.99 to 5.89 (m, 1H, H₁₀), 5.11 to 5.04 (m, 4H, H₁₁+₁₂), 4.47 (d, 1H, J = 8.0 Hz, H₁), 4.24 (t, 1H, J = 8.0 Hz, H₂₁), 3.80 to 3.73 (m, 8H, H₁₄+₁₆+₁₈+₂₀), 3.70 (m, 2H, H₄+₅), 3.66 to 3.54 (m, 5H, H₂+₆+₉), 3.44 (dd, 1H, J = 10.0 Hz, J = 3.6 Hz, H₃); ¹³C NMR (100 MHz, CD₃OD): 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 C₃₇H₄₄N₅O₁₃ 766.2936 [M+H]⁺); m.p. 145-147°C.

Thread 16 (200 mg, 0.21 mmol) and Et₃N (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 CHCl₃/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 CH₂Cl₂/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 CHCl₃/MeOH (50/50) as eluent to give rotaxane S1 as a pale brown powder (161 mg, 0.11 mmol, 51%).
NMR (400 MHz, CDCl3): 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, NH15+17+19), 5.89 to 5.79 (m, 1H, H 10), 5.73 (bs, 1H, NH13), 5.46 (t, 1H, J = 8.0 Hz, H 2), 5.37 (bs, 1H, H 4), 5.14 to 5.05 (m, 3H, H3+11), 4.84 (d, 1H, J = 8.0 Hz, H 1), 4.74 (bs, 2H, H12), 4.48 to 4.39 (m, 8H, HE), 4.05 (t, 1H, J = 8.0 Hz, H21), 4.02 (d, 2H, J = 6.0 Hz, H 6), 3.80 (t, 1H, J = 6.0 Hz, H 5), 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, 

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 (CH2Cl2/MeOH: 2 to 10% MeOH) to afford 22 (75 mg, 0.058 mmol, 87%) as a pale brown powder. 1H NMR (400 MHz, CD3OD): δ 8.34 (bs, 2H, Hc), 8.05 to 8.03 (m, 4H, Hb), 7.59 (t, 2H, J = 7.6 Hz, Ha), 7.23 to 7.01 (m, 18H, H22+23+24+F), 5.97 to 5.87 (m, 1H, H10), 5.09 (dd, 1H, J = 8.8 Hz, J = 1.6 Hz, H10), 5.05 (bs, 1H, H11), 4.57 (bs, 2H, H12), 4.48 to 4.39 (m, 9H), 3.93 (t, 1H, J = 8.0 Hz, H21), 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,
Thread 16 (400 mg, 0.43 mmol) and Et$_3$N (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-azidoisophthaloyl 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%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.88 (bs, 2H, H$_B$), 7.62 (bs, 4H, H$_C$), 7.54 (bs, 4H, H$_A$), 7.34 (s, 1H, H$_1$), 7.18 to 7.17 (m, 1H, NH), 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, NH), 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$_9$), 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,
To a solution of peracetylated β-D-glucose (317 mg, 0.81 mmol) and alkyne 17 (283 mg, 1.5 equiv.) in anhydrous CH₂Cl₂ (2 mL) under N₂ at 0°C, was added BF₃·Et₂O (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 NaHCO₃ (10 mL) was added, the layers were separated and the aqueous layer was extracted with CH₂Cl₂ (3×15 mL). The combined organic fractions were dried (Na₂SO₄) and concentrated under reduced pressure. The resulting brown residue was purified by flash column chromatography on silica gel with CH₂Cl₂/acetone (93/7) as eluent to give pure 18 as a colourless oil (378 mg, 0.67 mmol, 83%). ¹H NMR (400 MHz, CDCl₃): δ 5.13 (t, 1H, J = 9.6 Hz, H₃), 5.01 (t, 1H, J = 9.6 Hz, H₄), 4.91 (dd, 1H, J = 8.0 Hz, J = 9.6 Hz, H₂), 4.55 (d, 1H, J = 8.0 Hz, H₁), 4.19 (dd, 1H, J = 4.7 Hz, J = 12.3 Hz, H₆), 4.14 (d, 2H, J = 2.4 Hz, H₁₅), 4.06 (dd, 1H, J = 2.3 Hz, J = 12.3 Hz, H₆'), 3.87 (dt, 1H, J = 4.0 Hz, J = 11.0 Hz, H₇), 3.71 to 3.52 (m, 16H, H₅+7'+8+9+10+11+12+13+14), 2.40 (t, 1H, J = 2.3 Hz, H₁₆), 2.01, 1.97, 1.95, 1.93 (4s, 12H, H-Ac); ¹³C NMR (100 MHz, CDCl₃): 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 C₂₅H₄₂O₁₄N 580.2600 [M+ NH₄]⁺).
To a solution of rotaxane 1 (100 mg, 64.60 μmol) and alkyne 17 (30 mg, 2.0 equiv.) in CH₂Cl₂ (10 mL) was added Cu(CH₃CN)₄PF₆ (12 mg, 0.5 equiv.) and Et₃N (20 μL, 2.2 equiv.) and the solution stirred overnight at room temperature. A saturated NH₄Cl 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 MgSO₄, filtrated and concentrated under reduced pressure. The resulting solid was purified by column chromatography on silica gel using CHCl₃/acetone (30 to 55% acetone) to give rotaxane 19 as a pale yellow powder (31 mg, 15.40 μmol, 24%). ¹H NMR (400 MHz, CDCl₃): 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, HE+22+23+24+15+17+19), 5.96 (bs, 1H, H13), 5.79 to 5.69 (m, 1H, H10), 5.36 (dd, 1H, J = 10.4 Hz, J = 8.0 Hz, H2), 5.30 (d, 1H, H4), 5.03 to 4.98 (m, 3H, H1+12), 4.68 (bs, 4H, H G), 4.39 to 4.35 (m, 8H, H D), 4.39 to 4.35 (m, 8H, H D), 3.77 to 3.94 (m, 3H, H 6+21), 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, H14+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); ¹³C NMR (100 MHz, CDCl₃): 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 C₉₈H₉₈¹³CN₁₅O₃₁ 2013.8152 [M+H]⁺).
To a solution of rotaxane 1 (70 mg, 45.20 μmol) and alkyne 18 (51 mg, 2.0 equiv.) in CH₂Cl₂ (7 mL) was added Cu(CH₃CN)₄PF₆ (8 mg, 0.5 equiv.) and Et₃N (14 μL, 2.2 equiv.) and the solution was stirred overnight at room temperature. A saturated NH₄Cl 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 MgSO₄, filtrated and concentrated under reduced pressure. The resulting solid was purified by preparative TLC on silica eluted 4 times with CHCl₃/acetone (1:1) as eluent to give rotaxane 20 as a pale yellow powder (84 mg, 31.6 μmol, 70%). ¹H NMR (400 MHz, CDCl₃): 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, H7), 7.22 (s, 1H, H8), 7.18 to 6.95 (m, 21H, H 15+17+19+22+23+24+E), 5.85 to 5.75 (m, 2H, H10+13), 5.43 (dd, 1H, J = 10.4 Hz, J = 8.0 Hz, H2), 5.37 (d, 1H, J = 3.6 Hz, H4), 5.20 (t, 2H, J = 9.6 Hz, H6), 5.10 to 5.04 (m, 5H, H3+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, H6+21), 3.77 to 3.76 (m, 3H, H5+7), 3.72 to 3.56 (m, 36H, H 14+20+H+1+J+K+L+M+N+O), 3.42 (d, 2H, J = 6.4 Hz, H6), 3.34 to 3.25 (m, 2H, H18), 2.84 (bs, 2H, H16), 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); ¹³C NMR (100 MHz, CDCl₃): 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), 20.7
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\(_{126}\)H\(_{131}\)N\(_{15}\)O\(_{49}\)Na calcd. 2695.9873 [M+Na]^+); m.p. 133-135°C.

![Image of rotaxane 2](image_url)

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\(_3\)CN (containing 6.6 mM of HCOOH) in H\(_2\)O (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\(_3\)OD): \(\delta\) 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\(_3\)OD): \(\delta\) 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.
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 H₂O (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%. ¹H NMR (400 MHz, CD₃OD): δ 8.69 (s, 2H, HB), 8.47 (s, 4H, HA), 8.39 (s, 2H, HF), 7.41-6.92 (m, 20H, H₇+₈+₂₂+₂₃+₂₄+E), 5.92-5.77 (m, 1H, H₁₀), 5.07-4.98 (m, 2H, H₁₁), 4.78 (s, 4H, H₁₂), 4.56-4.38 (m, 8H, HD), 4.32 (d, J = 7.7 Hz, 2H, H₁), 4.29 (d, J = 7.8 Hz, 2H, HP), 4.04-3.93 (m, 3H, HO+21), 3.90-3.10 (m, 52H, H₁₃+J+K+L+M+N+O'+U+2+3+4+5+6+9+12+14+16+18+2₀); ¹³C NMR (100 MHz, CD₃OD): 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 C₁₀₃H₁₃₁N₁₅O₇; m.p. 129-131°C (decomposition).
To a solution of 5-amino-pentan-1-ol (3.0 g, 29.10 mmol) in dry DMF (40 mL) at 0 °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 redissolved in CH2Cl2 (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%). 1H NMR (400 MHz, DMSO d6): δ 8.36 (t, 2H, J = 5.6 Hz, H6), 6.80 (s, 2H, H7), 3.36 (t, 4H, J = 6.4 Hz, H1), 3.12 (q, 4H, J = 6.0 Hz, H5), 1.47-1.34 (m, 8H, H2+H4), 1.32-1.21 (m, 4H, H3); 13C 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 C14H27N2O4 287.1965 [M+H]+); m.p. = 219-222°C.

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 CH2Cl2 (30 mL) at room temperature, was added bis-alcohol S5 (0.49 g, 1.71 mmol). The reaction mixture was stirred overnight. CH2Cl2 (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 CH2Cl2 (2×25 mL). The combined organic fractions were washed with an aqueous saturated solution of NaHCO3, dried (MgSO4) and concentrated under reduced pressure. Purification by flash column chromatography on silica using CH2Cl2/MeOH (98/2) as eluent gave S6 as a colourless solid (1.50 g, 1.41 mmol, 84%). 1H NMR (400 MHz, CDCl3/CD3OD: 9/1): δ 8.01 (t, 2H, J = 5.6 Hz, H9), 7.23 to 7.16 (m, 12H, H1), 7.12 to 7.04 (m, 12H, H2), 6.71 (s, 2H, H10), 3.75 (t, 4H, J = 6.5 Hz, H4), 3.59 (s, 4H, H3), 3.23 to 3.17 (m, 4H, H8), 1.51-1.26 (m, 8H, H5+7), 1.19 to 1.05 (m, 4H, H6); 13C NMR (400 MHz, CDCl3/CD3OD: 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}H_{52}D_{35}Cl_{6}N_{2}O_{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 Et3N (2 mL, 30.0 equiv.) in 1 L of dry CHCl3 under nitrogen, was simultaneously added a solution of p-xylylenediamine (1.00 g, 15.0 equiv.) in CHCl3 (50 mL) and a solution of 5-azido-isophthaloyl dichloride (1.81 g, 15.0 equiv.) in CHCl3 (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/CH2Cl2 (10/90) as eluent to give rotaxane S7 (0.69 g, 4.12 mmol, 83%). 1H NMR (400 MHz, CDCl3): 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); 13C NMR (100 MHz, CDCl3): 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}Cl_{6}N_{13}O_{10} 1694.4439 [M+NH4]^+); isotopic distribution matches that calculated for C_{88}H_{82}Cl_{6}N_{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(CH3CN)_4PF6 (0.5 equiv.) in dry CH2Cl2 (5 mL) and under N2 at room temperature was added Et3N (2.2 equiv.) and the mixture was stirred overnight. Air was bubbled through the mixture for 15 min.
then a saturated solution of NH₄Cl was added (15 mL). The layers were separated and
the aqueous layer was extracted with CH₂Cl₂ (10 mL). The combined organic
fractions were washed with a 1 M solution of EDTA/K₂CO₃ (2x10 mL) and brine (10
mL), then dried (MgSO₄) and concentrated under reduced pressure.

Using the general method, rotaxane S7 (97 mg, 0.060 mmol), alkyne 17 (27 mg, 2.0
equiv.), Cu(CH₃CN)₄PF₆ (11 mg, 0.5 equiv.) and Et₃N (16 μL, 2.2 equiv.) in CH₂Cl₂
(15 mL) at room temperature overnight gave S8 as a pale yellow solid (115 mg, 0.054
mmol, 93%). ¹H NMR (400 MHz, CDCl₃): δ 8.50 to 8.23 (m, 8H, H A, H B+H 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, H₂+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 7+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); ¹³C NMR (100 MHz, CDCl₃): 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
C₁₁₀H₁₁₈Cl₆N₁₂O₂₀Na; m.p. 100-102°C.
Using the general method, rotaxane S7 (250 mg, 0.150 mmol), alkyne 18 (168 mg, 2.0 equiv.), Cu(CH3CN)4PF6 (28 mg, 0.5 equiv.) and Et3N (455 µL, 2.2 equiv.) in CH2Cl2 (15 mL) at room temperature overnight gave S9 as a pale yellow solid (296 mg, 0.105 mmol, 78%). 1H NMR (400 MHz, CDCl3): δ 8.41 (s, 2H, Hb), 8.36 (s, 4H, Ha), 8.26 (s, 2H, Hf), 7.98-7.77 (m, 4H, Hc), 7.74-7.56 (m, 2H, Hg), 7.22-7.12 (m, 12H, H1), 7.12-6.97 (m, 20H, H2 and H5), 5.75 (s, 2H, H10) 5.21 (t, 2H, J = 9.4 Hz, Hr), 5.08 (t, 2H, J = 9.6 Hz, Hs), 4.98 (t, 2H, J = 8.8 Hz, Hz), 4.80 (t, 4H, Hg), 4.60 (d, 2H, J = 7.9 Hz, Hp), 4.47-4.30 (m, 8H, HD), 4.25 (dd, 2H, J = 4.4 Hz and J = 12.2 Hz, Hu), 4.13 (d, 2H, J = 11.5Hz, Hu'), 3.93 (dt, 2H, J = 4.3Hz and J = 11.1 Hz, Hh), 3.81-3.45 (m, 40H, H3+4+H'1+2), 3.20-297 (m, 4H, H8), 2.07, 2.04, 2.01, 1.99 (4s, 24H, HAc), 1.51-1.17 (m, 8H, H5 and H7), 1.17-1.00 (m, 4H, H6); 13C NMR (100 MHz, CDCl3): 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 C138H154C6N12O38Na; m.p. 96-98°C.
Rotaxane S8 (40 mg, 0.018 mmol) was stirred for 4 days at room temperature in a 1 M solution of NaOH in THF/H2O/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 CH2Cl2/MeOH (15:85) to give macrocycle 9 as a waxy solid (18 mg, 0.016 mmol, 90%). 1H NMR (400 MHz, CD3OD/CDCl3 : 1/1): δ 8.41 (2s, 4H, H A), 8.35 (bs, 2H, H B), 8.11 (t, 2H, J = 1.4 Hz, HF), 7.28 (s, 8H, H E), 4.53 (s, 8H, H D), 4.70 (s, 2H, H G), 3.72-3.49 (m, 32H, HH+I+J+K+L+M+N+O); 13C NMR (100 MHz, CD3OD/CDCl3: 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 C54H66N10O14Na 1101.4652 [M+Na]+).
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$_2$O/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$_3$CN/H$_2$O (10 mL), filtered by gravity and the filtrate used for purification by preparative RP-HPLC using a linear gradient of CH$_3$CN (25 to 40% in 20 min) in H$_2$O to give macrocycle 10 as a colourless solid (18 mg, 0.012 mmol, 25%). $^1$H NMR (400 MHz, CD$_3$OD/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$_3$OD/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, CDCl$_3$)
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$_3$
$\text{S8}$

$\text{1H NMR of S8, 400 MHz, 298 K, CDCl}_3$

$\text{13C NMR (pendant) of S8, 100 MHz, 298 K, CDCl}_3$
$^1$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$_3$OD/CDCl$_3$: 1/1

$^{13}$C NMR (pendant) of 9, 100 MHz, 298 K, CD$_3$OD/CDCl$_3$: 1/1
$^1$H NMR of 10, 400 MHz, 298 K, CD$_3$OD/CDCl$_3$: 1/1

$^{13}$C NMR (pendant) of 10, 100 MHz, 298 K, CD$_3$OD/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$_3$OD

$^{13}$C NMR of 21, 100 MHz, 298 K, CD$_3$OD
1H NMR of S1, 400 MHz, 298 K, CDCl₃

13C NMR of S1, 100 MHz, 298 K, CDCl₃
$^1$H NMR of 22, 400 MHz, 298 K, CD$_3$OD

$^{13}$C NMR of 22, 100 MHz, 298 K, CD$_3$OD
**1H NMR of** 1, 400 MHz, 298 K, CDCl₃

**13C NMR of** 1, 100 MHz, 298 K, CDCl₃
\[ ^1\text{H NMR of 19, 400 MHz, 298 K, CDCl}_3 \]

\[ ^{13}\text{C NMR of 19, 100 MHz, 298 K, CDCl}_3 \]
$^1$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$_3$OD

$^{13}$C NMR of 2, 100 MHz, 298 K, CD$_3$OD
$^1$H NMR of 3, 400 MHz, 298 K, CD$_3$OD

$^{13}$C NMR (pendant) of 3, 100 MHz, 298 K, CD$_3$OD
6. References
