Supplementary Information

Fluorescence Resonance Energy Transfer labels with interlocked molecule

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1. General Experimental

^{1}H NMR spectra were recorded at 600 MHz using a Bruker DRX-600 spectrometer or at 300 MHz using a Varian Mercury 300 spectrometer, and ^{13}C NMR spectra were recorded at 151 MHz using a Bruker DRX-600 spectrometer or at 75.5 MHz using a Varian Inova 300 spectrometer. Chloroform-*d* with an isotopic purity of 99.8%, dimethyl sulfoxide (DMSO)–d_6 with an isotopic purity of 99.9% and Methanol-d_4 with isotopic purity of 99.8% were purchased from Cambridge Isotope Laboratories Inc., MA., and were referenced to δ_H = 7.26 and δ_C = 77.23 for chloroform-*d*, δ_H = 2.50 and δ_C = 39.51 for DMSO–d_6, δ_H = 3.31 for methanol–d_4 with respect to the resonance of Me_4Si.

Mass spectrometric analyses were carried out by Scripps Center for Mass Spectrometry. Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry experiments were performed on an Applied Biosystems Voyager-STR mass spectrometer with delayed extraction. MALDI-FT mass spectrometry experiments were performed on an IonSpec FTMS mass spectrometer. Electrospray ionization time-of-flight reflectron (ESI-TOF) experiments were performed on an Agilent ESI-TOF mass spectrometer. Electrospray ionization (ESI) experiments were performed on a Waters-Micromass LCT mass spectrometer.

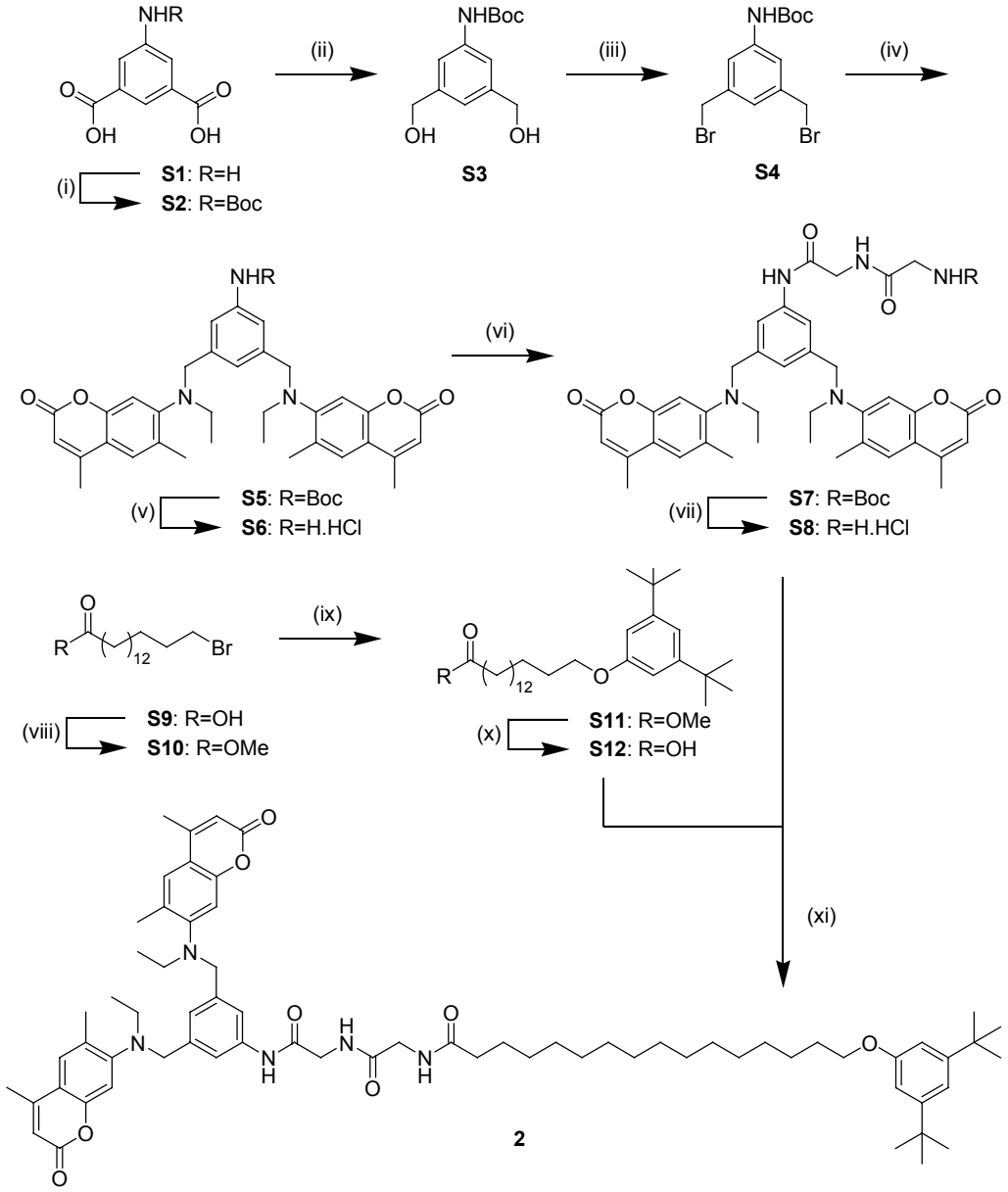
Thin-layer chromatography (TLC) was performed on Kieselgel 60 F_{254} coated plates (Merck). Developed plates were visualized using UV light and/or by dipping the plate into a solution of phosphomolybdic acid (PMA) followed by heating with a heat-gun. Flash column chromatography was carried out on Silicycle silica gel R10030B 60 (230-400 mesh).

Analytical and semi-preparative HPLC was carried out using a Waters controlling module 600 with a Waters 996 photodiode array detector or a Waters 486 tunable absorbance detector, connected to a Waters 717 plus autosampler. The system was controlled with a Waters Millennium operating system. A Waters NovaPak Silica column was used for both analytical and preparative purpose.

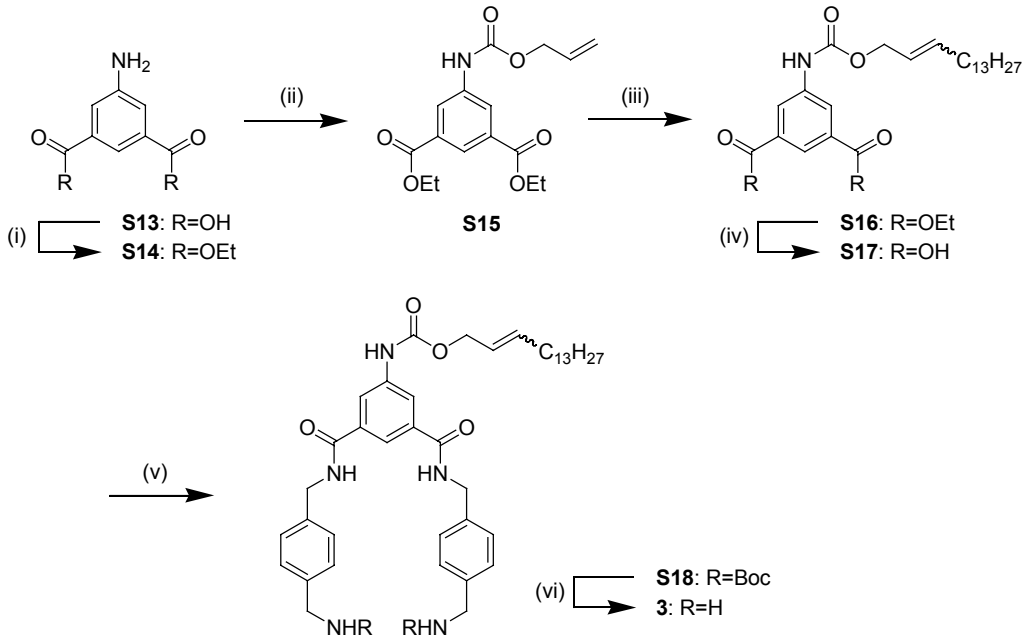
Dichloromethane (CH_2Cl_2) and THF were passed through columns of activated aluminum oxide as described by Grubbs and coworkers.[1] Anhydrous chloroform (amylene stabilized), anhydrous dimethylformamide (DMF), Extra dry CH_2Cl_2 (water <50ppm) were purchased from Acros Organics, Pittsburgh, PA. All the other solvents were purchased from Fisher Scientific International Inc., Hampton, NH. Coumarin 2, coumarin 343, Benzotriazole-1-yl-oxy-trispyrrolidinophosphonium hexafluorophosphate (PyBOP), and 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) were purchased from Acros Organics, Pittsburgh, PA. N-[(1,1-dimethylethoxy)carbonyl]glycylglycine (BOC-Gly-Gly-OH) was purchased from Bachem California Inc., Torrance, CA. All the other reagents were purchased from Sigma-Aldrich, Milwaukee, WI and were used without further purification. Unless otherwise stated, all reactions were performed under an anhydrous nitrogen atmosphere.

[1] A. B. Pangborn, M. A. Giardello, R. H. Grubbs, R. K. Rosen and F. J. Timmers, *Organometallics* 1996, **15**, 1518.

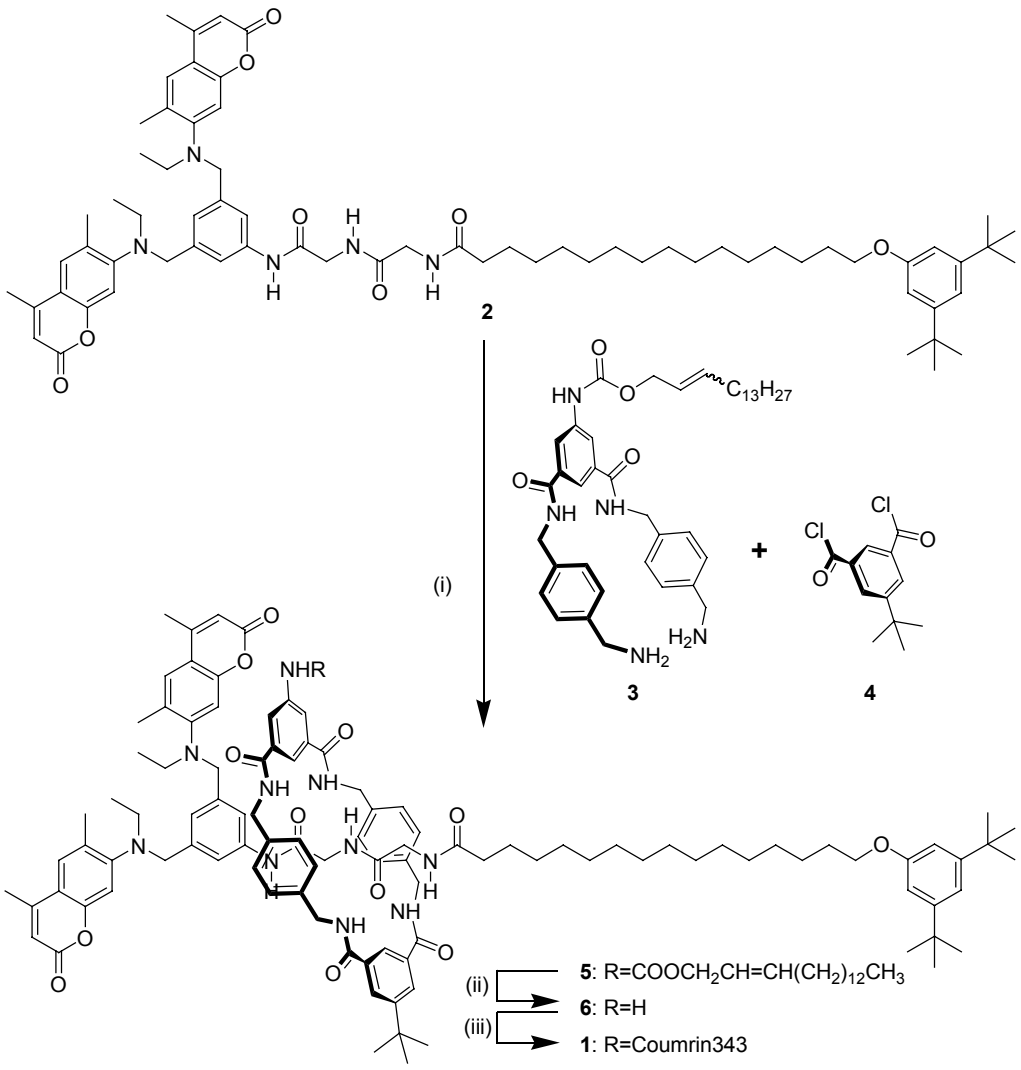
2. Synthetic Schemes



Scheme S1. Preparation of the dumbbell unit **2**: i) Di-*tert*-butyl dicarbonate (BOC), NaOH, Water/DMF, RT, 24 h, (see ref. i); ii) Borane-THF complex, THF, 25 °C, 20 h, 82%; iii) CBr_4, PPh_3, THF, RT, 2 h, 65%; iv) Coumarin 2, K_2CO_3, DMF, 85 °C, 3 days, 69%; v) 4M HCl in dioxane, 0 °C→RT, 30 min, quant.; vi) BOC-Gly-Gly-OH, PyBOP, DIEA, DMF, RT, 10 h, 76%; vii) 4M HCl in dioxane, 0 °C→RT, 30 min, quant.; viii) MeOH, TosOH.H_2O, Toluene, Reflux, 10 h, 73%; ix) 3,5-di-tert-butylphenol, K_2CO_3, CH_3CN, Reflux, 3 days; x) LiOH, THF/MeOH/H_2O, RT, 10 h, 44% (from **S10**); xi) PyBOP, DIEA, DMF, RT, 10 h, 26%.



Scheme S2. Preparation of the macrocycle precursor **3**: i) $SOCl_2$, EtOH, Reflux, 5 h, (see ref. iii); ii) allyl chloroformate, pyridine, 0 °C-1 h→RT-10 h, 81%; iii) 1-pentadecene, Grubbs Catalyst, 1st Generation (10% mol), dry CH_2Cl_2, Reflux, 12 h, 40%; iv) LiOH, THF/MeOH/H_2O, RT, 10 h, quant.; v) PyBOP, DIEA, DMF, RT, 12 h, 67%; vi) 4M HCl in dioxane, 0 °C→RT, 30 min, followed by base work up, (see synthetic procedure).



Scheme S3. Preparation of the [2]rotaxane **1**: i) Et_3N, anhydrous $CHCl_3$ (amylene stabilized), RT, **3** and **4** were added to a solution of **2** by syringe pump over 4 h then stirred for 10 h; ii) $Pd(PPh_3)$, $PhSiH_3$, CH_2Cl_2, RT, 10 h 8.4% (from **2**); iii) EDCI, CH_2Cl_2, RT, 10h, 69%.

3. Synthetic Procedures

5-(*N*-BOC-amino)-1,3-benzenedicarboxylic acid (S2)

The synthetic procedure was as described by Blackburn and Wentworth.[2] The ^{1}H NMR spectral data of the obtained product was consistent with the literature.

1,3-Bis(hydroxymethyl)-5-(*N*-BOC-amino)-benzene (S3)

Borane-THF complex (30 mL of a 1 M solution, 30 mmol) was slowly added to a stirred and heat controlled (ca. 25 °C) solution of **S2** (1.70 g, 6.0 mmol) in THF (60 mL) over 10 min. After being stirred overnight for 20 hours, the reaction mixture was quenched by the addition of water (30 mL), and the product was extracted with ethyl acetate (2 × 60 mL). The organic layers were combined and washed sequentially with water (30 mL), 5% aqueous sodium hydrogencarbonate (2 × 30mL), and water (30 mL), then dried with $MgSO_4$. The solution was filtered, and concentrated under reduced pressure to yield a white solid. This was recrystallised from hexane/CH_2Cl_2 (80:20 mL) to afford the title compound **S3** (1.25 g, 82%). LRMS: *m/z* (ESI) 276 $(M + Na)^+$; HRMS: (MALDI-FTMS) Found: $(M + Na)^+$, 276.1206. $C_{13}H_{19}NO_4$ requires $(M + Na)^+$, 276.1207; ^{1}H NMR (DMSO, 300 MHz): δ_H 9.25 (s, 1H; NH), 7.27 (s, 2H; ArH), 6.84 (s, 1H; ArH), 5.11 (t, J = 5.7 Hz, 2H; OH), 4.39 (d, J = 5.7 Hz, 4H; Benzyl), 1.46 (s, 9H; *t*-Butyl) ppm; ^{13}C NMR (DMSO, 75.5 MHz): δ_C 152.5 (C=O), 142.4, 138.9 (all ArC, quaternary), 118.1, 114.5 (all ArC, CH), 78.7 (*t*-Butyl, quaternary), 63.0 (Benzyl), 28.2 (*t*-Butyl, CH_3) ppm.

1,3-Bis(bromomethyl)-5-(*N*-BOC-amino)-benzene (S4)

Compound **S3** (0.5 g, 2.0 mmol) and CBr_4 (1.65 g, 5.0 mmol) were dissolved in dry THF (20 mL), and triphenylphosphine (1.30 g, 5.0 mmol) was added to the solution in two parts. The reaction mixture was stirred at room temperature under argon for 2 hours, and solvent was evaporated under reduced pressure. The residue was passed through a short plug of silica gel using a solvent mixture hexane/CH_2Cl_2 (v/v, 60:40) as mobile phase to give **S4** as white solid (490 mg, 65%). TLC (6:4 v/v Hexane-CH_2Cl_2) R_f 0.16; LRMS: *m/z* (ESI) 378 $(M - H)^-$; HRMS: (ESI-TOF) Found: $(M + Na)^+$, 399.9522. $C_{13}H_{17}Br_2NO_2$ requires $(M + Na)^+$, 399.9518; ^{1}H NMR (DMSO, 300 MHz): δ_H 9.51 (s, 1H; NH), 7.46 (s, 1H; ArH), 7.45 (s, 1H; ArH), 7.09 (s, 1H; ArH), 4.62 (s, 4H; Benzyl), 1.47 (s, 9H; *t*-Butyl) ppm; ^{13}C NMR (DMSO, 75.5 MHz): δ_C 152.5 (C=O), 139.9, 138.7 (all ArC, quaternary), 123.4, 118.6 (all ArC, CH), 79.3 (*t*-Butyl, quaternary), 34.2 (Benzyl), 28.1 (*t*-Butyl, CH_3) ppm.

Modified Coumarin 2 (S6)

To coumarin 2 (750 mg, 3.4 mmol) was added dry DMF (10 mL), compound **S4 (**500 mg, 1.3 mmol), and K_2CO_3 (475 mg, 3.4 mmol). The mixture was heated at 85 °C for 3 days. The resulting mixture was poured into ether (ca. 250 mL) and washed with brine

[2] G. M. Blackburn and P. Wentworth, (Zeneca Limited, UK). EP 745673, 1996.

(100 ml) and water (3 × 100 ml). The organic layer was dried under $MgSO_4$, then concentrated to yield a crud product under reduced pressure. The crude product was subjected to a silica gel chromatography (CH_2Cl_2/EtOAc, v/v, 95:5). Fractions containing the product were concentrated under reduced pressure to give the BOC-protected modified coumarin 2 **S5** as a yellow powder (590 mg, 69%). TLC (9:1 v/v $CHCl_3$-EtOAc) R_f 0.64 (relative to the solvent front), 0.88 (relative to coumarin 2); LRMS: *m/z* (ESI) 652 $(M + H)^+$; HRMS: (MALDI-FTMS) Found: $(M + Na)^+$, 674.3204. $C_{39}H_{45}N_3O_6$ requires $(M + Na)^+$, 674.3200; ^{1}H NMR ($CHCl_3$, 600 MHz): δ_H 7.32 (s, 2H; Coumarin CH), 7.16 (s, 2H; ArH), 6.92 (s, 1H; ArH), 6.84 (s, 2H; Coumarin CH), 6.50 (s, 1H; NH) 6.08 (s, 2H; Coumarin CH), 4.10 (s, 4H; Benzyl), 2.99 (q, J = 6.9 Hz, 4H; Ethyl CH_2) 2.35 (s, 6H; Coumarin CH_3), 2.33 (s, 6H; Coumarin CH_3), 1.47 (s, 9H; *t*-Butyl), 0.98 (t, J = 6.9 Hz, 6H; Ethyl CH_3) ppm. ^{13}C NMR ($CDCl_3$, 150 MHz): δ_C 161.7, 153.9, 152.8, 152.5, 139.5, 138.8, 129.6, 126.8, 122.5,117.2, 114.9, 112.8, 109.3, 80.8, 56.9, 46.5, 28.5, 19.0, 18.8, 12.0 ppm.

The protected amine **S5** was subsequently treated with a solution of 4M HCl in dioxane (5 mL) at 0 °C. After the complete dissolution of **S5**, the mixture was stirred for 30 min while it was allowed to warm up to room temperature. The solvent was then removed under reduced pressure to give a hydrochloride salt of the title compound **S6** as a tan powder in quantitative yield. HRMS: (MALDI-FTMS) Found: $(M + H)^+$, 552.2876. $C_{34}H_{37}N_3O_4$ requires $(M + H)^+$, 552.2857; ^{1}H NMR ($CHCl_3$, 300 MHz): δ_H 7.32 (s, 2H; Coumarin CH), 6.86 (s, 2H; ArH), 6.64 (s, 1H; ArH), 6.48 (s, 2H; Coumarin CH), 6.09 (s, 2H; Coumarin CH), 4.05 (s, 4H; Benzyl), 3.00 (q, J = 7.2 Hz, 4H; Ethyl CH_2), 2.28 (s, 12H; Coumarin CH_3), 0.92 (t, J = 7.2 Hz, 6H; Ethyl CH_3) ppm.

***N*-BOC-Glycylglycine-Modified Coumarin 2 (S8)**

To the modified coumarin **S6** (700 mg, 1.2 mmol) was added dry DMF (20 mL), BOC-Gly-Gly-OH (550 mg, 2.4 mmol), PyBOP (1.25 g, 2.4 mmol), and DIEA (2 mL, 11 mmol). The reaction mixture was stirred at room temperature under N_2. After 10 hours, the solvent was removed under reduced pressure, and the residue was subjected to column chromatography (CH_2Cl_2/MeOH, v/v, gradient elution from 97:3 to 95:5) to yield BOC-protected product **S7** as a dark tan solid (690 mg, 76%). TLC (95:5 v/v $CHCl_3$-MeOH) R_f 0.30 (relative to the solvent front), 0.36 (relative to **S5**); LRMS: *m/z* (ESI) 766 $(M + H)^+$; HRMS: (ESI-TOF) Found: $(M + H)^+$, 766.3804. $C_{43}H_{51}N_5O_8$ requires $(M + H)^+$, 766.3810; ^{1}H NMR ($CHCl_3$, 600 MHz): δ_H 8.59 (s, 1H; NH), 7.45 (s, 2H; ArH), 7.34 (s, 2H; Coumarin CH), 7.10 (br-t, 1H; NH), 7.02 (s, 1H; ArH), 6.86 (s, 2H; Coumarin CH), 6.12 (s, 2H; Coumarin CH), 5.43 (s, 1H; NH), 4.13-4.11 (m, 6H; Benzyl and Glycine CH_2), 3.88 (d, J = 5.4 Hz, 2H; Glycine CH_2), 3.01 (br-q, 4H; Ethyl CH_2), 2.38 (s, 6H; Coumarin CH_3), 2.37 (s, 6H; Coumarin CH_3), 1.44 (s, 9H; *t*-Butyl), 1.01 (t, J = 7.2 Hz, 6H; Ethyl CH_3) ppm. ^{13}C NMR ($CDCl_3$, 150 MHz): δ_C 170.6, 167.3, 161.8, 156.7, 153.9, 152.7, 152.7, 139.4, 138.2, 129.7, 126.8,123.8, 118.9, 115.0, 112.7, 109.3, 80.9, 56.6, 46.8, 46.6, 44.1, 28.5, 19.0, 18.8, 12.0 ppm.

The protected amine **S7** was subsequently treated with a solution of 4M HCl in dioxane (5 mL) at 0 °C. After complete dissolution of **S7**, the mixture was stirred for 30 min while it was allowed to warm up to room temperature. The solvent was then removed under reduced pressure to give a hydrochloride salt of the title compound **S8** as a tan powder in quantitative yield. HRMS: (ESI-TOF) Found: $(M + H)^+$, 666.3279. $C_{38}H_{43}N_5O_6$ requires $(M + H)^+$, 666.3286.

Methyl 16-bromododecanoate (S10)

16-Bromododecanoic acid **S9** (10 g, 29.8 mmol), methanol (12.1 mL, 299 mmol) and *p*-toluenesulfonic acid monohydrate (85 mg, 0.45 mmol) were heated under reflux in toluene (20 mL) for 10 hours. After the completion of the reaction, the mixture was dried using $MgSO_4$, and was subjected to vacuum distillation to give the title compound **S10** as a colorless oil (7.6 g, 73%). LRMS: *m/z* (ESI) 349 $(M + H)^+$; HRMS: (ESI-TOF) Found: $(M + H)^+$, 349.1737. $C_{17}H_{33}BrO_2$ requires $(M + H)^+$, 349.1737; ^{1}H NMR ($CDCl_3$, 600 MHz): δ_H 3.61 (s, 3H, OCH_3), 3.35 (t, J = 6.8 Hz, 2H; CH_2), 2.25 (t, J = 7.5 Hz, 2H; CH_2), 1.80 (m, 2H; CH_2), 1.56 (m, 2H; CH_2), 1.37 (m, 2H; CH_2), 1.24-1.20 (m, 20H; CH_2) ppm. ^{13}C NMR ($CDCl_3$, 150 MHz): δ_C 174.4 (C=O), 51.5 (-OCH_3), 34.2, 34.1, 33.0, 29.7^7, 29.7^5, 29.7^2, 29.6^9, 29.6, 29.4, 29.3, 28.9, 28.3, 25.1 (all CH_2) ppm.

16-(3,5-di-*tert*-butylphenoxy)hexadecanoic acid (S12)

3,5-Di-*tert*-butylphenol (1.18 g, 5.7 mmol), methyl 16-bromododecanoate **S10** (2.00 g, 5.7 mmol) and K_2CO_3 (1.02 g, 7.4 mmol) were heated under reflux in CH_3CN (6 mL). After 3 days, the solvent was removed under reduced pressure to give a crude product containing **S11** as a white solid. TLC (50:50 v/v Hexane-$CHCl_3$) R_f 0.67 (plate stained by PMA). The solid was then dissolved in a mixture of THF/Methanol/Water (50/15/10 mL), and LiOH (0.41 g, 17 mmol). The reaction mixture was stirred at room temperature for 10 hours. The resulting solution was acidified with 1.0 M aq. HCl and the product was extracted with ethyl acetate. The organic layers were combined and dried over $MgSO_4$. The solvent was removed under reduced pressure, and the residue was subjected to the silica gel chromatography (CH_2Cl_2/MeOH, v/v, gradient elution from 100:0 to 99:1). Fractions containing products were concentrated under reduced pressure to give the title compound **S12** as a white solid (1.16 g, 44%). TLC (95:5 v/v $CHCl_3$-MeOH) R_f 0.43 (plate stained by PMA); LRMS: *m/z* (ESI) 461.4 $(M + H)^+$; HRMS (MALDI-FTMS) Found: $M^{+\bullet}$, 460.3929. $C_{30}H_{52}O_3$ requires $M^{+\bullet}$, 460.3916; ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H 6.99 (t, J = 1.5 Hz, 1H; ArH), 6.73 (d, J = 1.5Hz, 2H; ArH), 3.94 (t, J = 6.6 Hz, 2H; CH_2), 2.33 (t, J = 7.2 Hz, 2H; CH_2), 1.76, 1.61, 1.44 (all m, 2H; CH_2), 1.29-1.24 (m, 38H) ppm; ^{13}C NMR ($CDCl_3$, 150 MHz): δ_C 180.1 (COOH), 158.9 (ArC, quaternary), 152.3 (ArC, quaternary), 115.0 (ArC, CH), 109.0 (ArC, CH), 68.0 (-OCH_2-), 35.2 (*t*-Butyl, quaternary), 34.3 (-$\underline{C}H_2$-COOH), 31.7 (*t*-Butyl, CH_3), 29.8^8, 29.8^6, 29.8^4, 29.8^2, 29.6^8, 29.6^6, 29.4, 29.3, 26.4, 24.9 (all CH_2) ppm.

Dumbbell-shaped Guest Unit (2)

To compound **S12** (690 mg, 1.5 mmol) was added dry DMF (30 mL), modified coumarin **S8** (700 mg, 1.0 mmol), PyBOP (780 mg, 1.5 mmol), and DIEA (1.74 mL, 10 mmol). The reaction mixture was stirred at room temperature under argon. After 10 hours, the solvent was removed under reduced pressure, and the residue was then subjected to column chromatography (CH_2Cl_2/MeOH, v/v, gradient elution from 97:3 to 95:5) Fractions containing products were concentrated under reduced pressure to give the dumbbell-shaped guest unit **2** as a tan solid (820 mg, 74%). LRMS: *m/z* (ESI) 1108.5 $(M + H)^+$; HRMS: (MALDI-FTMS) Found: $(M + Na)^+$, 1130.6941. $C_{68}H_{93}N_5O_8$ requires $(M + Na)^+$, 1130.6916; ^{1}H NMR ($CDCl_3$, 600 MHz): δ_H 7.43 (s, 1H; ArH), 7.31 (s, 2H; ArH), 6.98 (s, 2H; ArH), 6.97 (s, 1H; ArH), 6.84 (s, 2H; ArH), 6.73 (s, 2H; ArH), 6.08 (s, 2H; ArH), 4.13 (s, 4H; Benzyl), 4.05 (d, J = 5.4 Hz, 2H; Glycine CH_2), 3.95-3.92 (m, 4H; -CH_2- and Glycine CH_2), 2.98 (q, J = 7.0 Hz, 4H; Ethyl CH_2), 2.34 (s, 6H; Coumarin CH_3), 2.33

(s, 6H; Coumarin CH_3), 2.26 (t, J = 7.7 Hz, 2H; -CH_2-), 1.74 (m, 2H; -CH_2-), 1.61 (m, 2H; -CH_2-), 1.43 (m, 2H; -CH_2-),1.32-1.18 (m, 38H; -CH_2- and *t*-Butyl), 0.99 (t, J = 7.0 Hz, 6H; Ethyl CH_3) ppm; ^{13}C NMR ($CDCl_3$, 150 MHz): δ_C 174.7, 170.1, 167.3, 161.8, 158.6, 153.7, 152.6, 152.5, 152.0, 139.1, 138.1, 129.4, 126.5, 123.2, 118.5, 114.7, 114.5, 112.2, 108.9, 108.8, 67.7, 56.2, 46.5, 46.3[1], 46.2[7], 43.9, 43.6, 36.2, 34.9, 31.4, 29.7, 29.6[1], 29.5[8], 29.5[1], 29.4[6], 29.4, 29.3, 26.4[1], 26.3[5], 26.1, 25.6, 18.7, 18.5, 11.9 ppm.

5-Amino-isophthalic acid diethyl ester (S14)

The synthetic procedure was as described by Aujard et al.[3] The ^{1}H NMR spectral data of the obtained product was consistent with the literature.

5-Allyloxycarbonylamino-isophthalic acid eiethyl ester (S15)

Allyl chloroformate (10.0 g, 83.0 mmol) was added to a stirred mixture of 5-aminoisophthalic acid diethyl ester **S14** (15.0 g, 63.2 mmol) in pyridine (100 mL) at 0 ºC. After the addition, mixture was kept at 0 ºC for 1 hour, and then gradually warmed up to room temperature. The reaction was stirred for 10 hours at room temperature, then the solvent was removed under reduced pressure. The brown residue was redissolved in CH_2Cl_2 and the solution was washed with water. The organic phase was dried using $MgSO_4$ and then concentrated under the reduced pressure until the volume of the mixture was approximately 50 mL. The solution was slowly added to vigorously stirring ether (300 mL). A white precipitate quickly formed and collecting it by filtration gave the title compound (16.5 g, 81%). TLC (95:5 v/v $CHCl_3$-EtOAc) R_f 0.54; LRMS: *m/z* (ESI) 322 $(M + H)^+$, 344 $(M + Na)^+$; HRMS: (ESI-TOF) Found: $(M + H)^+$, 322.1291. $C_{16}H_{19}NO_6$ requires $(M + H)^+$, 322.1285; ^{1}H NMR ($CDCl_3$, 600 MHz): δ_H 8.38 (s, 2H + 1H; two different ArHs, coincidental), 7.53 (br-s, 1H; NH), 6.00-5.94 (m, 1H; Allyl), 5.38 (apparent d, J_{trans} = 17.4 Hz, 1H; Allyl), 5.28 (apparent d, J_{cis} = 10.2 Hz, 1H; Allyl), 4.70 (d, J = 5.4 Hz, 2H; Allyl), 4.42 (q, J = 7.2 Hz, 4H; Ethyl), 1.41 (t, J = 7.2 Hz, 6H; Ethyl) ppm; ^{13}C NMR ($CDCl_3$, 150 MHz): δ_C 166.0 (COOEt), 153.4 (NHC=O), 139.2 (ArC, quaternary), 132.4 (ArC, CH), 131.7 (ArC, quaternary), 125.3 (all ArC, CH), 123.7 (Allyl, CH) 118.7, 66.2 (all Allyl, CH_2), 67.8 (Ethyl, CH_2), 14.4 (Ethyl, CH_3) ppm.

5-(Hexadec-2-enoxycarbonylamino)-isophthalic acid (S17)*

To the dry flask filled with 20 mL dry CH_2Cl_2 kept under argon atmosphere, the compound **S15** (1.0 g, 3.1 mmol), pentadec-1-ene (1.0 g, 4.8 mmol) and 10 mol% of Grubbs 1st generation catalyst were added. Reaction apparatus was sealed with PTFE tape and then heated at reflux. After 12 hours, the reaction mixture was cooled down to room temperature, and the mixture was subjected to a silica gel chromatography (Hexane/EtOAc, v/v, 85:15%). Appropriate fractions were collected and concentrated under reduced pressure to give diester **S16** as a white solid (620 mg, 40%). TLC (85:15 v/v Hexane-EtOAc) R_f 0.41 (relative to the solvent front), 1.23 (relative to **S15**); LRMS: *m/z* (ESI) 504 $(M + H)^+$, 526 $(M + Na)^+$; HRMS: (ESI-TOF) Found: $(M + H)^+$, 504.3330. $C_{29}H_{45}NO_6$ requires (M +

[3] I. Aujard, J. P. Baltaze, J. B. Baudin, E. Cogne, F. Ferrage, L. Jullien, E. Perez, V. Prevost, L. M. Qian and O. Ruel, *J. Am. Chem. Soc*. 2001, **123**, 8177.

H)$^+$, 504.3319; ^{1}H NMR ($CDCl_3$, 600 MHz): δ_H 8.36 (s, 1H; ArH), 8.27 (s, 2H; ArH), 6.95 (br-s, 1H; NH), 5.69-5.53 (m, 2H; -C<u>H</u>=C<u>H</u>-), 4.73 (d, *J* = 6.6 Hz, 1.6H*; -O-C<u>H</u>$_2$-CH=), 4.61 (d, *J* = 6.6 Hz, 0.4H*; -O-C<u>H</u>$_2$-CH=), 4.39 (q, *J* = 7.2 Hz, 4H; Ethyl), 2.11 (q, *J* = 7.2 Hz, 1.6H*; =CH-C<u>H</u>$_2$-CH$_2$-), 2.05 (q, *J* = 7.2 Hz, 0.4H*; =CH-C<u>H</u>$_2$-CH$_2$-), 1.39 (t, *J* = 7.2 Hz, 6H, Ethyl), 1.37-1.34 (m, 2H, -CH$_2$-), 1.26-1.23 (m, 18H, -CH$_2$-), 0.86 (t, *J* = 6.6 Hz, 3H, -CH$_2$-C<u>H</u>$_3$) ppm; ^{13}C NMR ($CDCl_3$, 150 MHz): δ_C 165.8 (<u>C</u>OOEt), 153.6 (NHC=O), 138.9 (quaternary), 137.5, 136.2 (all CH), 132.0 (quaternary), 125.6, 123.8, 123.7, 123.3 (all CH), 66.8 (CH$_2$), 61.9 (Ethyl, CH$_2$), 61.8, 32.5, 32.1, 29.89, 29.87, 29.83, 29.71, 29.66, 29.57, 29.4, 29.1, 27.8, 22.9 (all CH$_2$), 14.5 (Ethyl, CH$_3$), 14.3 (CH$_3$) ppm.

The diester **S16** (1.6 g, 3.2 mmol) was dissolved in a mixture of THF/MeOH/Water (75/25/25 mL), and LiOH (400 mg, 17 mmol) was added. The reaction mixture was stirred at room temperature for 10 hours. The resulting solution was acidified with citric acid until the solution became pH 1. The precipitated product was collected by filtration, and the solid obtained was resuspended in the solution of EtOH/H_2O (10/50 mL) and filtered. The solid was washed with water and dried to give title compound **S17** as white powder in quantitative yield. LRMS: *m/z* (ESI) 470 (M + Na)$^+$; HRMS: (ESI-TOF) Found: (M + Na)$^+$, 470.2522. $C_{25}H_{37}NO_6$ requires (M + Na)$^+$, 470.2513; ^{1}H NMR (DMSO, 600 MHz): δ_H 10.04 (s, 1H; NH), 8.30 (s, 2H; ArH), 8.10 (s, 1H; ArH), 5.84-5.54 (m, 2H; -C<u>H</u>=C<u>H</u>-), 4.67 (d, *J* = 7.2 Hz, 0.7H*; -O-C<u>H</u>$_2$-CH=), 4.61 (d, *J* = 7.2 Hz, 1.3H*; -O-C<u>H</u>$_2$-CH=), 2.11 (q, *J* = 7.2 Hz, 0.7H*; =CH-C<u>H</u>$_2$-CH$_2$-), 2.03 (q, *J* = 7.2 Hz, 1.3H*; =CH-C<u>H</u>$_2$-CH$_2$-), 1.35-1.19 (m, 22H, -CH$_2$-), 0.84 (t, *J* = 6.6 Hz, 3H, -CH$_2$-C<u>H</u>$_3$) ppm; ^{13}C NMR (DMSO, 150 MHz): δ_C 166.4 (COOH), 153.4, 153.3 (NHC=O), 139.9 (quaternary), 135.8, 135.0 (all CH), 131.7 (quaternary), 124.4, 123.9 123.8, 122.5 (all CH), 64.9, 60.1, 31.5, 31.3, 29.0, 28.8, 28.7, 28.5, 28.3, 26.8, 22.1 (all CH$_2$), 13.9 (CH$_3$) ppm.

* This product is a mixture of *cis* and *trans* isomers.

Macrocycle Precursor (3)*

To isophthalic acid derivative **S17** (250 mg, 0.6 mmol) was added to dry DMF (20 mL), 1-(N-BOC-aminomethyl)-4-(aminomethyl)benzene (345 mg, 1.5 mmol), PyBOP (760 mg, 1.5 mmol), and DIEA (2.5 mL, 14.4 mmol). The reaction mixture was stirred at room temperature under N_2. After 12 hours, the solvent was removed under reduced pressure, and the residue was then subjected to silica gel chromatography (Hexane/EtoAc, v/v, 50:50). Fractions containing products were concentrated under reduced pressure to give the BOC-protected macrocycle precursor **S18** as a off white solid (330 mg, 67%). TLC (50:50 v/v Hexane-EtOAc) R_f 0.38 (relative to the solvent front); LRMS: *m/z* (ESI) 907 (M + Na)$^+$; HRMS: (ESI-TOF) Found: (M + Na)$^+$, 906.5350. $C_{51}H_{73}N_5O_8$ requires (M + Na)$^+$, 906.5351; ^{1}H NMR ($CDCl_3$, 600 MHz): δ_H 8.181 (s, 1H, NH), 8.175 (s, 2H; ArH), 7.89 (s, 1H; ArH), 7.17 (d, *J* = 7.8 Hz, 4H; ArH), 7.12 (d, *J* = 7.8 Hz, 4H; ArH), 5.76-5.45 (m, 2H; -C<u>H</u>=C<u>H</u>-), 5.04 (t, *J* = 5.4 Hz, 2H, NH), 4.65 (d, *J* = 6.6 Hz, 0.7H*; -O-C<u>H</u>$_2$-CH=), 4.53-4.50 (m, 5.3H*; -O-C<u>H</u>$_2$-CH= and Benzyl, overlapping), 4.21 (d, *J* = 5.4 Hz, 4H; Benzyl), 2.05 (q, *J* = 7.2 Hz, 0.7H*; =CH-C<u>H</u>$_2$-CH$_2$-), 1.97 (q, *J* = 7.2 Hz, 1.3H*; =CH-C<u>H</u>$_2$-CH$_2$-), 1.44 (s, 18H, *t*-Butyl) 1.25 (m, 22H, -CH$_2$-), 0.88 (t, *J* = 6.6 Hz, 3H, -CH$_2$-C<u>H</u>$_3$) ppm; ^{13}C NMR ($CDCl_3$, 150 MHz): δ_C 166.6, 156.3, 154.1, 154.0 (all NHC=O), 139.7, 138.5 (quaternary), 137.3 (CH), 137.1 (quaternary) 135.8 (CH) 135.3 (quaternary), 128.3, 127.8, 123.8, 123.4, 120.4, 119.9 (all CH), 79.8 (*t*-Butyl,

quaternary), 66.4, 61.5 (all CH_2), 44.4, 44.1 (Benzyl, CH_2), 32.5, 32.1, 29.9, 29.8, 29.71, 29.66, 29.6, 29.5, 29.1 (all CH_2), 28.6 (*t*-Butyl, CH_3), 27.8, 22.9 (all CH_2), 14.3 (CH_3) ppm

The protected amine **S18** was subsequently treated with 4M HCl in dioxane (10 mL) at 0 °C. After the complete dissolution of **S18**, the mixture was stirred for 30 min while it was allowed to warm up to room temperature which gave a hydrochloride salt in quantitative yield. The title compound **3** can be obtained after base work up, however the free base **3** was generated just before the synthesis of the rotaxane **5**. See the next procedure. LRMS: *m/z* (ESI) 684 $(M + H)^+$; HRMS (ESI-TOF) Found: $(M + H)^+$, 684.4472. $C_{41}H_{57}N_5O_4$ requires $(M + H)^+$, 684.4483;

* This product is a mixture of *cis* and *trans* isomers.

[2]Rotaxane (6)

Anhydrous $CHCl_3$ (24 mL, amylene stabilized) and triethylamine (1 mL) were added to the dihydrochloride of the macrocycle precursor **3** (410 mg, 0.54 mmol) under argon atmosphere. The resulting milky solution/suspension (the mixture will never become a clear solution, but reaction works as long as large particles are not present) were charged into a gas-tight syringe and placed on the syringe pump. A solution of *tert*-butylisophthaloyl diacid chloride **4** (140 mg, 0.54 mmol) in anhydrous $CHCl_3$ (25 mL, amylene stabilized) was filled in a separate gas-tight syringe and placed on the same syringe pump. These two separate solutions were simultaneously delivered to a stirred solution of the dumbbell unit **2** (200 mg, 0.18 mmol) and triethylamine (150 μL, 1.08 mmol) in anhydrous $CHCl_3$ (20 mL, amylene stabilized) under argon atmosphere over 4 hours using syringe pump. After addition was completed, the reaction mixture was stirred for further 10 hours, then the solvent was removed under reduced pressure. The solid was redissolved in CH_2Cl_2 (ca. 10 mL), and was subjected to silica gel chromatography (CH_2Cl_2/MeOH, v/v, gradient from 3:97 to 5:95), and fractions containing a material showing slightly higher TLC spot than the axle were collected to obtain approximately 140 mg of crude product which contains the [2]rotaxane **5** and the [2]catenane. TLC (95:5 v/v $CHCl_3$-MeOH) R_f 0.63 (relative to the solvent front), 1.13 (relative to the axle **1**); LRMS: *m/z* (MALDI-TOF) 1978 $(M + H)^+$, 2000 $(M + Na)^+$; HRMS: (ESI-TOF) Found: $(M + H)^+$, 1978.2170. $C_{121}H_{160}N_{10}O_{14}$ requires $(M + H)^+$, 1978.2188.

To the crude mixture (ca. 140 mg) of **5** in 10 mL of dry CH_2Cl_2, $Pd(PPh_3)_4$ (4.6 mg, 4 μmol) and phenylsilane (43 mg, 0.4 mmol) were added. The reaction mixture was stirred at room temperature for 4 hours. The reaction mixture was then subjected to silica gel chromatography (CH_2Cl_2/MeOH, v/v, gradient from 97:3 to 95:5). Fractions containing products were concentrated under reduced pressure to give the [2]rotaxane **6** as an off white solid (26 mg, 8.4% from moles of axle **2**). TLC (95:5 v/v $CHCl_3$-MeOH) R_f 0.55 (relative to the solvent front), 0.90 (relative to the axle **2**); HPLC t_R 3.4 min [column: Nova-Pak Silica 7.8×300 mm; 90% $CHCl_3$-10% MeOH; flow rate: 5 cm^3 min^{-1}]; LRMS: *m/z* (MALDI-TOF) 1734 $(M + Na)^+$; HRMS: (ESI-TOF) Found: $(M + H)^+$, 1711.9920. $C_{104}H_{130}N_{10}O_{12}$ requires $(M + H)^+$, 1711.9942; ^1H NMR ($CDCl_3$ 600 μL + CD_3OD 50 μL, 600 MHz): δ_H 9.29 (br-s), 8.28 (br-s), 8.21 (s), 8.20 (br-s), 8.12 (s), 7.65 (br-s), 7.33 (br-s), 7.16 (s), 7.06 (s), 6.99 (s, 8H; ArH), 6.95 (s), 6.94 (s), 6.91 (s), 6.69 (s, 2H, ArH,), 6.57 (s), 6.02 (s, 2H; Coumarin 2), 4.40-4.27 (m, 8H), 4.00 (s, 4H; Benzyl), 3.90 (t, J = 6.6, 2H; -O-C$\underline{H}_2$-CH_2-), 2.95 (q, J = 6.0 Hz,

4H; Ethyl), 2.31 (s, 6H, Methyl), 2.2 (s, 6H, Methyl), 1.90 (t, J = 6.0 Hz, 2H; -NHCO-C<u>H</u>$_2$-CH$_2$-), 1.72 (m, 2H; -O-CH$_2$-C<u>H</u>$_2$-), 1.40 (m, 2H; -O-CH$_2$-CH$_2$-C<u>H</u>$_2$-), 1.32-1.057 (m), 0.95 (t, J = 6.6 Hz, 6H; Ethyl) ppm; ^{13}C NMR ($CDCl_3$, 150 MHz): δ_C 174.7, 170.0, 167.9, 167.7, 166.6, 162.5, 158.7, 153.5, 152.5, 152.4, 152.2, 139.4, 137.8, 137.4, 137.3, 136.0, 135.4, 135.3, 134.0,129.7, 129.0, 128.8, 128.3, 126.8, 125.5, 123.7, 123.4, 118.5, 114.9, 114.7, 112.0, 108.9, 68.0, 55.9, 47.4, 44.29, 44.25, 42.5, 42.1, 36.0, 35.2, 35.0, 31.5, 31.2, 30.3, 29.8, 29.7, 29.7, 29.6, 29.5, 29.34, 29.33, 26.2, 25.4, 18.7, 18.6, 12.0 ppm

[2]Rotaxane (1)

To the [2]rotaxane **4** (10 mg, 5.8 μmol) was added dry CH_2Cl_2 (1 mL), coumarin 343 (3.3 mg, 11.6 μmol), EDCI (2.22 mg, 11.6 μmol). The reaction mixture was stirred at room temperature under argon atmosphere. After 10 hours, the solvent was removed under reduced pressure, and the residue was then subjected to silica gel chromatography ($CHCl_3$/MeOH, v/v, 97:3) to yield the desired compound as a yellow solid (8 mg, 69%). TLC (95:5 v/v $CHCl_3$-MeOH) R_f 0.47; HPLC t_R 2.27 min [column: Nova-Pak Silica 7.8×300 mm; 96% $CHCl_3$-4% MeOH; flow rate: 5 cm^3 min^{-1}], LRMS: *m/z* (MALDI-TOF) 2001 $(M + Na)^+$; HRMS: (ESI-TOF) Found: $(M + H)^+$, 1979.0837. $C_{120}H_{143}N_{11}O_{15}$ requires $(M + H)^+$, 1979.0830; ^{1}H NMR (DMSO, 600 MHz): δ_H 9.89 (s, 1H; NH), 8.74 (br-t, 1H; NH), 8.68 (br-t, 1H; NH), 8.64 (s, 1H; Coumarin 343), 8.29 (s, 2H; Macrocycle), 8.18 (br-t, 1H; NH), 8.05 (s, 1H; Macrocycle), 8.00 (s, 1H; Macrocycle), 7.98 (s, 2H; Macrocycle), 7.48 (s, 2H; Coumarin 2), 7.46 (s, 1H; ArH), 7.32 (s, 1H; Coumarin 343), 7.15 (s, 8H; Macrocycle), 6.98 (s, 1H; ArH), 6.94 (s, 1H; ArH), 6.93 (s, 2H; Coumarin2), 6.68 (s, 2H; ArH), 6.15 (s, 2H; Coumarin 2), 4.47 (m, 4H; Benzyl, Macrocycle), 4.23 (m, 4H; Macrocycle), 4.13 (s, 4H; Benzyl) 3.78 (m, 4H; Glycine CH$_2$ and Ar-O-C<u>H</u>$_2$-CH$_2$-), 3.61 (br-d, 2H; Glycine CH$_2$), 2.97 (q, J = 7.2 Hz,4H; Ethyl, Coumarin 2), 2.77 (t, J = 6.6 Hz,2H; Coumarin 343), 2.71 (t, J = 6.6 Hz, 2H; Coumarin 343), 2.35 (s, 6H; CH$_3$, Coumarin 2), 2.30 (s, 6H; CH$_3$, Coumarin 2), 2.02 (m, 2H; -NHCO-C<u>H</u>$_2$-CH$_2$-), 1.90 (br-t, 2H; Coumarin 343), 1.86 (br-t, 2H; Coumarin 343), 1.46 (m, 2H; Ar-O-CH$_2$-C<u>H</u>$_2$-), 1.32-1.24 (m; 29H), 1.05 (m, 2H; -CH$_2$-), 0.94 (t, J = 6.6 Hz, 6H; Ethyl, Coumarin2), 0.87 (m, 8H; -CH$_2$-), 0.80 (m, 2H; -CH$_2$-), 0.71 (m, 2H; -CH$_2$-), 0.68 (m, 2H; -CH$_2$-), 0.56 (m, 2H, -CH$_2$-), 0.47 (m, 2H, -CH$_2$-), 0.39 (m, 2H, -CH$_2$-) ppm.

4. ^{1}H, ^{13}C, and 2D NMR spectra with partial assignment of proton resonances

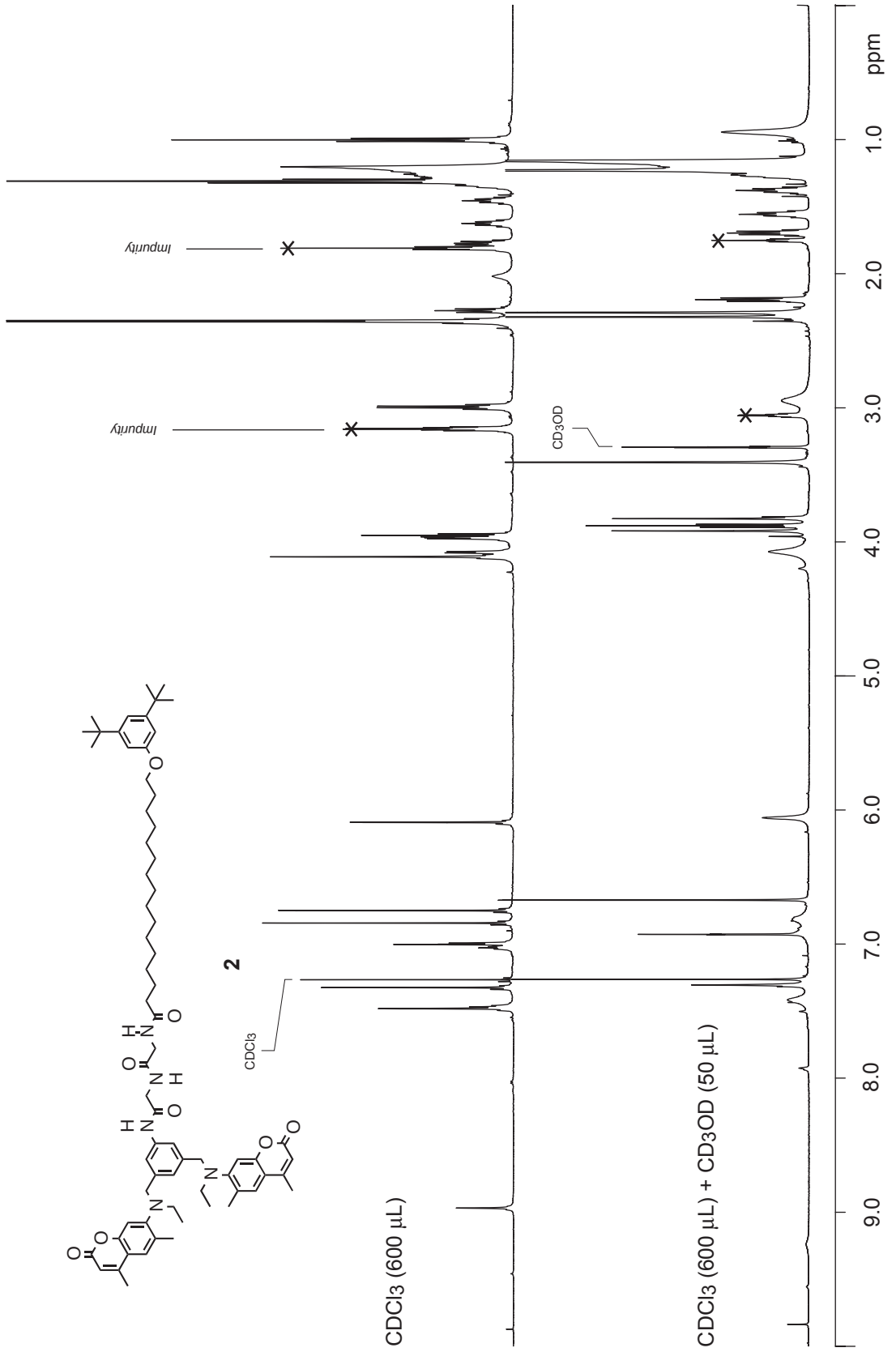


Figure S1. The 600 MHz ^{1}H NMR spectrum of the dumbbell **2**.

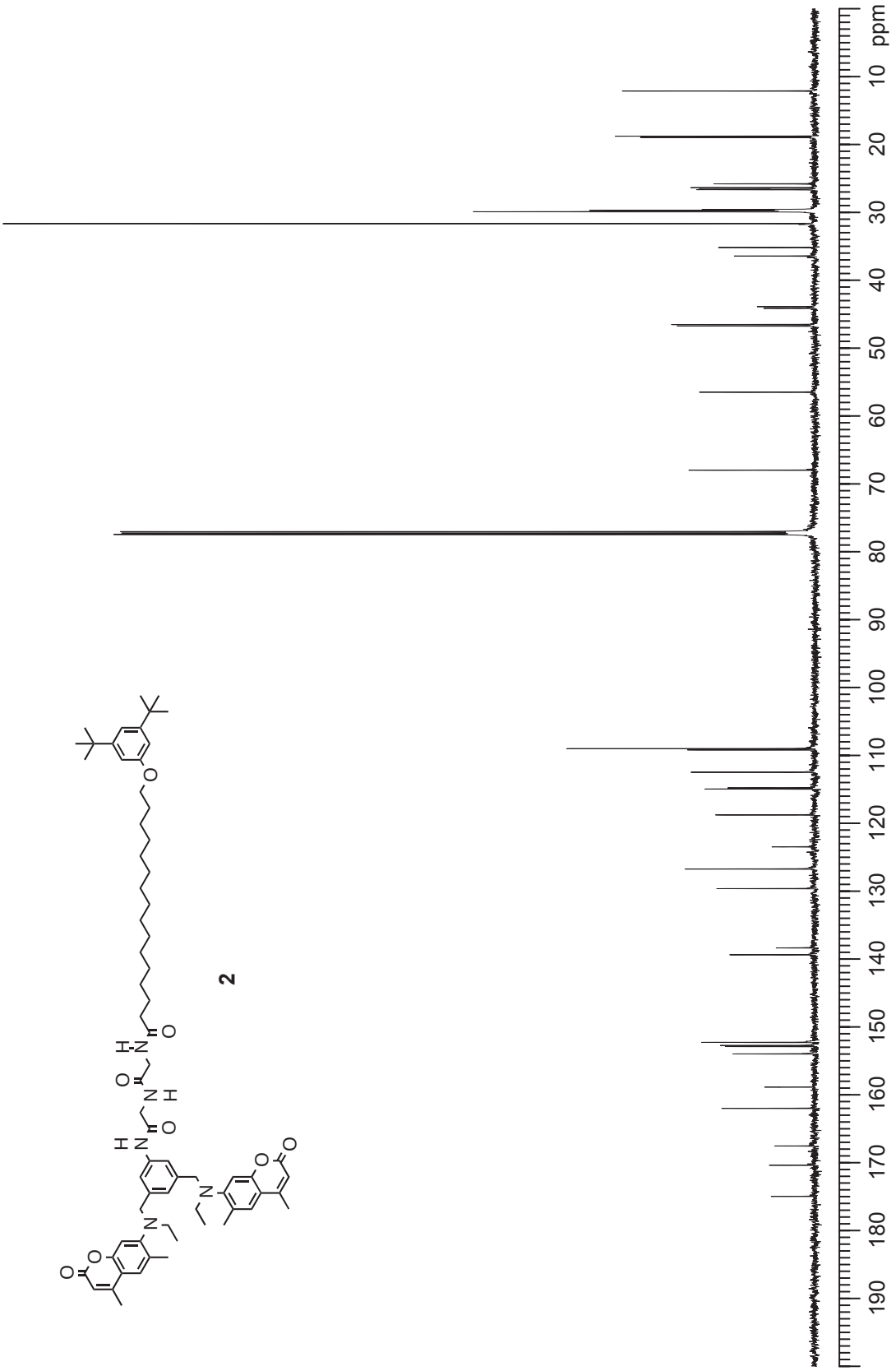


Figure S2. The 150 MHz ^{13}C NMR spectrum recorded in $CDCl_3$ of the dumbbell **2**.

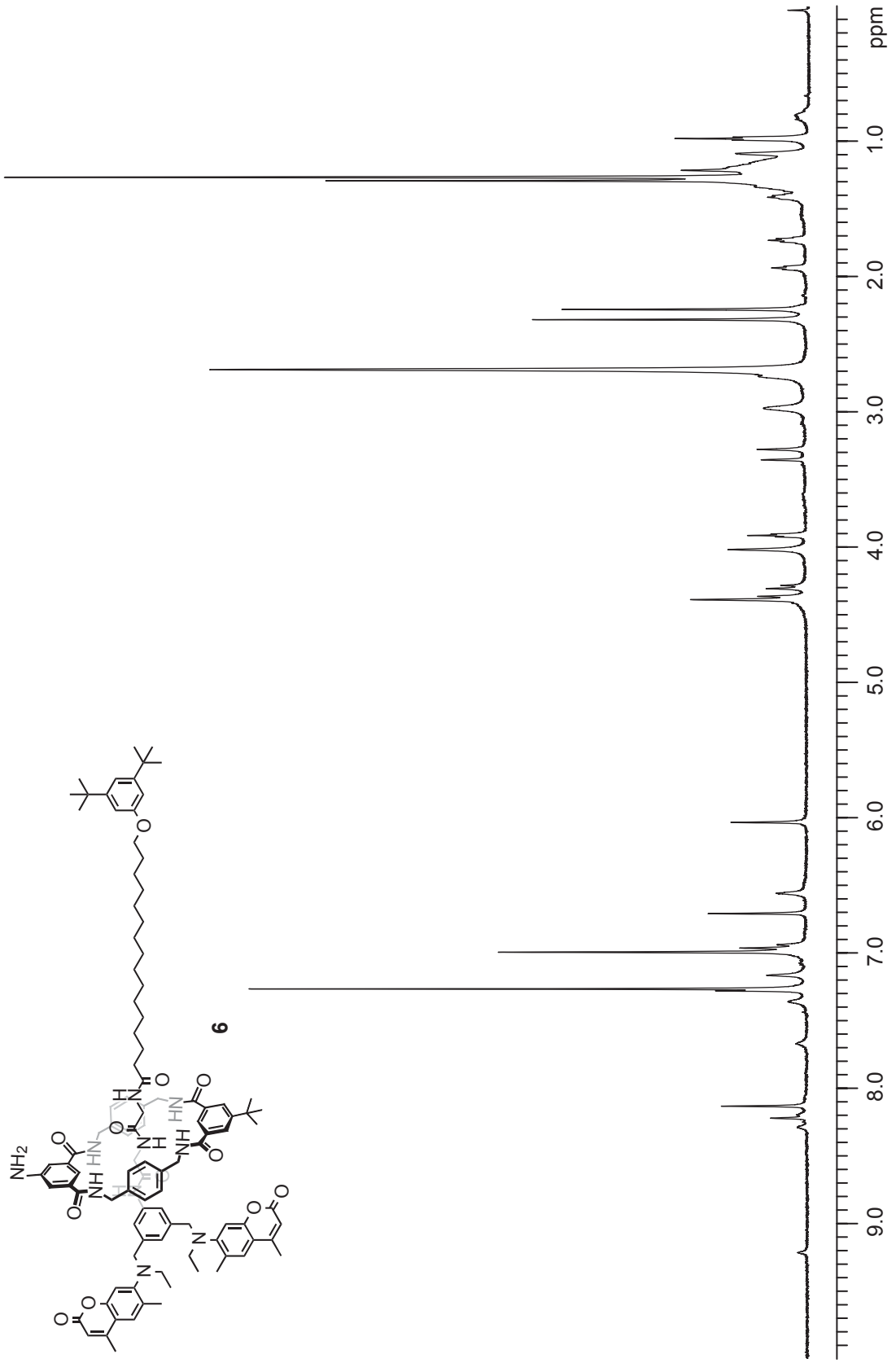


Figure S3. The 600 MHz ^{1}H NMR spectrum of the [2]rotaxane **6** recorded in $CDCl_3$ (600 μL) and CD_3OD (75 μL).

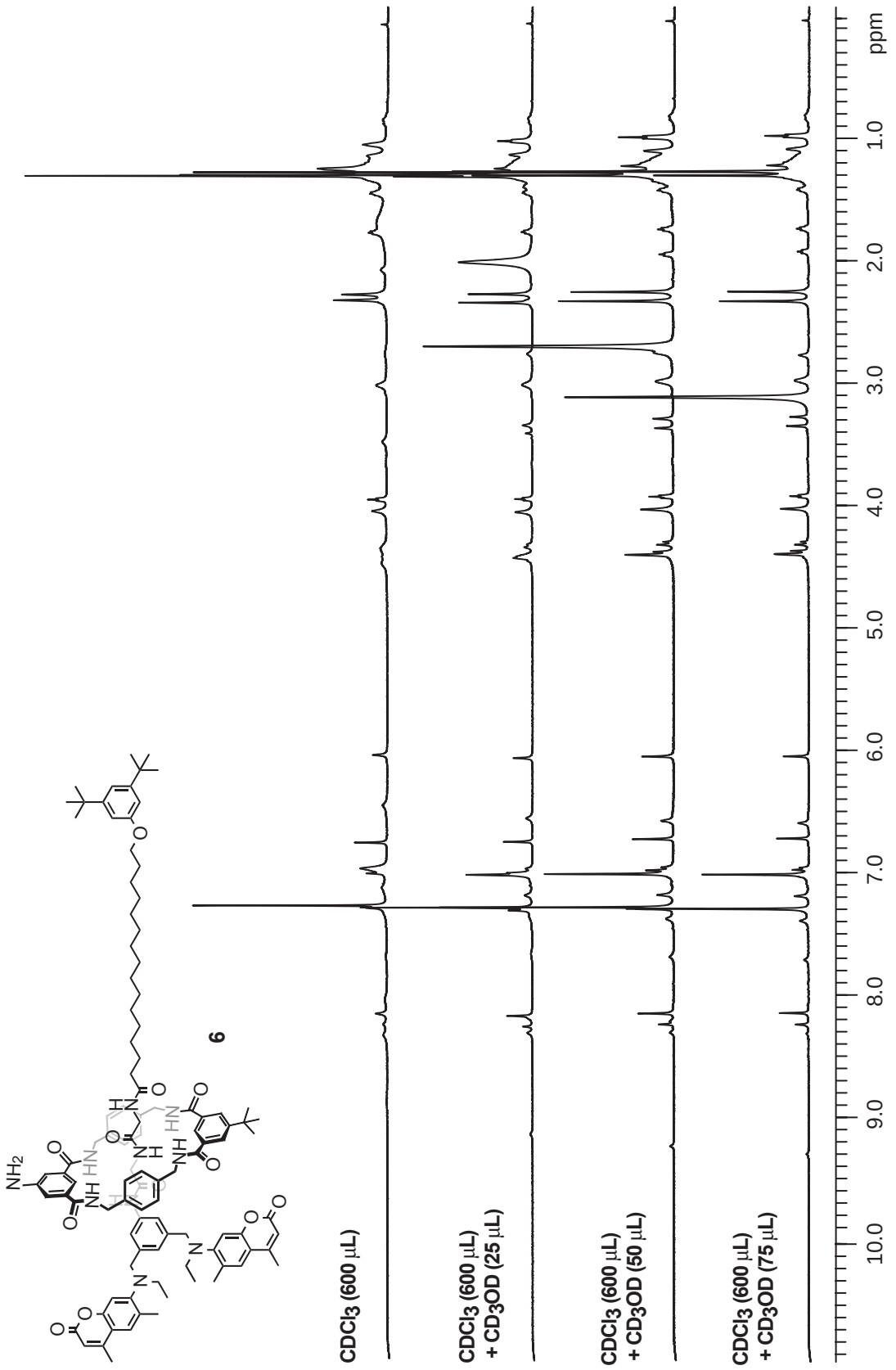


Figure S4. The 600 MHz ^{1}H NMR spectrum of the [2]rotaxane **6** recorded in $CDCl_3$ (600 μL) and CD_3OD (0-75 μL).

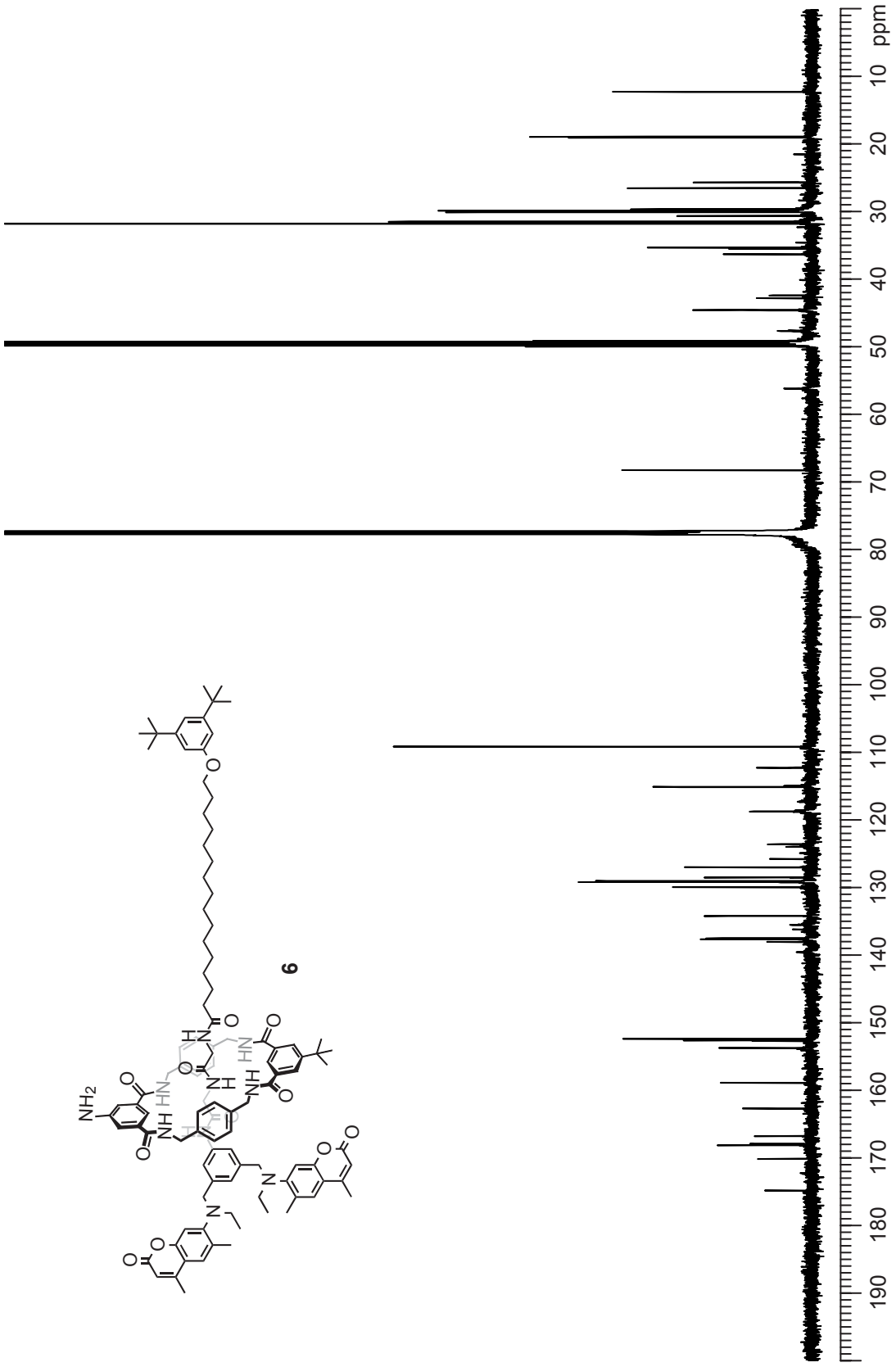


Figure S5. The 150 MHz ^{13}C NMR spectrum of the [2]rotaxane **6** recorded in $CDCl_3$ (600 μL) and CD_3OD (50 μL).

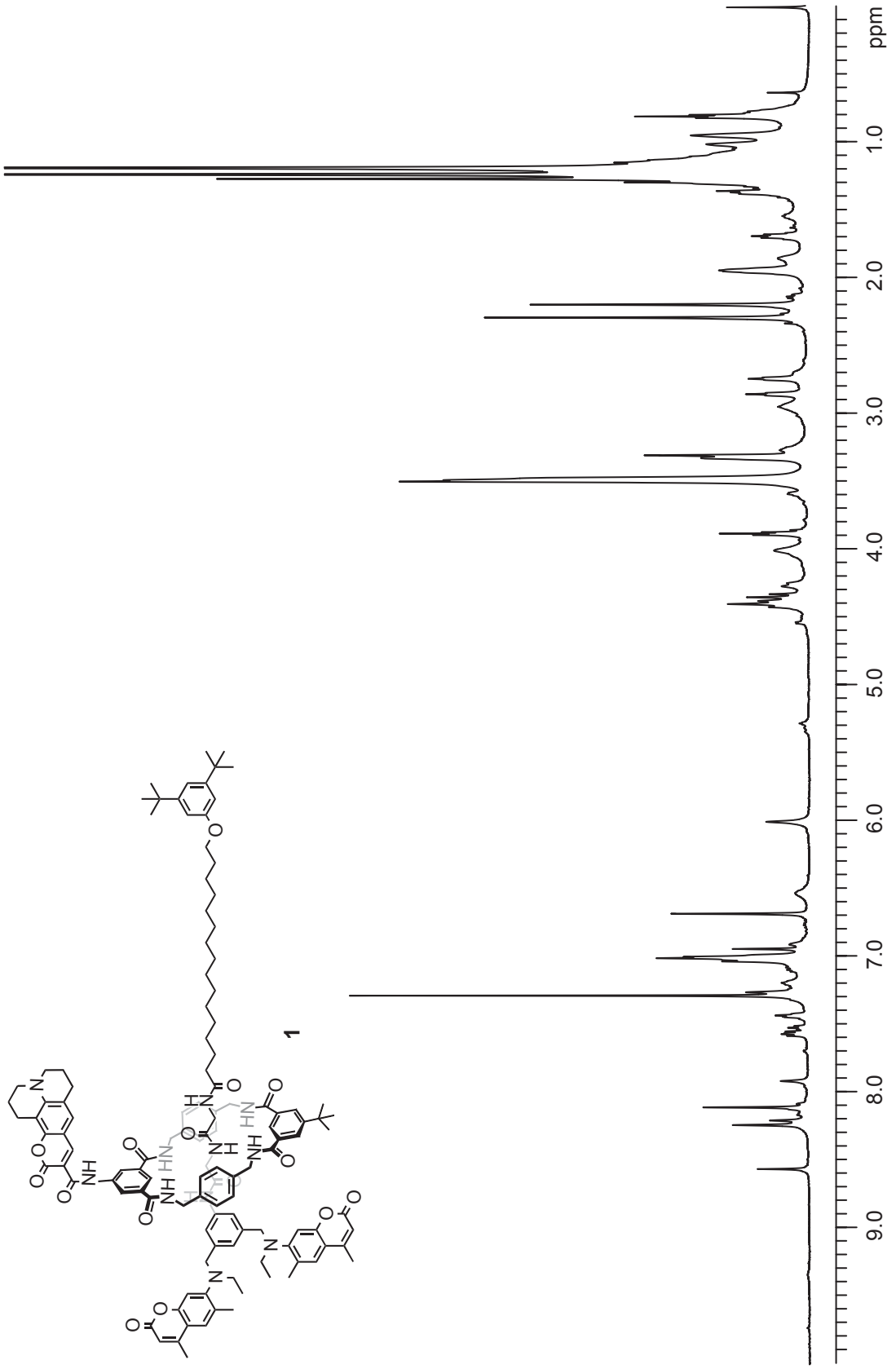


Figure S6. The 600 MHz ^{1}H NMR spectrum of the [2]rotaxane **1** recorded in $CDCl_3$ (600 μL) and CD_3OD (50 μL).

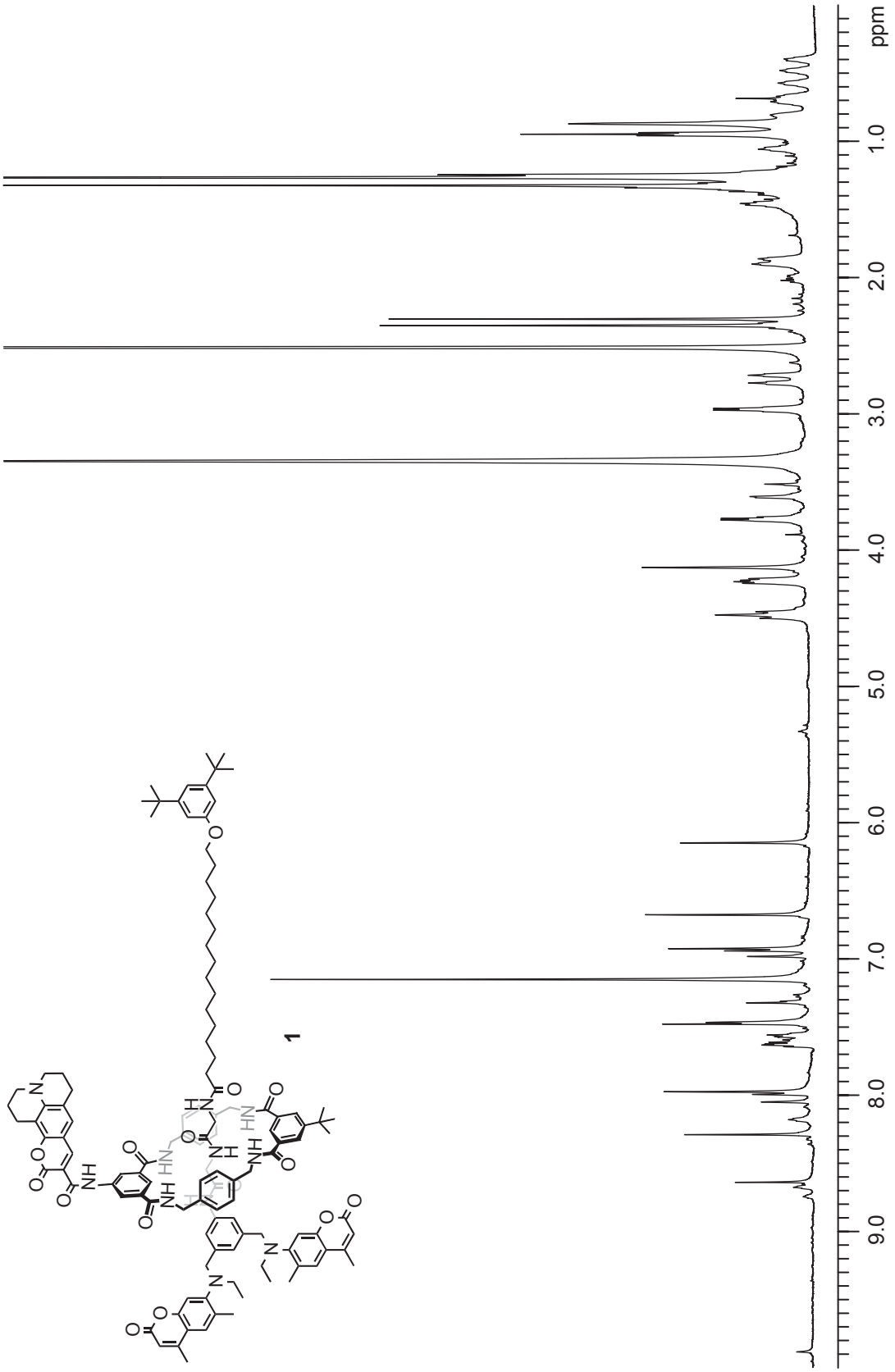


Figure S7. The 600 MHz ^{1}H NMR spectrum of the [2]rotaxane **1** recorded in DMSO-d_6.

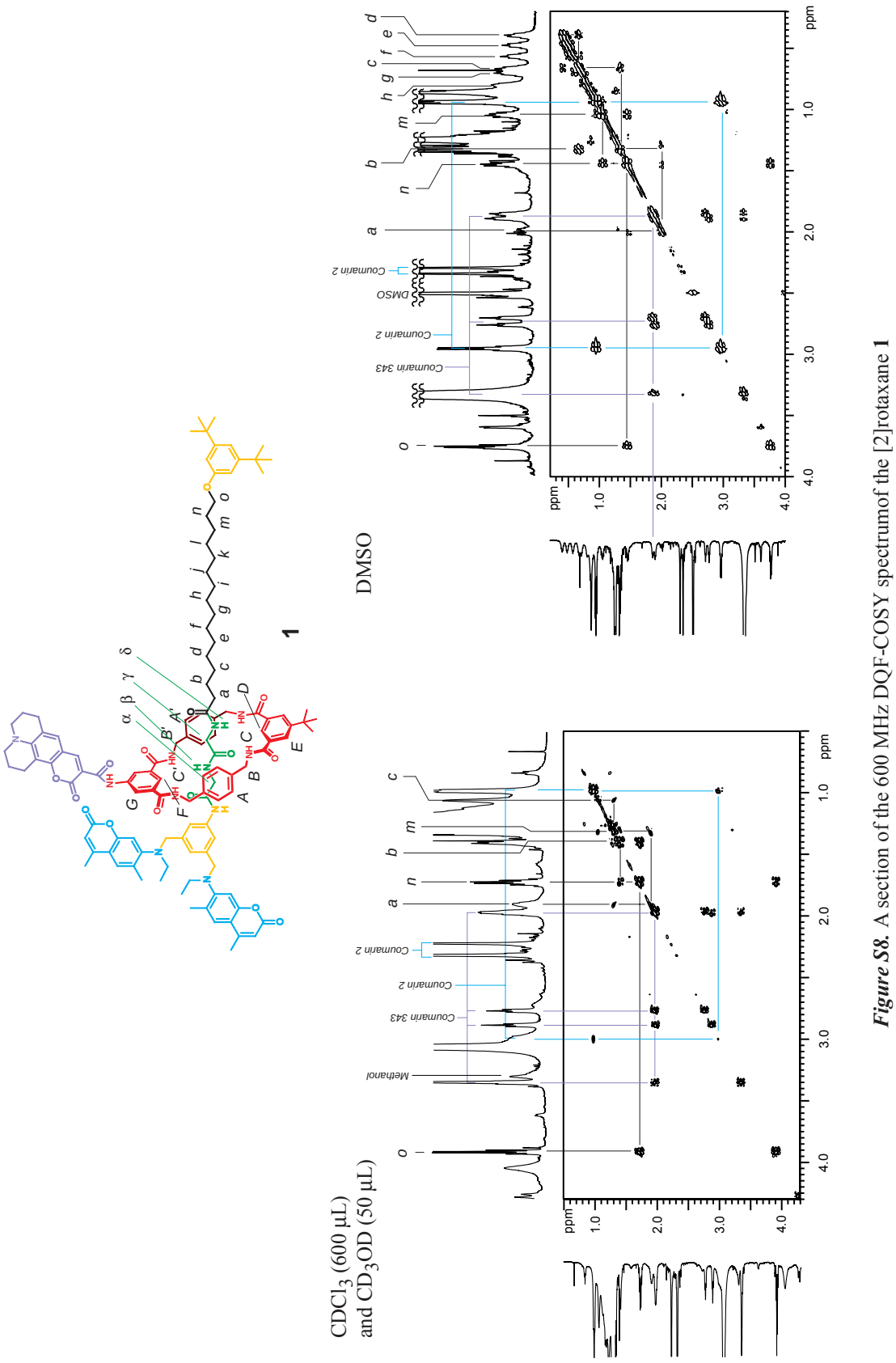
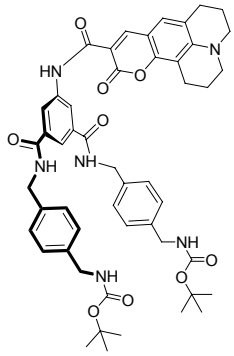


Figure S8. A section of the 600 MHz DQF-COSY spectrumof the [2]rotaxane **1**

5. UV/vis spectroscopy

Measurements were performed on a Varian Cary 50 Bio Spectrometer. The system was controlled with Varian Cary Win UV scan application version 3.00. Spectrophotometric grade chloroform and DMSO were purchased from Acros Organics, Pittsburgh, PA and used as received.

For the comparison of absorption and fluorescence spectra of both interlocked and non-interlocked species, it was attempted to prepare a macrocycle alone tagged with an acceptor coumarin. However, the synthesis was not successful, and therefore, precursor **S19** was prepared as a closest analog to the acceptor fluorophore unit.



S19

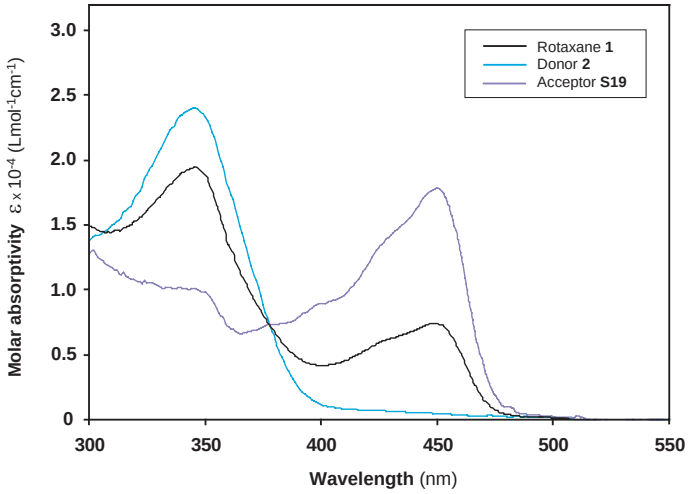


Figure S9. UV/vis spectra of the [2]rotaxane **1**, the donor **2** and the acceptor **S19** in $CHCl_3$ (50 μM, 298K).

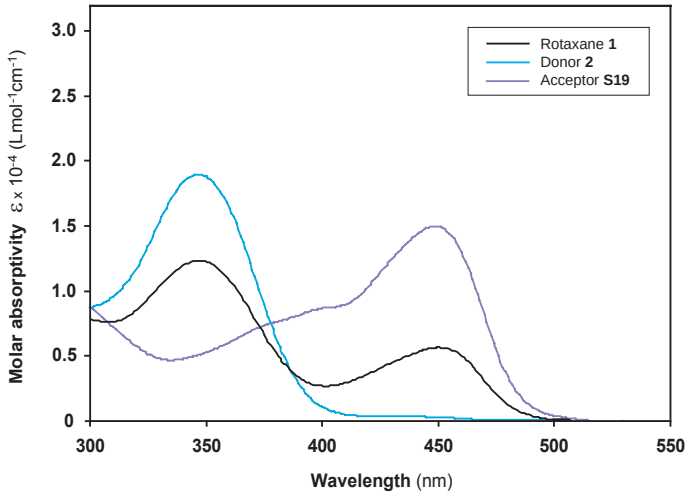


Figure S10. UV/vis spectra of the [2]rotaxane **1**, the donor **2** and the acceptor **S19** in DMSO (50 μM, 298K).

6. Fluorescence spectroscopy

Measurements were performed on a Spex Fluorolog 3 spectrofluorometer equipped with a 450W xenon lamp, a Czerny Turner design double grating excitation monochromator (BP 2 nm), and Czerny Turner design single grating emission monochromator (BP 1.1 nm). Fluorescence is presented in arbitrary units calculated as the ratio of observed emission divided by n internal reference. Spectrophotometric grade chloroform and DMSO were purchased from Acros Organics, Pittsburgh, PA and used as received.

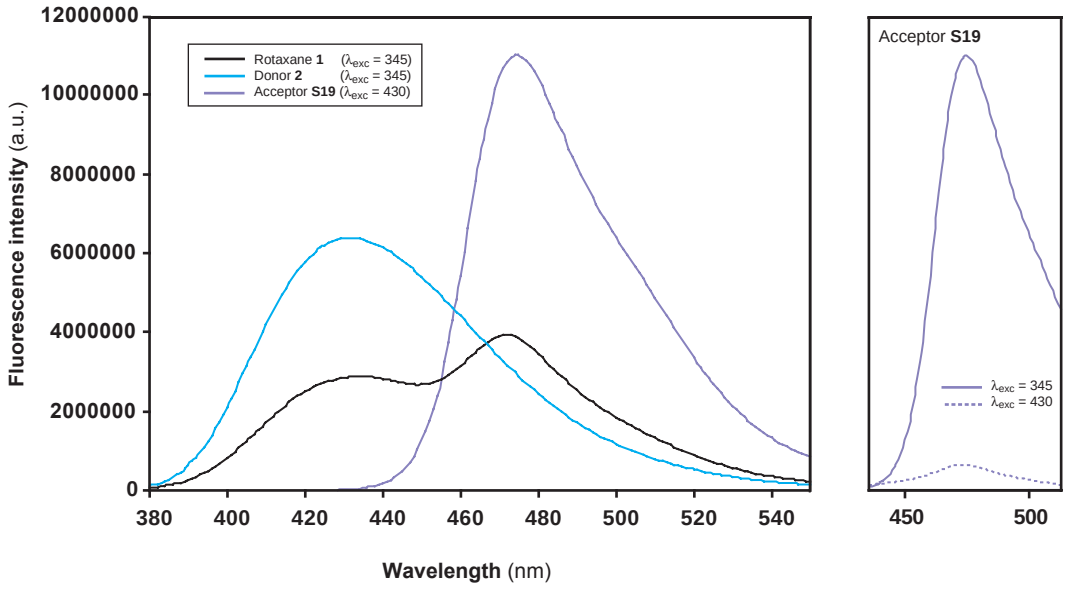


Figure S11. Fluorescence spectra of the [2]rotaxane **1**, the donor **2** and the acceptor **S19** in $CHCl_3$ (50 μM, 298K). The section at right shows the difference in two different λ_{exc}.

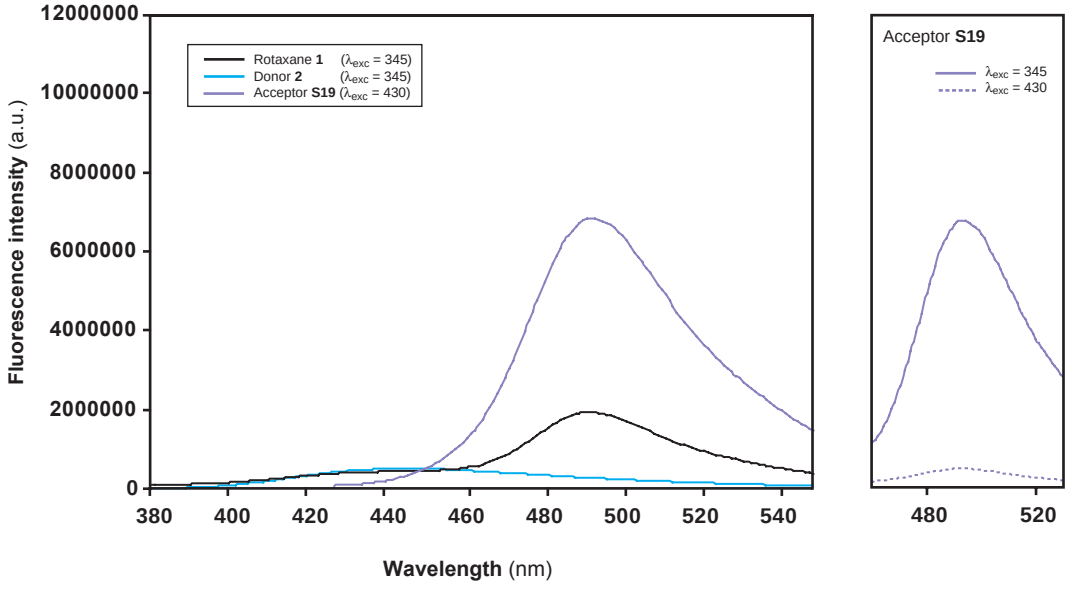


Figure S12. Fluorescence spectra of the [2]rotaxane **1**, the donor **2** and the acceptor **S19** in $CHCl_3$ (50 μM, 298K). The section at right shows the difference in two different λ_{exc}.

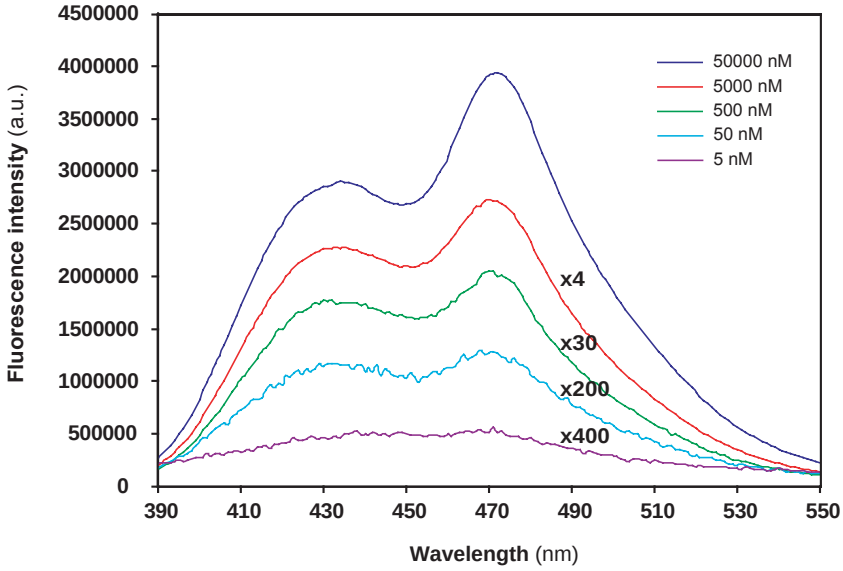


Figure S13. Fluorescence spectra showing the detection limit of FRET of the [2]rotaxane **1**. ($CHCl_3$, 298K)

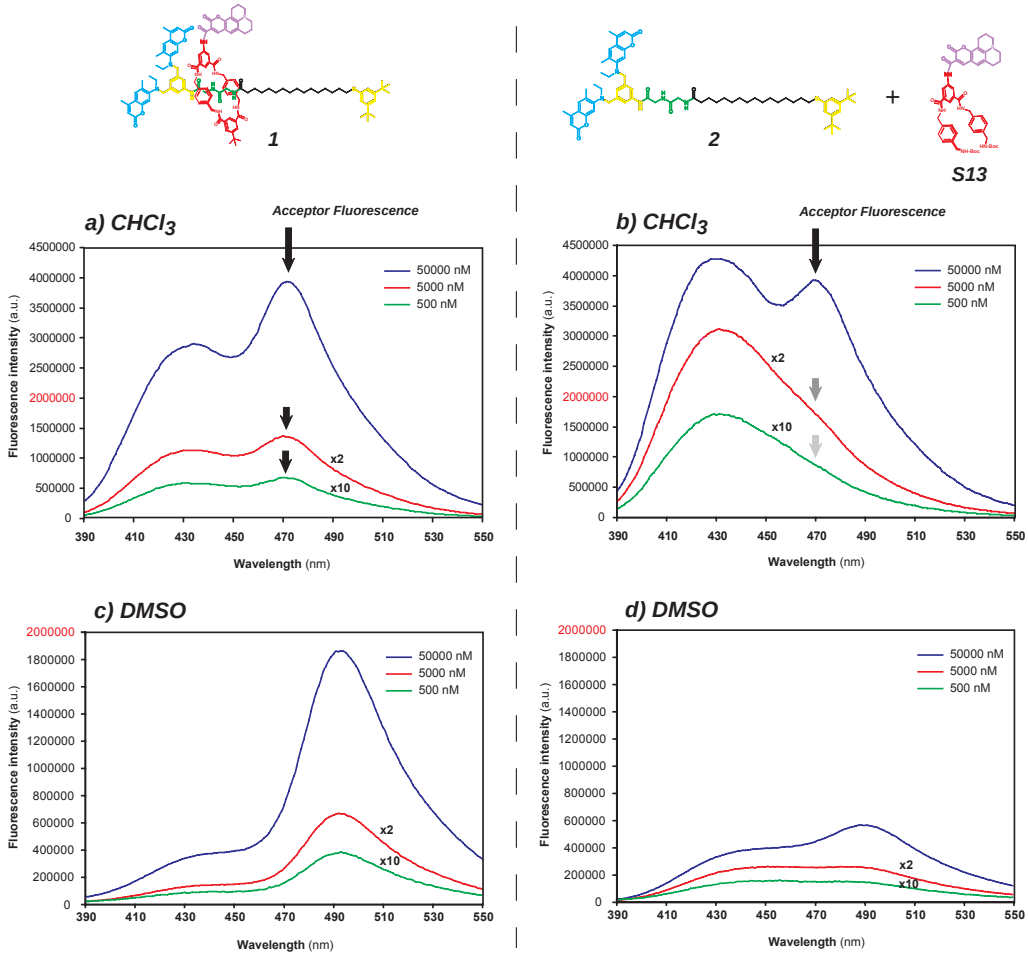


Figure 14. A series of dilution experiments showing the evidence of interlocking of a donor dumbbell and an acceptor macrocycle.