ELECTRONIC SUPPLEMENTARY INFORMATION FOR:

The foundation of a light driven molecular muscle based on stilbene and α-cyclodextrin

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General

^{1}H NMR spectroscopy and 2D NMR spectroscopy were conducted using a Varian Inova 500 spectrometer operating at 500 MHz, or a Varian Inova 600 spectrometer operating at 600 MHz. ROESY 2D ^{1}H NMR spectroscopy experiments were conducted using a Varian Inova 500 spectrometer operating at 500 MHz, using a spin lock time of 0.25 seconds, a relaxation delay of 2.0 – 2.1 seconds and a B1 field strength of 2129 Hz. ^{13}C NMR spectroscopy was conducted on a Varian Inova 500 spectrometer operating at 125 MHz. and 2D NMR spectroscopy were conducted using a Varian Inova 500 spectrometer operating at 500 MHz, d_4-Methanol with an isotopic purity of 99.8% was purchased from Apollo Scientific Ltd. D_2O with an isotopic purity of 99.75% was

purchased from Cambridge Isotope Laboratories Inc. d_4-Methanol was referenced at δ = 3.31 for ^{1}H NMR spectroscopy. When D_2O was used, 3-(trimethylsilyl)-3,3,2,2-tetradeuteropropionic acid sodium salt (d_4-TSP) was used as an external standard and referenced at $\delta = 0$ for ^{1}H and ^{13}C NMR spectroscopy.

Low resolution electrospray ionisation (ESI) mass spectrometry was conducted using a Micromass-Waters LC-ZMD single quadrupole liquid chromatograph mass spectrometer. High resolution ESI mass spectrometry was carried out on a Bruker Apex 4.7T FTICR mass spectrometer. Mass spectral data are reported as a mass-to-charge ratio (*m/z*).

Elemental analyses were performed by the Australian National University microanalytical service based at the Research School of Chemistry.

Thin layer chromatography (TLC) was carried out using aluminium backed 0.2 mm thick silica gel 60 F254 plates purchased from Merck. The mobile phase used was either *i*-PrOH:EtOH:H_2O:AcOH (5:4:3:2 v/v/v/v) or *n*-BuOH:EtOH:H_2O (5:4:3 v/v/v). TLC plates were visualised with a 254 nm UV lamp and by treatment with a naphthalene-1,3-diol solution (0.1% w/v) in EtOH:H_2O:H_2SO_4 (200:157:43 v/v/v). The R_f values are reported relative to the solvent front.

Analytical high performance liquid chromatography (HPLC) was performed with a Waters 2695 Separation Module with a Waters 2996 Photodiode Array Detector running with Empower Pro Empower 2 software. Semi-preparative and preparative HPLC was performed with a Waters 600 Controller, a Waters 717 Plus Autosampler and a Waters 2996 Photodiode Array Detector running with Empower Pro Empower 2 software. Fractions were collected with a Waters Fraction Collector III.

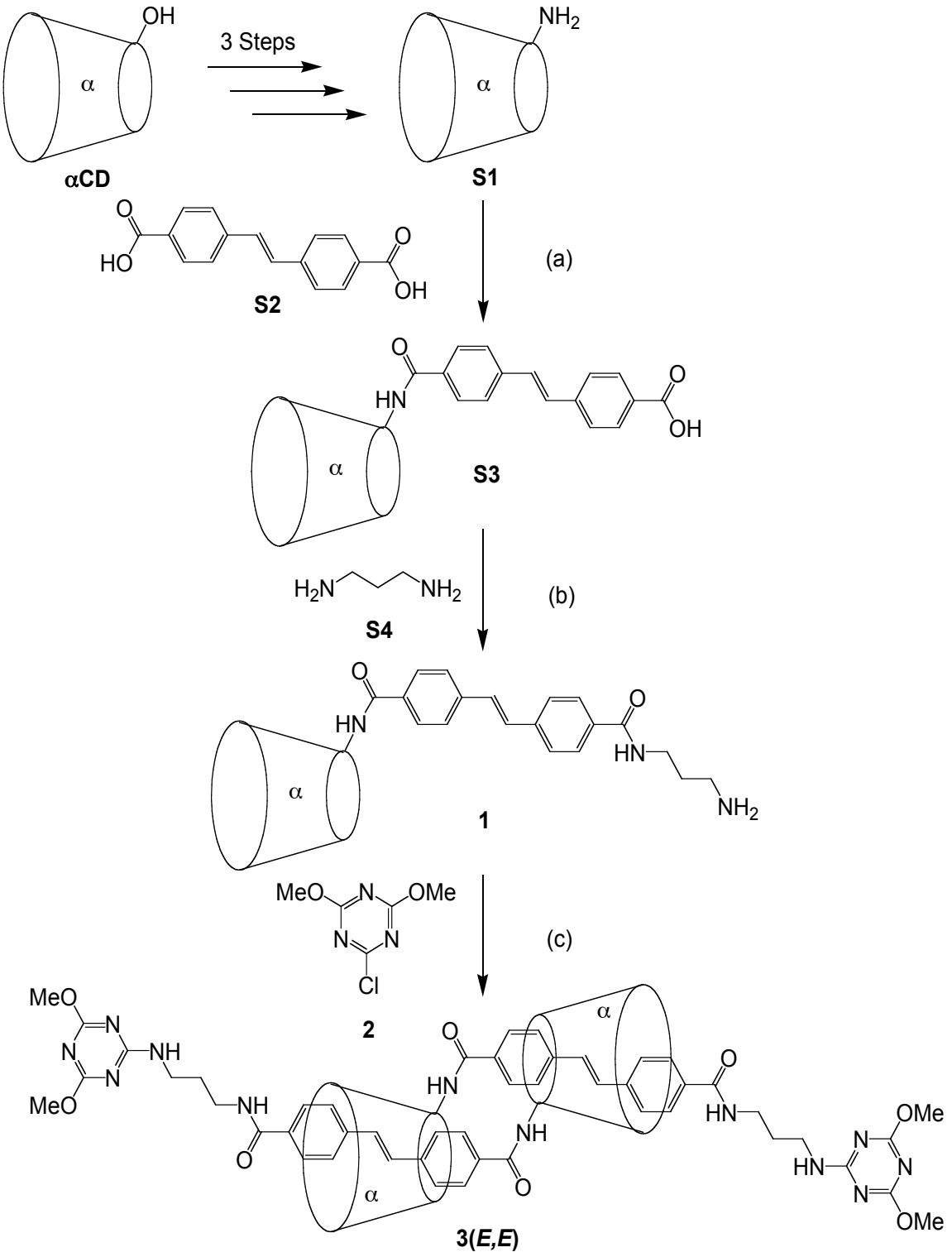
UV/visible studies were conducted using a Shimadzu UV-2450 UV/visible spectrophotometer with a Shimadzu CPS-temperature controller running with UV Probe Version 2.10 software.

A Luzchem photoreactor was used to irradiate samples in conjunction with UV-A fluorescent lamps (for 350 nm irradiation) and UV-C fluorescent lamps (for 254 nm irradiation).

Melting points were determined using a Reichert hot-stage microscope and are not corrected.

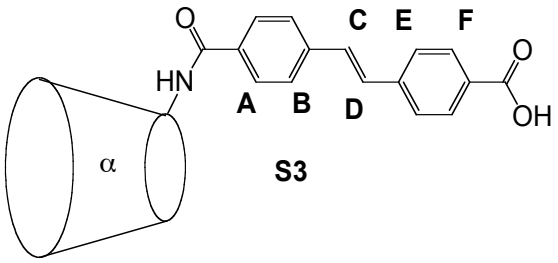
αCD was acquired from the Nihon Shokuhin Kako Co. Japan in 99.1% purity and was dried under reduced pressure at 60 °C to constant weight before use. The Diaion HP-20 resin was purchased from Supelco, PA. HPLC grade acetonitrile was purchased from J. T. Baker and H_2O was purified using an ELGA system. The amino-CD **S1**[1–4] and the stilbene **S2**[5] were synthesised according to literature procedures. All other starting materials, solvents and reagents were commercially available.

Synthesis of 3(*E,E*)



Scheme 1. Reagents and conditions: (a) BOP, TEA, DMF, 20 h, 24%; (b) BOP, TEA, DMF, 2 days, 33%; (c) TEA, H_2O, 24 h, 30%.

(*E*)-*N*-(6^{A}-Deoxy-α-cyclodextrin-6^{A}-yl)-4-aminocarbonyl-4'-carboxystilbene (S3)

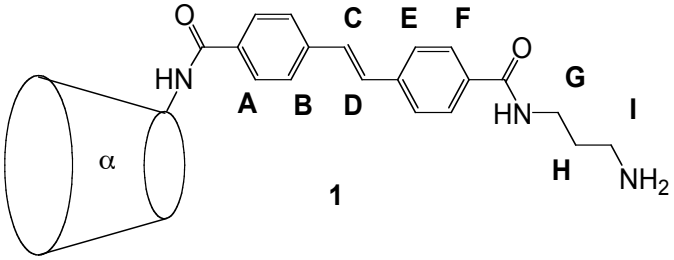


S3

Following the procedure of Cieslinski,[6] the stilbene **S2** (1.32 g, 4.91 mmol) and BOP (0.80 g, 1.80 mmol) were dissolved in anhydrous DMF (20 cm^3) and TEA (2 cm^3, 14.3 mmol) under an atmosphere of nitrogen. A solution of the CD **S1** (1.59 g, 1.64 mmol) dissolved in anhydrous DMF (30 cm^3) and TEA (1 cm^3, 7.2 mmol) was then added dropwise over a period of 20 min and the mixture was stirred for 20 h. The reaction mixture was then treated with acetone (600 cm^3) and the precipitate that formed was collected and dissolved in H_2O (800 cm^3). That solution was acidified to pH 2 with 1 M HCl and the resulting precipitate was collected by filtration. The solid was suspended in H_2O (600 cm^3) and the solution was adjusted to pH 12 with 1 M NaOH where upon the solid dissolved. The solution was applied to a Diaion HP-20 resin column (30 cm × 2.5 cm^3 i.d.) running a methanol/H_2O gradient. The title compound **S3** eluted with 50–70% methanol/H_2O with the desired fractions being combined and lyophilised to give the hermaphroditic CD **S3** as a colourless powder (0.49 g, 24%): R_f = 0.58 (*n*-BuOH:EtOH:H_2O 5:4:3); m.p. 246–249 ^{o}C (dec.); ^{1}H NMR (500 MHz, D_2O, 25 °C, TSP): δ = 8.10 (d, ^{3}J(H,H) 8.5 Hz, 2H; **F**), 7.97 (d, ^{3}J(H,H) 8.5 Hz, 2H; **E**), 7.52 (d, ^{3}J(H,H) 8.5 Hz, 2H; **A**), 7.32 (d, ^{3}J(H,H) 8.5 Hz, 2H; **B**), 7.26 (d, ^{3}J(H,H) 16.5 Hz, 1H; **C** or **D**), 7.14 (d, ^{3}J(H,H) 16.5 Hz, 1H; **C** or **D**), 5.18–4.94 (m, 6H; CD-H1), 4.58–3.06 ppm (m, 36H; CD-H2, CD-H3, CD-H4, CD-H5, CD-H6^{A,B}); ^{13}C NMR (125 MHz; D_2O, 25

°C, TSP): δ =174.8, 168.3, 138.6, 137.6, 136.9, 132.6, 130.3, 129.1, 128.2, 128.1, 127.3, 125.7, 102.6, 102.4, 102.3, 102.2, 102.1, 84.2, 81.2, 81.0, 80.9, 73.7, 73.6, 73.5, 72.8, 72.8, 72.5, 72.4, 72.2, 72.1, 72.0, 71.8, 71.7, 60.2, 59.9, 59.6, 59.3 and 40.9 ppm; MS (ESI): *m/z* (%) 1222.7 (20) [M^+ + H], 1244.7 (100) [M^+ + Na]; elemental analysis calcd (%) for $C_{52}H_{71}NO_{32}.7H_2O$: C 46.32, H 6.35, N 1.04; found: C 46.04, H 5.96, N 1.21.

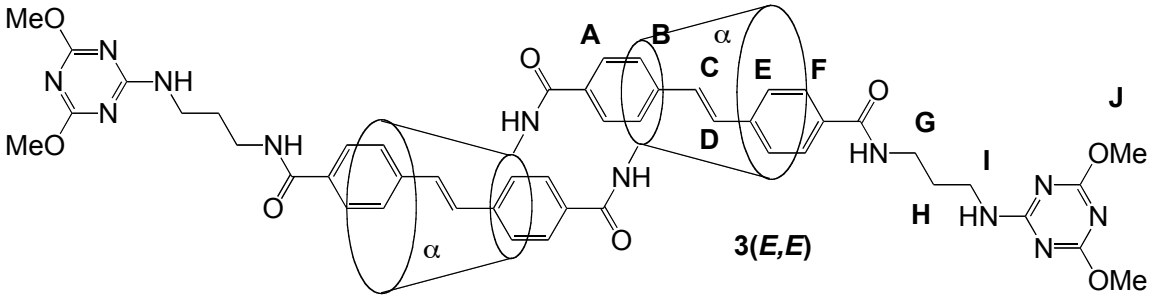
(*E*)-*N*-(6^A-Deoxy-α-cyclodextrin-6^A-yl)-4-aminocarbonyl-4'-(3-aminopropylaminocarbonyl)stilbene (1)



Following the procedure of Cieslinski,[6] the hermaphroditic CD **S3** (0.49 g, 0.40 mmol), the diamine **S4** (0.33 cm^3, 4.0 mmol) and BOP (0.53 g, 1.19 mmol) were dissolved in anhydrous DMF (30 cm^3) under an atmosphere of nitrogen. TEA (1.4 cm^3, 19.9 mmol) was then added and the mixture was stirred for 2 days. The reaction mixture was then treated with acetone (500 cm^3) and the precipitate that formed was collected by centrifugation. The solid was washed with acetone (2 × 200 cm^3), then dissolved in MeCN/H_2O (10 cm^3, 9:1) and that solution was subjected to preparative HPLC. The desired fractions were combined and lyophilised to give the hermaphroditic CD **1** (0.17 g, 33%) as a colourless powder: R_f = 0.40 (*i*-PrOH:EtOH:H_2O:AcOH 5:4:3:2); ^{1}H NMR (500 MHz, D_2O, 25 °C, TSP): δ = 7.99 (d, ^3J(H,H) 8.5 Hz, 2H; **E** or **F**), 7.97 (d, ^3J(H,H) 8.5 Hz, 2H; **E** or **F**), 7.53 (d, ^3J(H,H) 8.5 Hz, 2H; **A**), 7.29 (d, ^3J(H,H) 8.5 Hz, 2H; **B**),

7.25 (d, ^3J(H,H) 16.0 Hz, 1H; **C** or **D**), 7.15 (d, ^3J(H,H) 16.0 Hz, 1H; **C** or **D**), 5.15–4.90 (m, 6H; CD-H1), 4.50–3.00 (m, 40H; CD-H2, CD-H3, CD-H4, CD-H5, CD-H6^{A,B}, **G**, **I**), 2.01 ppm (p, ^3J(H,H) 7.0 Hz, 2H; **H**); ^{13}C NMR (125 MHz; D_2O, 25 °C, TSP): δ =170.0, 168.1, 138.7, 138.1, 134.1, 132.8, 129.6, 128.9, 128.4, 127.4, 127.3, 125.6, 102.4, 102.1, 102.0, 101.8, 84.3, 81.3, 81.2, 81.0, 80.8, 73.6, 73.5, 73.4, 73.3, 72.5, 72.4, 72.3, 71.7, 71.6, 71.5, 71.4, 60.0, 59.7, 59.4, 59.1, 40.7, 37.0, 36.6 and 26.8 ppm; MS (ESI): *m/z* (%): 1278.7 (100) [M^+ + H], 1300.7 (15) [M^+ + Na]; HPLC: t_R 13.6 min (column: YMC-PACK ODS-AQ, 250 × 20 mm; eluting with H_2O:MeCN:TFA, 84:16:0.1 v/v/v).

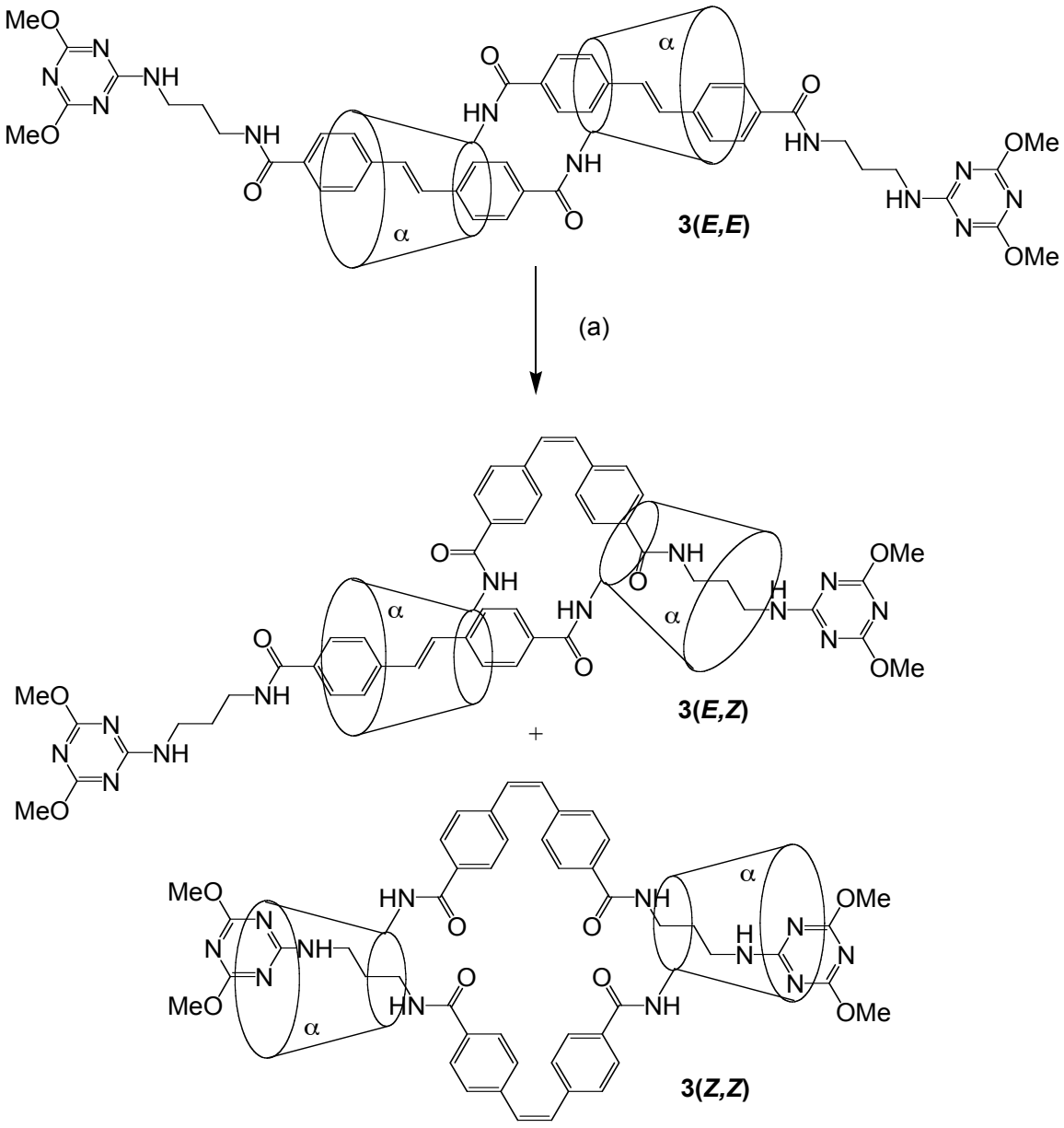
[(*E*)-*N*-(6^A-Deoxy-α-cyclodextrin-6^A-yl)-4-aminocarbonyl-4'-(3-(4,6-dimethoxy-1,3,5- triazin-2-ylamino)propylaminocarbonyl)stilbene]-[c2]-[daisy chain] (3(*E,E*)).



TEA was added to a stirring solution of the hermaphroditic CD **1** (94 mg, 74 μmol) in H_2O (5 cm^3) until the pH of the solution reached 9. The triazine **2** (13 mg, 74 μmol) was then added and the resulting suspension was stirred. After 24 hours, the reaction mixture was subjected to preparative reverse-phase HPLC. Fractions containing the hermaphroditic [2]-rotaxane **3(*E,E*)** were lyophilised to give a colourless powder (31 mg, 30%): R_f = 0.41 (*i*-PrOH:EtOH:H_2O:AcOH 5:4:3:2); m.p. 239 °C (dec.); ^{1}H NMR (500 MHz, D_2O, 25 °C, TSP): δ = 8.02–7.97 (m, 8H; **E** and **F**), 7.58 (d, ^3J(H,H) 8.0 Hz, 4H; **A**), 7.32 (d, ^3J(H,H) 8.0 Hz, 4H; **B**), 7.29 (d, ^3J(H,H) 16.5 Hz, 2H; **C** or **D**), 7.19 (d,

^{3}J(H,H) 16.5 Hz, 2H; **C** or **D**), 5.18–4.92 (m, 12H; CD-H1), 4.53–3.01 (m, 92H; CD-H2, CD-H3, CD-H4, CD-H5, CD-H6^{A,B}, **G**, **I**, **J**), 1.97 ppm (p, ^{3}J(H,H) 6.5 Hz, 4H; **H**); ^{13}C NMR (125 MHz; D_2O, 25 °C, TSP): δ =174.9, 174.3, 172.6, 171.0, 170.2, 141.5, 141.1, 137.4, 135.7, 132.6, 131.7, 131.3, 130.4, 130.1, 128.6, 105.3, 105.0, 104.9, 104.7, 87.2, 84.2, 84.0, 83.9, 83.7, 76.6, 76.5, 76.4, 76.3, 75.5, 75.3, 75.1, 74.6, 74.5, 74.4, 62.9, 62.6, 62.3, 62.1, 57.7, 57.6, 43.7, 40.9, 40.2, 33.1 and 31.0 ppm; UV/vis (H_2O): λ_{max} (ε) = 334 nm (105100); MS (ESI): *m/z* 2836.3 [M^+ + H], 2858.3 [M^+ + Na]; elemental analysis calcd (%) for $C_{120}H_{168}N_{12}O_{66}.13H_2O$: C 46.96, H 6.37, N 5.48; found: C 47.07, H 6.26, N 5.15; HPLC: t_R 7.6 min (column: YMC-PACK ODS-AQ, 250 × 20 mm; gradient eluting with H_2O:MeCN, 82:18 to 90:10 (20 min), to 0:100 v/v (25 min).

Synthesis of 3(*E,Z*) and 3(*Z,Z*)

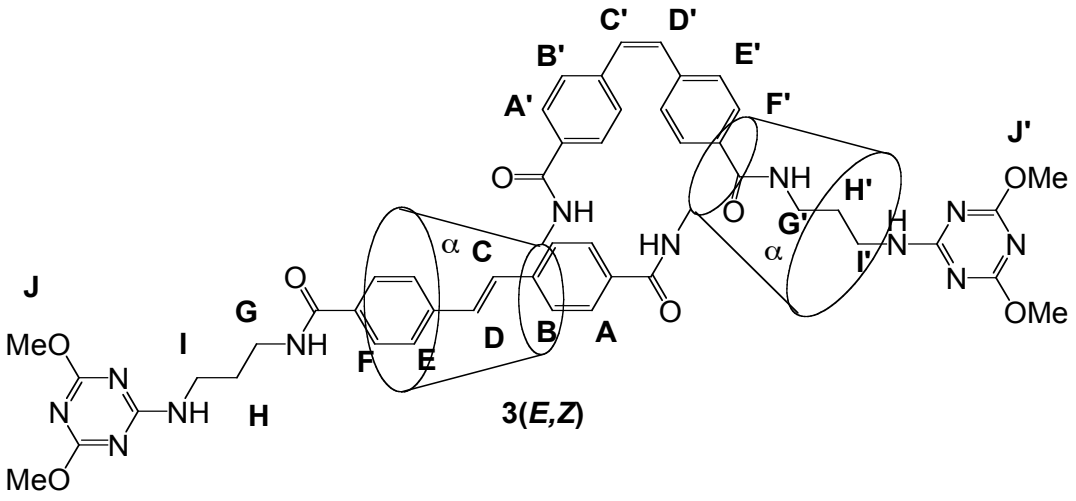


Scheme 2. Reagents and conditions: (a) H_2O, *hν* = 350nm, 2 min.

The hermaphroditic [2]-rotaxane **3(*E,E*)** (15 mg, 5.3 μmol) was made up to a concentration of 9.8×10^{-6} M in H_2O (540 cm^3) in a 1 dm^3 pyrex conical flask. The solution was irradiated with light of wavelength 350 nm for two minutes and the solvent

was removed by lyophilisation. The crude product was dissolved in H_2O (10 cm^3) and the solution was subjected to preparative HPLC. Desired fractions were combined and lyophilised to afford the hermaphroditic [2]-rotaxanes **3(*E,Z*)** (3.2 mg, 21%) and **3(*Z,Z*)** (1.7 mg, 11%), and recovered starting material **3(*E,E*)** (5.4 mg, 36%) as colourless powders.

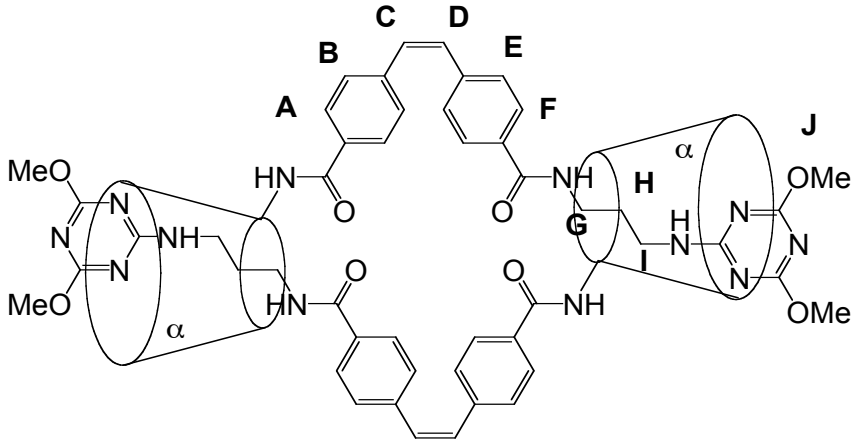
[(*E*)-*N*-(6^A-Deoxy-α-cyclodextrin-6^A-yl)-4-aminocarbonyl-4'-(3-(4,6-dimethoxy-1,3,5-triazin-2-ylamino)propylaminocarbonyl)stilbene]-[(*Z*)-*N*-(6^A-deoxy-α-cyclodextrin-6^A-yl)-4-aminocarbonyl-4'-(3-(4,6-dimethoxy-1,3,5-triazin-2-ylamino)propylaminocarbonyl)stilbene]-[c2]-[daisy chain] (3(*E,Z*)).



R_f = 0.51 (*i*-PrOH:EtOH:H_2O:AcOH 5:4:3:2); ^{1}H NMR (500 MHz, D_2O, 25 °C, TSP): δ = 7.96 (d, ^2J(H,H) 8.0 Hz, 2H; **E** or **F**), 7.91 (d, ^3J(H,H) 8.0 Hz, 2H; **E** or **F**), 7.73 (d, ^3J(H,H) 8.0 Hz, 2H; **F'**), 7.67 (d, ^3J(H,H) 8.0 Hz, 2H; **A**), 7.54 (d, ^3J(H,H) 8.0 Hz, 2H; **B**), 7.51 (d, ^3J(H,H) 8.0 Hz, 2H; **E'**), 7.39 (d, ^3J(H,H) 16.5 Hz, 1H; **C** or **D**), 7.33–7.24 (m, 5H; **B'**, **C** or **D**, **A'**), 6.93 (d, ^3J(H,H) 12.0 Hz, 1H; **C'** or **D'**), 6.80 (d, ^3J(H,H) 12.0 Hz, 1H; **C'** or **D'**), 5.34–4.84 (m, 12H; CD-H1), 4.26–2.98 (m, 92H; CD-H2, CD-H3,

CD-H4, CD-H5, CD-H6^{A,B}, **G**, **G'**, **I**, **I'**, **J**, **J'**), 2.12–1.92 ppm (m, 4H; **H**, **H'**); UV/vis (H_2O): λ_{max} (ε) = 330 nm (40000); HPLC: t_R 14.9 min (column: YMC-PACK ODS-AQ, 250 × 20 mm; gradient eluting with H_2O:MeCN, 82:18 to 90:10 (20 min), to 0:100 v/v (25 min).

[(*Z*)-*N*-(6^A-Deoxy-α-cyclodextrin-6^A-yl)-4-aminocarbonyl-4'-(3-(4,6-dimethoxy-1,3,5-triazin-2-ylamino)propylaminocarbonyl)stilbene]-[c2]-[daisy chain] (3(*Z*,*Z*)).



3(*Z*,*Z*)

R_f = 0.68 (*i*-PrOH:EtOH:H_2O:AcOH 5:4:3:2); ^{1}H NMR (500 MHz, d_4-Methanol, 25 °C): δ = 7.52 (d, ^3J(H,H) 8.5 Hz, 4H; **A** or **F**), 7.47 (d, ^3J(H,H) 8.5 Hz, 4H; **A** or **F**), 7.18 (d, ^3J(H,H) 8.5 Hz, 4H; **B** or **E**), 7.01 (d, ^3J(H,H) 8.5 Hz, 4H; **B** or **E**), 6.70 (d, ^3J(H,H) 12.5 Hz, 2H; **C** or **D**), 6.66 (d, ^3J(H,H) 12.5 Hz, 2H; **C** or **D**), 5.08–4.84 (m, 12H; CD-H1), 4.58–3.00 (m, 92H; CD-H2, CD-H3, CD-H4, CD-H5, CD-H6^{A,B}, **G**, **I**, **J**), 2.17–1.96 ppm (m, 4H; **H**); UV/vis (H_2O): λ_{max} (ε) = 305 nm (10300); HPLC: t_R 28.0 min (column: YMC-PACK ODS-AQ, 250 × 20 mm; gradient eluting with H_2O:MeCN, 82:18 to 90:10 (20 min), to 0:100 v/v (25 min).

Photochemical isomerisation of the hermaphroditic [2]-rotaxane 3(*E,E*)

The hermaphroditic [2]-rotaxane **3(*E,E*)** (1.8 mg, 0.64 μmol) was made up to a concentration of 6.4×10^{-6} M in H_2O (10 cm^3). A 1 cm^3 aliquot of the solution in a quartz UV/visible cell was irradiated with two 350 nm UV-A lamps for three minutes in the photoreactor. An aliquot of the irradiated solution was then analysed using HPLC. The solution was then irradiated with two 254 nm UV-C lamps for three minutes and an aliquot of the solution was analysed using HPLC. The cycle of irradiation with 350nm and 254 nm light was then repeated a further two times.

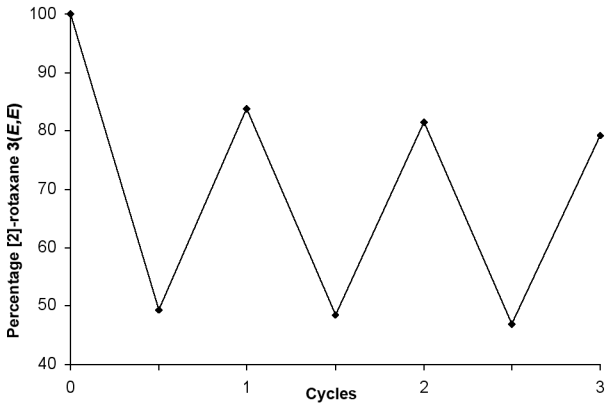


Fig. S1. Percentage of the hermaphroditic [2]-rotaxane **3(*E,E*)** remaining after cycles of irradiation of a 6.4×10^{-6} M solution in H_2O with 350 nm light followed by 254 nm light, each for 3 min.

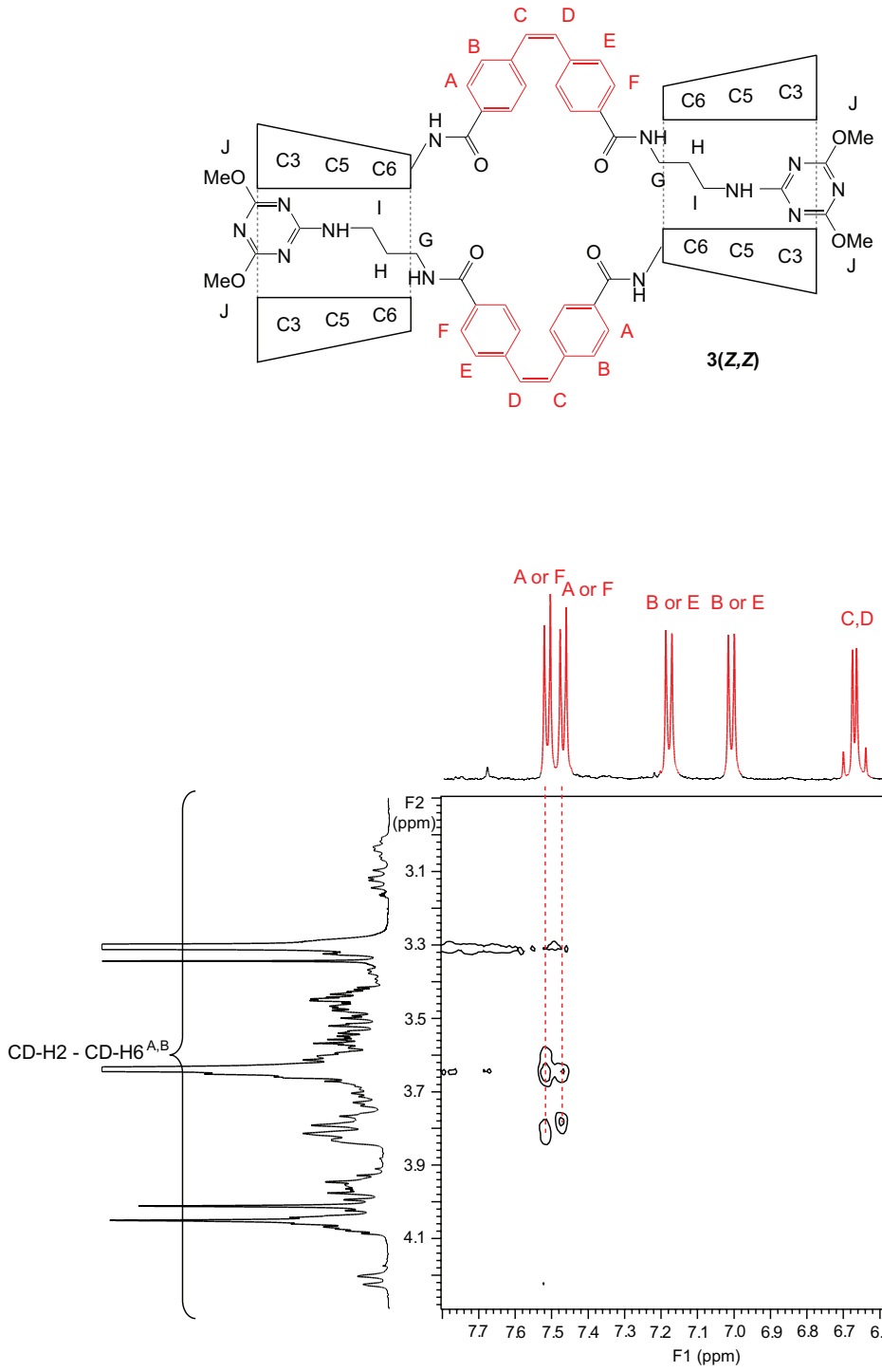


Fig. S2. A section of the 500 MHz ROESY NMR spectrum of the hermaphroditic [2]-rotaxane **3(*Z*,*Z*)** in d_4-methanol at 25 °C, of the region where crosspeaks are observed between aromatic and cyclodextrin proton signals.

References

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