Supplementary Material

Controlling the Morphology of Aggregates of an Amphiphilic Synthetic Receptor through Host-Guest Interactions

Chomchai Suksai,[a,e] Sergio Figueiras Gómez,[b] Anjali Chhabra,[c] Jingyuan Liu,[a] Jeremy N. Skepper,[d] Thawatchai Tuntulani[e] and Sijbren Otto[*a]

[a] *Department of Chemistry, University of Cambridge, Lensfield Road,Cambridge, CB2 1EW, UK Fax: +44 1223 336017; Tel: +44 1223 336509; E-mail: so230@cam.ac.uk*

[b] *Current address:Departamento de Química Orgánica y Unidad Asociada al CSIC, Universidad de Santiago de Compostela, 15706 Santiago de Compostela, Spain.*

[c] *Current address:Department of Chemistry, Indian Institute of Technology Bombay, India.*

[d] *Multi-Imaging Center, Department of Anatomy, University of Cambridge, Downing Street, Cambridge, CB2 3DY, UK.*

[e] *Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand.*

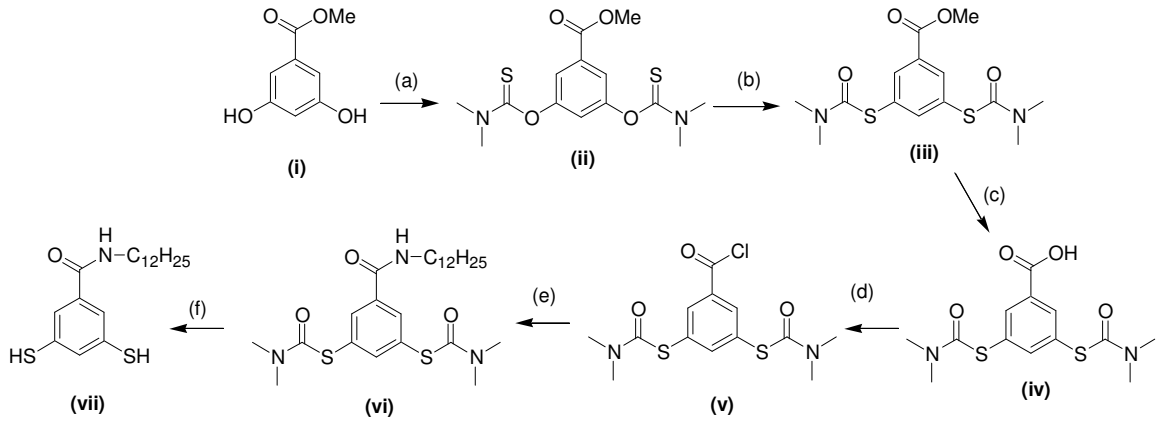
Contents

General Procedures. Receptor **1a**,[1] building block **2**,[1] and guests **4**[2] and **5**[3] were prepared following literature procedures. ^{1}H-NMR and 2D-NMR spectra were recorded on a Bruker ATM 500 MHz Spectrometer at room temperature. VT ^{1}H-NMR spectra were recorded on a Bruker DPX 500 Spectrometer.

Analytical HPLC was carried out on a Hewlett Packard 1100 instrument coupled to a UV analyser, set to 250 nm. The data was processed using HP Chemstation software. Separations were performed using a Nucleodur C_{18} column (4.6 cm × 250 mm, 100 Å, 5 μm), flow rate 1 mL/min, 75:25:0.1 acetonitrile:water:trifluoroacetic acid as mobile phase at 45^0 C.

LC-MS Method and Parameters. LC was performed using an Agilent 1100 series HPLC equipped with an online degasser, binary pump, autosampler, heated column compartment and diode array detector. MS was performed using an Agilent XCT ion trap MSD mass spectrometer. The mixture of **2**, **3** and **4** (see main text) was analyzed by injecting 10 μl of the solution onto a Nucleodur C_{18} column (4.6 cm × 250 mm, 100 Å, 5 μm) using a 75:25:0.1 acetronitrile:water:trifluoroacetic acid mixture as the mobile phase at a flow rate of 1 mL/min. Mass spectra (negative ion mode) were acquired in ultra scan mode using a drying temperature of 350^0C, a nebuliser pressure of 55.00 psi, a drying gas flow of 121 mL/min, a capillary voltage of 4000 V and an ICC target of 200,000 ions. We tuned for a target mass of 1200.

Synthetic Procedures for building block 3 are outlined in Scheme S1.



Scheme S1. Synthesis of building block **3**. Chemicals and reagents: (a) DMF, *N*,*N*-dimethylthiocarbamoyl chloride, DABCO, 15 hours,[1] (b) diphenyl ether, 230^0C, 3 hours,[1] (c) Me_3SiI, reflux 100^0C, 15 hours, (d) oxalyl chloride, DCM, 4 hours, (e) dodecylamine, NEt_3, DCM, 12 hours and (f) diethylene glycol, KOH, 105^0C, 4 hours.

***3,5-Bis(dimethylcarbamoylsulfanyl)benzoic acid* (iv)**

A mixture of 3,5-bis(dimethylcarbamoylsulfanyl)benzoic acid methyl ester (**iii**)[1] (2.8 g, 8.18 mmol) and Me_3SiI (6.22 mL, 45 mmol) was refluxed at 105°C for 15 hours. Diethyl ether (200 mL) was added and the solution was washed with 10% NaOH and dried over Na_2SO_4. A white solid (2.42 g, 7.36 mmol) was obtained in 90% yield after removing the diethyl ether. ^{1}H NMR ($CDCl_3$, 400 MHz, (ppm)): δ = 8.19 (s, 2H, *ArH*), 7.85 (s, 1H, *ArH*), 3.09 (m, 12H, -N*(CH₃)₂*); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm); δ = 166.68, 164.12, 144.85, 135.73, 128.90, 128.05, 34.98. Exact mass: calcd: 329.0630; found: 329.0630 ($M+H^+$).

***3,5-Bis(dimethylcarbamoylsulfanyl)benzoyl chloride* (v)**

Oxalyl chloride (1.3 mL, 15mmol) was added to a solution of (**iv**) (0.98 g; 3 mmol) in 8 mL of dry dichloromethane with 4 drops of DMF under a nitrogen atmosphere. The solution was stirred at room temperature for 3 hours. The solvent was removed under vacuum and the crude product was used in the next step without further purification.

***N-dodecyl-bis(3,5-dimethylcarbamoylsulfanyl)benzamide* (vi)**

To a solution of dodecylamine (0.64g, 3.5 mmol) and triethylamine (0.55 mL, 4 mmol) in dry dichloromethane (50 mL) was added a solution of acid chloride **v** (1.09 g; 3 mmol) in dry dichloromethane (15 mL). The mixture was stirred at room temperature for 15 hours. Then the solution was washed with 10% HCl and water, respectively. After drying over $MgSO_4$ and removal of the solvent, the residue was recrystallised from MeOH, giving 1.33 g (2.7 mmol; 90%) of a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ = 7.86 (s, 2H, *ArH*), 7.73 (s, 1H, *ArH*), 6.22 (m, 1H, -*NH*-), 3.39 (q, J = 6 Hz, 2H, -NH*CH₂*-), 3.05 (m, 12H, -N(*CH₃*)₄), 1.59 (m, J = 7.6 Hz, 2H, -NHCH₂*CH₂*-), 1.25 (m, 18H, *alkyl chain CH₂*), 0.87 (t, J = 6.8 Hz, 3H, -*CH₃*); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ = 164.89, 143.42, 135.19, 133.51, 129.12, 44.78, 39.25, 35.98, 30.90, 28.61, 28.53, 28.32, 25.99, 21.67, 13.10. Exact mass: calcd: 518.2487; found: 518.2487($M+Na^+$).

***N-dodecyl-3,5-dimercaptobenzamide* (vii)**

Compound **vi** (0.3 g; 0.60 mmol) was dissolved in 35 mL of a degassed solution of KOH (1.75 M) in diethylene glycol and heated at 105^0C for 30 min under a nitrogen atmosphere. The solution was cooled to room temperature and 250 mL of degassed H_2O

was added rapidly, followed by 10% HCl (30 mL). The white precipitate (0.20 g; 0.58 mmol; 97%) was filtered and extensively washed with water. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.39 (s, 1H, *ArH*), 7.26 (d, J = 7.2 Hz, 2H, *ArH*), 5.9 (m, 1H, -*NH*-), 3.42 (q, J = 7.2 Hz, 2H, -NH*CH$_2$*-), 1.55 (m, 2H, -NHCH$_2$*CH$_2$*-), 1.25 (m, 18H, -*CH$_2$*-), 0.87 (t, J = 7.2 Hz, 3H, -*CH$_3$*); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 166.99, 135.66, 132.14, 130.65, 123.89, 39.41, 31.05, 28.77, 28.73, 28.68, 28.46, 26.13, 21.82, 13.25. Exact mass: calcd: 354.1925; found: 354.1925(M+H$^+$).

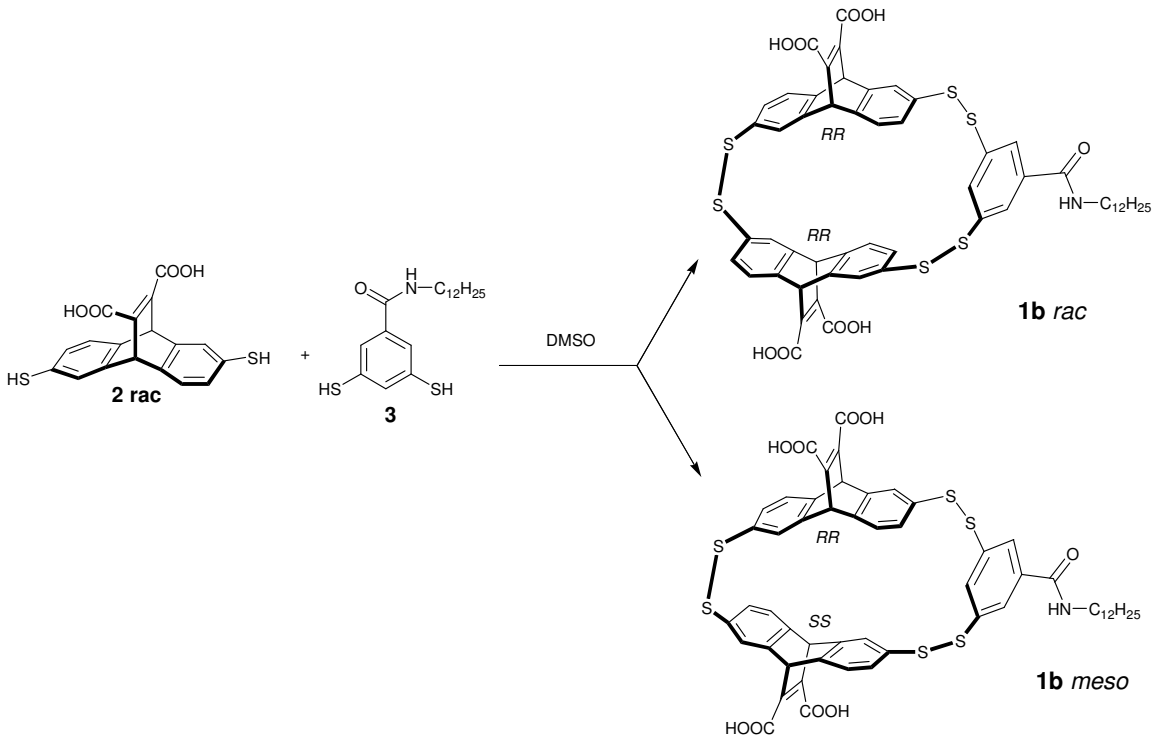
Synthesis and Purification of Receptor 1b

Receptor **1b** was prepared by stirring a mixture of building blocks **2** (6.67 mM), **3** (3.33 mM) and template **4** (10 mM) in DMSO for 24 h at room temperature. The receptor was isolated by using a Gilson preparative HPLC. Aliquots of 2 mL of the solution were chromatographed using a Nucleodur C_{18} preparative column (25.0 cm × 2.1 cm, 100 Å, 5 μm) with a Nucleodur C_{18} guard column (5.0 cm × 2.1 cm, 100 Å, 5 μm), at a flow rate of 20 mL/min, using 75:25:0.1 acetonitrile:water:trifluoroacetic acid as mobile phase. The chromatography was performed at 45^0 C by immersing the column, the guard column and a length of HPLC tubing (to ensure heat exchange) into a water bath. Retention times were 35-40 minutes for the major *rac* diastereomer. The collected fractions of 10 runs were combined and dried *in vacuo*, and redissolved in 10 mL of DMSO and rechromatographed in 5 injections. The collected product was dried *in vacuo*, filtered and washed extensively with water (250 mL).

^{1}H NMR of **1b** *rac* (D_2O:CD_3CN 1:1, 500 MHz, ppm) δ 8.59 (s, 1H, *ArH*), 8.10 (m, 6H, *ArH*), 7.78 (d, J = 7.5 Hz , 2H, *ArH*), 7.69 (m, 4H, *ArH*), 7.55 (m, 2H, *ArH*), 6.37 (s, 2H, -*CH*-), 6.29 (s, 2H, -*CH*-), 3.78 (q, J = 6.6 Hz, 2H, -NH*CH$_2$*-), 2.02 (t, J = 6.1 Hz, 2H, - NHCH$_2$*CH$_2$*-), 1.63 (m, 18H, -*CH$_2$*-), 1.21 (t, J = 7.1 Hz, 3H, -*CH$_3$*).

^{1}H NMR of **1b** *meso* (D_2O:CD_3CN 1:1, 500 MHz, ppm) δ 8.28 (s, 2H, *ArH*), 8.06 (s, 2H, *ArH*), 8.02 (s, 2H, *ArH*), 7.98 (s, 1H, *ArH*), 7.74 (d, J = 7.5 Hz, 2H, *ArH*), 7.66 (m, 4H, *ArH*), 7.55 (d, J = 7.5 Hz, 2H, *ArH*), 6.33 (s, 2H, -*CH*-), 6.27 (s, 2H, -*CH*-), 3.82 (q, J = 6.5 Hz, 2H, -NH*CH$_2$*-), 2.05 (t, J = 7.0 Hz, 2H, - NHCH$_2$*CH$_2$*-), 1.75 (m, 18H, -*CH$_2$*-), 1.11 (t, J = 7.5 Hz, 3H, -*CH$_3$*).

Characterization of the Solution of Receptors 1b by LC-MS



Scheme S2 Thiol oxidation to form receptor **1b** as a mixture of stereoisomers.

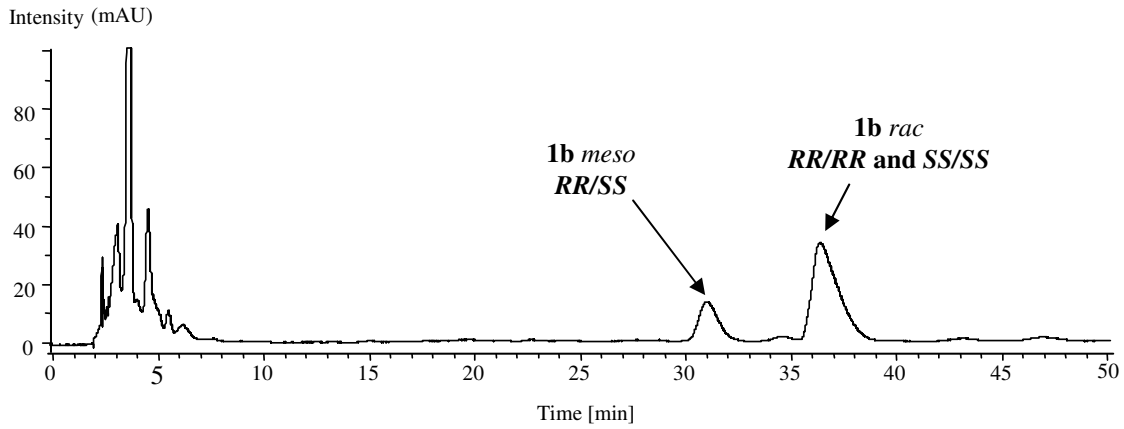


Figure S1. LC-MS analysis (negative ion mode) of the solution of receptors **1b** in DMSO.

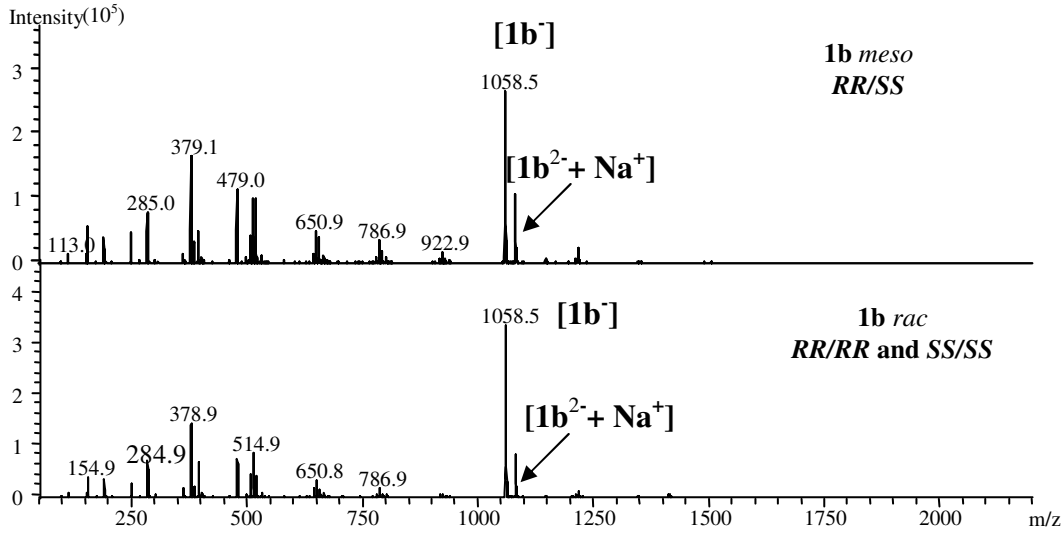


Figure S2. A mass spectrum retention time 31 min (top) and 36.5 min (bottom) for receptor **1b** *meso* and **1b** *rac*, respectively.

Characterization of Receptor 1b *rac* after Purification by Preparative HPLC

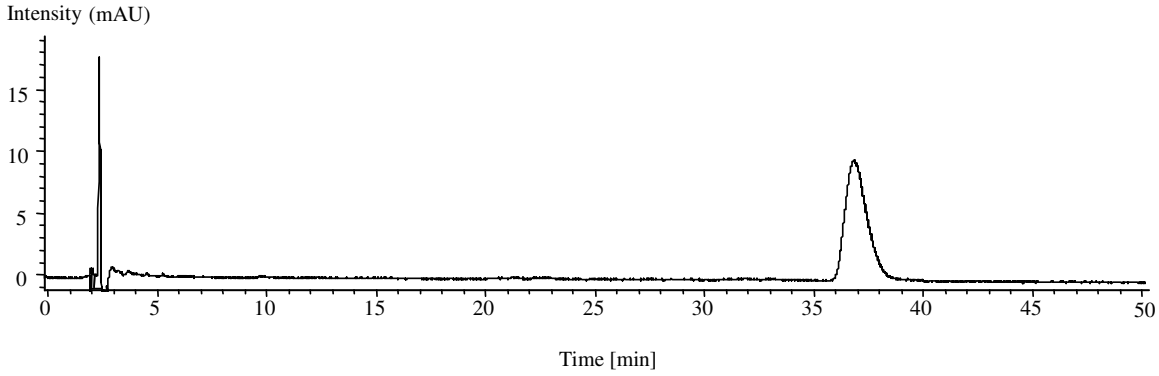


Figure S3. LC-MS analysis (negative ion mode) of the solution of receptor **1b** *rac* after purification by preparative HPLC.

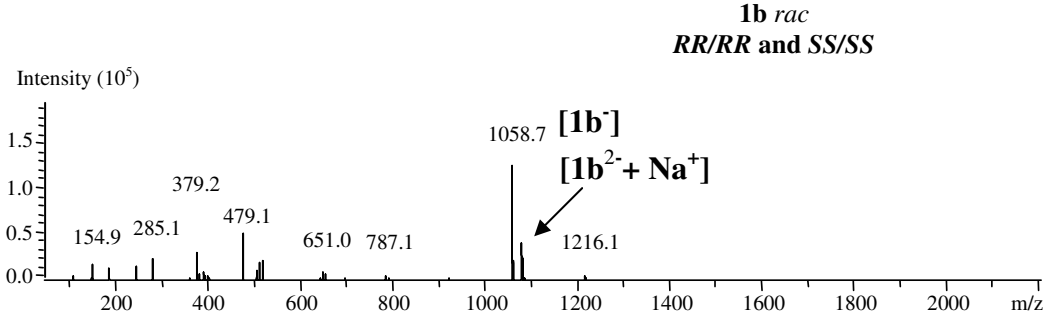


Figure S4. A mass spectrum t = 37 min of receptor **1b** *rac* after purification by preparative HPLC.

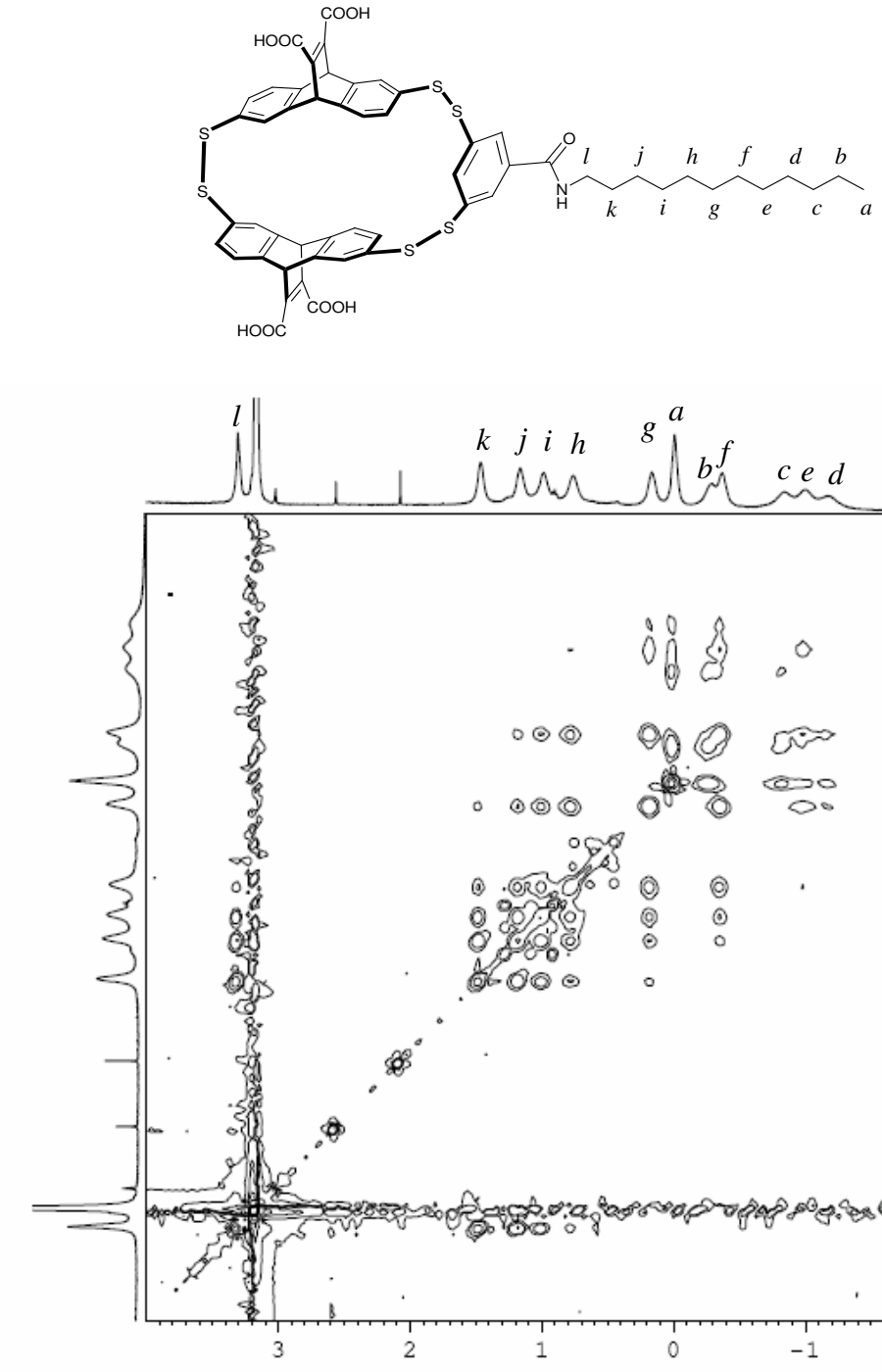


Figure S5. Part of the TOCSY NMR spectrum of receptor **1b** *rac* (0.6 mM in D_2O (pD 8.7) : MeOD-d_4 2:1).

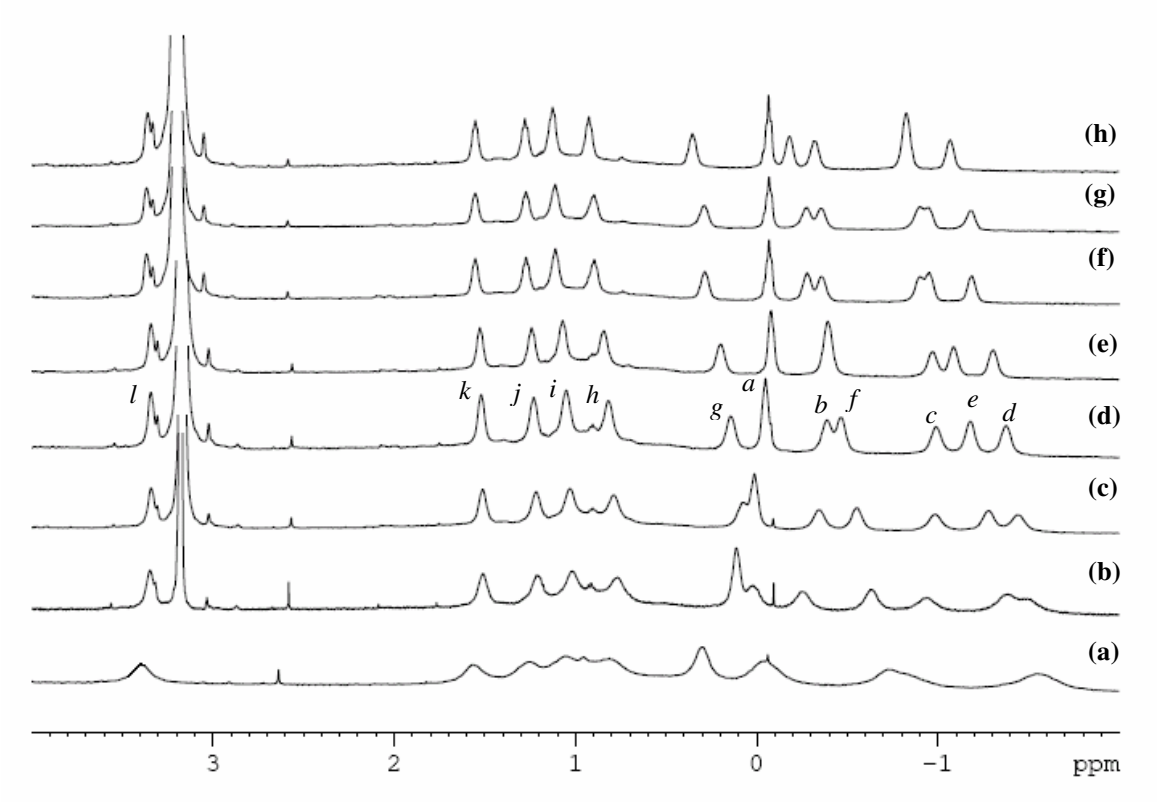


Figure S6. Part of the ^{1}H NMR titration of MeOD-*d*$_4$ into a solution of receptor **1b** *rac* (1 mM in D_2O (pD 8.7)). The volume ratio of D_2O (pD 8.7) to MeOD-*d*$_4$ was: (a) 1:0; (b) 1:0.16; (c) 1:0.32; (d) 1:0.5; (e) 1:0.64; (f) 1:0.8; (g) 1:1; and (h) 1:1.16.

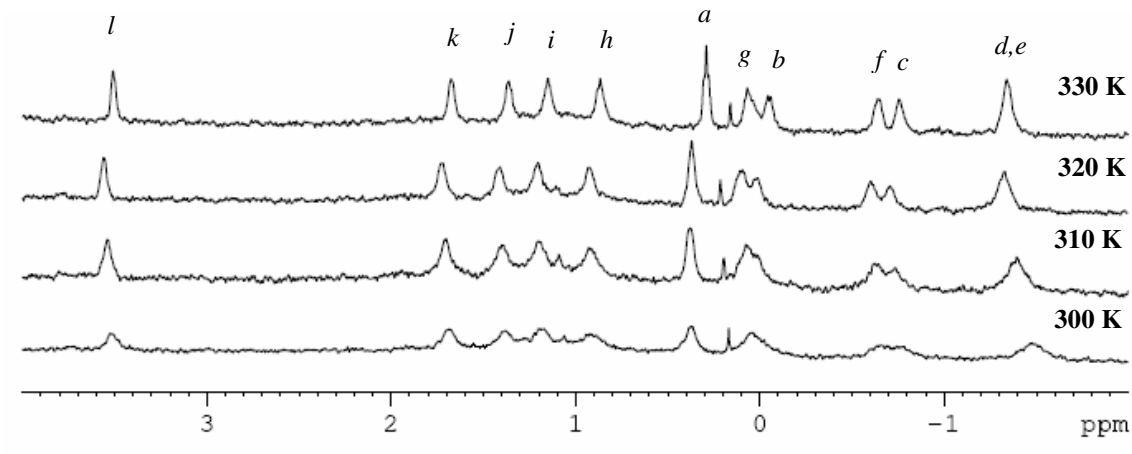


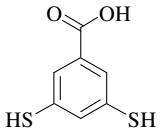
Figure S7. Part of the VT-^{1}H NMR spectrum of receptor **1b** *rac* at 1 mM in D_2O (pD 8.7).

Re-Equilibration Experiments

To assign the diastereomers of receptor **1b** (*rac RR/RR* and *SS/SS*, *meso RR/SS*), we have studied the early stages of the re-equilibration of **1b** and its transformation into receptor **1a** of which we have previously assigned the diastereomers.[4] Each diastereomer of receptor **1b** was isolated by preparative HPLC and dissolved in 1:1 solvent mixture of 10 mM borate buffer pH 9: acetonitrile (0.1 mg/mL of major or minor isomer). To this solution was added 1 equivalent of dithiol building block **viii**. The build up of small amounts of the expected **1a** isomers (Scheme S3) was monitored with time by HPLC. Separations were achieved by injecting 5 µl of the mixture onto a Nucleodur C_{18} column (4.6 cm × 250 mm, 100 Å, 5 µm) at 45^0C using a flow rate 1 mL/min. The following gradient was used:

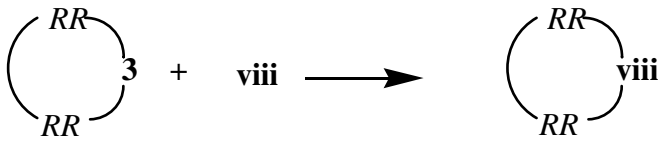
Time (minutes)	**Acetronitrile + 0.1% Trifluoroacetic acid**	**H_2O + 0.1% Trifluoroacetic acid**
0	50	50
20	50	50
25	75	25
60	75	25
65	50	50
75	50	50

The results were compared with the HPLC analysis of an independently generated mixture made from building blocks **2***rac* and **viii**, which contains all stereoisomers of macrocycles **1a** which we have assigned previously (Scheme S3 and Figure S8).[4]



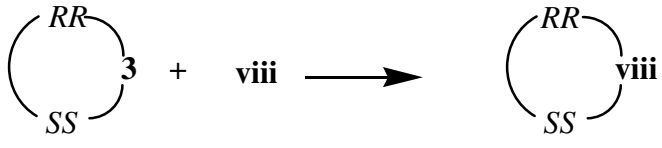
viii

(A) Receptor 1b *rac*



1b *rac* **1a** *rac*

(B) Receptor 1b *meso*



1b *meso* **1a** *meso*

Scheme S3 Correlation between the diastereomers of **1b** with those of **1a** that are formed in the early stages of re-equilibration of receptor **1b** with building block **xiii**.

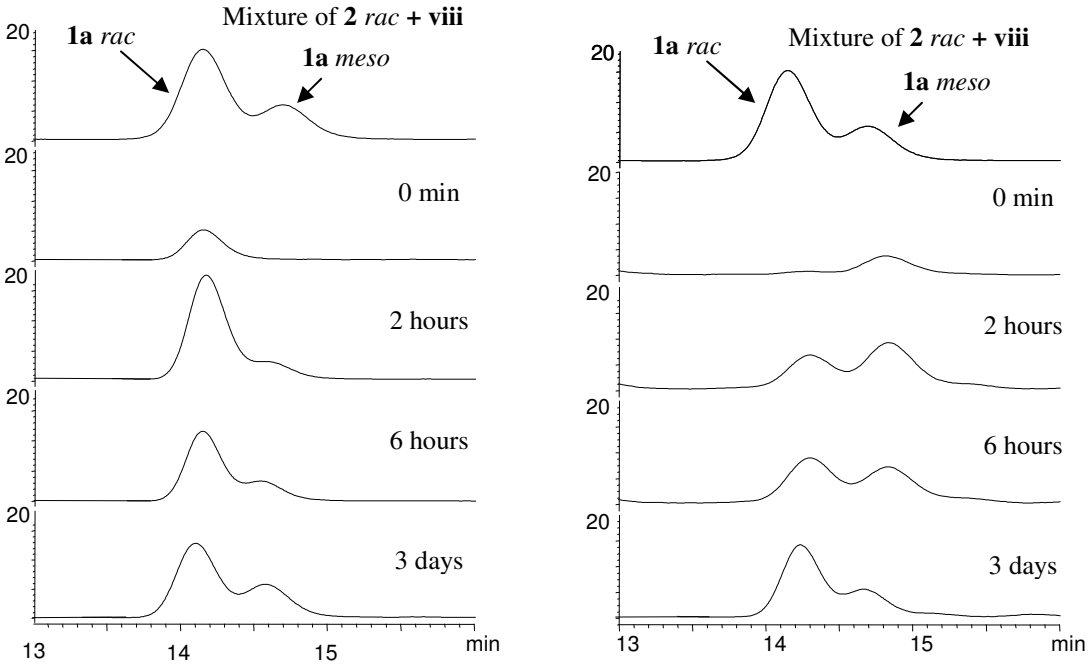


Figure S8. HPLC chromatograms of the product mixture obtained upon re-equilibration of **(A)** receptor **1b** (major *rac* isomer; 0.1 mM) and **(B)** receptor **1b** (minor *meso* isomer; 0.1 mM) in the presence of building block **viii** (1 equivalent) in H_2O (pH 9):CH_3CN 1:1. The top traces represent the HPLC analysis of an independently generated mixture made from building blocks **2***rac* and **viii**, which contains all stereoisomers of macrocycles **1a** which we have assigned previously.

Isothermal Titration Calorimetry Isothermal titration calorimetry was performed at 25^0C using a MCS isothermal titration calorimeter (MicroCal, Northampton, MA, USA). All solutions were degassed before titrations. Titrations were performed in 10 mM borate buffer pH 9. The host concentration in the cell was 0.075 mM and the guest (0.75 mM) was titrated in 10 μL steps using 30 injections spaced at intervals of 200 seconds with a syringe stirring speed at 300 rpm. The binding constants were obtained using the one-site binding model provided in the ORIGIN software (version 2.9).

Dynamic Light Scattering Studies Dynamic light scattering measurements were performed on a Malvern Instruments Zetasizer Nano ZS. Samples were filtered through a membrane filter (0.45 μm) prior to measurements to remove any dust particles – control experiments without filtration gave essentially identical results confirming that this procedure does not remove parts of the sample.

Dynamic Light Scattering Data

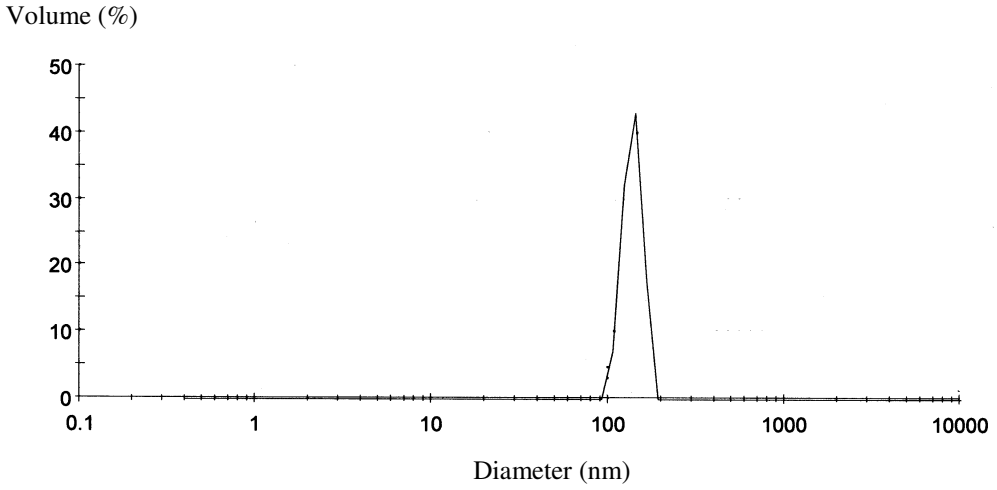


Figure S9. Dynamic Light Scattering data for a solution of **1b** *rac* (0.1 mg/mL in 10 mM borate buffer pH 9). The graph represents the volume average taken over 14 measurements.

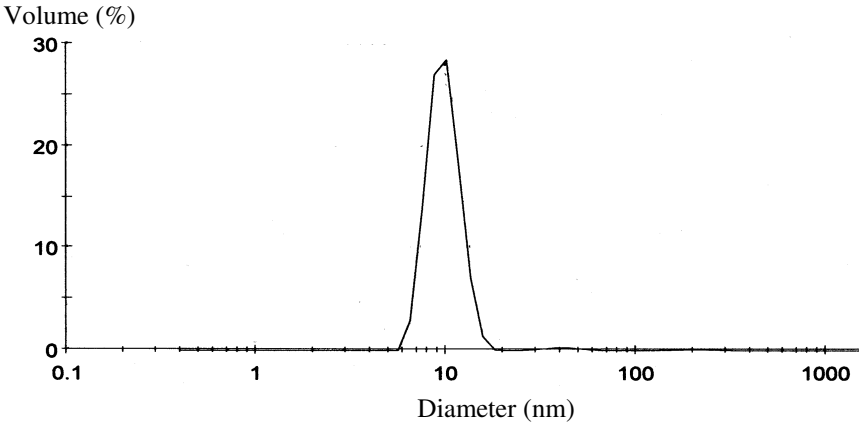


Figure S10. Dynamic Light Scattering data for a solution of **1b·5** (0.1 mg/mL of **1b** *rac* and 4 equivalent of **5**) in 10 mM borate buffer pH 9. The graph represents the volume average taken over 14 measurements.

Transmission Electron Microscopy TEM was conducted on a Philips CM100 electron microscope operating at 80 kV. A 300 mesh carbon-coated copper grid was placed on top of a drop of sample solution (0.1 mg/mL) for 30 minutes and then gently blotted with filter paper. The specimen was negatively stained using a drop of 2% PTA (pH 7) solution, incubated for 5 minutes, finally gentle blotted and dried at room temperature.

Nile Red Solubilisation Nile red (0.10 mg) was added to 3mL of a solution of **1b·5** and equilibrated for 2 hours. The solution was filtered before measurements. UV-Vis experiments were carried out on a Hewlett Packard 8452A spectrophotometer. Fluorescence experiments were measured on an Aminco-Bowman spectrometer.

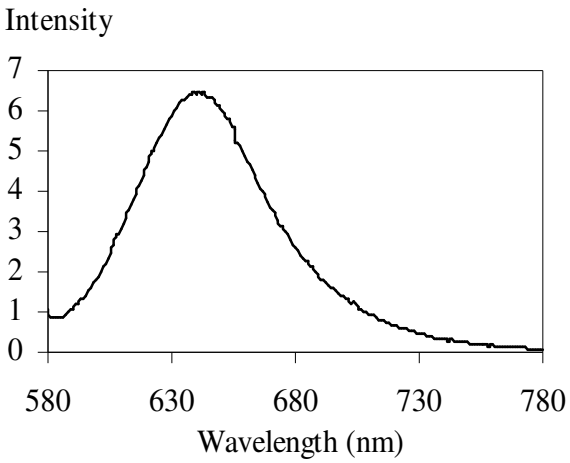


Figure S11. The emission spectrum of Nile Red in a solution of **1b·5** (0.1 mg/mL of **1b** *rac* and 4 equivalent of **5**) in 10 mM borate buffer pH 9 (λ_{ex} = 570 nm).

References

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2. M. A. Petti, T. J. Sheppod, R. E. Barrans and D. A. Dougherty, *J. Am. Chem. Soc.,* 1988, **110**, 6825,
3. G. K. van der Wel, J. W. Wijnen and J. F. B. N. Engberts, *J. Org. Chem.,* 1996, **61**, 9001.
4. P. T. Corbett, manuscript in preparation