Selective Disaccharide Binding by a Macrotetracyclic Receptor

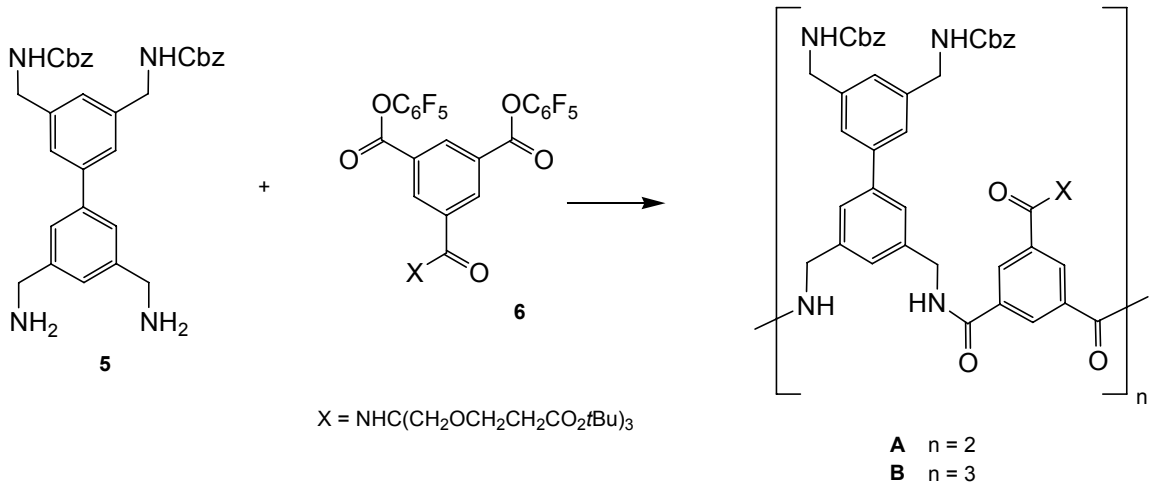
Emmanuel Klein, Yann Ferrand, Elizabeth K. Auty, and Anthony P. Davis*

School of Chemistry, University of Bristol, Cantock's Close, Bristol, UK BS8 1TS.
Fax: +44 117 9298611; Tel: +44 117 9546334. E-mail: Anthony.Davis@bristol.ac.uk

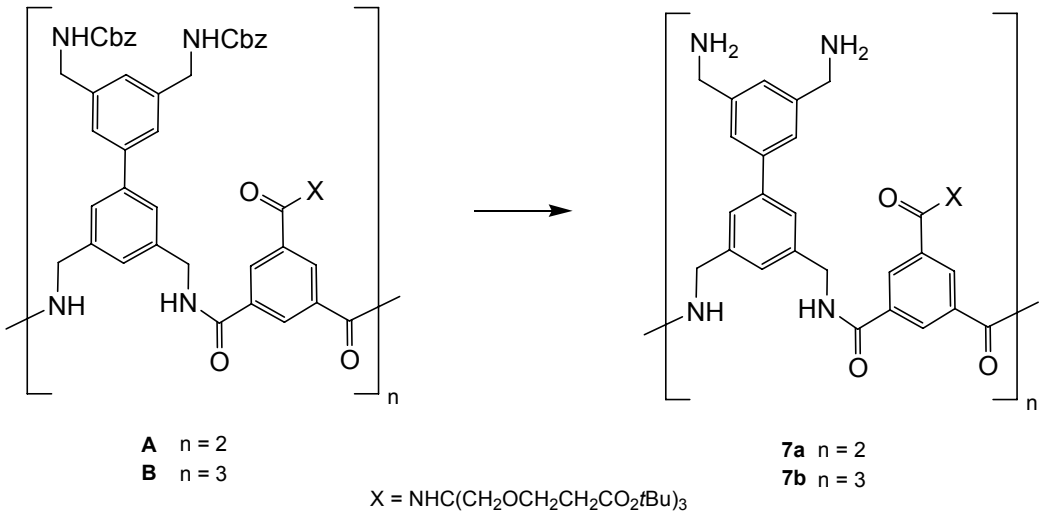
Supplementary Information

Synthesis of Receptor 3

General: ^{1}H-NMR spectra were recorded on a JEOL/Delta GX-400 MHz, a JEOL/Eclipse-400 MHz or a Varian *INOVA* 600 MHz spectrometer. ^{13}C-NMR spectra were recorded at 100 MHz on the first two instruments. Chemical shifts (δ) are quoted in parts per million and are calibrated relative to solvent residual peaks. Low resolution mass spectra were recorded on an Applied Biosystems Mariner API-TOF spectrometer or an Applied Biosystems Voyager MALDI-TOF spectrometer. Reactions were monitored by thin layer chromatography (TLC) on Merck silica gel 60-F_{254} plates. Flash column chromatography was carried out on Fisher Scientific silica gel 60 (particle size 35-70 μm). Commercial reagents were purchased from Aldrich, Lancaster, Sigma, Strem or Fluka and were used without further purification unless otherwise specified.



Macrocyclisation of diamine 5 with bis-pentafluorophenyl ester 6. A solution of bis-pentafluorophenyl ester **6**[1](1.79 g, 1.74 mmol) in dry THF (55 mL) was added dropwise over 30 h to a solution of diamine **6**[1] (853 mg, 1.58 mmol) and *N*,*N*-diisopropylethylamine (1.7 mL, 9.50 mmol) in dry THF (1700 mL) under nitrogen and at RT. The reaction mixture was stirred vigorously for 24 h at RT. The solvent was removed under reduced pressure to give a crude solid that was taken up in chloroform (200 mL) and washed with saturated aqueous NH_4Cl (100 mL), water (100 mL), saturated aqueous $NaHCO_3$ (100 mL) and brine (100 mL). The organic layer was dried (Na_2SO_4), filtered and the solvent was removed at reduced pressure. Flash column chromatography (eluent toluene/dichloromethane/ethyl acetate/ethanol, 35:30:30:0 to 35:30:30:6) afforded the mixture of macrocycles **7** (1.68 g, 90 %), which was used without further purification for the following step. Analysis by HPLC indicated that cyclodimer **A**[1] and cyclotrimer **B** formed ~65 % and ~25 % of the total respectively. Repeated purification by flash chromatography yielded a small sample of cyclotrimer **B**. R_f = 0.70 (dichloromethane/ethyl acetate/methanol, 65:30:5); ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ 92:8): δ = 8.62, 8.42 (2 br s, 9 H, Ar*H*), 7.42-7.03 (m, 48 H, Ar*H*), 5.04 (s, 12 H, OCH_2Ph), 4.62 (s, 12 H, CH_2NH), 4.26 (s, 12 H, CH_2NHCbz), 3.78 (s, 18 H, CCH_2O), 3.63 (t, ^3J(H,H) = 6.0 Hz, 18 H, CH_2CH_2O), 2.37 (t, ^3J(H,H) = 6.0 Hz, 18 H, CH_2CO), 1.34 (s, 81 H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$ 92:8): δ = 171.0 (*C*O_2t-Bu), 166.6, 166.3 (*C*ONHC, *C*ONHCH$_2$), 156.9 (*C*OOCH$_2$Ph), 141.1, 139.5, 139.4, 139.2, 139.1 (Ar*C*), 136.3 (Cbz-Ar*C*), 135.9, 135.0 (Ar*C*), 128.8, 128.7, 128.5, 128.3 (Ar*C*H), 128.3, 127.9, 127.8 (Cbz-Ar*C*H), 125.7, 125.6, 125.4, 125.1, 125.0 (Ar*C*H), 80.6 (*C*(CH$_3$)$_3$), 68.7 (C*C*H$_2$O), 66.9 (O*C*H$_2$Ph), 66.6 (CH_2CH_2O), 60.3 (*C*CH$_2$O), 44.5, 44.4 (*C*H$_2$NHCbz, *C*H$_2$NHCOAr), 36.0 (CH_2CH_2CO), 27.8 (*C*H$_3$); IR: ν_{max} = 3300, 2979, 1713, 1651, 1367, 1265, 1155, 907, 729 cm^{-1}; MS(MALDI$^+$): *m/z* 3623 [M+Na]$^+$, 3639 [M + K]$^+$.

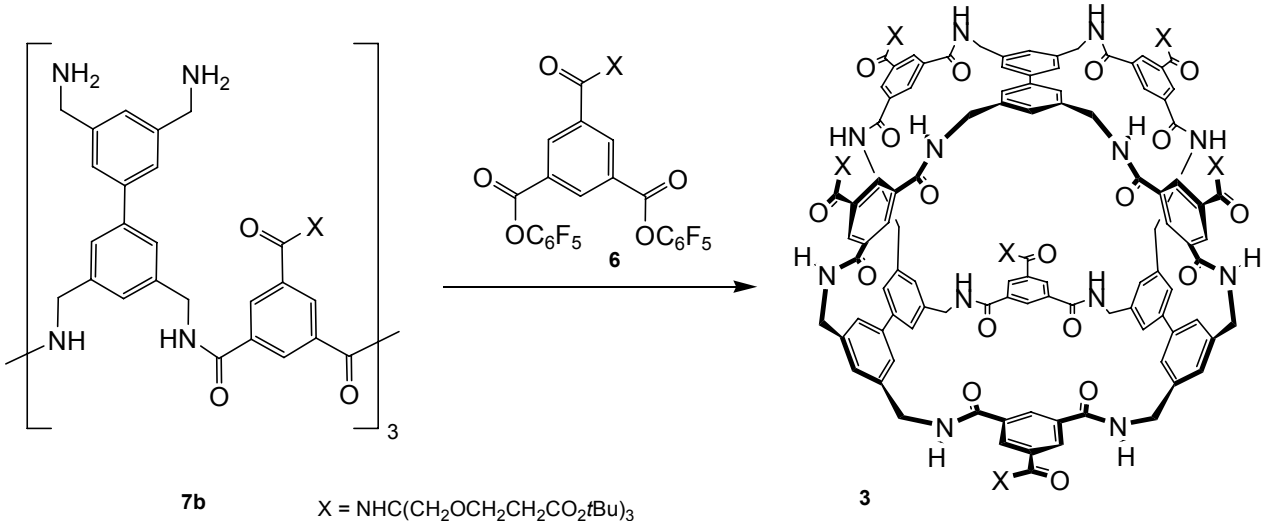


A n = 2
B n = 3

7a n = 2
7b n = 3

X = $NHC(CH_2OCH_2CH_2CO_2tBu)_3$

Macrocyclic hexa-amine 7b. The mixture of Cbz-protected macrocycles (**A**, **B** etc., 1.39 g, *ca.* 579 μmol) from the above cyclisation was dissolved in a mixture of THF and methanolic ammonia[2] 1:1 (100 mL). Pd on C (10 % w/w, *Aldrich* catalogue number 33,010-8, 1.38 g), previously activated by heating under vacuum with a heat gun, was added. The flask was evacuated and filled with hydrogen (1 atm) and the reaction mixture was stirred for 2 h 30 at RT. The Pd catalyst was removed by filtration, washed with ethyl acetate and the filtrate was evaporated under reduced pressure. The material was purified by flash column chromatography (eluent dichloromethane/methanolic ammonia,[2] 100:0 to 70:30) to yield the cyclotrimeric hexa-amine **7b** (215 mg, ~18 % based on **5**). R_f = 0.05 (dichloromethane/methanolic ammonia, 90:10); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.40 (s, 3H, spacer-Ar*H*), 8.19 (s, 6H, spacer-Ar*H*), 7.80-7.25 (m, 18 H, Ar*H*), 4.66 (s, 12 H, CH_2NH), 3.45 (s, 24 H, CH_2NH_2, NH_2), 3.82 (s, 18 H, CCH_2O), 3.68 (t, ^3J(H,H) = 5.9 Hz, 18 H, CH_2CH_2O), 2.46 (t, ^3J(H,H) = 5.9 Hz, 18 H, CH_2CO), 1.40 (s, 81 H, CH_3); IR: ν_{max} = 3310, 2929, 1726, 1264, 1159, 907, 728 cm^{-1}. Also isolated was the cyclodimeric tetra-amine **7a** (593 mg, 55 %; R_f = 0.15), as described in ref. 1.



Macrotetracyclic receptor 3. To a stirred solution of hexaamine **7b** (232 mg, 83 μmol) and *N*,*N*-diisopropylethylamine (440 μL, 2.5 mmol) in dry THF (500 mL) under nitrogen and at RT was added a solution of bispentafluorophenyl ester **6** (282 mg, 270 μmol) in dry THF (50 mL), dropwise over 25 h. After a further 14 h the mixture was concentrated under vacuum to give a yellow solid. The solid was taken up in chloroform (200 mL) and washed with saturated aqueous NH_4Cl (75 mL), water (75 mL), saturated aqueous $NaHCO_3$ (75 mL) and brine (75 mL). The organic layer was dried (Mg_2SO_4), filtered and the solvent was removed at reduced pressure. The residue was purified by flash column chromatography (eluent dichloromethane/ethyl acetate/ethanol, 65:30:0 to 65:30:8) and afforded crude macrocyclic trimer **3** (318 mg, 79 %). The obtained product was subjected to further purification by preparative high performance liquid chromatography (Vydac Protein C4 column, 250 x 10 mm, 5 μm, eluent methanol/water, 70:30 to 90:10 in 20 min then methanol/water, 90:10, flow rate 4 mL.min^{-1}). The pure macrotricycle **3** (59 mg, 15 %) was isolated as a white solid. R_f = 0.40 (dichloromethane/ethyl acetate/ethanol, 60:35:5); ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD 85:15): δ = 8.22 (s, 12 H, spacer-Ar*H*), 8.00 (s, 6 H, spacer-Ar*H*), 7.32 (s, 12 H, Ar*H* α,γ to $CCH_2NHCOAr$), 7.15 (s, 6 H, Ar*H* α to $CCH_2NHCOAr$), 4.47 (A part of AB syst., ^2J(H,H) = 14.4 Hz, 12 H, CH_2NH), 4.38 (B part of AB syst., ^2J(H,H) = 14.4 Hz, 12 H, CH_2NH), 3.74 (s, 36 H, CCH_2O), 3.62 (t, ^3J(H,H) = 6.3 Hz, 36 H, CH_2CH_2O), 2.39 (t, ^3J(H,H) = 6.1 Hz, 36 H, CH_2CO), 1.42 (s, 162 H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD 92:8): δ = 171.5 (*C*O_2t-Bu), 167.2 (*C*ONHC), 167.1 (*C*ONHCH$_2$), 141.2, 139.7 (Ar*C*-Ar, Ar*C*CH$_2$NHCOAr), 136.8 (spacer-Ar*C*CONHC), 135.2 (spacer-Ar*C*CONHCH$_2$Ar), 129.1 (spacer-Ar*C*H α to CCONHC), 128.1 (spacer-Ar*C*H γ to CCONHC), 125.2 (Ar*C*H α to $CCH_2NHCOAr$), 126.1 (Ar*C*H α,γ to $CCH_2NHCOAr$), 80.9 (*C*$(CH_3)_3$), 69.1 (CCH_2O), 67.2 (CH_2CH_2O), 60.8 (*C*CH_2O), 43.9 (*C*H_2NH),

36.4 (CH_2CH_2CO), 28.1 (CH_3); IR: ν_{max} = 3389, 2977, 2931, 2865, 1726, 1632, 1450, 1366, 1255, 1154, 1104 cm^{-1}; MS(MALDI$^+$): *m/z* 4820 $[M + K]^+$.

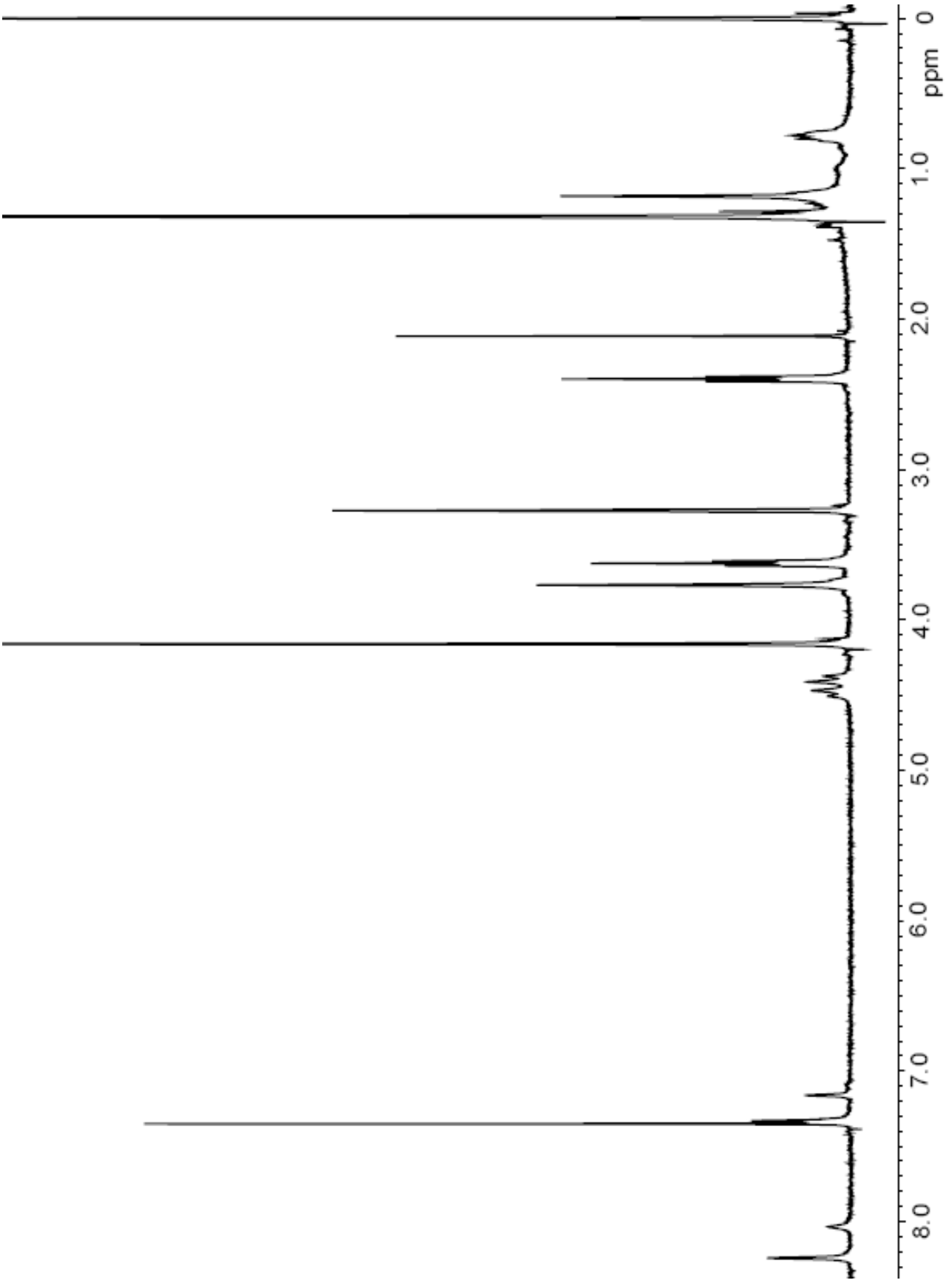


Figure S1. ^{1}H NMR spectrum of receptor **3** (1 mM) in $CDCl_3/CD_3OD$ (75/25).

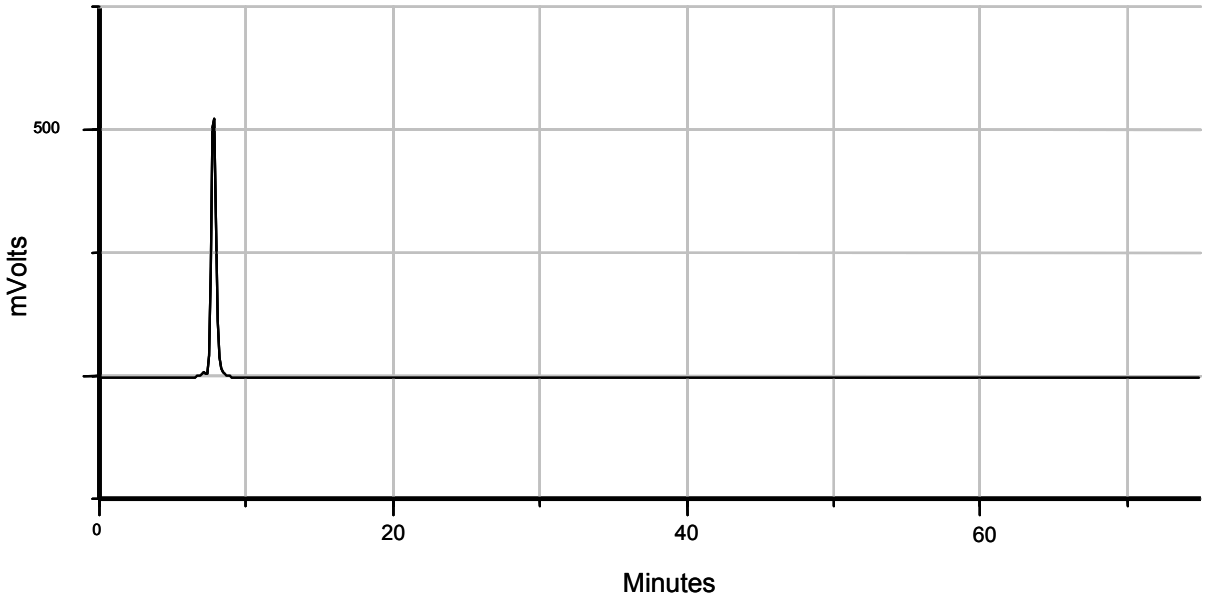


Figure S2. Chromatogram of receptor **3** on Hichrom ODS-2 column (250 x 4.6 mm, 5 µm). Eluent : methanol/water 70:30 to 90:10 in 10 minutes then methanol/water 90:10, flow rate 0.8 mL.min-1. Retention time = 7.77 min

Reaction of 7b + 6 - further discussion.

As **7b** contains 6 free amino groups, it might appear that reaction with **6** at high dilution could lead to a large number of polycyclic and/or polymeric products. However, free rotation about the Ar-Ar' bonds means that the amino groups on a given $C_6H_3(CH_2NH_2)_2$ unit are equivalent. Also, **6** cannot cyclise across a single $C_6H_3(CH_2NH_2)_2$ unit as the product is too strained. This limits the range of outcomes at each stage of the process. In the following analysis we assume (i) that 1:1 cyclisations are exclusively favoured (tending to truth at high dilution), and (ii) that all NH_2 groups are equally reactive so that the choice between alternatives is random. The structures of the intermediates are shown in Scheme S1 on the following page.

Cyclisation 1: According to the above assumptions, the reaction of **7b** with the first equivalent of **6** can lead to just one 1:1 cyclisation product, the macrobicycle **C** (see Scheme S1).

Cyclisation 2: In this case two macrotricycles **D** and **E** are possible, of which **D** represents the productive pathway (leading to **3** via a third 1:1 cyclisation). Considering the reactions in detail, **C** possesses two types of NH_2, labelled * and †. Statistically, half the molecules of **6** should react at NH_2*, and must then cyclise to form **D**. The other 50% react at NH_2† and are then faced with a choice of 2 × NH_2* groups (leading to **D**) or the second NH_2† (leading to **E**). Statistically, two thirds should yield **D**, while a third should lead to **E**. As shown in Figure S3, a total of 83% of **C** is expected to form **D**, while just 17% ends up as **E**.

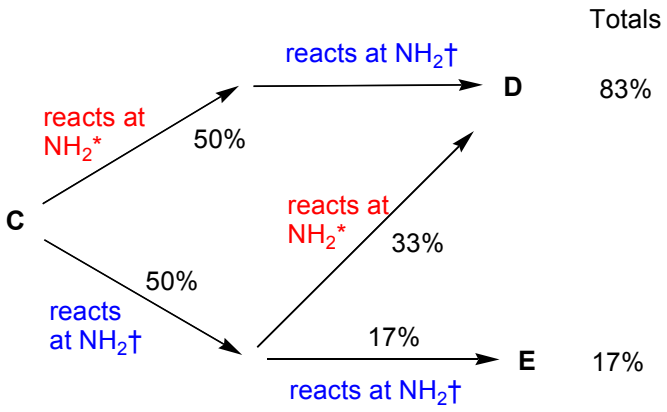
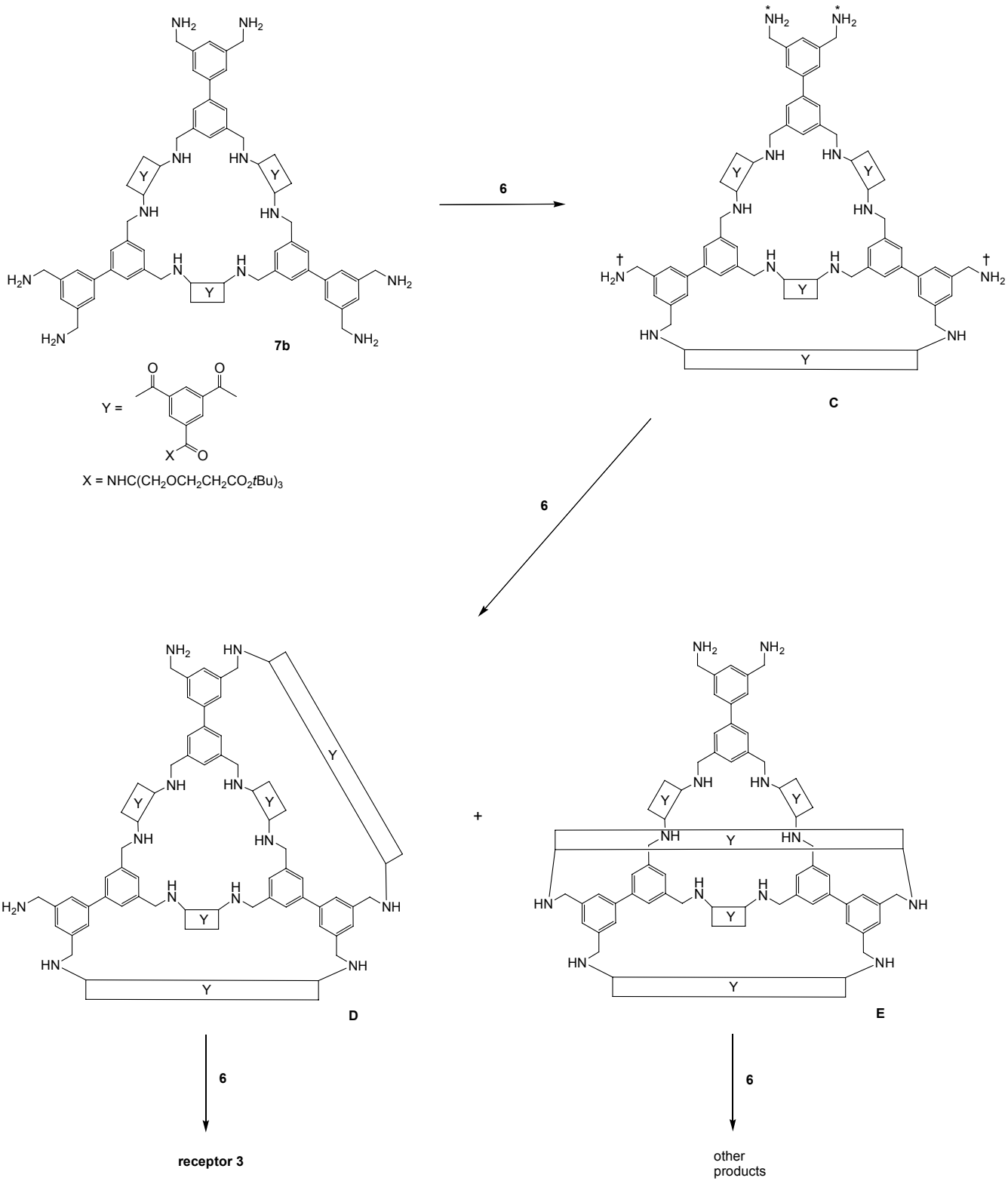


Figure S3. Pathways from bicycle **C** to tricycles **D** and **E**, with expected populations. For structures see Scheme S1.

Although the assumptions are not strictly justified, the prospects of a high yield of **3** appear good. The actual yield of 15% might even seem disappointing, although this may reflect losses in purification rather than unproductive reaction pathways.



Scheme S1. Structures formed from the cyclisation of hexa-amine **7b** with active ester **6**.

Binding Studies

Substrates dodecyl β-D-maltoside **4a**, dodecyl α-D-maltoside **9**, octyl β-D-glucoside **10**, and octyl α-D-glucoside **11** were obtained commercially (Sigma-Aldrich). Octyl β-D-cellobioside **2a** was prepared as described previously.[3]

^{1}H NMR titrations. Solutions of glycoside substrates were made up in $CDCl_3/CD_3OD$ 75:25, and added as aliquots to an NMR tube containing receptor **3** in the same solvent (0.7 μmol in 700 μL, $[\mathbf{3}]_{initial}$ = 1.0 mm). The sample tube was shaken carefully after each addition and ^{1}H-NMR spectra were recorded at 296 K. Spectra for the additions of β-D-maltoside **4a** and β-D-cellobioside **2a** are shown in Figures S4 and S5 respectively. In the case of **2a**, the chemical shift changes ($\Delta\delta$) were analysed according to a 1:1 binding model, using a non-linear least squares curve-fitting program implemented within Excel. The programme yields binding constants K_a and limiting $\Delta\delta$ as output. The resulting binding curve (experimental *vs.* calculated data) is shown in Figure S4.

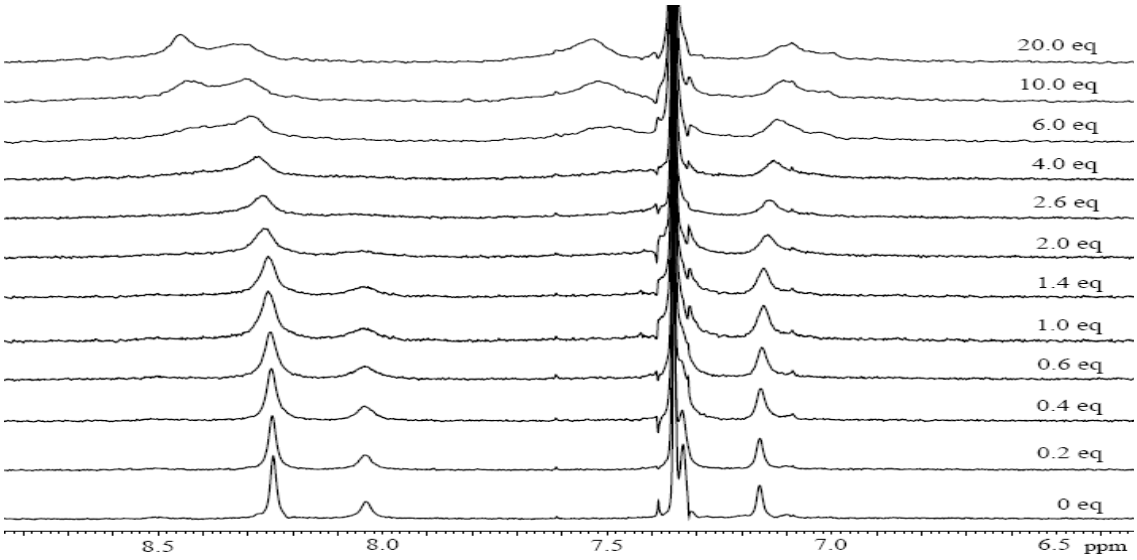


Figure S4. NMR spectra recorded for the binding study of receptor **3** vs dodecyl β-D-maltoside **4a** in $CDCl_3/CD_3OD$ (75/25). $[\mathbf{3}]_{initial}$ = 1 mM.

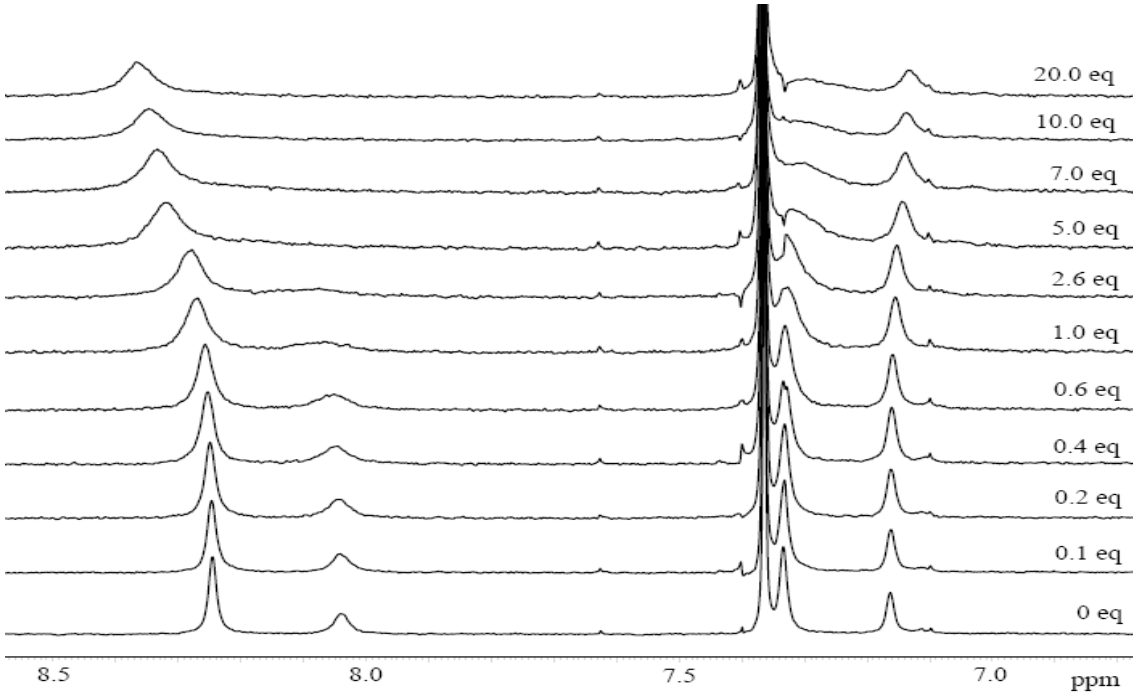


Figure S5. NMR spectra recorded for the binding study of receptor **3** vs octyl β-D-cellobioside **2a** in $CDCl_3/CD_3OD$ (75/25). $[\mathbf{3}]_{initial}$ = 1 mM.

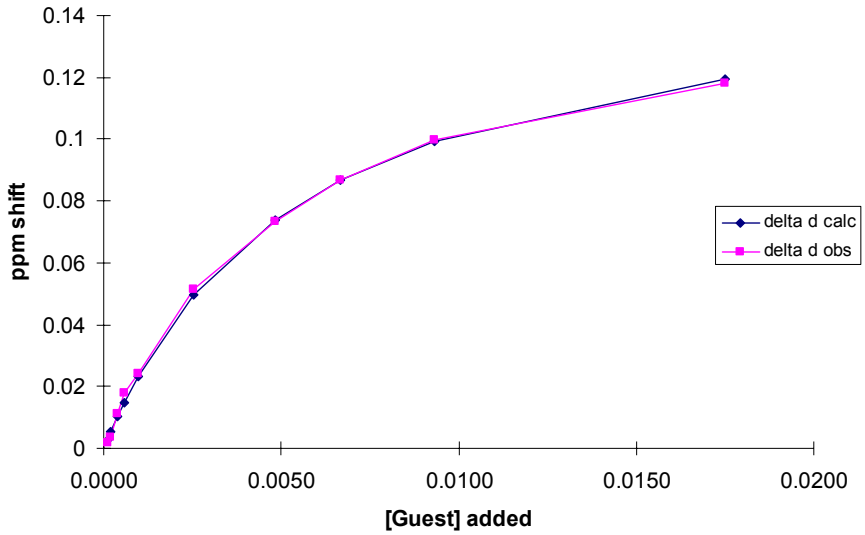


Figure S6. Analysis of data from Figure S5 (signal starting at 8.25 p.p.m.) according to a 1:1 binding model. K_a = 215 M^{-1}, limiting $\Delta\delta$ = 0.153 p.p.m.

Circular Dichroism titrations. Solutions of glycoside substrates were made up in $CDCl_3/CD_3OD$ 75:25, and added as aliquots with stirring to a 10 mm path length cell containing receptor **3** in the same solvent (1 mL, $[\mathbf{3}]_{initial}$ = 40 - 60 μM). Stirring was performed after each addition and CD spectra were recorded on a Jasco J-810 spectropolarimeter at 25°C. Spectra for the additions of *β*-D-maltoside **4a**, *β*-D-cellobioside **2a** and β-D-glucoside **10** are shown in figures S7, S9 and S11 respectively. In the case of **2a** and **4a** ellipticity changes (Δθ) were analysed according to a 1:1 binding model, using a non-linear least squares curve-fitting program implemented within Excel. The programme yields binding constants K_a and limiting Δθ as output. The resulting binding curves (experimental *vs.* calculated data) are shown in Figures S8 and S10. In the case of **10**, an attempt to analyse the data according to the 1:1 binding model yielded a very poort fit (Figure S12). Instead, the ellipticity changes were analysed according to a 1:1 + 1:2 binding model, using the programm WinEqNMR. The resulting binding curve is shown in Figure S13. The programme yields equilibrium constants K_{a1} and K_{a2} for the binding of successive substrate molecules to the receptor, i.e.:

$$H + G \overset{K_{a1}}{\rightleftharpoons} HG$$

$$HG + G \overset{K_{a2}}{\rightleftharpoons} HG_2$$

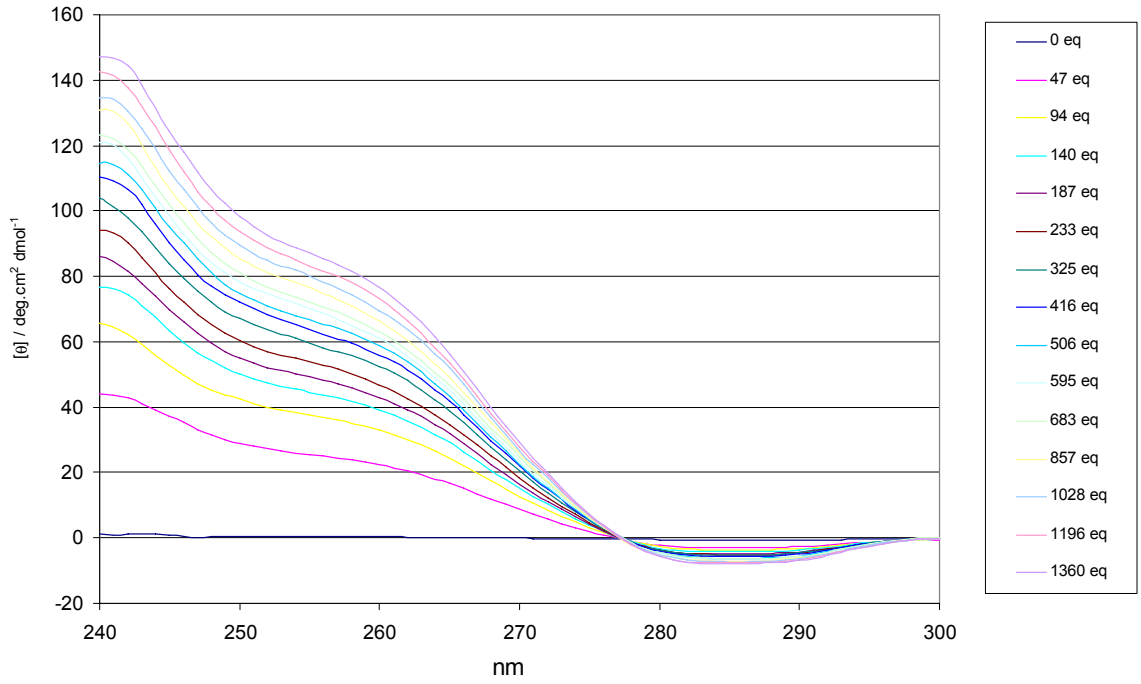


Figure S7. CD spectra recorded for the binding study of receptor **3** vs dodecyl β-D-maltoside **4a** in chloroform/methanol (75/25). $[Host]_{initial}$ = 40 μM, $[Guest]_{titrant}$ = 0.094 M.

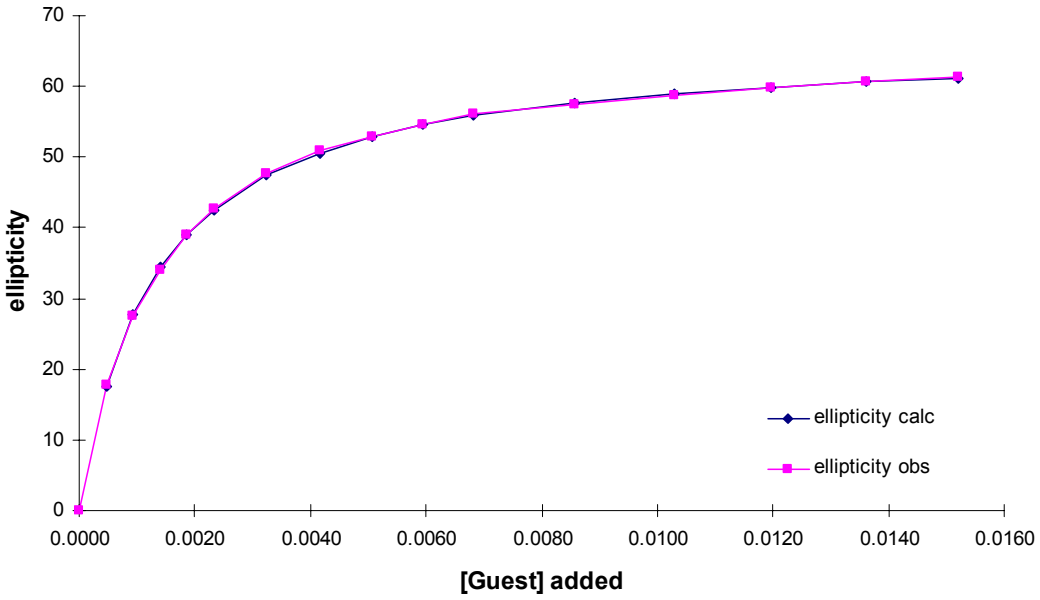


Figure S8. Experimental and calculated values for the CD binding study (1:1) of receptor **3** + dodecyl β-D-maltoside **4a** in chloroform/methanol (75/25). $[Host]_{initial}$ = 40 μM, $[Guest]_{titrant}$ = 0.094 M. Ellipticities measured at 265 nm. K_a = 780 M^{-1}, limiting ellipticity = 67 deg cm^2 $dmol^{-1}$.

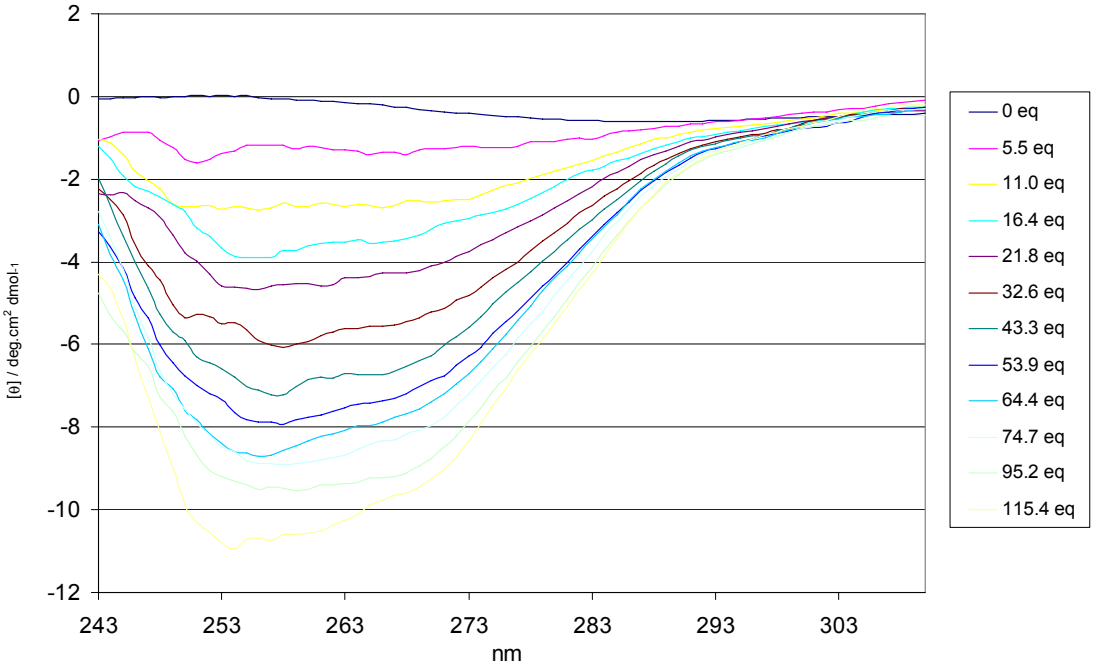


Figure S9. CD spectra recorded for the binding study of receptor **3** vs. octyl β-D-cellobioside **2a** in chloroform/methanol (75/25). $[Host]_{initial}$ = 60 μM, $[Guest]_{titrant}$ = 0.066 M.

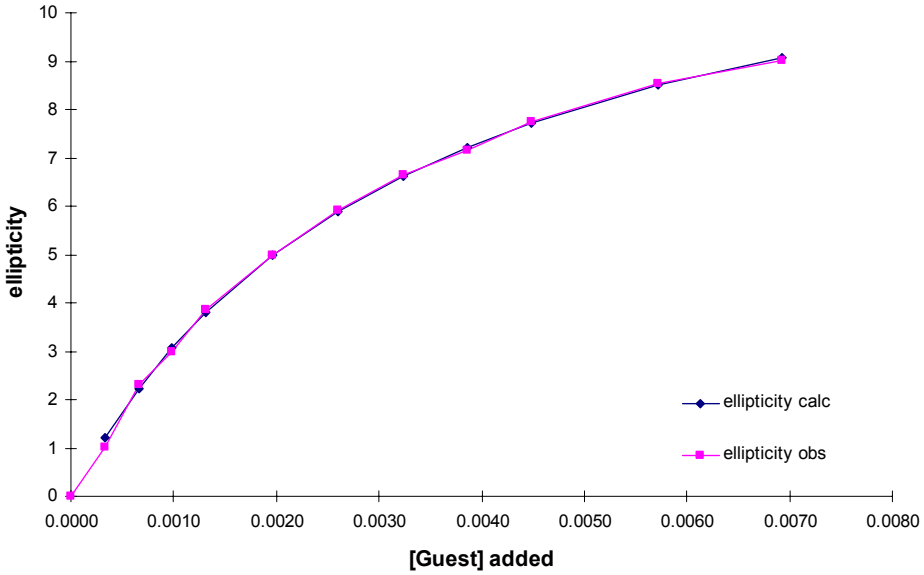


Figure S10. Experimental and calculated values for the CD binding study (1:1) of receptor **3** + octyl β-D-cellobioside **2a** in chloroform/methanol (75/25). $[Host]_{initial}$ = 60 μM, $[Guest]_{titrant}$ = 0.066 M. Ellipticities measured at 270 nm. K_a = 310 M^{-1}, limiting ellipticity = 13.6 deg cm^2 $dmol^{-1}$.

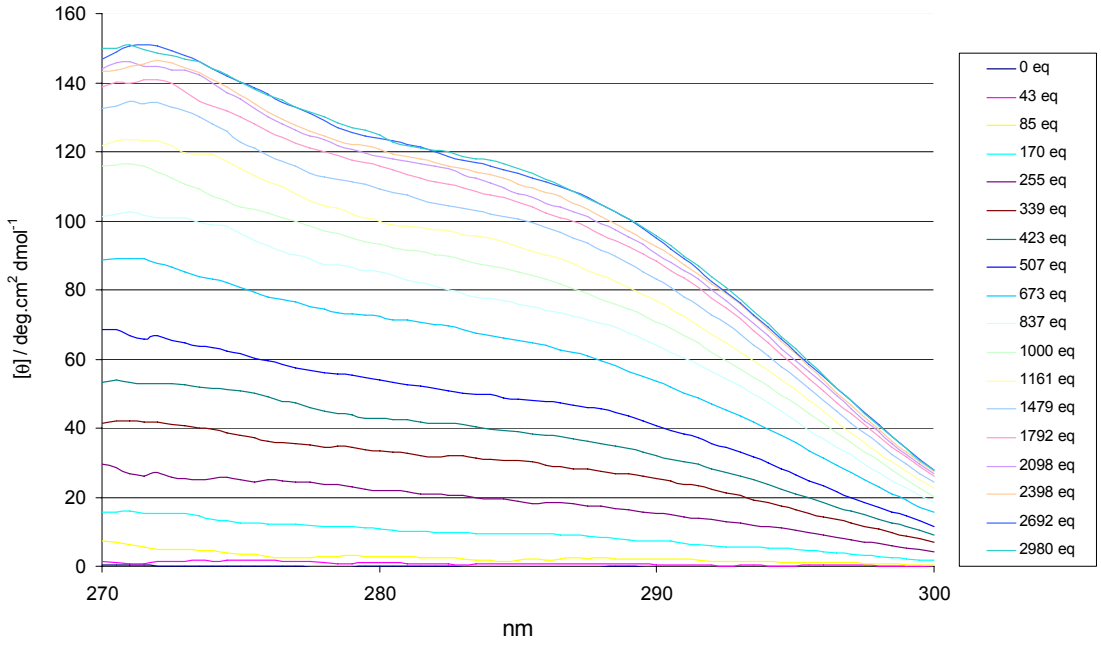


Figure S11. CD spectra recorded for the binding study of receptor **3** vs octyl β-D-glucoside **10** in chloroform/methanol (75/25). $[Host]_{initial}$ = 40 μM, $[Guest]_{titrant}$ = 0.68 M.

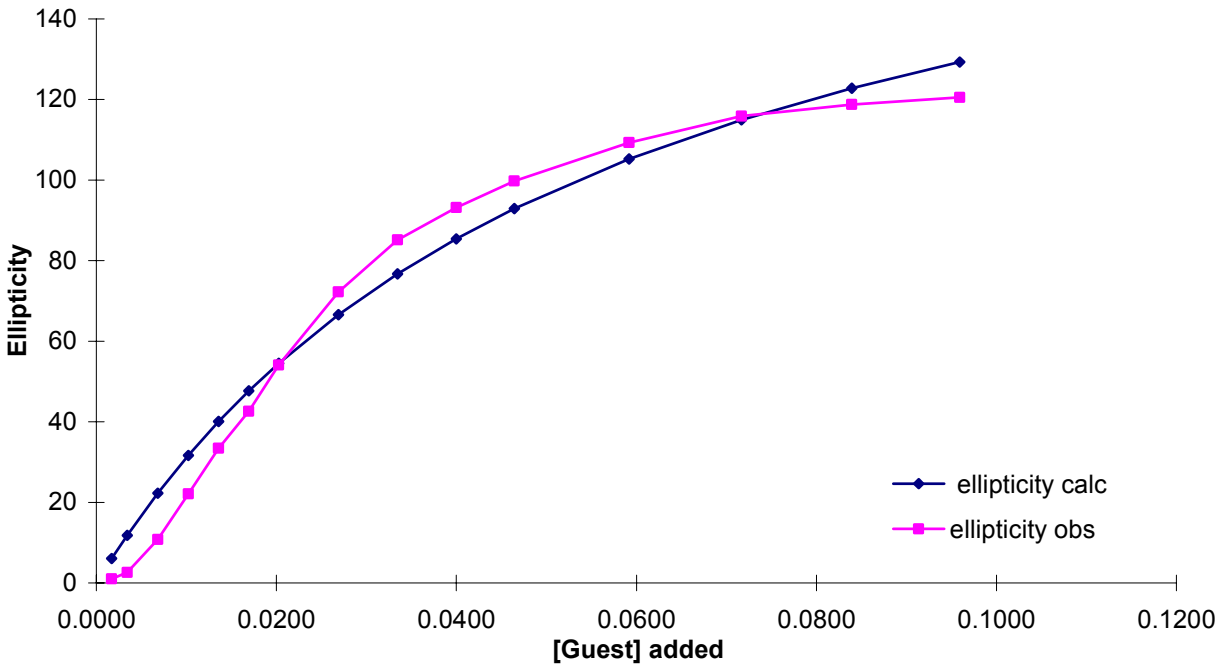


Figure S12. Attempted analysis of the the CD binding study of receptor **3** + octyl β-D-glucoside **10** according to a 1:1 model. Solvent = chloroform/methanol (75/25). $[Host]_{initial}$ = 40 μM, $[Guest]_{titrant}$ = 0.7 M. Ellipticities measured at 280 nm.

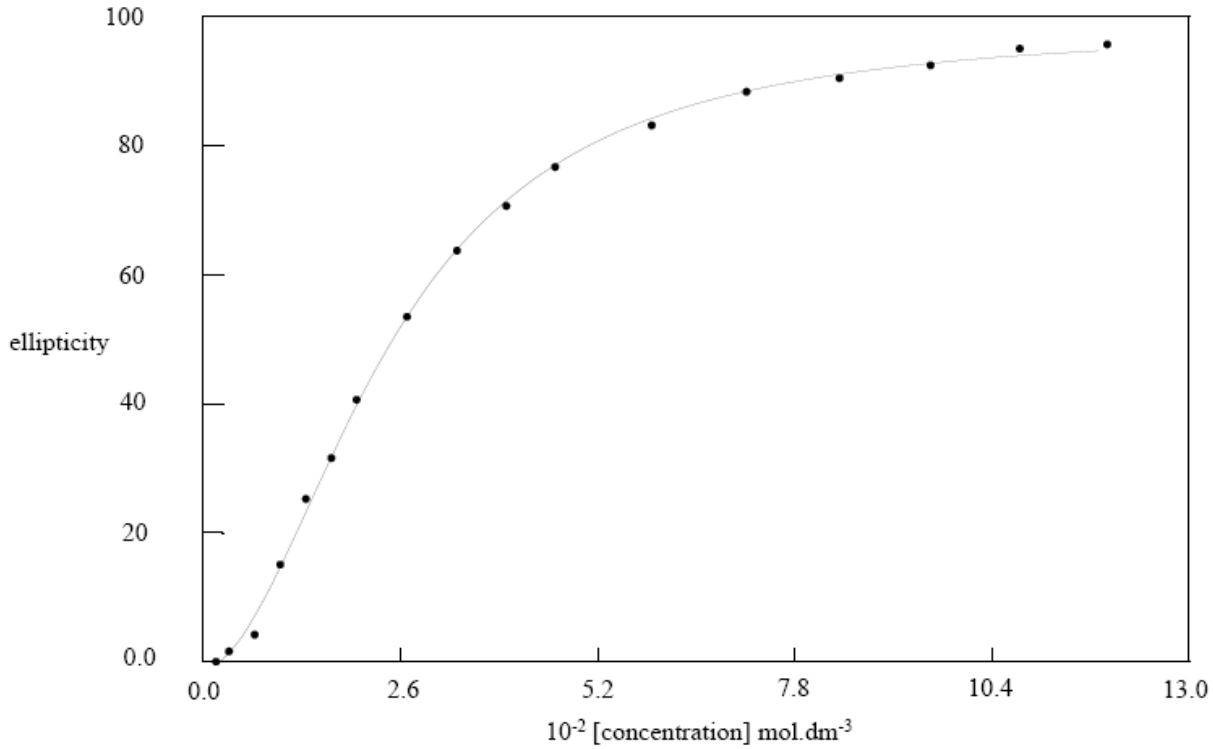


Figure S13. Analysis of the data from Figure 12 according to a 1:1 + 1:2 binding model (experimental points and calculated curve). K_{a1} = ~1 M^{-1}; K_{a2} = 1470 M^{-1}. Limiting ellipticity for 1:2 complex = 98.04 deg cm^2 $dmol^{-1}$. *Errors:* The error in K_{a2} as estimated by WinEqNMR, is ± 16%. K_{a1} is relatively uncertain, as expected for very weak binding. WinEqNMR estimates an upper limit of 4 M^{-1}.

References and Notes

1. E. Klein, M. P. Crump and A. P. Davis, *Angew. Chem., Int. Ed.*, 2005, **44**, 298.
2. Methanolic ammonia was prepared by passing ammonia through ice-cooled methanol until no further increase in volume was observed.
3. G. Lecollinet, A. P. Dominey, T. Velasco and A. P. Davis, *Angew. Chem., Int. Ed.*, 2002, **41**, 4093.