Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

New H-bonding patterns in biphenyl-based synthetic lectins; pyrrolediamine bridges enhance glucose-selectivity

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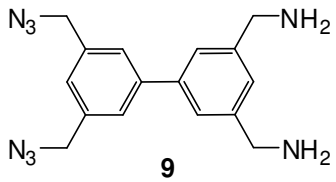
Synthesis and Characterisation

General Methods

All commercially available compounds were purchased from Aldrich, Alfa-Aesar, or Sigma and were used without further purification except where stated. Solvents for synthesis were dried by passing through a modified Grubbs system, of alumina column, manufactured by Anhydrous Engineering. Routine monitoring of reactions was performed using precoated silica gel TLC plates (Merck silica gel 60 F_{254}). Spots were visualized by either UV light, ethanolic solution of phosphomolybdic acid, potassium permanganate, or ninhydrin. R_f values are given under these conditions. Flash column chromatography was performed using silica gel (Fisher brand silica 60Å particle size 35-70 micron) as the absorbent. Both analytical and preparative High Performance Liquid Chromatography (HPLC) were performed on reversed phase-HPLC (RPHPLC) instruments, using C_{18} columns and a binary solvent system (CH_3OH and H_2O). ^1H and ^{13}C NMR spectra were recorded on a Varian 400-MR spectrometer, or on respectively VNMRSYS 500 (proton sensitive) or VNMRSYS 500 (carbon sensitive) spectrometers. Chemical shifts were reported in ppm downfield from tetramethylsilane for proton and carbon or relative to the signals corresponding to the residual non-deuterated solvents ($CDCl_3$: δ 7.26 ppm, D_2O: δ 4.79 ppm / 298 K). Mass spectra were recorded on a VG Analytical Autospec for Electron Impact (EI), a VG Analytical Quattro for ESI and Nanospray, or an Applied Biosystems 4700 spectrometer for MALDI.

.3,5-Bis(azidomethyl)-3',5'-bisaminomethyl biphenyl 9



9

Boronic ester **8**[1] (0.80 g, 1.73 mmol) and 3,5-bis(azidomethyl)-iodobenzene **7**[2] (0.55 g, 1.73 mmol) were suspended in DMSO (10 mL). To this was added [1,1'-bis(diphenylphosphino)ferrocene]-dichloropalladium(II) (18 mg, 0.02 mmol) and sodium

[1] T. J. Ryan, G. Lecollinet, T. Velasco and A. P. Davis, *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 4863.

[2] B. Sookcharoenpinyo, E. Klein, Y. Ferrand, D. B. Walker, P. R. Brotherhood, C. Ke, M. P. Crump and A. P. Davis, *Angew. Chem., Int. Ed.*, 2012, **51**, 4586.

carbonate (0.448 g, 4.23 mmol) in water (2.0 mL). The resulting mixture was heated to 80°C for 6 hours before being cooled to room temperature. The reaction mixture was dissolved in water (30 mL) and extracted with ethyl acetate (3 × 25 mL). The combined organic fractions were washed with water (2 × 25 mL), brine (25 mL), dried over sodium sulfate, filtered and concentrated in *vacuo* to yield a black oil. The oil was purified by flash column chromatography (hexane/ethyl acetate 3:2) producing the expected bis-Boc-protected biphenyl as a white solid (0.7 g, 77%). Trifluoroacetic acid (0.5 mL) was added to a solution of this material (0.7 g, 1.3 mmol) in DCM (5 mL) at 5°C under nitrogen atmosphere and the mixture was stirred for 5 hours at room temperature. Evaporation under reduced pressure gave an oil which was purified by flash column chromatography (DCM/methanol saturated with NH_3 95:5) to yield di-amine **9** (0.42 g, 97%). $\boldsymbol{R_f}$ = 0.4 (DCM/methanol saturated NH_3 9:1). **^{1}H NMR** (500 MHz, CD_2Cl_2) δ = 2.56 (brs., 4H, N*H*$_2$), 3.91 (s, 4H, CH_2NH_2), 4.44 (s, 4H, C*H*$_2$N$_3$), 7.28 (s, 1H, Ar*H*) 7.32 (s, 1H, Ar*H*), 7.45 (d, *J* = 1.2 Hz, 2H, Ar*H*) 7.53 (d, *J* = 1.4 Hz, 2H, Ar*H*). **^{13}C NMR** (126 MHz, CD_2Cl_2) δ = 45.8 (CH_2NH_2), 54.5 (CH_2N_3), 124.7 (Ar*C*H), 125.7 (Ar*C*H), 126.7 (Ar*C*H), 136.9 (Ar*C*), 140.4 (Ar*C*), 142.1 (Ar*C*), 143.4 (Ar*C*). **HRMS (ESI)**: *m/z* calculated for $C_{16}H_{19}N_8^+$ $[M + H]^+$: 323.1733, found 323.1245.

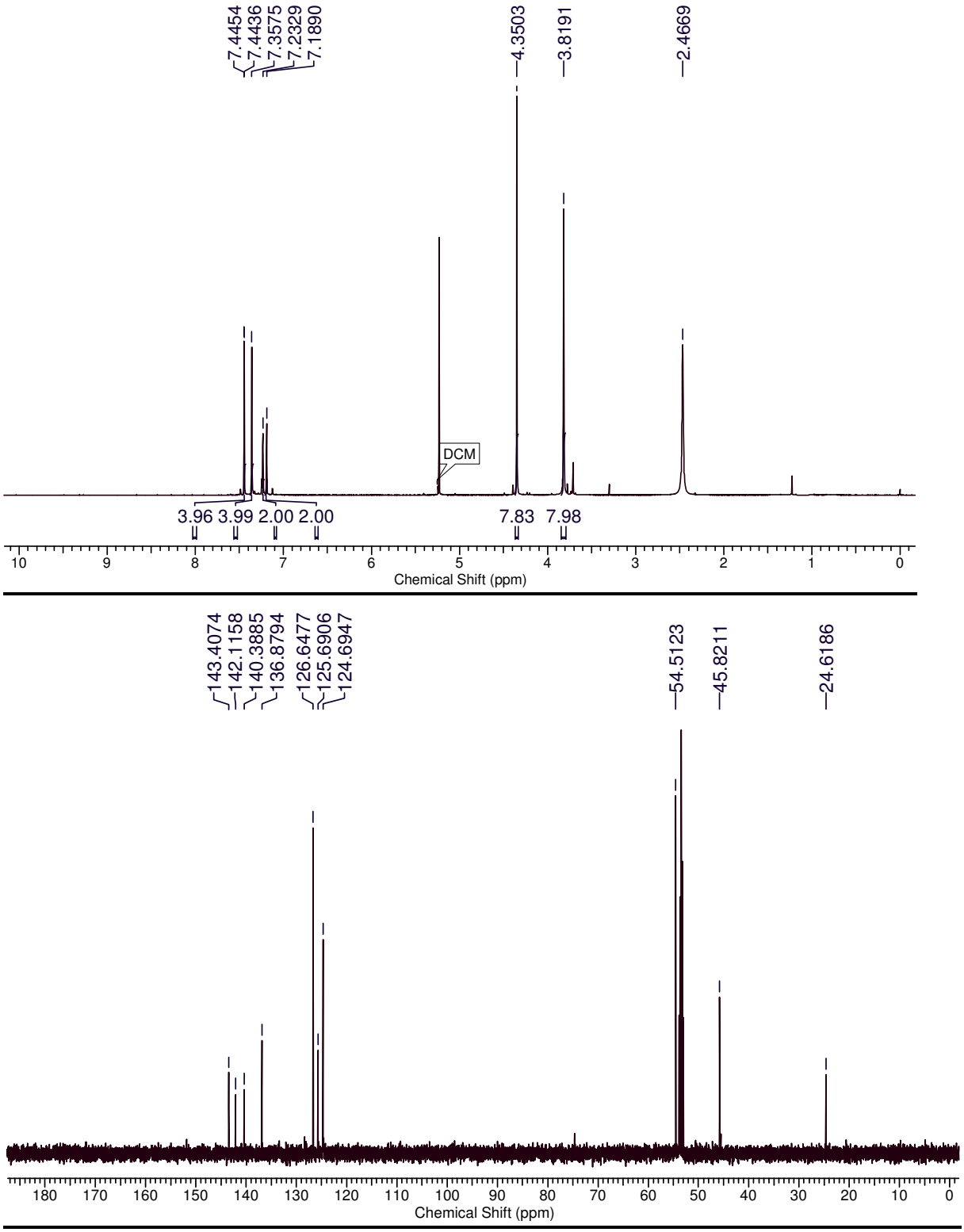
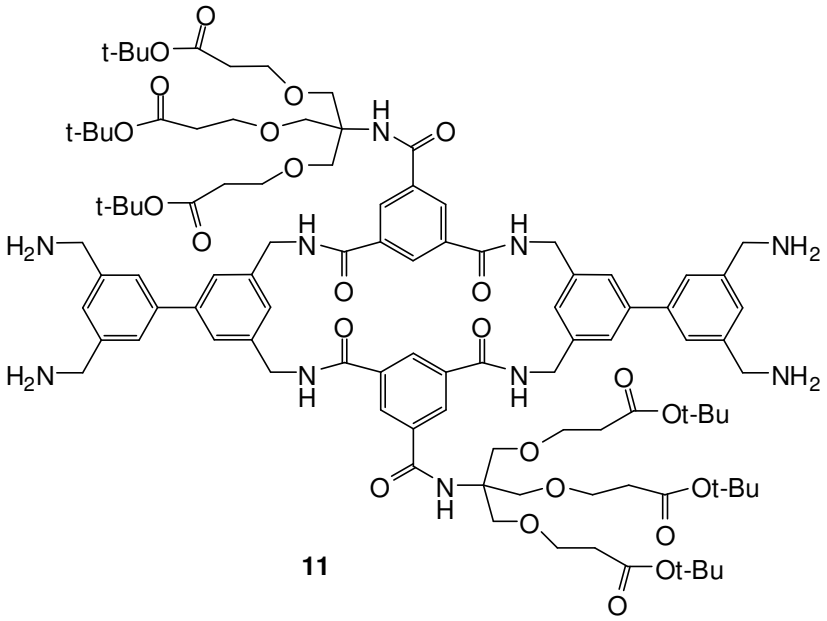


Figure S1. ^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of biphenyl **9** ($CDCl_3$, 400 MHz).

Macrocyclic tetra-amine 11



11

A solution of bis-pentafluorophenyl ester **10**[3] (1.5 g, 1.46 mmol) in dry THF (50 mL) was added drop-wise over 30 hours to a solution of di-amine **9** (0.42 g, 1.36 mmol) and DIPEA (1.5 mL, 8.3 mmol) in dry THF (*ca.* 1200 mL) under nitrogen atmosphere at room temperature. The reaction mixture was further stirred vigorously for 24 hours at room temperature. The solvent was removed under reduced pressure to give a solid that was taken up in chloroform (200 mL) and washed with saturated aqueous solution of NH_4Cl (100 mL), water (100 mL), saturated aqueous solution of $NaHCO_3$ (100 mL) and brine (100 mL). The solution was dried over sodium sulfate, filtered and concentrated in *vacuo* to yield an off-white solid which was purified by flash column chromatography (eluent toluene/DCM/ethyl acetate/EtOH 35:30:30:0 to 35:30:30:6) to afford a crude mixture of macrocycles. The mixture was further purified by preparative HPLC (Hichrom Kromasil column, 150 x 21.2 mm, 5 µm, eluent: methanol/water 80:20 to 95:5 in 10 min, then methanol/water 99:1 after 40 min, and 100:0 after 48 min, flow rate 21 $mL.min^{-1}$, retention time = 17 minutes) to give pure [2+2] macrocycle (0.7 g, 55%). Activated Pd on C (0.2 g) was added to the [2+2] macrocycle (0.2 g, 1.05 mmol) in dry THF/methanol saturated with NH_3 1:1 (15 mL) and the flask was evacuated and filled with H_2 (1 atm) gas. The reaction mixture was stirred at room temperature for an hour and the Pd catalyst was removed by filtration and washed with ethyl acetate. The filtrate and washings were concentrated and purified by flash column

[3] E. Klein, M. P. Crump and A. P. Davis, *Angew. Chem., Int. Ed.*, 2005, **44**, 298.

chromatography (DCM/methanol saturated with NH_3 95:5 to 4:1) to obtain tetra-amine **11** as a white solid (0.18 g, 94%). R_f = 0.15 (DCM/methanol saturated with NH_3 4:1). **^{1}H NMR** (500 MHz, CD_3OD) δ = 1.38 (s, 54H, $C(CH_3)_3$), 2.45 (t, J = 6.11 Hz, 12H, CH_2CH_2O), 3.71 (t, J = 6.11 Hz, 12H, CH_2CH_2O), 3.89 (s, 12H, $C(CH_2O)_3$), 3.93 (s, 8H, CH_2NH_2), 4.64 (s, 8H, CH_2NH), 7.34 (s, 2H, Ar*H*), 7.39 (s, 2H, Ar*H*), 7.56 (s, 4H, Ar*H*), 7.59 (s, 4H, Ar*H*), 8.23 (s, 6H, Linker- Ar*H*). **^{13}C NMR** (126 MHz, CD_3OD) δ = 27.8 ($C(CH_3)_3$), 36.1 (CH_2CH_2CO), 44.0 (CH_2NH), 45.6 (CH_2NH_2), 60.5 ($C(CH_2O)_3$), 67.0 (CH_2CH_2O), 68.8 ($C(CH_2O)_3$), 80.6 ($C(CH_3)_3$), 124.6 (Ar*C*H), 125.3 (Ar*C*H), 126.1 (Ar*C*H), 128.2 (Ar*C*H), 129.0 (Ar*C*H), 134.4 (Ar*C*), 136.0 (Ar*C*), 139.2 (Ar*C*), 140.9 (Ar*C*), 141.2 (Ar*C*), 142.8 (Ar*C*), 166.1 ($CONHCH_2$), 166.7 ($CONHC$), 171.1 ($COOC(CH_3)_3$). **HRMS (ESI)**: *m/z* calculated for $C_{100}H_{139}N_{10}O_{24}^+$ $[M + H]^+$ is 1863.9964, found 1863.9998.

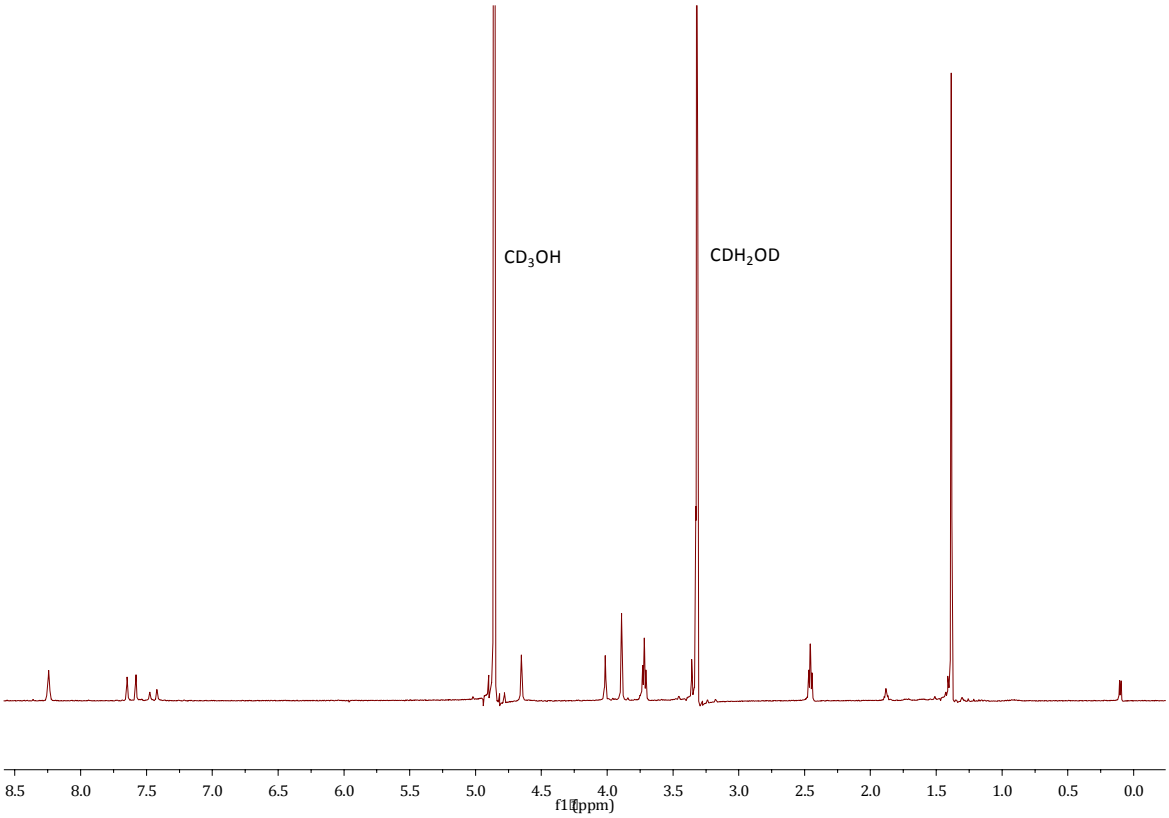
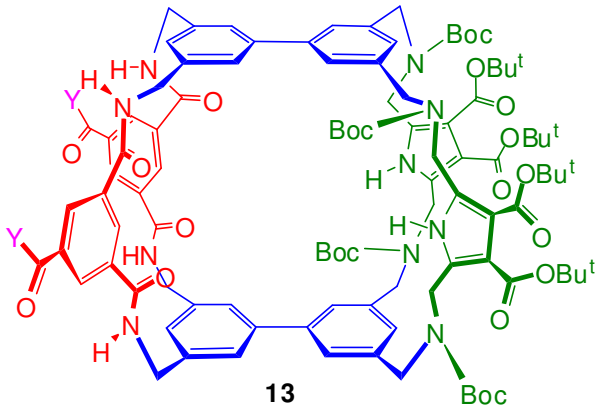


Figure S2. ^{1}H NMR spectrum of tetra-amine **11** (CD_3OD, 500 MHz).

Protected macrotricycle 13



Y = $NHC(CH_2OCH_2CH_2CO_2Bu^t)_3$

Pyrrolediadehyde **12**[4] (13.4 mg, 40 µmol) was added to a solution of tetra-amine **11** (38.5 mg, 20 µmol) in dry degassed $CHCl_3/CH_3OH$ (9:1, 20 mL) and stirred at room temperature overnight. Analysis by ^{1}H NMR suggested quantitative imine formation. The solvent was removed under reduced pressure to yield a yellow solid, presumed to be the corresponding macrotricyclic tetra-imine (50 mg, quantitative). This material was dissolved in $CHCl_3/CH_3OH$ (1:1, 10 mL), $NaBH_4$ (38 mg, 1 mmol) was added and the resultant mixture was stirred for an hour. The reaction was monitored by ^{1}H NMR (for the disappearance of imine-*H*). The solvent was removed under reduced pressure to yield a white solid. DCM (25 mL) was added to form a turbid solution which was washed with water (20 mL), HCl (0.1 N, 20 mL), brine (20 mL), dried over sodium sulfate, filtered and evaporated. The resulting solid was partially purified by flash column chromatography (DCM/methanol saturated with NH_3 100:0 to 98:2), collecting the fraction with $\boldsymbol{R_f}$ **=** 0.2 (DCM/methanol saturated with NH_3 99:1). The obtained amine was dissolved in dry THF (10 mL) and di-*tert*-butyl dicarbonate (50 mg, 23 mmol) was added. The mixture was heated under reflux overnight under a nitrogen atmosphere. The solvent was removed and the residue redissolved in DCM (25 mL) and washed with saturated aqueous $NaHCO_3$ (20 mL), water (20 mL), brine (20 mL), dried over Na_2SO_4, filtered and evaporated. The resulting solid was subjected to preparative HPLC (Hichrom Kromasil column, 250 x 21.2 mm, 5 µm, eluent: methanol/water 95:5 to methanol 100 in 10 min, flow rate 21 mL.min^{-1}, retention time = 24 minutes), to yield protected macrotricycle **13** as a white solid (20 mg, 34% over two steps). $\boldsymbol{R_f}$ **=** 0.6 (hexane/ethyl acetate

[4] M. Galezowski and D. T. Gryko, *J. Org. Chem.*, 2006, **71**, 5942.

3:2). **^{1}H NMR** (500 MHz, CD_2Cl_2/CD_3OD) δ = 1.24 -1.38 (s, 90H, $C(CH_3)_3$), 1.58 (brs., 36H, $C(CH_3)_3$), 2.45 (t, *J* = 6.26 Hz, 12H, CH_2CH_2O), 3.67 (t, *J* = 6.26 Hz, 12H, CH_2CH_2O), 3.81 (s, 12H, $C(CH_2O)_3$), 4.43 (brs., 24H, $ArCH_2NHCO$, $ArCH_2NH$, $Pyr\text{-}CH_2NH$), 6.57 (brs., 2H, Ar*H*), 7.00 (brs., 4H, Ar*H*), 7.39 (brs., 6H, Ar*H*), 7.89 (brs., 2H, Linker-Ar*H*), 8.37 (brs., 4H, Ar*H*). **HRMS (ESI)**: *m/z* calculated for $C_{152}H_{212}N_{12}O_{40}Na^+$ $[M + Na]^+$ is 2868.4821, found 2868.3006.

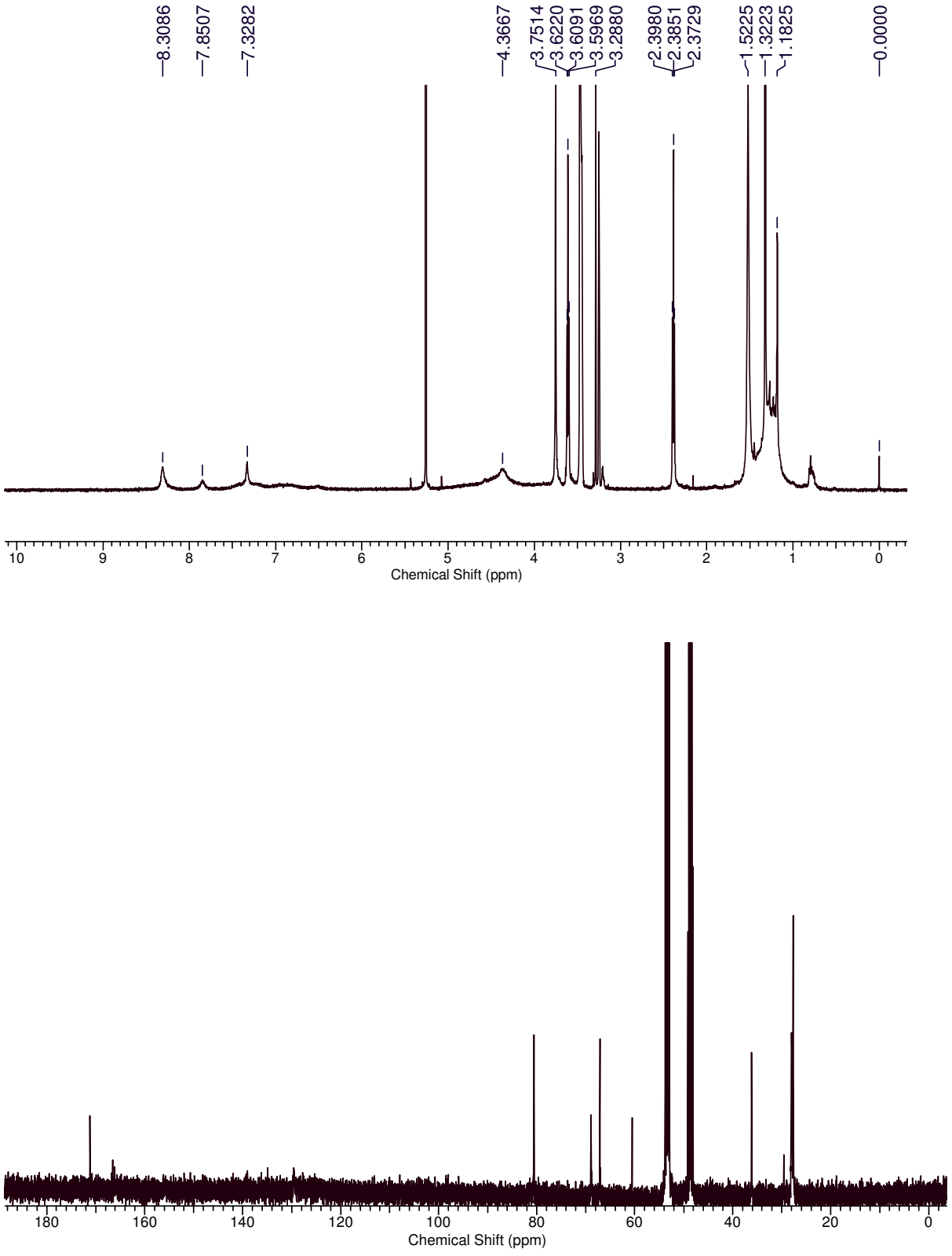
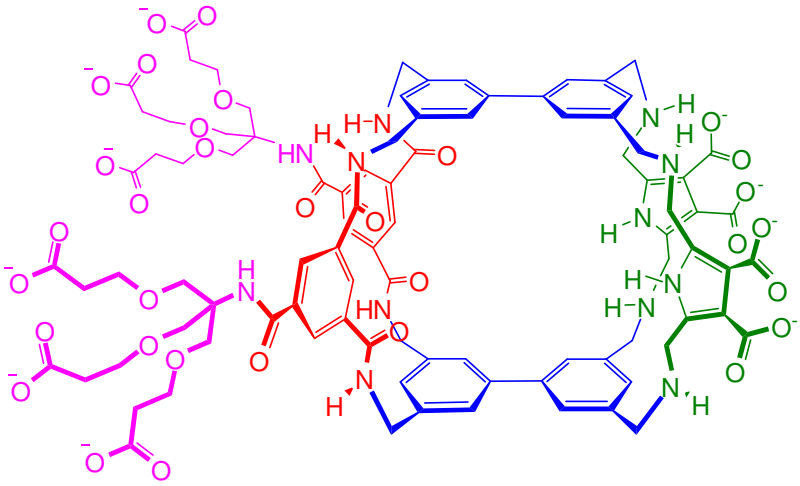


Figure S3. ^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of protected macrotricycle **13** ($CDCl_3$, 400 MHz).

Receptor 6



6

Protected macrotricycle **13** (10 mg, 3.5 μmol) was dissolved in DCM (2 mL) and cooled to 5°C under a nitrogen atmosphere. Trifluoroacetic acid (200 μL) was added and the solution was stirred at room temperature for 6 hours. Evaporation under reduced pressure gave a residue which was dissolved in methanol/water (3:2, 5 mL). Sodium hydroxide (0.5 M) was added till pH 7.0. The solvent was removed by freeze drying to obtain pure macrotricycle **6** as a white solid (7 mg, 93%). The solid, however, was not soluble in D_2O at pD 7.0. NaOD was added to the turbid solution slowly while stirring until the solution was clear at pD 13. **^{1}H NMR** (500 MHz, D_2O at pD 13.0) δ = 2.37 (t, *J* = 6.72 Hz, 12H, C*H*$_2$CH$_2$O), 3.30 (AB quartet, d, *J* = 13.69 Hz, 4H, Pyr-C*H*$_2$NH), 3.40 (AB quartet, d, *J* =13.69 Hz, 4H, Pyr-C*H*$_2$NH), 3.59 (AB quartet, d, *J* = 14.92 Hz, 4H, ArC*H*$_2$NHCH$_2$), 3.67 (t, *J* = 6.72 Hz, 12H, CH$_2$C*H*$_2$O), 3.70 - 3.85 (m, 16H, C(C*H*$_2$O)$_3$ and ArC*H*$_2$NHCH$_2$), 4.39 (AB quartet, d, *J* = 14.43 Hz, 4H, ArC*H*$_2$NHCO) 4.52 (AB quartet, d, *J*=14.18 Hz, 4H, ArC*H*$_2$NHCO), 6.87 (s, 2H, Ar*H*), 7.12 (s, 4H, Ar*H*), 7.27 (s, 2H, Ar*H*), 7.43 (s, 4H, Ar*H*), 7.75 (s, 2H, Linker-Ar*H*), 8.17 (d, *J* = 1.47 Hz, 4H, Linker-Ar*H*). **^{13}C NMR** (126 MHz, D_2O) δ = 37.7 (*C*H$_2$CH$_2$O), 42.8 (Pyr-*C*H$_2$NH), 44.0 (Ar*C*H$_2$NH), 48.8 (Ar*C*H$_2$NH), 61.1 (*C*(CH$_2$O)$_3$), 68.6 (CH$_2$*C*H$_2$O), 68.8 (C(*C*H$_2$O)$_3$), 115.1 (Pyr-*C*CO$_2$Na), 117.4 (Ar*C*H), 118.2 (Ar*C*H), 120.0 (Ar*C*H), 125.3 (Ar*C*H), 126.1 (Ar*C*H), 129.2 (Pyr-*C*CH$_2$), 134.8 (Ar*C*), 136.0 (Ar*C*), 139.1 (Ar*C*-Ar), 139.9 (Ar*C*-Ar), 168.8 (Linker-*C*ONHC), 169.4 (Ar*C*ONHCH$_2$), 174.5 (Pyr-*C*O$_2$Na), 180.3 (*C*O$_2$Na). **HRMS (ESI)**: *m/z* calculated for $C_{46}H_{50}N_6O_{16}Na_2^{2+}$ [M + 2Na]$^{2+}$ (corresponding deca-carboxylic acid macrotricycle) is 965.3175, found 966.3170.

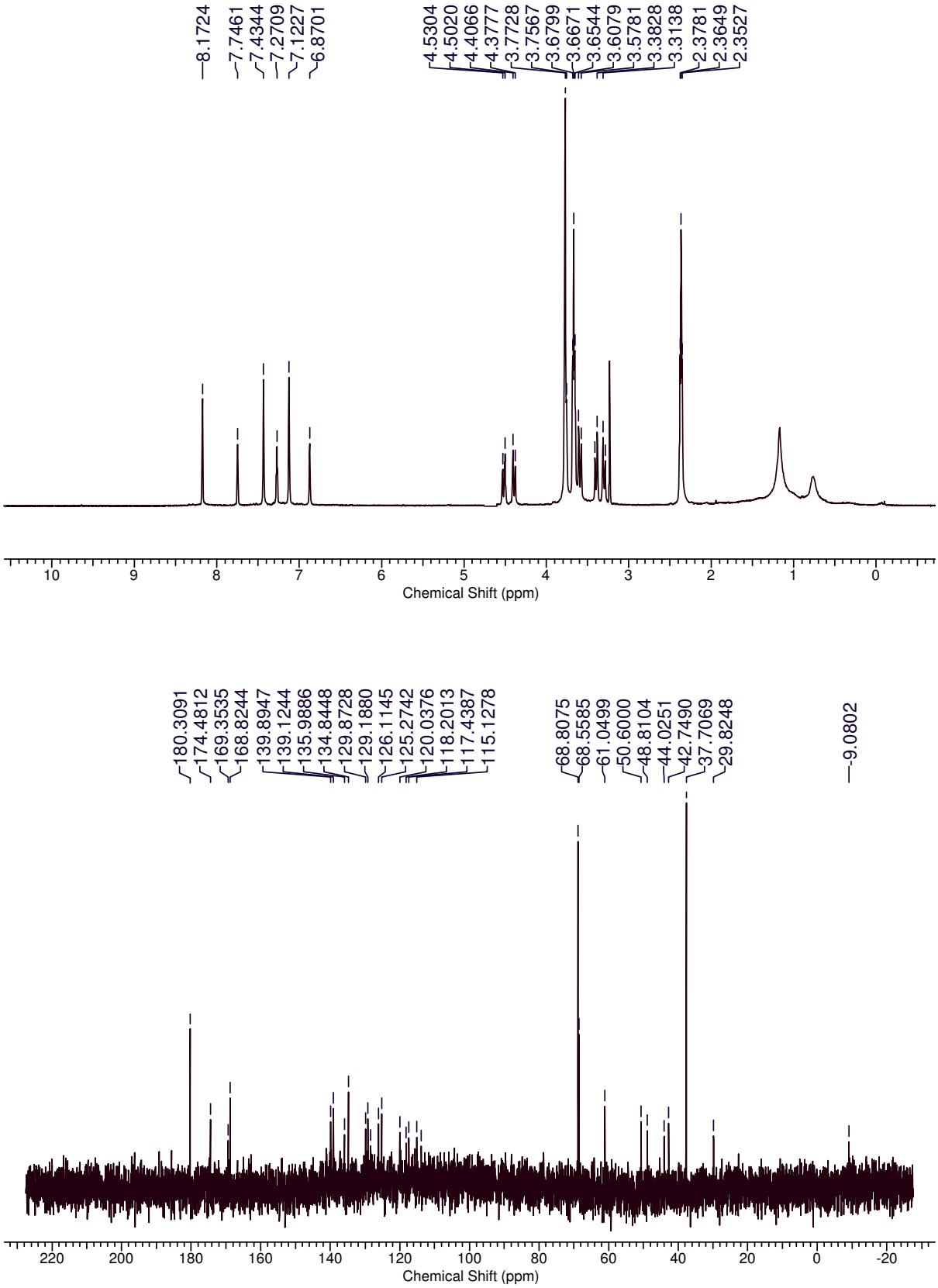


Figure S4. ^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of receptor **6** (D_2O, 400 MHz). The HOD peak is suppressed in the ^{1}H NMR spectrum.

NMR Binding Studies

Standard procedure for NMR titrations.

Solutions of guest in were prepared in phosphate buffer (pD = 13, 50 mM, prepared using D_2O*) and allowed to equilibrate overnight. Aliquots were added to 500 μl of a solution receptor **6** (1.15 mmol, $[\mathbf{6}]_{initial}$ = 2.3 mM) in phosphate buffer (pD = 13, 50 mM, prepared using D_2O*) in an NMR sample tube. The tube was shaken carefully after each addition and ^1H NMR spectra were recorded at 296 K. Variations in chemical shifts for receptor protons were entered into a specifically written non-linear least squares curve-fitting program implemented within Excel. Assuming 1:1 stoichiometry, the programme calculates K_a and the limiting change in chemical shift Δδ. The assumption of 1:1 stoichiometry is supported by the generally good fits between observed and calculated data.

*The phophate buffer solution (PBS) was prepared as follows: A 1 M PBS stock solution was prepared by dissolving disodium hydrogen phosphate (2.68 g) in D_2O (10 mL). The pD was adjusted to 13.0 by addition of 4% NaOD solution. A portion of this solution (500 μL) was diluted to 10 mL using D_2O and the pD readjusted to 13.0 to give the 50 mM PBS used for the titrations.

Titration of receptor 6 with GlcNAc 3

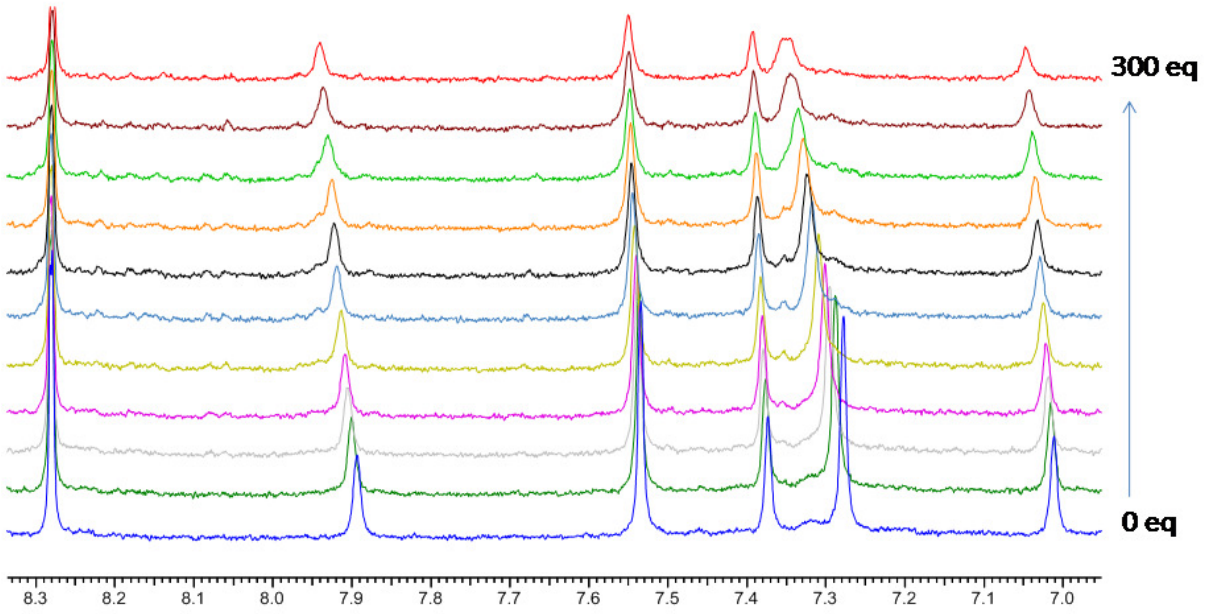


Figure S5. Partial ^{1}H NMR spectra from the addition of **3** to **6**. The signals shown are due to receptor aromatic protons. [**3**] = 0.50 M.

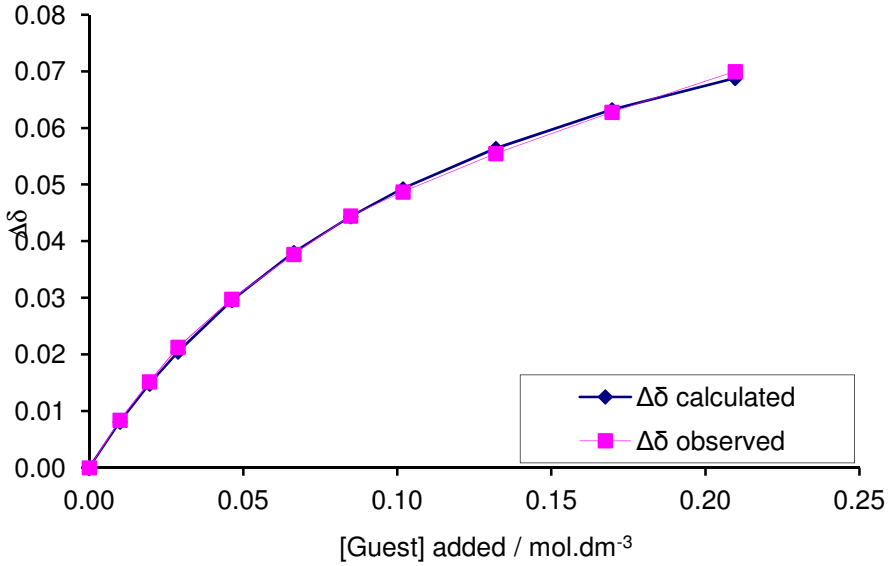


Figure S6. Experimental and calculated values for the ^{1}H NMR binding study of **6** + **3**. $[\mathbf{6}]_{initial}$ = 2.3 mmol, $[\mathbf{3}]_{titrant}$ = 0.5 M. K_a = 6.8 M^{-1}. Peak at 7.28 ppm used for analysis. Limiting Δδ = 0.07 ppm.

Titration of receptor 6 with methyl β-D-glucoside 14

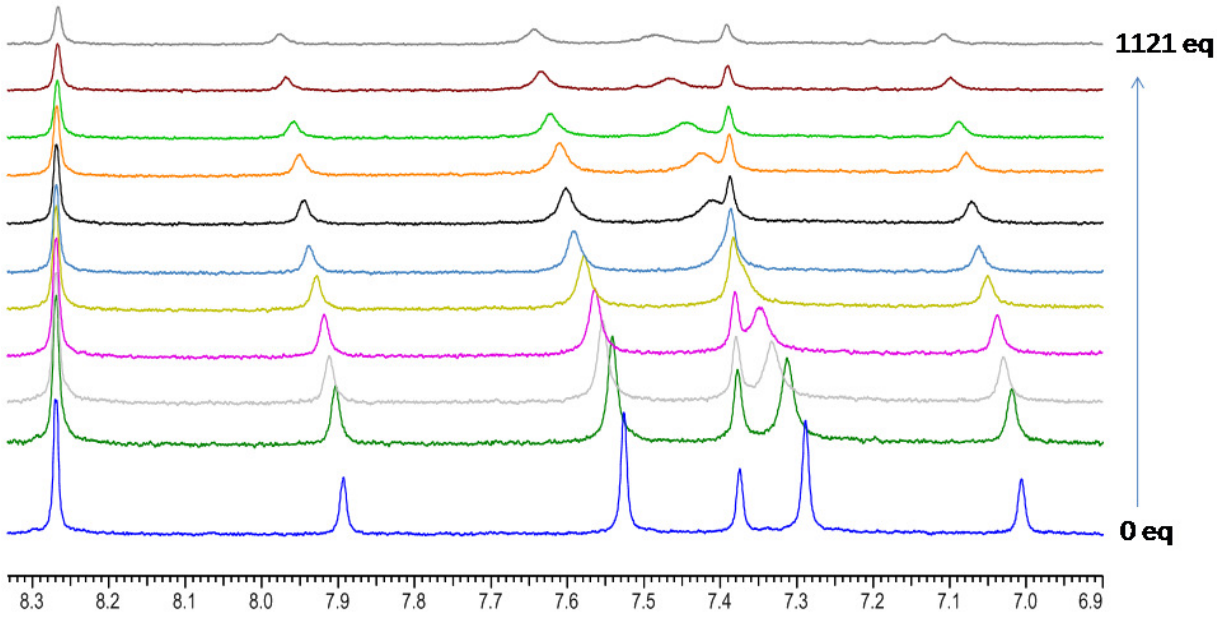


Figure S7. Partial ^{1}H NMR spectra from the addition of **14** to **6**. The signals shown are due to receptor aromatic protons. [**14**] = 0.96 M.

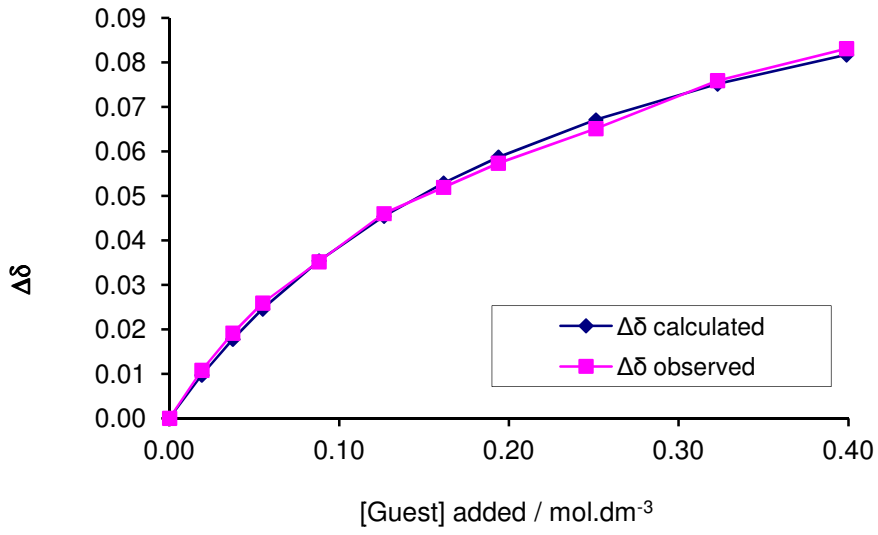


Figure S8. Experimental and calculated values for the ^{1}H NMR binding study of **6** + **14**. $[\mathbf{6}]_{initial}$ = 2.3 mmol, $[\mathbf{14}]_{titrant}$ = 0.96 M. K_a = 4.2 M^{-1}. Peak at 7.89 ppm used for analysis. Limiting Δδ = 0.08 ppm.

Titration of receptor 6 with D-galactose 16

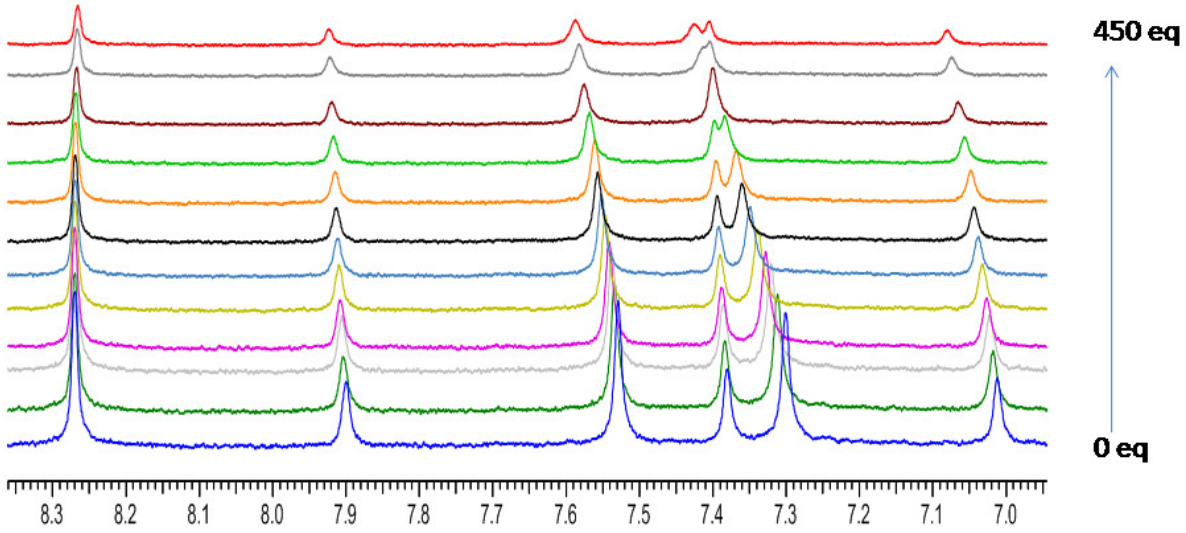


Figure S9. Partial ^{1}H NMR spectra from the addition of **16** to **6**. The signals shown are due to receptor aromatic protons. [**16**] = 0.61 M.

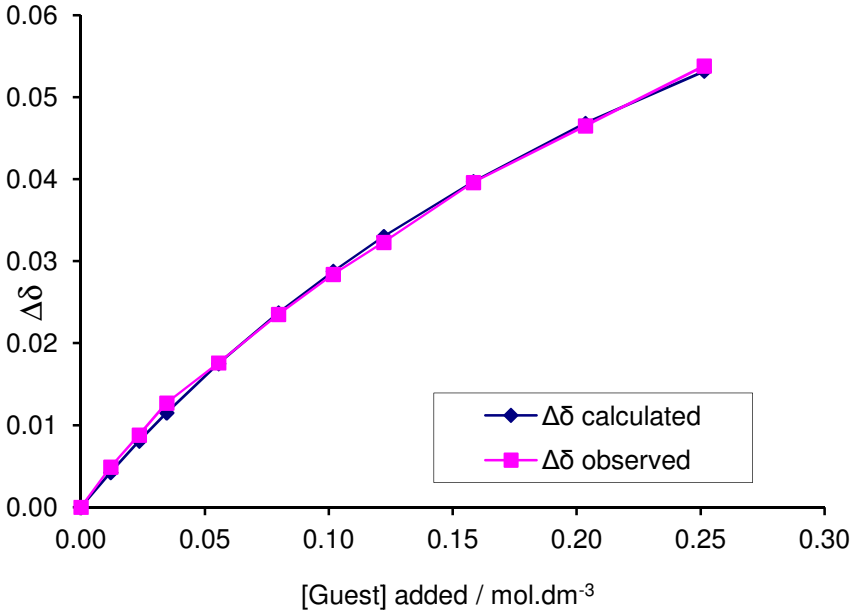


Figure S10. Experimental and calculated values for the ^{1}H NMR binding study of **6** + **16**. $[\mathbf{6}]_{initial}$ = 0.9 mmol, $[\mathbf{16}]_{titrant}$ = 0.61 M. K_a = 2.9 M^{-1}. Peak at 7.53 ppm used for analysis. Limiting Δδ = 0.053 ppm.

Titration of receptor 6 with D-xylose 17

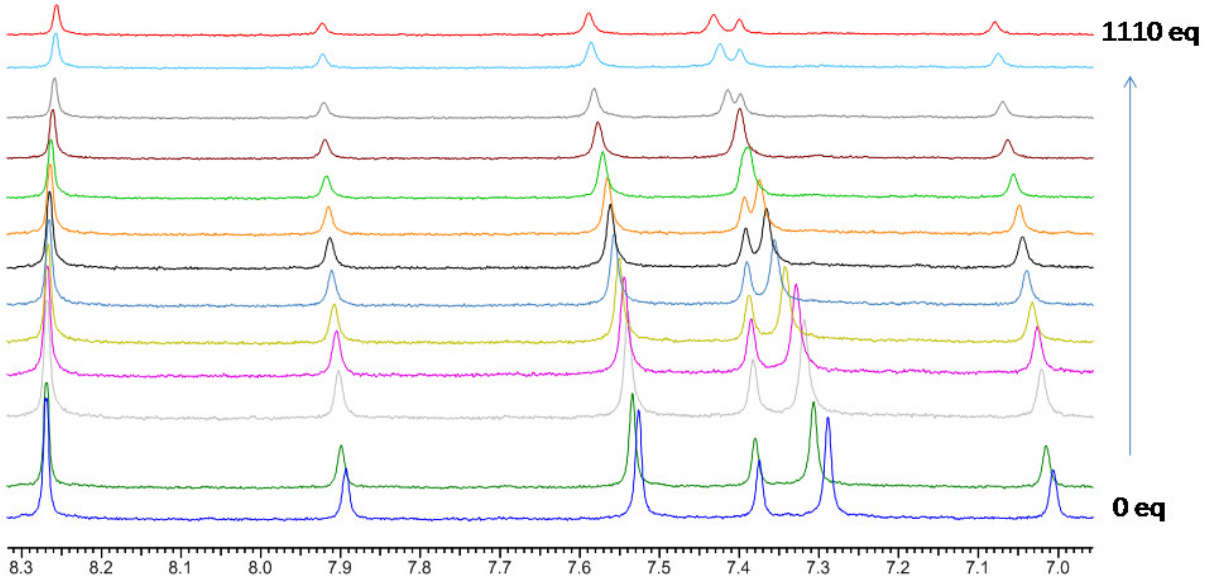


Figure S11. Partial ^{1}H NMR spectra from the addition of **17** to **6**. The signals shown are due to receptor aromatic protons. [**17**] = 0.96 M.

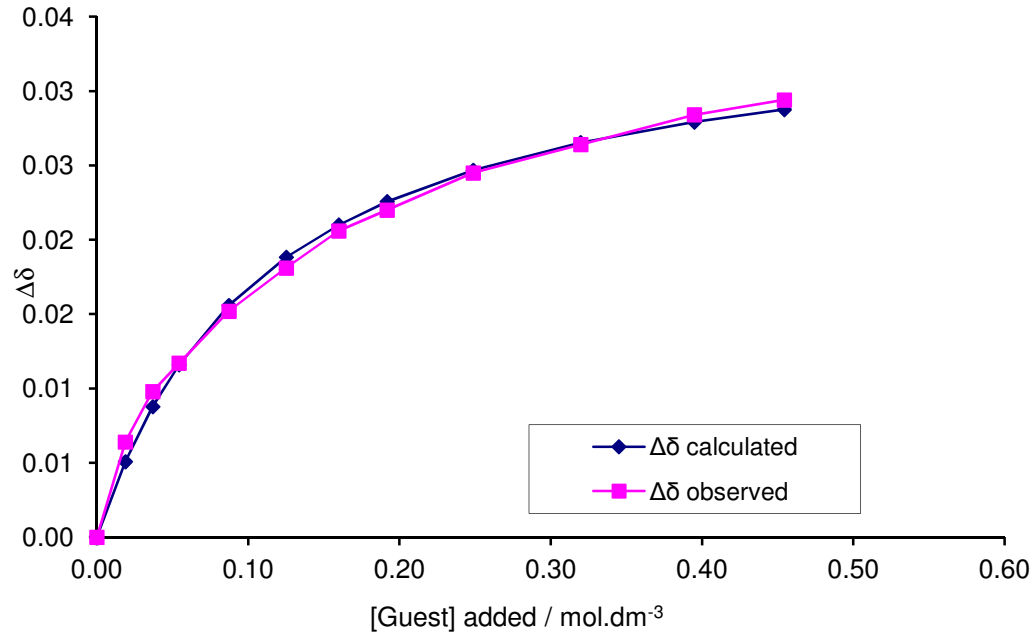


Figure S12. Experimental and calculated values for the ^{1}H NMR binding study of **6** + **17**. $[\mathbf{6}]_{initial}$ = 2.3 mmol, $[\mathbf{17}]_{titrant}$ = 0.96 M. K_a = 4.2 M^{-1}. Peak at 7.89 ppm used for analysis. Limiting Δδ = 0.029 ppm.