Supporting Information

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Highly Selective Disaccharide Recognition by a Tricyclic Octaamide Cage

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Supporting Information
Figure S1. Partial $^1$H NMR spectra in CDCl$_3$/CD$_3$OH (92:8) of macrotricycles 4 + 7, synthesized as shown in Scheme 1, before a) and after b) addition of 11 (3 equivalents). Resonances marked * are due due to 4, while the remaining peaks in spectrum a) are assigned to 7.
Figure S2. Fluorescence emission spectra recorded in the binding study of receptor 4 vs. n-octyl-β-D-cellobioside 11 in CHCl₃/MeOH 92:8. [4] = 0.03 mM, [11] = 0.065 - 2.3 mM.
Figure S3. Fluorescence emission spectra recorded in the binding study of receptor 4 vs. n-octyl-β-D-cellobioside 11 in CHCl₃/MeOH 98:2. [4] = 0.029 mM, [11] = 0.036 - 0.19 mM.
Figure S4. Experimental and calculated values for the fluorescence binding study of 4+11 in CHCl$_3$/CH$_3$OH (98:2). [4] = 0.029 mM, [11] = 0.036 - 0.19 mM. $K_a = 64,000$ M$^{-1}$. 
**Figure S5.** Fluorescence emission spectra of 4 (0.030 mM) in CHCl₃/MeOH (98:2), alone and in the presence of celllobioside 11 (6 equiv.), lactoside 12 (10 equiv.) and maltoside 13 (10 equiv.).
**Figure S6.** CD spectra recorded in the binding study of receptor 4 vs. n-octyl-\(\beta\)-D-cellobioside 11 in CHCl\(_3\)/MeOH 92:8. [4] = 0.09 mM, [11] = 0.011 - 0.4 mM.
Experimental details

$^1$H and $^{13}$C spectra were recorded on a Jeol Eclipse 400 MHz spectrometer. Chemical shifts ($\delta$) are quoted in parts per million downfield from tetramethysilane. Fluorescence studies were performed on a Perkin Elmer LS 50B luminescence spectrometer. Circular dichroism spectra were recorded on a Jobin Yvon CD6 spectrometer. HPLC grade solvents were used for the fluorescence and CD experiments.

Glucosides 2 and 3 and maltoside 14 were purchased from Sigma while disaccharides 11, 12 and 13 were prepared according to known procedures.\[1\]

3,5,3',5''-tetra[(N-tert-butyloxycarbonyl)aminomethyl]terphenyl (C): A flask was charged with [1,1'-bis(diphenylphosphino)ferrocene]-dichloropalladium(II) (17 mg, 0.02 mmol), 1,4-benzenediboronate B (94 mg, 0.31 mmol), 3,5-bis[(N-tert-butyloxycarbonyl)aminomethyl]-iodobenzene A [2] (431 mg, 0.93 mmol) and sodium carbonate (327 mg, 3.09 mmol). DMF (6 mL) and water (1.5 mL) were added, and the reaction was heated to 80 °C. After 19 h the reaction was cooled to room temperature and poured into water (40 mL). The aqueous solution was extracted with diethyl ether (3 × 20 mL), the combined organic extracts were dried (MgSO₄) and the solvent was removed at reduced pressure. Purification by flash column chromatography (SiO₂, ethyl acetate-toluene, 1:4) afforded 3,5,3',5''-tetra[(N-tert-butyloxycarbonyl)aminomethyl]-1,1',4',1''-terphenyl C (154 mg, 0.21 mmol, 66 %). Rᵣ = 0.27 (ethyl acetate-toluene, 1:2); m.p. 195-197 °C; ¹H NMR (400 MHz, CDCl₃, 30 °C): δ = 7.65 (s, 4 H; ArH), 7.46 (s, 4 H; ArH), 7.21 (s, 2 H; ArH), 4.92 (br, 4 H; NH), 4.40 (d, ³J(H,H) = 5.5 Hz, 8 H; CH₂), 1.50 (s, 36 H; CH₃); ¹³C NMR (100.6 MHz, CDCl₃, 30 °C): δ = 155.4, 140.9, 139.6, 139.3, 127.1, 125.0, 124.7, 79.1, 44.2, 28.0; IR (Nujol): ʋ = 3340, 1691 cm⁻¹; MS (ES⁺, %): 764 (25) [M+H₂O]+; elemental analysis calcd (%) for C₄₂H₅₈N₄O₈ (747.0): C 67.54, H 7.83, N, 7.50; found C 67.42, H 7.80, N 7.42.

Macrotricycles (4) and (7): To a stirred solution of tetra-Boc terphenyl 5 (109 mg, 0.14 mmol) in dichloromethane (5 mL) was added trifluoroacetic acid (5 mL). After 21.5 h the mixture was concentrated at reduced pressure and the crude product was purified by triturating with dichloromethane to yield the tetra TFA salt of tetra-amine 5 (112 mg, 0.14 mmol, 100 %). To a stirred solution of this salt (112 mg, 0.14 mmol), N,N-diisopropylethylamine (1.4 mL, 1.0 g, 7.7 mmol) and N,N-dimethylformamide (11 mL) in tetrahydrofuran (ca. 300 mL) was added a solution of triester 6 [3] (160 mg, 0.26 mmol) in tetrahydrofuran (ca. 250 mL), dropwise over ca. 6.5 h. After 20.5 h the reaction was concentrated at reduced pressure to a brown oil. The residue was taken up in ethyl acetate (35 mL) and the solution was washed with hydrochloric acid (0.5 mol dm$^{-3}$, 15 mL), saturated aqueous sodium hydrogen carbonate (15 mL) and brine (15 mL). The organic layer was dried (MgSO$_4$) and the solvent was removed at reduced pressure. Flash column chromatography (SiO$_2$, methanol-chloroform, 1:99) afforded crude macrotricycles 4 and 7 as a mixture of isomers (67 mg, 0.04 mmol, 57 %). Small portions were subjected to purification by preparative thin layer chromatography (SiO$_2$, methanol-chloroform, 1:19). $R_f = 0.26$ (methanol-chloroform, 1:49); $^1$H

NMR (400 MHz, [D$_6$]DMSO, 30 °C): $\delta = 9.26$ (t, $^3$$J$(H,H) = 6.0 Hz, 8 H; NH), 9.16 (t, $^3$$J$(H,H) = 5.3 Hz, 8 H; NH), 8.61 (s, 8 H; ArH), 8.53 (s, 8 H; ArH), 8.27 (s, 4 H; ArH), 7.50 (s, 8 H; ArH), 7.45 (s, 8 H; ArH), 7.34 (s, 4 H; ArH), 7.29 (s, 4 H; ArH), 4.53 (m, 32 H; CH$_2$), 4.36 (m, 16 H; CH$_2$), 1.77 (m, 16 H; CH$_2$), 1.39-1.24 (m, 32 H; CH$_2$), 0.93-0.84 (m, 24 H; CH$_3$); MS (FAB): 1670 [MH$^+$].
4-Bromo-3',5'-Bis[(N-tert-butyloxycarbonyl)aminomethyl]-biphenyl (E): The reaction flask was charged with bis-Boc derivative A (0.5 g, 1.08 mmol), 4-bromobenzeneboronic ester D (0.32 g, 1.19 mmol, 1.1 eq), Na₂CO₃ aq. (2 M, 1.2 mL, 2.38 mmol, 2.2 eq), and PdCl₂(dppf) (0.020 g, 0.027 mmol, 2.5 mol %), and was flushed with argon. DMF (8 mL) was added, and the mixture was stirred at 80 °C for 15 h then cooled to room temperature. After filtration through Celite and removal of the solvents in vacuo, the residue is dissolved in CH₂Cl₂ and passed through a short silicagel plug to obtain, after evaporation of the solvent, a grey solid. Recrystallisation in EtOAc/toluene (1:3) afforded pure biaryl E as a white/grey solid (0.398 g, 0.81 mmol, 75 %). R₆ = 0.3 (EtOAc/toluene 1:9); ¹H NMR (400 MHz, CDCl₃): δ = 7.56 (d, 3J(H,H) = 8.5 Hz, 2 H; H-3, H-5), 7.42 (d, 3J(H,H) = 8.6 Hz, 2 H; H-2, H-6), 7.36 (s, 2 H; H-2’, H-6’), 7.18 (s, 1 H; H-4’), 4.88 (br t, 2 H; NH), 4.37 (d, 3J(H,H) = 6.0 Hz, 4 H; CH₂NHBoc), 1.48 (s, 18 H; CH₃); ¹³C NMR (100 MHz, CDCl₃): δ = 155.3 (COO), 139.9, 139.3, 138.8, 131.1, 127.9, 124.8, 124.1, 120.9 (ArC), 77.7 (CCH₃), 43.4 (CH₂), 28.1 (CH₃); MS (FAB, %): 513 (50), 515 (50) [MNa]+.
3,5-Bis[(N-benzyloxycarbonyl)aminomethyl]-3′,5′-bis[(N-tert-butyloxycarbonyl)amino-
methy]terphenyl (9): Biaryl E (0.680 g, 1.38 mmol) was dissolved in a solution of anhydrous,
deoxygenated DMF (15 mL) along with bis(pinacolato)diboron (0.782 g, 2.77 mmol, 2 eq), KOAc
(0.811 g, 8.28 mmol, 6 eq), and PdCl₂(dppf) (0.050 g, 0.069 mmol, 5 mol %). The mixture was
heated at 80 °C for 24 h, cooled to room temperature, filtered through Celite and concentrated in
vacuo to an oil. Purification by flash column chromatography eluting with EtOAc/toluene (2:98 then
10:90) yielded boronic ester F as a white solid (0.545 g, 1.03 mmol, 75 %). \( R_f = 0.30 \)
(EtOAc/toluene 15:85); \(^1\)H NMR (400 MHz, CDCl₃): \( \delta = 7.88 \) (d, \(^3\)J(H,H) = 7.8 Hz, 2 H; H-3, H-5),
7.58 (d, \(^3\)J(H,H) = 8.1 Hz, 2 H; H-2, H-6), 7.40 (s, 2 H; H-2’, H-6’), 7.19 (s, 1 H; H-4’), 4.91 (br t, 2
H; NH), 4.37 (d, \(^3\)J(H,H) = 6.0 Hz, 4 H; CH₂NHBoc), 1.48 (s, 12 H; C(CH₃)₂), 1.30 (s, 18 H,
C(CH₃)₃); \(^{13}\)C NMR (100 MHz, CDCl₃): \( \delta = 155.3 \) (COO), 139.9, 139.3, 138.8, 131.1, 127.9, 124.8,
124.1, 120.9 (ArC), 77.7 (CCH₃), 43.4 (CH₂), 28.1 (CH₃).

Boronate G (0.444 g, 0.84 mmol) was placed in a flask along with bis-Cbz derivative 8(E)
(0.89 g, 1.68 mmol, 2 eq), Na₂CO₃ aq. (2 m, 2.2 mL, 4.4 mmol, 5 eq), and PdCl₂(dppf) (0.061 g, 0.083
mmol, 10 mol %), and was flushed with argon. DMF (8 mL) was added, and the mixture was stirred
at 80 °C for 19 h then cooled to room temperature. After filtration through Celite and removal of the
solvents in vacuo, the residual oil was purified by flash column chromatography eluting with
EtOAc/toluene (1:9 then 25:75) then with MeOH/DCM (2:98). After evaporation of the solvents,
the solid obtained was recrystallised from boiling acetone to afford pure terphenyl 9 (0.297 g, 0.365
mmol, 43 %) \( R_f = 0.35 \) (MeOH/DCM 2:98); m.p. : 160-162 °C; \(^1\)H NMR (400 MHz, CDCl₃): \( \delta =\)
7.61-7.28 (m, 18 H; ArH), 7.20-7.16 (br s, 2 H; H-4, H-4’’), 5.18 (br t, 2 H, NHCbz), 5.15 (s, 4 H, OCH$_2$), 4.92 (br t, 2 H; NHBoc), 4.50-4.30 (2d, 8 H; CH$_2$NHBoc, CH$_2$NHCbz), 1.48 (s, 18 H; CH$_3$); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 155.5 (COOCH$_2$Ph), 155.0 (COOC(CH$_3$)$_3$), 140.7, 140.5, 139.2, 138.8, 135.5, 127.8, 127.2, 126.8, 124.5, 124.2 (ArC), 78.1 (C(CH$_3$)$_3$), 66.4 (CH$_2$Ph), 43.8 (CH$_2$NHCbz), 43.2 (CH$_2$NHBoc), 27.8 (CH$_3$); HRMS (FAB): m/z calcd for C$_{48}$H$_{54}$N$_4$O$_8$ 814.3942, found 814.3978 and C$_{48}$H$_{54}$N$_4$O$_8$Na 837.3839, found 837.3844.
Tetra(N-tert-butyloxycarbonyl) protected macrocycle (10): Palladium on carbon (10 % wt. (Aldrich catalogue number 33,010-8), 50 mg before activation) was heated at 200 °C for 2 h under vacuum. The flask was allowed cool to room temperature under nitrogen before a solution of bis-Cbz bis-Boc terphenyl 9 (0.200 g, 0.245 mmol) in 10 mL MeOH/DCM (1:1) was added. The flask was evacuated and filled with hydrogen (1 atm) and the mixture was stirred for 2 days under hydrogen at room temperature. The mixture was filtered through Celite and then evaporated in vacuo to a white solid. The material was purified by passing through a short silica plug, eluting initially with MeOH/DCM (5:95) followed by MeOH.NH$_3$/DCM (12:88). The desired fractions were evaporated in vacuo to yield the free diamine G as a white amorphous solid (0.090 g, 0.184 mmol, 75 %). $R_f = 0.26$ (MeOH.NH$_3$/DCM 10:90); $^1$H NMR (400 MHz, CD$_3$OH/CDCl$_3$ 5:95): $\delta = 7.72$-$7.62$ (m, 4 H; H-2’, H-3’, H-5’, H-6’), 7.46 (s, 2 H; H-2, H-6), 7.42 (s, 2 H; H-2”, H-6”), 7.28 (s, 2 H; H-2”, H-6”), 7.28

[3] MeOH.NH$_3$ refers to methanol which has been pre-saturated with ammonia gas.
(s, 1 H; H-4), 7.19 (s, 1 H; H-4’’), 5.41-5.30 (br s, 2 H; NHBoc), 4.38 (d, 4 H; CH$_2$NHBoc), 3.95 (s, 4 H; CH$_2$NH$_2$), 1.48 (s, 18 H; CH$_3$); $^{13}$C NMR (100 MHz, CD$_3$OH/CDCl$_3$ 5:95): $\delta$ = 155.2 (COO), 142.5, 140.3, 139.0, 138.8, 126.5, 124.5, 124.0, 123.5 (ArC), 78.9 (C(CH$_3$)$_3$), 45.2 (CH$_2$NHBoc), 43.6 (CH$_2$NH$_2$), 27.5 (CH$_3$).

To a solution of diamine G (0.125 g, 0.23 mmol, 1 eq) in dry THF (70 mL), dry DMF (6 mL) and N,N-di-isopropylethylamine (0.70 mL, 4.6 mmol, 20.0 eq) was added a solution of the triester 6 (0.14 g, 0.23 mmol, 1 eq) in dry THF (130 mL) under nitrogen with rapid stirring at room temperature. After addition, the reaction mixture was stirred for 20 h. The solvents were evaporated in vacuo and the crude solid obtained was extracted several times with boiling acetone. After cooling down the filtrate, the desired macrocycle 10 was obtained as a white precipitate (0.070 g, 0.044 mmol, 40 %). $R_f = 0.25$ (MeOH/DCM 3:97); $^1$H NMR (400 MHz, [D$_6$]DMSO): $\delta$ = 9.40 (t, $^3$J(H,H) = 5.3 Hz, 4 H; NHCOAr), 8.59 (br s, 2 H, ARCH spacer), 8.56 (m, 4 H, ArCH spacer), 7.83 (d, $^3$J(H,H) = 8.3 Hz, 4 H; ArCH terphenyl), 7.75 (d, $^3$J(H,H) = 8.3 Hz, 4 H; ArCH), 7.65 (s, 4 H; NHCCCH$_2$), 7.48 (s, 4 H; ArCH terphenyl), 7.46 (t, $^3$J(H,H) = 6.2 Hz, 4 H; NHBoc), 7.22 (s, 2 H; ArCH terphenyl $\alpha$ to CCH$_2$NHBoc), 7.14 (s, 2 H; ArCH terphenyl $\alpha$ to CCH$_2$NHCOAr), 4.58 (d, 8 H; CH$_2$NHCOAr), 4.34 (t, $^3$J(H,H) = 6.8 Hz, 4 H; OCH$_2$CH$_2$), 4.21 (d, 8 H; CH$_2$NHBoc), 1.78 (q, $^3$J(H,H) = 7.1 Hz, 4 H; OCH$_2$CH$_2$), 1.47-1.32 (br m, 44 H; CH$_2$CH$_2$CH$_3$ and C(CH$_3$)$_3$), 0.92 (t, $^3$J(H,H) = 7.3 Hz, 6H, CH$_3$CH$_3$); $^{13}$C NMR (100 MHz, CD$_3$OD/CDCl$_3$ 5:95): $\delta$ = 165.1 (CONHCCH$_2$), 155.2 (COOC(CH$_3$)$_3$), 138.8, 138.4, 138.2, 133.7, 130.4, 130.3, 128.5, 126.5, 126.4, 125.2, 124.1, 124.0 (ArC), 78.8 (C(CH$_3$)$_3$), 65.0 (OCH$_2$), 43.2 (CH$_2$NHBoc), 43.1 (CH$_2$NHCOAr), 27.3 ((CH$_3$)$_3$), 27.2, 27.0, 21.2 (CH$_3$), 12.9 (CH$_3$).
Macrotricycle (4): Trifluoroacetic acid (2 mL, 26.2 mmol) was added to a solution of macrocycle 10 (0.063 g, 0.04 mmol) in DCM (2 mL) under nitrogen. The resultant mixture was stirred at room temperature for 2 h. The volatiles were removed in vacuo and the resultant oil triturated and washed with ether to give a white solid. Remaining solvent was removed under vacuum to give the corresponding tetra ammonium salt as a white solid. A solution of triester 6 (0.049 g, 0.08 mmol, 2 eq) in dry THF (160 mL) was added dropwise over ca. 5 h to a solution of the tetra ammonium salt (0.04 mmol, 1 eq) in dry THF (160 mL), dry DMF (10 mL) and N,N-di-isopropylethylamine (0.20 mL, 0.8 mmol, 10.0 eq) under nitrogen with rapid stirring at room temperature. After addition, the reaction mixture was stirred for 24 h and concentrated in vacuo. The crude oil was triturated with toluene to obtain a grey-white solid which was collected by filtration and washed several times in
boiling diethyl ether. A pale yellow solid which appeared to be fairly clean macrotricycle 4 was obtained (0.030 g, 0.018 mmol, 45 %). Small portions were subjected to further purification by preparative TLC eluting with MeOH/DCM 5:95. $R_f = 0.45$ (MeOH/DCM 5:95); $^1$H NMR (400 MHz, CD$_3$OH/CDCl$_3$ 8:92): $\delta = 8.68$ (d, $^4$J(H,H) = 1.0 Hz, 8 H; ArCH $\alpha$ to COOC$_2$H$_{11}$), 8.31 (t, $^3$J(H,H) = 4.9 Hz, 8 H; NH), 8.08 (s, 4 H; ArCH $\alpha$ to CCONH), 7.46 (s, 8 H; ArCH terphenyl), 7.43 (s, 8 H; ArCH terphenyl), 7.33 (s, 4 H; ArCH), 4.69 (A part of ABX, $J$(A,B) = 13.6 Hz, $J$(A,X) = 4.9 Hz, 8 H; CH$_2$NH), 4.43 (B part of ABX, $J$(A,B) = 13.6 Hz, $J$(A,X) = 4.9 Hz, 8 H; CH$_2$NH), 4.35 (t, $^3$J(H,H) = 6.9 Hz, 8 H; OCH$_2$), 1.80 (quintet, $^3$J(H,H) = 6.2 Hz, 8 H; OCH$_2$CH$_2$), 1.48-1.32 (br m, 16 H; CH$_2$CH$_2$CH$_3$), 0.92 (t, $^3$J(H,H) = 7.0 Hz, 12 H; CH$_3$); $^{13}$C NMR (100 MHz, CD$_3$OH/CDCl$_3$ 8:92): $\delta = 166.2$ (CONH), 165.9 (COCOCH$_2$), 141.3, 139.4, 139.3, 135.0, 132.2, 131.8, 129.1, 128.8, 127.5, 127.0 (ArC), 66.1 (OCH$_2$), 42.9 (CH$_2$NH), 28.6 (CH$_2$), 28.2 (CH$_2$), 22.5 (CH$_2$CH$_3$), 14.0 (CH$_3$); MS (FAB): 1692 [MNa]$^+$, 1714 [M+2Na-H]$^+$; HRMS (FAB): $m/z$ calcd for C$_{100}$H$_{100}$N$_8$O$_{16}$Na 1691.7155, found 1691.7106.