Supporting Information

Synthesis of 1,2,3-Triazole linked Galactopyranosides and Evaluation of Cholera Toxin Inhibition

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2-[2-(2-Prop-2-ynoxyethoxy)ethoxy]ethanol (3). Compound 3 was prepared as described in the literature, but at two-thirds the scale and in a yield of 58% (lit.1 75%). Data for compound 3 were identical to literature values.1

2-[2-[2-(2-prop-2-ynoxyethoxy)ethoxy]ethoxy]ethanol (4). Compound 4 was prepared as described for compound 3, but using tetraethylene glycol (20 g) and it was obtained in a yield of 41% (lit.2 65%). Data for compound 4 were identical to literature values.2

2-[2-[2-(2-prop-2-ynoxyethoxy)ethoxy]ethoxy]ethyl 4-methylbenzenesulfonate (6). Compound 6 was prepared as described for compound 5, using compound 4 (2.8 g) giving 6 {51% (lit.2 81%)}. Data for compound 6 were identical to literature values.2

1,4-Bis[2-[2-(2-prop-2-ynoxyethoxy)ethoxy]ethoxy]ethyl]piperazine (8). Compound 8 was obtained by the same procedure used for compound 7 with the following modifications: to a solution of piperazine (248 mg, 2.9 mmol) in dry THF (40 mL) was added sodium hydride (60% dispersion in oil, 483 mg) and the reaction mixture was stirred at room temperature for 1 h before a solution of 2-[2-[2-(2-prop-2-ynoxyethoxy)ethoxy]ethoxy]ethyl 4-methylbenzenesulfonate 6 (2.33 g, 6.0 mmol) in dry THF (10 mL + 10 mL rinse) was added. After 40 min, 4-dimethylaminopyridine (DMAP, 17 mg) was added to the reaction mixture, which was then refluxed at 77 °C for 25 h. The crude oil was purified by column chromatography on silica gel eluting with 5-50% MeOH:EtOAc containing 1% Et3N to yield compound 8 as a yellow oil (690 mg, 47%). IR (ATR, cm−1) 3248, 2866, 2809, 2113, 1456, 1349, 1296, 1249, 1090, 1011, 930, 845. 1H NMR (CDCl3, 300 MHz), 4.16 (4H, t, J = 2.4 Hz), 3.66-3.55 (28H, m), 2.57-2.51 (12H, m), 2.40 (2H, d, J = 2.3 Hz); 13C NMR (CDCl3, 75 MHz), 79.6, 74.5, 70.5, 70.34, 70.30, 69.0, 68.8, 58.3, 57.6 and 53.4. MS-ESI: m/z [M + H]+ for C29H47N2O8+ calculated 515.3; observed 515.3. HRMS-ESI: m/z [M + H]+ for C29H47N2O8+ calculated 515.3332; observed 515.3350.

[(1R,3S,4S,5R,6R)-4,5-diacetoxy-2-(acetoxyethyl)-6-[(1R)-4-[2-[2-[2-[2-[2-[2-[(1R)-1-[(2R,3R,4S,5S,6R)-3,4,5-triacetoxy-6-(acetoxyethyl)tetrahydropyran-2-yl]triazol-4-yl]methoxy]ethoxy]ethoxy]ethoxy]ethyl]piperazin-1-yl]ethoxy]ethoxy]ethoxy[methyl(triazol-1-yl)tetrahydropyran-3-yl]acetate (10). Compound 10 was obtained by the same procedure used for compound 9 with the following modifications. Compound 8 (298 mg, 0.58 mmol) was suspended in a 2:1 t-BuOH:H2O mixture (6 mL) to which was added 2,3,4,6-tetraacetate-β-D-galactopyranosyl azide (476 mg) and copper(0) (368 mg). The reaction mixture was allowed to stir vigorously for 16 h (not under inert atmosphere). The crude material was then purified by column chromatography on silica gel eluting with 17-20% MeOH:EtOAc containing 1% Et3N to yield compound 10 as a thick brown/yellow oil (431 mg, 59%). [α]D20 -4 (c 4.04, MeOH); IR (ATR, cm−1) 2869, 2859, 1746, 1454,
1432, 1368, 1210, 1089, 1042, 947, 922, 1H NMR δ (CDCl₃, 300 MHz), 7.82 (2H, s), 5.82 (2H, d, J = 9.3 Hz), 5.55-5.49 (4H, m), 5.27-5.19 (2H, m), 4.66 (4H, s), 4.22-4.09 (6H, m), 3.64-3.58 (28H, m), 2.61 (12H, br s), 2.19 (6H, s), 2.01 (6H, s), 1.98 (6H, s), 1.86 (6H, s); 13C NMR δ (CDCl₃, 75 MHz), 170.3, 169.9, 169.8, 169.0, 145.8, 121.1, 86.2, 74.0, 70.8, 70.5, 70.3, 69.7, 67.9, 66.9, 64.5, 61.2, 57.5, 53.1, 20.6, 20.5 and 20.2. MS-ESI: m/z [M + H]+ for C₅₈H₉₁N₆O₂₅⁺ calculated 1261.6; observed 1261.5. HRMS-ESI: m/z [M + H]+ for C₅₈H₉₁N₆O₂₅⁺, calculated 1261.5575; observed 1261.5526.

2-[2-(2-Azidoethoxy)ethoxy]ethoxy]ethanol (16). Compound 16 was prepared as described in the literature, using 14 (10.08 g) and sodium azide (5 equivalents) in a yield of 81% (lit.\textsuperscript{3} 76%). Data for compound 16 were identical to literature values.\textsuperscript{3}

2-[2-(2-Azidoethoxy)ethoxy]ethyl 4-methylbenzenesulfonate (17). Compound 17 was prepared as described for compound 14, using compound 15 (2.06 g), TsCl (2 equiv.) and Et\textsubscript{3}N (2.7 equiv.). The reaction was allowed to stir at room temperature for 3 d to give 17 (90%), after work-up. Data for compound 17 were identical to literature values.\textsuperscript{5}

2-[2-(2-Azidoethoxy)ethoxy]ethoxy]ethyl 4-methylbenzenesulfonate (18). Compound 18 was prepared as described for compound 17, using 16 (6.22 g) and the reaction was allowed to stir at room temperature for 23 h to give 18 (96%) after work-up. Data for compound 18 were identical to literature values.\textsuperscript{6}

1,4-Bis[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethyl]piperazine (20). Compound 20 was obtained by the same procedure as used for compound 19 with the following modifications: 2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethyl 4-methylbenzenesulfonate 17 (5.12 g, 13.7 mmol), piperazine (576 mg, 6.7 mmol) and sodium hydride 60% oil dispersion (1.10 g, 4.1 eq) in dry THF (30 mL). The crude product was purified via column chromatography on silica gel eluting with 0-9% MeOH:EtOAc containing 1% Et\textsubscript{3}N to yield compound 20 as a pale yellow oil (2.06 g, 63%). \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 500 MHz), 3.61-3.54 (24H, m), 3.32 (4H, t, J = 5.0 Hz), 2.55-2.51 (12H, m); \textsuperscript{13}C NMR (CDCl\textsubscript{3}, 125 MHz), 70.6, 70.51, 70.47, 70.2, 69.9, 68.6, 57.6, 53.2 and 50.5. MS-ESI: m/z [M + H]\textsuperscript{+} for C\textsubscript{20}H\textsubscript{41}N\textsubscript{8}O\textsubscript{6}\textsuperscript{+} calculated 489.3; observed 489.3. HRMS-ESI: m/z [M + H]\textsuperscript{+}, calculated 489.3149; observed 489.3161.

3,7-Anhydro-4,5,6,8-tetra-\textit{O}-benzyl-1,1,2,2-tetra-dehydro-1,2-\textit{d}-glycero-\textit{L}-mannooctitol (21). Compound 21 was prepared as described in the literature.\textsuperscript{7} Data for compound 21 were identical to literature values.\textsuperscript{8}

[(2\textit{R},3\textit{S},4\textit{R},5\textit{S},6\textit{S})-3,4,5-Triacetoxy-6-ethylhly-tetrahydropyran-2-yl]methyl acetate (24). Compound 24 was prepared as described in literature, using compound 21 (191 mg) and the reaction was stirred at room temperature for 7 d and 15 h to give 24 (54% (lit.\textsuperscript{8} 81%)). Data for compound 24 were identical to literature values.\textsuperscript{8}

Trimethyl(2-tributylstannylethynyl)silane (27). Compound 27 was prepared as described in the literature.\textsuperscript{9} Data for compound 27 were identical to literature values.\textsuperscript{9}
Acetyl 2,3,4,6-tetra-O-benzyl-D-galactopyranoside (28). To a solution of 2,3,4,6-tetra-O-benzyl-D-galactopyranoside (3.13 g, 5.8 mmol) in dry pyridine (35 mL) at 0 °C was slowly added acetic anhydride (32 mL). The reaction mixture was stirred at 0 °C for 1 h, after which DMAP (22 mg, 0.2 mmol) was added. The reaction mixture was then allowed to stir at room temperature for another 1 h, after which the reaction mixture was slowly poured into ice/water. After ethyl acetate extraction, evaporation of the solvent, and co-evaporations with distilled toluene the resulting crude oil was dry loaded onto silica and purified by column chromatography eluting with 50-83% EtOAc:Hexane to yield compound 28 as a colourless oil (3.37 g, 100%). Data for compound 28 were identical to literature values.\(^\text{10,11}\) Compound 28 was obtained as 1:1 α:β anomic mixture as evident by the equal intensity of the anomic signals in the ¹H NMR spectrum [δ 6.50 ppm, \(J = 3.6\) Hz (α-anomeric H) and 5.68 ppm, \(J = 8.1\) Hz (β-anomeric H)].

Trimethyl-[2-[(2R,3S,4R,5S,6R)-3,4,5-tribenzyloxy-6-(benzyloxymethyl)tetrahydropyran-2-yl]ethynyl]silane (29). Compound 29 was prepared as described in the literature, using compound 28 (3.37 g) to give 29 (65% (lit.\(^\text{12}\) 87%)). Data for compound 29 were identical to literature values.\(^\text{12}\)

(2R,3S,4R,5S,6R)-3,4,5-Tribenzyloxy-2-(benzyloxymethyl)-6-ethynyl-tetrahydropyran (30). Compound 30 was prepared as described in the literature, using 29 (1.41 g) to give 30 (68% (lit.\(^\text{12}\) 100%)). Data for compound 30 were identical to literature values.\(^\text{12}\)

**Scheme S1. Reagents and conditions**

- **a** Et₃N, TsCl, CH₂Cl₂
- **b** EtOH, NaN₃, 85 °C
- **c** Cu(0), TBTA, 2:1:1 tBuOH:H₂O:CH₂Cl₂
- **d** H₂, 5% Pd/C, EtOH/AcOH
**2-Methoxyethyl 4-methylbenzenesulphonate (39).** To a solution of 2-methoxyethanol 38 (3.06 g, 40.2 mmol) in dry CH₂Cl₂ (50 mL) was added Et₃N (15.10 mL, 109 mmol) and the solution was cooled to 0 °C. TsCl was added to this solution and the reaction mixture was allowed to stir at 0 °C for 40 min and then 25 h at room temperature. The resulting orange mixture was diluted with CH₂Cl₂ and washed with water. The aqueous fractions were backwashed with CH₂Cl₂ and the combined organic layers were washed with 2% AcOH and dried over MgSO₄. The crude oil was dry loaded onto silica and purified by column chromatography on silica gel eluting with 10-67% EtOAc:Hexane to yield compound 39 as a pale yellow oil (7.90 g, 85%). Data for compound 39 were identical to literature values.13

**1-Azido-2-methoxy-ethane (40).** Compound 40 was prepared as described for compound 16, using 39 (1.03 g). (Note: compound 40 is extremely volatile and most of the CH₂Cl₂ was evaporated at 0 °C with a little bit of CH₂Cl₂ remaining, which prevented a yield being obtained). **WARNING:** No further purification of 40 was attempted due to the hazards involved in distilling low molecular weight azides. Data for compound 40 were identical to literature values.14

**1-(2-Methoxymethyl)-4-[(2S,3S,4R,5S,6R)-3,4,5-tribenzyloxy-6-(benzyloxyethyl)tetrahydropyran-2-yl]triazole (41).** Compound 41 was obtained by the same procedure used for compound 22, compound 21 (94 mg, 0.17 mmol), compound 40 (102 mg, excess, not accurate due to volatility of the azide), Cu(0) (54 mg, 5 eq.) and TBTA (1.8 mg, 2mol%). The crude material was dry loaded onto silica and purified by column chromatography on silica gel eluting with 10-67% EtOAc:Hexane to yield compound 41 as a colourless oil (69 mg, 62%). [α]D²⁰ = +7 (c 1.26, DCM); IR (ATR, cm⁻¹) 3028, 2868, 1722, 1497, 1452, 1362, 1103, 1H NMR δ (CDCl₃, 300 MHz), 7.61 (1H, s), 7.41-7.18 (18H, m), 7.06-7.03 (2H, m), 5.03 (1H, d, J = 11.7 Hz), 4.77-4.65 (4H, m), 4.56-4.35 (6H, m), 4.27 (1H, t, J = 9.6 Hz), 4.08 (1H, d, J = 2.1 Hz), 3.76-3.60 (6H, m), 3.24 (3H, s); ¹³C NMR δ (CDCl₃, 75 MHz), 145.9, 138.7, 138.3, 138.2, 137.8, 128.3, 128.1, 128.0, 127.9, 127.7, 127.5, 127.4, 127.3, 123.5, 84.3, 78.4, 77.4, 74.9, 74.6, 74.5, 73.9, 73.4, 72.4, 70.6, 68.6, 58.8 and 50.1. MS-ESI: m/z [M + H]⁺ for C₃₀H₄₅N₅O₉⁺ calculated 650.3; observed 650.2. HRMS-ESI: m/z [M + Na]⁺ for C₃₀H₄₅N₅O₉Na⁺, calculated 672.3050, observed 672.3046.

**45 mg, 5 eq.) and**

**2-(Hydroxymethyl)-6-[(2-methoxymethyl)triazol-4-yl]tetrahydropyran-3,4,5-triol (36).** Compound 36 was obtained by the same procedure used for compound 23 using compound 41 (60 mg, 0.1 mmol) and 5% Pd/C catalyst (341 mg) in 90% EtOH/AcOH (5.5 mL). Compound 36 was obtained as a pale yellow oil (27 mg, 100%). ¹H NMR δ (D₂O, 300 MHz), 8.36 (1H, s), 5.42 (1H, br s), 4.53-4.45 (1H, m), 4.20-3.88 (9H, m), 3.39 (3H, s); ¹³C NMR δ (D₂O, 75 MHz), 146.4, 126.4, 79.0, 73.6, 73.3, 70.1, 69.2, 68.7, 60.7, 57.8 and 51.3. MS-ESI: m/z [M + H]⁺ for C₁₁H₁₆N₃O₆⁺ calculated 290.1; observed 290.1. HRMS-ESI: m/z [M + Na]⁺ for C₁₁H₁₆N₃O₆Na⁺, calculated 312.1172; observed 312.1146.
1-(2-Methoxyethyl)-4-[(2R,3S,4R,5S,6R)-3,4,5-tribenzyloxy-6-(benzylomethyl)tetrahydropyran-2-yl]triazole (42). Compound 42 was obtained by the same procedure used for compound 22 using compound 30 (101 mg, 0.18 mmol), compound 40 (165 mg, excess, not accurate due to volatility of the azide), Cu(0) (58 mg, 5 eq.), TBTA (2 mg, 2% mol). The crude material was dry loaded onto silica and purified by column chromatography on silica gel eluting with 10-67% EtOAc:Hexane to yield compound 42 as a colourless oil (71 mg, 59%). $[\alpha]_{D}^{20}+43$ (c 0.93, DCM); IR (ATR, cm$^{-1}$) 3028, 2870, 1722, 1495, 1452, 1096, 1302, 1260, 1033, 973, 852, 711, 629 cm$^{-1}$; $^{1}$H NMR (CDCl$_{3}$, 300 MHz), 7.68 (1H, s), 7.30-7.13 (20H, m), 5.29 (1H, d, J = 4.2 Hz), 4.75-4.43 (9H, m), 4.28-3.87 (5H, m), 3.73-3.67 (3H, m), 3.29 (3H, s); $^{13}$C NMR (CDCl$_{3}$, 75 MHz), 144.7, 138.1, 138.0, 137.8, 127.9, 127.8, 127.6, 127.5, 127.4, 127.3, 127.2, 127.1, 124.3, 76.3, 73.7, 73.4, 73.2, 72.84, 72.78, 72.5, 70.3, 67.0, 66.8, 58.6 and 49.9. MS-ESI: $m/z$ [M + H]$^+$ for C$_{39}$H$_{42}$N$_{3}$O$_{6}$ calculated 650.3; observed 650.3. HRMS-ESI: $m/z$ [M + Na]$^+$ for C$_{39}$H$_{42}$N$_{3}$NaO$_{6}$, calculated 672.3050; observed 672.3036.

(2R,3R,4R,5R,6R)-2-(Hydroxymethyl)-6-[1-(2-methoxyethyl)triazol-4-yl]tetrahydropyran-3,4,5-triol (37). Compound 37 was obtained by the same procedure used for compound 23 using compound 42 (145 mg, 0.22 mmol) and 5% Pd/C catalyst (888 mg). The crude material was purified via column chromatography on silica gel eluting with 1% AcOH:MeOH. Compound 37 was obtained as a thick yellow oil (33 mg, 51%). $^{1}$H NMR (D$_{2}$O, 300 MHz), 8.39 (1H, s), 6.05 (1H, br s), 4.75-4.67 (2H, m), 4.51 (1H, br s), 4.31 (1H, br s), 4.17 (1H, br s), 4.05-3.85 (4H, m), 3.63 (1H, t, J = 6.1 Hz), 3.40 (3H, s); $^{13}$C NMR (D$_{2}$O, 75 MHz), 143.5, 127.8, 73.7, 69.7, 69.3, 67.6, 67.2, 59.4, 57.9 and 51.2. MS-ESI: $m/z$ [M + H]$^+$ for C$_{13}H$_{20}N$_{2}$O$_{6}$$^+$ calculated 290.1; observed 290.1. HRMS-ESI: $m/z$ [M + Na]$^+$ for C$_{13}H$_{20}N$_{2}$NaO$_{6}$$^+$, calculated 312.1172; observed 312.1136.

**Biological studies**

Control plots were obtained by plotting [(CT concentration) divided by (10$^{3}$ × rate of absorbance change)] vs (CT concentration). A typical example is shown in Fig. S1, which illustrates the linear nature of the plot. Knowing the intercept and gradient of this plot, the concentration of free CT (i.e., CT not bound to immobilised G$_{M1}$) could be determined from the rate of
absorbance change at any given concentration of inhibitor of CT binding to immobilised GM1. An example of a polynomial fit to a plot of free [CT] vs concentration of inhibitor (in this case compound 37) is shown in Fig. S2.

**Fig. S1.** Linear form of the relationship of rate of absorbance change with concentration of CT in control wells.
Fig. S2. Inhibition of the binding of 10 ng/ml CT to immobilised GM1 by compound 37 (α-C-GT).

References
