Supporting information

Direct Evaluation of Live Uropathogenic *Escherichia coli* Adhesion and Efficiency of Anti-adhesive Compounds Using a Simple Microarray Approach

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EXPERIMENTAL SECTION

Synthesis and characterization of monomeric glycoside derivatives and glycofullerenes.

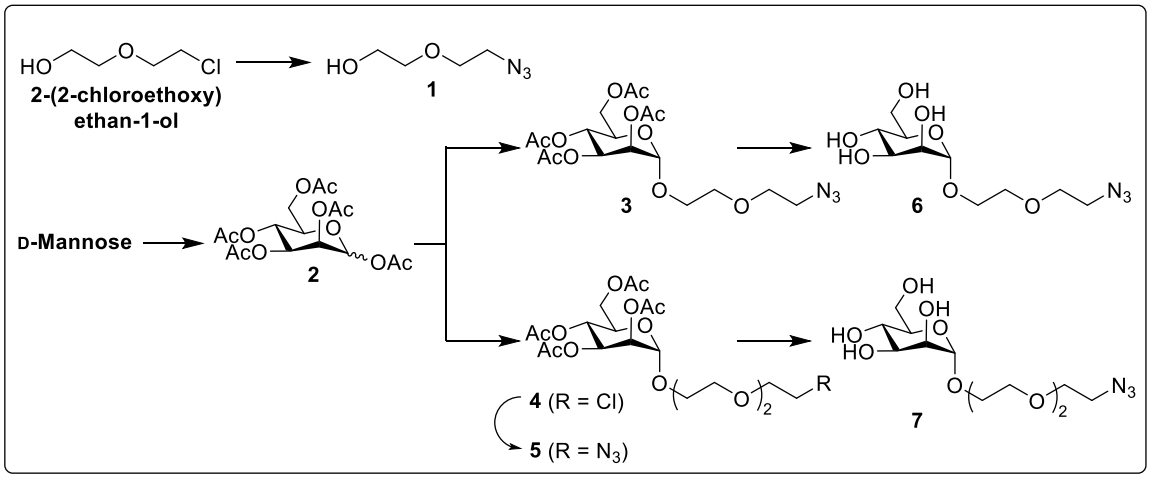
General materials and methods. ACS-grade reagents and chemicals were obtained from Fluka, Aldrich, or Acros Organics, and used without further purification unless otherwise stated. When necessary, methanol (MeOH) was refluxed over magnesium; tetrahydrofuran (THF) over sodium-benzophenone; dichloromethane (CH_2Cl_2) and acetonitrile (CH_3CN) over calcium hydride and stored over KOH pellets. N,N-dimethylformamide (DMF) was bought anhydrous and stored over molecular sieves (4 Å).

Thin layer chromatography was performed on aluminum-baked 0.2 mm thick Merck silica gel 60 F254 plates. The compounds were detected either by fluorescence quenching at 254 nm, by dipping into a solution of 1 g ceric (IV) sulfate tetrahydrate and 25 g ammonium molybdate tetrahydrate in 100 mL sulfuric acid/900 mL H_2O and subsequent heating, or by dipping into a solution of 5% sulfuric acid in ethanol and heating. Retention factors (*Rf*) are indicated with the corresponding solvent mixture in brackets. Flash column chromatography was performed either on a Reveleris® Flash System (Grace) with evaporative light scattering detection (ELSD) or manually under 1.5-3 bar pressure, using silica gel 60 (Merck, typically 30 g of silica per g of crude). Solvents were at least of technical grade and distilled prior to use.

Specific rotation was measured in a Perkin-elmer 241 polarimeter at a wavelength of 589 nm and room temperature ($[\alpha]_D$). ATR-IR spectroscopy (cm^{-1}) was performed on a Perkin–Elmer Spectrum One Spectrophotometer. UV spectra were obtained using an Analytik Jena Specord 205 spectrometer. MALDI-MS spectra were recorded using a Waters QToF Premier mass spectrometer equipped with a nitrogen laser, operating at 337 nm with a maximum output of 500 J m^{-2} delivered to the sample in 4 ns pulses at 20 Hz repeating rate. Time-of-flight mass analyses were performed in the reflectron mode at a resolution of about 10 000. All the samples were analyzed using 20 mg/mL dihydroxybenzoic acid in acetone as matrix. High-resolution mass spectra (HRMS) were acquired in a Bruker maXis mass spectrometer Q-TOF by the "Fédération de Recherche" ICOA/CBM (FR2708) platform of Orléans in France.

NMR spectra were obtained using JEOL JNM EX-400 or EX-500 spectrometers and resolved with JEOL's Delta software. Chemical shifts (δ) were referred to the partially deuterated nuclei of the solvents used. All spectra were described in the first order. The chemical shifts of signals featuring defined multiplicity were determined by the arithmetic mean of the signal lines. The following abbreviations were used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and their combinations. Assignment of protons was accomplished by ^1H-^1H correlation by COSY experiments and assignment of carbons by ^1H-^{13}C correlation (HMQC), HMBC and DEPT experiments.

Synthesis of monomeric glycosides. The synthesis of azido-functionalized mannosides **6** and **7** is illustrated in Scheme S-1. The synthesis of azido heptyl mannoside **12** is depicted in Scheme S-2. The synthesis of azido-functionalized galactoside **17** is shown in Scheme S-3. These syntheses were based on slightly modified literature procedures. Modified and new procedures are described below.



Scheme S-1. Synthesis of azido-functionalized mannosides **6** and **7**.

Compound **1**. 2-(2-chloroethoxy)ethan-1-ol (10 mL, 94.70 mmol) was dissolved in H_2O (60 mL) and sodium azide (15.4 g, 236.75 mmol, 2.5 eq) was added. The reaction mixture was stirred at 80 ºC under argon for 16 h, and then poured into sodium hydroxide (5%, 100 mL) and extracted with diethyl ether (100 mL × 3). The organic layer was dried over $MgSO_4$ and evaporated to dryness to afford **1** (12.4 g, 99%) as a colorless oil. The analytical data of **1** were in complete agreement with previous reports.[1] *Rf* : 0.49 (ethyl acetate/MeOH 9:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.76 (t, *J* = 4.0 Hz, 2H, *CH*$_2$O), 3.70 (t, *J* = 4.4 Hz, 2H,*CH*$_2$O), 3.61 (t, *J* = 4.8 Hz, 2H, *CH*$_2$O), 3.41 (t, *J* = 4.8 Hz, 2H, CH_2O), 2.01 (s, 1H, *OH*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 72.5 (*CH*$_2$O), 70.2 (*CH*$_2$O), 61.9 (*CH*$_2$O), 50.8 (*CH*$_2$N$_3$)*;* HRMS (ESI$^+$-MS, m/z) calcd. for $C_4H_9N_3O_2Na$ $[M+Na]^+$: 154.0587, found: 154.0587.

Compound **2**. 4-Dimethylaminopyridine (1.355 g, 11.1 mmol, 0.1 eq) was added slowly to a solution of D-mannose (20 g, 111.01 mmol, 1 eq) and Ac_2O (78.6 mL, 832.58 mmol, 7.5 eq) in dry pyridine (110 mL). The reaction mixture was stirred overnight at room temperature under argon and then diluted in ethyl acetate (150 mL), washed with HCl (1M, 50 mL × 5), NH_4Cl (50 mL), and brine (50 mL). The organic layer was dried over $MgSO_4$ and evaporated to dryness to afford **2** (43 g, quant.) as a sticky colorless oil. The analytical data of **2** were in complete agreement with literature data.[2] *Rf* : 0.64 (Cy/ EtOAc 4:6); $[\alpha]_D$ (CH_2Cl_2, c = 1, 20°C) = +53.5°; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.06 (d, $J_{1,2}$ = 1.8 Hz, 1 H, H-1), 5.33 (m, 2 H, H-3, H-4), 5.23 (d, $J_{2,3}$ = 2.3 Hz, 1 H, H-2), 4.24 (m, 1 H, H-6a), 4.09-4.05 (m, 2 H, H-6b, H-5), 2.15 (s, 3H, CH_3), 2.15 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 1.98 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.7 (CO), 170.1 (CO), 169.8 (CO), 169.6 (CO), 168.0 (CO), 90.6 (C-1), 70.6 (C-5), 68.8 (C-3), 69.7 (C-2), 68.4 (C-2), 65.5 (C-4), 62.1 (C-6), 20.9 (CH_3), 20.9 (CH_3), 20.8 (CH_3), 20.7 (CH_3), 20.7 (CH_3).

Compound **3**. To an ice-cold (0 °C) solution of D-mannose peracetate **2** (4 g, 10.25 mmol, 1 eq) and **1** (2.68 g, 20.49 mmol, 2 eq) in dry acetonitrile (50 mL) under argon atmosphere at 0 °C was added dropwise boron trifluoride diethyl etherate (2.52 mL, 20.49 mmol, 2 eq) and trimethylsilyl trifluoromethanesulfonate (0.38 mL, 2.05 mmol, 0.2 eq). The reaction mixture was allowed to warm to room temperature and then stirred overnight under argon. The solution was quenched with a saturated solution of $NaHCO_3$ (50 mL) and diethyl ether (50 mL). The organic phase was then washed with $NaHCO_3$ (2 × 50 mL), H_2O (50 mL) and brine (50 mL). It was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification of the residue by flash chromatography on silica gel (Cy/EtOAc 7:3) afforded the desired mannoside **3** (3.975 g, 84%) as a yellow oil. The analytical data of **3** were in complete agreement with literature data.[3] *Rf* : 0.63

(Cy/EtOAc 4:6); $[\alpha]_D$ ($CHCl_3$, c=1, 20°C) = +36.6°; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.36 (dd, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 9.8 Hz, 1 H, H-3), 5.29 (t, $J_{4,5}$ = 10.1 Hz, 1 H, H-4), 5.28 (dd, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.4 Hz, 1 H, H-2), 4.88 (d, 1 H, $J_{1,2}$ = 1.8 Hz, H-1), 4.29 (dd, $J_{5,6a}$ = 5.0 Hz, $J_{6a,6b}$ = 7.3 Hz, 1 H, H-6a), 4.11 (dd, $J_{5,6b}$ = 2.5 Hz, $J_{6a,6b}$ = 7.3 Hz, 1 H, H-6b), 4.09 (m, 1 H, H-5), 3.84-3.82 (m, 1 H, SugOC*H*H), 3.77-3.62 (m, 1 H, SugOCH*H*), 3.69 (m, 4 H, 2 x CH_2O), 3.39 (t, J = 4.8 Hz, 2 H, CH_2N_3), 2.17 (s, 3 H, CH_3), 2.16 (s, 3 H, CH_3), 2.11 (s, 3 H, CH_3), 2.04 (s, 3 H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.5 (CO), 170.9 (CO), 170.1 (CO), 169.9 (CO), 97.9 (C-1), 70.4 (CH_2O), 70.2 (CH_2O), 69.7 (C-2), 69.2 (C-3), 68.5 (C-5), 67.4 (SugOCH_2), 66.2 (C-4), 62.6 (C-6), 50.9 (CH_2N_3), 21.1-20.9 (4 CH_3); HRMS: (ESI$^+$-MS, m/z) calcd for $C_{18}H_{27}N_3NaO_{11}$ $[M+Na]^+$: 484.1538, found: 484.1539.

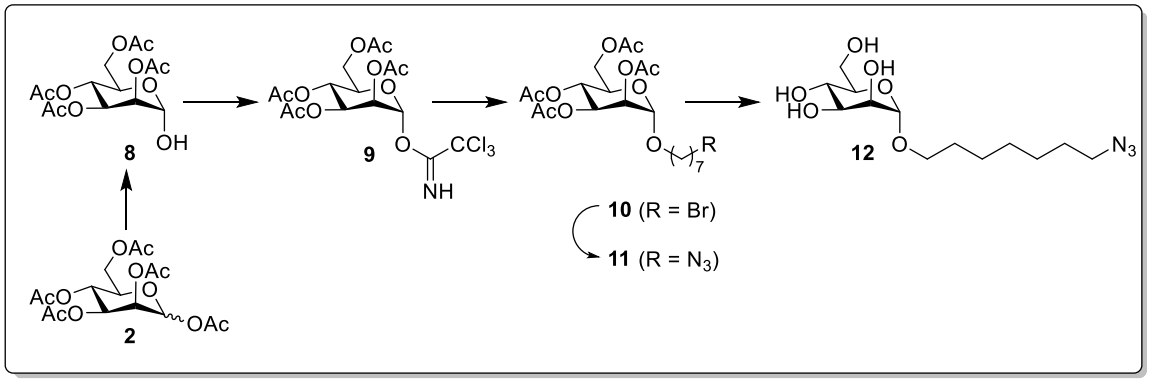
Compound **4**. To an ice-cold (0 °C) solution of D-mannose peracetate **2** (10 g, 25.62 mmol, 1 eq) and 2-(2-(2-chloroethoxy)ethoxy)ethan-1-ol (7.5 mL, 51.24 mmol, 2 eq) in dry acetonitrile (130 mL) under argon atmosphere at 0 °C was added dropwise boron trifluoride diethyl etherate (6.30 mL, 51.24 mmol, 2 eq) and trimethylsilyl trifluoromethanesulfonate (1 mL, 5.124 mmol, 0.2 eq). The reaction mixture was allowed to warm to room temperature and then stirred overnight under argon. The solution was quenched with a saturated solution of $NaHCO_3$ (100 mL) and diethyl ether (100 mL). The organic phase was then washed with $NaHCO_3$ (100 mL × 2), H_2O (100 mL), and brine (100 mL). It was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification of the residue by flash chromatography on silica gel (Cy/EtOAc 7:3) afforded the desired mannoside **4** (8.718 g, 68%) as a yellow oil. The analytical data of **4** were in complete agreement with literature data.[4] *Rf* : 0.52 (Cy/EtOAc 4:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.34 (dd, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 6.6 Hz, 1 H, H-3), 5.27 (t, $J_{4,5}$ =10.1 Hz, 1 H, H-4), 5.25 (dd, $J_{1,2}$ = 1.6 Hz, 1 H, H-2), 4.86 (d, 1 H, H-1), 4.29 (dd, $J_{5,6a}$ = 5.0 Hz, $J_{6a,6b}$ = 7.3 Hz, 1 H, H-6a), 4.10 (dd, $J_{5,6b}$ = 2.5 Hz, 1 H, H-6b), 4.04 (m, 1 H, H-5), 3.84-3.78 (m, 2 H, SugOCH_2), 3.77-3.62 (m, 10 H, 5 x CH_2O), 2.14 (s, 3 H, CH_3), 2.09 (s, 3 H, CH_3), 2.02 (s, 3 H, CH_3), 1.97 (s, 3 H, CH_3); ^{13}C NMR (100 MHz, CDCl3): δ = 170.2-169.8 (CO), 97.8 (C-1), 71.5 (CH_2O), 70.8 (CH_2O), 70.7 (CH_2O), 70.1 (CH_2O), 69.6 (C-2), 69.1 (C-3), 68.5 (C-5), 67.5 (SugOCH_2), 66.2 (C-4), 62.5 (C-6), 42.9 (CH_2N_3), 20.9-20.8 (CH_3). HRMS (ESI$^+$-MS, m/z) calcd for: $C_{20}H_{31}NaClO_{12}$ $[M+Na]^+$: 521.1396, found: 521.1406.

Compound **5**. Mannoside **4** (5.5 g, 10.98 mmol) was dissolved in dry DMF (50 mL) and sodium azide (2.86 g, 43.92 mmol, 4 eq) was added. The reaction mixture was stirred at 80 ºC under argon for 48 h and then poured into icy H_2O (50 mL) and extracted with ethyl acetate (50 mL × 3). The organic layer was washed with brine (50 mL), dried over $MgSO_4$ and evaporated to dryness. Purification of the crude by column chromatography (Cy/EtOAc 7:3 to 1:1) afforded **5** (3.57 g 64%) as a yellow oil. The analytical data of **5** were in complete agreement with literature data.[4] *Rf* : 0.37 (Cy/EtOAc 4:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.37 (dd, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 6.9 Hz, 1 H, H-3), 5.29 (t, $J_{4,5}$ =9.6 Hz, 1 H, H-4), 5.27 (dd, $J_{1,2}$ = 1.6 Hz, 1 H, H-2), 4.87 (d, 1 H, H-1), 4.31 (dd, $J_{5,6a}$ = 5.0 Hz, $J_{6a,6b}$ = 7.3 Hz, 1 H, H-6a), 4.12 (dd, $J_{5,6b}$ = 3.7 Hz, 1 H, H-6b), 4.08 (m, 1 H, H-5), 3.85-3.78 (m, 1 H, SugOCH*H*), 3.71-3.65 (m, 9 H, SugO*CH*H, 4 x CH_2O), 3.40 (t, J = 4.8 Hz, 2 H, CH_2N_3), 2.15 (s, 3 H, CH_3), 2.10 (s, 3 H, CH_3), 2.04 (s, 3 H, CH_3)., 1.99 (s, 3 H, CH_3).; ^{13}C NMR (100 MHz, CDCl3): δ = 170.9 (CO), 170.2 (CO), 170.1 (CO), 169.9 (CO), 97.9 (C-1), 70.9 (CH_2O), 70.8 (CH_2O), 70.3 (CH_2O), 70.2 (CH_2O), 69.7 (C-2), 69.2 (C-3), 68.5 (C-5), 67.5 (SugOCH_2), 66.2 (C-4), 62.5 (C-6), 50.8 (CH_2N_3), 21.1, 20.9 (CH_3); HRMS: (ESI$^+$-MS, m/z) calcd for**:** $C_{20}H_{31}NaN_3O_{12}$ $[M+Na]^+$: 528.1800, found: 528.1817.

Compound **6**. To a solution of **3** (1 g, 2.17 mmol, 1 eq) in dry MeOH (20 mL) under argon atmosphere was added sodium methoxide (0.468 g, 8.67 mmol, 4 eq) at room temperature and the solution was stirred during 2 h. The reaction mixture was concentrated under reduced pressure until the volume was 10 mL and passed through a short column of Dowex® 50WX8-200 (H^+ form). The resin was washed with a solution of

H_2O/MeOH 99:1 (50 mL). The fractions containing the product were concentrated under reduced pressure to afford the desired product **6** (0.651 g, quant.) as a yellow oil. The analytical data of **6** were in complete agreement with literature data.[3] *Rf* : 0.1 (Cy/EtOAc 4:6); $[\alpha]_D$ (MeOH, c=1, 20°C) = +38.5°; ^{1}H NMR (400 MHz, D_2O) δ =4.1 (s, 1H, H-1), 3.79 (dd, $J_{1,2}$ = 1.6 Hz, $J_{2,3}$ = 1.8 Hz, 1H, H-2), 3.81-3.72 (m, 2H, H-6a,), 3.68-3.61 (m, 3H, H-3, H-6b), 3.73-3.67 (m, 2H, H-4, H-5), 3.40 (t, *J* = 5.0 Hz, 2H, C*H*$_2$N$_3$); ^{13}C NMR (100 MHz, D_2O) δ = 99.9 (C-1), 72.6, 70.5, 70.0, 66.7 (C-2, C-3, C-4, C-5), 69.4 (SugO*CH*$_2$), 69.3 (*CH*$_2$O), 66.3 (*CH*$_2$O), 60.9 (C-6), 50.1 (*C*H$_2$N$_3$); HRMS: (ESI$^+$-MS, m/z) calcd for $C_{10}H_{19}N_3KO_7$ $[M+K]^+$: 332.0855, found: 332.0863.

Compound **7**. To a solution of **5** (3.5 g, 6.92 mmol, 1 eq) in dry MeOH (60 mL) under argon atmosphere was added sodium methoxide (1.5 g, 27.70 mmol, 4 eq) at room temperature and the solution was stirred during 15 h. The reaction mixture was concentrated under reduced pressure until the volume was 10 mL and passed through a short column of Dowex® 50WX8-200 (H^+ form). The resin was washed with a solution of H_2O/MeOH 99:1 (100 mL). The fractions containing the product were concentrated under reduced pressure to afford the desired product **7** (2.40 g, quant.) as a yellow oil. The analytical data of **7** were in complete agreement with literature data.[4] *Rf* : 0.1 (Cy/EtOAc 4:6); $[\alpha]_D$ (MeOH, c=1, 20°C) = +38.7°; ^{1}H NMR (400 MHz, D_2O) δ = 4.83 (d, $J_{1,2}$ = 1.6 Hz, 1H, H-1), 3.90 (dd, $J_{2,3}$ = 1.2 Hz, 1H, H-2), 3.86-3.10 (m, 2H, H-6), 3.78-3.74 (m, 1H, H-3), 3.72-3.60 (m, 11H, H-5, SugO*CH*$_2$, 4 x *CH*$_2$O), 3.60-3.58 (m, 1H, H-4), 3.40 (t, *J* = 5.0 Hz, 2H, C*H*$_2$N$_3$); ^{13}C NMR (100 MHz, D_2O) δ = 99.9 (C-1), 72.7, 70.5, 69.9, 66.7 (C-2, C-3, C-4, C-5), 69.6 (*CH*$_2$O), 69.5 (*CH*$_2$O), 69.4 (*CH*$_2$O), 69.3 (*CH*$_2$O), 66.3(C-9), 60.9 (C-6), 50.1 (C*H*$_2$N$_3$); HRMS: (ESI$^+$-MS, m/z) calcd for $C_{12}H_{23}N_3KO_8^+$ $[M+K]^+$: 376.1117, found: 376.1125.



Scheme S-2. Synthesis of azido heptyl mannoside **12**.

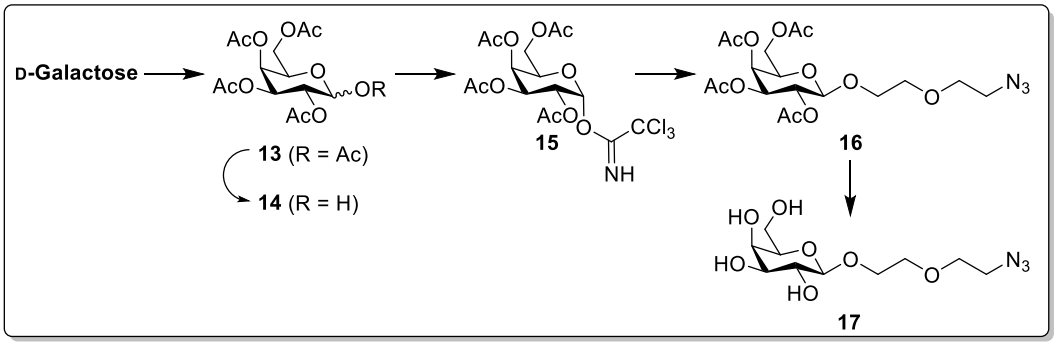
Compound **8**. Peracetylated mannose **2** (5 g, 12.81 mmol) was dissolved in dry DMF (25 mL) and hydrazine acetate (1.30 g, 14.09 mmol, 1.1 eq) was added dropwise. The reaction mixture was stirred overnight at room temperature under argon and then diluted in ethyl acetate (100 mL) and washed with sat. $NHCl_4$ (100 mL × 3) and brine (50 mL). The organic layer was dried over $MgSO_4$ and evaporated to dryness. The crude material was purified by column chromatography on silica gel (Cy/EtOAc 7:3) to provide **8** (3.7g, 83%) as a white powder. The analytical data of **8** were in complete agreement with literature data.[5] *Rf* : 0.50 (Cy/EtOAc 4:6); $[\alpha]_D$ (CH_2Cl_2, c=1, 20°C) = +19.3°; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.36 (dd, $J_{2,3}$= 4.6, $J_{3,4}$= 10.0 Hz, 1 H, H-3), 5.26 (d, $J_{3,4}$= 9.7 Hz, 1 H, H-4), 5.18 (d, $J_{1,2}$= 1.9, 1 H, H-2), 5.17 (d, 1 H, H-1), 4.64 (s, 1 H, -OH), 4.22-4.16 (m, 2 H, H-5, H-6a), 4.09-4.01 (m, 1 H, H-6b), 2.09 (s, 3 H, CH_3), 2.04 (s, 3 H, CH_3), 1.99 (s, 3 H, CH_3), 1.93 (s, 3 H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 171.1-170.0 (CO), 92.1 (C-1), 70.3 (C-2), 68.9 (C-3), 68.2 (C-5), 66.2 (C-4), 62.6 (C-6), 21.1 (*C*H_3), 21.0 (*C*H_3), 20.8 (*C*H_3), 20.7 (*C*H_3).

Compound **9**. Mannoside **8** (3.5 g, 10.05 mmol, 1 eq) and trichloroacetonitrile (10.1 mL, 100.48 mmol, 10 eq) were dissolved in dry dichloromethane (18 mL) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.15 mL, 1.01 mmol, 0.1 eq) was added dropwise. The reaction mixture was stirred overnight at room temperature under argon and then evaporated to dryness. Purification of the crude by column chromatography (Cy: EtOAc 8:2) afforded **9** (4.32 g, 87%) as a white powder. The analytical data of **9** were in complete agreement with literature data.[5] *Rf* : 0.72 (Cy/EtOAc 4:6); $[\alpha]_D$ (CH_2Cl_2, c=1, 20°C) = +50.7°; ^{1}H NMR (270 MHz, $CDCl_3$) δ = 8.79 (s, 1 H, NH), 6.02 (d, $J_{1,2}$ = 1.35 Hz, 1 H, H-1), 5.19 (m, 1 H, H-2), 5.12-5.10 (m, 2 H, H-3, H-4), 3.98-3.82 (m, 3 H, H-5, H-6), 1.94 (s, 3 H, CH_3), 1.81 (s, 3 H, CH_3), 1.76 (s, 3 H, CH_3), 1.74 (s, 3 H, CH_3); ^{13}C NMR (270 MHz, $CDCl_3$) δ = 170.0 (CO), 169.3 (CO), 169.2 (CO), 169.2 (CO), 159.0 (C=N), 94.1 (C-1), 90.1 (*C*Cl_3), 70.8 (C-5), 68.4 (C-3), 67.4 (C-2), 64.9 (C-4), 61.6 (C-6), 20.4-20.3 (4 x *C*H_3); HRMS: (ESI^+-MS, m/z) calcd for $C_{16}H_{20}NKO_{10}^+$ $[M+K]^+$: 529.9784, found: 529.9784.

Compound **10**. To a solution of mannose trichloroacetimidate **9** (1 g, 2.030 mmol, 1 eq) and 7-bromoheptan-1-ol (0.37 mL, 2.435 mmol, 1.2 eq) in dry dichloromethane (12 mL) under argon atmosphere was added dropwise boron trifluoride diethyl etherate (0.25 mL, 2.030 mmol, 1 eq). The reaction mixture was stirred for 5 h under argon and then quenched with $NaHCO_3$ (160 mg) and stirred for 20 min. Then it was filtered through Celite® and concentrated under reduced pressure. Purification of the residue by flash chromatography on silica gel (Cy/EtOAc 95:5) afforded the desired mannoside **10** (0.460 g, 43%) as a colorless oil. The analytical data of **10** were in complete agreement with literature data.[6] *Rf* : 0.67 (Cy/EtOAc 4:6); $[\alpha]_D$ (CH_2Cl_2, c=1, 20°C) = +34.9°; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.35 (dd, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$= 10.1 Hz, 1 H, H-3), 5.29 (d, $J_{3,4}$ = 9.8 Hz, 1 H, H-4), 5.22 (dd, $J_{1,2}$=1.84 Hz, $J_{2,3}$=3.44 Hz, 1 H, H-2), 4.79 (d, $J_{1,2}$= 1.6 Hz, 1 H, H-1), 4.30-4.25 (dd, $J_{5,6}$ = 5.28 Hz, $J_{5,6}$ = 12.12 Hz, 1 H, H-6a), 4.11-4.08 (dd, $J_{5,6}$ = 2.32 Hz, $J_{5,6}$ = 12.16 Hz, 1 H, H-6b), 3.97 (m, 1 H, H-5), 3.68-3.64 (m, 1 H, SugO*CH*H), 3.45-3.38 (m, 3 H, SugO*CH*, *CH_2Br*), 2.15 (s, 3 H, CH_3), 2.09 (s, 3 H, CH_3), 2.04 (s, 3 H, CH_3), 1.99 (s, 3 H, CH_3), 1.87-1.82 (m, 2 H, *CH_2CH_2Br*), 1.62-1.57 (m, 2 H, SugOCH_2*CH_2*), 1.46-1.41 (m, 2 H, *CH_2*), 1.36-1.34 (m, 4 H, 2 x *CH_2*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 170.8 (CO), 170.3 (CO), 170.1 (CO), 169.9 (CO), 97.7 (C-1), 69.8 (C-2), 69.2 (C-3), 68.5 (C-5), 68.5 (SugO*CH_2*), 66.4 (C-4), 62.6 (C-6), 34.1 (*CH_2Br*), 32.8 (*CH_2CH_2Br*), 29.3 (SugOCH_2*CH_2*), 28.6 (*CH_2*), 28.1 (*CH_2*), 26.1 (*CH_2*), 21.1-20.8 (4 x *C*H_3); HRMS: (ESI^+-MS, m/z) calcd for $C_{21}H_{33}BrNaO_{10}^+$ $[M+Na]^+$: 547.1149, found: 547.1149.

Compound **11**. To a solution of **10** (400 mg, 0.761 mmol, 1 eq) in dry DMF (6 mL) under argon atmosphere was added sodium azide (396 mg, 6.091 mmol, 8 eq). The reaction mixture was then stirred at 80 °C for 20 h under argon. After cooling to room temperature, the solution was poured into icy H_2O (50 mL), extracted with EtOAc (50 mL × 3), and washed with brine. Then it was concentrated under reduced pressure and the residue was purified by flash chromatography on silica gel (Cy/EtOAc 8:2) affording the desired mannoside **11** (371 mg, 98%) as a colorless oil. The analytical data of **11** were in complete agreement with literature data.[6] *Rf* : 0.60 (Cy/EtOAc 6:4); $[\alpha]_D$ (CH_2Cl_2, c=1, 20°C) = +25.1°; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.35 (dd, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$= 10.1 Hz, 1 H, H-3), 5.27 (t, $J_{3,4}$ = 9.9 Hz, 1 H, H-4), 5.22 (dd, $J_{1,2}$=1.6 Hz, $J_{2,3}$=3.2 Hz, 1 H, H-2), 4.79 (d, $J_{1,2}$=1.4 Hz, 1 H, H-1), 4.27 (dd, $J_{5,6}$ = 12.16 Hz, $J_{6a,6b}$ = 5.28 Hz, 1 H, H-6a), 4.11-4.08 (dd, 1 H, H-6b), 3.97 (m, 1 H, H-5), 3.68-3.64 (m, 1 H, SugO*CH*H), 3.43 (m, 1 H, SugO*CH*H), 3.27 (t, $J_{12,13}$=6.9 Hz, 2 H, *CH_2*N_3), 2.15 (s, 3 H, CH_3), 2.10 (s, 3 H, CH_3), 2.04 (s, 3 H, CH_3), 1.99 (s, 3 H, CH_3), 1.60 (m, 4 H, 2 x *CH_2*), 1.36 (m, 6 H, 3 x *CH_2*); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 170.5 (CO), 170.0 (CO), 169.8 (CO), 169.6 (CO), 97.4 (C-1), 69.6 (C-2), 69.0 (C-3), 68.3 (SugO*CH_2*), 68.3 (C-5), 66.1 (C-4), 62.4 (C-6), 51.3 (*CH_2*N_3), 29.0 (*CH_2*), 28.8 (*CH_2*), 28.7 (*CH_2*), 26.5 (*CH_2*), 25.9 (*CH_2*), 20.8-20.6 (4 x *C*H_3); HRMS: (ESI^+-MS, m/z) calcd for $C_{21}H_{33}N_3NaO_{10}$ $[M+Na]^+$: 510.2058, found: 510.2058.

Compound **12**. To a solution of **11** (135 mg, 0.277 mmol, 1 eq) in MeOH (3 mL) under argon atmosphere was added NaOMe (15 mg, 0.277 mmol, 1eq). The reaction mixture was then stirred at room temperature for 2 h under argon. The reaction mixture was concentrated under reduced pressure and passed through a short column of Dowex® 50WX8-200 (H^+ form). The resin was washed with a solution of H_2O/MeOH 99:1 (10 mL). The fractions containing the product were concentrated under reduced pressure to afford the desired product 12 (62 mg, 70%) as a colorless oil. *Rf* : 0.01 (Cy/EtOAc 4:6); $[\alpha]_D$ (MeOH, c=1, 20°C) = +7.4°; ^{1}H NMR (400 MHz, D_2O) δ = 3.71-3.34 (m, 9 H*)*, 3.10 (t, $J_{a,b}$= 7.2 Hz, 2 H, CH_2N_3), 1.39 (m, 4 H, 2 x CH_2), 1.15 (m, 6 H, 3 x CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 100.4 (C-1), 73.5, 71.4, 70.9, 68.7, 67.6 (C-2, C-3, C-4, C-5, SugOCH_2), 61.7 (C-6), 52.0 (CH_2N_3), 29.2 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 26.6 (CH_2), 26.1 (CH_2); HRMS: (ESI^+-MS, m/z) calcd for $C_{13}H_{25}N_3O_6Na$ $[M+Na]^+$: 342.1641, found: 342.1637.



Scheme S-3. Synthesis of azido galactoside **17**.

Compound **13**. 4-Dimethylaminopyridine (0.678 g, 5.55 mmol, 0.1 eq) was added slowly to a solution of D-galactose (10 g, 55.51 mmol) and Ac_2O (39.3 mL, 416.297 mmol, 7.5 eq) in dry pyridine (55 mL). The reaction mixture was stirred overnight at room temperature under argon, and then diluted in ethyl acetate (150 mL) and washed with HCl (1M, 50 mL × 5), NH_4Cl (50 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$ and evaporated to dryness to afford **13** (21.9 g, quant., all α anomer) as a colorless oil.[7] *Rf* : 0.64 (Cy/EtOAc 4:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.35 (d, $J_{1,2}$ = 1.6 Hz, 1 H, H-1α), 5.47 (dd, $J_{3,4}$ < 1.0 Hz, $J_{4,5}$ = 1.2 Hz, 1 H, H-4), 5.31 (m, 2 H, H-2, H-3), 4.32 (dt, $J_{5,6}$ = 6.4 Hz, 1 H, H-5), 4.09-4.06 (m, 2 H, H-6a, H-6b), 2.13 (s, 3 H, CH_3), 2.13 (s, 3 H, CH_3), 2.01 (s, 3 H, CH_3), 1.99 (s, 3 H, CH_3), 1.98 (s, 3 H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.7-169.8 (5 CO), 89.8 (C-1), 68.8 (C-5), 67.5 (C-3), 67.4 (C-2), 66.5 (C-4), 61.3 (C-6), 30.0 (CH_3), 20.7 (CH_3), 20.7 (CH_3), 20.7 (CH_3), 20.6 (CH_3).

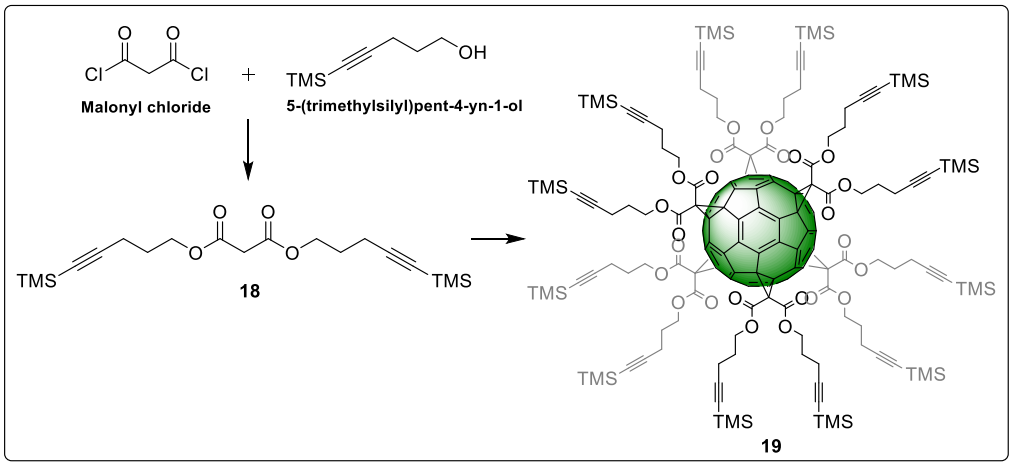
Compound **14**. Peracetylated galactose **13** (10 g, 25.62 mmol, 1 eq) was dissolved in dry DMF (50 mL) and hydrazine acetate (2.59 g, 28.18 mmol, 1.1 eq) was added dropwise. The reaction mixture was stirred overnight at room temperature under argon and then diluted in diethyl ether (150 mL), and washed with H_2O (100 mL x 3), HCl (1M, 100 mL × 3), and brine (50 mL). The organic layer was dried over $MgSO_4$ and evaporated to dryness. The crude material was purified by column chromatography (Cy/EtOAc 8:2) to provide galactoside **14** (5.08 g, 57%) as a colorless oil.[8] *Rf* : 0.56 (Cy/EtOAc 4:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.42 (dd, $J_{3,4}$ = 3.4, $J_{4,5}$= 10.8 Hz, 1 H, H-4), 5.17 (dd, $J_{2,3}$ = 3.4 Hz, 1 H, H-2), 5.08 (m, 1 H, H-3), 4.70 (d, $J_{1,2}$ = 7.4, 1 H, H-1), 4.47 (t, $J_{5,6}$= 6.6 Hz, 2 H, H-6), 3.91 (m, 1 H, H-5), 2.14 (s, 3 H, CH_3), 2.09 (s, 3 H, CH_3), 2.04 (s, 3 H, CH_3), 1.98 (s, 3 H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.7-169.8 (5 CO), 90.6 (C-1), 70.6 (C-5), 68.8 (C-3), 68.4 (C-2), 65.5 (C-4), 62.1 (C-6), 20.9-20.7 (5 CH_3); HRMS: (ESI^+-MS, m/z) calcd for $C_{14}H_{20}KO_{10}$ $[M+K]^+$: 387.0688, found: 387.0698.

Compound **15**. Galactoside **14** (4.5 g, 12.92 mmol, 1 eq) and trichloroacetonitrile (12.9 mL, 129.19 mmol, 10 eq) were dissolved in dry dichloromethane (23 mL) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.19 mL, 1.29 mmol, 0.1 eq) was added dropwise. The reaction mixture was stirred overnight at room temperature under argon and then evaporated to dryness. Purification of the crude by column chromatography (Cy/EtOAc 8:2) afforded **15** (4.74 g, 75%) as a white powder. The analytical data of **15** were in complete agreement with literature data.[8] *Rf* : 0.77 (Cy/EtOAc 4:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.65 (s, 1 H, H-7), 6.50 (d, $J_{1,2}$ = 3.4 Hz, 1 H, H-1), 5.46 (dd, $J_{2,3}$ = 1.2 Hz, $J_{3,4}$ = 1.8 Hz, 1 H, H-3), 5.30-5.26 (m, $J_{4,5}$ =10.8 Hz, 2 H, H-2, H-4), 4.35 (dt, $J_{5,6}$ = 6.6 Hz, 1 H, H-5), 4.07-3.98 (m, 2 H, H-6), 2.07 (s, 3 H, CH_3), 1.93 (s, 3 H, CH_3), 1.92 (s, 3 H, CH_3), 1.91 (s, 3 H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.1 (CO), 170.0 (CO), 169.9 (CO), 169.8 (CO), 160.6 (CN), 93.3 (C-1), 90.6 (CCl_3), 68.9 (C-5), 67.4, 67.2, 66.8 (C-2, C-3, C-4), 61.2 (C-6), 20.5-20.4 (4 CH_3); HRMS: (ESI^+-MS, m/z) calcd for $C_{16}H_{20}NKO_{10}$ $[M+K]^+$: 529.9784, found: 529.9794.

Compound **16.** To a solution of galactoside **15** (1.2 g, 2.44 mmol, 1 eq) and **1** (0.64 g, 4.87 mmol, 2 eq) in dry dichloromethane (12 mL) under argon atmosphere was added dropwise boron trifluoride diethyl etherate (0.6 mL, 4.87 mmol, 2 eq). The reaction mixture was then stirred for 15 min under argon. $NaHCO_3$ (300 mg) was added to the reaction mixture and stirred for 20 min. Subsequently, it was filtered over Celite® and concentrated under reduced pressure. Purification of the residue by chromatography on silica gel (Cy/EtOAc 8:2) afforded the desired galactoside **16** (0.59 g, 53%) as a clear oil. The analytical data of **16** were in complete agreement with literature data.[8] *Rf* : 0.68 (Cy/EtOAc 4:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.24 (d, *J* = 3.2 Hz, 1 H, H-4), 5.05 (dd, *J* = 8.0 Hz, *J* = 10.4 Hz, 1 H, H-2), 4.89 (dd, *J* = 3.6, J = 10.4 Hz, 1 H, H-3), 4.47 (d, *J* = 8.0 Hz, 1 H, H-1), 4.00 (m, 2 H, H-6), 3.82 (m, 2 H, H-5, H-7a), 3.63 (m, 1 H, H-7b), 3.52 (m, 4 H, 2 x CH_2O), 3.23 (m, 2 H, CH_2N), 2.00 (s, 3 H, CH_3), 1.93 (s, 3 H, CH_3), 1.90 (s, 3 H, CH_3), 1.83 (s, 3 H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.1 (CO), 170.0 (CO), 169.8 (CO), 169.2 (CO), 101.0 (C-1), 70.6 (C-3), 70.4 (C-5), 70.2, 69.9 (2 x CH_2O), 68.8 (C-7), 68.6 (C-2), 66.9 (C-4), 61.1 (C-6), 50.5 (CH_2N), 20.5-20.3 (4 CH_3); HRMS: (ESI^+-MS, m/z) calcd for $C_{18}H_{27}N_3KO_{11}$ $[M+K]^+$: 500.1277, found: 500.1295.

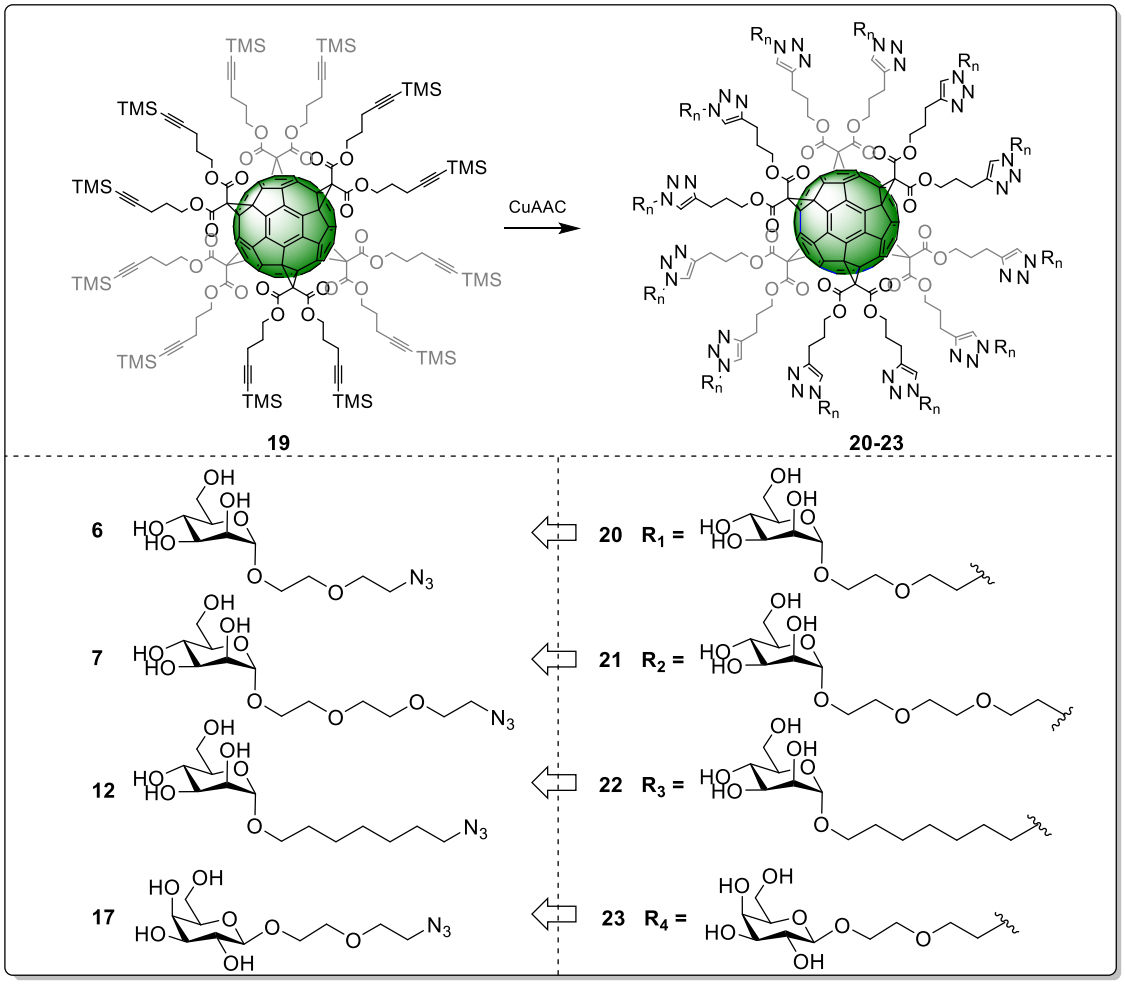
Compound **17**. To a solution of **16** (550 mg, 1.19 mmol, 1 eq) in dry MeOH (15 mL) under argon atmosphere was added sodium methoxide (129 mg, 2.38 mmol, 2 eq) at room temperature, and the solution was stirred during 3 h. The reaction mixture was concentrated under reduced pressure and passed through a short column of Dowex® 50WX8-200 (H^+ form). The resin was washed with a solution of H_2O/MeOH 99:1 (100 mL). The fractions containing the product were concentrated under reduced pressure to afford the desired product **17** (349 mg, quant) as colorless oil. The analytical data of **17** were in complete agreement with literature data.[9] *Rf* : 0.1 (Cy/EtOAc 4:6); ^{1}H NMR (400 MHz, D_2O) δ =4.40 (d, *J* = 7.6 Hz, 1H, H-1), 4.05 (m, 1 H), 3.89 (d, *J* = 3.32, 1 H), 3.857-3.61 (m, 9 H), 3.50 (m, 3H, H-2, C$H_2$$N_3$); ^{13}C NMR (100 MHz, D_2O) δ = 102.9 (C-1), 75.2, 72.7, 70.8, 68.7 (C-2, C-3, C-4, C-5), 69.7 (CH_2O), 69.2 (CH_2O), 68.6 (CH_2O), 61.0 (C-6), 50.2 (CH_2N_3); HRMS: (ESI^+-MS, m/z) calcd for $C_{10}H_{19}N_3KO_7$ $[M+K]^+$: 332.0855, found: 332.0853.

Synthesis of glycofullerenes. Trimethylsilyl (TMS)-protected polyalkyne **19** was synthesized according to literature procedure (Scheme S-4).[10]



Scheme S-4. Synthesis of TMS-protected polyalkyne C60 hexakis-adduct **19**.

This protected dodecaalkyne was grafted with azido functionalized glycosides **6, 7, 12** and **17** by CuAAC to obtain glycoclusters **20-23** (designated glycofullerenes **1-4** in the main text), as depicted in Scheme S-5.



Scheme S-5. Synthesis of dodecaglycoside fullerenes **20-23**.

For the click reaction, TMS-protected polyalkyne **19** (50 mg, 0.0167 mmol, 1 eq) was dissolved in 0.3 mL of tetrahydrofuran and the sugar (0.2172 mmol, 13 eq) was added dissolved in DMSO (0.5 mL). Tetra-n-butylammonium fluoride 1 M in tetrahydrofuran (0.25 mL; 0.2505 mmol, 15 eq) and a mixture of $CuSO_4$ (0.27 mg, 0.0017 mmol, 0.1 eq) and NaAsc (0.9500 mg, 0.0050 mmol, 0.3 eq) in H_2O (0.2 mL) were added and the reaction mixture was stirred under argon at room temperature for 72 h. Then, the product was precipitated by addition of 15 mL of acetone and washed/centrifuged with acetone (5 mL × 3). Purification of the crude was accomplished firstly by Cu scavenging (addition of 20 mg of QuadraSil™ Mercaptopropyl) and then size exclusion chromatography on Sephadex G25 in deionized H_2O:MeOH 95:5. All the fractions containing the products were lyophilized to give glassy orange-brown solids (**20** from **6**, 49%;[11] **21**, from **7**, 82%; **22**, from **12**, 35%; **23**, from **17**, 82%).

Manofullerene **20**. ^{1}H NMR (400 MHz, DMSO) δ = 7.80 (s, 12H), 4.74-3.35 (m, 204 H), 2.65 (br, 24H, H-h), 1.96 (br, 24H, H-g); ^{13}C NMR (100 MHz, DMSO) δ = 162.8 (C-e), 145.6 (C-i), 145.1 (C-a), 140.8 (C-b), 122.3 (C-j), 99.9 (C-1), 74.0 (C-4), 72.1 (C-c), 71.0 (C-3), 70.3 (C-2), 69.2 (C7), 68.8 (C-8), 67.0 (C-5), 65.6 (C-9), 61.3 (C-6), 60.1 (C-f), 49.2 (C-10), 45.6 (C-d), 27.8 (C-h), 21.3 (C-g); MS: TOF-ESI MS m/z: [M+3H] +3 1878.6, [M+3Na] +3 1903.6, [M+3K] +3 1919.6; ATR-IR: 3344 (O-H), 1738 (C=O) cm-1. UV (H2O) λmax (log ε) 244 (sh) (5.11), 271 (5.00), 317 (sh) (4.78), 338 (sh) (4.67) nm.

Mannofullerene **21**. ^{1}H NMR (400 MHz, D_2O) δ = 7.65 (bs, 12H, $CH_{triazole}$), 4.47 (bs, 12H, H-1), 4.28-3.50 (m), 2.60 (br, 24H, H-h,), 1.91 (br, 24 H, H-g); ^{13}C NMR (100 MHz, D_2O) δ = (400 MHz, H_2O) δ = 163.8 (C-e), 146.6 (C-i), 145.4 (C-a), 141.2 (C-b), 123.3 (C-j), 99.9 (C-1), 72.7 (C-4), 71.9 (C-c), 70.6 (C-3), 70.0 (C-2), 69.7, 69.6, 69.5,68.9, 66.6 (C-5), 66.3, 60.9 (C-6), 60.4 (C-f), 50.0 (C-12), 46.0 (C-d), 27.6 (C-h), 21.4 (C-g); MS: TOF-ESI MS m/*z*: $[M+Na]^+$ 6197.7, $[M–C_6H_{11}O_5+Na]^+$ 6034.6, $[M–2(C_6H_{11}O_5)+Na]^+$ 5875.5, $[M–C_{18}H_{30}N_3O_{10}+Na]^+$ 5749.4, $[M–(C_6H_{11}O_5) – (C_{18}H_{30}N_3O_{10})+Na]^+$ 5588.3, $[M–5(C_6H_{11}O_5)+Na]^+$ 5381.1, $[M– C_{37}H_{60}N_6O_{20}+Na]^+$ 5288.2, $[M–(C_{37}H_{60}N_6O_{20}) –(C_6H_{11}O_5)+Na]^+$ 5125.1; ATR-IR: 3367 (O-H), 1738 (C=O) cm^{-1}. UV (H_2O) λ_{max} (log ε) 245 (sh) (5.10), 270 (5.00), 319 (sh) (4.77), 339(sh) (4.65) nm.

Mannofullerene **22**. ^{1}H NMR (400 MHz, DMSO) δ = 7.79 (bs, 12H, H-j), 4.74-4.25 (m), 3.61-3.56 (m), 3.28 (m), 2.62 (m), 1.95-1.25 (m); ^{13}C NMR (100 MHz, DMSO) δ = 162.8 (C-e), 145.6 (C-i), 145.1 (C-a), 140.8 (C-b), 121.8 (C-j), 99.8 (C-1), 74.0 (C-4), 71.1 (C-3), 70.4 (C-2), 68.8 (C-c), 67.0 (C-7), 66.2 (C-5), 61.3 (C-6), 61.2 (C-f), 49.3 (C-10), 45.6 (C-d), 29.8, 28.9, 28.3, 27.8 (C-h), 25.9, 25.6, 21.4 (C-g); MS: TOF-ESI MS m/*z*: $[M+Na]^+$ 5985.1, $[M–C_6H_{11}O_5+Na]^+$ 5824.0, $[M–2(C_6H_{11}O_5)+K]^+$ 5668.9, $[M–3(C_6H_{11}O_5)+K]^+$ 5509.6, $[M–4(C_6H_{11}O_5)+K]^+$ 5344.6, $[M–5(C_6H_{11}O_5)+K]^+$ 5183.5; ATR-IR: 3343 (O-H), 1740 (C=O) cm^{-1}. UV (H_2O) λ_{max} (log ε) 243 (sh) (5.11), 270 (5.00), 319 (sh) (4.79), 338 (sh) (4.69) nm.

Galactofullerene **23**. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.83 (bs, 12H, $CH_{triazole}$), 4.45-3.27 (m), 2.64 (br, 24H, H-h), 1.96 (br, 24H, H-g); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 162.8 (C-e), 145.7 (C-i), 145.1 (C-a), 140.8 (C-b), 122.5 (C-j), 103.6 (C-1), 75.3 (C-5), 73.5 (C-3), 71.9 (C-c), 70.6 (C-2), 69.6 (C-7), 68.8 (C-8), 68.2 (C-4), 67.8 (C-9), 66.8 (C-f), 60.5 (C-6), 49.3 (C-10), 45.6 (C-d), 27.8 (C-h), 21.4 (C-g); MS: TOF-MALDI MS m/*z*: $[M+Na]^+$ 5667.8, $[M–C_{10}H_{19}N_3O_7+Na]^+$ 5376.8, $[M–C_{16}H_{30}N_3O_9+Na]^+$ 5265.7, $[M–2(C_{10}H_{19}N_3O_7)+Na]^+$ 5083.6, $[M–C_{10}H_{19}N_3O_7– C_{16}H_{30}N_3O_9+Na]^+$ 4973.6, $[M–2(C_{16}H_{30}N_3O_9)+Na]^+$ 4848.5; HRMS: (MALDI-TOF, m/z) calcd for $C_{258}H_{312}N_{36}O_{108}Na^+$ $[M+Na]^+$: 5668.0, found: 5667.8; ATR-IR: 3367 (O-H), 1738 (C=O) cm^{-1}. UV (H_2O) λ_{max} (log ε) 244 (sh) (4.95), 271 (4.84), 318 (sh) (4.63), 337 (sh) (4.53) nm.

NMR and MS spectra of new compounds.

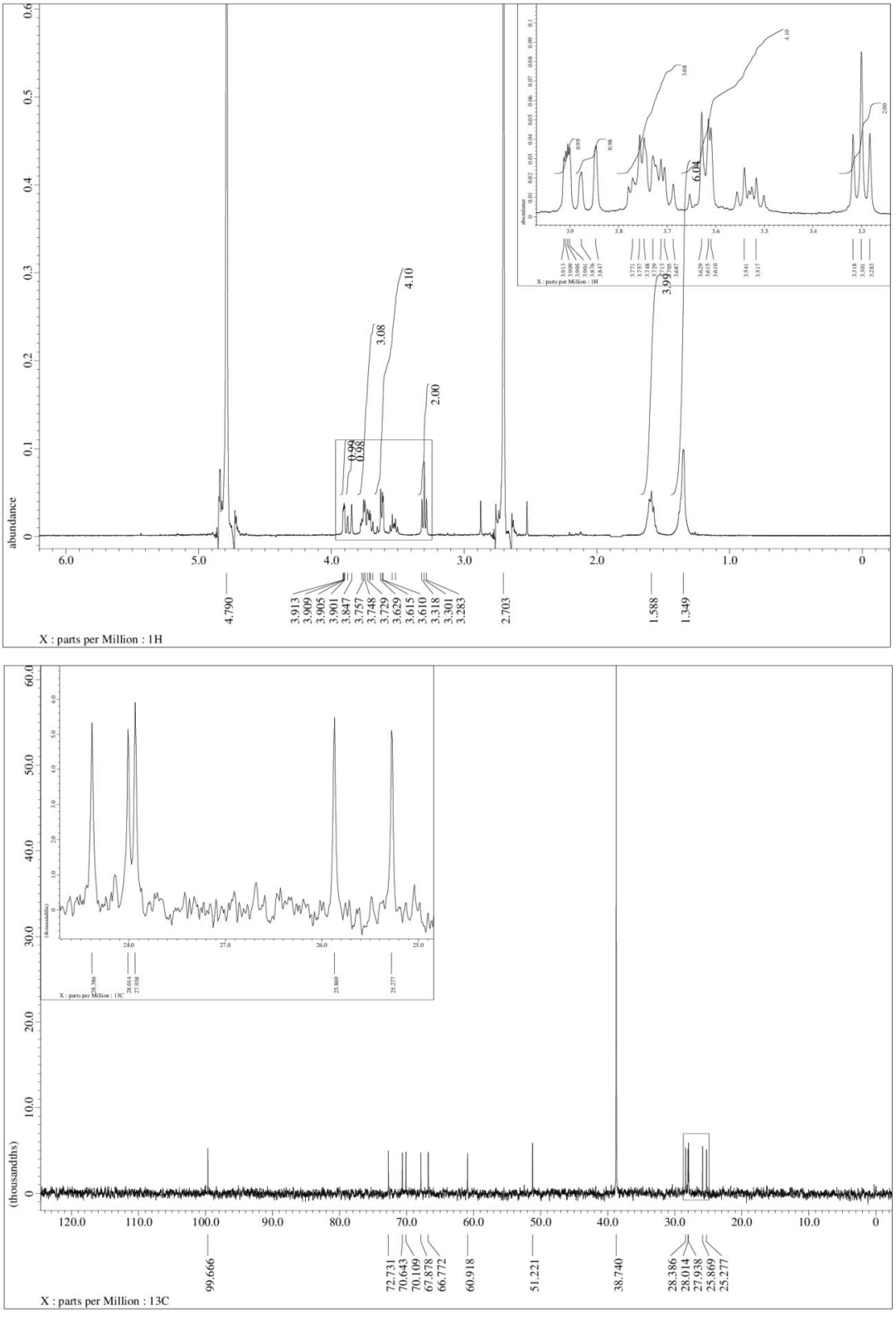


Figure S-1. ^{1}H NMR (top panel) and ^{13}C NMR (bottom panel) spectra of compound **12** recorded in D_2O and DMSO-d_6, respectively.

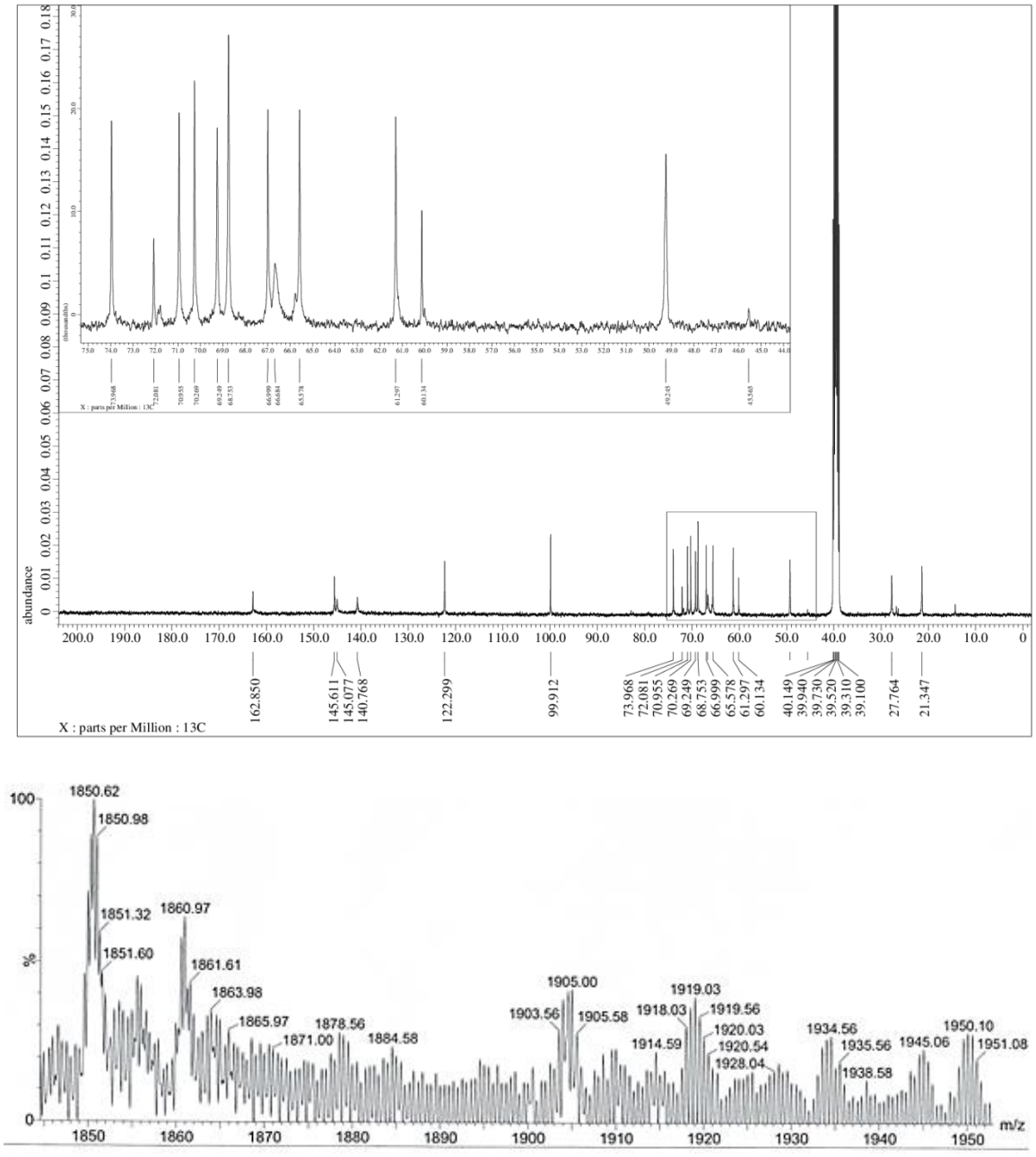


Figure S-2. ^{13}C NMR spectrum recorded in DMSO-d_6 (top panel) and mass spectrum (bottom panel) of mannofullerene **20**.

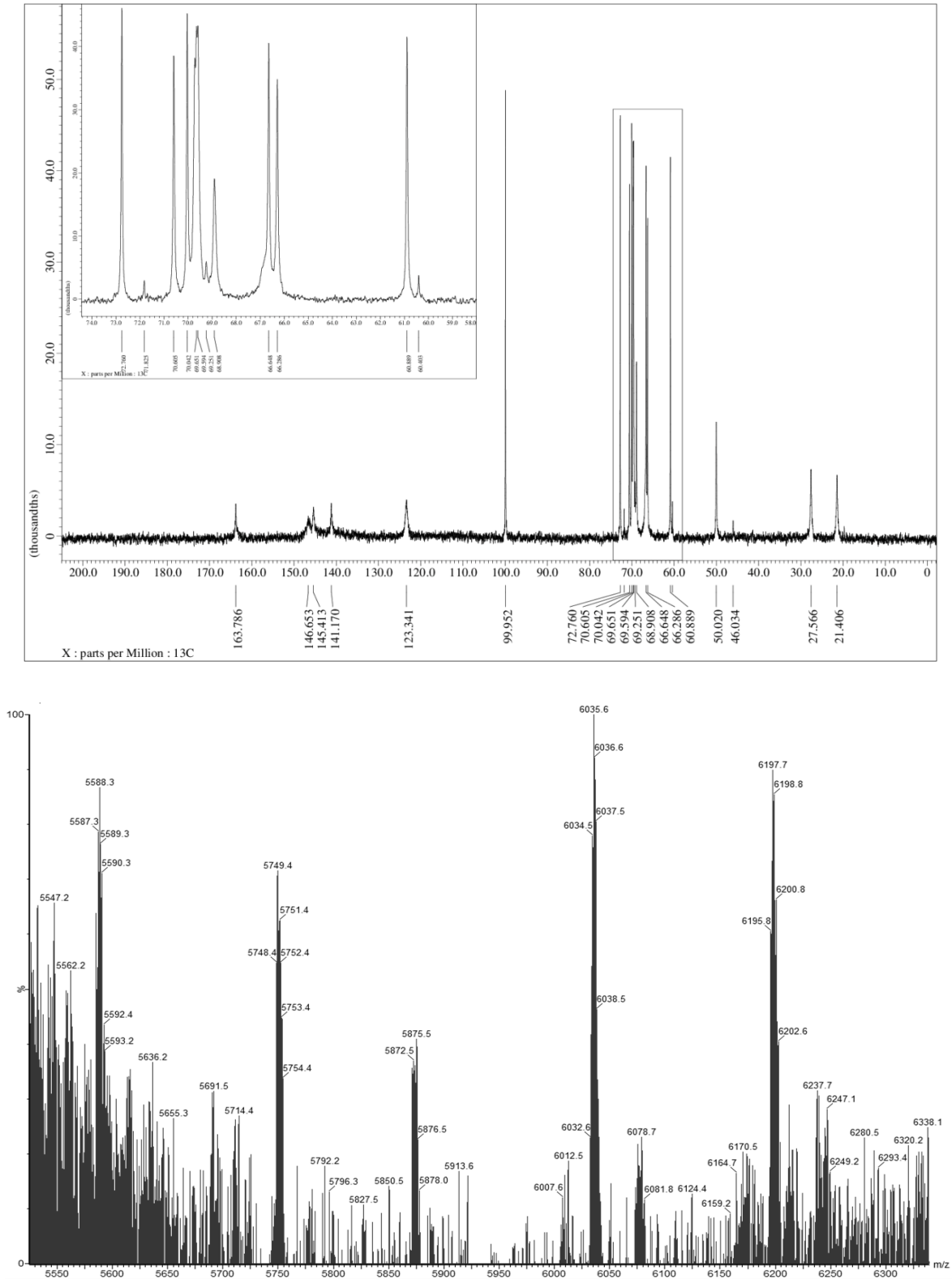


Figure S-3. ^{13}C NMR spectrum in D_2O (top panel) and mass spectrum (bottom panel) of mannofullerene **21**.

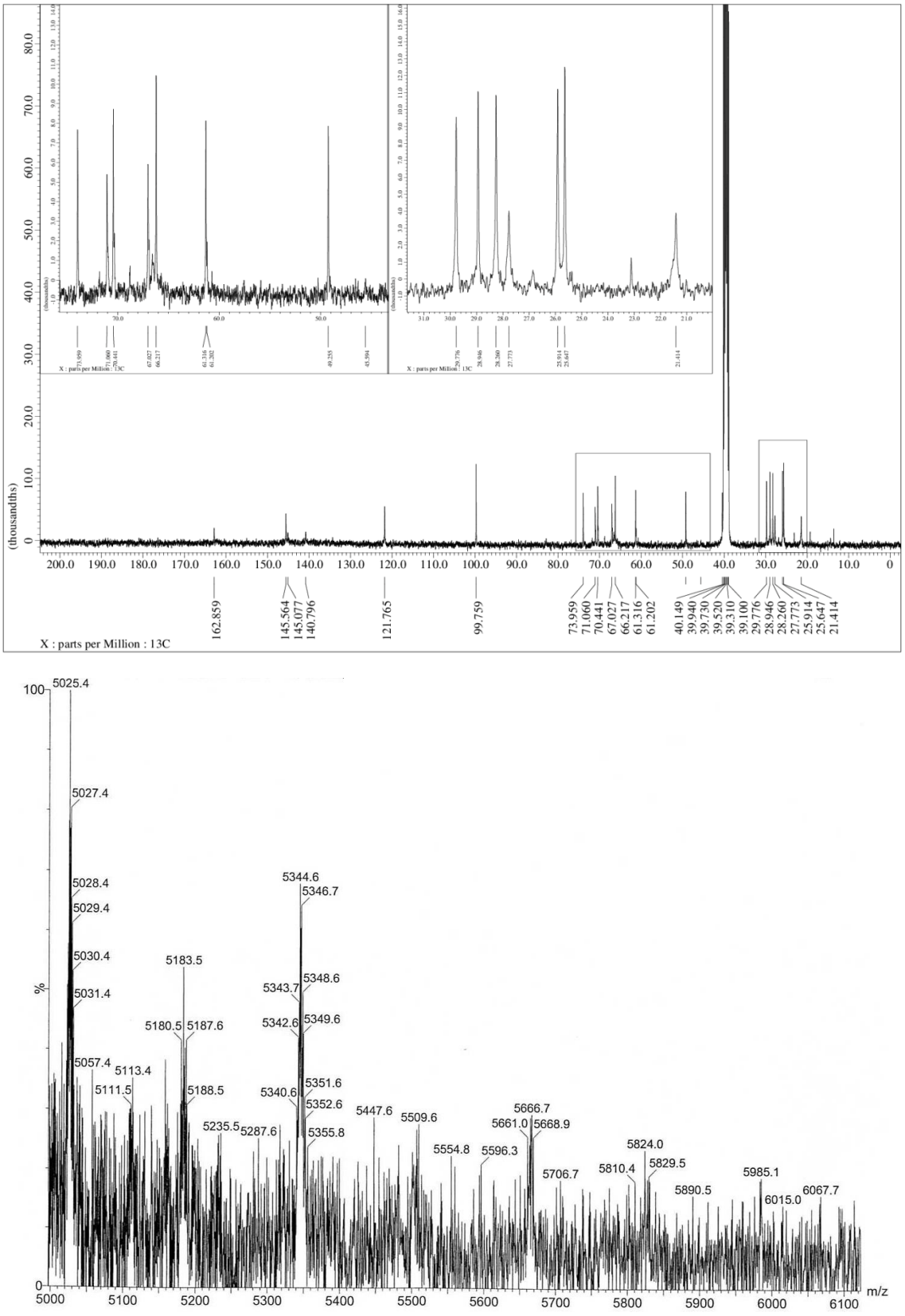
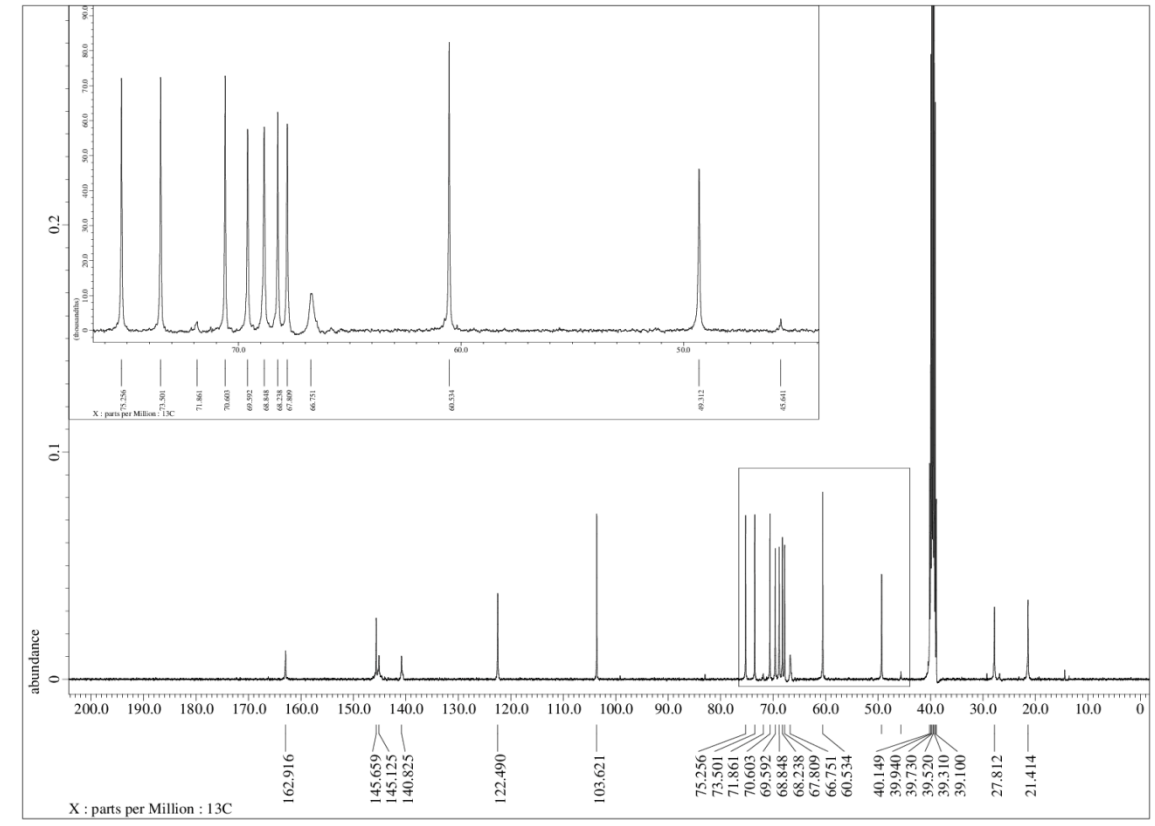


Figure S-4. ^{13}C NMR spectrum in DMSO-d_6 (top panel) and mass spectrum (bottom panel) of mannofullerene **22**.



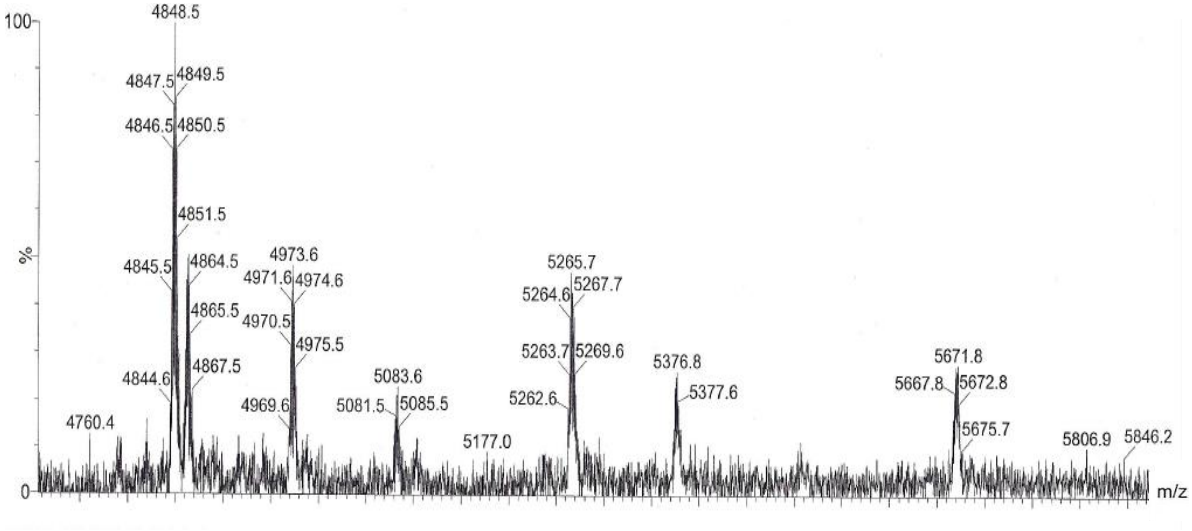


Figure S-5. ^{13}C NMR spectrum in DMSO-d_6 (top panel) and mass spectrum (bottom panel) of galactofullerene **23**.

RESULTS AND DISCUSSION

Microarray evaluation of mannofullerenes as inhibitors of ConA. Mannofullerenes **1** and **2** (corresponding to compounds **20** and **21** in the Experimental Section of this Supporting Information) were tested as inhibitors of the binding of ConA to RNaseB printed onto hydrogel-coated slides (Figure S-6). Selection of this probe was based on the superior affinity of this lectin for high-mannose oligosaccharide chains and its known binding to RNaseB Man_{5-9} glycoforms.[12]

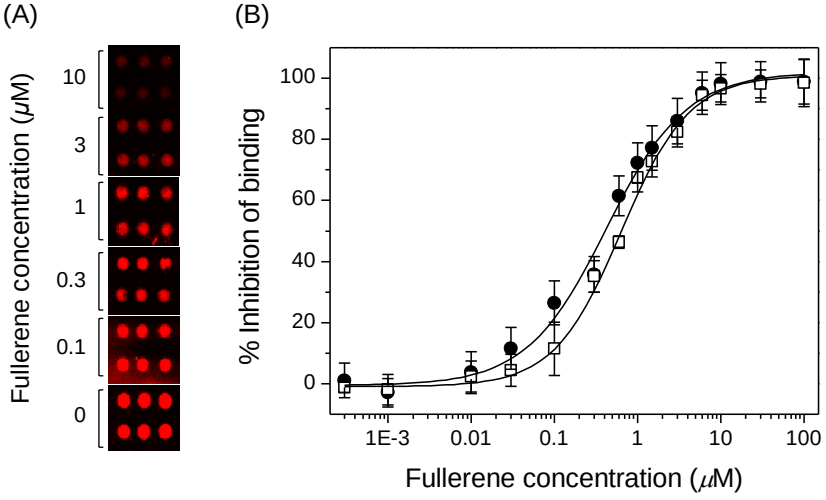


Figure S-6. Mannofullerene inhibition of ConA binding to RNaseB-printed microarray slides. (A) Representative scans showing inhibition of the binding of 0.4 μM ConA to RNase B, printed as sextuplicates at 1 mg/mL, by increasing concentrations of mannofullerene **1**. (B) Inhibitory activity of mannofullerenes **1** (□) and **2** (●) (0% inhibition = 6850 ± 150 rfu). Data shown are the mean of at least four different experiments and error bars indicate standard deviations. Solid lines correspond to sigmoidal fits to experimental data. All experiments were carried out in HBS, pH 7.4.

ITC analysis of ConA–mannofullerene interactions. Calorimetric titration of ConA with mannofullerenes **1** and **2** generated significant exothermic signals (shown for mannofullerene **1** in Figure S-7A). In contrast, only small endothermic signals, similar to those obtained for fullerene dilution, were observed for galactofullerene **4** (Figure S-7A), consistent with the absence of interaction. The thermodynamic parameters of ConA–mannofullerene interactions were calculated from the fit to experimental data of a one-set-of-sites model (Figure S-7B) using the "ligand-in-cell" option of ITC-Origin software, which yielded the number of binding sites for ConA per dodecamannofullerene molecule. Interestingly, 6.8 ± 0.7 sites for mannofullerene **1** and 7 ± 1 for mannofullerene **2** were determined, indicating that simultaneous binding of ConA to the 12 mannose residues did not take place. As rough approximation, considering the glycofullerene as a sphere of 1.5-2.5 nm radio and a symmetrical homogenous distribution of sugar substituents over the fullerene surface, an area of 2.4-6.5 nm^2 per mannose residue could be estimated. This area would correspond to average distances between adjacent mannoses in the range of 1.7-2.8 nm. Since dimensions of the ConA tetramer are ~ 6.0 × 7.5 × 7.5 nm and the distance between carbohydrate-binding sites is 6.5 nm,[13] it seems highly likely that binding of ConA to the 12 fullerene-displayed mannose residues is not possible due to steric hindrance.

To assess the effect of multivalent presentation of Man residues and the influence of the aglycon chain

and triazole ring in the binding affinity, titrations with MeαMan and the monovalent R_1 Man-derivative bearing a terminal azide (compound **6** in Scheme S-1, designated R_1–azide in the main text) or triazole group (designated R_1–triazole), as present in the glycofullerenes (Figure 3), were also performed (illustrated for compound **6** and MeαMan in Figure S-7C). The K_d values obtained for the derivatives were comparable to that obtained for MeαMan (Table 1). However, for compound **6** the binding stoichiometry approached 1.5 molecules per ConA subunit, resulting in lower enthalpy and entropy changes. Although no aggregation was perceptible in the solutions, this behavior could be attributed to a moderate self-association of compound **6**. Recalculation of ΔH and ΔS per mole of ConA monomer yielded values very similar to those obtained for MeαMan, indicating that the spacer has neither detrimental nor beneficial meaningful effects on ConA binding.

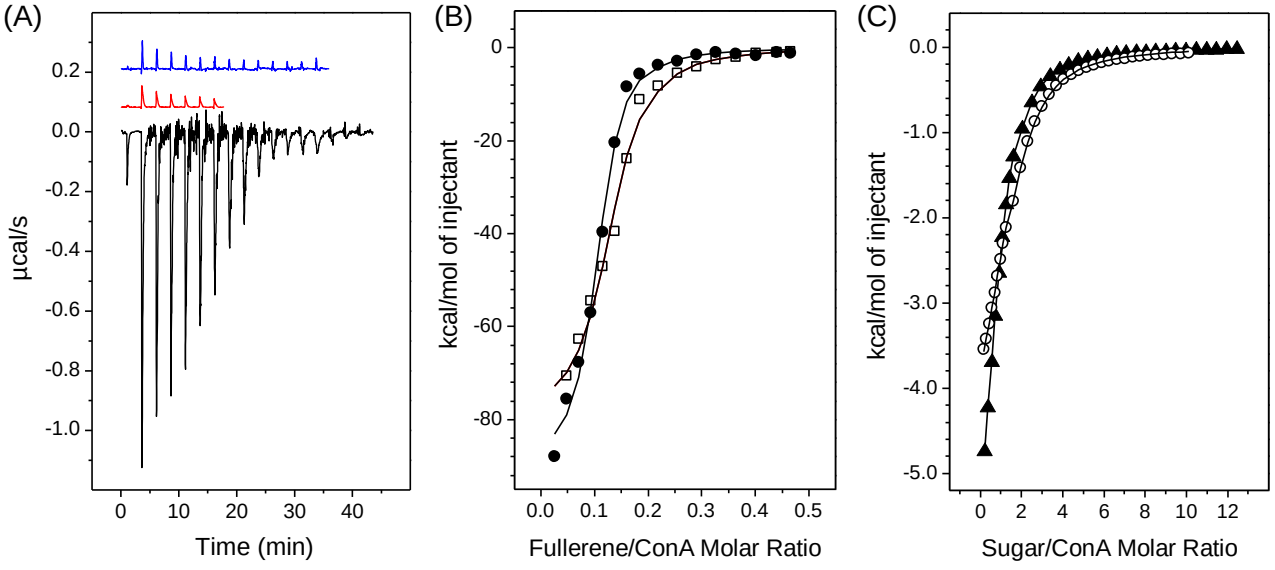


Figure S-7. Calorimetric titration of ConA with glycofullerenes and monovalent mannose compounds. (A) Representative raw data obtained upon step-wise injection of 0.2 mM mannofullerene **1** (black) or galactofullerene (red) onto 90 μM ConA. Blue peaks correspond to the injection of mannofullerene **1** onto the cell filled with buffer. (B) Plot of the total heat released during titrations with mannofullerene **1** (□) and **2** (●) as a function of the fullerene/ConA monomer molar ratio**.** Solid lines correspond to the best fit to experimental data obtained using a one-set-of-sites model with the "ligand-in-cell" analysis option, and the functional monomer concentration determined for ConA (*N* for MeαMan in Table 1) as input for ligand concentration. (C) Titration of 239 μM ConA with 15 mM MeαMan (▲) and compound **6** (○). Continuous lines correspond to the fit to one-set-of-sites model. All titrations were carried out in HBS, pH 7.4, at 22 ºC.

REFERENCES

(1) Sinha, J.; Sahoo, R.; Kumar, A. Processable, regioregular, and "click"able monomer and polymers based on 3,4-propylenedioxythiophene with tunable solubility. *Macromolecules* **2009**, *42*, 2015-2022.
(2) Levene, P. A. Preparation of α-mannose: Second paper. *J. Biol. Chem.* **1924**, *59*, 129-134.
(3) Roy, R.; Page, D.; Figueroa Perez, S.; Verez Bencomo, V. Effect of shape, size, and valency of multivalent mannosides on their binding properties to phytohemagglutinins. *Glycoconj. J.* **1998**, *15*, 251-263.
(4) Otman, O.; Boullanger, P.; Lafont, D.; Hamaide, T. New amphiphilic glycopolymers based on a polycaprolactone-maleic anhydride copolymer backbone: Characterization by 15n nmr and application to colloidal stabilization of nanoparticles. *Macromol. Chem. Phys.* **2008**, *209*, 2410-2422.
(5) Mukhopadhyay, B.; Maurer, S. V.; Rudolph, N.; van Well, R. M.; Russell, D. A.; Field, R. A. From solution phase to "on-column" chemistry: Trichloroacetimidate-based glycosylation promoted by perchloric acid−silica. *J. Org. Chem.* **2005**, *70*, 9059-9062.
(6) Yan, X.; Delgado, M.; Fu, A.; Alcouffe, P.; Gouin, S. G.; Fleury, E.; Katz, J. L.; Ganachaud, F.; Bernard, J. Simple but precise engineering of functional nanocapsules through nanoprecipitation. *Angew. Chem. Int. Ed.* **2014**, *53*, 6910-6913.
(7) Fiandor, J.; García-López, M. T.; De Las Heras, F. G.; Méndez-Castrillón, P. P. A facile regioselective 1-O-deacylation of peracylated glycopyranoses. *Synthesis* **1985**, *1985*, 1121-1123.
(8) Cheng, H.; Cao, X.; Xian, M.; Fang, L.; Cai, T. B.; Ji, J. J.; Tunac, J. B.; Sun, D.; Wang, P. G. Synthesis and enzyme-specific activation of carbohydrate−geldanamycin conjugates with potent anticancer activity. *J. Med. Chem.* **2005**, *48*, 645-652.
(9) Artner, L. M.; Merkel, L.; Bohlke, N.; Beceren-Braun, F.; Weise, C.; Dernedde, J.; Budisa, N.; Hackenberger, C. P. R. Site-selective modification of proteins for the synthesis of structurally defined multivalent scaffolds. *Chem. Commun.* **2012**, *48*, 522-524.
(10) Iehl, J.; Nierengarten, J.-F. A click–click approach for the preparation of functionalized [5:1]-hexaadducts of C60. *Chem. Eur. J.* **2009**, *15*, 7306-7309.
(11) Timmer, B. J. J.; Flos, M. A.; Jorgensen, L. M.; Proverbio, D.; Altun, S.; Ramstrom, O.; Aastrup, T.; Vincent, S. P. Spatially well-defined carbohydrate nanoplatforms: synthesis, characterization and lectin interaction study. *Chem. Commun.* **2016**, *52*, 12326-12329.
(12) González, L.; Bruix, M.; Díaz-Mauriño, T.; Feizi, T.; Rico, M.; Solís, D.; Jiménez-Barbero, J. Conformational studies of the Man8 oligosaccharide on native ribonuclease B and on the reduced and denatured protein. *Arch. Biochem. Biophys.* **2000**, *383*, 17-27.
(13) Derewenda, Z.; Yariv, J.; Helliwell, J. R.; Kalb, A. J.; Dodson, E. J.; Papiz, M. Z.; Wan, T.; Campbell, J. The structure of the saccharide-binding site of concanavalin A. *EMBO J.* **1989**, *8*, 2189-2193.