Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Tetraphenylethylene-based glycoclusters with aggregation-induced emission (AIE) properties as high affinity ligands of bacterial lectins

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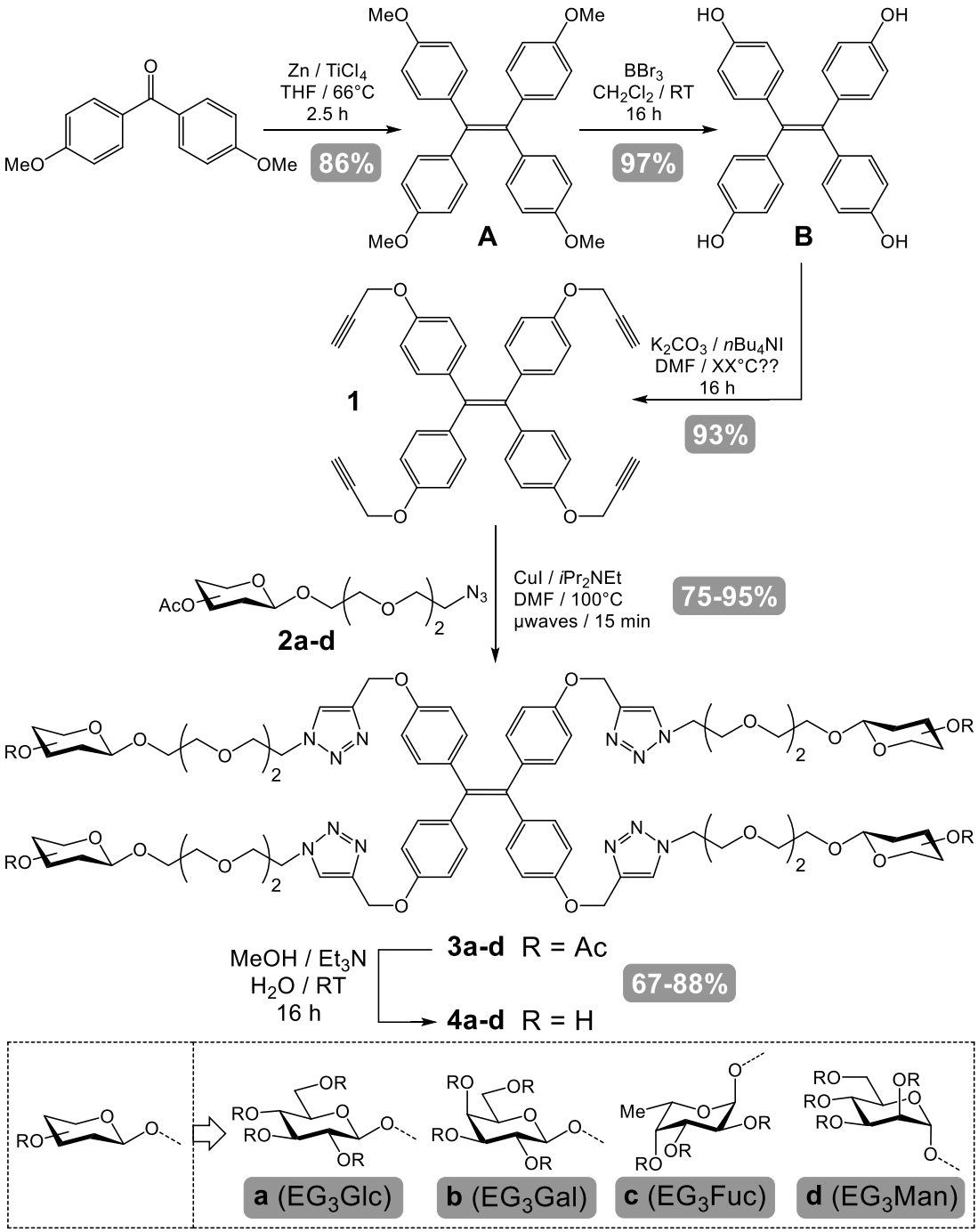
General Methods

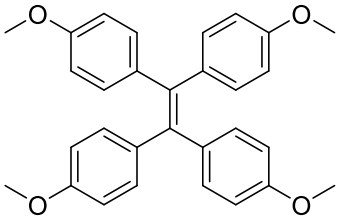
All reagents for synthesis commercially available (highest purity available for reagent grade compounds) were used without further purification. Solvents were distilled over CaH_2 (CH_2Cl_2), Mg/I_2 (MeOH), Na/benzophenone (THF) or purchased dry. Reactions under microwave activation were performed on a Biotage Initiator system. Thin-layer chromatography (TLC) was carried out on aluminum sheets coated with silica gel 60 F_{254} (Merck). TLC plates were inspected by UV light (λ = 254 nm, 365 nm) and developed by treatment with a mixture of 10% H_2SO_4 in $EtOH/H_2O$ (95:5 v/v) followed by heating. Silica gel column chromatography was performed with silica gel Si 60 (40–63 μm). Optical rotation was measured using a Perkin Elmer polarimeter. NMR spectra were recorded at 293 K, unless stated otherwise. Chemical shifts are referenced relative to deuterated solvent residual peaks. The following abbreviations are used to explain the observed multiplicities: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; p, pseudo and b, broad. Complete signal assignments were based on 1D and 2D NMR correlations COSY and HSQC. High resolution (HR-ESI-QToF) mass spectra were recorded using a Bruker MicroToF-Q II XL spectrometer. The glycoclusters tested in bioassays were purified using automated purification systems with medium pressure chromatography on reverse C_{18} silica gel. Their purity was verified by ^{1}H and ^{13}C NMR techniques, indicating ca. 95% purity.

General procedure A for 1,3-dipolar cycloadditions: Compound **1** (100 mg, 0.18 mmol, 1 eq), copper iodide (17 mg, 0.09 mmol, 0.5 eq), DIPEA (159 µL, 0.91 mmol, 5 eq) and azido-functionalized glycoside **2a-d** (0.91 mmol, 5 eq) in dimethylformamide (4 mL) were introduced into a Biotage Initiator 2-5 mL vial. The vial was sealed with a septum cap and heated at 110°C for 25 min under microwave irradiation (solvent absorption level: High). The crude mixture was then concentrated and co-evaporated with toluene 3 times before purification by silica gel column chromatography using as eluent EtOAc to remove impurities and azido derivative and then $CHCl_3/CH_3OH$ (99:1 to 90/10, v/v) to collect the desired acetylated glycoclusters **3a-d**.

General procedure B for deacetylation: A solution of acetylated glycoclusters **3a-d** in a mixture of $CH_3OH/H_2O/Et_3N$ (4/1/1, v/v) was stirred at room temperature overnight. The solvent was then removed under reduced pressure and the residue was dissolved in 5 mL of saturated EDTA solution. The resulting mixture was purified by flash chromatography on C_{18} silica gel column using H_2O/CH_3OH (100/0 to 0/100, v/v) as eluent to afford the corresponding hydroxylated glycoclusters **4a-d**.



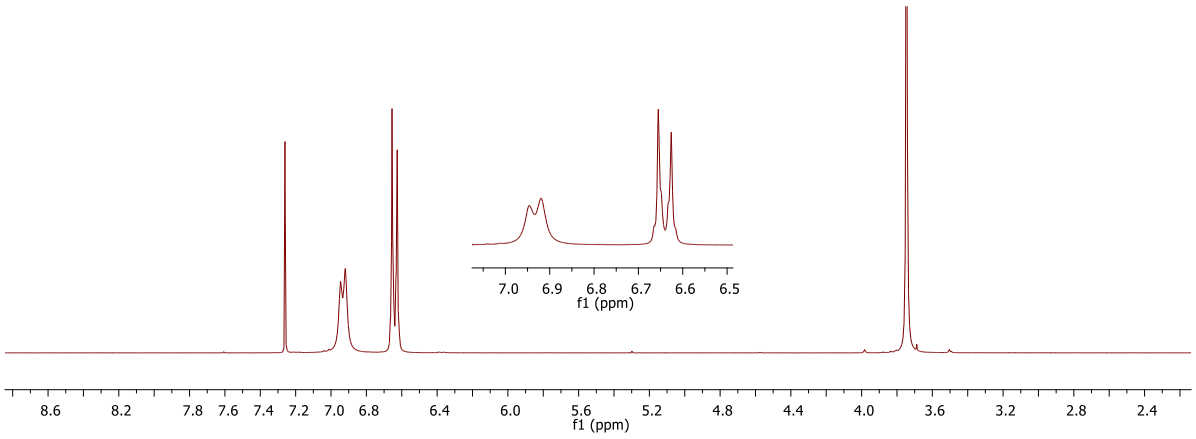
Scheme S1. Complete synthesis of the TPE-based glycoclusters

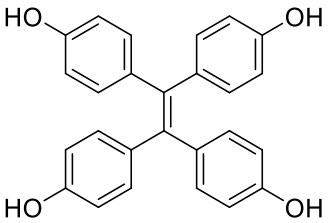


Chem. Asian J. 2011, 6, 2376-2381 / RSC Adv. 2013, 3, 7996-8002 / New J. Chem. 2013, 37, 1696-1699

1,1,2,2-Tetrakis-(4-methoxyphenyl)-ethylene (A): Zn dust was activated by a solution of 5% hydrochloric acid. After 15 minutes, the powder was filtered and washed successively with distilled water (4×100 mL), ethanol (50 mL), acetone (100 mL) and diethyl ether (50 mL). The zinc was finally dried at 100°C for 10 min in oven, cooled at room temperature and used immediately. A suspension of Zn dust (2.20 g, 33.02 mmol, 4 eq) in anhydrous THF (20 mL) was cooled to 0°C and $TiCl_4$ (16.5 mL, 16.5 mmol, 2 eq, 1 M in toluene) was added dropwise under inert atmosphere. The resulting mixture was refluxed for 2.5 h and then cooled to 0°C. A solution of 4,4'-dimethoxybenzophenone (2.00 g, 8.25 mmol, 1 eq) in anhydrous THF (20 mL) was added to the reacting mixture. After heating to reflux for 20 h, the reaction was quenched with saturated aqueous NH_4Cl solution (150 mL) and extracted with diethyl ether (3×60 mL). The combined organic layer was washed with brine (60 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by recrystallization from methanol/dichloromethane solution to afford compound **1** as a white solid (1.60 g, 3.53 mmol, 86%).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 6.93 (d, *J* = 8.7 Hz, 8H, H-ar), 6.63 (d, *J* = 8.8 Hz, 8H, H-ar), 3.74 (s, 12H, OCH_3).

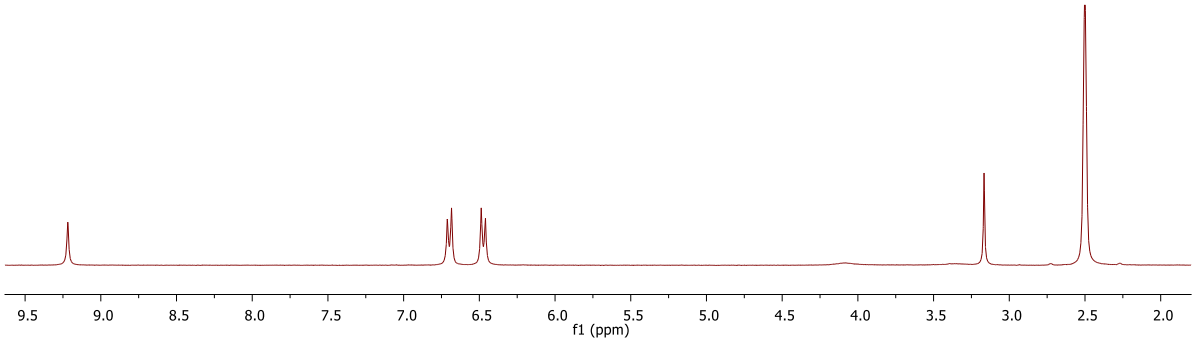


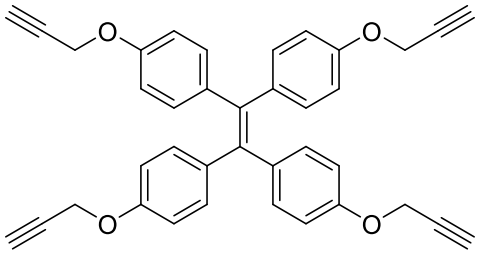


Chem. Asian J. 2011, 6, 2376-2381 / Chem. Commun. 2014, 50, 9122-9125

1,1,2,2-Tetrakis-(4-hydroxyphenyl)-ethylene (B): Under inert atmosphere, a solution of compound **A** (2.40 g, 5.3 mmol, 1 eq) in anhydrous dichloromethane (20 mL) was cooled to -45°C, then BBr_3 (50 mL, 50 mmol, 9.5 eq, 1 M in dichloromethane) was added. After stirring at -45°C for 30 min, the deep red solution was allowed to warm to room temperature and was stirred for a further 18 h. The reacting mixture was then hydrolyzed by dropwise addition into cold water with vigorous stirring until no more precipitate was formed. The purple precipitate was collected by filtration and washed with water (250 mL) and dichloromethane (250 mL). The solid was then dissolved in methanol, concentrated and dried under *vaccuo.* The desired compound **2** was obtained as a purple solid (2.05 g, 5.2 mmol, 97%).

^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) = 9.22 (s, 4H, OH), 6.69 (d, *J* = 8.4 Hz, 8H, H-ar), 6.47 (d, *J* = 8.4 Hz, 8H, H-ar).

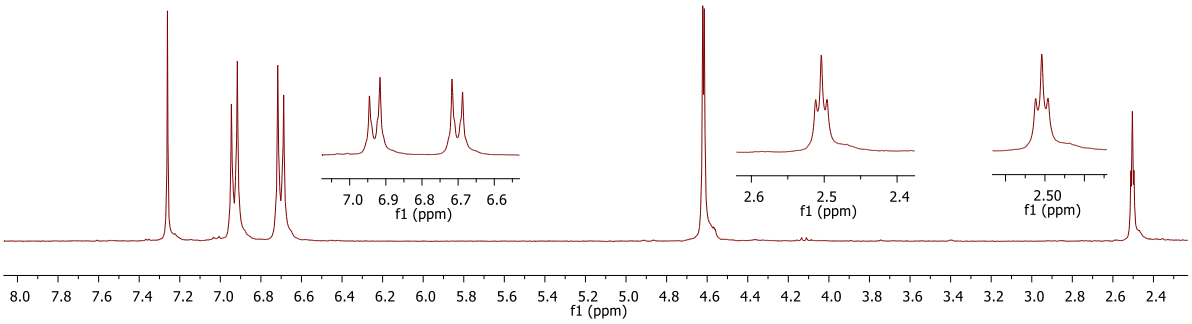


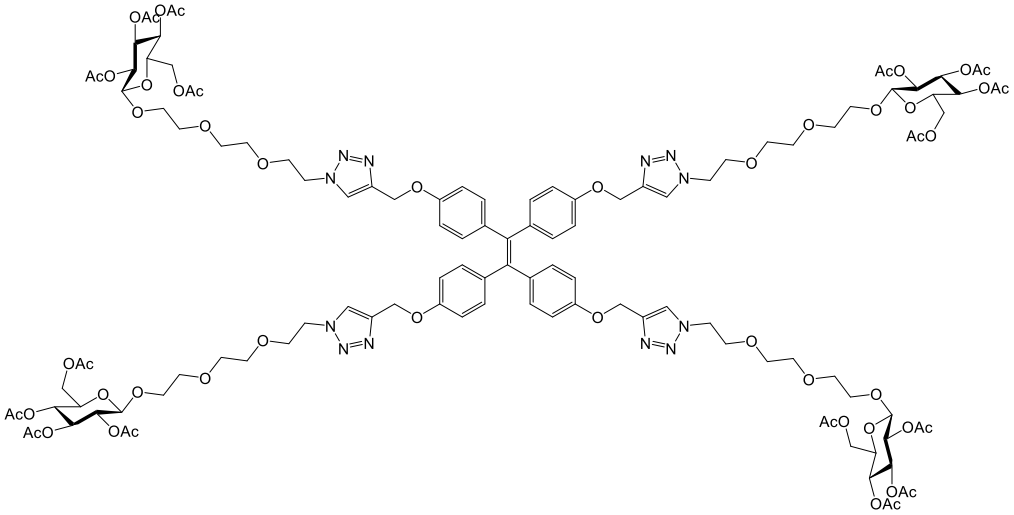


Chem. Asian J. 2011, 6, 2376-2381 / Chem. Commun. 2014, 50, 9122-9125

1,1,2,2-Tetrakis-[4-(prop-2-ynyloxy)phenyl]-ethylene (1): A mixture of compound **B** (1.0 g, 2.52 mmol, 1 eq), K_2CO_3 (2.79 g, 20.16 mmol, 8 eq), NBu_4I (3.72 g, 10.08 mmol, 4 eq) and propargyl bromide (2.82 mL, 25.2 mmol, 10 eq, 80 wt% in toluene) in anhydrous dimethylformamide (50 mL) was heated at 70°C under inert atmosphere. The mixture was then cooled to room temperature and filtered. After removal of the solvent under reduced pressure, the crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:5, v/v) as eluent to afford compound **1** as a beige solid (1.29 g, 2.35 mmol, 93%).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 6.93(d, *J* = 8.8 Hz, 8H, H-ar), 6.70 (d, *J* = 8.8 Hz, 8H, H-ar), 4.61 (d, *J* = 2.3 Hz, 8H, C*H*$_2$≡CH), 2.50 (t, *J* = 2.3 Hz, 4H, CH_2≡C*H*).



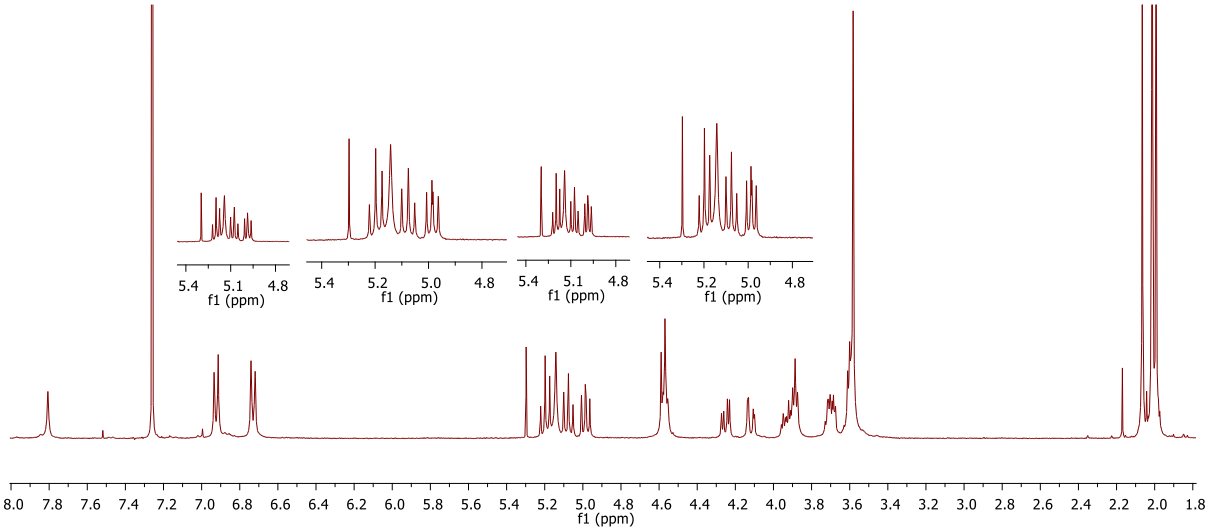


1,1,2,2-Tetrakis-(4{1-[1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-3,6-dioxaoct-8-yl]-1,2,3-triazol-4-ylmethoxy}phenyl)-ethylene TPE-(EG$_3$GlcOAc)$_4$ (3a): Obtained according to the general procedure **A** as a beige solid (353 mg, 0.14 mmol, 75%).

R_f = 0.60 ($CHCl_3$/CH_3OH, 90/10, v/v)

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 7.81 (s, 4, H-triaz), 6.92 (d, *J* = 8.7 Hz, 8H, H-ar), 6.73 (d, *J* = 8.8 Hz, 8H, H-ar), 5.20 (t, *J* = 9.5 Hz, 4H, H-3), 5.14 (s, 8H, OC*H*$_2$-triaz), 5.08 (t, *J* = 9.8 Hz, 4H, H-4), 4.98 (dd, *J* = 8.0 Hz, *J* = 9.5 Hz, 4H, H-2), 4.60-4.56 (m, 12H, H-1, OCH_2C*H*$_2$-triaz), 4.25 (dd, *J* = 4.6 Hz, *J* = 12.3 Hz, 4H, H-6), 4.12 (dd, *J* = 2.3 Hz, *J* = 12.9 Hz, 4H, H-6'), 3.97-3.91 (m, 4H, Glc-OC*H*$_2$), 3.89 (t, *J* = 5.1 Hz, 8H, OC*H*$_2$$CH_2$-triaz), 3.73-3.67 (m, 8H, H-5, Glc-OC*H*$_2$), 3.61-3.58 (m, 24H, OCH_2), 2.07 (s, 12H, $COCH_3$), 2.02 (s, 12H, $COCH_3$), 2.01 (s, 12H, $COCH_3$), 1.99 (s, 12H, $COCH_3$).
^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 170.7, 170.3, 169.4, 169.3 (4×*C*OCH_3), 156.7 (C^{IV}-ar), 144.0 (C^{IV}-triaz), 138.6 (C=C), 137.2 (C^{IV}-ar), 132.6 (CH-ar), 124.1 (CH-triaz), 113.9 (CH-ar), 100.8 (C-1), 72.8 (C-3), 71.8 (C-5), 71.2 (C-2), 70.62 (OCH_2), 70.58 (OCH_2), 70.2 (OCH_2), 69.4 (O*C*H_2CH_2-triaz), 69.2 (Glc-O*C*H_2), 68.4 (C-4), 61.91 (C-6), 61.88 (O*C*H_2-triaz), 50.4 (OCH_2*C*H_2-triaz), 20.8, 20.7, 20.64, 20.61 (4×CO*C*H_3).

HR-ESI-QTof (positive mode) *m*/*z*: calcd for $C_{118}H_{155}N_{12}O_{52}$ $[M+3H]^{3+}$ 857.3279, found 857.3314.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f1 (ppm)
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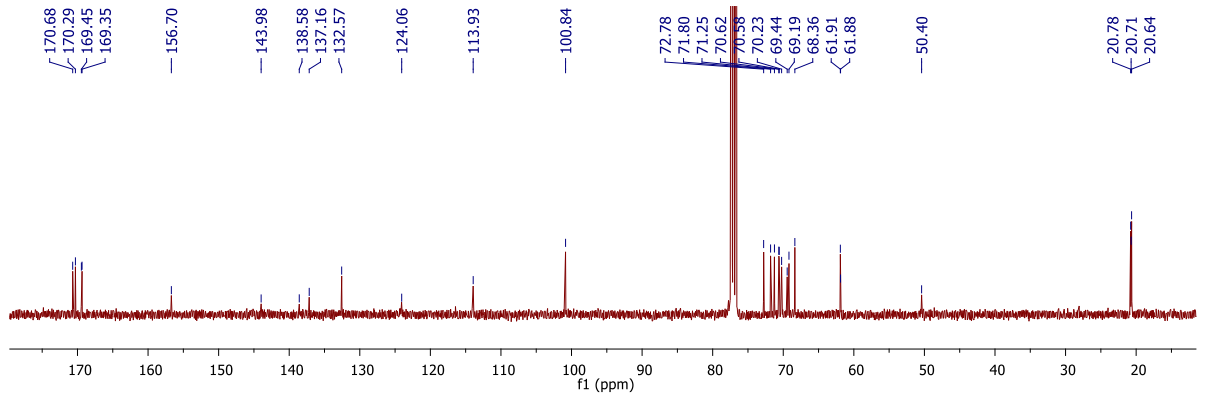
f1 (ppm)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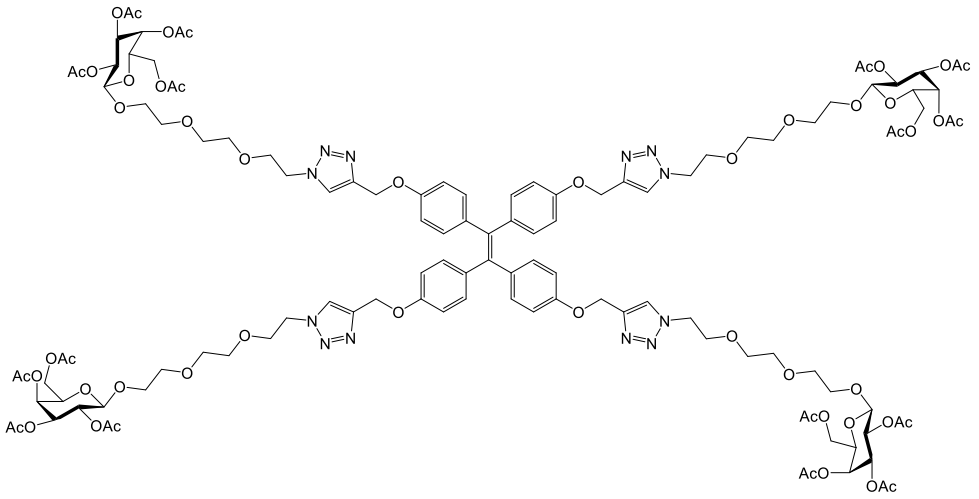


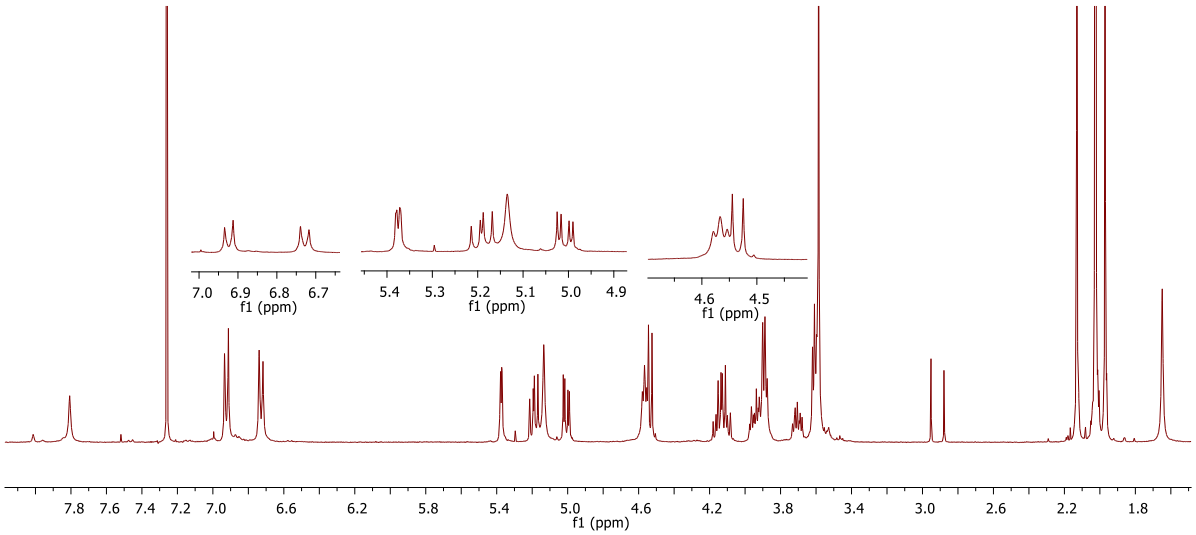
1,1,2,2-Tetrakis-(4{1-[1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)-3,6-dioxaoct-8-yl]-1,2,3-triazol-4-ylmethoxy}phenyl)-ethylene TPE-(EG$_3$GalOAc)$_4$ (3b): Obtained according to the general procedure **A** as a beige solid (393 mg, 0.15 mmol, 85%).

R_f = 0.50 ($CHCl_3$/CH_3OH, 92/8, v/v)

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 7.81 (s, 4H, H-triaz), 6.92 (d, *J* = 8.7 Hz, 8H, H-ar), 6.73 (d, *J* = 8.7 Hz, 8H, H-ar), 5.37 (dd, *J* = 0.8 Hz, *J* = 3.4 Hz, 4H, H-4), 5.19 (dd, *J* = 8.0 Hz, *J* = 10.5 Hz, 4H, H-2), 5.13 (s, 8H, OC*H$_2$*-triaz), 5.01 (dd, *J* = 3.4 Hz, *J* = 10.5 Hz, 4H, H-3), 4.57 (t, *J* = 4.9 Hz, 8H, OCH_2C*H$_2$*-triaz), 4.53 (d, *J* = 8.0 Hz, 4H, H-1), 4.17-4.10 (m, 8H, H-6, H-6'), 3.96-3.92 (m, 16H, H-5, Gal-OC*H$_2$*), 3.89 (t, *J* = 4.9Hz, 8H, OC*H$_2$*CH_2-triaz), 3.73-3.68 (m, 4H, Gal-OC*H$_2$*), 3.63-3.58 (m, 24H, OCH_2), 2.13 (s, 12H, $COCH_3$), 2.03 (s, 12H, $COCH_3$), 2.02 (s, 12H, $COCH_3$), 1.97 (s, 12H, $COCH_3$).

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 170.5, 170.4, 170.3, 169.6 (4×*C*OCH_3), 156.8 (C^{IV}-ar), 144.1 (C^{IV}-triaz), 138.7 (C=C), 137.3 (C^{IV}-ar), 132.7 (CH-ar), 124.2 (CH-triaz), 114.0 (CH-ar), 101.5 (C-1), 71.0 (C-3), 70.78 (C-5), 70.76 (OCH_2), 70.7 (OCH_2), 70.3 (OCH_2), 69.6 (OCH_2CH_2-triaz), 69.3 (C-6), 68.9 (C-2), 67.1 (C-4), 62.0 (O*C*H_2-triaz), 61.4 (C-6), 50.5 (OCH_2CH_2-triaz), 20.9, 20.84, 20.82, 20.74 (4×CO*C*H_3).

HR-ESI-QTof (positive mode) *m*/*z*: calcd for $C_{118}H_{155}N_{12}O_{52}$ $[M+3H]^{3+}$ 857.3279, found 857.3311.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f1 (ppm)
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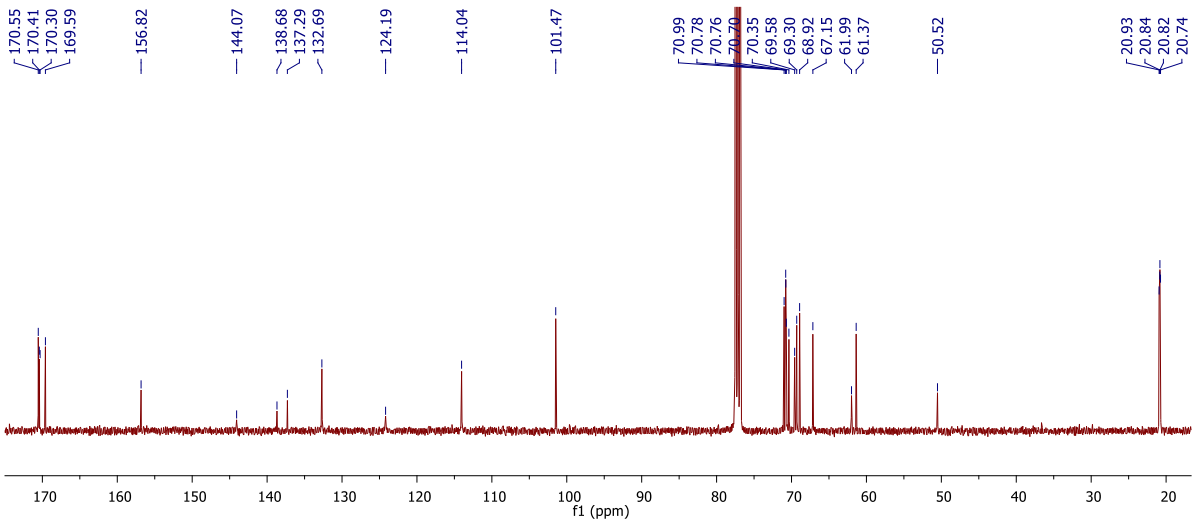
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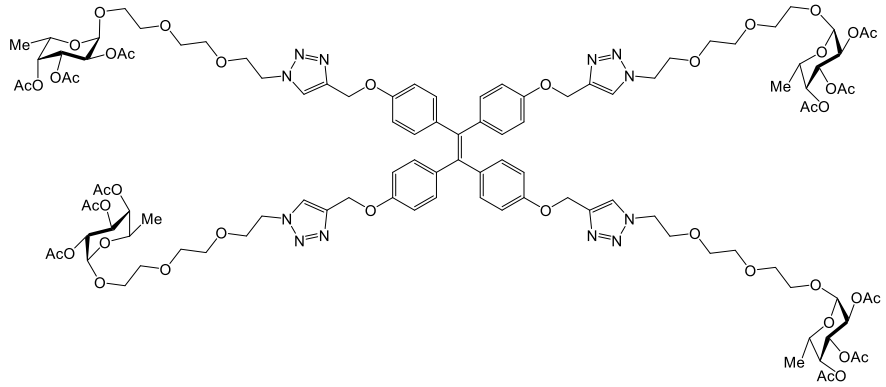
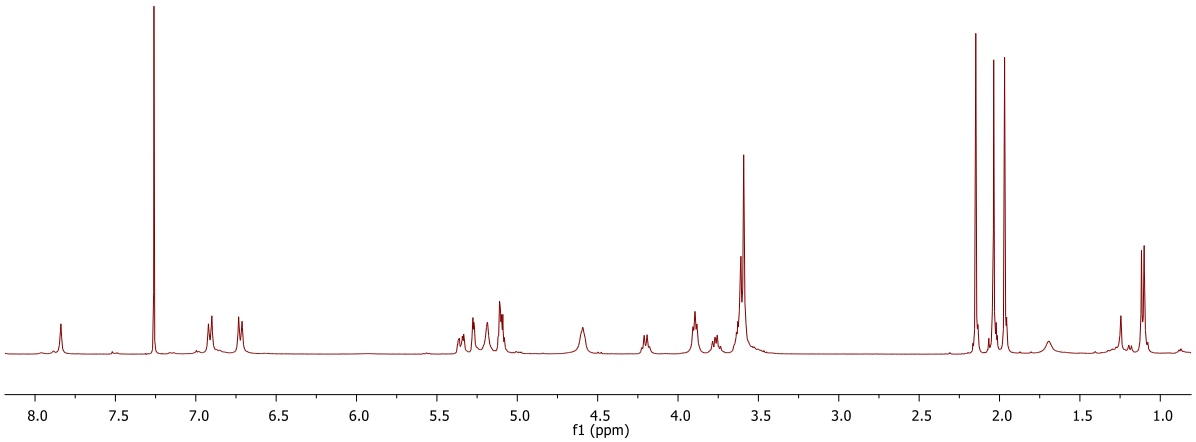


1,1,2,2-Tetrakis-(4{1-[1-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyloxy)-3,6-dioxaoct-8-yl]-1,2,3-triazol-4-ylmethoxy}phenyl)-ethylene TPE-(EG$_3$FucOAc)$_4$ (3c): Obtained according to the general procedure **A** as a beige solid (425 mg, 0.18 mmol, 95%).

R_f = 0.60 ($CHCl_3$/CH_3OH, 90/10, v/v)

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = δ 7.84 (s, 4H, H-triaz), 6.91 (d, *J* = 8.7 Hz, 8H, H-ar), 6.72 (d, *J* = 8.7 Hz, 8H, H-ar), 5.37-5.33 (m, 4H, H-3), 5.28 (dd, *J* = 3.3 Hz, *J* = 1.1 Hz, 4H, H-4), 5.19 (s, 8H, OC*H*$_2$-triaz), 5.12-5.08 (m, 8H, H-1 and H-2), 4.59 (s, 8H, CH_2C*H*$_2$-triaz), 4.20 (q, *J* = 6.1 Hz, 4H, H-5), 3.89 (t, *J* = 4.8 Hz, 8H, C*H*$_2$$CH_2$-triaz), 3.78-3.74 (m, 4H, Fuc-OC*H*$_2$), 3.64-3.59 (m, 28H, Fuc-OC*H*$_2$ and OCH_2), 2.15 (s, 12H, $COCH_3$), 2.04 (s, 12H, $COCH_3$), 1.97 (s, 12H, $COCH_3$), 1.11 (d, *J* = 6.5 Hz, 12H, H-6).
^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 170.7, 170.6, 170.3 (3×*C*OCH_3), 156.8 (C^{IV}-ar), 144.2 (C^{IV}-triaz), 138.7 (C=C), 137.2 (C^{IV}-ar), 132.7 (CH-ar), 124.4 (CH-triaz), 114.1 (CH-ar), 96.3 (C-1), 71.3 (C-4), 70.71 (OCH_2), 70.70 (OCH_2), 70.4 (OCH_2), 69.5 (O*C*$H_2$$CH_2$-triaz), 68.3 (C-2), 68.1 (C-3), 67.4 (Fuc-O*C*H_2), 64.5 (C-5), 62.1 (O*C*H_2-triaz), 50.7 (OCH_2*C*H_2-triaz), 21.0, 20.9, 20.8 (3×CO*C*H_3), 16.0 (C-6).

HR-ESI-QTof (positive mode) *m*/*z*: calcd for $C_{110}H_{146}N_{12}O_{44}$ $[M+2H]^{2+}$ 1169.4772, found 1169.4731.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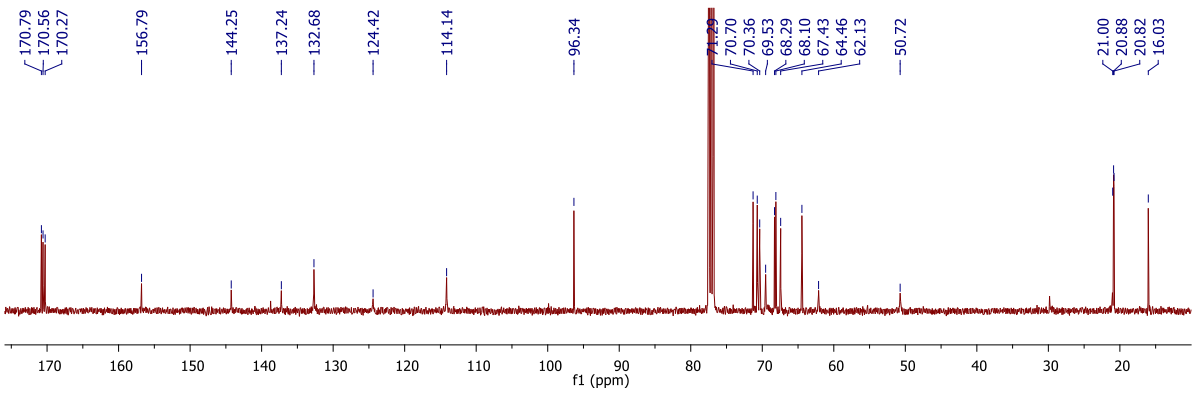
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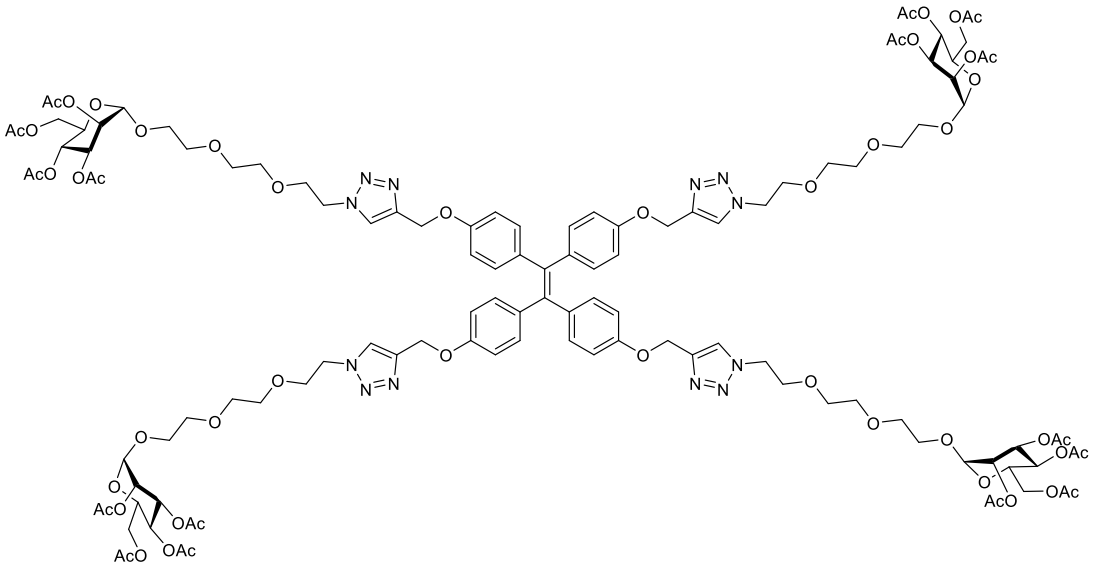
f1 (ppm)



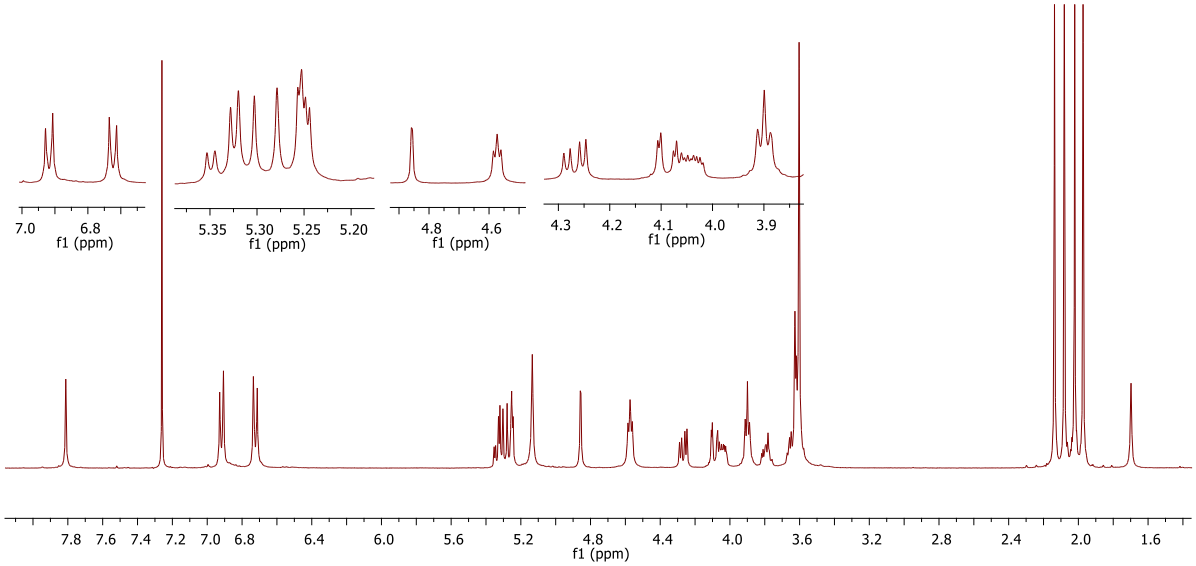
1,1,2,2-Tetrakis-(4{1-[1-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)-3,6-dioxaoct-8-yl]-1,2,3-triazol-4-ylmethoxy}phenyl)-ethylene TPE-(EG$_3$ManOAc)$_4$ (3d): Obtained according to the general procedure **A** as a beige solid (382 mg, 0.15 mmol, 82%).

R_f = 0.30 ($CHCl_3$/CH_3OH, 96/4, v/v)

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 7.81 (s, 4H, H-triaz), 6.92 (d, *J* = 8.7 Hz, 8H, H-ar), 6.72 (d, *J* = 8.8 Hz, 8H, H-ar), 5.34 (dd, *J* = 3.3 Hz, *J* = 10.0 Hz, 4H, H-3), 5.28 (t, *J* = 9.6 Hz, 4H, H-4), 5.25 (dd, *J* = 1.5 Hz, *J* = 3.2 Hz, 4H, H-2), 5.13 (s, 8H, OC*H*$_2$-triaz), 4.86 (d, *J* = 1.5 Hz, 4H, H-1), 4.57 (t, *J* = 4.8 Hz, 8H, OCH_2C*H*$_2$-triaz), 4.27 (dd, *J* = 5.0 Hz, *J* = 12.2 Hz, 4H, H-6), 4.09 (dd, *J* = 2.3 Hz, *J* =12.2 Hz, 4H, H-6'), 4.06-4.01 (m, 4H, H-5), 3.90 (t, *J* = 5.1 Hz, 8H, OC*H*$_2$CH$_2$-triaz), 3.83-3.76 (m, 4H, Man-OC*H*$_2$), 3.68-3.58 (m, 28H, Man-OC*H*$_2$, OCH_2), 2.14 (s, 12H, $COCH_3$), 2.08 (s, 12H, $COCH_3$), 2.02 (s, 12H, $COCH_3$), 1.97 (s, 12H, $COCH_3$).

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 170.8, 170.2, 170.1,169.8 (4×*C*OCH_3), 156.8 (C^{IV}-ar), 144.1 (C^{IV}-triaz), 138.7 (C=C), 137.3 (C^{IV}-ar), 132.7 (CH-ar), 124.2 (CH-triaz), 114.0 (CH-ar), 97.8 (C-1), 70.8 (O*C*H$_2$), 70.7 (O*C*H$_2$), 70.1 (O*C*H$_2$), 69.65 (O*C*H$_2$CH$_2$-triaz), 69.0 (C-2), 69.2 (C-3), 68.6 (C-5), 67.5 (Man-O*C*H$_2$), 66.2 (C-4),62.5 (C-6), 62.0 (O*C*H$_2$-triaz), 50.5 (OCH_2*C*H$_2$-triaz), 21.0, 20.90, 20.86, 20.84 (4×CO*C*H_3).

HR-ESI-QTof (positive mode) *m*/*z*: calcd for $C_{118}H_{155}N_{12}O_{52}$ $[M+3H]^{3+}$ 857.3279, found 857.3300.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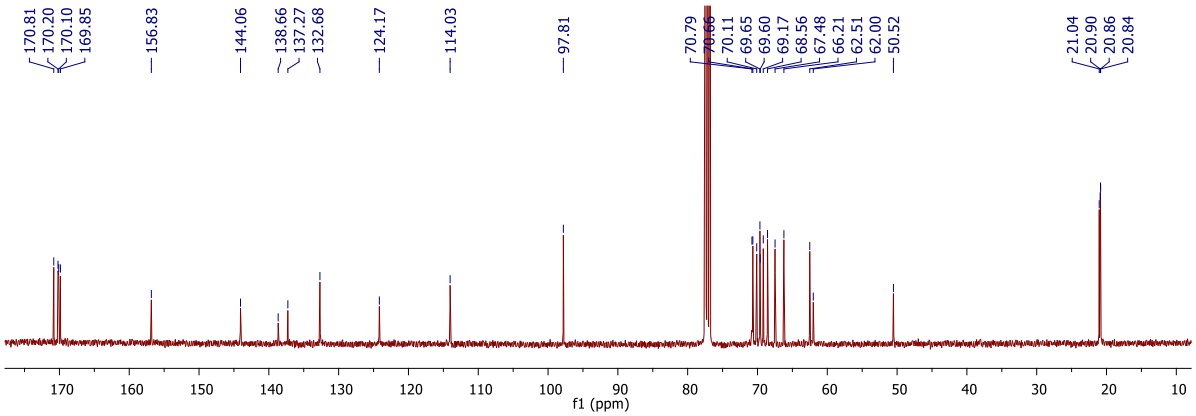
f1 (ppm)
4.8 4.6
f1 (ppm)
4.3 4.2 4.1 4.0 3.9
f1 (ppm)
7.8 7.6 7.4 7.2 7.0 6.8 6.4 6.0 5.6 5.2 4.8 4.4 4.0 3.6 3.2 2.8 2.4 2.0 1.6
f1 (ppm)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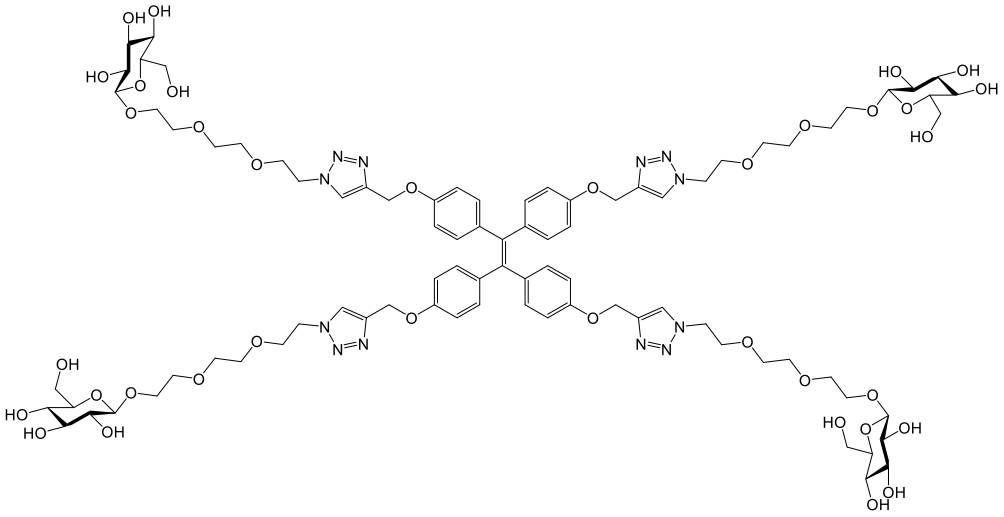
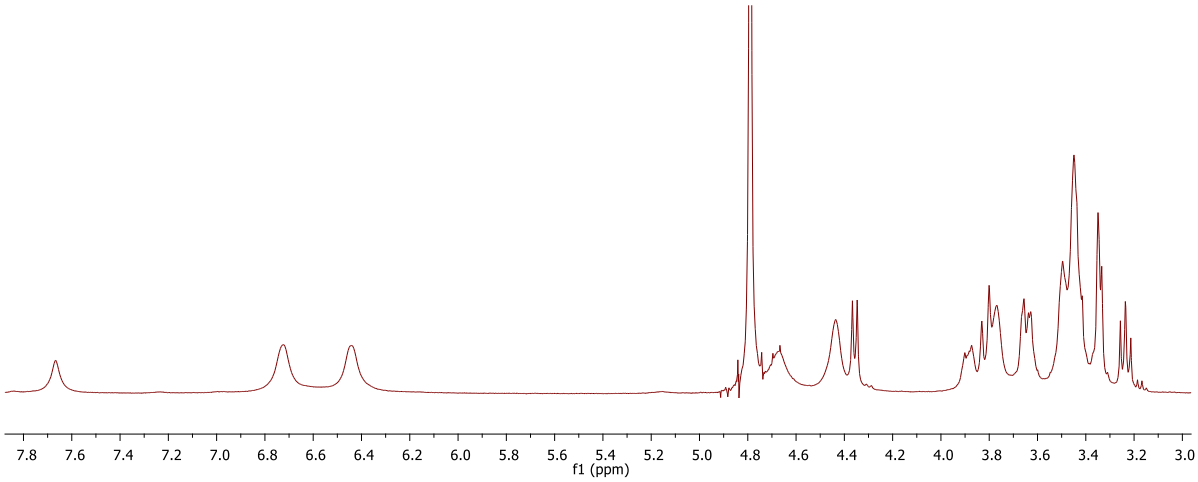
70.66
70.11
69.65
69.60
69.17
68.56
67.48
66.21
62.51
62.00
50.52
21.04
20.90
20.86
20.84
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)



1,1,2,2-Tetrakis-(4{1-[1-(β-D-glucopyranosyloxy)-3,6-dioxaoct-8-yl]-1,2,3-triazol-4-ylmethoxy}phenyl)-ethylene TPE-(EG$_3$Glc)$_4$ (4a): Obtained according to the general procedure **B** as a beige solid (165 mg, 0.087 mmol, 67%).

^{1}H NMR (400 MHz, D_2O) δ (ppm) = 7.67 (bs, 4H, H-triaz), 6.85-6.62 (m, 8H, H-ar), 6.54-6.32 (m, 8H, H-ar), 4.70-4.77 (m, 8H, OC*H*$_2$-triaz), 4.50-4.39 (m, 8H, OCH$_2$C*H*$_2$-triaz), 4.36 (d, *J* = 7.9 Hz, 4H, H-1), 3.91-3.77 (m, 16H, H-6, Glc-OC*H*$_2$ and OC*H*$_2$CH$_2$-triaz), 3.69-3.59 (m, 8H, H-6' and Glc-OC*H*$_2$), 3.55-3.31 (m, 36H, H-3, H-4, H-5 and OCH$_2$), 3.24 (t, *J* = 8.6 Hz, 4H, H-2).
^{13}C NMR (100 MHz, D_2O) δ (ppm) = 156.2 (C^{IV}-ar), 142.9 (C^{IV}-triaz), 138.6 (C=C), 137.0 (C^{IV}-ar), 132.3 (CH-ar), 125.0 (CH-triaz), 114.0 (CH-ar), 102.2 (C-1), 75.9 (C-4), 75.6 (C-3), 73.0 (C-2), 69.6 (OCH$_2$), 69.5 (OCH$_2$), 69.4 (OCH$_2$ and C-5), 68.7 (O*C*H$_2$CH$_2$-triaz), 68.5 (Glc-O*C*H$_2$), 60.7 (C-6 and O*C*H$_2$CH$_2$-triaz), 50.0 (OCH$_2$*C*H$_2$-triaz).

HR-ESI-QTof (positive mode) *m*/*z*: calcd for $C_{86}H_{122}N_{12}O_{36}$ $[M+2H]^{2+}$ 949.4037, found 949.4036.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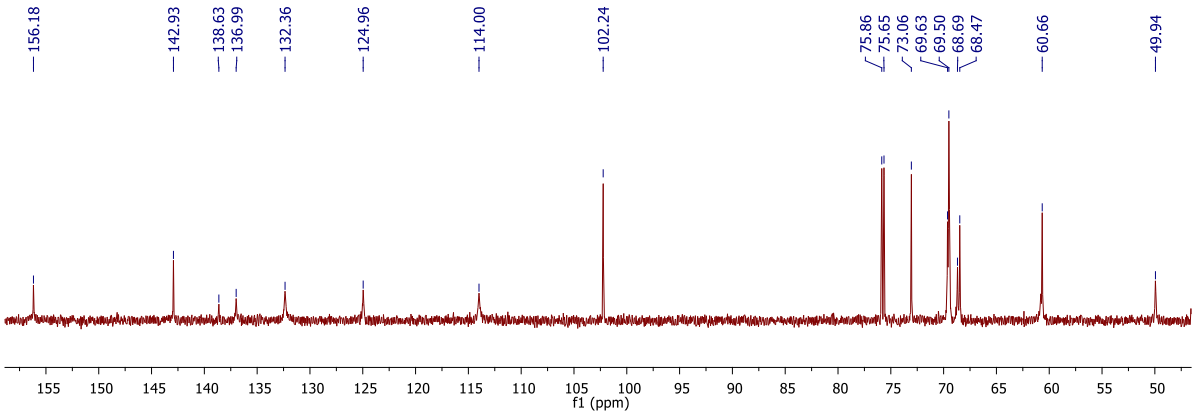
f1 (ppm)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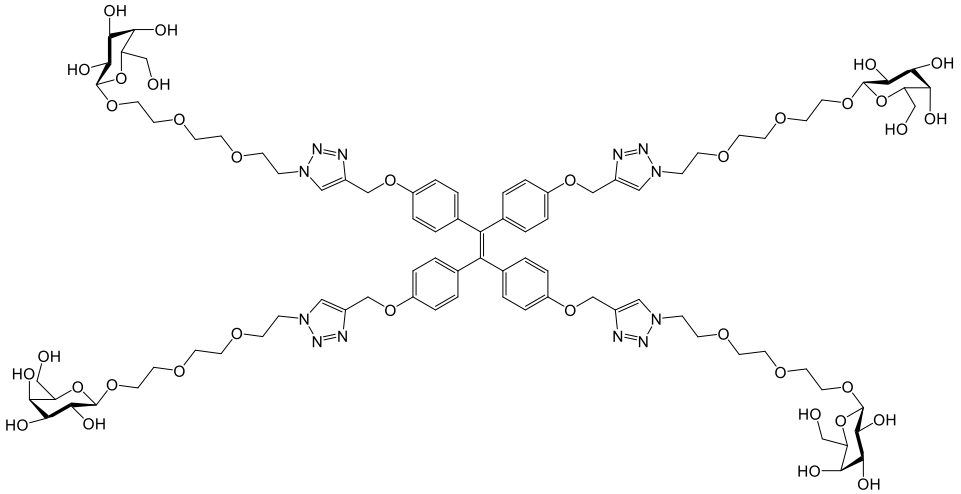
f1 (ppm)

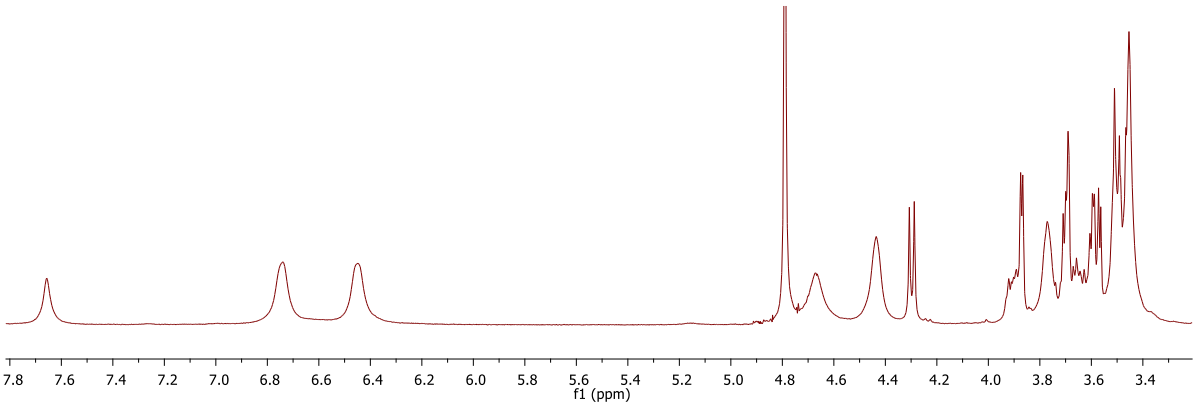


1,1,2,2-Tetrakis-(4{1-[1-(β-D-galactopyranosyloxy)-3,6-dioxaoct-8-yl]-1,2,3-triazol-4-ylmethoxy}phenyl)-ethylene TPE-(EG$_3$Gal)$_4$ (4b): Obtained according to the general procedure **B** as a beige solid (315 mg, 0.17 mmol, 83%).

^{1}H NMR (400 MHz, D_2O) δ (ppm) = 7.66 (bs, 4H, H-triaz), 6.85-6.67 (m, 8H, H-ar), 6.50-6.38 (m, 8H, H-ar), 4.74-4.61 (m, 8H, OC*H*$_2$-triaz), 4.50-4.36 (m, 8H, OCH$_2$C*H*$_2$-triaz), 4.30 (d, *J* = 7.9 Hz, 4H, H-1), 3.92-3.83 (m, 8H, H-4 and Gal-OC*H*$_2$), 3.80-3.74 (m, 8, OC*H*$_2$CH$_2$-triaz), 3.72-3.68 (m, 8H, H-6), 3.67-3.60 (m, 4H, Gal-OC*H*$_2$), 3.59-3.55 (m, 8H, H-3 and H-5), 3.53-3.38 (m, 28H, H-2 and OCH$_2$).

^{13}C NMR (100 MHz, D_2O) δ (ppm) = 156.2 (C^{IV}-ar), 142.9 (C^{IV}-triaz), 138.8 (C=C), 137.0 (C^{IV}-ar), 132.3 (CH-ar), 124.9 (CH-triaz), 114.0 (CH-ar), 102.8 (C-1), 75.0 (C-3), 72.7 (C-5), 70.7 (C-2), 69.7 (OCH$_2$), 69.5 (OCH$_2$), 69.4 (OCH$_2$), 68.7 (O*C*H$_2$CH$_2$-triaz), 68.5 (C-4), 68.4 (Gal-O*C*H$_2$), 60.8 (C-6 and O*C*H$_2$CH$_2$-triaz), 49.9 (OCH$_2$*C*H$_2$-triaz).

HR-ESI-QTof (positive mode) *m*/*z*: calcd for $C_{86}H_{120}Na_2N_{12}O_{36}$ $[M+2Na]^{2+}$ 971.8873, found 971.3844.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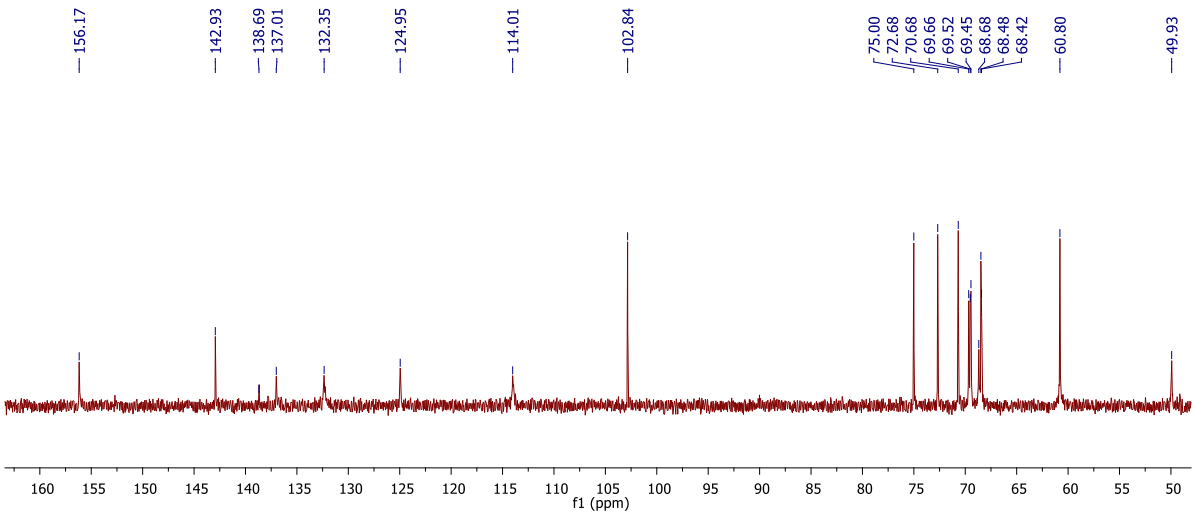
f1 (ppm)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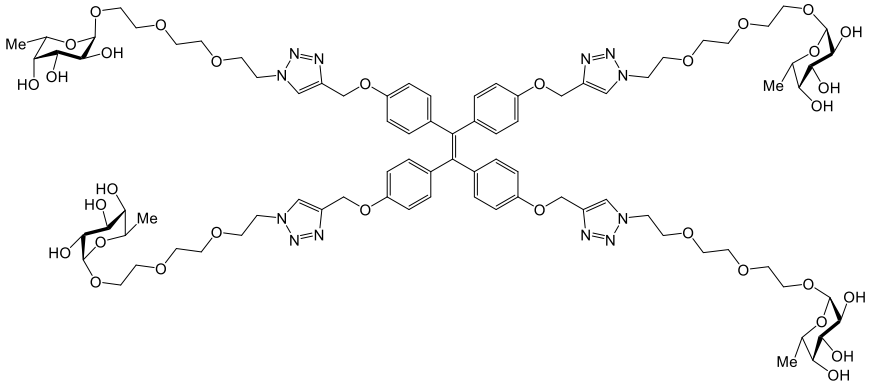
70.68
69.66
69.52
69.45
68.68
68.48
68.42
60.80
49.93
160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50
f1 (ppm)

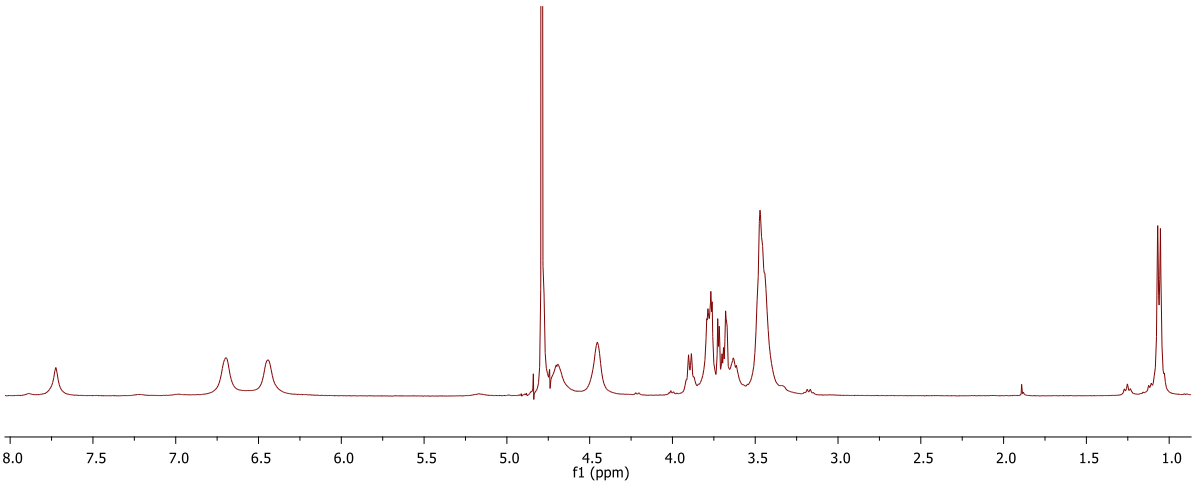


1,1,2,2-Tetrakis-(4{1-[1-(α-L-fucopyranosyloxy)-3,6-dioxaoct-8-yl]-1,2,3-triazol-4-ylmethoxy}phenyl)-ethylene TPE-(EG$_3$Fuc)$_4$ (4c): Obtained according to the general procedure **B** as a beige solid (246 mg, 0.13 mmol, 77%).

^{1}H NMR (400 MHz, D_2O) δ (ppm) = 7.72 (bs, 4H, H-triaz), 6.76-6.63 (m, 8H, H-ar), 6.52-6.36 (m, 8H, H-ar), 4.79-4.77 (m, 4H, H-1), 4.74-4.65 (m, 8H, OC*H*$_2$-triaz), 4.53-4.36 (m, 8H, OCH$_2$C*H*$_2$-triaz), 3.89 (dd, *J* = 12.9 Hz, *J* = 6.2 Hz, 4H, H-5), 3.85-3.56 (m, 24H, H-2, H-3, H-4, Fuc-OC*H*$_2$ and OC*H*$_2$CH$_2$-triaz), 3.56-3.33 (m, 28H, Fuc-OC*H*$_2$ and OC*H*$_2$), 1.06 (d, *J* = 6.5 Hz, 12H, H-6).

^{13}C NMR (100 MHz, D_2O) δ (ppm) = 156.2 (C^{IV}-ar), 142.9 (C^{IV}-triaz), 138.5 (C=C), 136.9 (C^{IV}-ar), 132.2 (CH-ar), 125.0 (CH-triaz), 114.0 (CH-ar), 98.6 (C-1), 71.7 (C-3 or C-4), 69.7 (OCH$_2$), 69.6 (C-3 or C-4 and OCH$_2$), 69.5 (OCH$_2$), 68.7 (O*C*H$_2$CH$_2$-triaz), 68.0 (C-2), 66.6 (Fuc-O*C*H$_2$), 66.4 (C-5), 60.8 (O*C*H$_2$CH$_2$-triaz), 50.0 (OCH$_2$*C*H$_2$-triaz), 15.4 (C-6).

HR-ESI-QTof (positive mode) *m*/*z*: calcd for $C_{86}H_{122}N_{12}O_{32}$ $[M+2H]^{2+}$ 917.4139, found 917.9203.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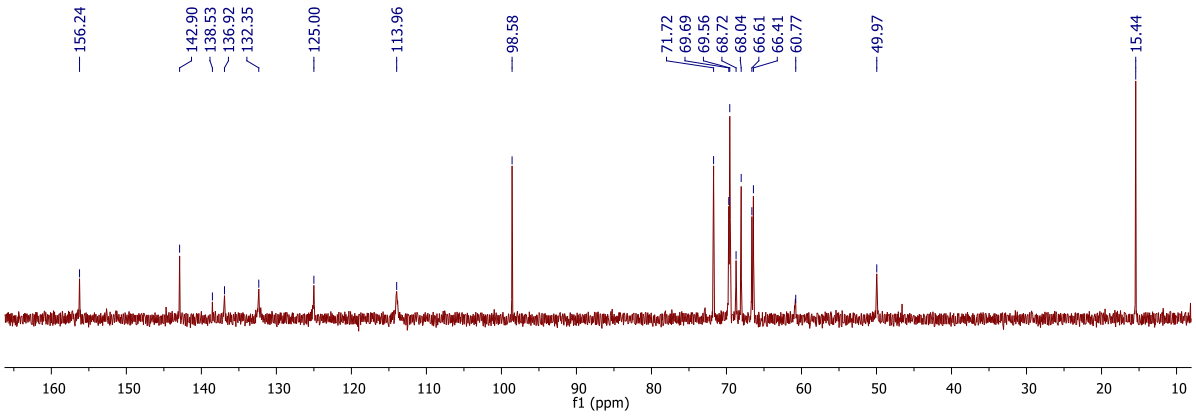
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)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60
50
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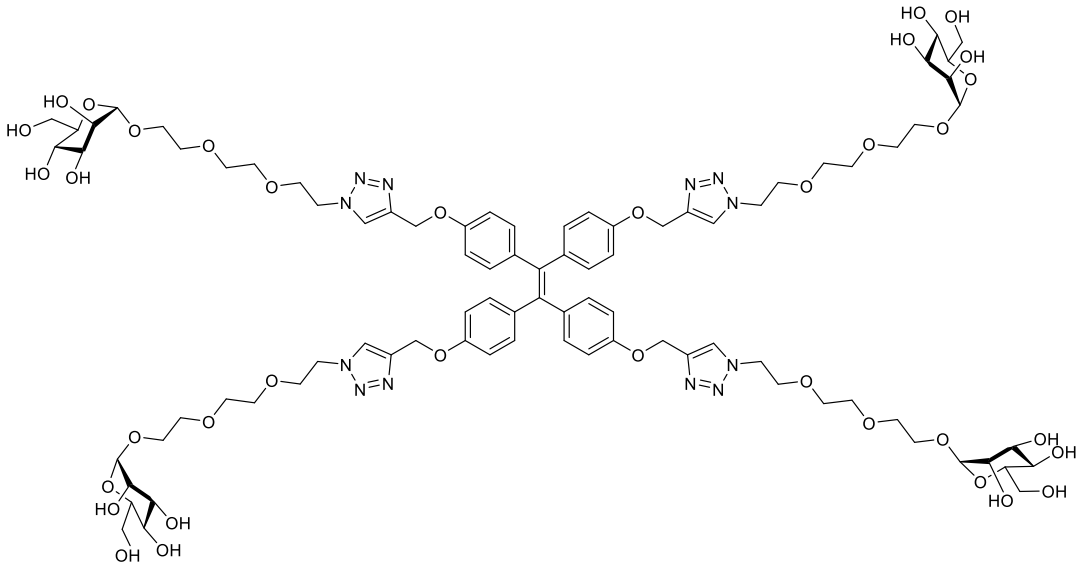
30
20
10
f1 (ppm)

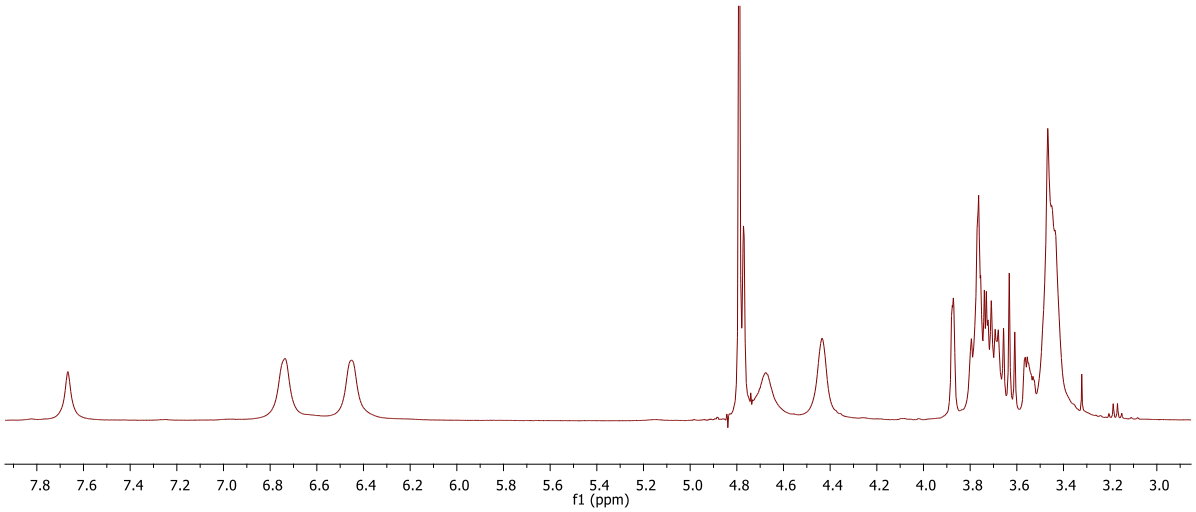


1,1,2,2-Tetrakis-(4{1-[1-(α-D-mannopyranosyloxy)-3,6-dioxaoct-8-yl]-1,2,3-triazol-4-ylmethoxy}phenyl)-ethylene TPE-(EG$_3$Man)$_4$ (4d): Obtained according to the general procedure **B** as a beige solid (200 mg, 0.10 mmol, 75%).

^{1}H NMR (400 MHz, D_2O) δ (ppm) = 7.67 (bs, 4H, H-triaz), 6.82-6.68 (m, 8H, H-ar), 6.53-6.38 (m, 8H, H-ar), 4.79-4.74 (m, 4H, H-1), 4.73-4.59 (m, 8H, OC*H*$_2$-triaz), 4.50-4.36 (m, 8H, OCH$_2$C*H*$_2$-triaz), 3.91-3.84 (m, 4H, H-2), 3.83-3.60 (m, 28H, H-3, H-4 or H-5, H-6, Man-OC*H*$_2$ and OC*H*$_2$CH$_2$-triaz), 3.56-3.52 (m, 4H, H-4 or H-5), 3.51-3.36 (m, 28H, Man-OC*H*$_2$ and OCH$_2$).

^{13}C NMR (100 MHz, D_2O) δ (ppm) = 156.2 (C^{IV}-ar), 142.9 (C^{IV}-triaz), 138.6 (C=C), 136.9 (C^{IV}-ar), 132.3 (CH-ar), 124.9 (CH-triaz), 114.0 (CH-ar), 99.87 (C-1), 72.7 (C-4 or C-5), 70.5 (C-3), 70.0 (C-2), 69.6 (OCH$_2$), 69.52 (OCH$_2$), 69.47 (OCH$_2$), 68.7 (O*C*H$_2$CH$_2$-triaz), 66.6 (C-4 or C-5), 66.2 (Man-O*C*H$_2$), 60.8 (C-6 and O*C*H$_2$CH$_2$-triaz), 50.0 (OCH$_2$*C*H$_2$-triaz).

HR-ESI-QTof (positive mode) *m*/*z*: calcd for $C_{86}H_{122}N_{12}O_{36}$ $[M+2H]^{2+}$ 949.4037, found 949.4005.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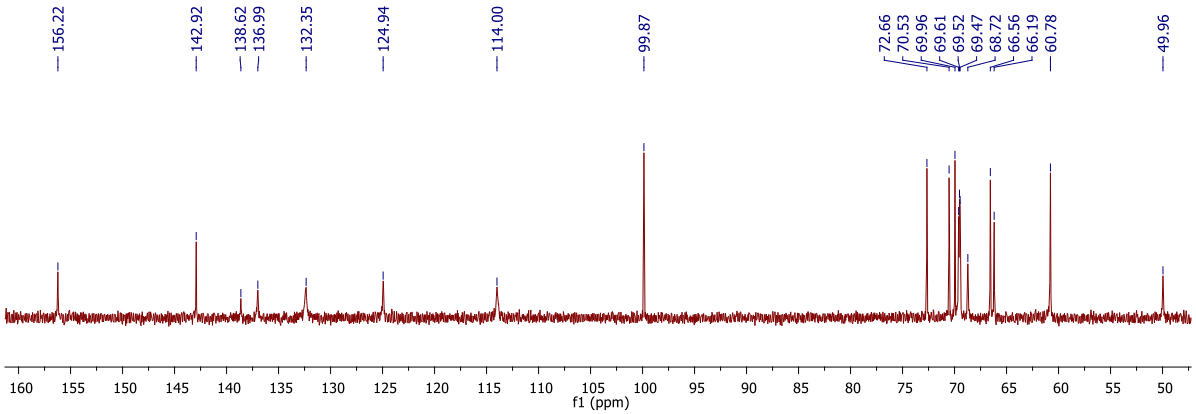
f1 (ppm)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69.47
68.72
66.56
66.19
60.78
49.96
160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50
f1 (ppm)

Fluorescence spectroscopy measurements

Preparation of stock solution. TPE-glycoclusters **4a-d** were suspended in ultrapure water at an initial concentration of 20 mM.

UV-vis. In a typical UV-vis assay, a TPE-glycocluster **4a-d** (with a final concentration of 50 μM) was dissolved in different solvents (DMF, DMSO, EtOH, MeOH or H_2O), followed by an incubation for 30 s. Then, the UV-vis spectrum was measured on a Varian Cary 500 spectrophotometer.

Fluorescence spectroscopy. In a typical fluorescence assay, a TPE-glycocluster **4a-d** (with a final concentration of 50 μM) was dissolved in different solvents (DMF, DMSO, EtOH, MeOH or H_2O), followed by an incubation for 30 s. Then, the fluorescence was measured on a Varian Cary Eclipse fluorescence spectrophotometer.

Concentration-dependence fluorescence spectroscopy of 4b. Compound **4b** at different concentrations (15 μM, 30 μM, 45 μM, 60 μM, 90 μM, 120 μM, 180 μM, 240 μM, 360 μM, 480 μM and 600 μM) was dissolved in deionized water. The fluorescence emission spectra of the resulting glycocluster solutions were measured on an Agilent Cary Eclipse fluorescence spectrophotometer with an excitation wavelength of 340 nm at room temperature.

Size distribution of 4b. Compound **4b** at different concentrations (20 μM, 60 μM, 180 μM, 540 μM) was dissolved in deionized water. The size distribution of the resulting glycocluster solutions was determined on a Horiba LB-550 DLS Nano-Analyzer at room temperature.

High-resolution transmission electron microscopy (HR-TEM). A droplet of Compound **4b** at different concentrations (20 μM, 60 μM, 180 μM, 540 μM) was dropped onto carbon copper grids for HRTEM. JEOL 2100 equipped with a Gatan Orius charged-coupled device camera and Tridiem energy filter operating at 200 kV was used for recording the TEM images, and the data obtained were processed using Image J software.

Isothermal titration microcalorimetry (ITC)

Recombinant lyophilized LecA was dissolved in buffer (20 mM TRIS-HCl, 100 μM $CaCl_2$, 100 mM NaCl, pH 7.5) and degassed. Protein concentration (100 μM) was checked by measurement of optical density by using a theoretical molar extinction coefficient of 28000. Glycoclusters were dissolved directly into the same buffer, degassed, and placed in the injection syringe (concentrations: 240 and 360 μM). ITC was performed using a VP-ITC MicroCalorimeter from MicroCal Incorporated. LecA was placed into the 1.4478 mL sample cell, at 25°C. Titration was performed with 10 μL injections of carbohydrate ligands every 300 s. Data were fitted using the "one-site model" using MicroCal Origin 7 software according to standard procedures. Fitted data yielded the stoichiometry (n), the association constant (K_a), and the enthalpy of binding (ΔH). Other thermodynamic parameters (i.e., changes in free energy ΔG and entropy ΔS) were calculated from the equation $\Delta G = \Delta H - T\Delta S = -RT\ln K_a$ in which T is the absolute temperature and R = 8.314 $J.mol^{-1}.K^{-1}$. Two or three independent titrations were performed for each ligand tested.

Recombinant lyophilized LecB was dissolved in buffer (20 mM TRIS-HCl, 100 μM $CaCl_2$, 100 mM NaCl, pH 7.5) and degassed. Protein concentration (50 μM) was checked by measurement of optical density by using a theoretical molar extinction coefficient of 28000. Glycoclusters were dissolved directly into the same buffer, degassed, and placed in the injection syringe (concentration: 120 μM). ITC was performed using a VP-ITC MicroCalorimeter from MicroCal Incorporated. LecB was placed into the 1.4478 mL sample cell, at 25°C. Titration was performed with 10 μL injections of carbohydrate ligands every 300 s. Data were fitted using the "one-site model" using MicroCal Origin 7 software according to standard procedures. Fitted data yielded the stoichiometry (n), the association constant (K_a), and the enthalpy of binding (ΔH). Other thermodynamic parameters (i.e., changes in free energy ΔG and entropy ΔS) were calculated from the equation $\Delta G = \Delta H - T\Delta S = -RT\ln K_a$ in which T is the absolute temperature and R = 8.314 $J.mol^{-1}.K^{-1}$. Two or three independent titrations were performed for each ligand tested.

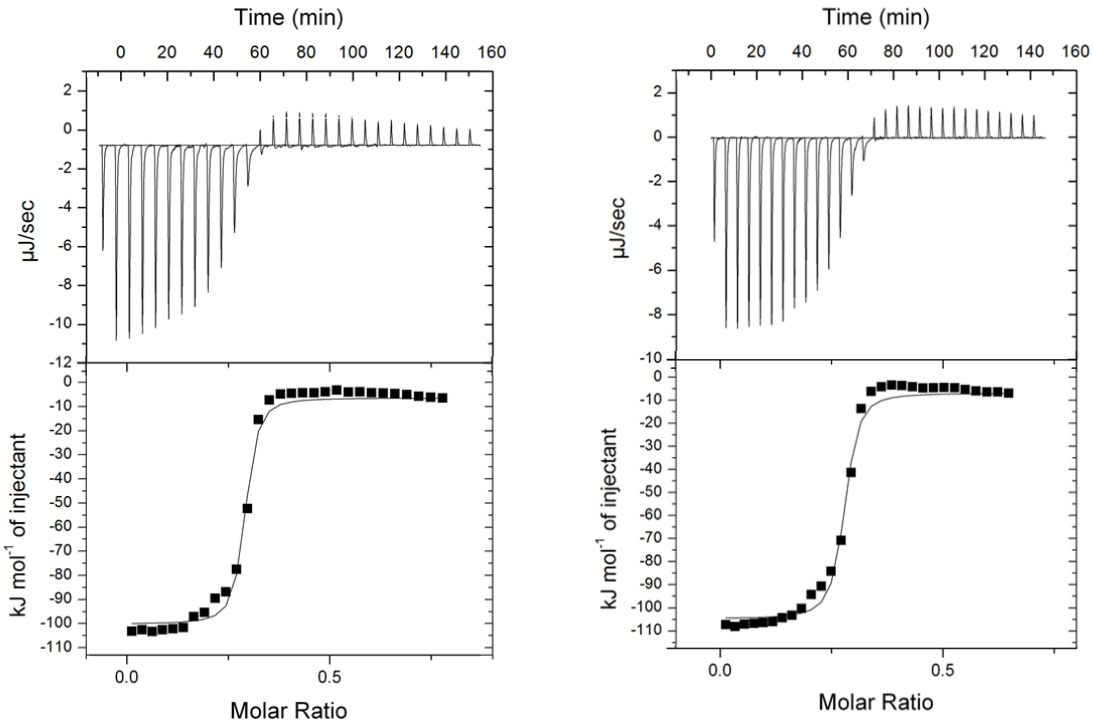

Figure S1. Raw ITC data (top) obtained by injections of glycocluster **4b**: TPE-$(EG_3\text{-Gal})_4$ (0.36 mM for the left panel and 0.30 mM for the right panel) to a solution of LecA (0.1 mM) and the corresponding integrated titration curve (bottom)

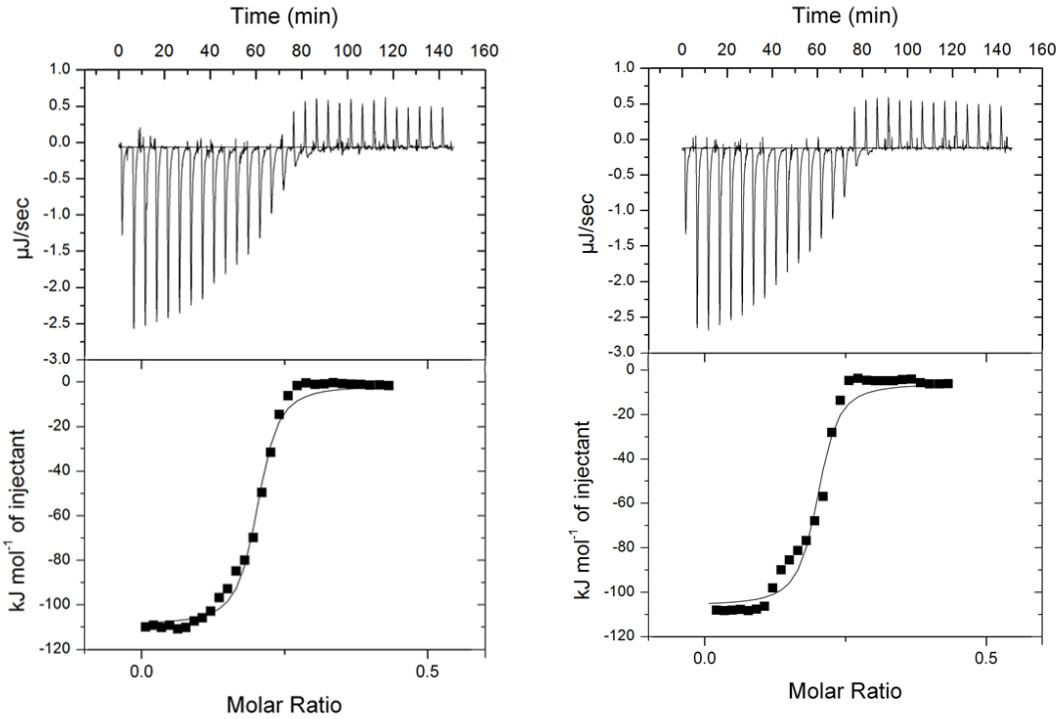

Figure S2. Raw ITC data (top) obtained by injections of glycocluster **4c**: TPE-$(EG_3\text{-Fuc})_4$ (0.1 mM) to a solution of LecB (0.05 mM) and the corresponding integrated titration curve (bottom)

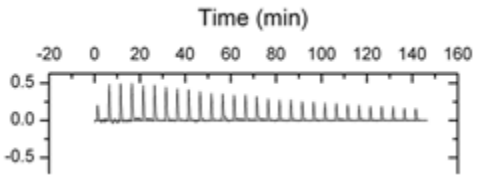

Figure S3. Raw ITC data obtained by injections of glycocluster **4a**: TPE-$(EG_3\text{-}Glc)_4$ (0.1 mM) to a solution of LecA (0.05 mM)

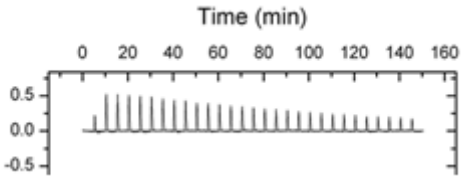

Figure S4. Raw ITC data obtained by injections of glycocluster **4a**: TPE-$(EG_3\text{-}Glc)_4$ (0.1 mM) to a solution of LecB (0.05 mM)

Cell adhesion assays

Adherent A549 cells were cultured in cell culture flasks containing Iscove' s modified Dulbecco' s medium supplemented with 10% (v/v) fetal calf serum (Dustcher, Vilmorin, France) and 1% penicillin/streptomycin. Cells were incubated at 37°C with a 5% (v/v) CO_2 until they formed a confluent monolayer. Cells were seeded in 96-well cell culture plates containing IMDM (10^5 cells/well) and incubated at 37°C. *Pseudomonas aeruginosa* PAO1 strain was used as reference strain. A single colony was inoculated into Lysogeny Broth Lennox media (LB) and grown overnight at 37°C with orbital shaking. Thirty minutes before the infection, cells were washed twice with PBS and IMDM with or without PDI-based glycoclusters (0 or 10 mM) was added to the wells. Bacteria calibrated at a density of 10^8 CFU/mL were incubated with the cells at a multiplicity of infection of 10 bacteria for a cell (MOI 10) for 1 h at 37°C with a 5% (v/v) CO_2. Nonadherent bacteria were removed by washing five times with PBS. Cells were lysed by incubation during 30 min at 37°C with PBS 1% triton-X100 (v/v). Live CFU were enumerated after a 24 h incubation at 37°C, after serial dilution and plating on BCP agar plates.