

Supplementary Information

Combining carbohydrate substitutions at bioinspired positions with multivalent presentation towards optimising lectin inhibitors: case study with calixarenes

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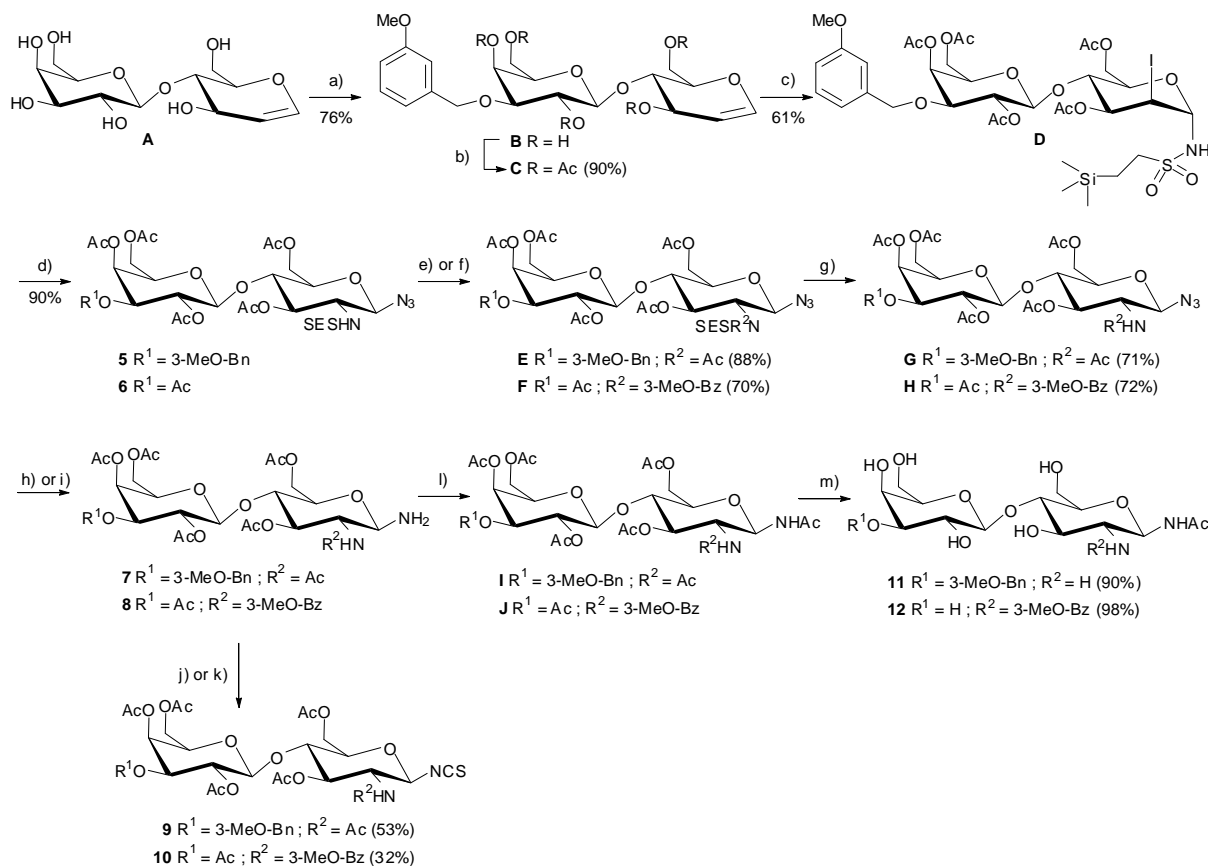
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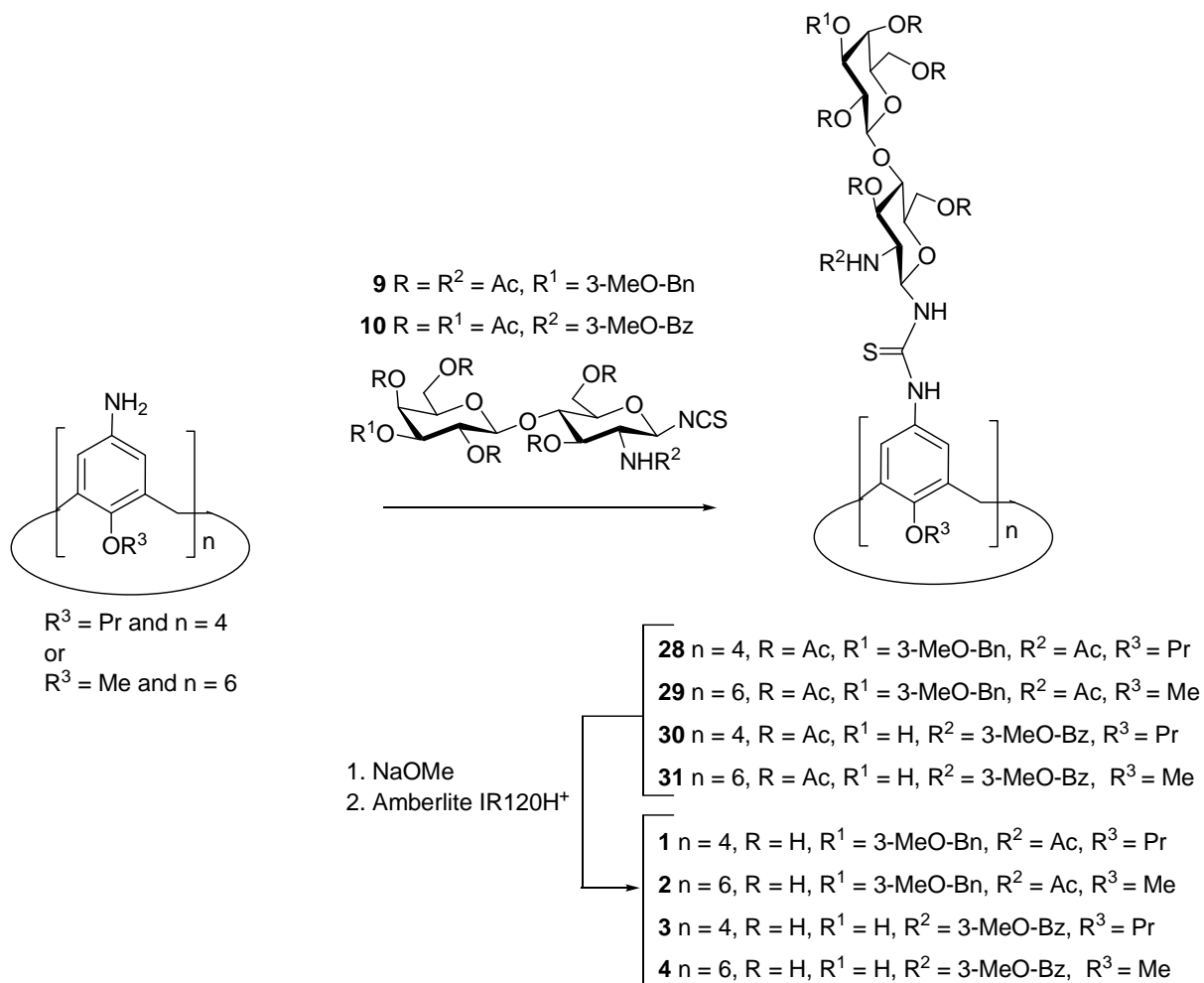
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I. Synthetic route for the preparation of the isothiocyanates and the monovalent references



Scheme. Synthesis of isothiocyanates and reference ligands. *Reagents and conditions:* a) Bu_2SnO (1.1 equiv), MeOH, reflux, 5 h then 3-MeOBnBr (1.2 equiv), DMF, rt, 48 h; b) Ac_2O (7 equiv), Et_3N (6 equiv), DMAP (0.1 equiv), CH_2Cl_2 , rt, 24 h; c) NIS (1 equiv), I_2 (1 equiv), 4 Å MS, CH_2Cl_2 , -10°C to rt, overnight; d) NaN_3 (2 equiv), DMF, 0°C to rt, 3 h; e) Ac_2O (6 equiv), Et_3N (2 equiv), DMAP (0.1 equiv), CH_2Cl_2 , 0°C to 30°C , 3 h; f) 3-MeOBzCl (3 + 3 equiv), Et_3N (2 equiv), DMAP (0.1 equiv), CH_2Cl_2 , 0°C to 30°C , 24 + 24 h; g) Bu_4NF (3 equiv), THF, reflux, 16 h; h) NaBH_4 (2 equiv), cat. $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{EtOH}/\text{CH}_2\text{Cl}_2$ (4.8:1), rt, 1 h; i) H_2 , Pd/C (10%), AcOEt/MeOH (1:1), rt, 3 h; j) thiophosgene (2 equiv), CaCO_3 (3 equiv), THF, 0°C to rt, overnight; k) 1,1'-thiocarbonyldiimidazole (1.5 equiv), CH_2Cl_2 , rt, 6 h; l) Ac_2O (3 equiv), Et_3N (2 equiv), CH_2Cl_2 , 0°C to rt, 2 h; m) MeONa, MeOH, rt, 2 h.

Ib. Synthetic scheme for the preparation of the calixarene based glycoclusters



IIa. Figure 1

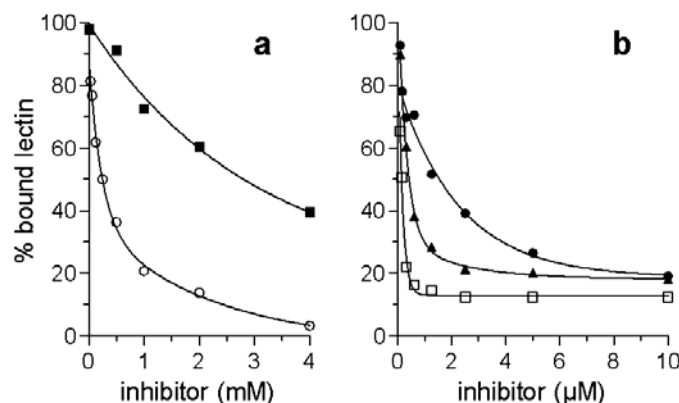


Fig. 1 SI Dependence of inhibition of binding of labelled human galectin-3 to the *N*-glycans of surface-immobilized asialofetuin on concentration of inhibitor (a: ■, lactose / ○, **11**; b: □, **1** / ▲, **2** / ●, **4**). The concentration at an inhibition level of 50% (IC₅₀-value) is determined by this graph (please see Table 1 for complete compilation).

IIb. Figure 2

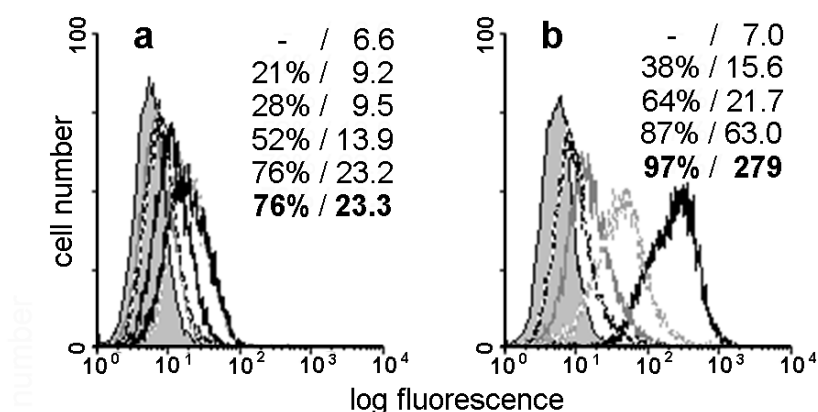


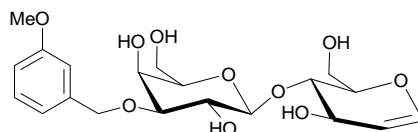
Fig. 2 SI Inhibition of lectin binding (quantitatively expressed as percentage of positive cells/mean fluorescence intensity; the data are listed in each panel in the order of compound listing from bottom to top) to human colon adenocarcinoma SW480 cells by the test compounds, the background control given as gray area, 100% -value as solid black line. (a) VAA (2 μg/ml) in the presence of 1 mM **12**, 1 mM lactose, 1 mM **3** and 1 mM **4**; (b) galectin-3 (10 μg/ml) in the presence of 500 μM **11**, 10 μM **2** and 10 μM **1**. Assays were routinely performed in duplicates with up to five independent series on aliquots of cell suspensions of the same or next passage with standard deviations not exceeding 13.7%.

III. Experimental Details and Characterization Data

General

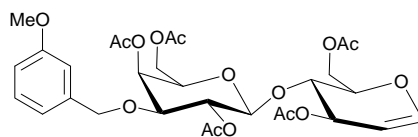
Reaction solvents were purchased anhydrous or distilled using standard procedures (CH₂Cl₂, CH₃CN, MeOH, DMF and THF). Moisture sensitive reactions were performed under nitrogen atmosphere. Solvents for chromatography were distilled before use. Reactions were monitored by TLC using pre-coated silica gel 60 F₂₅₄ plates. Compounds were detected by UV absorption and by staining with vanillin or by oxidation with a 5% (V/V) H₂SO₄ solution in ethanol and subsequent heating at 120 °C. Flash-column chromatography was performed on 230-400 mesh silica gels. ¹H NMR, ¹³C NMR and all multidimensional NMR spectra were recorded on Bruker DPX300, AV300, AV400 or DRX500 spectrometers. Chemical shifts were referenced to the residual proton or carbon resonance of the deuterated solvent. for ¹H NMR spectra recorded in D₂O at 90 °C correction of chemical shifts was performed using the expression $\delta = 5.060 - 0.0122 \times T(^{\circ}\text{C}) + (2.11 \times 10^{-5}) \times T^2(^{\circ}\text{C})$ (H. E. Gottlieb, V. Kotlyar, A. Nudelman, *J. Org. Chem.* **1997**, 62, 7512-7515). HR-ESI MS spectra were recorded on a Waters-Micromass Q-TOF or on a LTQ Orbitrap XL instruments in positive mode with MeOH as solvent. Optical rotations were determined at 589 nm on a Perkin-Elmer Model 343 polarimeter.

1,5-Anhydro-2-deoxy-4-*O*-(3-*O*-(3-methoxybenzyl)-β-D-galactopyranosyl]-D-arabino-hex-1-enitol (**B**)



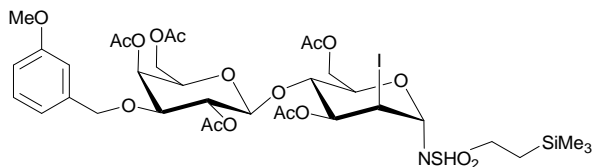
A mixture of D-lactal [1,5-anhydro-2-deoxy-4-*O*-(β-D-galactopyranosyl)- D-arabino-hex-1-enitol **A** (1.75 g, 5.7 mmol) and dibutyltin oxide (1.57 g, 6.25 mmol) in dry MeOH (50 mL) was refluxed for 5 h. Then the solvent was distilled to give a syrup, which was evaporated to dryness under reduced pressure. The residue was dissolved in DMF (10 mL) in the presence of 3-methoxybenzyl bromide (0.96 mL, 6.8 mmol) and stirred under argon at rt for 48 h. The reaction mixture was concentrated under reduced pressure and the crude residue purified by flash-chromatography on silica gel (eluent: CH₂Cl₂:MeOH 99:1 then 95:5) to give compound **B** (1.85 g, 76%); TLC *R_f* = 0.56 (CH₂Cl₂:MeOH 90:10); [α]_D +42 (*c* 0.25 in MeOH); ¹H NMR (300 MHz, CDCl₃) δ 7.30 (1 H, t, *J* = 7.9 Hz), 7.10 (1 H, br s), 7.04 (1 H, d, *J* = 7.6 Hz), 6.88 (1 H, dd, *J* = 1.7 and 8.1 Hz), 6.42 (1 H, dd, *J* = 1.7 and 5.9 Hz), 4.77 (1 H, br d, *J* = 2.1 Hz), 4.81-4.62 (3 H, m), 4.50 (1 H, d, *J* = 7.9 Hz), 4.37 (1 H, dt, *J* = 2.1 and 6.8 Hz), 4.06 (1 H, d, *J* = 2.9 Hz), 3.96 (1 H, dd, *J* = 2.6 and 7.9 Hz), 3.91-3.71 (m, 6 H), 3.83 (3 H, s), 3.60 (1 H, dd, *J* = 4.2 and 7.9 Hz), 3.46 (1 H, dd, *J* = 3.1 and 9.7 Hz); ¹³C NMR (75 MHz, CD₃OD) δ 159.7, 143.9, 139.8, 129.1, 120.0, 113.1 (2 C), 103.6, 102.0, 80.7, 78.5, 77.3, 75.5, 71.1, 70.5, 65.7, 65.7, 61.2, 60.2, 54.5; HR-ESI-MS *m/z* calculated for C₂₀H₂₈O₁₀ (M + Na)⁺ 451.1581, found 451.1591.

1,5-Anhydro-3,6-di-*O*-acetyl-4-*O*-[2,4,6-tri-*O*-acetyl-3-*O*-(3-methoxybenzyl)- β -D-galactopyranosyl]-2-deoxy-D-*arabino*-hex-1-enitol (C)



A mixture of compound **B** (1.02 g, 2.38 mmol), Ac₂O (1.57 mL, 16.7 mmol) Et₃N (2 mL, 14.3 mmol) and DMAP (29 mg, 0.24 mmol) in dry CH₂Cl₂ (20 mL) was reacted at rt for 24 h. The crude reaction mixture was then diluted with CH₂Cl₂ and successively washed with 0.1 N aqueous HCl, aqueous saturated NaHCO₃ and brine. The extract was dried over Na₂SO₄, filtered and concentrated under reduced pressure. Flash-chromatography (eluent: ethyl acetate:cyclohexane 1:1) of the resulting residue provided the peracetylated derivative **C** (1.37 g, 90%); TLC *R*_f = 0.37 (ethyl acetate:cyclohexane 1:1); [α]_D +27.4 (*c* 1 in CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 7.28-7.22 (1H, m), 6.85-6.82 (m, 3 H), 6.40 (1 H, br d, *J* = 6.2 Hz), 5.48 (1 H, d, *J* = 3.3 Hz), 5.39 (1 H, br t, *J* = 3.7 Hz), 5.11 (1 H, dd, *J* = 8.1 and 10.1 Hz), 4.83 (1 H, dd, *J* = 3.3 and 6.2 Hz), 4.66 (1 H, d, *J* = 12.5 Hz), 4.57 (1 H, d, *J* = 8.1 Hz), 4.44-4.36 (2 H, m), 4.20-4.08 (4 H, m), 3.96 (1 H, dd, *J* = 5.5 and 7.3 Hz), 3.84-3.75 (1 H, m), 3.81 (3 H, s), 3.53 (1 H, dd, *J* = 3.3 and 10.1 Hz), 2.16 (3 H, s), 2.10 (3 H, s), 2.09 (3 H, s), 2.07 (3 H, s), 2.04 (3 H, s); ¹³C NMR (75 MHz, CD₃OD) δ 170.5, 170.4, 170.3 (2 C), 169.9, 169.4, 159.8, 145.4, 139.0, 129.4, 119.9, 113.5, 113.2, 101.1, 99.0, 76.9, 74.6, 74.2, 71.1, 70.9, 70.5, 69.0, 65.5, 61.9, 61.6, 55.2, 21.0, 20.8 (2 C), 20.7, 20.6; HR-ESI-MS *m/z* calculated for C₃₀H₃₈O₁₅ (M + Na)⁺ 661.2108, found 661.2133.

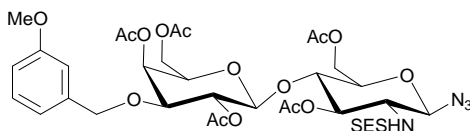
3,6-Di-*O*-acetyl-4-*O*-[2,4,6-tri-*O*-acetyl-3-*O*-(3-methoxybenzyl)- β -D-galactopyranosyl]-1,2-dideoxy-2-iodo-1-[2-(trimethylsilyl)ethanesulfonamido]- α -D-mannopyranose (D)



To a suspension of compound **C** (1.37 g, 2.15 mmol), 2-(trimethylsilyl)ethanesulfonamide (428 mg, 2.36 mmol) and 4 Å MS in dry CH₂Cl₂ (4 mL) was added NIS (483 mg, 2.15 mmol) at -10 °C followed by iodine (545 mg, 2.15 mmol) 2 h later. The reaction mixture was allowed to warm to rt overnight. The mixture was then filtered and diluted with CH₂Cl₂. The filtrate was washed with saturated aqueous Na₂S₂O₃, and brine, dried (Na₂SO₄), filtered and concentrated under reduced pressure. Iodosulfonamide **D** (1.20 g, 61%) was obtained following flash-chromatography (eluent: ethyl acetate:cyclohexane 1:1); TLC *R*_f = 0.60 (ethyl acetate:cyclohexane 6:4); [α]_D +36 (*c* 1 in CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 7.28-7.22 (1 H, m), 6.85-6.82 (3 H, m), 6.34 (1 H, d, *J* = 9.7 Hz), 5.50 (1 H, br d, *J* = 3.4 Hz), 5.32-5.36 (1 H, m), 5.21 (1 H, br t, *J* = 4.0 Hz), 5.09 (1 H, dd, *J* = 8.1 and 10.1 Hz), 4.64 (1 H, d, *J* = 12.4 Hz), 4.50 (1 H, dd, *J* = 3.0 and 7.5 Hz), 4.45 (1 H, d, *J* = 7.2 Hz), 4.37 (1 H, d, *J* = 12.4 Hz), 4.28-4.05 (5 H, m), 3.86 (1 H, br t, *J* = 6.3 Hz), 3.82 (3 H, s), 3.74 (1 H, t, *J* = 4.8 Hz), 3.55 (1 H, dd, *J* = 3.4 and 10.1 Hz), 3.10-2.97 (2 H, m), 2.16 (3 H, s), 2.14 (3 H, s), 2.08 (3 H, s), 2.02 (3 H, s), 1.99 (3 H, s), 1.11-1.01 (2 H, m), 0.04 (9 H, s); ¹³C NMR (75 MHz, CDCl₃) δ 170.8, 170.5, 170.4, 170.0, 169.4, 159.8, 138.9, 129.5, 120.0, 113.6, 113.2, 101.5, 80.5,

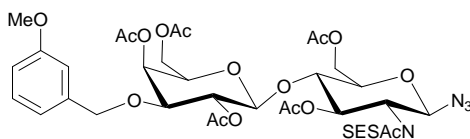
76.2, 75.0, 73.3, 72.1, 71.2 (2 C), 70.5, 65.8, 61.9, 61.3, 55.2, 51.2, 25.8, 20.9 (5 C), 10.3, -1.9 (3 C); HR-ESI-MS m/z calculated for $C_{35}H_{52}INO_{17}SSi$ ($M + Na$)⁺ 968.1668, found 968.1669.

3,6-di-*O*-Acetyl-1-azido-4-*O*-[2,4,6-tri-*O*-acetyl-3-*O*-(3-methoxybenzyl)-β-D-galactopyranosyl]-1,2-dideoxy-2-[2-(trimethylsilyl)ethanesulfonamido]-β-D-glucopyranose (5)



To a solution of compound **D** (1.92 g, 2.02 mmol) in dry DMF (8 mL), cooled at 0 °C, was added NaN₃ (264 mg, 4.04 mmol) as a solid. The mixture was allowed to warm at rt and stirred for 3 h. The reaction mixture was diluted in CH₂Cl₂, washed with 5% aqueous NaHCO₃ and brine. The organic phase was dried over Na₂SO₄, filtrated and concentrated under reduced pressure. Flash-chromatography (eluent: ethyl acetate:cyclohexane 4:6) of the resulting residue provided the azide **5** (1.66 g, 90%); TLC R_f = 0.68 (ethyl acetate:cyclohexane 6:4); $[\alpha]_D$ +9.6 (c 1 in CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 7.26 (1 H, t, J = 7.7 Hz), 6.87-6.82 (3 H, m), 5.53 (1 H, d, J = 9.6 Hz), 5.48 (1 H, br d, J = 3.1 Hz), 5.07 (1 H, d, J = 9.6 Hz), 5.00 (1 H, dd, J = 8.1 and 9.9 Hz), 4.79 (1 H, d, J = 9.2 Hz), 4.66 (1 H, d, J = 12.2 Hz), 4.47 (1 H, br d, J = 11.8 Hz), 4.37 (2 H, dd, J = 2.0 and 10.3 Hz), 4.17-4.06 (2 H, m), 3.99-3.92 (1 H, m), 3.81 (3 H, s), 3.81-3.72 (3 H, m), 3.52 (1 H, dd, J = 3.3 and 9.9 Hz), 3.35 (1 H, q, J = 9.6 Hz), 3.15-2.99 (2 H, m), 2.16 (3 H, s), 2.15 (3 H, s), 2.11 (6 H, s), 2.03 (3 H, s), 1.07-0.96 (2 H, m), 0.05 (9 H, s); ¹³C NMR (75 MHz, CDCl₃) δ 171.3, 170.5, 170.4, 170.2, 169.7, 159.8, 138.8, 129.5, 120.0, 113.5, 113.3, 101.0, 88.9, 76.6, 75.3, 74.2, 73.0, 71.4, 70.9, 70.6, 65.6, 62.0, 61.4, 57.6, 55.2, 50.8, 21.1, 20.8 (2 C), 20.7 (2 C), 10.3, -2.1 (3 C); HR-ESI-MS m/z calculated for $C_{35}H_{52}N_4O_{17}SSi$ ($M + Na$)⁺ 883.2715, found 883.2681.

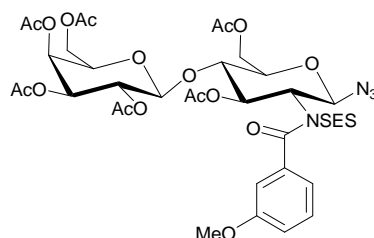
2-*N*-Acetyl-3,6-di-*O*-acetyl-4-*O*-[2,4,6-tri-*O*-acetyl-3-*O*-(3-methoxybenzyl)-β-D-galactopyranosyl]-1-azido-1,2-dideoxy-2-[2-(trimethylsilyl)ethanesulfonamido]-β-D-glucopyranose (E)



To a solution of compound **5** (1.57 g, 1.82 mmol) in dry CH₂Cl₂ (10 mL), cooled at 0 °C, were successively added Ac₂O (690 μL, 7.28 mmol), Et₃N 510 μL, 3.64 mmol) and DMAP (24 mg, 0.18 mmol). The mixture was heated at 30 °C and stirred for 3 h. The reaction mixture was diluted in CH₂Cl₂, washed with 1 N aqueous HCl, saturated aqueous NaHCO₃ and brine. The organic phase was dried over Na₂SO₄, filtrated and concentrated under reduced pressure. Flash-chromatography (eluent: ethyl acetate:cyclohexane 4:6) of the resulting residue provided the acylated derivative **7** (1.85 g, 88%); TLC R_f = 0.56 (ethyl acetate:cyclohexane 6:4); $[\alpha]_D$ +46 (c 1 in CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 7.24-7.18 (1 H, m), 6.84-6.76 (3 H, m), 5.77 (1 H, d, J = 8.7 Hz), 5.72 (1 H, dd, J = 7.8 and 10.1 Hz), 5.46 (1 H, br d, J = 3.2 Hz), 5.02 (1 H, dd, J = 8.1 and 10.1 Hz), 4.64 (1 H, d, J = 12.3 Hz), 4.46-4.31 (3 H, m), 4.19-4.04 (3 H, m), 3.88 (1 H, t, J = 9.8 Hz), 3.79 (3 H, s), 3.80-3.68 (3 H, m), 3.58 (1 H, dd, J = 3.2 and 10.1 Hz), 3.39-3.27 (2 H, m), 2.48 (3 H, s), 2.14 (3 H, s), 2.13

(3 H, s), 2.11 (3 H, s), 2.09 (3 H, s), 2.02 (3 H, s), 1.08-0.94 (2 H, m), 0.06 (9 H, s); ^{13}C NMR (75 MHz, CDCl_3) δ 171.6, 170.5 (2 C), 170.3, 170.1, 169.3, 159.7, 138.9, 129.4, 119.9, 113.4, 113.2, 101.0, 86.4, 77.2, 76.7, 74.6, 71.2, 70.8, 70.7, 69.8, 65.4, 62.3, 62.2, 61.3, 55.2, 52.4, 26.4, 20.8 (4 C), 20.7, 9.97, -2.1 (3 C); HR-ESI-MS m/z calculated for $\text{C}_{37}\text{H}_{54}\text{N}_4\text{O}_{18}\text{SSi}$ ($\text{M} + \text{Na}$) $^+$ 925.2821, found 925.2823.

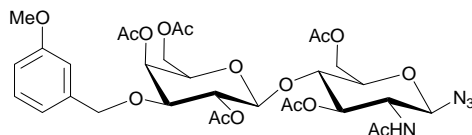
3,6-Di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl)-1-azido-1,2-dideoxy-2-*N*-(3-methoxybenzoyl)-[2-(trimethylsilyl)ethanesulfonamido]- β -D-glucopyranose (F)



To a solution of known compound **6**^a (966 mg, 1.24 mmol) in dry CH_2Cl_2 (13 mL), cooled at 0 °C, were successively added 3-methoxybenzoyl chloride (505 μL , 3.72 mmol), Et_3N (344 μL , 2.48 mmol) and DMAP (15 mg, 0.12 mmol). The mixture was heated at 30 °C and stirred for 24 h. 3-Methoxybenzoyl chloride (505 μL , 3.72 mmol) was then added and the reaction mixture was further heated for another 24 h. The reaction mixture was diluted in CH_2Cl_2 , washed with 1 N aqueous HCl, saturated aqueous NaHCO_3 and brine. The organic phase was dried over Na_2SO_4 , filtered and concentrated under reduced pressure. Flash-chromatography (eluent: ethyl acetate:cyclohexane 4:6) of the resulting residue provided the aromated derivative **F** (794 mg, 70%); TLC R_f = 0.56 (ethyl acetate:cyclohexane 6:4); $[\alpha]_D$ -15.8 (c 1 in CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ 7.65 (1 H, br d, J = 7.7 Hz), 7.56 (1 H, br s), 7.36-7.29 (1 H, m), 7.10 (1 H, dd, J = 2.4 and 8.1 Hz), 5.57 (1 H, d, J = 9.5 Hz), 5.33 (1 H, d, J = 2.6 Hz), 5.07-4.93 (3 H, m), 4.74 (1 H, d, J = 9.2 Hz), 4.52-4.46 (2 H, m), 4.11-4.02 (3 H, m), 3.91-3.75 (2 H, m), 3.82 (3 H, s), 3.33 (1 H, q, J = 9.7 Hz), 3.08-2.94 (2 H, m), 2.13 (3 H, s), 2.11 (3 H, s), 2.09 (3 H, s), 2.03 (3 H, s), 2.01 (3 H, s), 1.94 (3 H, s), 1.04-0.97 (2 H, s), 0.02 (9 H, s); ^{13}C NMR (75 MHz, CDCl_3) δ 171.3, 170.4, 170.4, 170.1, 169.9 (2 C), 169.5, 159.6, 130.8, 129.5, 122.5, 120.1, 114.4, 101.3, 88.8, 75.6, 74.2, 73.0, 70.7, 70.1, 68.7, 61.9, 60.8, 57.6, 55.4, 50.8, 21.1, 20.8, 20.6 (3 C), 20.5, 10.2, -2.5 (3 C); HR-ESI-MS m/z calculated for $\text{C}_{37}\text{H}_{52}\text{N}_4\text{O}_{19}\text{SSi}$ ($\text{M} + \text{Na}$) $^+$ 939.2613, found 939.2617.

^aF.-M. Gautier, F. Djedaini-Pilard and C. Grandjean, *Carbohydr. Res.*, 2011, in press (doi: 10.1016/j.carres.2011.01.022).

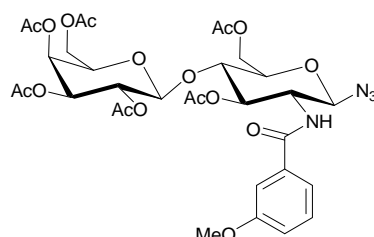
2-Acetamido-3,6-di-*O*-acetyl-4-*O*-[2,4,6-tri-*O*-acetyl-3-*O*-(3-methoxybenzyl)- β -D-galactopyranosyl]-1-azido-1,2-dideoxy- β -D-glucopyranose (G)



To a solution of compound **E** (1.15 g, 1.28 mmol) in dry THF (18 mL) was added Bu_4NF (1.21 g, 3.84 mmol). The mixture was heated at reflux and stirred for 16 h under an argon atmosphere. The reaction mixture was then concentrated under reduced pressure. Flash-chromatography (eluent: ethyl acetate:cyclohexane 6:4 to 8:2) of the resulting residue

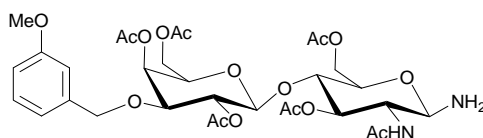
provided the acetamido derivative **G** (670 mg, 71%); TLC R_f = 0.38 (ethyl acetate:cyclohexane 8:2); $[\alpha]_D +7.5$ (c 1 in CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ 7.22 (1 H, t, J = 8.1 Hz), 6.85-6.81 (3 H, m), 6.22 (1 H, d, J = 9.7 Hz), 5.47 (1 H, d, J = 2.7 Hz), 5.07 (1 H, dd, J = 7.8 and 10.1 Hz), 5.02 (1 H, dd, J = 8.2 and 10.1 Hz), 4.60 (1 H, d, J = 12.3 Hz), 4.56-4.46 (2 H, m), 4.42 (1 H, d, J = 8.2 Hz), 4.36 (1 H, d, J = 12.3 Hz), 4.23 (1 H, dd, J = 3.5 and 5.9 Hz), 4.15-4.04 (2 H, m), 3.81 (3 H, s), 3.81-3.68 (2 H, m), 3.52 (1 H, dd, J = 3.2 and 9.0 Hz), 2.17 (3 H, s), 2.10 (6 H, s), 2.09 (3 H, s), 2.04 (3 H, s), 2.04 (3 H, s), 1.99 (3 H, s); ^{13}C NMR (75 MHz, CDCl_3) δ 171.0, 170.5 (3 C), 170.2, 169.4, 159.8, 138.8, 129.5, 119.9, 113.5, 113.2, 101.4, 88.4, 76.6, 75.8, 74.6, 73.1, 71.3, 71.0, 70.7, 68.2, 65.4, 62.1, 61.3, 55.2, 53.0, 23.1, 20.9, 20.7 (4 C); HR-ESI-MS m/z calculated for $\text{C}_{32}\text{H}_{42}\text{N}_4\text{O}_{16}$ ($\text{M} + \text{Na}$) $^+$ 761.2494, found 761.2500.

3,6-Di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl)-1-azido-1,2-dideoxy-2-*N*-(3-(methoxybenzoyl)- β -D-glucopyranose (H**)**



To a solution of compound **F** (750 mg, 0.96 mmol) in dry THF (7 mL) was added Bu_4NF (907 mg, 2.88 mmol). The mixture was heated at reflux and stirred for 16 hours under an argon atmosphere. The reaction mixture was then concentrated under reduced pressure. Flash-chromatography (eluent: ethyl acetate:cyclohexane 1:1) of the resulting residue provided compound **H** (519 mg, 72%); TLC R_f = 0.43 (ethyl acetate:cyclohexane 6:4); $[\alpha]_D -10.8$ (c 0.5 in CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ 7.37-7.18 (2 H, m), 7.15 (1 H, t, J = 7.9 Hz), 5.37-2.26 (2 H, m), 5.04 (1 H, dd, J = 7.6 and 10.3 Hz), 4.96 (1 H, dd, J = 3.3 and 10.3 Hz), 4.62 (1 H, d, J = 9.2 Hz), 4.60-4.48 (2 H, m), 4.36 (1 H, q, J = 9.6 Hz), 4.14-4.02 (3 H, m), 3.96-3.81 (2 H, m), 3.80-3.64 (2 H, m), 3.74 (3 H, s), 2.13 (3 H, s), 2.11 (3 H, s), 2.04 (3 H, s), 2.01 (6 H, s), 1.94 (3 H, s); ^{13}C NMR (75 MHz, CDCl_3) δ 171.4, 170.5, 170.3, 170.2, 170.0, 169.3, 167.5, 159.5, 135.0, 129.5, 118.9, 117.8, 112.7, 101.6, 88.5, 76.6, 74.6, 73.5, 70.9, 70.7, 69.1, 66.6, 61.9, 60.7, 55.3, 53.4, 20.9, 20.8, 20.6 (3 C), 20.5; HR-ESI-MS m/z calculated for $\text{C}_{32}\text{H}_{40}\text{N}_4\text{O}_{17}$ ($\text{M} + \text{Na}$) $^+$ 775.2286, found 775.2278.

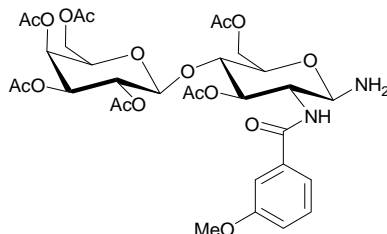
2-Acetamido-3,6-di-*O*-acetyl-4-*O*-[2,4,6-tri-*O*-acetyl-3-*O*-(3-methoxybenzyl)- β -D-galactopyranosyl]-1,2-dideoxy- β -D-glucopyranosyl amine (7**)**



To a solution of compound **G** (650 mg, 0.89 mmol) in CH_2Cl_2 :EtOH (7.7:37 mL) were added NaBH_4 (62 mg, 1.77 mmol) and a catalytic amount of NiCl_2 . The mixture was stirred at rt for 1 h. The reaction mixture was then concentrated under reduced pressure and the residue diluted with CH_2Cl_2 , washed with water (twice) and brine. The organic layer was dried over Na_2SO_4 , filtered and concentrated under reduced pressure to give the amino intermediate **13**

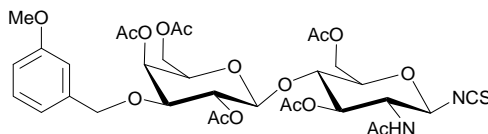
(275 mg, 95%) which was used without further purification; TLC R_f = 0.07 (ethyl acetate:cyclohexane 8:2); ESI-MS m/z ($M + Na$)⁺ 735.1.

3,6-Di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl)-1,2-dideoxy-2-*N*-(3-methoxybenzoyl)- β -D-glucopyranosyl amine (8)



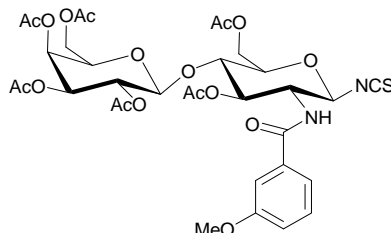
To a solution of compound **H** (400 mg, 0.53 mmol) in ethyl acetate:MeOH 1:1 (18 mL) was added 10% Pd/C (60 mg). The mixture was stirred under a hydrogen atmosphere at rt for 3 h. The reaction mixture was then filtered through a pad of Celite®, washed with MeOH and the filtrate concentrated under reduced pressure to give 385 mg of crude amine **15** which was used without further purification. TLC R_f = 0.22 (ethyl acetate); ESI-MS m/z calculated for $C_{32}H_{42}N_2O_{17}$ ($M + Na$)⁺ 749.2.

2-Acetamido-3,6-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl)-1,2-dideoxy-1-isothiocyanato- β -D-glucopyranose (9)



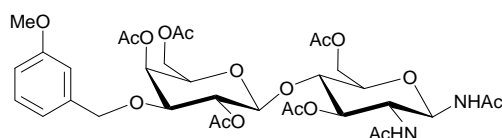
To a suspension of compound **7** (185 mg, 0.29 mmol) and $CaCO_3$ (90 mg, 0.87 mmol) in THF (3 mL), cooled at 0 °C, was added thiophosgene (44 μ L, 0.58 mmol) under an inert atmosphere. The mixture was allowed to warm to rt overnight under stirring. The reaction mixture was then diluted with CH_2Cl_2 and successively washed with cold H_2O , 5% aqueous $NaHCO_3$ and brine. The extract was dried over Na_2SO_4 , filtered and concentrated under reduced pressure. Flash-chromatography (eluent: ethyl acetate:cyclohexane 6:4 then ethyl acetate) of the resulting residue provided the isothiocyanate **9** (105 mg, 53%); TLC R_f = 0.16 (ethyl acetate); $[\alpha]_D^{+21}$ (c 0.5 in $CHCl_3$); 1H NMR (300 MHz, $CDCl_3$) δ 7.26 (1 H, t, J = 8.1 Hz), 6.87-6.81 (3 H, m), 6.29 (1 H, d, J = 9.7 Hz), 5.50 (1 H, dd, J = 0.8 and 3.2 Hz), 5.11 (1 H, dd, J = 7.9 and 8.9 Hz), 5.01 (1 H, dd, J = 8.1 and 10.1 Hz), 4.99 (1 H, d, J = 8.6 Hz), 4.66 (1 H, d, J = 12.2 Hz), 4.48 (1 H, dd, J = 2.7 and 11.8 Hz), 4.41 (1 H, d, J = 8.1 Hz), 4.37 (1 H, d, J = 12.2 Hz), 4.30-4.20 (1 H, m), 4.18-4.08 (3 H, m), 3.82 (3 H, s), 3.82-3.66 (3 H, m), 3.53 (1 H, dd, J = 3.2 and 10.1 Hz), 2.18 (3 H, s), 2.12 (6 H, s), 2.07 (3 H, s), 2.04 (3 H, s), 2.02 (3 H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 170.5 (3 C), 170.2, 169.5, 159.8, 142.5, 138.8, 129.5, 120.0, 113.6, 113.2, 101.3, 84.0, 76.6, 75.4, 74.5, 72.4, 71.3, 71.0, 70.7, 65.4, 62.0, 61.4, 55.2, 54.4, 23.1, 20.8 (5 C); HR-ESI-MS calculated for $C_{33}H_{42}N_2O_{16}S$ ($M + Na$)⁺ 777.2153, found 777.2169.

3,6-Di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl)-1,2-dideoxy-1-isothiocyanato-2-*N*-(3-methoxybenzoyl)- β -D-glucopyranose (10**)**



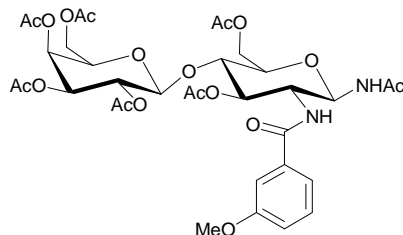
To a suspension of compound **8** (600 mg, 0.82 mmol) in CH_2Cl_2 (8 mL) was added thiocarbonyldiimidazole (220 mg, 1.2 mmol) under an inert atmosphere. The mixture was stirred at rt for 6 h under stirring. The reaction mixture was then diluted with CH_2Cl_2 and successively washed with cold HCl 0.1 N, 5% aqueous NaHCO_3 and brine. The extract was dried over Na_2SO_4 , filtrated and concentrated under reduced pressure. Flash-chromatography (eluent: ethyl acetate:cyclohexane 6:4 then ethyl acetate) of the resulting residue provided the isothiocyanate **10** (208 mg, 32%); TLC R_f = 0.64 (ethyl acetate); $[\alpha]_D^{+21.1}$ (c 4 in CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ 7.47-7.17 (3 H, m), 7.14 (1 H, dt, J = 2.3 and 10.2 Hz), 6.98-6.89 (1 H, m), 5.34-5.27 (2 H, m), 5.12-5.04 (2 H, m), 4.96 (1 H, dd, J = 3.2 and 10.5 Hz), 4.58-4.43 (3 H, m), 4.14-4.01 (3 H, m), 3.94-3.74 (2 H, m), 3.76 (3 H, s), 3.70-3.61 (1 H, m), 2.15 (3 H, s), 2.14 (3 H, s), 2.05 (3 H, s), 2.04 (3 H, s), 2.03 (3 H, s), 1.96 (3 H, s); ^{13}C NMR (75 MHz, CDCl_3) δ 171.3, 170.5, 170.3, 170.2, 170.0, 169.3, 167.7, 159.7, 142.7, 135.1, 129.5, 118.9, 117.9, 112.6, 101.7, 84.2, 76.6, 74.5, 73.3, 70.9, 70.7, 69.1, 66.5, 61.9, 60.6, 55.3, 55.0, 21.0, 20.9, 20.6 (3 C), 20.5; HR-ESI-MS m/z calculated for $\text{C}_{33}\text{H}_{40}\text{N}_2\text{O}_{17}\text{S}$ ($\text{M} + \text{Na}$) $^+$ 791.1945, found 791.1940.

1,2-Di-acetamido-3,6-di-*O*-acetyl-4-*O*-[2,4,6-tri-*O*-acetyl-3-*O*-(3-methoxybenzyl)- β -D-galactopyranosyl]-1,2-dideoxy- β -D-glucopyranose (I**)**



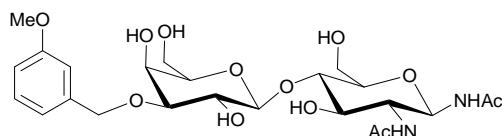
To a suspension of compound **7** (30 mg, 0.042 mmol) and Et_3N (13 μL , 0.09 mmol) in CH_2Cl_2 (500 μL), cooled at 0 $^\circ\text{C}$, was added Ac_2O (13 μL , 0.14 mmol). The reaction mixture was stirred at rt for 2 h and then concentrated under reduced pressure. Flash-chromatography (eluent: ethyl acetate) of the resulting residue provided the di-acetamido derivative **I** (29 mg, 90%); TLC R_f = 0.17 (ethyl acetate); $[\alpha]_D^{+12}$ (c 1 in MeOH); ^1H NMR (300 MHz, CDCl_3) δ 7.27 (1 H, t, J = 8.1 Hz), 7.00 (1 H, d, J = 8.3 Hz), 6.90-6.78 (3 H, m), 6.21 (1 H, d, J = 7.8 Hz), 5.49 (1 H, br d, J = 3.1 Hz), 5.10-4.95 (3 H, m), 4.67 (1 H, d, J = 8.3 Hz), 4.45-4.31 (3 H, m), 4.21-3.96 (4 H, m), 3.83 (3 H, s), 3.80-3.65 (3 H, m), 3.52 (1 H, dd, J = 3.2 and 9.1 Hz), 2.18 (3 H, s), 2.12 (9 H, s), 2.05 (3 H, s), 1.98 (6 H, s); ^{13}C NMR (75 MHz, CDCl_3) δ 172.3, 171.8, 170.8, 170.5 (2 C), 170.2, 169.3, 159.8, 138.9, 129.5, 119.9, 113.4, 113.3, 101.1, 80.2, 76.6, 75.5, 74.3, 73.2, 71.3, 71.0, 70.6, 65.5, 62.3, 61.5, 55.2, 53.6, 23.4, 23.1, 20.9, 20.8 (2 C), 20.7 (2 C); HR-ESI-MS m/z calculated for $\text{C}_{34}\text{H}_{46}\text{N}_2\text{O}_{17}$ ($\text{M} + \text{Na}$) $^+$ 777.2694, found 777.2711.

1-Acetamido-3,6-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1,2-dideoxy-2-(3-methoxy)benzamido-β-D-glucopyranose (J**)**



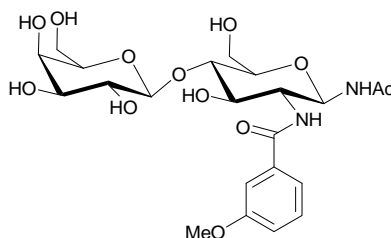
To a suspension of compound **8** (41 mg, 0.056 mmol) and Et₃N (16 μL, 0.11 mmol) in CH₂Cl₂ (500 μL), cooled at 0 °C, was added Ac₂O (16 μL, 0.17 mmol). The reaction mixture was stirred at rt for 2 h and then concentrated under reduced pressure. Flash-chromatography (eluent: ethyl acetate then ethyl acetate:MeOH 95:5) of the resulting residue provided the acetamido derivative **J** (42 mg, 98%); TLC *R*_f = 0.32 (ethyl acetate); [α]_D +42 (*c* 2 in CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 7.36 (1 H, t, *J* = 8.0 Hz), 7.30 (1 H, br s), 7.24 (1 H, br d, *J* = 8.0 Hz), 7.08 (1 H, dd, *J* = 2.4 and 8.0 Hz), 6.96 (1 H, d, *J* = 8.1 Hz), 5.38 (1 H, br d, *J* = 3.3 Hz), 5.25-5.11 (3 H, m), 4.99 (1 H, dd, *J* = 3.5 and 10.5 Hz), 4.55 (1 H, d, *J* = 7.9 Hz), 4.46 (1 H, br d, *J* = 11.8 Hz), 4.30-4.05 (4 H, m), 3.92 (1 H, t, *J* = 6.4 Hz), 3.87-3.82 (1 H, m), 3.86 (3 H, s), 3.78-3.72 (1 H, m), 2.17 (3 H, s), 2.15 (3 H, s), 2.10 (3 H, s), 2.08 (3 H, s), 2.07 (3 H, s), 1.99 (3 H, s), 1.95 (3 H, s); ¹³C NMR (75 MHz, CDCl₃) δ 172.0, 170.9, 170.5, 170.4, 170.1 (2 C), 169.2, 169.0, 159.9, 134.1, 130.0, 119.0, 118.7, 112.2, 101.1, 80.3, 75.9, 74.3, 73.3, 70.9, 70.7, 69.0, 66.7, 62.2, 60.9, 55.4, 54.1, 23.4, 20.9 (2 C), 20.6 (3 C), 20.5; HR-ESI-MS *m/z* calculated for C₃₄H₄₄N₂O₁₈ (M + Na)⁺ 791.2487, found 791.2481.

1,2-Di-acetamido-1,2-dideoxy-4-*O*-[3-*O*-(3-methoxybenzyl)-β-D-galactopyranosyl]-β-D-glucopyranose (11**)**



Compound **I** (28 mg, 0.041 mmol) was dissolved in 0.2 M MeONa in MeOH (1.5 mL) and stirred at rt for 2 h. The reaction mixture was neutralized upon addition of Amberlite IR120 (H⁺) and filtrated. The filtrate was concentrated under reduced pressure and purified by RP-HPLC to provide the di-acetamido derivative **11** (16 mg, 80%); [α]_D +22 (*c* 1 in MeOH); ¹H NMR (300 MHz, CD₃OD/D₂O 4:1) δ 7.25 (1 H, br t, *J* = 8.0 Hz), 6.98-6.92 (3 H, m), 6.88-6.84 (1 H, m), 4.92 (1 H, d, *J* = 9.4 Hz), 4.62 (1 H, d, *J* = 11.7 Hz), 4.51 (1 H, d, *J* = 11.7 Hz), 4.34 (d, 1 H, *J* = 7.7 Hz), 3.97 (1 H, br s), 3.83-3.47 (10 H, m), 3.72 (3 H, s), 3.40 (1 H, br d, *J* = 9.9 Hz), 1.88 (6 H, s); ¹³C NMR (75 MHz, CD₃OD/D₂O 4:1) δ 175.1, 174.9, 159.4, 139.6, 130.2, 121.4, 114.1 (2 C), 103.2, 80.3, 78.7, 78.3, 76.8, 75.6, 73.2, 71.4, 70.5, 65.6, 61.4, 60.2, 55.6, 54.2, 22.3, 22.2; HR-ESI-MS *m/z* calculated for C₂₄H₃₆N₂O₁₂ (M + Na)⁺ 567.2166, found 567.2173.

1-Acetamido-1,2-dideoxy-4-*O*-(β -D-galactopyranosyl)-2-(3-methoxy)benzamido- β -D-glucopyranose (12**)**



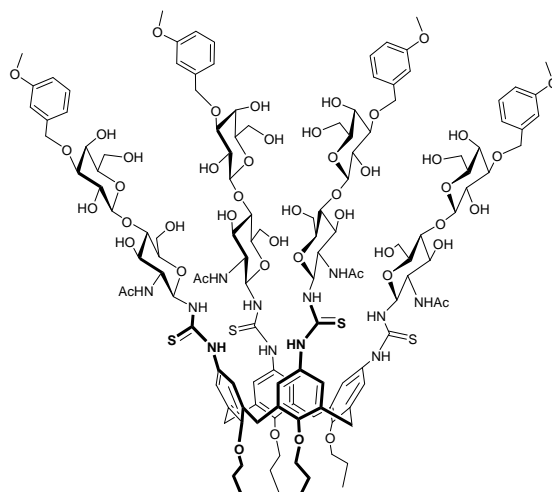
Compound **J** (40 mg, 0.041 mmol) was dissolved in 0.2 M MeONa in MeOH (1.56 mL) and stirred at rt for 2 h. The reaction mixture was neutralized upon addition of Amberlite IR120 (H^+) and filtrated. The filtrate was concentrated under reduced pressure and purified by RP-HPLC to provide compound **12** (20 mg, 74%); $[\alpha]_D^{25} +28.3$ (c 1 in MeOH); 1H NMR (300 MHz, CD_3OD) δ 7.42-7.32 (3 H, m), 7.11 (1 H, dt, $J = 2.2$ and 7.0 Hz), 5.20 (1 H, d, $J = 9.9$ Hz), 4.45 (1 H, d, $J = 7.4$ Hz), 4.12 (1 H, t, $J = 9.9$ Hz), 3.94-3.51 (11 H, m), 3.87 (3 H, s), 1.94 (3 H, s); ^{13}C NMR (75 MHz, CD_3OD) δ 172.5, 169.2, 159.9, 13.7, 129.2, 119.2, 117.2, 112.3, 103.8, 79.4, 79.0, 77.0, 75.8, 73.4, 73.0, 71.2, 69.0, 61.2, 60.4, 54.8, 54.5, 21.4; HR-ESI-MS calculated for $C_{22}H_{32}N_2O_{12}$ ($M + Na$) $^+$ 539.1853, found 539.1835.

General procedure for conjugation between aminocalix[4/6]arenes and glycosylisothiocyanates **9 and **10****

In a two-neck round bottom flask 1 equivalent of the aminocalixarene (5,11,17,23-tetrakis-amino-26,26,27,28-tetrapropoxycalix[4]arene (F. Sansone, E. Chierici, A. Casnati, R. Ungaro, *Org. Biomol. Chem.* **2003**, *1*, 1802-1809) or 5,11,17,23,29,35-hexakis-amino-25,26,27,28,29,30-hexamethoxycalix[6]arene (M. Dudič, A. Colombo, F. Sansone, A. Casnati, G. Donofrio, R. Ungaro, *Tetrahedron* **2004**, *60*, 11621-11626)) was dissolved in 3 ml of CH_2Cl_2 dry under N_2 . Then for each amino group of the calixarene 1.25 equivalent of glycosylisothiocyanate **9** or **10** and 1 equivalent of triethylamine were added. The mixture was allowed to react at room temperature under N_2 overnight and then the solvent evaporated under reduced pressure.

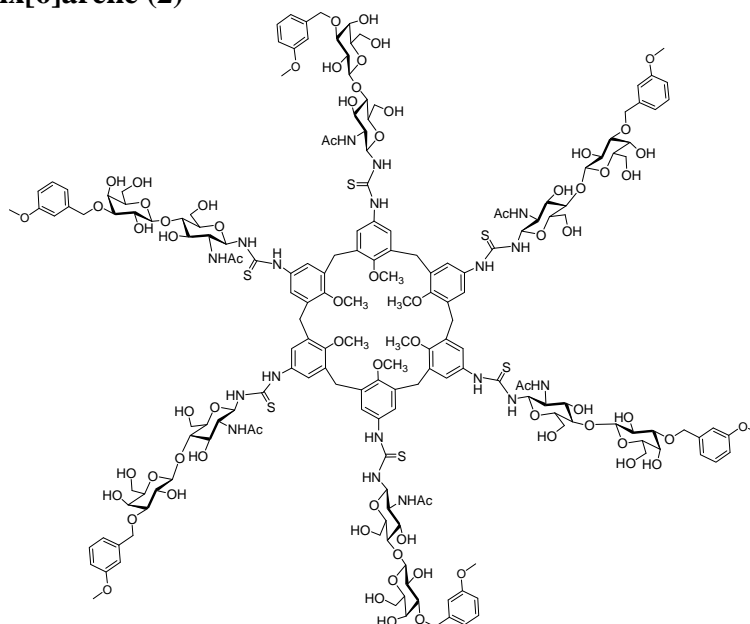
Each compound obtained as follows: **28**, via preparative layer on silica gel (hexane/AcOEt/MeOH 5:5:1); **29**, via preparative layer on silica gel (CH_2Cl_2 /MeOH 95:5 and then CH_2Cl_2 /AcOEt/MeOH 5:5:0.7; **30**, via flash column chromatography (CH_2Cl_2 /MeOH 97.5:2.5) followed by preparative layer on silica gel (hexane/AcOEt/MeOH 5:5:1); **31**, via flash column chromatography (hexane/AcOEt/MeOH from 5:5:1 to 6:3:1) followed by preparative layer on silica gel (hexane/AcOEt/MeOH 6:4:1). Purified acetylated products **28-31** were then dissolved in MeOH and drops of a freshly prepared methanol solution of MeONa were added till pH 8-9. The mixture was stirred at room temperature overnight. When a precipitate was observed, H_2O was added to help complete solubilisation. Amberlite resin IR120 (H^+) was subsequently added for quenching. After neutralization, the resin was filtered off and the solvent removed from the filtrate under vacuum to give pure products (**1-4**).

5,11,17,23-Tetrakis[2-acetamido-1,2-dideoxy-4-*O*-[3-*O*-(3-methoxybenzyl)- β -D-galactopyranosyl]]- β -D-glucopyranosyl]thioureido-25,26,27,28-tetrapropoxycalix[4]arene (1)



Compound **1** was obtained as a white solid in 44% yield. $^1\text{H-NMR}$ (300MHz, CD_3OD , 333K): δ 7.21 (t, $J = 7.8\text{Hz}$, 4H), 7.06 (s, 4H), 6.99 (d, $J = 6.9\text{Hz}$, 4H), 6.88-6.82 (m, 8H), 6.63 (sb, 4H), 5.61 (d, 4H, $J = 9.3\text{Hz}$, H-1), 4.77-4.64 (m, 8H), 4.50 (m, 8H), 4.05-3.50 (m, 64H), 3.41 (d, $J = 6\text{Hz}$, 4H), 3.24 (d, $J = 13.2$, 4H), 1.98 (m, 20H), 1.05 (t, $J = 6.9\text{Hz}$, 12H); $^{13}\text{C-NMR}$ (400MHz, CD_3OD , 298K): δ 180.9, 173.1, 159.8, 154.4, 140.0, 135.5, 131.0, 128.9, 124.1, 119.7, 112.9, 103.8, 83.9, 80.8, 79.5, 76.7, 75.5, 72.9, 70.9, 70.5, 65.6, 61.1, 60.4, 54.3, 30.6, 23.0, 22.0, 9.4; HR-ESI-MS m/z calculated for $\text{C}_{132}\text{H}_{180}\text{N}_{12}\text{O}_{48}\text{S}_4\text{Na}_2$ ($\text{M} + 2\text{Na}$) $^{2+}$ 1438.03569, found 1438.03564.

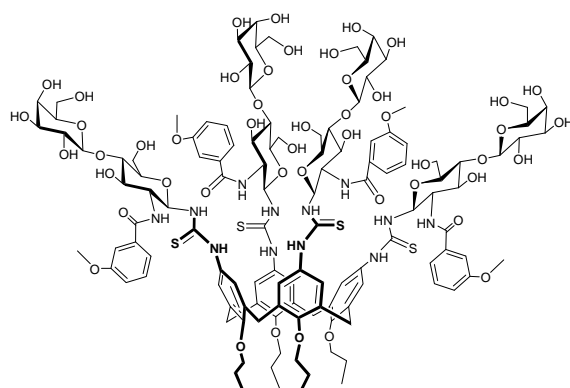
5,11,17,23,29,35-Exakis[2-acetamido-1,2-dideoxy-4-*O*-[3-*O*-(3-methoxybenzyl)- β -D-galactopyranosyl]]- β -D-glucopyranosyl]thioureido-25,26,27,28,29,30-hexamethoxycalix[6]arene (2)



Compound **2** was obtained as a white solid in 22% yield. $^1\text{H-NMR}$ (300MHz, CD_3OD , 333K): δ 7.20 (t, $J = 7.5\text{Hz}$, 6H), 7.10-6.85 (m, 24H), 6.81 (d, $J = 9\text{Hz}$, 6H), 5.54 (d, 6H, $J =$

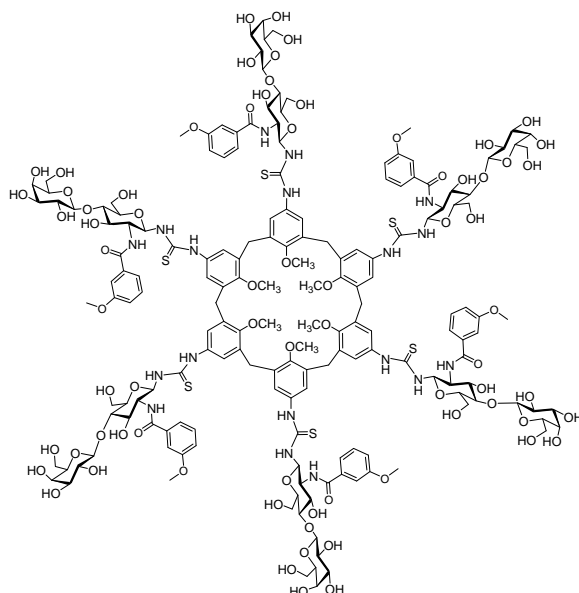
9Hz, H-1), 4.68 (q, $J = 12\text{Hz}$, 12H), 4.42 (bs, 6H), 4.08-3.45 (m, 114H), 3.39 (dd, $J = 6\text{Hz}$, $J = 3\text{Hz}$, 6H), 2.01 (bs, 18H). ^{13}C -NMR (400MHz, CD_3OD , 298 K): δ 182.02, 173.2, 172.9, 159.8, 154.0, 140.0, 134.6, 128.9, 125.8, 119.7, 112.9, 103.9, 83.3, 80.8, 79.8, 76.6, 75.5, 72.8, 71.0, 70.4, 66.7, 65.6, 61.1, 60.6, 60.1, 54.3, 29.2, 21.7; HR-ESI-MS m/z calculated for $\text{C}_{186}\text{H}_{245}\text{N}_{18}\text{O}_{72}\text{S}_6\text{Cl}$ ($\text{M} - \text{H} + \text{Cl}$) $^{2-}$ 2055.70770, found 2055.71074.

5,11,17,23-Tetrakis[1,2-dideoxy-4-*O*-(β -D-galactopyranosyl)-2-(3-methoxy)benzamido- β -D-glucopyranosyl]thioureido-25,26,27,28-tetrapropoxycalix[4]arene (3)



After filtering off the Amberlite resin IR120 H^+ , the crude was purified via trituration in ether to give compound **3** as a white solid in 55% yield. ^1H -NMR (300MHz, D_2O , 348K): δ 7.21-6.86 (m, 12H), 6.61 (bs, 4H), 6.21 (bs, 4H), 6.00 (bs, 4H), 5.68 (bs, 4H, H-1), 4.37 (bs, 4H, H-1'), 4.01-3.21 (m, 72H), 2.57 (bs, 4H), 1.58 (bs, 8H), 0.69 (bs, 12H). ^{13}C -NMR (400MHz, $\text{CD}_3\text{OD}/\text{D}_2\text{O}$ 9:1, 298K): δ 181.8, 169.7, 159.6, 155.2, 135.3, 129.7, 125.6, 125.1, 119.5, 117.5, 113.0, 103.6, 83.9, 79.4, 76.7, 75.6, 73.2, 72.8, 71.2, 68.8, 62.8, 61.1, 60.5, 55.0, 54.3, 30.1, 23.0, 9.5. HR-ESI-MS m/z calculated for $\text{C}_{124}\text{H}_{164}\text{N}_{12}\text{O}_{48}\text{S}_4\text{Na}_2$ ($\text{M} + 2\text{Na}$) $^{2+}$ 1381.97309, found 1381.97449.

5,11,17,23,29,35-Exakis[1,2-dideoxy-4-*O*-(β -D-galactopyranosyl)-2-(3-methoxy)benzamido- β -D-glucopyranosyl]thioureido-25,26,27,28,29,30-hexamethoxycalix[6]arene (4)



Compound **4** was obtained as a white solid in 42% yield. ^1H -NMR (400MHz, $\text{CD}_3\text{OD}/\text{D}_2\text{O}$ 3:1, 333K): δ 7.40-7.20 (m, 18H, Ar), 7.04 (bs, 6H, Ar), 6.67 (bs, 12H), 5.77(d, 6H, $J = 7.2\text{Hz}$, H-1), 4.49 (bs, 6H, H-1'), 4.16 (t, 6H, $J = 9.6\text{Hz}$), 4.05–3.45 (m, 102H), 2.67 (bs, 12H). ^{13}C -NMR (400MHz, $\text{CD}_3\text{OD}/\text{D}_2\text{O}$ 3:1, 298 K): δ 182.0, 170.1, 159.4, 155.1, 134.9, 129.8, 126.3, 119.6, 118.0, 112.8, 103.4, 84.0, 79.2, 76.7, 75.6, 73.0, 71.2, 68.8, 61.1, 60.2, 55.2, 30.1. HR-ESI-MS m/z calculated for $\text{C}_{174}\text{H}_{221}\text{N}_{18}\text{O}_{72}\text{S}_6\text{Cl}_2$ ($\text{M} - \text{H} + 2\text{Cl}$) $^{3-}$ 1326.39781, found 1326.40006.

