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Synthesis of Gal determinant epitopes, their glycomimetic variants, and trimeric clusters—relevance to tumor associated antigens and to discordant xenografts

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Abstract—The synthesis of Galα1→3Gal-quinic acid pseudo-trisaccharides and their elaboration into trimeric clusters is described. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Carbohydrates play a critical role in cell surface phenomena during the proliferation of cancer.[1] The presence of so-called tumor associated antigens can in fact be related to tumor progression.[2] The terminal α-Gal residue, particularly in conjunction with other sugar containing oligosaccharides, plays a dominant role in recognition and antibody response in autoimmune processes. For example, Galα1→3Gal (B disaccharide) **1** epitopes are more abundantly expressed on cell surfaces of human breast cancers, compared to normal cells.[3] The overexpression of other tumor associated antigens such as GalNAc (Tn)[4,5] and Galβ1→3GalNAc (T),[3,6] has been linked to certain types of tumors.[7] In a recent paper, we reported on the synthesis of trimeric, clustered Tn and T motifs on a pentaerythritol scaffold.[8,9] The potential importance of clustered antigens for cooperative binding to protein receptors such as antibodies has been previously reviewed.[10] Another relevant epitope is associated with the Galα1→3Galβ1→4GlcNAc (linear B type 2 trisaccharide) which interacts with natural IgG, IgM and IgA antibodies.[11] The overexpression of this trisaccharide epitope on cell surface glycoproteins may illicit an anti-Gal antibody mediated autoimmune response.[12]

Of great interest is the identification of the Galα1→3Gal and Galα1→3Galβ1→4GlcNAc, epitopes on all tissue of pig.[13] Thus, the prospects of this epitope being recognized by natural anti-αGal antibodies will have major consequences on the success of pig-to-human organ xenotransplantation.[14] In an ideal scenario, the depletion of anti-αGal antibodies from an organ recipient's blood before (if at all) using a suitable formulation of the above mentioned disaccharide and trisaccharide would then set the stage for the administration of immunosuppressant drugs, thus minimizing the risk of humoral immune rejection. The involvement of Gal epitopes in important cell surface phenomena with obvious practical therapeutic consequences has revived an interest in their synthesis.

In this paper, we report on a synthesis of the 3-aminopropyl glycoside of the linear B type 2 trisaccharide **1**, an analog in which the GlcNAc unit is replaced by a carbocyclic variant derived from quinic acid **2**, and the deployment of the pseudo-trisaccharide **2** as a trimeric cluster on a pentaerythritol scaffold,[8,15] **3** (Fig. 1).

Several syntheses of the linear B type 2 trisaccharide have been recorded during the past two decades.[16–22] For example in one case, the Galα1→3Gal unit was prepared first using halide ion catalyzed α-galactosylation.[16] A shorter sequence[19] involving 15 steps relied on D-lactal as a precursor to the Galβ1→4GlcNAc unit. Enzymes, galactosidase[21] or α(1→3)Gal transferase,[22] were used as well for the construction of the Galα(1→3) linkage. In the preceding paper, we reported on practical syntheses of 6-aminohexyl glycosides of Galα1→3Gal and Galα3Gal→4GlcNAc.[23]

The synthesis of the Galα1→3Gal donor **6**[24] was realized utilizing methodology developed in our laboratory for α-glycosylations.[25] Thus, treatment of the acceptor **5**[16] with a slight excess of donor **4** in the presence of $Cu(OTf)_2$ as promoter led to the formation of the desired disaccharide **6** as a single anomer in 75% yield (Scheme 1). Coupling to a

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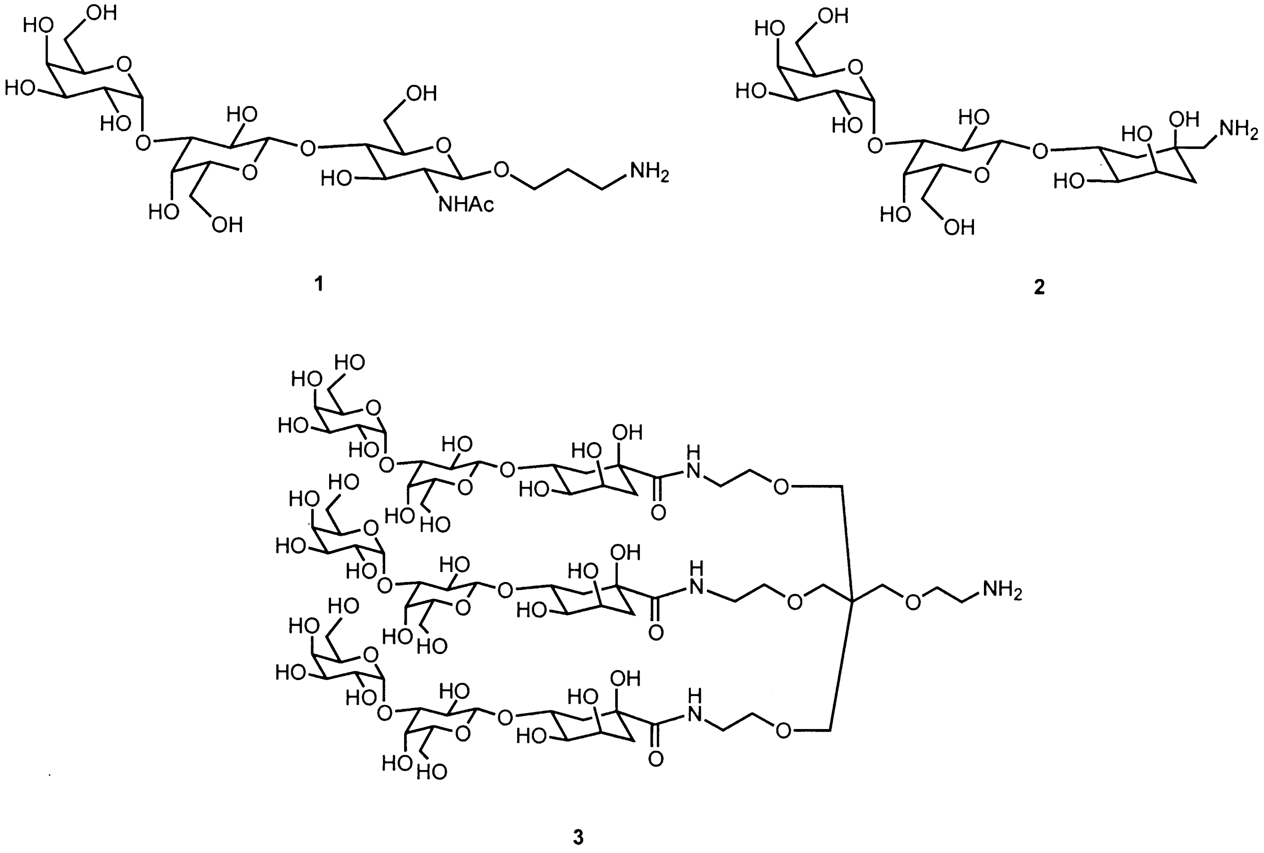


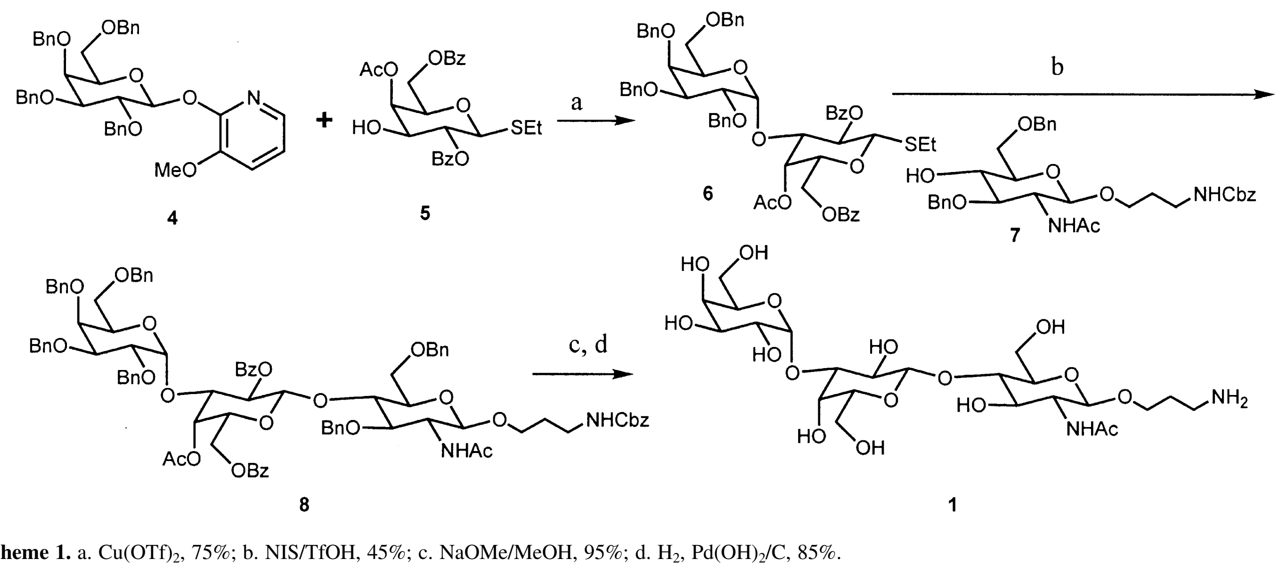
1 **2** **3**

Figure 1.

pre-formed GlcNAc acceptor **7** using NIS as a promoter[26] afforded the protected trisaccharide **8** in modest yield. Finally, deprotection under standard conditions gave the desired 3-aminopropyl trisaccharide **1** as an amorphous solid.

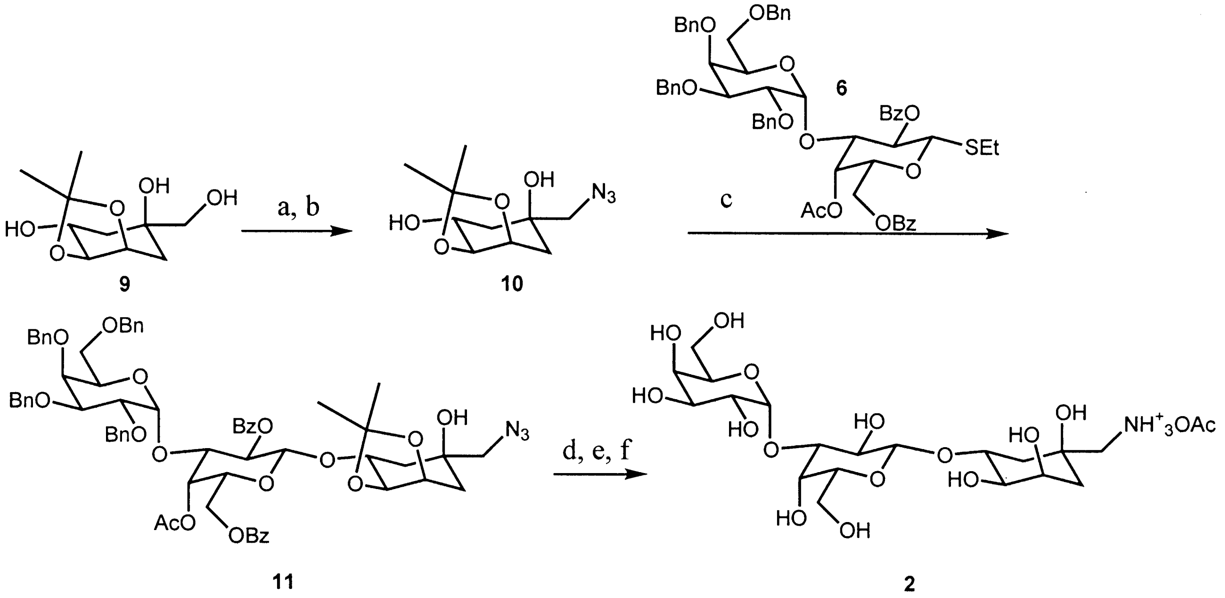
We next considered the replacement of the GlcNAc unit in **2** with a carbocyclic spatial mimic derived from quinic acid (Scheme 2).[27] The known intermediate **9**,[27,28] readily available in two steps, was transformed into the azidomethyl derivative **10** using tin-mediated selective tosylation[29] and displacement of the primary tosylate with sodium azide. Coupling with the thioethyl disaccharide **6** as a glycosyl donor proceeded in excellent yield to afford the pseudo-trisaccharide **11**. Systematic removal of the protective groups gave the intended carbocyclic analog **2** in excellent overall yield.

The recognition of compound **2** by natural anti-α-Gal antibodies of pooled human AB-serum was tested by a

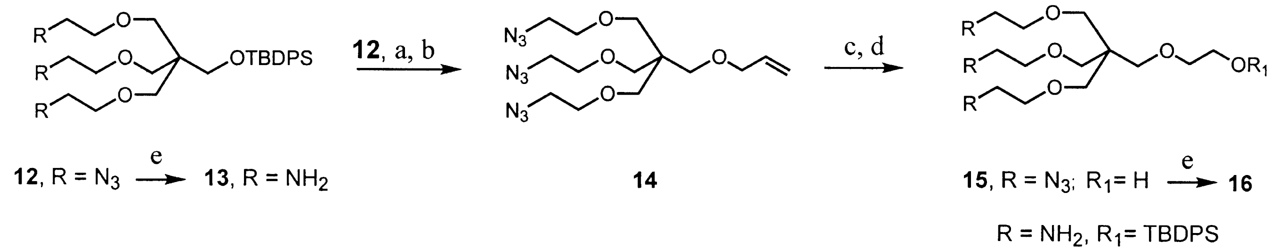


4 **5** **6** **7** **8** **1**

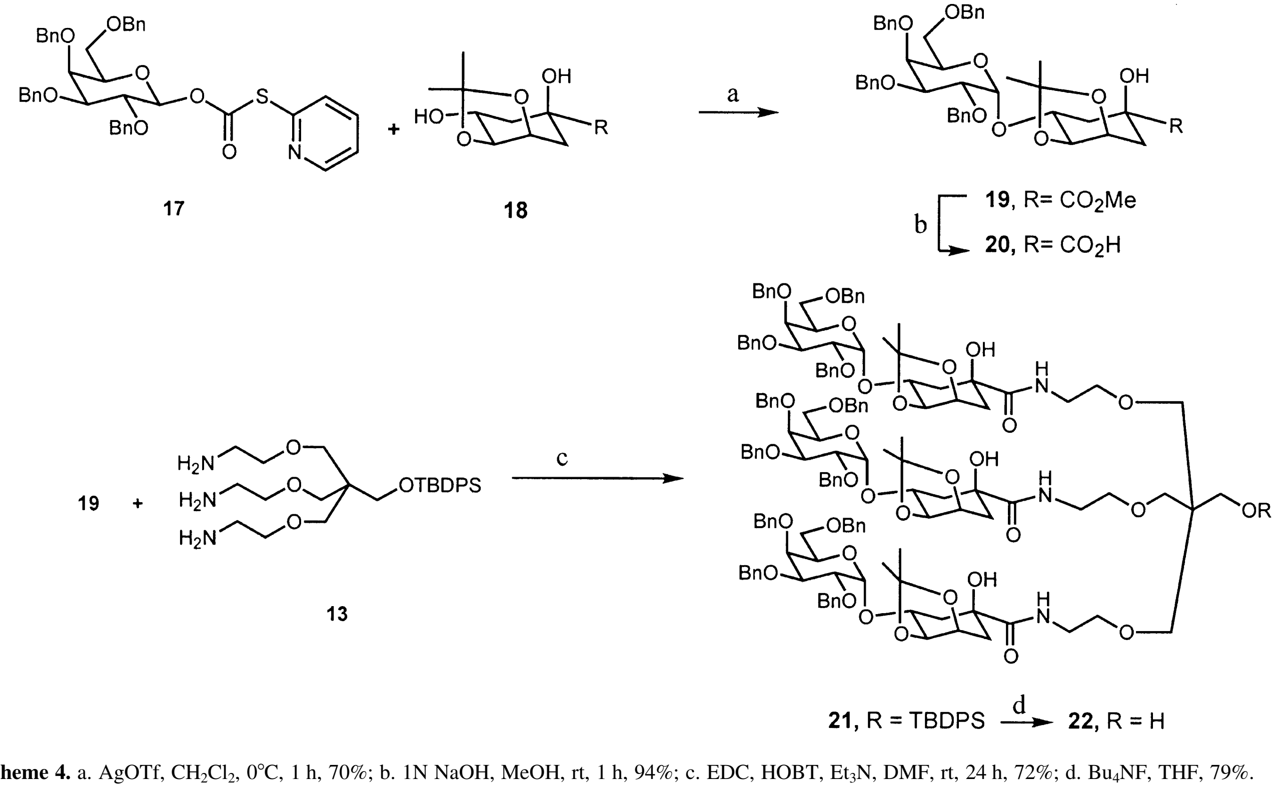
Scheme 1. a. $Cu(OTf)_2$, 75%; b. NIS/TfOH, 45%; c. NaOMe/MeOH, 95%; d. H_2, $Pd(OH)_2/C$, 85%.



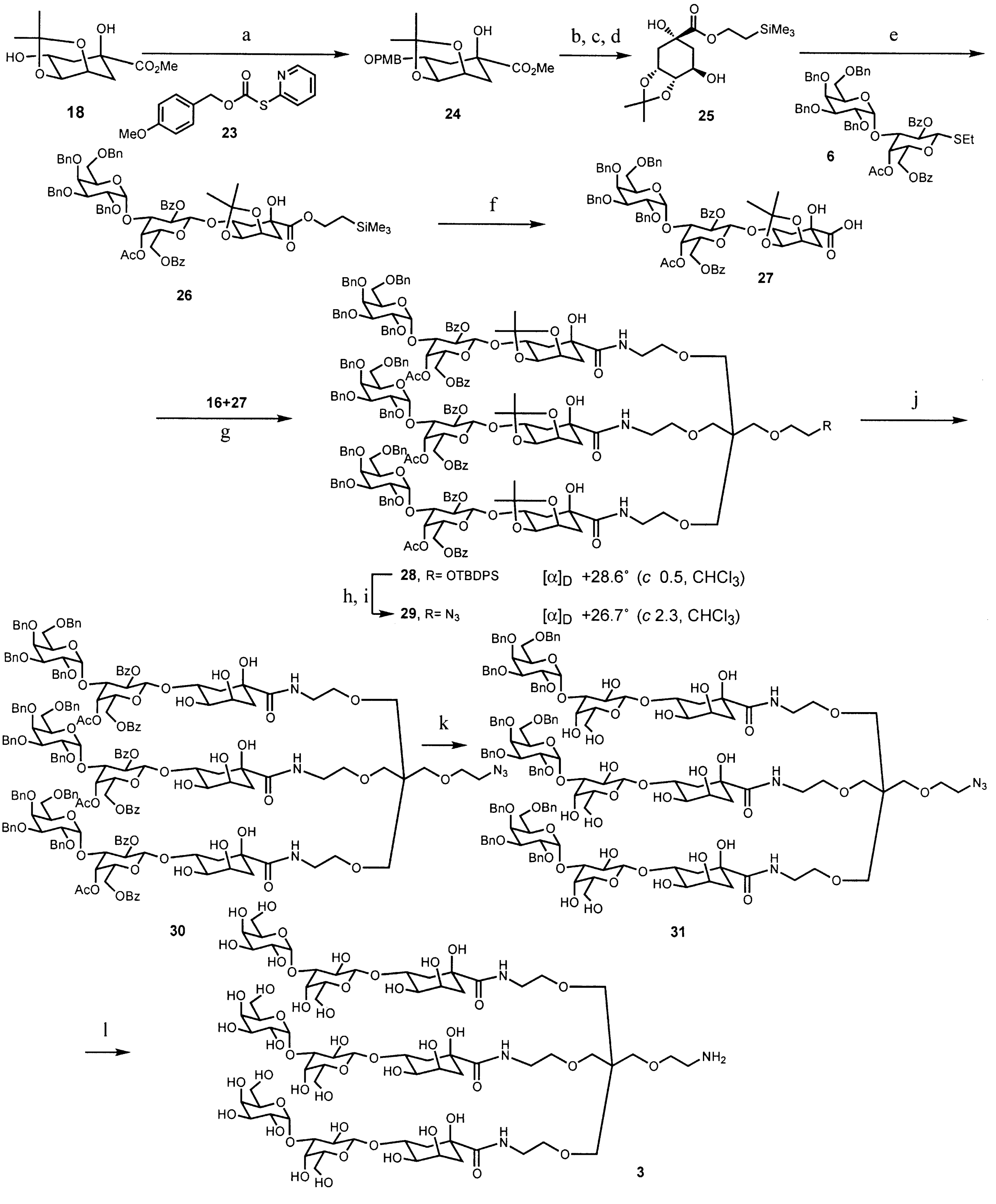
Scheme 2. a. Bu_2SnO, then TsCl, 90%; b. NaN_3, 65%; c. NIS/TfOH, 90%; d. 80% aq., AcOH, 88%; e. NaOMe/MeOH, 85%; f. H_2, $Pd(OH)_2/C$, 90%.



Scheme 3. a. TBAF, THF, rt, 24 h, 88%; b. allyl bromide, NaH, DMF, rt, 10 h, 78%; c. O_3, CH_2Cl_2, then $NaBH_4$, MeOH, 30 min., 81%; d. TBDPSCl, Imidazole, DMAP, rt, 12 h, then Pd/C, H_2, MeOH, rt, 10 h, 71%; e. Pd/C, H_2, MeOH, rt, 10 h, 70%.



Scheme 4. a. AgOTf, CH_2Cl_2, 0°C, 1 h, 70%; b. 1N NaOH, MeOH, rt, 1 h, 94%; c. EDC, HOBT, Et_3N, DMF, rt, 24 h, 72%; d. Bu_4NF, THF, 79%.



Scheme 5. a. AgOTf, CH_2Cl_2, 0°C, 3 h, 70%; b. 1 N NaOH, MeOH, rt, 1 h, 90%; c. Trimethylsilylethanol, EDC, HOBT, Et_3N, DMF, rt, 24 h, 71%; d. DDQ, CH_2Cl_2, H_2O, rt, 1 h, 87%; e. NIS, TfOH, CH_3CN, −40°C, 1 h, 71%; f. TBAF, THF, rt, 3 h, 90%; g. EDC, HOBT, Et_3N, DMF, rt, 24 h, 74%; h. TBAF, THF, rt, 12 h, 75%; i. MsCl, Pyridine, CH_2Cl_2, then NaN_3, DMF, 60°C, 6 h, 81%; j. AcOH-H_2O (4:1), 60°C, 75%; k. LiOH, eq. MeOH, 60°C, 68%; l. $Pd(OH)_2/C$, H_2, aq. dioxane, 67%.

competitive enzyme-linked immunosorbent assay (ELISA), with a high-affinity polyvalent α-galactoside conjugate as primary coating reagent, and horseradish peroxidase labeled anti-IgG or anti-IgM antibodies as secondary reagents. Interestingly, the pseudo-trisaccharide **2** (IC_{50} 19 μM/IgG, 2000 μM/IgM) was found to be qualitatively equal in its binding properties compared to the trisaccharide **1** (IC_{50} 15 μM/IgG, 1100 μM/IgM).[30]

The design of an oligomeric cluster that deploys pseudo-diand trisaccharides related to B disaccharide and linear B type 2 trisaccharide was based on previous experience in our group using pentaerythritol as a scaffold (Scheme 3).[8,31] Pentaerythritol was transformed to the tri-azidoethyl ether derivative **12** as previously described.[15] Reduction of the azido groups led to the triamine **13**. Desilylation of **12** and attachment of the allyl group afforded **14**, which was transformed into **15** using standard methodology. Protection of the primary alcohol and reduction of the azide groups gave the triamine **16**. To explore an alternative α-galactosylation method, we utilized the crystalline 2-pyridylthiocarbonate (TOPCAT) donor **17**[32] in conjunction with an acceptor derived from quinic acid **18**. Using AgOTf as promoter,[33] the pseudo-disaccharide **19** was obtained in 70% yield (Scheme 4). Hydrolysis to the acid **20** and coupling with the triamine **13** in the presence of EDC and HOBT led to the trimeric cluster **21** in 72% yield. Removal of the TBDPS group with fluoride ion afforded the corresponding alcohol, **22**.

Having established the conditions for amide coupling with the triamine **13** and the pseudo-disaccharide **20**, we proceeded to elaborate the corresponding pseudo-trisaccharide trimer (Scheme 5). *p*-Methoxybenzylation of the quinic ester derivative **18** under mild conditions, with *p*-methoxybenzyl 2-pyridylthiocarbonate in the presence of silver triflate,[34] afforded the ether **24**, which was further elaborated to the corresponding trimethylsilylethyl ester **25**. Coupling with the acceptor disaccharide **6** in the presence of NIS as described above led to the expected pseudo-trisaccharide analog **26** in 75% yield. Hydrolysis to the acid **27** and coupling with the triamine **16**, in the presence of EDC and HOBT, gave the fully protected trimer **28** in 74% yield. There remained to introduce an amino group on the pendant hydroxyethyl side-chain, and to remove the protective groups. Desilylation of **28** followed by mesylation and displacement with sodium azide gave the desired azido derivative **29** in excellent overall yield. Sequential removal of the acetonide groups led to **30**, which upon treatment with sodium methoxide gave the partially benzylated target compound **31** in good overall yield. Complete debenzylation with simultaneous reduction of the azido group in **31** proved to be problematic at first. Eventually, it was found that catalytic reduction with 20% $Pd(OH)_2/C$ (Pearlman's catalyst),[35] in aqueous dioxane, and repeating the process again led to the desired unprotected trimeric cluster **3**. The structure of **3** ($C_{70}H_{122}N_4O_{49}$) was ascertained from spectroscopic studies including two-dimensional $^1H-^{13}C$ correlation (HSQC).

In conclusion, we have described methods for the stereoselective α-galactosylations in conjunction with the synthesis of biologically relevant oligosaccharides. In addition, we have shown that the synthesis of pseudo-oligosaccharides related to Galα1→3Gal (B disaccharide) and Galα1→3Galβ1→4GlcNAc (linear B type 2 trisaccharide) provides alternative approaches to the design of glycomimetic motifs. Finally, we have constructed a trimeric clustered 'pseudo-hapten' that can be conjugated to appropriate carriers for the study of their possible immunogenic responses. Further studies in the area will be reported in due course.

2. Experimental

2.1. General information

Solvents were distilled under positive pressure of dry nitrogen before use and dried by standard methods: THF and ether, from Na/benzophenone; and CH_2Cl_2, from $CaCl_2$. All commercially available reagents were used without further purification. All reactions were performed under nitrogen atmosphere. NMR (^1H, ^{13}C) spectra were recorded on AMX-300 and ARX-400 spectrometers. Low- and high-resolution mass spectra were recorded on VG Micromass, Ael-MS902 or Kratos MS-50 spectrometers using fast atom bombardment (FAB) or electrospray techniques. Optical rotations were recorded on a Perkin–Elmer 241 polarimeter in a 1 dm cell at ambient temperature. Analytical thin-layer chromatography was performed on Merck $60F_{254}$ pre-coated silica gel plates. Visualization was performed by ultraviolet light and/or by staining with ceric ammonium molybdate or ninhydrine. Flash column chromatography was performed using (40–60 μm) silica gel at increased pressure.

2.1.1. Ethylthio 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→3)-4-*O*-acetyl-2,6-di-*O*-benzoyl-β-D-galactopyranoside, 6. To a mixture of the glycosyl donor **4**[25] (1.1 g; 1.69 mmol), 0.4 g (0.843 mmol) of the acceptor **5**,[24] activated 4 Å MS, and 30 mL of dry CH_2Cl_2 was added 1.22 g (3.37 mmol) of oven-dried (100°C) $Cu(OTf)_2$ under argon at room temperature. The mixture was stirred for 6 h, three drops of pyridine were added, then concentrated. The residue was purified by flash chromatography on silica gel column (hexanes/EtOAc 1:1) to give 600 mg (75%) of the desired disaccharide **8** as the α-anomer only; $[\alpha]_D=+314.9$ (*c* 0.28, $CHCl_3$); HRMS: *m/z* calcd for $C_{58}H_{60}O_{13}S$ (M+1) 998.1553; found 998.1573.

2.1.2. 3-Benzyloxycarbonylaminoethyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→3)-4-*O*-acetyl-2,6-di-*O*-benzoyl-β-(1→4)-2-acetamido-2-deoxy-3,6-di-*O*-benzyl-β-D-glucopyranoside, 8. To a solution of disaccharide **6** (80 mg; 0.079 mmol) and glycosyl acceptor **7** (45 mg; 0.076 mmol) in dichloromethane (15 mL) were added successively NIS (45 mg; 0.197 mmol) and TfOH (1.5 μL; 0.019 mmol) at −40°C. The reaction mixture was stirred for 1 h, warmed up to room temperature and filtered through Celite, then washed with dichloromethane. The filtrate was washed successively with $Na_2S_2O_3$, $NaHCO_3$, brine, dried over Na_2SO_4, the filtrate concentrated and purified by flash chromatography on silica gel (hexanes/EtOAc 2:1) to give the desired trisaccharide **8** (53 mg; 45%). $[\alpha]_D=+29.6$ (*c* 0.57, $CHCl_3$); HRMS: *m/z* calcd for $C_{89}H_{94}O_{21}N_2$ (M+1) 1528.6996; found 1528.6349.

2.1.3. 3-Aminoethyl α-D-galactopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)β-D-2-acetamido-2-deoxy-D-glucopyranoside, 1. To a solution of trisaccharide **8** (50 mg; 0.0324 mmol) in dry methanol (2 mL) was slowly added freshly prepared sodium methoxide at 0°C and the pH of the solution was kept at 9. The solution was stirred at room temperature for 5 h, then neutralized with Amberlite IR-120 (H^+), filtered and concentrated. The residue was purified by flash chromatography on silica gel ($MeOH–CH_2Cl_2$ 1:20) to give 40 mg of the desired product (95%); $[\alpha]_D=+34.8$ (*c*

2.38, $CHCl_3$); HRMS: *m*/*z* calcd for $C_{72}H_{82}O_{18}N_2$ (M+1) 1263.4320; found 1263.1230.

The product (36.0 mg; 0.027 mmol) in methanol (3 mL) was hydrogenolyzed in the presence of 20% $Pd(OH)_2/C$ (20 mg) under pressure (50 psi) for 12 h at room temperature. The mixture was filtered through Celite, the latter washed with methanol and water and the filtrate was lyophilized to give the title product **1** (14.5 mg; 85%); $[\alpha]_D=+59.8$ (*c* 0.59, $CHCl_3$); HRMS: *m*/*z* calcd for $C_{65}H_{78}O_{16}N_2$ (M+1) 1144.3250; found 1144.6330.

2.1.4. (1*R*,3*R*,4*S*,5*R*)-1-Azidomethyl-1,3,4,5-tetrahydroxy-3,4-*O*-isopropylidene-1-cyclohexane, 10. A solution of **9** (0.5 g, 2.29 mmol) in benzene containing Bu_2SnO (0.38 g, 1.5 mmol) was azeotropically distilled at 100°C for 6 h. The mixture was cooled down to 0°C and was treated with tosyl chloride (0.48 g, 2.5 mmol). After being stirred for 30 min at this temperature, the mixture was evaporated to dryness and purified by column chromatography using EtOAc/hexanes (1:1) to give 0.77 g of the corresponding tosylate in 90% yield.

To a solution of the tosylate (0.5 g, 1.63 mmol) in dry DMF (10 mL) was added NaN_3 (0.16 g, 2.4 mmol), and the contents were stirred at 90°C for 12 h. The reaction mixture was cooled to room temperature, diluted with EtOAc (50 mL), the solids were filtered, and the filtrate was washed with brine (3×10 mL). The organic layer was dried, evaporated, and the resultant residue was purified on silica gel using EtOAc/hexanes (3:2) to afford the azido compound **10** (0.40 g) in 65% yield; $[\alpha]_D=107.7$ (*c* 1.0, $CHCl_3$). IR ($CHCl_3$): 2110 cm^{-1} (N_3); ^1H NMR (400 MHz, $CDCl_3$): δ 4.44 (m, 1H, H-5), 4.10 (m, 1H, H-3), 3.92 (t, 1H, *J*=6.0, 12.0 Hz, H-4), 3.3–2.89 (m, 4H), 2.28–1.97 (m, 2H, CH_2), 1.80 (dd, 1H, *J*=3.9, 15.6 Hz), 1.51, 1.35 [2s, 6H, $C(CH_3)_2$]. ^{13}C NMR (100 MHz, $CDCl_3$): δ 109.2, 80.3, 74.9, 72.8, 68.7, 60.5, 39.0, 33.7, 28.2 and 25.6. HRMS: *m*/*z* calcd for $C_{10}H_{18}N_3O_4$ (M^+) 244.1297; found 244.1305.

2.1.5. Pseudo-trisaccharide 11. Compound **10** (35 mg, 0.14 mmol) and disaccharide **6** (170 mg, 0.17 mmol) in dry CH_2Cl_2 (20 ml) were stirred at room temperature in the presence of 4 Å molecular sieves for 5 h. The solution was cooled down to −10°C and NIS (44 mg, 0.19 mmol) followed by TfOH (3 μL, catalytic) were added. The contents were stirred at this temperature for 10 h under an inert atmosphere. The mixture was diluted with CH_2Cl_2 (20 mL), filtered over Celite, and the filtrates were washed successively with 10% $NaHCO_3$, 10% brine solution and the organic phase was dried. Evaporation and purification over silica gel using EtOAc/hexanes (1:3) as eluent gave 150 mg of the title compound **11** in 90% yield; $[\alpha]_D=+51.0$ (*c* 1.0, $CHCl_3$); IR ($CHCl_3$): 2110 cm^{-1} (N_3); ^1H NMR (400 MHz, $CDCl_3$): δ 8.08–8.02 (m, 4H), 7.5–7.13 (m, 26H, aromatic), 5.65 (bs, 1H, H-4′), 5.52 (t, 1H, H-2′), 5.23 (d, 1H, *J*=3.3 Hz, H-1″), 4.79–4.32 (m, 11H), 4.21 (m, 1H), 4.14 (m, 2H), 3.98–3.91 (m, 3H), 3.61 (dd, 1H), 3.45 (t, 1H), 3.27 (m, 2H), 2.96 (q, 2H), 2.04–1.65 (m, 4H), 1.90 (s, 3H), 1.45, 1.26 [2s, 6H, $C(CH_3)_2$]; ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 165.9, 164.7, 138.5, 138.3, 138.2, 133.2, 133.1, 129.7, 129.7, 129.3, 128.4, 128.3, 128.1, 128.0, 127.8, 127.6, 127.5, 127.4, 127.3, 127.2, 108.8, 100.8, 93.5, 78.7, 76.7, 75.5, 74.8, 73.5, 73.3, 73.1, 73.0, 71.6, 71.3, 70.6, 69.8, 69.5, 64.9, 62.3, 60.5, 60.2, 36.1, 33.4, 27.4, 24.8, 20.4 and 14.1; HRMS: *m*/*z* calcd for $C_{66}H_{71}N_3O_{17}$ (M^+) 1177.4783; found 1177.4795.

2.1.6. Pseudo-trisaccharide 2. A solution of **13** (120 mg, 0.1 mmol) in 80% aqueous AcOH was heated at 60°C for 4 h. The solvents were evaporated to dryness and the resultant material was purified on silica gel using EtOAc/hexanes (1:2) as eluent to provide 100 mg of the diol in 88% yield.

To a solution of this compound (100 mg, 0.088 mmol) in MeOH (5 mL) was slowly added freshly prepared NaOMe in MeOH until pH of the reaction reached ∼10. After stirring at 40°C for 16 h, the mixture was cooled down to room temperature, and carefully neutralized at 0°C with Amberlite IR (H^+) resin and filtered through a bed of Celite which was washed with a small amount of water several times. The combined filtrates were evaporated at low bath temperatures and the resulting concentrate was freeze-dried to obtain the polyol precursor (66 mg) in 85% yield. $[\alpha]_D=+43.6$ (*c* 2.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.4–7.16 (m, 20H, aromatic), 4.9–4.6 (m, 7H), 4.54–4.34 (m, 3H), 4.32 (d, 1H, *J*=7.9 Hz, H-1), 4.32 (m, 1H), 4.20 (bs, 1H), 4.15 9m, 1H), 4.06 (dd, 1H), 4.01 (m, 2H), 3.93 (dd, 1H, *J*=7.6, 12.3 Hz), 3.78 (bs, 1H), 3.71 (q, 2H, *J*=9.2, 17.0 Hz), 3.57–3.43 (m, 5H), 3.06 (q, 2H), 2.12.0 (m, 2H), 1.44–1.35 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.3, 137.6, 128.4–127.2, 102.7, 95.7, 79.2, 77.8, 75.8, 74.7, 74.5, 74.4, 74.3, 74.1, 73.3, 72.4, 70.3, 69.2, 68.7, 66.3, 62.3, 60.3 and 39.7. HRMS: *m*/*z* calcd for $C_{47}H_{57}N_3O_{14}$ (M^+) 887.3810; found 887.3740.

To a solution of the above precursor (60 mg, 0.068 mmol) in 80% aq. AcOH/EtOH (1:9, 10 ml) was added 10% $Pd(OH)_2$/C (50 mg) and the suspension was stirred under H_2 at 45 psi in a medium pressure hydrogenator for 16 h. Filtration of the catalyst over Celite, washing the residue with water, evaporation of the combined filtrates, evaporation and freeze-drying gave compound **2** (30 mg) in 90% yield; $[\alpha]_D=+47.1$ (*c* 1.0, H_2O). ^1H NMR (D_2O): δ 5.14 (d, 1H, *J*=3.1 Hz, H-1″), 4.53 (d, 1H, *J*=7.6 Hz, H-1′), 4.23–4.0 (m, 4H), 3.96 (bs, 1H), 3.83 (m, 1H), 3.79–3.62 (m, 9H), 3.29–3.12 (m, 3H), 2.95 (m, 2H), 2.2–1.68 (m, 5H), 1.97 (s, 3H). ^{13}C NMR (D_2O): δ 101.8, 96.0, 77.9, 75.7, 73.3, 72.2, 71.5, 69.9, 69.8, 68.8, 65.5, 64.8, 62.1, 61.8, 61.6, 56.5, 51.3, 44.6, 43.9, 37.4 and 10.8. HRMS: *m*/*z* calcd for $C_{19}H_{35}N_3O_{14}$ (M+1) 501.2057; found 501.2566.

2.1.7. Tri-*O*-(2-azidoethyl)-1-(2-hydroxyethyl)-pentaerythritol, 15. Ozone was bubbled through a solution of **14**[8] (88 mg; 0.2297 mmol) in 4 mL dichloromethane–methanol (1:1) at −78°C until the solution was saturated (10 min). Argon was bubbled through the solution for 15 min to remove the residual ozone and an excess of methyl sulfide was added. The solution was allowed to warm to room temperature and concentrated to a syrup that was dissolved in ethanol (4 mL). Sodium borohydride (35 mg; 0.919 mmol) was added to a cooled solution at 0°C. After stirring for 24 h at room temperature, the solution was acidified with 20% HCl to pH 6, then concentrated to a syrup that was partitioned between ethyl acetate and water. After usual work-up, a syrupy material was purified by silica gel flash chromatography (EtOAc–MeOH 20:1) to give the title product **15** as a syrup (72.1 mg; 81%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.69 (t, 2H), 3.62 (t, 6H), 3.54 (t, 2H), 3.49 (s, 8H), 3.32 (t, 6H), 2.30 (s, 1H, OH). ^{13}C NMR (100 MHz, $CDCl_3$). δ 72.1, 70.4, 69.6, 69.1, 61.3, 50.6, 44.9. HRMS (FAB), calcd for $C_{13}H_{25}O_5N_9$ (M+1) 387.1979; found 387.1995.

2.1.8. Tri-*O*-(2-aminoethyl)-1-*O*-*tert*-butyldiphenylsilyloxyethyl-pentaerythritol, 16. To a solution of **3a** (72 mg; 0.186 mmol) in 4 mL DMF/THF (1:1) were added successively imidazole (76 mg; 1.12 mmol), a catalytic amount of DMAP and *tert*-butyldiphenylchlorosilane (65 μL; 0.372 mmol) at room temperature. The solution was stirred for 24 h, poured into 10 mL water, and extracted twice with diethyl ether. The organic phase was washed with brine and water, and the extracts were dried ($MgSO_4$), filtered, concentrated, and purified by silica gel flash chromatography to give a syrup. This was dissolved in methanol (3 mL) and hydrogenated in the presence of 10% Pd/C (40 mg) for 12 h at room temperature. The mixture was filtered through Celite, washed with methanol, then concentrated to give **16** (72 mg; 71%). ^{1}H NMR (400 MHz, CD_3OD): δ 7.72–7.70 (m, 4H), 7.46–7.38 (m, 6H), 3.82 (t, J=4.53 Hz, 2H), 3.57–3.31 (m, 22H), 2.77 (s, 6H). ^{13}C NMR (100 MHz, CD_3OD): δ 136.7, 134.8, 130.9, 128.8, 74.1, 73.3, 71.4, 70.9, 64.8, 46.8, 41.9, 27.4, 20.2. HRMS (FAB), calcd for $C_{29}H_{49}O_5N_3Si$ (M+1) 548.3441; found 548.3462.

2.1.9. Pseudo-disaccharide methyl ester 19. To a mixture of the glycosyl donor **17**[25] (4.1 g; 6.1 mmol), glycosyl acceptor **18**[8] (1.3 g; 5.28 mmol), and activated powdered 4 Å molecular sieves (200 mg) in 50 mL of CH_2Cl_2 was added silver triflate (2.3 g; 9.1 mmol). The resulting suspension was stirred for 1 h under nitrogen at 0°C, pyridine (100 μL) was added, and the mixture was filtered through Celite and washed with CH_2Cl_2. Concentration of the filtrate and purification by flash chromatography on silica gel (hexanes/EtOAc 1:1) gave the desired glycoside **19** (2.85 g; 70%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45–7.22 (m, 20H, Ph), 5.54 (d, J=3.85 Hz, 1H, H-1, α), 4.97 (d, J=11.41 Hz, 1H), 4.88 (d, J=11.63 Hz, 1H), 4.81 (d, J=11.66 Hz, 1H), 4.78 (d, J=11.70 Hz, 1H), 4.74 (d, J=11.64 Hz, 1H), 4.59 (d, J=11.44 Hz, 1H), 4.51 (d, J=11.51 Hz, 1H), 4.46 (m, 1H), 4.42 (d, J=11.60 Hz, 1H), 4.32 (m, 1H, H-5), 4.22 (dd, J=5.6, 7.3 Hz, 1H), 4.08 (dd, J=3.83, 10.1 Hz, 1H, H-2), 3.98 (m, 2H), 3.93 (dd, J=2.77, 10.2 Hz, 1H, H-3), 3.77 (s, 3H, OMe), 3.60 (m, 2H), 3.45 (s, 1H, OH), 2.26 (m, 2H), 2.07 (m, 1H), 1.55 (s, 3H, Me), 1.39 (s, 3H, Me); ^{13}C NMR (100 MHz, $CDCl_3$); δ 174.9, 138.9, 138.7, 138.6, 137.9, 128.3, 128.2, 128.2, 128.1, 127.9, 127.7, 127.6, 127.5, 127.4, 109.0, 97.3, 79.5, 78.8, 76.3, 75.4, 75.1, 74.7, 74.1, 73.9, 73.4, 73.1, 72.9, 72.6, 69.5, 68.9, 68.6, 60.3, 52.8, 38.4, 34.3, 28.2, 27.9, 26.0, 25.7, 20.9, 14.2. HRMS: m/z calcd for $C_{46}H_{56}O_{11}$ (M+1) 785.9420; found 785.5833.

2.1.10. Pseudo-disaccharide free acid, 20. To the solution of **19** (1.14 g; 1.48 mmol) in 30 mL of methanol was added dropwise 5 mL of aq. NaOH (1N) at 0°C. The solution was stirred for 3 h at room temperature, then neutralized with Amberlite (IR-120$^+$), filtered and concentrated to give desired product **20** as a syrup (1.05 g; 94%). HRMS: m/z calcd for $C_{44}H_{50}O_{11}$ (M+1) 754.6290; found 754.5679.

2.1.11. Trimeric pseudo-disaccharide cluster, 21. To a solution of **20** (749 mg; 0.994 mmol) in DMF (20 mL) were added successively HOBT (161 mg; 1.19 mmol), EDC (228 mg; 1.19 mmol) and triethylamine (207 μL; 1.49 mmol) at 0°C. The solution was stirred for 1 h, then the triamine **13** (100 mg; 0.199 mmol) in 3 mL of DMF was added dropwise to the reaction mixture. The solution was warmed up to room temperature, the stirring was continued for 24 h, DMF was removed by evaporation, the resulting residue was dissolved in EtOAc (300 mL), then washed successively with 5% aqueous HCl (50 mL), $NaCHO_3$ sat. (50 mL), water and brine, dried over Na_2SO_4, and concentrated. Purification by flash chromatography on silica gel (hexane/EtOAc 1:2) gave the desired product **21** (390 mg; 72%): LRMS (MALDI): calcd for $C_{159}H_{189}O_{34}N_3Si$ (M+Na) 2737; found 2740.

2.1.12. Trimeric pseudo-disaccharide cluster, 22. To a solution of **21** (91 mg; 0.038 mmol) in THF (2 mL) was added TBAF (168 μL; 0.167 mmol) at room temperature. The resulting solution was stirred for 12 h, then poured into ice–water and extracted with CH_2Cl_2. Concentration and purification by flash chromatography on silica gel (hexane/EtOAc 1:12) gave 66.0 mg (79%) of the desired product **22** as a syrup in 79% yield; LRMS (MALDI): calcd for $C_{143}H_{171}O_{34}N_3$ (M+Na) 2497; found 2500.

2.1.13. Methyl 5-*O*-*p*-methoxybenzyl-3,4-*O*-isopropylidene quinate, 24. To a solution of **18** (0.88 g; 3.58 mmol) and **23** (1.06 g; 4.3 mmol) in CH_2Cl_2 (20 mL) was added silver triflate (1.6 g; 6.46 mmol) at room temperature. The mixture was stirred for 3 h and the suspension was filtered through Celite, washed with dichloromethane, concentrated and purified by flash chromatography on silica gel (hexanes/EtOAc 1:1) to give **24** (0.92 g; 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29 (m, 2H), 6.87 (m, 2H), 4.66 (d, J=11.43 Hz, 1H), 4.58 (d, J=11.04 Hz, 1H), 4.47 (m, 1H), 4.12 (dd, J=5.42, 6.56 Hz, 1H), 3.92 (m, 1H), 3.78 (s, 3H, OMe), 3.77 (s, 3H, OMe), 3.44 (s, 1H, OH), 2.23 (m, 2H), 2.16 (m, 1H), 1.82 (dd, J=11.24, 13.35 Hz, 1H), 1.46(s, 3H, Me), 1.35 (s, 3H, Me). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.9, 159.0, 130.4, 129.3, 113.6, 108.9, 79.2, 74.5, 73.9, 73.8, 71.3, 55.1, 52.8, 37.2, 34.3, 28.2, 25.8. HRMS: m/z calcd for $C_{19}H_{26}O_7$ (M+1) 367.4102; found 367.4676.

2.1.14. 2-Trimethylsilylethyl 3,4-*O*-isopropylidene quinate, 25. To a solution of **24** (0.62 g; 1.7 mmol) in methanol (10 mL) was added 1N NaOH (2 mL) at 0°C. The mixture was stirred at room temperature for 1 h, then neutralized with Amberlite IR-120 (H$^+$) (pH 6), filtered and concentrated. The syrupy residue was dissolved in EtOAc (200 mL), then acidified with HCl (10%), dried over Na_2SO_4, and concentrated to give the desired acid (0.55 g; 90%); HRMS: m/z calcd for $C_{18}H_{24}O_7$ (M+1) 353.3834; found 353.3522.

To a solution of the acid (1.1 g; 3.1 mmol) in DMF (30 mL) were added successively HOBT (0.63 g; 4.63 mmol), EDC

(1.2 g; 6.2 mmol) and triethylamine (3 mL; 21.6 mmol) at 0°C. The solution was stirred for 1 h, then trimethysilylethanol (2.2 mL; 15.5 mmol) was added to the reaction mixture. The solution was warmed up to room temperature and the stirring was continued for 24 h. DMF was removed by evaporation, and the resulting residue was dissolved in EtOAc (300 mL), then washed successively with 5% aqueous HCl (50 mL), $NaHCO_3$ sat. (50 mL), water and brine, dried over Na_2SO_4, concentrated and purified by flash chromatography on silica gel (hexanes/EtOAc 2:1) to give the desired product (0.99 g; 71%). HRMS: *m/z* calcd for $C_{23}H_{36}O_7Si$ (M+1) 453.6190; found 453.6276.

To a solution of the above product (0.7 g; 1.66 mmol) in dichloromethane (20 mL) and water (2 mL) was added DDQ (0.75 g; 3.32 mmol) at 0°C. The suspension was stirred at room temperature for 2 h, then diluted with EtOAc (50 mL), washed with $NaHCO_3$ (three times) and brine, dried over Na_2SO_4, concentrated and purified by flash chromatography on silica gel (hexanes/EtOAc 1:1) to give the desired product **25** as an oil (0.48 g; 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.37 (dd, J=9.18, 3.78 Hz, 1H), 4.19 (m, 2H), 4.04 (m, 2H), 3.90 (t, J=6.13 Hz, 1H), 3.40 (s, 2H, OH), 2.17 (m, 2H), 1.96 (m, 1H), 1.75 (t, J=11.51 Hz, 1H), 1.44 (s, 3H, Me), 1.29 (s, 3H, Me), 0.96 (m, 2H), 0.03 (s, 9H, $SiMe_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.2, 108.9, 80.1, 73.5, 67.8, 64.4, 60.2, 38.9, 34.6, 28.0, 25.5, 20.8, 17.2, 13.9, −1.73. HRMS: *m/z* calcd for $C_{23}H_{36}O_7Si$ (M+1) 333.4686; found 333.4555.

2.1.15. Pseudo-trisaccharide 26. To a solution of disaccharide **6** (0.45 g; 1.35 mmol) and **25** (1.47 g; 1.48 mmol) in dichloromethane (15 mL) were added successively NIS (0.5 g; 2.22 mmol) and TfOH (26 μL; 0.27 mmol) at −40°C. The reaction mixture was stirred for 1 h, then warmed up to room temperature and filtered through Celite, then washed with dichloromethane. The filtrate was washed successively with $Na_2S_2O_3$, $NaHCO_3$, brine, dried over Na_2SO_4, concentrated, and purified by flash chromatography on silica gel (hexanes/EtOAc 2:1) to give the desired product **26** (1.2 g; 71%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07–7.98 (m, 4H), 7.59–7.11 (m, 26H, Ph), 5.66 (d, J=3.21 Hz, 1H), 5.57 (dd, J=10.19, 8.07 Hz, 1H), 5.23 (d, J=4.21 Hz, 1H), 4.47 (d, J=11.47 Hz, 1H), 4.67–4.64 (m, 3H), 4.55–4.50 (m, 2H), 4.43 (d, J=11.77 Hz, 1H), 4.36 (m, 2H), 4.36 (d, J=11.07 Hz, 1H), 4.31 (d, J=11.49 Hz, 1H), 4.18–4.08 (m, 6H), 3.96–3.93 (m, 2H), 3.85 (t, J=6.34 Hz, 1H), 3.59 (dd, J=10.14, 2.57 Hz, 1H), 3.47 (m, 2H), 3.22 (s, 1H, OH), 3.20 (m, 1H), 2.17–2.04 (m, 3H), 1.93 (s, 3H, OAc), 1.60 (dd, J=13.07, 11.90 Hz, 1H), 1.47 (s, 3H, Me), 1.29 (s, 3H, Me), 0.89 (m, 2H), 0.03 (s, 9H, $SiMe_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.4, 170.3, 165.9, 164.7, 163.3, 138.6, 138.4, 138.3, 138.2, 133.1, 133.0, 129.7, 129.6, 129.5, 129.5, 128.3, 128.2, 128.0, 127.9, 127.6, 127.5, 127.4, 127.3, 127.3, 108.9, 100.2, 93.4, 78.7, 77.8, 77.3, 77.0, 76.7, 75.3, 74.8, 74.4, 73.7, 73.5, 73.3, 73.1, 71.6, 71.3, 70.7, 69.6, 69.3, 64.8, 64.4, 62.3, 37.1, 34.3, 28.1, 25.7, 20.4, 17.1, −1.65. HRMS: *m/z* calcd for $C_{71}H_{82}O_{19}Si$ (M+1) 1268.5033; found 1268.5218.

2.1.16. Pseudo-trisaccharide 27. To a solution of **26** (1 g; 0.80 mmol) in THF (20 mL) was added TBAF (2.4 mL; 2.37 mmol) at 0°C. The solution was stirred at room temperature for 3 h, then diluted with EtOAc (50 mL). The reaction mixture was washed successively with 10% HCl (3×20 mL), water, brine, dried over Na_2SO_4, concentrated and purified by flash chromatography on silica gel ($MeOH/CH_2Cl_2$ 1:20) to give the desired product **27** (0.83 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06–8.04 (m, 4H), 7.60–7.08 (m, 26H, Ph), 5.66 (d, J=2.85 Hz, 1H), 5.44 (dd, J=10.02, 8.11 Hz, 1H), 5.17 (d, J=3.13 Hz, 1H), 4.81 (d, J=8.07 Hz, 1H, H_1'-β), 4.66–4.23 (m, 15H), 4.08 (t, J=5.27 Hz, 1H), 3.99 (m, 1H), 3.88 (m, 2H), 3.62 (dd, J=10.2, 2.65 Hz, 1H), 3.42 (dd, J=9.49, 3.11 Hz, 1H), 3.35–3.29 (m, 2H), 3.21 (m, 1H), 2.15–1.99 (m, 4H), 1.92 (s, 3H, OAc), 1.53 (t, J=9.00 Hz, 1H), 1.36 (s, 3H, Me), 1.21 (s, 3H, Me). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.2, 174.6, 163.7, 163.8, 138.9, 138.6, 138.2, 138.1, 133.5, 133.1, 129.5, 129.6, 129.5, 129.4, 128.3, 128.1, 128.0, 127.8, 127.6, 127.5, 127.4, 127.3, 127.1, 108.9, 100.2, 93.4, 78.7, 76.7, 75.3, 74.8, 74.4, 73.7, 73.5, 73.3, 73.1, 71.6, 71.3, 70.7, 69.6, 69.3, 64.8, 64.4, 37.1, 34.3, 28.1, 25.7, 20.4. HRMS: *m/z* calcd for $C_{66}H_{70}O_{19}$ (M+1) 1168.2676; found 1168.4570.

2.1.17. Trimeric cluster, 28. To a solution of **27** (600 mg; 0.558 mmol) in DMF (20 mL) were added successively HOBT (150 mg; 1.12 mmol), EDC (213 mg; 1.12 mmol) and triethylamine (155 μL; 1.12 mmol) at 0°C. The solution was stirred for 1 h, then the triamine **18** (61 mg; 0.112 mmol) in 3 mL of DMF was added dropwise to the reaction mixture. The solution was warmed up to room temperature, the stirring was continued for 24 h, DMF was removed by evaporation, the resulting residue was dissolved in EtOAc (300 mL), then washed successively with 5% aqueous HCl (50 mL), $NaHCO_3$ (50 mL), water and brine, dried over Na_2SO_4, and concentrated. Purification by flash chromatography on silica gel (hexanes/EtOAc 1:2) gave the desired product **28** (329 mg; 74%); $[\alpha]_D$=+29.6 (*c* 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05–7.95 (m, 12H), 7.68–7.10 (m, 88H, Ph), 5.64 (d, J=3.08 Hz, 3H), 5.60 (dd, J=10.14, 8.13 Hz, 3H), 5.21 (d, J=3.32 Hz, 3H), 4.75 (d, J=11.47 Hz, 3H), 4.69 (d, J=8.08 Hz, 3H, H_1'-β), 4.64 (m, 9H), 4.53 (m, 3H), 4.50 (d, J=11.50 Hz, 3H), 4.40–4.26 (m, 15H), 4.20–4.10 (m, 9H), 3.94–3.91 (m, 6H), 3.87 (t, J=6.43 Hz, 3H), 3.76 (t, J=4.43 Hz, 3H), 3.57 (dd, J=15.72, 3.77 Hz, 3H), 2.05–1.95 (m, 6H), 1.89 (s, 9H, 3Me), 1.85 (t, J=11.99 Hz, 3H), 1.42 (s, 9H, 3Me), 1.23 (s, 9H, 3Me), 1.03 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.4, 170.4, 165.9, 164.6, 138.6, 138.5, 138.3, 138.2, 135.4, 133.5, 133.2, 132.9, 129.7, 129.6, 129.5, 128.3, 128.2, 128.2, 128.0, 127.8, 127.5, 127.4, 127.2, 109.0, 98.8 (C-1′, α), 93.5(C-1″, β), 78.7, 77.5, 77.3, 76.9, 76.6, 75.4, 75.2, 74.6, 74.3, 73.2, 73.1, 73.0, 71.7, 71.1, 70.5, 69.7, 69.3, 64.8, 62.1, 45.4, 38.7, 35.9, 33.9, 28.1, 26.7, 25.7, 20.5, 19.1. LRMS (MALDI): calcd for $C_{227}H_{253}O_{59}N_3Si$ $(M+Na)^+$ 3992; found 4015.

2.1.18. Trimeric cluster, 29. To a solution of **28** (151 mg; 0.038 mmol) in THF (4 mL) was added TBAF (227 μL; 0.227 mmol) at room temperature. The resulting solution was stirred for 12 h, then poured into ice–water and extracted with CH_2Cl_2. Concentration and purification by flash chromatography on silica gel (hexanes/EtOAc 1:12) gave the desired product in 75% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05–7.95 (m, 12H), 7.59–7.06 (m, 78H, Ph), 5.64 (d, *J*=2.83 Hz, 3H), 5.59 (dd, *J*=10.15, 8.15 Hz, 3H), 5.20 (d, *J*=2.27 Hz, 3H), 4.74 (d, *J*= 11.45 Hz, 3H), 4.70 (d, *J*=9.05 Hz, 3H), 4.67–4.61 (m, 9H), 4.54 (m, 3H), 4.51 (d, *J*=11.40 Hz, 3H), 4.39 (d, *J*=11.62 Hz, 3H), 4.32–4.23 (m, 9H), 4.19–4.07 (m, 15H), 3.94 (m, 9H), 3.86 (t, *J*=6.16 Hz, 3H), 3.62 (m, 3H), 3.56 (dd, *J*=10.20, 6.26 Hz, 3H), 3.50–3.14 (m, 39H), 2.28 (dd, *J*=15.85, 3.75 Hz, 3H), 2.03–1.94 (m, 6H), 1.89 (s, 9H, 3OAc), 1.80 (t, *J*=11.75 Hz, 3H), 1.44 (s, 9H, 3Me), 1.22 (s, 9H, 3Me). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.6, 170.4, 165.9, 164.6, 138.6, 138.5, 138.3, 138.2, 133.1, 132.9, 129.7, 129.6, 129.5, 128.3, 128.1, 127.9, 127.8, 127.5, 127.4, 127.2, 108.9, 98.9 (C-1′, β), 93.5 (C-1″, α), 78.7, 77.6, 77.3, 76.9, 76.6, 75.5, 75.2, 74.6, 74.3, 73.2, 73.1, 72.3, 71.7, 71.1, 70.5, 69.9, 69.7, 69.3, 64.8, 62.1, 61.3, 38.6, 36.0, 33.9, 29.6, 28.1, 25.7, 20.4. LRMS (MALDI): calcd for $C_{211}H_{234}O_{58}N_6$ (M+Na) 3757; found 3780.

To the above product (123 mg; 0.033 mmol) in a mixture of pyridine (1 mL) and CH_2Cl_2 (1 mL) was added MsCl (7.5 μL; 0.098 mmol) at 0°C. The solution was stirred at room temperature for 2 h, then poured into ice–water (2 mL), washed with CH_2Cl_2 and concentrated. The resulting residue was dissolved in DMF (2 mL) and treated with NaN_3 (11 mg; 0.163 mmol) at 60°C for 6 h, then diluted with EtOAc (20 mL). The solution was washed successively with HCl (5%), water and brine, and dried over Na_2SO_4. Concentration and purification by flash chromatography on silica gel (hexanes/EtOAc 1:7) gave 100 mg of desired product **29** in 81% yield; $[\alpha]_D$=+226.7 (*c* 2.3, $CHCl_3$). IR ($CHCl_3$): 2110 cm^{-1} (N_3). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05–7.95(m, 12H), 7.59–7.10 (m, 78H), 5.64(d, *J*= 2.9 Hz, 3H), 5.59 (dd, *J*=10.01, 8.14 Hz, 3H), 5.20 (d, *J*=3.27 Hz, 3H), 4.75 (d, *J*=11.50 Hz, 3H), 4.71 (d, *J*= 9.1 Hz, 3H), 4.68 (m, 9H), 4.55 (m, 3H), 4.52(d, *J*= 11.60 Hz, 3H), 4.40 (d, *J*=11.39 Hz, 3H), 4.34–4.25 (m, 9H), 4.19–4.09 (m, 12H), 3.99–3.93 (m, 9H), 3.85 (t, *J*=6.1 Hz, 3H), 3.58 (dd, *J*=10.4, 6.3 Hz, 3H), 3.51–3.35 (m, 24H), 3.29–3.16 (m, 12H), 2.26 (dd, *J*=15.2, 3.68 Hz, 3H), 2.04–1.96 (m, 6H), 1.90 (s, 9H, 3OAc), 1.85 (t, *J*=11.33 Hz, 3H), 1.44 (s, 9H, 3Me), 1.25 (s, 9H, 3Me). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.5, 170.4, 165.9, 164.4, 138.6, 138.5, 138.3, 138.2, 133.1, 132.9, 129.7, 129.6, 129.5, 129.3, 128.3, 128.3, 128.2, 128.0, 127.8, 127.5, 127.4, 127.3, 127.2, 109.0, 98.9 (C-1′, β), 93.5 (C-1″, α), 78.7, 77.6, 77.3, 76.9, 76.6, 75.5, 75.3, 74.7, 74.3, 73.2, 73.1, 71.7, 71.1, 70.5, 69.7, 69.3, 64.8, 62.1, 50.5, 45.2, 38.7, 36.0, 33.9, 28.1, 25.7, 20.5. LRMS (MALDI): calcd for $C_{211}H_{234}O_{58}N_6$ (M+Na) 2779; found 2802.

2.1.19. Trimeric cluster, 31. A solution of **29** (95.6 mg, 0.0255 mmol) in AcOH/H_2O (4:1; 3 mL) was heated to 60°C for 2 h. Volatiles were evaporated at reduced pressure, followed by coevaporation with toluene and chloroform. Chromatography of the residue (93 mg) on silica gel (15 g) afforded 70 mg (75%) of **30** upon elution with ethyl acetate. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.72 (b, 3H, 3× OH), 1.7–2.05 (m, 12H, 3× 2H-C(3), 2H-C(7)), 1.9 (s, 9H, 3× H_3CCO_2–C(4′)), 3.07 (b, 3H, 3× OH), 3.1–3.3 (m, 9H), 3.3–3.6 (m, 27H), 3.8–4.0 (m, 12H), 4.09 (dd, *J*=11, 3 Hz, 3H, 3× H-C(2″)), 4.2-4.8 (m, 39H), 4.88 (b, 3H, 3× NH), 5.18 (d, *J*=4 Hz, 3× H-C(1″)), 5.53 (dd, *J*=11, 8 Hz, 3H, 3× H-C(2′)), 5.63 (d, *J*=3 Hz, 3H, 3× H-C(4′)), 7.1–7.15 (m, 6H), 7.2–7.55 (m, 69H), 7.55–7.65 (m, 3H), 7.98 (d, *J*=7 Hz, 6H), 8.12 (d, *J*=7 Hz, 6H). Partial assignments relate to two-dimensional ^{1}H–^{13}C-correlation spectroscopy (HSQC).

To a solution of the above product (70 mg, 0.019 mmol) in methanol (2 mL), H_2O (0.2 mL) and $CHCl_3$ (1 mL) was added LiOH-monohydrate (24 mg, 0.57 mmol) and the mixture was heated to 60°C for 3 h. After addition of AcOH (38 μL) volatiles were evaporated under reduced pressure. The residue (105 mg) was chromatographed on silica gel (10 g, $CHCl_3$/MeOH/H_2O 15:2:0.2) affording 41 mg of partially saponified material. Treatment with LiOH in CH_3OH/H_2O/$CHCl_3$ as above at 60°C was repeated for 15 h. After evaporation of solvents the residue was partitioned between $CHCl_3$ (25 mL) and H_2O (10 mL, emulsions!). The aqueous phase was extracted with $CHCl_3$ (15 mL), and the combined organic phases were washed with 0.1N NaOH (15 mL). Chromatography of the residue of the organic phases on silica gel (9 g) eluting with $CHCl_3$/CH_3OH/H_2O (15:2:0.2) yielded 38 mg (68%) of **31**; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.75–2.2 (m, 12H, 3× 2H-C(3), 2H-C(7)); 3.2–3.85 (m, ~51H); 3.85–3.95 (m, 3H); 3.95–4.1 (m, 12H); 4.25–4.35 (m, 3H, 3×H-C(5′); 4.35–4.55 (m, 12H, 3×H-C(1′), 9× $OCHHC_6H_5$); 4.6–4.8 (m, 9H, 9×OC*H*HC_6H_5); 4.8–5.0 (m, 9H, 3×H-C(1″), 6× OC*H*HC_6H_5); 5.1–5.4 (b, 3H, 3×OH); 7.15–7.4 (m, 60H, 12×C_6H_5); 7.4–7.55 (b, 3H, 3×NH). Partial assignments relate to two-dimensional ^{1}H–^{1}H- and ^{1}H–^{13}C-correlation spectroscopy (COSY and HSQC). HRMS: *m*/*z* calcd for $C_{154}H_{192}N_6O_{49}$ (M+Na). 2911.2581; found 2934.1

2.1.20. Trimeric cluster, 3. To a solution of **31** (29 mg, 9.81 mmol) in water/dioxane (2:3; 4 mL) was added Pearlman's catalyst[30] (20% $Pd(OH)_2$ on C, 10 mg) under argon and the mixture was stirred for 18 h under hydrogen at room temperature. After addition of more catalyst (10 mg) stirring under hydrogen was continued for 18 h. Filtration and evaporation of volatiles gave **3** (12 mg, 67%). ^{1}H NMR (400 MHz, D_2O): 1.8–2.2 (m, 12H, 3× 2H-C(3), 2H-C(7)); 3.2–3.45 (m, 8H, 4×CH_2-NHR); 3.45–3.8 (m, ∽34H); 3.85–3.9 (m, 6H); 3.9–4.0 (m, 9H); 4.05–4.15 (m, 6H); 4.15–4.25 (m, 6H); 4.44 (d, *J*=8 Hz, 3H, 3×H-C(1′)); 5.05 (d, *J*=4 Hz, 3H, 3×H-C(1″)). Partial assignments relate to two-dimensional ^{1}H–^{13}C-correlation spectroscopy (HSQC).

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