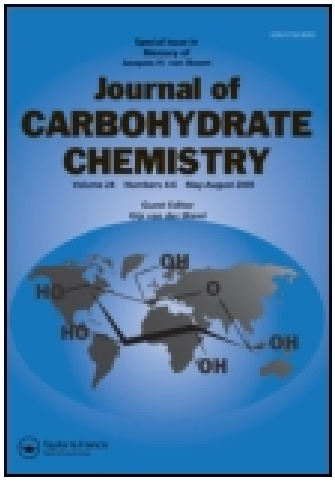

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Synthesis of an α-Gal epitope α-D-Galp-(1→3)-β-D-Galp-(1→4)-β-D-Glcp NAc-lipid conjugate

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Synthesis of an α-Gal epitope α-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p* NAc–lipid conjugate

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The synthesis of a neoglycoconjugate containing the Galili epitope trisaccharide connected to a spacer-lipid entity is described. The α-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc trisaccharide, equipped with a 3-aminopropyl spacer, is efficiently assembled from easily accessible building blocks in a one-pot procedure. Global deprotection of the trisaccharide and ensuing introduction of a bis(palmitamido)- propanamido moiety afforded title compound **1** as depicted in Scheme 1.

Keywords α-Gal epitope, Glycolipid, Oligosaccharide synthesis

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Dedicated to the memory of Prof. Dr. Jacques H. van Boom, our teacher, colleague, and friend.

INTRODUCTION

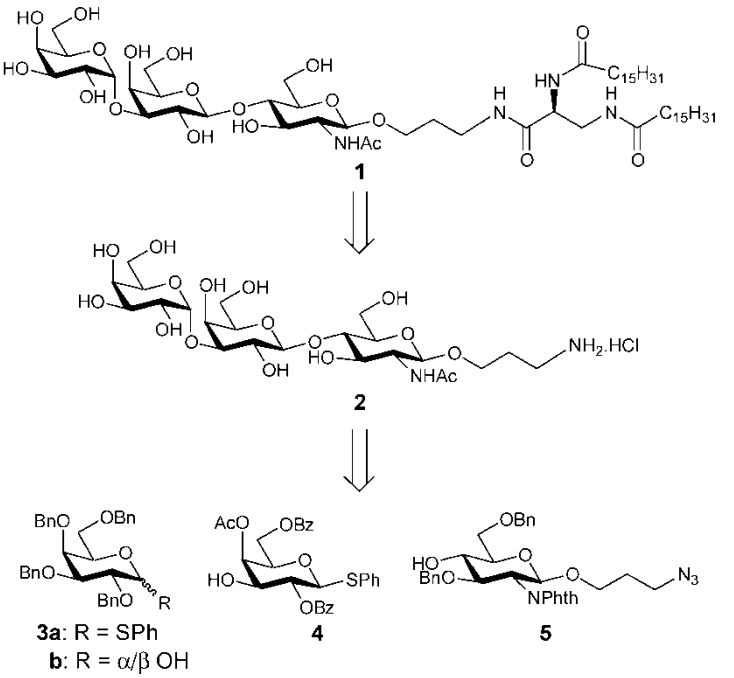
The carbohydrate structure α-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc, in the biologic literature also referred to as "Galili antigen" or simply "α-Gal," is expressed on many cells and tissues of nonprimate mammals and New World monkeys.[1] However, the Galili epitope trisaccharide is nonself to Old World monkeys, apes, and humans due to evolutionary inactivation of the gene encoding α1,3-galactosyltransferase. The Galili epitope is a major obstacle in the field of xenotransplantation of tissues or organs from pigs to monkeys (or humans).[2,3] On the other hand, the strong immunologic response to the Galili trisaccharide could be beneficial in vaccinology or immune therapy. It was demonstrated that covalent introduction of the Galili trisaccharide onto hepatitis B virus hemagglutinin[4,5] or tumor cells[6–10] enhanced the immunogenicity. To this end, the β-D-Gal*p*-(1 → 4)-GlcNAc epitopes present on the hemagglutinin or liberated (with neuraminidase) on the tumor cell surface were modified with uridine diphosphogalactose (UDP-Gal) and a suitable α1,3-galactosyltransferase. It is obvious that such an enzymatic modification of antigens will not always be possible or practical. We envisage that a noncovalent association of the Galili trisaccharide and the target particle should be possible via lipid anchors attached to the carbohydrate. With this objective in mind, we prepared the artificial glycolipid **1** as described in this paper.

RESULTS AND DISCUSSION

Our synthetic strategy to target compound **1** comprises the construction of the hydrophilic trisaccharide **2**, followed by the introduction of the lipophilic tails (Sch. 1). Following this strategy, 3-aminopropyl equipped trisaccharide **2** should be functionalized with diaminopropionic acid, allowing the introduction of two palmitic acid moieties to give amphiphile **1**. Construction of the spacer containing trisaccharide **2** was envisaged to proceed by either a chemoselective or an orthogonal coupling sequence of monosaccharide building blocks **3a** or **3b**, **4**, and **5** followed by a global deprotection of the formed trisaccharide.[11,12]

Initially, attention was focussed on the synthesis of the intermediate thiodisaccharide **6** employing benzylated and acylated galactosides **3a**/**b** and **4**. Based on the findings in armed-disarmed,[13] chemoselective,[14,15] and orthogonal glycosylations,[16] several condensation conditions were investigated (Table 1).

Iodonium *sym*-collidine perchlorate (IDCP)-mediated chemoselective glycosidation of armed phenyl 2,3,4,6-tetra-*O*-benzyl-β-D-thiogalactoside[17] **3a** with disarmed phenyl 4-*O*-acetyl-2,6-di-*O*-benzoyl-β-D-thiogalactoside[18] **4** in a mixture of dichloroethane and diethyl ether gave α-linked disaccharide **6** in 50% yield as the sole isomer (entry 1). Executing the IDCP protocol in



Scheme 1: Retrosynthetic analysis of target glycolipid **1**.

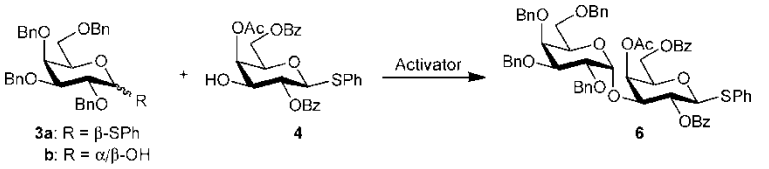
toluene/dioxane, as advocated by Zhu and Boons, gave α-disaccharide **6** in 54% (entry 2).[19] Condensation of the same donor and acceptor with the aid of 1-benzenesulfinylpiperidine (BSP)/trifluoromethanesulfonic anhydride (Tf_2O),[20] the activation system of the Crich group, followed by quenching with triethyl phosphite (TEP)[15] (entry 3) resulted in a 52% isolated yield of **6**. Diphenyl sulfoxide (DPS)/Tf_2O[21] promoted orthogonal condensation of galactosyl donor **3b** with **4** afforded **6** in 64% yield (entry 4).

Having thiodisaccharide **6** in hand, the elongation with acceptor **5** was examined.[a] DPS/Tf_2O[15a] mediated condensation of donor disaccharide **6** with acceptor **5** afforded trisaccharide **7** with the expected equatorial orientation of the newly introduced glycosidic bond in 69% yield (Sch. 2).

On the basis of the above described glycosylation experiments, it was investigated whether the construction of trisaccharide **7** could be improved by performing the condensation of **3b**, **4**, and **5** in a one-pot procedure.[16]

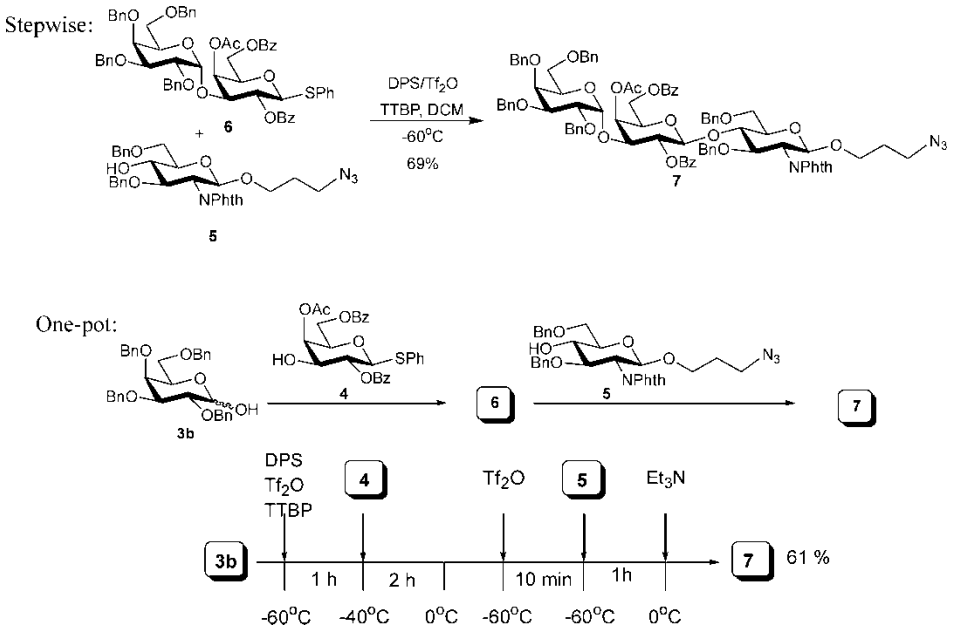
[a] Acceptor **5** was readily prepared in two steps starting from ethyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[23] (first BSP/Tf_2O-mediated β-glycosylation of 3-azido-propan-1-ol,[24] then regioselective reductive opening of the benzylidene in 3-azido-1-propyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside under the agency of TES/TfOH)

Table 1: Synthesis of thiodigalactoside **6**.



Entry	Donor	Activator	Solvent	Yield of 6 (%)
1	**3a**	IDCP	DCE/Et_2O (1 : 5 v/v)	50
2	**3a**	IDCP	Toluene/dioxane (3 : 1 v/v)	54
3	**3a**	BPS/Tf_2O TEP quench	DCM	52
4	**3b**	DPS/Tf_2O	DCM	64

Therefore, hemiacetal **3b** was activated with DPS/Tf_2O followed by adding acceptor thiogalactoside **4** to the reaction mixture to afford transient dimer **6** with concomitant regeneration of DPS. Ensuing activation of the thio function in **6** was affected by the addition of another equivalent of triflic anhydride. Subsequent introduction of acceptor **5** to the reaction vessel led to the one-pot construction of **7** in 61% yield. The yield of this operation is a

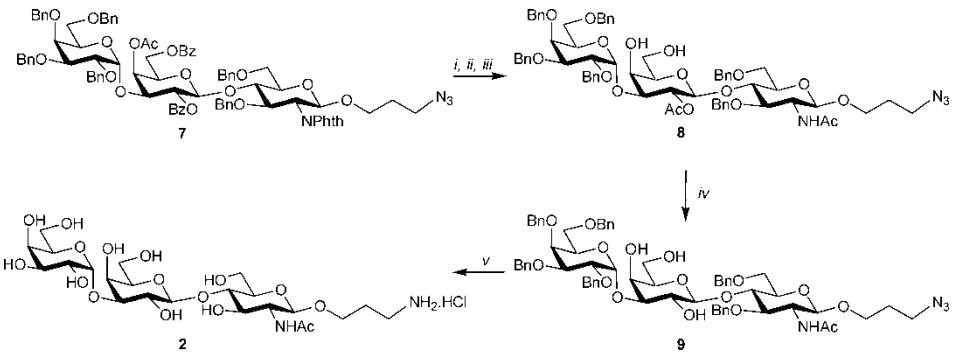


Scheme 2: Stepwise and one-pot construction of trisaccharide **7**.

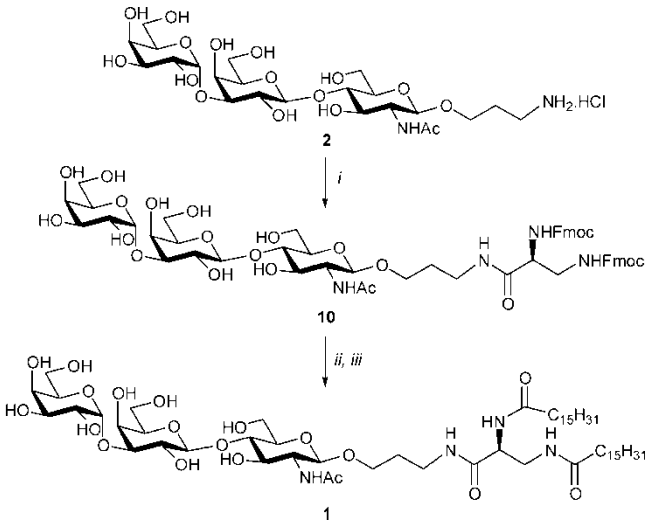
significant improvement in comparison with the overall yield of 44% from the stepwise approach.

The introduction of the lipophilic tails started with the global deprotection of trisaccharide **7** (Sch. 3). First, the phthalimide in **7** was transformed into the free amine using ethylenediamine[22] (EDA) in refluxing *n*BuOH under strictly anhydrous conditions. H was followed by acetylation (Ac_2O in pyridine) and subsequent saponification of the ester moieties by treatment with catalytic KO*t*Bu in MeOH to give **8** with the 2′-*O*-acetate in the central galactose residue unaffected. Ensuing treatment with stoichiometric KO*t*Bu in refluxing MeOH resulted in clean removal of the acetate, furnishing the desired trisaccharide **9** in 94%. Hydrogenolysis of **9** with palladium on carbon under a hydrogen atmosphere afforded 3-aminopropyl trisaccharide **2** in quantitative yield. The identity of **2** was ascertained by NMR spectroscopy and HRMS spectrometry and was in accordance with the reported data.[11a] The overall yield of trisaccharide **2** starting from monosaccharides **3b**, **4**, and **5** is 52% over seven steps (based on **4**). In a related synthesis by Hanessian et al., **2** was prepared in 27% over four steps starting from three monosaccharide building blocks.[11b]

The stage was now set for the introduction of the lipid anchor moiety (Sch. 4). The free amine in **2** was acylated with bis-Fmoc-diaminopropionic acid under the influence of benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate (BOP reagent) and di-isopropylethylamine (DiPEA), to afford bis-Fmoc trisaccharide **10**, which was purified by a sequence of trituration steps. Removal of the Fmoc groups in **10** by treatment with piperidine and purification of the resulting diamine proved to be very tedious. This problem could be circumvented by the following sequence of steps. The diamine was released by treatment of **10** with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), and the generated fluorene was quenched with ethanethiol to avoid any side reactions. The reaction mixture was concentrated and the



Scheme 3: *i.* EDA, *n*BuOH, reflux; *ii.* Ac_2O, pyridine; *iii.* KO*t*Bu (cat.), MeOH, 92% over the three steps; *iv.* KO*t*Bu (1 eq.), MeOH, reflux, 94%; *v.* H_2, Pd/C, HCl, *t*BuOH/H_2O 11/4 v/v, quant.



Scheme 4: *i.* 2(*S*),3-bis-(9*H*-fluoren-9-ylmethoxycarbonylamino)-propionic acid, BOP, DiPEA, DMSO/DMF 1 : 2 v/v; *ii.* DBU, DMSO/DMF 1 : 2 v/v; *iii.* palmitic acid *N*-hydroxysuccinimide ester, DiPEA, DMF/DMSO/$CHCl_3$ 2 : 1 : 1 v/v, 35% over the three steps.

residue dissolved in a mixture of DMSO, DMF, and $CHCl_3$. Subsequent treatment with excess palmitic acid *N*-hydroxysuccinimide ester in the presence of DiPEA followed by concentration and precipitation from hot methanol provided homogeneous glycolipid **1** in 35% over three steps. The identity of **1** was ascertained by ^{1}H NMR, LCMS, and HRMS. The purity was determined from the ^{1}H NMR spectrum and was $>$95%.

CONCLUSION

Artificial glycolipid **1**, designed for incorporation into liposomes and membranes, was assembled in two stages. Aminopropyl spacer containing trisaccharide **7** was synthesized in a one-pot procedure from easily available orthogonal protected building blocks. After removal of the protecting groups in **7**, the lipid anchor was introduced via a procedure, in which the number of chromatographic purification steps was minimized. The application of neoglycoconjugate **1** in immunologic experiments is currently being examined.

EXPERIMENTAL SECTION

General Methods

Dichloromethane was refluxed with P_2O_5 and distilled before use. 1-Benzenesulfinylpiperidine (BSP) and tri-*tert*-butylpyrimidine (TTBP) were

synthesized as described by Crich et al.[20,25] Trifluoromethanesulfonic anhydride (Tf_2O) was stirred for 3 hr on P_2O_5 and subsequently distilled. All other chemicals (Fluka, Acros, Merck, Aldrich, Sigma) were used as received. Hyflo Super Cel filter aid was purchased from Fluka and used as received. Reactions were performed under an inert atmosphere under strictly anhydrous conditions unless stated otherwise. Traces of water from reagents used in reactions that require anhydrous conditions were removed by coevaporation with toluene and dichloroethane. Molecular sieves (3Å and 5′) were flame dried before use. Column chromatography was performed on Fluka Silica gel 60 (0.04–0.063 mm, 230–400 mesh ASTM). TLC analysis was conducted on DC-alufoil (Merck, Kieselgel 60 F_{254}). Compounds were visualized by UV absorption (254 nm) and by spraying with 20% H_2SO_4 in ethanol, with a solution of ninhydrin 0.4 g in EtOH (100 mL) containing acetic acid (3 mL) or with a solution of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 25 g/L, followed by charring at $\pm 140^\circ$C. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker DPX 300 (300 and 75.1 MHz), a Bruker AV 400 (400 and 100 MHz), or a Bruker DMX 600 (600 and 125 MHz). NMR spectra were recorded in $CDCl_3$ with chemical shifts (δ) relative to tetramethylsilane unless stated otherwise. Mass spectra were recorded on a PE/SCIEX API 165 equipped with an Electrospray Interface (Perkin-Elmer) or a Finnigan LTQ-FT (Thermo Electron). Optical rotations were recorded on a Propol automatic polarimeter. The purity of the synthesized compounds was >95% as judged by ^{1}H NMR.

Phenyl (2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)-(1 → 3)-4-*O*-acetyl-2,6-di-*O*-benzoyl-1-thio-β-D-galactopyranoside (6).

IDCP: To a solution of **3a** (190 mg, 0.3 mmol) and **4** (131 mg, 0.25 mmol) in DCE/Et_2O (5 mL, 1 : 5 v/v) containing powdered 5Å MS was added IDCP (280 mg, 0.6 mmol). After stirring for 1 hr, the reaction mixture was diluted with ethyl acetate and washed with a 10% aq. Na_2SO_4 solution. The organic layer was dried ($MgSO_4$), filtered, and concentrated. Column chromatography of the residue (ethyl acetate/light petroleum, 1 : 9 → 1 : 5 v/v) afforded **6** (130 mg, 125 μmol, 50%) as a colorless oil.

BSP/Tf_2O; $(EtO)_3P$ quench: To a solution of thiodonor **3a** (0.2 mmol, 1.0 equiv), BSP (46 mg, 0.22 mmol), and TTBP (124 mg, 0.5 mmol) in dichloromethane (5 mL) containing 3′ MS at −60°C was added trifluoromethanesulfonic anhydride (37 μL, 0.22 mmol). The reaction mixture was stirred for 5 min, after which a solution of acceptor thioglycoside **4** (115 mg, 0.22 mmol) in dichloromethane (2 mL) was added. The mixture was stirred at −60°C for 1 hr followed by slowly warming to −10°C. The reaction was quenched with triethyl phosphite (1.0 equiv.) and triethylamine (5 equiv.). Sat. aq. $NaHCO_3$ was added and the organic layer was separated, washed with saturated NaCl

solution, dried ($MgSO_4$), and concentrated. Purification by silica gel chromatography (light petroleum → ethyl acetate/light petroleum 1 : 4 v/v) gave disaccharide **6** (109 mg, 104 μmol, 52%) as an oil.

DPS/Tf_2O: To a solution of the 1-hydroxyl donor **3b** (140 mg, 0.26 mmol), DPS (120 mg, 0.57 mmol), and TTBP (140 mg, 0.57 mmol) in DCM (5 mL) was added trifluoromethanesulfonic anhydride (0.27 mmol, 46 μL) at −60°C. The temperature was raised to −40°C, and the reaction mixture was stirred at this temperature for 1 hr. Then, a solution of acceptor **4** (104 mg, 0.2 mmol) in DCM (2 mL) was added, and the reaction mixture was allowed to warm to rt. Dry Et_3N (10 equiv to donor) was added, and the reaction mixture was washed with saturated $NaHCO_3$ and water. After drying ($MgSO_4$) and concentration, the residue was purified by column chromatography (ethyl acetate/light petroleum) to give disaccharide **6** (134 mg, 128 μmol, 64%) as a colorless syrup.

6: R_f 0.80 (33% ethyl acetate in light petroleum). $[\alpha]_D^{25}$ +90.8 ($c = 1$). ^{1}H NMR: δ (ppm) 8.12–7.14 (m, 35 H, CH_{arom}), 5.67 (d, 1H, $J = 2.8$ Hz, H-4), 5.61 (t, 1H, $J = 9.9$ Hz, H-2), 5.22 (d, 1H, $J = 3.2$ Hz, H-1′), 4.81 (d, 1H, $J = 10.1$ Hz, H-1), 4.76 (d, 1H, $J = 11.4$ Hz, −C*H*Ph), 4.65 (s, 2H, −C*H*Ph), 4.64 (d, 1H, $J = 12.2$ Hz, −C*H*Ph), 4.48 (m, 2H, H-6, −C*H*Ph), 4.36 (m, 4H, H-6, −C*H*Ph), 4.16 (dd, 1H, $J = 3.0$, 9.7 Hz, H-3), 3.93 (m, 3H, H-2′, H-5, H-5′), 3.75 (dd, 1H, $J = 2.6$, 10.1 Hz, H-3′), 3.44 (dd, 1H, $J = 7.3$, 9.6 Hz, H-6′), 3.23 (bs, 1H, H-4′), 3.20 (dd, 1H, $J = 5.2$, 9.6 Hz, H-6′), 1.89 (s, 3H, Ac). ^{13}C NMR: δ (ppm) 170.3, 165.9, 164.8 (C═O), 138.6, 138.5, 138.3 (C_q Bn), 133.4 (C_q SPh), 129.5, 129.4 (C_q Bz), 133.2–127.4 (CH_{arom}), 93.3 (C-1′), 87.0 (C-1), 78.7 (C-3′), 75.5 (C-2′), 74.8 (C-5′), 74.7 (C-4′), 74.3, 74.2, 73.1, 73.0 (CH_2 Bn), 72.7 (C-3), 69.9 (C-5), 69.4 (C-6′), 68.9 (C-2), 65.1 (C-4), 62.7 (C-6), 20.4 (CH_3 Ac). ESI-MS: m/z 1068.1 $[M + Na]^+$.

3-Azidopropyl 3,6-Di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (5).

A solution of ethyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalamido-1-thio-β-D-glucopyranoside (3.7 g, 7 mmol), BSP (1.77 g, 8.5 mmol), and TTBP (3.8 g, 15.4 mmol) containing 3Å MS in DCM (50 mL) at −60°C was treated with Tf_2O (1.44 mL, 8.5 mmol) for 10 min, after which 3-azidopropan-1-ol (2.12 g, 21 mmol) in DCM (10 mL) was added. The mixture was allowed to warm to 0°C, and Et_3N (2 mL) was added. The reaction mixture was filtered and washed with sat. aq. $NaHCO_3$. The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. Column chromatography of the residue (light petroleum → ethyl acetate/light petroleum 1 : 5 v/v) afforded 3-azidopropyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (3.35 g, 5.9 mmol, 84%) as a white foam. ^{1}H NMR: δ (ppm)

7.74–6.89 (m, 14H, H_{arom}), 5.62 (s, 1H, –C*H*Ph), 5.20 (d, 1H, $J = 8.8$ Hz, H-1), 4.80 (d, 1H, $J = 12.4$ Hz, –C*H*Ph), 4.50 (d, 1H, $J = 12.4$ Hz, –C*H*Ph), 4.43 (m, 2H, H-2, H-3), 4.20 (dd, 1H, $J = 11.0$, 8.8 Hz, H-2), 3.82 (m, 3H, H-4, 2 × H-6), 3.72 (m, 1H, H-5), 3.41 (m, 2H, O–CH_2–CH_2), 3.12 (m, 2H, CH_2N_3), 1.71 (m, 2H, CH_2–CH_2–CH_2). ^{13}C NMR: δ (ppm) 167.1, 137.4, 136.9, 133.4, 131.6, 130.8, 128.5, 128.3, 127.6, 127.4, 126.8, 125.5, 124.5, 122.7, 100.5, 98.3, 82.3, 74.0, 73.3, 68.0, 65.7, 65.5, 60.5, 58.5, 55.2, 47.2, 28.1. ESI-MS: m/z 593.2 $[M + Na]^+$.

3-Azidopropyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyrano-side (2.85 g, 5 mmol) was treated with TfOH (1.27 mL, 15.0 mmol) in the presence of triethylsilane (2.6 mL, 16.6 mmol) in DCM (50 mL) at –78°C. After 20 min the reaction was quenched by the subsequent addition of MeOH and triethylamine. After the reaction mixture was washed with saturated aqueous $NaHCO_3$, dried, and concentrated, the residue was purified by column chromatography (ethyl acetate/light petroleum, 1 : 20 → 1 : 4 v/v) to provide compound **5** (2.0 g, 3.55 mmol, 71%) as a slightly yellow oil. ^{1}H NMR: δ (ppm) 7.79–6.92 (m, 14 H, H_{arom}), 5.14 (d, 1H, $J = 8.3$ Hz, H-1), 4.75 (d, 1H, $J = 12.2$ Hz, –C*H*Ph), 4.64 (d, 1H, $J = 12.0$ Hz, –C*H*Ph), 4.58 (d, 1H, $J = 12.0$ Hz, –C*H*Ph), 4.53 (d, 1H, $J = 12.2$ Hz, –C*H*Ph), 4.24 (dd, 1H, $J = 8.4$, 10.8 Hz, H-3), 4.15 (dd, 1H, $J = 8.4$, 10.8 Hz, H-2), 3.81 (m, 4H, H-4, H-6, O–CH_2–CH_2), 3.66 (m, 1H, H-5), 3.45 (m, 1H, H-6), 3.11 (m, 2H, CH_2N_3), 1.66 (m, 2H, CH_2–CH_2–CH_2). $^{[13]}$C NMR: δ (ppm) 138.0, 137.6 (C_q Bn), 133.8, 128.4, 128.0, 127.7, 127.6, 127.3, 123.2 (CH_{arom}), 131.4 (C_q, Phth), 98.2 (C-1), 78.6 (C-3), 74.2 (CH_2 Bn), 74.2 (C-5), 74.0 (C-4), 73.6 (CH_2 Bn), 70.3 (O–CH_2–CH_2), 68.1 (C-6), 55.2 (C-2), 47.8 (CH_2N_3), 28.7 (OCH_2–CH_2–CH_2). ESI-MS: m/z 595.3 $[M + Na]^+$.

3-Azidopropyl (2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)-(1 → 3)-(4-O-acetyl-2,6-di-O-benzoyl-β-D-galactopyranosyl)-(1 → 4)-3,6-di-O-benzyl-2-deoxy-2-phtalimido-β-D-glucopyranoside (7).

Stepwise procedure: A solution of disaccharide **6** (209 mg, 0.2 mmol), DPS (81 mg, 0.4 mmol), and TTBP (124 mg, 0.45 mmol) was treated with Tf_2O (37 μL, 0.22 mmol) for 10 min at –60°C. Then, acceptor **5** (179 mg, 0.3 mmol) was added and the reaction mixture was slowly warmed to 0°C. Standard workup and purification gave trisaccharide **9** (211 mg, 138 μmol, 69% yield) as a colorless oil.

One-pot procedure: 2,3,4,6-Tetra-*O*-benzyl-α,β-D-galactopyranose **3b** (1.36 g, 2.5 mmol), DPS (1.02 g, 5.0 mmol), and TTBP (1.86 g, 7.5 mmol) were dissolved in DCM (50 mL), powdered 3Å MS (500 mg) was added, and

the reaction mixture was cooled to −60°C. Tf_2O (440 μL, 2.6 mmol) was added, and the reaction mixture was brought to −40°C. Stirring was continued for 1 hr at this temperature, after which phenyl 4-*O*-acetyl-2,6-di-*O*-benzoyl-1-thio-β-D-galactopyranoside **4** (1.04 g, 2.0 mmol in 5 mL DCM) was added. The reaction mixture was kept at −40°C for 1 hr after which it was slowly warmed to 0°C. After 30 min at 0°C the reaction mixture was cooled to −60°C, and Tf_2O (400 μL, 2.4 mmol) was added. After the reaction was kept at −60°C for 10 min, 3-azidopropyl 3,6-di-*O*-benzyl-2-deoxy-2-phtalimido-β-D- glucopyranoside **5** (1.64 g, 3 mmol) was added. The mixture was slowly warmed to 0°C, after which Et_3N (2 mL) was added. Standard workup and purification gave compound **7** (1.85 g, 1.23 mmol, 61%) as a colorless oil. R_f 0.60 (33% ethyl acetate in light petroleum). $[\alpha]_D^{25}$ +39.2 ($c = 0.75$, $CHCl_3$). ^{1}H NMR: δ (ppm) 8.10–6.83 (m, 44H, H_{arom}), 5.54 (m, 2H, H-2′, H-4′), 5.14 (d, 1H, $J = 3.3$ Hz, H-1″), 5.00 (d, 1H, $J = 8.5$ Hz, H-1), 4.91 (d, 1H, $J = 12.4$ Hz, −C*H*Ph), 4.78 (d, 1H, $J = 8.1$ Hz, H-1′), 4.74 (d, 1H, $J = 11.4$ Hz, −C*H*Ph), 4.63 (s, 2H, −C*H*Ph), 4.62 (d, 1H, $J = 11.8$ Hz, −C*H*Ph), 4.55 (d, 1H, $J = 12.0$ Hz, −C*H*Ph), 4.51 (d, 1H, $J = 12.4$ Hz, −C*H*Ph), 4.44 (d, 1H, $J = 11.8$ Hz, −C*H*Ph), 4.42 (d, 1H, $J = 11.8$ Hz, −C*H*Ph), 4.35 (d, 1H, $J = 12.0$ Hz, −C*H*Ph), 4.29 (m, 3H, 2 × −C*H*Ph, H-3), 4.22 (dd, 1H, $J = 6.5$ Hz, $J = 11.3$ Hz, H-6′), 4.13 (m, 2H, H-6′, H-2), 4.05 (dd, 1H, $J = 8.5$ Hz, $J = 9.9$ Hz, H-4), 3.99 (dd, 1H, $J = 3.4$ Hz, $J = 10.2$ Hz, H-3′), 3.91 (dd, 1H, $J = 3.3$, 10.2 Hz, H-2″), 3.85 (bt, 1H, $J = 6.9$ Hz, H-5″), 3.75 (m, 1H, O−C*H*H−CH_2), 3.67 (m, 2H, H-5′, H-6), 3.58 (m, 2H, H-6, H-3″), 3.41 (m, 1H, H-5), 3.38 (m, 2H, H-6″, O-CH*H*-CH_2), 3.25 (dd, 1H, $J = 1.2$ Hz, $J = 2.6$ Hz, H-4″), 3.21 (dd, 1H, $J = 5.9$ Hz, $J = 9.4$ Hz, H-6″), 3.08 (m, 2H, CH_2−C*H*$_2$−N_3), 1.81 (s, 3H, Ac), 1.64 (m, 2H, CH_2−C*H*$_2$−;CH_2). ^{13}C NMR: δ (ppm) 170.2, 166.0, 164.6 (C═O), 138.7, 138.4, 138.1, 138.0 (C_q Bn), 131.5 (C_q Phth), 129.3, 129.7 (C_q Bz), 133.7–123.2 (CH_{arom}), 100.8 (C-1′), 98.3 (C-1), 94.1 (C-1″), 78.8 (C-3″), 78.3 (C-4), 76.9 (C-3), 75.5 (C-2″), 74.8 (C-4″), 74.7 (C-5), 74.5, 73.5, 73.3, 73.2, 73.1 (CH_2 Bn), 72.3 (C-3′), 71.4 (C-2′), 71.0 (C-5′), 69.8 (C-5″), 69.2 (C-6″), 67.8 (C-6), 65.9 (O−*C*H$_2$−CH_2), 65.0 (C-4′), 61.7 (C-6′), 55.7 (C-2), 48.0 (CH_2−*C*H$_2$−N_3), 28.8 (CH_2-*C*H$_2$-CH_2), 20.4 (Ac). ESI-MS: m/z 1529.8 [M + Na] +. ESI-HRMS calcd for $C_{87}H_{86}N_4O_{20}$ (M + H): 1507.5914. Found: 1507.5908.

3-Azidopropyl (2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)-(1 → 3)-(2-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (8).

To a solution of **7** (320 mg, 0.21 mmol) in dry *n*BuOH (2 mL) was added ethylene diamine (1 mL), and the mixture was heated to 90°C and stirred

overnight. After concentration and coevaporation with toluene (2×), the resulting solid was dissolved in pyridine (2 mL), and Ac_2O (200 μL, 2.12 mmol) was added. After 3 hr, TLC analysis (ethyl acetate/light petroleum, 2 : 1 v/v) showed formation of a single product, and the reaction mixture was concentrated and then concentrated from toluene (2×). The resulting oil was dissolved in dry MeOH (2 mL) and a catalytic amount of KO*t*Bu was added. After overnight reaction, TLC analysis (ethyl acetate/light petroleum, 2 : 1 v/v) showed full consumption of the starting material into one lower running spot. The reaction mixture was neutralized with Amberlite IR 120 H^+-resin, filtered, and concentrated. The crude product was purified by column chromatography (30% ethyl acetate/light petroleum → ethyl acetate), affording the title compound (234 mg, 0.19 mmol, 92%) as a white foam. R_f 0.40 (ethyl acetate/light petroleum, 2 : 1 v/v). [1]H NMR: δ (ppm) 7.28 (m, 30H, H_{arom}), 6.03 (d, 1H, $J = 8.5$ Hz, H-1), 5.10 (t, 1H, $J = 8.3$ Hz, H-2′), 4.91 (d, 1H, $J = 11.4$ Hz, −C*H*Ph), 4.86 (d, 1H, $J = 11.5$ Hz, −C*H*Ph), 4.74 (bs, 4H, −C*H*Ph), 4.59 (m, 5H, −C*H*Ph, H-1″), 4.42 (m, 3H, −C*H*Ph), 4.33 (d, 1H, $J = 8.4$ Hz, H-1′), 4.07 (dd, 1H, $J = 9.6$, 3.7 Hz, H-3′), 3.90 (m, 2H, H-4, H-6″), 3.83 (m, 3H, H-6, H-5″, H-3), 3.73 (m, 4H, H-2, H-2″, H-6, H-6), 3.69 (d, 1H, $J = 3.3$ Hz, H-4′), 3.61 (m, 2H, H-6′, H-5), 3.50 (m, 3H, H-3′, O−C*H*$_2$−CH$_2$), 3.34 (m, 3H, H-5′, CH$_2$−C*H*$_2$-N$_3$), 2.02 (s, 3H, Ac), 1.89 (s, 3H, Ac), 1.78 (m, 2H, CH$_2$−C*H*$_2$−;CH$_2$). ESI-MS: m/z 1233.5 $[M + Na]^+$. ESI-HRMS calcd for $C_{67}H_{78}N_4O_{17}$ (M + H): 1211.5440. Found: 1211.5443.

3-Azidopropyl (2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)-(1 → 3)-β-D-galactopyranosyl-(1 → 4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (9).

To a solution of **8** (234 mg, 0.19 mmol) in dry MeOH (2 mL) was added KO*t*Bu (22 mg, 0.19 mmol) and the mixture was refluxed for 16 hr after which TLC analysis (MeOH/$CHCl_3$, 1 : 9 v/v) showed full conversion of the starting material into one lower running spot. The reaction mixture was neutralized by the addition of Amberlite IR 120 H^+-resin, filtered, and concentrated under reduced pressure. Column chromatography gave compound **9** (210 mg, 0.18 mmol, 94%) as a white foam. R_f 0.56 (MeOH/$CHCl_3$, 1 : 9 v/v). $[\alpha]_D^{25}$ +69.1 ($c = 0.1$, $CHCl_3$). ^{1}H NMR: δ (ppm) 7.31 (m, 30H, H_{arom}), 4.90 (m, 3H, −C*H*Ph), 4.85 (d, 1H, $J = 8.2$ Hz, H-1), 4.74 (s, 2H, −C*H*Ph), 4.67 (d, 1H, $J = 11.6$ Hz, −C*H*Ph), 4.59 (m, 4H, −C*H*Ph, H-1′), 4.47 (m, 3H, −C*H*Ph), 4.34 (d, 1H, $J = 7.6$ Hz, H-1″), 4.28 (t, 1H, $J = 3.3$ Hz, H-3″), 4.13 (dd, 1H, $J = 10.2$, 6.0 Hz, H-2), 3.99 (m, 3H, H-3, H-4, H-6″), 3.88 (m, 3H, H-6, H-6′, H-6), 3.74 (bs, 1H, H-4′), 3.62 (m, 6H, H-5, H-6′, H-6″, H-5″,

O–CH_2–CH_2), 3.38 (m, 3H, CH_2–CH_2–N_3, H-3′), 3.28 (m, 1H, H-5′), 1.88 (s, 3H, Ac), 1.80 (m, 2H, CH_2–CH_2–CH_2). ^{13}C NMR: δ (ppm) 170.80, 138.18, 138.02, 137.92, 137.76, 137.51, 137.39, 102.25, 100.38, 95.87, 80.14, 79.21, 78.97, 76.22, 75.89, 75.05, 74.69, 74.68, 74.53, 74.35, 73.15, 73.14, 72.53, 71.6, 70.01, 69.65, 68.85, 68.29, 66.19, 65.72, 62.19, 55.19, 47.93, 28.77, 22.82. ESI-HRMS calcd for $C_{65}H_{76}N_4O_{16}$ (M + Na): 1191.5153. Found: 1191.5141.

3-Aminopropyl α-D-Galactopyranosyl-(1 → 3)-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside (2).

Pd/C (10 wt. % on activated carbon, 100 mg) was added to a solution of **9** (154 mg, 0.13 mmol) in *t*BuOH/H_2O (2 mL, 11 : 4 v/v) and HCl (1 M in H_2O, 500 μL), after which H_2 was bubbled through the solution for 1 hr followed by stirring under an H_2 atmosphere for 16 hr. TLC analysis (ethyl acetate/pyridine/AcOH/H_2O, 8 : 7 : 1.6 : 1 v/v) showed full transformation of the starting material into one lower running spot. The mixture was filtered over Hyflo, concentrated in vacuo, and lyophilized to afford compound **2** (96 mg, 0.13 mmol, quant.) as a white powder. R_f 0.32 (ethyl acetate/pyridine/AcOH/H_2O, 8 : 7 : 1.6 : 1 v/v). $[\alpha]_D^{25}$ +64.3 ($c = 0.5$, H_2O). ^{1}H NMR (D_2O, TSP as internal standard at $\delta = 0$ ppm): δ (ppm) 5.15 (bs, 1H, H-1″), 4.54 (2H, m, H-1, H-1′), 4.18 (2H, H-1″, H-4′), 4.03–3.95 (m, 4°H), 3.89–3.47 (m, 16H), 3.09 (t, 2H, CH_2–CH_2–NH_2, $J = 6.4$ Hz), 2.06 (s, 3H, Ac), 1.96 (m, 2H, CH_2–CH_2–CH_2). ^{13}C NMR (D_2O, TSP as internal standard at $\delta = 0$ ppm): δ (ppm) 176.0, 104.4, 102.7, 97.1, 80.4, 78.9, 76.6, 76.3, 75.2, 73.8, 72.4, 71.2, 70.9, 70.8, 69.8, 69.5, 66.5, 62.6, 61.7, 65.6, 39.2, 28.3, 23.8. ESI-HRMS calcd for $C_{23}H_{42}O_{16}N_2$ (M + H): 603.2607. Found: 603.2646.

3-(2(*S*),3-Bis(palmitamido))-propanamido)-propyl α-D-Galactopyranosyl-(1 → 3)-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside (1).

To a mixture of 2(*S*),3-bis-(9*H*-fluoren-9-ylmethoxycarbonylamino)-propionic acid (26 mg, 46 μmol) and amine **2** (15 mg, 23 μmol) in DMF/DMSO (2/1 v/v, 0.75 mL) were added BOP (46 μmol, 21 mg) and DiPEA (16 μL, 92 μmol), and the mixture was stirred for 6 hr. Aminoacetaldehyde dimethyl acetal (5 μL, 46 μmol) was added, and the mixture was stirred for one-half hr, after which DiPEA (8 μL, 46 μmol) was added, and the reaction mixture was concentrated in vacuo. The resulting syrup was triturated with Et_2O and centrifuged, and the supernatant was removed. The precipitate was treated with DCM/Et_2O (1/1 v/v) and centrifuged, and the supernatant removed. The resulting precipitate was mixed with $CHCl_3$/MeOH/H_2O (63/33/4 v/v), and the slurry

was centrifuged and the liquids removed. The resulting solid was dissolved in DMF/DMSO (1 mL, 2 : 1 v/v), and DBU (14 μL, 92 μmol) was added. After 10 min, HOBt (16 mg, 115 μmol) was added. This mixture was stirred for 5 min, after which EtSH (15 μL, 200 μmol) was added, and stirring was continued for 5 min. Subsequently, the mixture was concentrated under vacuum, and the residue was dissolved in DMF/DMSO/$CHCl_3$ (1 mL, 2 : 1 : 1 v/v). Palmitic acid *N*-hydroxysuccinimide ester (40 mg, 115 μmol) and DiPEA (20 μL, 69 μmol) were added. After stirring for 16 hr, the reaction mixture was concentrated under reduced pressure. The resulting solid was dissolved in boiling MeOH, and after cooling down the solution to 0°C the formed precipitate was isolated to provide the compound **1** (9.3 mg, 8 μmol, 35% over three steps) as an amorphous white solid. ^{1}H NMR (DMSO-d_6 with internal standard at 2.54 ppm, T = 303 K): δ (ppm) 5.05 (d, 1H, J = 5.2 Hz, H-1), 4.83 (d, 1H, J = 3.5 Hz, H-1″), 4.63 (t, 1H, J = 5.1, C*H*-DAP), 4.64 (d, 1H, J = 7.7 Hz, H-1′), 4.59 (t, 1H, J = 6.1 Hz, H-3′), 4.55 (d, 1H, J = 5.0 Hz, H-4″), 4.42 (t, 1H, J = 6.0 Hz, H-6), 4.35 (s, 1H, H-6′), 4.29 (m, 2H, H-6, H-6″), 3.99 (t, 1H, J = 8.1 Hz, H-3), 3.84 (s, 1H, H-6′), 3.76 (m, 4H, H-6″, O−CH_2−CH_2, H-5′), 3.63 (m, 2H, H-5″, H-3″), 3.58 (m, 2H, H-2′, H-2″), 3.51 (m, 4H, H-4, CH_2−DAP, H-5), 3.37 (m, 2H, H-2, H-5′), 3.11 (m, 1H, CH_2−C*H*H−NH_2), 3.03 (m, 1H, CH_2−CH*H*−NH_2), 2.10 (t, 2H, J = 7.26 Hz, COCH_2 palmitoyl), 2.02 (t, 2H, J = 7.20 Hz, COCH_2 palmitoyl), 1.80 (s, 3H, Ac), 1.58 (t, 2H, J = 6.3 Hz, CH_2−CH_2−CH_2), 1.45 (m, 4H, $COCH_2$CH_2 palmitoyl), 1.23 (m, 48H, −CH_2− palmitoyl), 0.84 (t, 6H, J = 6.7 Hz, −CH_3 palmitoyl). ESI-HRMS calcd for $C_{58}H_{108}N_4O_{19}$ (M + Na): 1187.7500. Found: 1187.7501.

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