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A Ferrier glycosylation/*cis*-dihydroxylation strategy to synthesize *Leishmania* spp. lipophosphoglycan-associated βGal(1,4)Man disaccharide†

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The Galβ(1→4)Man disaccharide, found in the cell surface lipophosphoglycan (LPG) of *Leishmania* species, has been synthesized by a Ferrier glycosylation/*cis*-dihydroxylation strategy. This stereoselective method proved efficient for synthesizing the target saccharide in good yield. In addition, we prepared two clickable *O*-glycoside and phospho-glycoside versions of Galβ(1→4)Man to enable conjugation to protein carriers for further immunological and antibody-binding studies.

Introduction

Leishmaniasis is a poverty-associated parasitic disease that imposes a heavy burden on the public health systems of least developed countries, particularly those of South Asia, East Africa, and Latin America. The protozoan *Leishmania* spp. (Trypanosomatidae), the etiological agent of leishmaniasis, is a vector-borne obligate intracellular parasite transmitted by hematophagous sandflies. More than 20 human-infective *Leishmania* species have been described to date, together with 70 different types of sandflies (*Phlebotomus* and *Lutzomyia*) identified as vectors.[1,2] Depending on the infecting *Leishmania* species, individuals can present one of the three disease phenotypes: self-resolvent cutaneous leishmaniasis (CL), disfiguring mucocutaneous leishmaniasis (MCL), or potentially fatal visceral leishmaniasis (VL).[2] Although leishmaniasis is an underreported infection, current estimates for CL range from 700 000 to 1.2 million worldwide cases each year, and yearly VL cases are about 100 000.[3] Available anti-leishmaniasis treatments include pentavalent antimonials, liposomal amphotericin B, miltefosine, and paromomycin.[4] These drugs are effective in treating leishmaniasis; however, side effects, poor patient compliance, and parasite resistance motivate the search for new and improved drugs. Moreover, the lack of an effective anti-leishmaniasis vaccine to prevent and treat the infection is a critical need.

A dense glycocalyx covers the *Leishmania* spp. cell membrane playing a pivotal role in the parasite's survival and infectivity. The chemical structure of this sugar coat varies according to the parasite species, its life cycle stage, and the host type.[5] Fig. 1A shows the structure of Leishmania's lipophosphoglycan (LPG), the predominant membrane-glycan in promastigotes, the human-infective form.[6] Its structure comprises a phosphatidylinositol anchor, a conserved oligosaccharide core, a linear phosphoglycan (PG), and a neutral mannose-rich oligosaccharide cap. The chemical structure of LPG varies among *Leishmania* species. The differences are typically found in the structures of the capping oligosaccharides and the type and sequence of the branching sugars connected to the conserved linear PG, a phospho-polysaccharide comprising the [-6)-β-D-Gal(1→4)α-D-Man-(1→PO_3O] repeating unit. For example, in *Leishmania donovani*, the etiological agent of VL in the Old World, promastigotes PG is unsubstituted and remains linear.[7] In contrast, in *L. infantum* (*L. chagasi*), the causative agent of New World's VL, PG carries Glcβ(1→3) substitutions.[8]

Given its role as an immune determinant, LPG is an attractive target for developing glycoconjugate vaccine candidates against leishmaniasis.[9] The groups of Nikolaev, Vishwakarma, and Seeberger have accomplished the synthesis of different LPG fragments and proved the suitability of protein-conjugated LPG subunits as immunogens.[10] However, despite the promising preliminary immunization results,[11] an effective anti-leishmaniasis vaccine remains elusive.

Because of our interest in anti-parasitic immunotherapies[12] and the development of virus-like particles (VLP) glycoconjugates to boost anti-carbohydrate immune responses,[13] we have centered our attention on LPG to explore VLP-conjugated anti-leishmaniasis vaccine candidates. Here, we report an expeditious synthetic route to prepare Galβ(1→4)Man, a disaccharide exclusively found in the linear phosphoglycan and the capping oligosaccharides of *Leishmania's* LPG. Two clickable versions of Galβ(1→4)Man (Fig. 1B), namely 3-azidopropyl 4-*O*-β-D-galactopyranosyl-α-D-mannopyranoside (1) and 3-azidopropyl 4-*O*-β-D-galactopyranosyl-α-D-mannopyranosyl

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† Electronic supplementary information (ESI) available: ^{1}H and ^{13}C{^{1}H} NMR spectra for synthesized compounds. See https://doi.org/10.1039/d2ra05158c

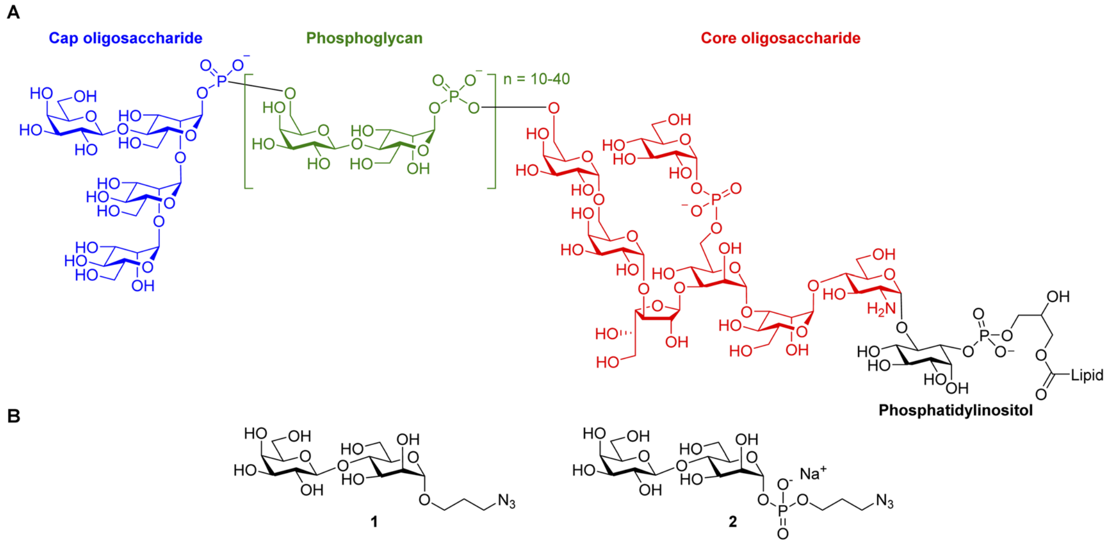


Fig. 1 (A) Structure of *Leishmania donovani* lipophosphoglycan; (B) structures of the clickable Galβ(1→4)Man *O*- and phospho-glycosides **1** and **2** synthesized in this work.

phosphate (2), were prepared to enable conjugation to the protein carrier and subsequent performance of immunological studies.

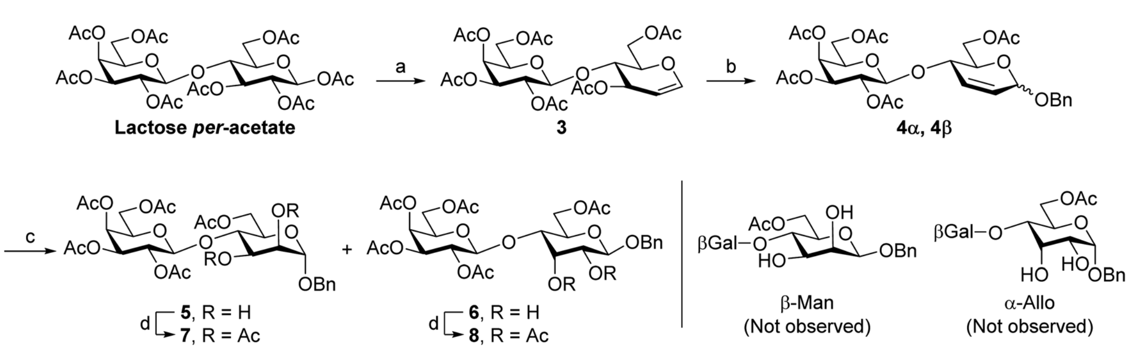
Results and discussion

Synthesis of Galβ(1→4)Man disaccharide

Scheme 1 shows the synthetic strategy to prepare Galβ(1→4)Man. We envisioned the combination of the Ferrier glycosylation[14] and osmium-catalyzed *cis*-dihydroxylation,[15] both high-yielding and stereoselective reactions, as a convenient sequence to synthesize the target disaccharide from the inexpensive and readily available starting material D-lactose *per*-acetate. First. we synthesized the Ferrier glycosyl donor hexa-*O*-acetyl-D-lactal **3** in 42% yield by adjusting the procedure of Zhao *et al.*[16] which involves forming the lactosyl bromide from lactose *per*-acetate followed by reduction with Zn dust. The subsequent Ferrier glycosylation[14,17] of benzyl alcohol with lactal **3**, catalyzed by $BF_3 \cdot Et_2O$, afforded a mixture of 2,3-unsaturated benzyl glycosides **4α** and **4β** (α/β = 6 : 1) in 92% yield. The osmium-catalyzed *cis*-dihydroxylation of the anomeric mixture gave diols **5** (major product) and **6** in excellent yield, which were readily separated by normal-phase flash chromatography. The treatment of diols **5** and **6** with Ac_2O in pyridine yielded the respective *per*-acetates **7** and **8**. Given the convenient peak separation in their ^{1}H NMR spectra, we performed the stereochemical configuration analysis of the oxidation adducts on their respective *per*-acetylated derivatives.

In the ^{1}H NMR spectrum of acetate **7**, derived from the major *cis*-dihydroxylation product **5**, proton H3 (δ 5.32 ppm) appears as a doublet of doublets with coupling constants $^3J_{H3-H4}$ and $^3J_{H3-H2}$ of 8.8 and 3.6 Hz respectively.

The large value for the $^3J_{H3-H4}$ unambiguously identifies a *trans*-diaxial correlation between H3 and H4. The anomeric proton H1 (δ 4.74 ppm) appears as a doublet with a small $^3J_{H1-H2}$ (1.8 Hz). These attributes are consistent with an α-mannose configuration.[18] In acetate **8**, derived from the minor *cis*-dihydroxylation product **6**, H3 (δ 5.76 ppm) appears as a triplet with



Scheme 1 Synthesis of Galβ(1→4)αMan disaccharide *via* Ferrier glycosylation/*cis*-dihydroxylation sequence: (a) synthesis of D-lactal: (i) HBr (34% in AcOH), CH_2Cl_2, 0 °C to room temperature. (ii) Zn, NaH_2PO_4 (sat.), acetone, (42% two steps); (b) BnOH, $BF_3 \cdot Et_2O$, dioxane, room temperature. (66%, α/β = 6 : 1); (c) OsO_4, NMO, H_2O, acetone, room temperature (92%); (d) Ac_2O, pyridine, CH_2Cl_2, room temperature (88% for **7**, 81% for **8**).

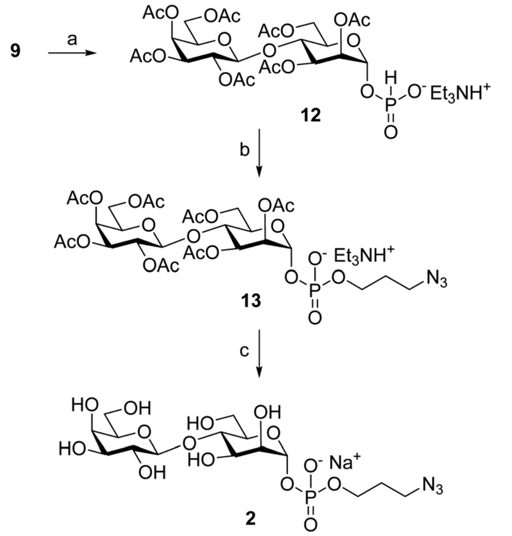
$^3J_{H3-H4}$ and $^3J_{H3-H2}$ values of ~2.5 Hz, indicating a *cis* correlation with H4. The proton H1 (δ 4.83 ppm) showed as a doublet with a large $^3J_{H1-H2}$ value (8.3 Hz), confirming a β-allose configuration for the minor oxidation product.

The results show that the osmium-catalyzed *cis*-dihydroxylation of disaccharide derivatives 2,3-dideoxy-4-*O*-β-D-galactopyranosyl-D-*erythro*-hex-2-enopyranosides exhibit the same stereospecificity reported for the oxidation of the monosaccharide 2,3-dideoxy-D-*erythro*-hex-2-enopyranoside congeners: oxidation of 2,3-unsaturated α-glycosides leads to the formation of α-mannosides whereas the oxidation of the respective β-anomers yields β-allosides.[19]

Since the formation of the β-glycoside in the Ferrier glycosylation limits the yield of the target Galβ(1→4)Man disaccharide **5**, we attempted to improve the α-stereoselectivity of this reaction by testing different solvents (MeCN, dioxane, THF, and Et_2O), common Lewis acid catalysts ($FeCl_3$,[20] TMSOTf,[21] and I_2 (ref. 22)), and Lewis acids reported to afford high α-glycoside yields in Ferrier glycosylations ($Y(OTf)_3$,[23] $Gd(OTf)_3$ (ref. 24)). However, in our hands, these attempts did not significantly improve the α-selectivity of this step using D-lactal **3** as a glycosyl donor.

Synthesis of azide-functionalized Galβ(1→4)αMan *O*-glycoside 1. To facilitate immobilization onto surfaces and conjugation with protein carriers for further immunological studies, we installed a clickable[25] azide-functionalized aglycone on Galβ(1→4)αMan (Scheme 2). The Pd-catalyzed hydrogenolysis of benzyl glycoside **7**, afforded the respective anomeric alcohol **9** in quantitative yield. The subsequent treatment of this intermediate with Cl_3CCN and DBU in MeCN generated the respective imidate **10** which was coupled to 3-azidopropanol by TMSOTf-catalyzed imidate activation in Et_2O at 0 °C. The azide-functionalized glycoside **11** was obtained in 70% yield as a single anomer. The removal of the acetyl protective groups *via* Zemplén transesterification[26] yielded the target azide-functionalized Galβ(1→4)Man α-glycoside **1**.

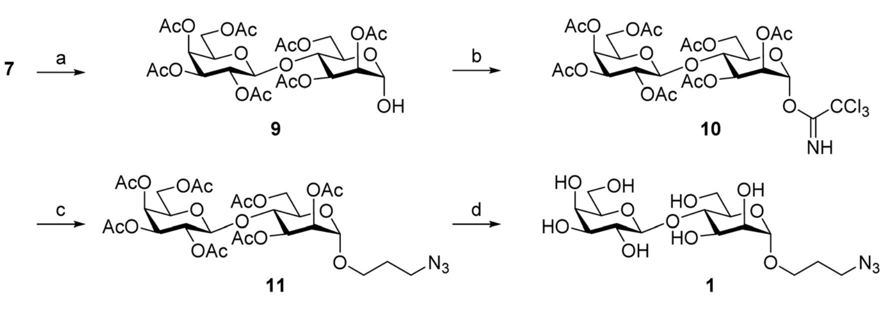
Synthesis of azide-functionalized Galβ(1→4)αMan phospho-glycoside 2. The H-phosphonate **12** was prepared by treating the anomeric alcohol **9** with a freshly prepared solution of the phosphorylating reagent triimidazolylphosphine (PIm_3),[27] made by reacting PCl_3 with imidazole in MeCN (Scheme 3). The addition of alcohol **9** to a PIm_3 solution forms the respective phosphorodiamidite intermediate which upon hydrolysis with triethylammonium bicarbonate buffer (TEAB, pH 7.2) affords the triethylammonium Galβ(1→4)αMan H-phosphonate **12** in 42% yield. The successful H-phosphonylation was confirmed by the ^{31}P NMR spectrum of **12**, which showed a characteristic H-phosphonate signal at δ_P −0.10 ppm (dd, $^1J_{P-H}$ = 638 Hz, $^1J_{P-H1}$ = 9.8 Hz). After confirming H-phosphonate identity, we coupled **12** to 3-azidopropanol in THF using pivaloyl chloride as a coupling reagent. The H-phosphonate diester intermediate, that is formed in this reaction, was oxidized *in situ* with I_2 and H_2O/pyridine to afford the respective phosphate diester **13** in 30% yield. The ^{31}P NMR spectrum of **13** showed phosphorus at δ_P −3.09 (d, $^3J_{P-H1}$ = 6.9 Hz) confirming the H-phosphonate oxidation to phosphate. Removal of the acetyl protecting groups afforded the target azide-functionalized Galβ(1→4)αMan phospho-glycoside **2**.



Scheme 3 Synthesis of azide-functionalized Galβ(1→4)αMan phospho-glycoside **2**. (a) PCl_3, imidazole, Et_3N, MeCN (42%); (b) (i) 3-azidopropanol, PivCl, THF; (ii) I_2, H_2O/pyridine (5 : 95) (30% two steps); (c) NaOMe, MeOH, Amberlite IR 120(+) (67%).

Conclusions

We have synthesized the βGal(1→4)αMan disaccharide, the phospho-polysaccharide repeating unit found in the cell surface



Scheme 2 Synthesis of clickable Galβ(1→4)Man *O*-glycoside. (a) H_2, Pd/C, EtOH (96%); (b) Cl_3CCN, DBU, CH_2Cl_2 (68%); (c) 3-azidopropanol, TMSOTf, CH_2Cl_2 (70%); (d) NaOMe, MeOH, Amberlite IR120 (H^+) (97%).

lipophosphoglycan of all Leishmania species. The Ferrier glycosylation/*cis*-dihydroxylation sequence proved efficient in affording the target βGal(1→4)Man core in a stereoselective fashion from readily available lactose *per*-acetate. Two clickable versions of βGal(1→4)Man, azide-functionalized *O*-glycoside and phospho-glycoside, were also prepared to enable coupling to protein carriers and immobilization onto surfaces for further immunological and binding studies.

Experimental

General information

D-Lactose and all reagents were purchased to the highest purity available and used without previous purification. Yields refer to isolated yields after chromatographic purification and anomeric ratios were determined from the ^{1}H NMR spectra of reaction crudes. Reactions were monitored by thin-layer chromatography (TLC) using SiliCycle SiliaPlate glass-backed TLC 250 mm with F-254 UV indicator. TLC visualization was performed under short wave UV light (254 nm) and charring with 5% H_2SO_4 in CH_3OH. Normal phase flash chromatography was performed using SiliCycle SiliaFlash P60 silica gel (40–63 μm) and mixtures of EtOAc-hexanes and CH_2Cl_2–MeOH (ACS-grade solvents) as eluents. 1D and 2D NMR spectra were recorded on a Bruker Avance 400 spectrometer at 298 K. ^{1}H NMR chemical shifts are reported in parts per million *vs.* tetramethylsilane (TMS) internal standard (δ_H 0.00 ppm). $^{13}C\{^1H\}$ NMR chemical shifts are reported in parts per million *vs.* the residual peak of the solvent.[28] LCMS data were collected in a Waters e2695 HPLC system coupled to a Waters Acquity QDa single quadrupole mass detector. HRMS data for new compounds were collected in a Waters high-resolution Xevo G2-XS Q-ToF mass spectrometer coupled with an Acquity UPLC H-Class in the positive electrospray ionization mode.

3,6-Di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1,5-anhydro-2-deoxy-D-arabino-hex-1-enitol (3). Following the procedure described by Zhao *et al.*[16] In a round-bottomed flask, a solution of HBr (147 ml, 0.62 moles, 34% in HOAc) in dry CH_2Cl_2 (200 mL) was cooled to 0 °C with an ice bath. Then, a solution of lactose *per*-acetate (141.2 g, 0.21 moles) in dry CH_2Cl_2 (100 mL) was added dropwise through addition funnel. After completing the addition, the system was allowed to warm to room temperature and stirring was kept until TLC showed total consumption of the sugar (∼3 hours). The mixture was then diluted with water, transferred to a separation funnel, and extracted with CH_2Cl_2. The organic layer was then successively washed with H_2O (3 times), and $NaHCO_3$ (sat. 2 times). The organic phase was collected, dried over Na_2SO_4, filtered, and the volatiles removed in rotary evaporator. The resulting material (hepta-*O*-acetyl lactose bromide) was then re-dissolved in acetone (420 mL), and a saturated aqueous solution of NaH_2PO_4 (830 mL) was added. To the resulting biphasic system, Zn (170 g, 2.6 moles) was added portion-wise, and the suspension was maintained at room temperature under strong stirring for 12 hours. After that period, TLC showed total consumption of the starting bromide and the predominance of a slightly less polar spot. The solids were then removed by filtration through a Celite pad, and the organic filtrate was concentrated in rotary evaporator. The residue was re-dissolved in EtOAc, washed with water (2 times), dried over Na_2SO_4, filtered, and the volatiles removed in rotary evaporator. Flash chromatography purification of the residue afforded hexa-*O*-acetyl D-lactal 2 (49.4 g, 88.2 mmol, 42%) as a colourless syrup. The identity of *per*-acetylated lactal 3 was confirmed by comparing its ^{1}H and $^{13}C\{^1H\}$ NMR spectra with reported data.[29] R_f = 0.56 (EtOAc/*n*-Hex 3 : 2), ^{1}H NMR (500 MHz, $CDCl_3$): 6.35 (dd, J = 1.0 and 6.1 Hz, H-1), 5.34 (t, J = 4.3 Hz, H-3), 5.30 (dd, J = 1.0 and 3.3 Hz, H-4′), 5.13 (dd, J = 7.9 and 10.5 Hz, H-2′), 4.94 (dd, J = 3.4 and 10.5 Hz, H-3′), 4.77 (dd, J = 3.4 and 6.1 Hz, H-2), 4.59 (d, J = 8.0 Hz, H-1′), 4.37 (dd, J = 2.6 and 11.7 Hz, H-6), 4.13 (dd, J = 6.2 and 11.7 Hz, H-6), 4.08 (m, H-6′, H-5), 4.01 (dd, J = 7.3 and 11.2 Hz, H-6′), 3.93 (dd, J = 5.4 and 7.4 Hz, H-4), 3.84 (ddd, J = 1.0, 6.0 and 7.0 Hz, H-5′), 2.09 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.91 (s, 3H); APT $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$): 170.4, 170.4, 170.2, 170.1, 169.9, 169.3, 145.4 (C-1), 101.0 (C-1′), 98.9 (C-2), 74.6 (C-4), 74.1 (C-5), 70.8 (C-3′), 70.7 (C-5′), 68.8 (C-3), 68.8 (C-4), 66.6 (C-4′), 61.8 (C-6), 60.9 (C-6′), 21.1, 20.8, 20.6, 20.6, 20.6, 20.5.

Benzyl 2,3-dideoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-6-*O*-acetyl-α-D-erythro-hex-2-enopyranoside (4α and 4β). D-Lactal (2.0 g, 3.5 mmol) and benzylic alcohol (563 μL, 1.5 equiv.) were dissolved in dry CH_2Cl_2 (10.0 mL mmol^{-1}) and cooled to 0 °C under nitrogen atmosphere. Then boron trifluoride (230 μL, 0.5 equiv.) was added dropwise, and the reaction was allowed to warm to room temperature. Reaction was stirred until TLC analysis showed total consumption of starting lactal (2 hours). Then the reaction is cooled down to 0 °C and quenched with Et_3N (500 μL). Crude was diluted with CH_2Cl_2, transferred to a separatory funnel, and washed with water. Aqueous layer was re-extracted with CH_2Cl_2, and organic fractions were gathered, dried over Na_2SO_4, filtered, and concentrated in rotary evaporator. Silica gel flash chromatography (EtOAc/*n*-Hex) afforded 1.4 g (2.3 mmol, 66%) of a mixture of **4α** and **4β**. Physical data for major product **4a** R_f = 0.5 (EtOAc/*n*-Hex, 1 : 3), ^{1}H NMR (500 MHz, $CDCl_3$): 7.35–7.26 (m, 5H), 6.11 (d, J = 10.2 Hz, H-3), 5.78 (ddd, J = 2.2, 2.2 and 10.2 Hz, H-2), 5.39 (d, J = 2.7 Hz, H-4′), 5.21 (dd, J = 8.0 and 10.4 Hz, H-2′), 5.08 (d, J = 2.2 Hz, H-1), 5.00 (dd, J = 3.4 and 10.4 Hz, H-3′), 4.79 (d, J = 11.8 Hz, OC*H*$_2$Ph), 4.57 (d, J = 8.9 Hz, H-1′), 4.58 (d, J = 11.8 Hz, OC*H*$_2$Ph), 4.20 (m, H-4, H-6′, H-6 and H-6), 4.11 (m, H-6′ and H-5), 3.92 (dd, J = 6.1 and 6.1 Hz, H-5′), 2.16 (s, 3H), 2.16 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H); APT $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$): 170.7, 170.4, 170.3, 170.1, 169.4, 137.7, 131.6 (C-3), 128.4 (2C), 127.9 (2C), 127.8, 127.1 (C-2), 102.2 (d, C-1′), 93.6 (d, C-1), 73.4, 70.9 (C-3′), 70.8 (C-5′), 70.1 (O*C*H$_2$Ph), 68.8 (C-2′), 67.6 (C-5), 66.9 (C-4′), 63.0 (C-6), 61.3 (C-6′), 20.9, 20.7, 20.7, 20.7, 20.6

Benzyl 4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-6-*O*-acetyl-α-D-mannopyranoside (5) and Benzyl 4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-6-*O*-acetyl-β-D-allopyranoside (6). The mixture of Ferrier adducts **4α** and **4β** (6.38 g, 10.5 mmol, α/β = 6 : 1) and *N*-methylmorpholine *N*-oxide (1.7 g, 12.5 mmol), were dissolved in acetone (40 mL) at room temperature. Then, OsO_4 (1.3 ml, 4% sol. in water, 0.20 mmol) was added and the system was maintained for 12 hours at room temperature under vigorous stirring. After that period, TLC showed total consumption of the starting olefin and the predominance of two more polar spots. Then, the volatiles were removed in rotary evaporator and the resulting syrup was purified by normal phase flash chromatography using an isocratic EtOAc/*n*-Hex (7 : 3) elution system. Chromatographic separation afforded the diol **5** (3.6 g, 5.6 mmol, 53%) as a white foam, the diol **6** (758.3 mg, 1.18 mmol, 11%) as a white foam, and a mixed fraction of **5** and **6** (1.83 g, **5**/**6** = 12 : 1). The combined isolated yield for this reaction was 92%. Physical data for major derivative **5** (Galβ(1→4)Man): R_f = 0.57 (EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): 7.30–7.22 (m, 5H), 5.33 (dd, *J* = 0.7 and 3.4 Hz, H-4′), 5.17 (dd, *J* = 8.0 and 10.5 Hz, H-2′), 4.93 (dd, *J* = 3.4 and 10.5, H-3′), 4.88 (d, *J* = 1.0 Hz, H-1), 4.64 (d, *J* = 11.8 Hz, 1H), 4.45 (d, *J* = 8.0 Hz, H-1′), 4.44 (d, *J* = 11.8 Hz, 1H), 4.22 (dd, *J* = 2.0 and 11.8 Hz, H-6′), 4.11 (dd, *J* = 4.8 and 11.6 Hz, H-6), 4.06 (dd, *J* = 7.6 and 11.3 Hz, H-6), 4.01 (dd, *J* = 5.5 and 11.7 Hz, H-6′), 4.03 (m, H-2 and H-5′), 3.94 (dd, *J* = 1.0 and 8.7 Hz, H-3), 3.96 (m, 2H, H-2 and H-5′), 3.88 (dd, *J* = 3.5 and 8.6 Hz, H-3), 3.82 (ddd, *J* = 1.6, 5.4, and 9.9 Hz, H-5), 3.67 (dd, *J* = 8.9 and 9.6 Hz, H-4), 2.10 (s, 3H), 2.06 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.91 (s, 3H); APT ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$): 170.6, 170.2, 169.9, 169.8, 169.4, 136.6, 128.4, 127.9 (x 2Cs), 127.8 (x 2Cs), 101.7 (C-1′), 97.9 (C-1), 80.4, 71.3, 70.7, 70.1, 69.4, 69.2, 68.5, 67.7, 66.7, 62.8, 61.6, 20.8, 20.5, 20.4, 20.4, 20.3; HRMS (ESI/Q-TOF) *m*/*z*: [M + Na]$^+$ calcd for $C_{29}H_{38}NaO_{16}$ 665.2058; found 665.2137; physical data for minor derivative **6** (Galβ(1→4)All): R_f = 0.48 (EtOAc/*n*-Hex, 4 : 1), ^{1}H NMR (400 MHz, $CDCl_3$): 7.35 (m, 5H), 5.39 (d, *J* = 2.8 Hz, H-4′), 5.24 (dd, *J* = 8.2 and 10.3 Hz, H-2′), 5.01 (dd, *J* = 3.3 and 10.5 Hz, H-3′), 4.91 (d, *J* = 11.8 Hz, 1H, $PhCH_2O$), 4.73 (d, *J* = 7.8 Hz, H-1), 4.64 (d, *J* = 11.8 Hz, 1H, $PhCH_2O$), 4.57 (d, *J* = 8.0 Hz, H-1′), 4.36 (d, *J* = 2.1 Hz, H-3), 4.32 (dd, *J* = 1.6 and 12.2 Hz, H-6), 4.21 (dd, *J* = 7.8 and 11.2 Hz, H-6′), 4.06 (m, 2H, H-6′, H-6), 3.96 (m, 2H, H-5, H-5′), 3.64 (dd, *J* = 2.3 and 9.5 Hz, H-4), 3.51 (ddd, *J* = 3.0, 7.6, and 10.0 Hz, H-2), 2.78 (s, 1H, OH), 2.59 (d, *J* = 7.6 Hz, 1H, OH) 2.17 (s, 3H), 2.11 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 1.99 (s, 3H); APT ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): 170.7, 170.5, 170.1, 170.0, 169.5, 137.1, 128.5, 128.1, 127.9, 101.8 (C-1′), 99.5 (C-1), 78.1 (C-4), 71.4 (C-5′), 71.2 (O*C*H$_2$Ph), 70.6 (C-2), 70.5 (C-3′), 69.6 (C-5), 69.5 (C-3), 68.4 (C-2′), 66.9 (C-4′), 63.2 (C-6), 61.4 (C-6′), 20.9, 20.6, 20.6, 20.5, 20.5; HRMS (ESI/Q-TOF) *m*/*z*: [M + Na]$^+$ calcd for $C_{29}H_{38}NaO_{16}$ 665.2058; found 665.2141.

General procedure for acetylation

Diol, Ac_2O (6.0 equiv.), and pyridine (3.0 equiv.) were dissolved in dry CH_2Cl_2 (5.0 mL mmol^{-1}) at room temperature and stirred until TLC showed total consumption of the starting diol (~15 min). Then, the mixture was cooled to 0 °C with an ice bath and the excess of Ac_2O was quenched by addition of EtOH (30 equiv.). The volatiles were then removed in rotary evaporator and the residue was re-dissolved in EtOAc, transferred to a separation funnel, and washed with H_2O followed by 1 M HCl, and $NaHCO_3$. The organic layer was collected, dried over Na_2SO_4, filtered, and concentrated in rotary evaporator. The crude acetate was purified by normal phase flash chromatography using EtOAc/*n*-Hex (1 : 1 to 7 : 3) gradient as eluent system.

Benzyl 2,3-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-6-*O*-acetyl-α-D-mannopyranoside (7). Following the general procedure for acetylation, diol **5** (2.11 g, 3.3 mmol), Ac_2O (1.9 mL, 19.8 mmol), and pyridine (797 μL, 9.9 mmol), afforded the respective octaacetate **7** (2.13 g, 2.9 mmol, 88%) as a white solid. R_f = 0.44 (EtOAc/*n*-Hex, 3 : 2), ^{1}H NMR (400 MHz, $CDCl_3$): 7.31–7.22 (m, 5H), 5.32 (dd, *J* = 3.6 and 8.8 Hz, H-3), 5.27 (dd, *J* = 1.0 and 3.4 Hz, H-4′), 5.20 (dd, *J* = 1.9 and 3.6 Hz, H-2), 5.07 (dd, *J* = 7.9 and 10.5 Hz, H-2′), 4.89 (dd, *J* = 3.4 and 10.4 Hz, H-3′), 4.74 (d, *J* = 1.8 Hz, H-1), 4.62 (d, *J* = 11.9 Hz, 1H, OC*H*$_2$Ph), 4.48 (d, *J* = 7.9 Hz, H-1′), 4.46 (d, *J* = 11.9 Hz, 1H, OC*H*$_2$Ph), 4.29 (dd, *J* = 1.7 and 11.9 Hz, H-6a), 4.10 (m, 2H, H-6b and H-6a′), 3.96 (dd, *J* = 7.7 and 11.9 Hz, H-6b′), 3.86 (m, 2H, H-5 and H-4), 3.80 (dd, *J* = 1.0 and 7.7 Hz, H-5′), 2.08 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.90 (s, 3H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): 170.5, 170.4, 170.2, 170.1, 169.8, 169.3, 169.2, 136.2, 128.6 (×2C), 128.2 (×2C), 128.2, 101.1 (C-1), 96.4 (C-1), 74.0, 71.0, 70.4, 69.7, 69.6, 69.6, 69.2, 69.1, 66.6, 62.6, 60.8, 20.9, 20.8, 20.8, 20.6, 20.6, 20.6, 20.5; HRMS (ESI/Q-TOF) *m*/*z*: [M + Na]$^+$ calcd for $C_{33}H_{42}NaO_{18}$ 749.2269; found 749.2399.

Benzyl 2,3-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-6-*O*-acetyl-β-D-allopyranoside (8). Following the general procedure for acetylation, diol **6** (124.2 mg, 0.19 mmol), Ac_2O (107 μL, 1.14 mmol), and pyridine (45 μL, 0.57 mmol) afforded the respective octaacetate **8** (109.0 mg, 0.15 mmol, 81%) as a white solid. R_f = 0.45 (EtOAc/*n*-Hex, 3 : 2), ^{1}H NMR (400 MHz, $CDCl_3$): 7.33 (m, 5H), 5.76 (t, *J* = 2.5 Hz, H-3), 5.33 (d, *J* = 2.6 Hz, H-4′), 5.15 (dd, *J* = 7.9 and 10.3 Hz, H-2′), 4.98 (dd, *J* = 3.2 and 10.4 Hz, H-3′), 4.89 (d, *J* = 12.1 Hz, 1H, OC*H*$_2$Ph), 4.83 (d, *J* = 8.3 Hz, H-1), 4.77 (dd, *J* = 2.5 and 8.2 Hz, H-2), 4.63 (d, *J* = 12.1 Hz, 1H, OC*H*$_2$Ph), 4.56 (d, *J* = 7.8 Hz, H-1′), 4.38 (d, *J* = 10.1 Hz, H-6), 4.03 (m, 4H, H-5, H-6′, H-6Gal, H-6′Gal), 3.91 (d, *J* = 6.4 and 6.4 Hz, H-5′), 3.80 (d, *J* = 2.5 and 9.3 Hz, H-4), 2.12 (s, 3H), 2.11 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): 170.6, 170.6, 170.1, 169.9, 169.6, 169.6, 169.5, 137.1, 128.3 (×2C), 127.8, 127.6 (×2C), 100.7 (C-1′), 97.4 (C-1), 74.9 (C-4), 70.9 (O*C*H$_2$Ph), 70.8 (C-5′), 70.7 (C-3′), 70.6 (C-5), 70.1 (C-2), 68.9 (C-3), 68.7 (C-2′), 66.8 (C-4′), 62.9 (C-6), 61.3 (C-6′), 20.8, 20.8, 20.6, 20.6, 20.6, 20.5, 20.5; HRMS (ESI/Q-TOF) *m*/*z*: [M + Na]$^+$ calcd for $C_{33}H_{42}NaO_{18}$ 749.2269; found 749.2330.

2,3-Di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl β-D-galactopyranosyl)-6-*O*-acetyl-α-D-mannopyranose (9). In a round-bottomed flask, benzyl glycoside **7** (1.92 g, 2.6 mmol) was dissolved in EtOH (300 mL) and Pd/C (260 mg) was added under inert atmosphere (N_2). The system was closed with a septum and the N_2 replaced by a reducing H_2 atmosphere *via* four vacuum/H_2 cycles. The reaction was vigorously stirred at room temperature until TLC showed complete consumption of the starting benzyl glycoside (~3 hours). The catalyst was removed by passing the suspension through a Celite® pad and the volatiles were removed by rotary evaporation. The resulting crude product was used in the next step without further purification. This procedure afforded the alcohol **9** (1.59 g, 2.5 mmol, 96%, α : β = 8 : 1) as a colorless syrup. Physical data for major anomer (α): R_f = 0.62 (EtOAc/*n*-Hex, 19 : 1), ^{1}H NMR (400 MHz, $CDCl_3$): 5.34 (dd, J = 3.5 and 9.5 Hz, H-3), 5.28 (d, J = 2.5 Hz, H-4′), 5.15 (dd, J = 1.5 and 3.3 Hz, H-2), 5.08 (d, J = 1.5 Hz, H-1), 5.05 (dd, J = 7.8 and 10.4 Hz, H-2′), 4.90 (dd, J = 3.3 and 10.5 Hz, H-3′), 4.49 (d, J = 7.9 Hz, H-1′), 4.38 (dd, J = 3.5 and 13.2 Hz, H-6_{Man}), 4.10 (m, 3H, H-5, H-6_{Gal}, and H-$6'_{Man}$), 3.96 (dd, J = 7.6 and 11.0 Hz, H-$6'_{Gal}$), 3.84 (m, 2H, H5′ and H4), 2.09 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 1.99 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H), 1.90 (s, 3H); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): 170.8, 170.5, 170.3, 170.2, 170.1, 169.6, 169.3, 100.9 (C-1′), 91.8 (C-1), 74.2 (C-4), 70.9 (C-3′), 70.4 (C-5′), 70.3 (C-2), 69.2 (C-2′), 69.1 (C-3), 68.9 (C-5), 66.7 (C-4′), 62.6 (C-6), 60.9 (C-6′), 20.9, 20.9, 20.8, 20.6, 20.6, 20.6, 20.5; HRMS (ESI/Q-TOF) *m*/*z*: $[M + Na]^+$ calcd for $C_{26}H_{36}NaO_{18}$ 659.1799; found 659.1939.

4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1-(2,2,2-trichloroethanimidate)-α-D-mannopyranose 2,3,6-triacetate (10). To a solution of alcohol **9** (535.2 mg, 0.84 mmol), and Cl_3CCN (400 mL, 5.04 mmol) in dry CH_2Cl_2 (4.0 mL), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 37 mL, 0.25 mmol) was added and the system was stirred at room temperature for 18 hours. After that period, TLC showed total consumption of the starting material. The volatiles were then removed in rotary evaporator and the residue was directly purified by normal phase flash chromatography. The imidate **10** (446.1 mg, 0.57 mmol, 68%) was obtained as a yellowish foam. R_f = 0.47 (EtOAc/*n*-Hex/Et_3N, 60 : 40 : 1), ^{1}H NMR (400 MHz, $CDCl_3$): 8.68 (s, 1H), 6.15 (d, J = 2.0 Hz, H-1), 5.37 (m, 2H, H-2, H-3), 5.29 (dd, J = 0.8 and 3.3 Hz, H-4′), 5.09 (dd, J = 8.0 and 10.5 Hz, H-2′), 4.91 (dd, J = 3.4 and 10.5 Hz, H-3′), 4.52 (d, J = 7.8 Hz, H-1′), 4.37 (dd, J = 1.9 and 11.9 Hz, H-6), 4.12 (dd, J = 6.1 and 11.1 Hz, H-6′), 4.10 (dd, J = 5.2 and 11.1 Hz, H-6), 4.05 (m, H-5), 3.97 (dd, J = 7.7 and 11.2 Hz, H-6′), 3.93 (dd, J = 9.6 and 9.6 Hz, H-4), 3.83 (dd, J = 7.4 and 7.4 Hz, H-5′), 2.11 (s, 3H), 2.09 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.98 (s, 3H), 1.91 (s, 3H). APT $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): 170.4, 170.4, 170.2, 170.1, 169.5, 169.3, 169.2, 160.0 ($\underline{C}$=NH), 101.4 (C-1′), 94.5 (C-1), 90.5 ($\underline{C}Cl_3$), 74.2 (C-4), 71.5 (C-5), 71.0 (C-3′), 70.5 (C-5′), 69.2 (C-3), 69.2 (C-2′), 67.9 (C-2), 66.5 (C-4′), 62.2 (C-6), 60.8 (C-6′), 20.8, 20.8, 20.8, 20.7, 20.7, 20.6, 20.6; HRMS (ESI/Q-TOF) *m*/*z*: $[M + Na]^+$ calcd for $C_{28}H_{36}Cl_3NNaO_{18}$ 802.0896; found 802.0851.

3-Azidopropyl 2,3-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-6-*O*-acetyl-α-D-mannopyranoside (11). Imidate **10** (367.0 mg, 0.47 mmol) and 3-azidopropanol (142.5 mg, 1.41 mmol) were placed in a round-bottomed flask. Prior to glycosyl donor activation, the system was dried *via* azeotropic removal of H_2O traces with toluene (3 cycles of ∼2.0 mL) by rotary evaporation. The reactants were then dissolved in dry CH_2Cl_2 (3.0 mL) and cooled to 0 °C with an ice bath. Imidate was activated by addition of $BF_3 \cdot Et_2O$ (30 mL, 0.24 mmol) and the reaction was allowed to warm at room temperature under stirring. After 3 hours, TLC showed a significant amount of remaining imidate and more BF_3Et_2O (50 mL, 0.4 mmol) was added. After 1 hour, TLC showed reaction completion and the excess of Lewis acid was quenched with Et_3N (1.0 mL). Then, the volatiles were removed by rotary evaporation and the residue directly purified by normal phase flash chromatography using EtOAc/*n*-Hex (3 : 7 to 3 : 2) gradient as eluent system. Glycoside **11** (240.0 mg, 0.33 mmol, 70%) was obtained as a colorless oil. R_f = 0.43 (EtOAc/*n*-Hex, 7 : 3), ^{1}H NMR (500 MHz, $CDCl_3$): 5.28 (dd, J = 1.0 and 3.4 Hz, H-4′), 5.25 (dd, J = 3.6 and 9.1 Hz, H-3), 5.15 (dd, J = 1.9 and 3.6 Hz, H-2), 5.07 (dd, J = 7.9 and 10.5 Hz, H-2′), 4.90 (dd, J = 3.4 and 10.4 Hz, H-3′), 4.68 (d, J = 1.9 Hz, H-1), 4.48 (dd, J = 8.0 Hz, H-1′), 4.35 (dd, J = 1.2 and 11.9 Hz, H-6_{Man}), 4.12 (dd, J = 6.0 and 11.1 Hz, H-$6'_{Man}$), 4.11 (dd, J = 1.3 and 11.6 Hz, H-6_{Gal}), 3.96 (dd, J = 7.8 and 11.1 Hz, H-$6'_{Gal}$), 3.82 (m, 3H, H-4, H-5, and H-5′), 3.71 (m, 1H), 3.43 (m, 1H), 3.35 (t, J = 6.5 Hz, 2H), 2.09 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H), 1.91 (s, 3H), 1.79 (m, 2H); APT $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$): 170.5, 170.4, 170.2, 170.1, 169.8, 169.3, 169.2, 101.2 (C-1′), 97.4 (C-1), 74.4, 70.9 (C-3′), 70.4, 69.6 (C-2), 69.4 (C-3), 69.2 (C-2′), 69.1, 66.6 (C-4′), 64.8 ($O\underline{C}H_2CH_2CH_2N_3$), 62.5 (C-6), 60.8 (C-6′), 48.1 ($OCH_2CH_2\underline{C}H_2N_3$), 28.6 ($OCH_2\underline{C}H_2CH_2N_3$), 20.9, 20.8, 20.8, 20.7, 20.6, 20.6, 20.5; HRMS (ESI/Q-TOF) *m*/*z*: $[M + Na]^+$ calcd for $C_{29}H_{41}N_3NaO_{18}$ 742.2283; found 742.2336.

3-Azidopropyl 4-*O*-β-D-galactopyranosyl-α-D-mannopyranoside (1). In a round-bottomed flask provided with a magnetic bar, *per*-acetylated disaccharide **11** (221.1 mg, 0.31 mmol) and NaOMe (∼10 mg) were dissolved in CH_3OH (3.0 mL) at room temperature. The solution was stirred until TLC showed total consumption of the starting disaccharide (∼12 h). The reaction was then diluted with CH_3OH (3.0 mL) and neutralized with Amberlite IR-120 (H form). The mixture was filtered, and the volatiles removed by rotary evaporation. This procedure afforded the target azide-derived Galβ(1→4)αMan disaccharide **1** as a white foam (127.6 mg, 0.30 mmol, 97%). R_f = 0.50 (MeOH/CH_2Cl_2, 1 : 4), ^{1}H NMR (400 MHz, CD_3OD): 4.78 (s, H-1), 4.36 (d, J = 7.4 Hz, H-1′), 3.90 (m, 4H), 3.81 (m, 4H), 3.73 (dd, J = 4.3 and 11.5 Hz, H-6), 3.63 (m, 2H), 3.53 (m, 3H), 3.41 (m, 2H), 1.88 (m, 2H); $^{13}C\{^1H\}$ NMR (100 MHz, CD_3OD): 103.8 (C-1′), 100.0 (C-1), 76.9, 75.8, 73.4, 71.7, 71.2, 70.1, 69.8, 69.0, 64.1, 61.2, 60.7, 48.1, 28.5; HRMS (ESI/Q-TOF) *m*/*z*: $[M + Na]^+$ calcd for $C_{15}H_{27}N_3NaO_{11}$ 448.1543; found 448.1654.

2,3-Di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-6-*O*-acetyl-α-D-mannopyranosyl H-phosphonate (12). In a round-bottomed flask provided with a magnetic bar, imidazole (2.6 g, 38.2 mmol) and Et_3N (5.4 mL, 38.7 mmol) were suspended in MeCN (30.0 mL) and cooled to 0 °C. PCl_3 (1.1 mL, 12.6 mmol) was then added, and the resulting suspension was vigorously stirred at 0 °C for 20 minutes. Then, a solution of alcohol **9** (1.0 g, 1.6 mmol) in MeCN (5.0 mL) was added dropwise over 30 minutes under vigorous stirring. The reaction was allowed to warm to room temperature. After 4 h, TLC showed complete consumption of the starting disaccharide, and the system was quenched by addition of 1 M TEAB buffer (27 mL, pH 7.2) and left stirring for 20 minutes. The volatiles were then removed by rotary evaporation and the residue was re-dissolved in $CHCl_3$, transferred to a separation funnel, and washed with 1 M TEAB buffer. The organic layer was collected, dried over Na_2SO_4, filtered, concentrated in rotary evaporator, and the resulting crude was purified by normal phase flash chromatography using a MeOH/CH_2Cl_2 gradient (1 : 24 to 1 : 9) containing 1% Et_3N as mobile phase. Purification afforded H-phosphonate **12** (409.1 mg, 0.50 mmol, 42%) as a white solid. R_f = 0.50 (MeOH/CH_2Cl_2, 1 : 4), ^{1}H NMR (400 MHz, $CDCl_3$): 6.92 (d, J = 638 Hz, P–$\underline{H}$), 5.47 (dd, $^3J_{H1-H2}$ = 2.3 and $^3J_{H-P}$ = 10.5 Hz, H-1), 5.32 (dd, J = 3.4 and 9.5 Hz, H-3), 5.29 (d, J = 3.0 Hz, H-4′), 5.21 (m, H-2), 5.07 (dd, J = 7.4 and 10.0 Hz, H-2′), 4.89 (dd, J = 3.5 and 10.0 Hz, H-3′), 4.47 (d, J = 10.5 Hz, H-1′), 4.35 (dd, J = 3.2 and 12.0 Hz, H-6$_{Man}$), 4.12 (m, 3H, H-5, H-6$_{Gal}$, and H-6′$_{Man}$), 3.96 (dd, J = 7.7 and 11.0 Hz, H-6′$_{Gal}$), 3.84 (t, J = 9.3 Hz, H-5′), 3.82 (dd, J = 7.2 and 7.2 Hz, H-4), 3.02 (q, J = 7.3 Hz, 6H, N(C$\underline{H}_2$CH$_3$)$_3$), 2.09 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.94 (s, 3H), 1.91 (s, 3H), 1.30 (t, J = 7.3 Hz, 9H, N(CH$_2$C$\underline{H}_3$)$_3$); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): 170.6, 170.4, 170.2, 170.1, 169.8, 169.6, 169.1, 101.1 (C-1′), 92.6 (d, $^2J_{C-P}$ = 3.7 Hz, C-1), 74.0 (C-5′), 70.9 (C-3′), 70.4 (C-4), 70.1 (d, $^3J_{C-P}$ = 7.2 Hz, C-2), 69.9 (C-5), 69.4 (C-3), 69.1 (C-2′), 66.7 (C-4′), 62.4 (C-6), 60.8 (C-6′), 45.7 (N($\underline{C}$H$_2$CH$_3$)$_3$), 20.8, 20.8, 20.8, 20.6, 20.6, 20.6, 20.5, 8.6 (N(CH$_2$$\underline{C}H_3$)$_3$); ^{31}P NMR (162 MHz, $CDCl_3$): −0.10 (dd, $^1J_{P-H}$ = 638 Hz, $^3J_{P-H1}$ = 9.8 Hz); HRMS (ESI/Q-TOF) m/z: [M − H]$^-$ calcd for $C_{26}H_{36}O_{20}P$ 699.1543; found 699.1545.

Triethylammonium 3-azidopropyl 2,3-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-6-*O*-acetyl-α-D-mannopyranosyl phosphate (13). In a round-bottomed flask provided with a magnetic bar, H-phosphonate **12** (100.1 mg, 0.12 mmol) and 3-azidopropanol (14 µL, 0.15 mmol) were dissolved in dry THF (2.8 mL) and cooled to 0 °C with an ice bath. To the resulting solution, PivCl (37 µL, 0.3 mmol) was added and the system was stirred allowing it to warm to room temperature. After 3 hours, TLC showed complete consumption of the starting H-phosphonate and a solution of I_2 (121.8 mg, 0.48 mmol) in pyridine/H_2O (19 : 1, 1.0 mL) was added. TLC showed the oxidation of H-phosphonate to phosphate completes in less than 30 minutes. The reaction was then quenched by addition of 10% $Na_2S_2O_3$ (6.0 mL), transferred to a separation funnel, and extracted with $CHCl_3$. The organic layer was then successively washed with brine, and 1 M TEAB buffer. The collected organic fraction was then dried over Na_2SO_4, filtered, and concentrated in rotary evaporator. The resulting crude was purified by normal phase flash chromatography using a MeOH/CH_2Cl_2 gradient (1 : 99 to 1 : 9) containing 1% of Et_3N. This procedure afforded the phosphodiester **13** (30.2 mg, 0.03 mmol, 30%) as a white solid. R_f = 0.5 (MeOH/CH_2Cl_2, 15 : 85), ^{1}H NMR (400 MHz, $CDCl_3$): 11.9 (br s, 1H, HN$^+$(CH$_2$CH$_3$)$_3$), 5.41 (dd, $^3J_{H1-H2}$ = <1.0 Hz and $^3J_{H1-P}$ = 7.9 Hz, H-1), 5.31 (dd, J = 3.5 and 9.4 Hz, H-3), 5.28 (d, J = 3.3 Hz, H-4′), 5.24 (s, H-2), 5.07 (dd, J = 8.2 and 10.4 Hz, H-2′), 4.89 (dd, J = 3.2 and 10.4 Hz, H-3′), 4.47 (d, J = 8.0 Hz, H-1′), 4.36 (d, J = 10.9 Hz, H-6$_{Man}$), 4.12 (m, 3H, H-5, H-6′$_{Man}$, H-6$_{Gal}$), 3.95 (dd, J = 7.9 and 11.0 Hz, H-6′$_{Gal}$), 3.92 (m, 2H, OC$\underline{H}_2$CH$_2$CH$_2$N$_3$), 3.87 (t, J = 9.7 Hz, H-4), 3.81 (dd, J = 6.8 and 8.0 Hz, H-5′), 3.37 (t, J = 6.9 Hz, OCH$_2$CH$_2$C$\underline{H}_2$N$_3$), 3.04 (m, 6H, HN$^+$(CH$_2$CH$_3$)$_3$), 2.09 (s, 3H), 2.06 (s, 3H), 2.06 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.94 (s, 3H), 1.91 (s, 3H), 1.83 (m, 2H, OCH$_2$C$\underline{H}_2$CH$_2$N$_3$), 1.33 (t, J = 7.3 Hz, 9H, HN$^+$(CH$_2$CH$_3$)$_3$). APT ^{13}C{^{1}H} (100 MHz, $CDCl_3$): 170.5, 170.4, 170.2, 170.1, 169.8, 169.6, 169.1, 101.1 (C-1′), 93.4 (d, $^2J_{C1-P}$ = 6.0 Hz, C-1), 73.8 (C-5′), 70.9 (C-3′), 70.4 (C-4), 69.8 (d, $^2J_{C2-P}$ = 6.8 Hz, C-2), 69.8 (C-5), 69.5 (C-3), 69.1 (C-2), 66.6 (C-4′), 62.7 (d, $^2J_{C1-P}$ = 5.3 Hz, OCH$_2$CH$_2$CH$_2$N$_3$), 62.5 (C-6), 60.8 (C-6′), 48.2 (OCH$_2$CH$_2$CH$_2$N$_3$), 45.8 (HN+(CH$_2$CH$_3$)$_3$), 30.1 (d, $^2J_{C-P}$ = 7.4 Hz, OCH$_2$CH$_2$CH$_2$N$_3$), 20.9, 20.9, 20.8, 20.6, 20.6, 20.6, 20.5, 8.6 (HN$^+$(CH$_2$CH$_3$)$_3$); ^{31}P NMR (162 MHz, $CDCl_3$): −3.09 (d, $^3J_{P-H1}$ = 6.9 Hz); HRMS (ESI/Q-TOF) m/z: [M − H]$^-$ calcd for $C_{29}H_{41}N_3O_{21}P$ 798.1976, found 798.1972.

3-Azidopropyl 4-*O*-β-D-galactopyranosyl-α-D-mannopyranosyl phosphate (2). In a round-bottomed flask provided with a magnetic bar, phosphodiester **13** (83.0 mg, 0.09 mmol) and NaOMe (14.6 mg, 0.27 mmol) were suspended in CH_3OH (5.0 mL) and stirred at room temperature for 48 hours. Then, the reaction was diluted with CH_3OH (5.0 mL) and NH_4Cl was added dropwise until reaching pH ∼7. The volatiles were then removed in rotary evaporator and the crude was subjected to purification by flash reverse phase chromatography using C-18 as stationary phase and MeCN/H_2O gradient (1 : 99 to 1 : 1) as eluent system. This procedure afforded the deprotected azido-derived Galβ(1→4)αMan phospho-glycoside **2** (33.7 mg, 0.06 mmol, 67%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD): 5.34 (dd, $^3J_{H1-H2}$ = 1.5 Hz, $^3J_{H1-P}$ = 7.9 Hz, H-1), 4.25 (d, J = 7.5 Hz, H-1′), 3.85 (m, 2H), 3.83 (m, 2H), 3.79 (m, 5H), 3.70 (m, 3H), 3.61 (dd, J = 4.1 and 11.5 Hz, H-6), 3.50 (dd, J = 4.1 and 7.3 Hz, 1H), 3.45 (dd, J = 7.6 and 9.7 Hz, 1H), 3.40 (d, J = 2.6 Hz, H-4′), 3.36 (m, 4H), 1.79 (m, 2H), 1.26 (t, J = 7.2 Hz, 3H); APT ^{13}C{^{1}H} (100 MHz, CD_3OD): 103.9 (C-1′), 96.1 (d, $^2J_{C1-P}$ = 5.5 Hz, C-1), 76.6, 75.7, 73.3, 72.6, 71.1, 70.5 (d, J_{C-P} = 8.6 Hz), 69.1, 69.0, 62.3 (d, J_{C-P} = 5.8 Hz, OCH$_2$CH$_2$CH$_2$N$_3$), 61.3, 60.4, 52.2, 29.7 (d, J_{C-P} = 7.6 Hz, OCH$_2$CH$_2$CH$_2$N$_3$); ^{31}P NMR (162 MHz, CD_3OD): −1.82 (q, J = 7.1 Hz); HRMS (ESI/Q-TOF) m/z: [M − H]$^-$ calcd for $C_{15}H_{27}N_3O_{14}P$ 504.1236; found 504.1239.

Conflicts of interest

The authors declare no competing financial interests or personal relationships that could have influenced the performance of this work.

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