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An anionic inositol phosphate glycan pseudotetrasaccharide exhibits high insulin-mimetic activity in rat adipocytes

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Abstract—Inositol phosphate glycan pseudotetrasaccharides consisting of man-(α1-6)-man-(α1-4)-glcN-(α,β1-6)-*myo*-inositol-1,2-cyclic phosphate possessing a sulfate group at either O-6 (compounds **3**α,β) or O-2 (compounds **4**α,β) of the terminal mannose have been prepared. Compound **4**α was able to stimulate lipogenesis in native rat adipocytes to 78% of the maximal insulin response (MIR) with an EC_{50} of 1.1 μM. The other compounds exhibited lower maximal stimulations (47–63% MIR) and higher EC_{50} values (9.5–10.6 μM).

1. Introduction

Insulin participates in glucose homeostasis by promoting uptake of glucose and synthesis of lipids and/or glycogen by insulin-sensitive cells. The mechanism by which insulin concentration in the blood is transduced into the metabolic effects within these cells is complex and still under active investigation. It is well established that binding of insulin to the extracellular domain of the insulin receptor, a transmembrane tyrosine kinase, results in phosphorylation of a number of intracellular proteins and lipids. Ultimately, this phosphorylation cascade results in the activation of the enzymes controlling the various metabolic effects, including glycogen and lipid synthesis and glucose transport. This part of the signal transduction pathway has been extensively reviewed.[1–5]

The insulin signal transduction pathway, however, appears to be even more complex since several cross-talk mechanisms have been identified that can activate the signaling cascade without requiring insulin receptor occupancy. Members of one class of compounds, the inositol phosphate glycans (IPGs), are capable of stimulating the insulin-signaling cascade in the absence of insulin. The IPGs, produced endogenously by insulin-sensitive cells upon insulin stimulation, are structurally similar to the glycosylphosphatidylinositol (GPI) membrane anchors. Indeed, IPGs capable of activating the insulin signaling pathway have been generated in vitro from *T. brucei*[6,7] and *S. cerevisiae*[8–10] by lipolytic and proteolytic degradation of their GPI-anchored proteins.

Although it has been established that endogenously generated IPGs mimic many metabolic actions of insulin, their exact chemical structures and the mechanism(s) of action remain largely unknown. Labeling and degradation studies of the natural IPGs have revealed that there are (at least) two classes of molecules with the ability to mimic some of insulin's activities. One class, referred to by Larner[11] as the pH 2 mediators and by Rademacher[12] as Type P IPGs, contains *chiro*-inositol and galactosamine, while the other class, known as the pH 1.3 mediators or Type A IPGs, contains *myo*-inositol and glucosamine. The chemistry and biology of the endogenous IPGs have been reviewed.[13–16]

To understand better the relationship between IPG structure and biological activity, a large number of IPG variants with known structures have been chemically synthesized.[17–33] Early work established that even a pseudodisaccharide containing a *myo*-inositol cyclic phosphate moiety glycosidically linked to a glucosamine (**1**, Fig. 1) was able to stimulate some insulin-like effects in insulin-sensitive cells, although the activity is weak and the concentration required for maximal stimulation of lipogenesis in rat adipocytes is high (40 μM).[20] In a very impressive study, Müller and his co-workers reported[27] the syntheses and insulin-mimetic activity of

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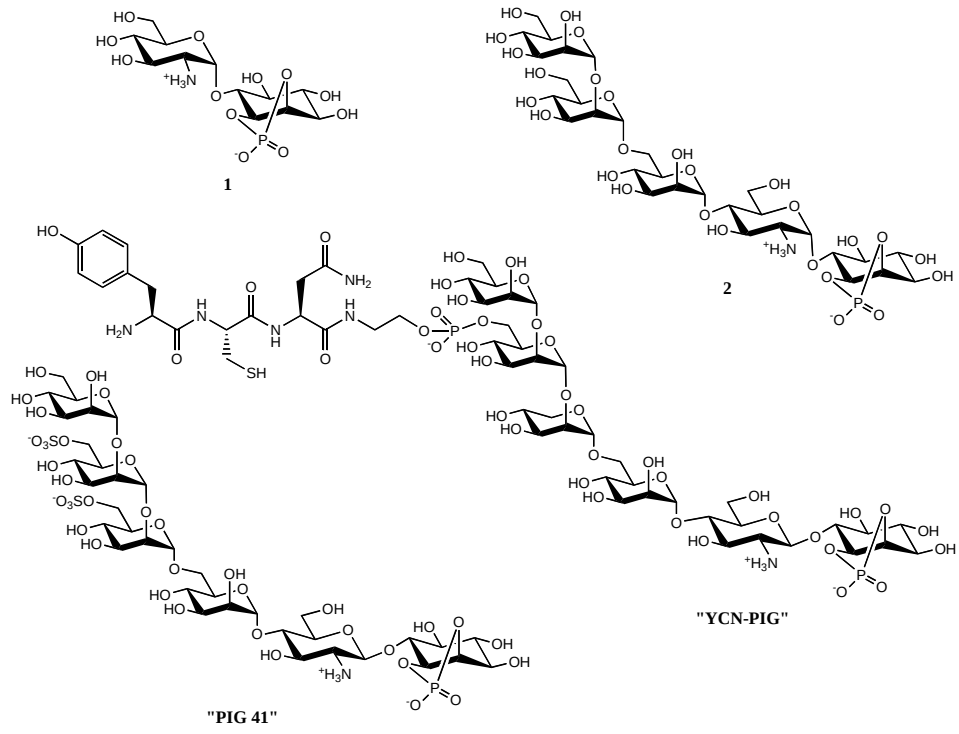

Figure 1. A selection of synthetic IPGs.

forty-six IPG variants. The IPG referred to by these workers as **PIG 41** (Fig. 1), a pseudohexasaccharide containing a *myo*-inositol-1,2-cyclic phosphate, a glucosamine, and four mannose residues with sulfate groups on two of the mannoses, was identified as the most actively insulin-mimetic synthetic IPG variant. Importantly, this compound was able to stimulate cells to almost the same level of activity as insulin, i.e., **PIG 41** showed >90% of the maximal insulin response (MIR) and an EC_{50} of 2.5 μM in stimulation of lipogenesis in rat adipocytes. Müller's work established that at least one anionic group on the mannose residues distal from the inositol moiety is required for achieving a high proportion of the MIR. This conclusion was confirmed by the observation that IPG **2**[30,32] (Fig. 1) has been shown to be almost inactive.[30] Müller's study, taken together with previously published observations,[6,7] established that the anomeric configuration between the glucosamine and the terminal inositol residue is relatively unimportant to the activity, since compounds with either anomeric configuration were found to be highly active. More recent work by Müller's group[34] demonstrated that attaching a tripeptide to the distal mannose via a phosphoethanolamine linkage, as in **YCN-PIG** (Fig. 1), further increased the activity of the compound.

While the syntheses of the complex oligosaccharides **PIG 41** and **YCN-PIG** are impressive, they are also rather lengthy. To simplify future work on the mechanism of IPG action, it would be desirable to establish the structurally simplest IPG variant that still possesses a high proportion of the MIR, thereby decreasing the difficulty of the synthesis and increasing the availability of the compound. Müller's most active IPGs were those containing four mannoses and two anionic groups on consecutive mannoses. Our goal was to investigate whether simpler IPGs containing only two mannose residues but retaining the requisite anionic group on the distal mannose, the inositol cyclic phosphate, and glucosamine retain significant insulin-mimetic activity.

In this correspondence we report the syntheses and biological activities of IPG variants **3**α, **3**β, **4**α, and **4**β (Fig. 2) that are considerably easier to synthesize than

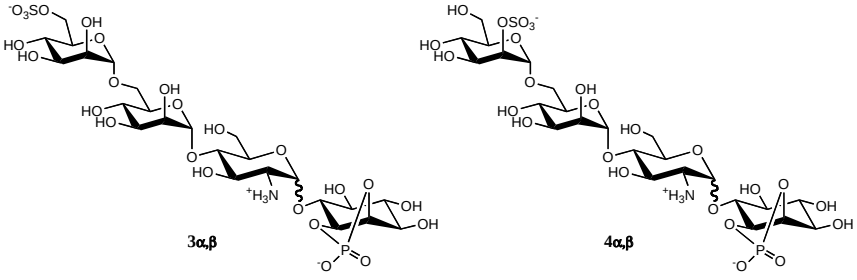

Figure 2. IPG pseudotetrasaccharides with high insulin-like activity.

PIG 41 and **YCN-PIG** but that showed high percentages of the MIR in stimulating lipogenesis in adipocytes in vitro.

2. Results and discussion

2.1. Syntheses of IPGs 3 and 4

The syntheses of **3** and **4** involved glycosidic coupling of the differentially protected mannoses **6** or **10** (Fig. 3), respectively, with disaccharide **11** (Fig. 4), and then glycosylation of *myo*-inositol **18**[32] with each of the resulting trisaccharides. Subsequent modification of the protective groups and addition of the sulfate and cyclic phosphate moieties, followed by complete deprotection in one step by dissolving metal reduction, yielded the final products (Fig. 4). The modular nature of the syntheses allowed room for modifications in the final structures, if necessary. The glycosidic couplings were carried out using the glycosyl fluorides as donors and silver triflate and bis(cyclopentadienyl)zirconium dichloride as the promoters.[35] This coupling method yielded the α-anomer almost exclusively in all cases where a mannosyl fluoride was the donor but produced a mixture of α and β anomers (the former predominating) when glucosyl fluorides were used in the final coupling reaction. Therefore, this coupling method was ideal for producing both anomers at the glucosamine-inositol linkage, as desired based on the earlier determination that both anomers at this position of IPGs are generally active.

Synthesis of the differentially protected mannosyl fluoride donor **6** was carried out in a two-step process from the known[36] mannosyl fluoride **5**. This transformation involved removal of the O-acetyl group with ammonia in methanol and then formation of the allyl ether[37] in its place using allyl bromide and sodium hydride in dry DMF. The mannosyl fluoride **6** was obtained in 77% yield over the two steps.

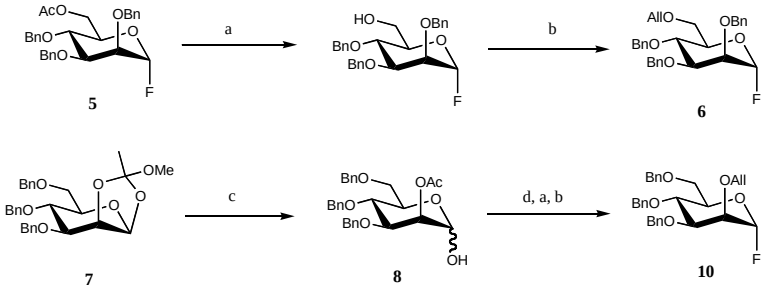

Figure 3. Synthesis of differentially protected mannose units. (a) NH_3, CH_3OH, 20 °C; (b) allyl bromide, NaH, DMF, 20 °C; (c) $NaHSO_4$, DME/H_2O (4:1), 20 °C; (d) DAST, THF, 20 °C.

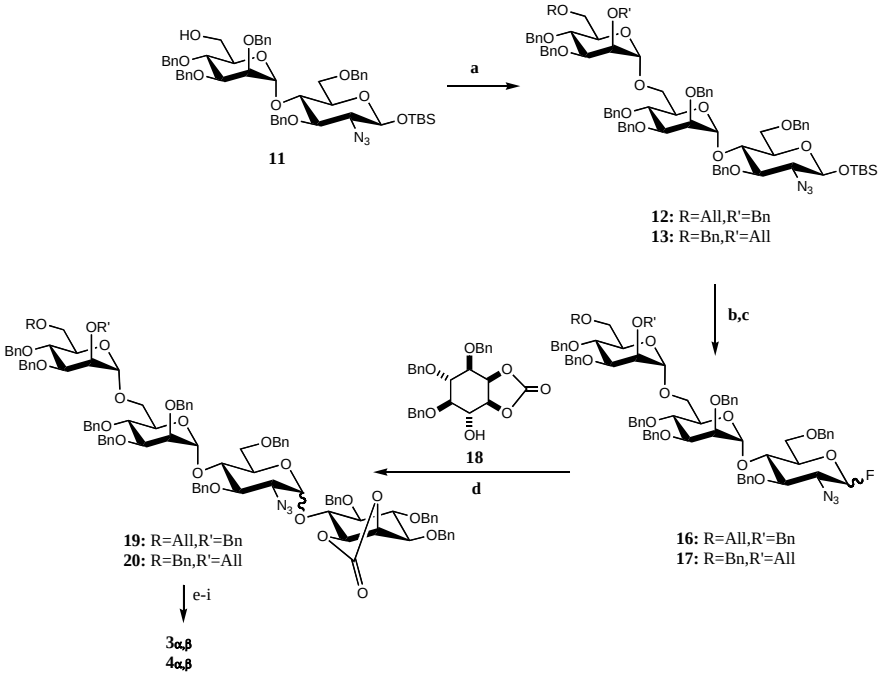

Figure 4. Completion of the synthesis of **3** and **4**. (a) **6** or **10**, Cp_2ZrCl_2, AgOTf, toluene, 20 °C; (b) *n*-Bu_4NF, HOAc, THF, 20 °C; (c) DAST, THF, 20 °C; (d) **18**, Cp_2ZrCl_2, AgOTf, toluene, 20 °C; (e) $(Ph_3P)_3RhCl$, DABCO, EtOH/H_2O (9:1); 80% HOAc; (f) SO_3·Py, 20 °C; (g) 1 M aq LiOH, THF, 0 °C; (h) $MeOPOCl_2$, Py, 20 °C; (i) Na, NH_3, −78 °C.

Synthesis of the mannosyl fluoride donor **10** was slightly more complex, but was achieved in four steps from the known[38] compound **7**. The methyl orthoester protecting group in **7** was removed by mild acid hydrolysis,[39] yielding a mixture of products. Mannose **8** was easy to separate from the 1-acetate that was also formed in the hydrolysis, and was obtained in 74% yield. It was further transformed by anomeric fluorination using DAST in dry THF in 88% yield,[40] after which removal of the O-acetyl group and allylation provided **10** in a 72% yield over the final two steps.

The disaccharide acceptor **11** (Fig. 4), prepared by deacetylation of the known corresponding acetate,[27] was glycosidically linked with either **6** or **10** using bis(cyclopentadienyl) zirconium dichloride and silver triflate in dry toluene to produce **12** and **13** in 70% and 76% yields, respectively. The α-configuration of the new glycosidic linkage in the products was confirmed by the ^{13}C–^{1}H coupling constant at the anomeric position of the mannose according to the observation of Bock and Pedersen[41] that α-mannosides exhibit anomeric $J_{C,H} = \sim 170$ Hz while β-mannosides exhibit anomeric $J_{C,H} = \sim 160$ Hz. In every case in our work where a glycosidic bond was formed from mannose, the anomeric configuration of the product(s) was assigned by this method.

Removal of the *tert*-butyldimethylsilyl protecting group was achieved by using tetrabutylammonium fluoride in THF under acidic conditions, yielding **14** and **15** in 95% and 92% yields, respectively. Anomeric fluorination with DAST in dry THF afforded the trisaccharide donors **16** and **17** in 71% and 73% yields, respectively. Glycosidic coupling of the trisaccharide donor with the protected *myo*-inositol acceptor **18**[32] using the same coupling method yielded a mixture of α and β anomers. Pseudotetrasaccharide **19**α was obtained in 38% yield together with 15% **19**β, while **20**α was obtained in 74% yield along with 18% **20**β. The anomers were separated at this stage.

Selective removal of the *O*-allyl protecting groups of **19**α, **19**β, **20**α, and **20**β was achieved with Wilkinson's catalyst in a 9:1 ethanol/water solution, followed by warming at reflux in 80% acetic acid.[42] Products **21**α,β and **22**α,β were obtained from this reaction in 75%, 46%, 70%, and 31% yields, respectively. The lower conversion in the case of the β-anomers was likely due to the lower solubility of these compounds in the ethanol/water solution. Sulfur trioxide–pyridine complex in dry pyridine solution was used to generate the sulfate ester of the free hydroxyl group,[43] yielding **23**α,β and **24**α,β in 55–61% yields. Removal of the cyclic carbonate group on the *myo*-inositol moiety and replacing it with a cyclic phosphate were crucial steps, but they were achieved by reactions that were successfully used previously in our laboratory.[32] The cyclic carbonate was hydrolyzed using 1 M LiOH (aq) in THF at 0 °C for 2 h, affording the pseudotetrasaccharide diols in 55–60% yields for the four compounds. Cyclic phosphorylation with MeO-$POCl_2$ in pyridine[44] and deprotection by dissolving metal reduction using the established procedure[32] yielded the final IPGs **3**α,β and **4**α,β, which were desalted using a Sephadex G-10 size exclusion column to afford pure products.

2.2. Lipogenesis in native rat adipocytes

Synthetic IPGs **3**α, **3**β, **4**α, and **4**β were tested for their ability to stimulate lipogenesis in isolated native rat adipocytes. For comparison, IPG **1** and insulin were also tested. The assays were carried out with slight modifications of the published procedures[45,46] by incubating [6-^{3}H]glucose with a cell suspension and the corresponding analyte (insulin: positive control, buffer: negative control, or the synthetic IPG) for 1.5 h at 37 °C and then measuring incorporation of tritium into toluene-extractable lipids (triglycerides).

The data obtained for the stimulation of lipogenesis by IPGs (Fig. 5) are reported as the percentage of the maximal stimulation (MIR) of lipogenesis above the basal activity (negative control) produced by insulin (positive control). In a typical assay, insulin (5 nM) resulted in a 2- to 3-fold increase over the negative control of toluene soluble tritium, under these conditions. The data relating IPG concentration to activity could be fit to a standard receptor model (Eq. 1, $n = 1$) with R values ranging from 0.81 to 0.98, but the curve fits were significantly better if the Hill constant n was allowed to be greater than 1, suggesting cooperativity in IPG binding. The parameters obtained from the best curve fits are presented in Table 1.

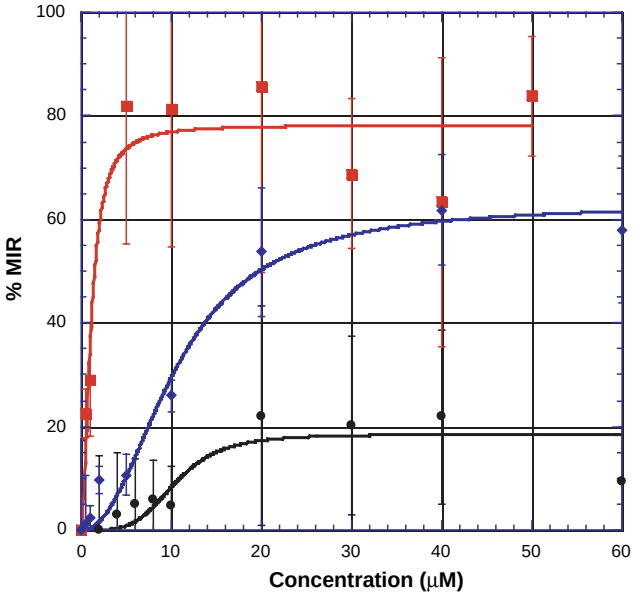

Figure 5. Stimulation of lipogenesis by synthetic IPGs **1**, **3**α, and **4**α. Isolated native rat adipocytes were incubated with 6-[^{3}H]glucose (0.55 mM) and various concentrations of the analyte for 1.5 h. Incorporation of tritium into lipids was measured and is expressed as a percent of the maximal insulin response (%MIR). Black circles (●): compound **1**, (0–60 μM); blue diamonds (♦): compound **3**α(0–60 μM); red squares (■): compound **4**α (0–50 μM). Each data point is the average of five replicates. Solid lines are the result of curve fitting as described in the text. Error bars represent ± 1 SD.

Table 1. Curve-fitting parameters for stimulation of lipogenesis by synthetic IPGs

IPG	E_{max} (% MIR)	EC_{50} (μM)	[Glucose] (mM)	*n* (Hill Constant)	*R* value for curve fit
1	18	10.5	0.55	4.2	0.8850
1	19	7.0	5.50	2.5	0.9909
3α	63	10.6	0.55	2.2	0.9897
3α	51	9.8	5.50	5.6	0.9974
3β	61	13.9	0.55	3.8	0.9981
4α	78	1.1	0.55	1.8	0.9630
4β	47	9.5	0.55	7.7	0.9906

$$\text{Activity}(\%\text{MIR}) = (E_{max} * [\text{IPG}]^n)/((\text{EC}_{50})^n + [\text{IPG}]^n). \quad (1)$$

At low glucose concentration (0.55 mM), IPG **1** exhibited only modest ability to stimulate lipogenesis with a maximal activity (E_{max}) of 18% that of the maximal insulin response (MIR) and an EC_{50} of 10.5 μM, confirming the earlier report.[20] In contrast, the pseudotetrasaccharide **4α** was extremely active, exhibiting an E_{max} = 78% MIR and an EC_{50} = 1.1 μM. The other synthetic pseudotetrasaccharides were less active (both lower E_{max} and higher EC_{50}) than **4α**, but still elicited significantly higher E_{max} than **1**.

It has been reported that at low glucose concentration the rate-limiting step in lipogenesis is glucose transport, while at [glucose] > 1 mM, other processes limit the rate.[47] Accordingly, we examined the ability of two representative IPGs, **1** and **3α**, to stimulate lipogenesis at 5.5 mM glucose. The resulting E_{max} and EC_{50} values (Table 1) are very similar to those obtained at 0.55 mM glucose, but the Hill constants differ. The molecular basis of this phenomenon, and indeed of the observation that the Hill coefficients differ amongst the compounds, is not clear and probably will have to await isolation of the IPG receptor for further study. Work toward this goal is underway.

3. Conclusions

Synthetic IPGs can mimic many of the metabolic actions of insulin, but to varying degrees, depending on the IPGs' structures. Müller's work[27] demonstrated the necessity of an anionic group on at least one of the distal mannose residues, but left open the question of how many mannose residues are necessary for high activity. In the current work, we have demonstrated that the pseudotetrasaccharides **3** and **4**, containing two mannose residues, can stimulate lipogenesis in rat adipocytes with high percentages of MIR. Furthermore, IPG **4α** has an EC_{50} value an order of magnitude lower than those of the other synthetic IPGs reported here and slightly lower than Müller's '**PIG 41**', although the latter compound has a slightly higher E_{max}.[27] In fact, to the best of our knowledge, IPG **4α** has the lowest EC_{50} for stimulation of lipogenesis in adipocytes of any synthetic IPG so far reported. Thus, we have successfully designed and synthesized highly active IPGs that are considerably simpler to synthesize than previously reported highly active IPGs. We anticipate that IPGs **3** and **4**, or their derivatives, will be useful tools for investigating the role of IPGs in the insulin signal transduction process.

4. Experimental

4.1. Lipogenesis in rat adipocytes

4.1.1. Adipocyte preparation. Epididymal fat pads were dissected from freshly sacrificed male Long–Evans rats and kept in 0.15 M NaCl solution at 37 °C until use; not longer than 0.5 h. The fat pads were cut into small pieces with scissors and incubated with collagenase (Sigma–Aldrich; Type 2 from *Clostridium histolyticum*; 1 mg/mL) in a KRB buffer (10 mM Hepes, 118 mM NaCl, 4.75 mM KCl, 1.87 mM $MgSO_4$, 1.9 mM $CaCl_2$, 1.2 mM $KHSO_4$, and 25 mM $NaHCO_3$, pH 7.4, saturated with 95% O_2/5% CO_2) containing 4% BSA and 5.5 mM glucose at 37 °C for 45 min. The digested fat pads were filtered through silk in order to separate the adipocytes from the cellular debris. The filtrate containing the cell suspension was centrifuged (Clay Adams model 151 bench-top centrifuge), the solution below the floating cells was discarded, and the cells were washed three times with KRB buffer containing 1% BSA and 0.55 mM glucose. After each wash, any oil floating above the cells on the surface of the suspension was removed by suction. The washed adipocytes were diluted to a final concentration of approximately 5×10^5 cells/mL with KRB buffer (resaturated with 95%O_2/5%CO_2) containing 1% BSA and 0.55 mM glucose, and incubated with 90 nM isoproterenol at 37 °C for 45 min before use in the lipogenesis assay.

4.1.2. Lipogenesis assay. To assay stimulation of lipogenesis at low glucose concentration, 40 μL of the cell suspension from above was added to a solution containing [6-^{3}H]glucose (0.12 μCi, 0.55 mM) and the analyte in 10 μL KRB buffer containing 1% BSA at 37 °C for 1.5 h. The assay was stopped by adding 450 μL water immediately followed by 5.0 mL of toluene-based scintillation cocktail (2.5 g PPO and 0.15 g POPOP per 500 mL toluene) and vortexing. The tubes were centrifuged to expedite phase separation, 3.0 mL of the toluene phase was removed, and [^{3}H] was measured by scintillation. For high glucose concentration assays, the procedure was identical, except that the wash buffer, isoproterenol incubation buffer, and the assay mixture contained 5.5 mM glucose, and 0.25 μCi of [6-^{3}H]glucose was used in the assay.

4.2. Synthesis of 6-*O*-allyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl fluoride (6)

Ammonia gas was bubbled through a solution of **5** (80 mg, 0.16 mmol) in methanol at 0 °C for 5 min. The resulting solution was stirred at 20 °C for 12 h in a heavy-walled vessel. The reaction vessel was re-cooled to 0 °C, opened, and the solution was concentrated. The crude product was dissolved in DMF (1 mL), and allyl bromide (20 μ L, 0.24 mmol) was added, followed by sodium hydride (24 mg of a 50% suspension in mineral oil, 0.5 mmol). The mixture was stirred at 20 °C until TLC (silica, 1:2 EtOAc/hexanes) showed completion of reaction, about 2 h. Methanol was then added slowly to quench the excess sodium hydride, and the solution was diluted with water (5 mL) and extracted with chloroform (3× 5 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated in vacuo. Chromatography of the residue (silica, 1:3 EtOAc/hexanes) gave **6** (61 mg, 77%). $R_f = 0.70$ (1:2 EtOAc/hexanes). ^{1}H NMR ($CDCl_3$): δ 3.59–4.08 (m, 8H), 4.61–5.03 (m, 6H, CH_2Ph), 5.17 (dd, 1H, $J = 1.3$, 10.4 Hz, allyl H-3a), 5.28 (dd, 1H, $J = 1.3$, 15.3 Hz, allyl H-3b), 5.57 (dd, 1H, $J = 1.8$, 50.6 Hz, H-1), 5.87–5.96 (ddt, 1H, $J = 10.4$, 10.4 and 15.3 Hz, allyl H-2), 7.06–7.40 (m, 15H, Ar*H*).

4.3. Synthesis of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-D-mannopyranose (8)

To a solution of **7** (105 mg, 0.21 mmol) in 4:1 DME/H_2O (2 mL) was added $NaHSO_4$ (32 mg, 0.27 mmol) and the mixture was stirred at 20 °C until TLC (silica, 1:1 EtOAc/hexanes) showed completion of reaction, approximately 30 min. The excess acid was quenched with LiOH (1 M, 0.5 mL), the reaction mixture was diluted with water (5 mL) and extracted with chloroform (3× 5 mL). The combined organic layers were dried ($MgSO_4$) and concentrated. The crude mixture of products was purified by flash column chromatography (silica, 1:2 EtOAc/hexanes) to yield **8** (76 mg, 74%). $R_f = 0.55$ (1:1 EtOAc/hexanes). ^{1}H NMR ($CDCl_3$): δ 2.14 (s, 3H, OCOCH_3), 3.58–3.75 (m, 3H, H-4, H-6a, H-6b), 4.03–4.10 (m, 2H, H-3, H-5), 4.44–4.87 (m, 6H, CH_2Ph), 5.20 (d, 1H, $J = 3$ Hz, H-1), 5.38 (dd, 1H, $J = 3$ Hz, H-2), 7.14–7.32 (m, 15H, Ar*H*).

4.4. Synthesis of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl fluoride (9)

DAST (40 μL, 0.23 mmol) was added to a solution of **8** (76 mg, 0.15 mmol) in dry THF (0.4 mL) at −42 °C. The reaction was stirred at 20 °C for approximately 30 min, until TLC (silica, 1:1 EtOAc/hexanes) showed completion of reaction. The mixture was cooled to −42 °C and the excess DAST was quenched with methanol (0.5 mL). The mixture was warmed up to 20 °C, followed by addition of $NaHCO_3$ (1 M, 2 mL), and extracted with ether (3× 3 mL). The combined organic layers were dried ($MgSO_4$) and concentrated. Purification by flash column chromatography (silica, 1:3 EtOAc/hexanes) gave **9** (67 mg, 88%). $R_f = 0.85$ (1:1 EtOAc/hexanes). ^{1}H NMR ($CDCl_3$): δ 2.12 (s, 3H, OCOCH_3), 3.63–3.99 (m, 5H), 4.43–4.89 (m, 6H, CH_2Ph), 5.47 (dd, 1H, $J = 2$ Hz, H-2), 5.61 (dd, 1H, $J = 2$, 50 Hz, H-1), 7.12-7.36 (m, 15H, Ar*H*).

4.5. Synthesis of 2-*O*-allyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl fluoride (10)

Deacetylation and then allylation of **9** (67 mg, 0.13 mmol) were carried out, as described above for the synthesis of **6**. The crude product from the reaction was purified by chromatography (silica, 1:3 EtOAc/hexanes), yielding **10** (48 mg, 72%). $R_f = 0.72$ (1:2 EtOAc/hexanes). ^{1}H NMR ($CDCl_3$): δ 3.68–4.29 (m, 8H), 4.48–4.90 (m, 6H, CH_2Ph), 5.23 (dd, 1H, $J = 1.3$, 10.4 Hz, allyl H-3a), 5.30 (dd, 1H, $J = 1.3$, 15.3 Hz, allyl H-3b), 5.56 (dd, 1H, $J = 1.8$, 50.6 Hz, H-1), 5.90 (ddt, 1H, $J = 10.4$, 10.4, 15.3 Hz, allyl H-2), 7.10–7.39 (m, 15H, Ar*H*).

4.6. Synthesis of 2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 4)-2-azido-2-deoxy-3,6-di-*O*-benzyl-1-*O*-*tert*-butyldimethylsilyl-α-D-glucopyranoside (11).

Deacetylation of the known[27] compound 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 4)-2-azido-2-deoxy-3,6-di-*O*-benzyl-1-*O*-*tert*-butyldimethylsilyl-α-D-glucopyranoside (0.47 g, 0.48 mmol) was carried out using the same procedure as described for the synthesis of **6**. Purification by flash column chromatography (silica, 1:3 EtOAc/hexanes) yielded **11** (0.33 g, 74%). $R_f = 0.5$ (1:3 EtOAc/hexanes). ^{1}H NMR ($CDCl_3$): δ 0.15 (s, 6H, OSiCH_3), 0.95 (s, 9H, OSiC(CH_3)$_3$), 2.26 (br s, 1H, O*H*), 3.21–3.39 (m, 3H), 3.58–3.71 (m, 7H), 3.73–3.85 (m, 3H), 4.21 (d, 1H, $J = 12$ Hz, *H*CHPh), 4.39 (d, 1H, $J = 12$ Hz, *H*CHPh), 4.48–4.64 (m, 6H), 4.85 (d, 1H, $J = 10.5$ Hz, *H*CHPh), 4.98 (d, 1H, $J = 10.5$ Hz, *H*CHPh), 5.25 (d, 1H, $J = 1.5$ Hz, H-1 man), 7.09–7.32 (m, 25H, Ar*H*).

4.7. Synthesis of 6-*O*-allyl-2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 6)-2,3,4-tri-*O*-benzyl-D-manno pyranosyl-(α1 → 4)-2-azido-2-deoxy-3,6-di-*O*-benzyl-1-*O*-*tert*-butyldimethylsilyl-α-D-glucopyranoside (12) or 2-*O*-allyl-3,4,6-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 6)-2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 4)-2-azido-2-deoxy-3,6-di-*O*-benzyl-1-*O*-*tert*-butyldimethylsilyl-α-D-glucopyranoside (13)

To a solution of the disaccharide **11** (63 mg, 0.07 mmol or 20 mg, 0.02 mmol, respectively) and either **6** (61 mg, 0.13 mmol) or **10** (21 mg, 0.04 mmol) in dry toluene (2 mL or 0.6 mL, respectively) was added 4Å molecular sieves, bis(cyclopentadienyl)zirconium dichloride (99 mg, 0.34 mmol or 31 mg, 0.11 mmol, respectively), and then silver trifloromethanesulfonate (174 mg, 0.68 mmol or 54 mg, 0.21 mmol, respectively) at −42 °C. The reaction mixture was stirred at 20 °C in the dark until TLC (silica, 1:2 EtOAc/hexanes) showed complete disappearance of the limiting starting material, approximately 12 h. The reaction was quenched with $NaHCO_3$ (1 M, 0.5 mL or 0.2 mL, respectively), diluted with methylene chloride (5 mL or 2 mL, respectively), and filtered through Celite. The layers were separated and the aqueous layer was washed with methylene

chloride (3× 5 mL or 3× 2 mL, respectively). The combined organic phase was washed with brine, dried ($MgSO_4$), and concentrated. Chromatographic purification (silica gel, 1:4 EtOAc/hexanes) yielded **12** (67 mg, 70%) or **13** (23 mg, 76%), from reactions performed with **6** or **10**, respectively. For **12**, $R_f = 0.60$ (1:2 EtOAc/hexanes). ^{1}H NMR ($CDCl_3$): δ 0.15 (s, 6H, OSiCH_3), 0.95 (s, 9H, OSiC(CH_3)$_3$), 3.20–4.20 (m, 24H), 4.31–4.64 (m, 9H), 4.80–5.29 (m, 8H), 5.78–5.91 (ddt, 1H, J = 10.4, 10.4, 15.3 Hz, allyl H-2), 7.03–7.39 (m, 40H, ArH). ^{13}C NMR ($CDCl_3$): δ 97.35 (d, 1H, J = 161 Hz, C-1 glc), 98.54 (d, 1H, J = 170 Hz, C-1 man-2), 100.04 (d, 1H, J = 167 Hz, C-1 man-1). For **13**, $R_f = 0.65$ (1:2 EtOAc/hexanes). ^{1}H NMR ($CDCl_3$): δ 0.17 (s, 6H, OSiCH_3), 0.95 (s, 9 H, OSiC(CH_3)$_3$), 3.20–3.40 (m, 4H), 3.47–4.02 (m, 16H), 4.09–4.61 (m, 13H), 4.79–5.32 (m, 8H), 5.77–5.90 (m, allyl H-2), 7.08–7.39 (m, 40H, ArH). ^{13}C NMR ($CDCl_3$): δ 98.14 (d, 1H, J = 159 Hz, C-1 glc), 99.32 (d, 1H, J = 170 Hz, C-1 man-2), 100.89 (d, 1H, J = 171 Hz, C-1 man-1).

4.8. Synthesis of 6-*O*-allyl-2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 6)-2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 4)-2-azido-2-deoxy-3,6-di-*O*-benzyl-D-glucopyranose (14) or 2-*O*-allyl-3,4,6-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 6)-2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 4)-2-azido-2-deoxy-3,6-di-*O*-benzyl-D-glucopyranose (15)

To a solution of **12** (67 mg, 0.05 mmol) or **13** (23 mg, 0.02 mmol) in dry THF (2 mL or 0.7 mL, respectively) was added glacial acetic acid (100 μL, 1.82 mmol or 34 μL, 0.73 mmol, respectively), followed by tetrabutylammonium fluoride (1 M solution in THF, 0.8 mL, 0.8 mmol or 260 μL, 0.26 mmol, respectively). The reaction mixture was stirred overnight at 20 °C and quenched with $NaHCO_3$ (1 M, 2 mL or 1 mL, respectively). The aqueous layer was extracted with chloroform (3× 5 mL or 3× 2 mL, respectively), rinsed with water (3× 5 mL or 3× 2 mL, respectively), brine, dried with $MgSO_4$ and concentrated. Chromatographic purifications (silica, 1:2 EtOAc/hexanes) produced **14** (61 mg, 95%) or **15** (20 mg, 92%), from reactions of **12** or **13**, respectively. For **14**, $R_f = 0.3$ (1:2 EtOAc/hexanes). ^{1}H NMR ($CDCl_3$): δ 3.18 (br s, 1H, OH), 3.55–4.09 (m, 19H), 4.44–5.31 (m, 22H), 5.79–5.95 (ddt, 1H, J = 10.4, 10.4, 15.3 Hz, allyl H-2), 7.09–7.38 (m, 40H, ArH). For **15**, $R_f = 0.35$ (1:2 EtOAc/hexanes). ^{1}H NMR ($CDCl_3$): δ 3.18–4.04 (m, 20H), 4.15–4.66 (m, 14H), 4.79–5.30 (m, 7H), 5.72–5.90 (ddt, 1H, J = 10.4, 10.4, 15.3 Hz, allyl H-2), 7.03–7.32 (m, 40H, ArH).

4.9. Synthesis of 6-*O*-allyl-2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 6)-2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 4)-2-azido-2-deoxy-3,6-di-*O*-benzyl-α-D-glucopyranosyl fluoride (16) or 2-*O*-allyl-3,4,6-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 6)- 2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 4)-2-azido-2-deoxy-3,6-di-*O*-benzyl-α-D-glucopyranosyl fluoride (17)

Anomeric fluorination of **14** (61 mg, 0.05 mmol) or **15** (20 mg, 0.02 mmol) was carried out following the same procedure as described above for the synthesis of **9**. Purification by flash column chromatography (silica, 1:4 EtOAc/hexanes) yielded **16** (43 mg, 71%) or **17** (15 mg, 73%). For **16**, $R_f = 0.52$ (1:3 EtOAc/hexanes). ^{1}H NMR ($CDCl_3$): δ 3.55–3.73 (m, 19H), 3.80–4.09 (m, 12H), 4.46–5.29 (m, 9H), 5.50 (dd, 1H, J = 2, 50 Hz, H-1 glc-α), 5.79–5.95 (ddt, 1H, J = 10.4, 10.4, 15.3 Hz, allyl H-2), 7.18–7.48 (m, 40H, ArH). HRMS (ESI): m/z calcd for $C_{77}H_{82}FN_3O_{14}$: 1291.5781. Found: 1291.5775. For **17**, $R_f = 0.55$ (1:3 EtOAc/hexanes). ^{1}H NMR ($CDCl_3$): δ 3.37 (ψt, 0.6H, J = 7.5 Hz, H-2 glc-β), 3.45 (dd, 0.4H, J = 3, 10 Hz, H-2 glc-α), 3.50–3.95 (m, 17H), 4.00–4.05 (m, 2H, allyl H-1), 4.28 (dd, 1H, J = 2, 15 Hz), 4.38–4.68 (m, 13H), 4.79–5.19 (m, 7H), 5.61 (dd, 0.4H, J = 2, 60 Hz, H-1 glc-α), 5.77–5.92 (ddt, 1H, J = 10.4, 10.4, 15.3 Hz, allyl H-2), 7.08–7.30 (m, 40H, ArH). HRMS (ESI): m/z calcd for $C_{77}H_{82}FN_3O_{14}$: 1291.5781. Found: 1291.5772.

4.10. Synthesis of 6-*O*-allyl-2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 6)-2,3,4-tri-*O*-benzylD-mannopyranosyl-(α1 → 4)-2-azido-2-deoxy-3,6-di-*O*-benzyl-D-glucopyranosyl-(α and β1 → 6)-3,4,5-tri-*O*-benzyl-1-D-*myo*-inositol-1, 2-cyclic carbonate (19α and 19β)

Trisaccharide **16** (43 mg, 0.03 mmol) was coupled with inositol **18** (29 mg, 0.06 mmol), following the procedures described for the synthesis of **12** and **13**. The products were purified by flash column chromatography (silica, 1:3 EtOAc/hexanes), providing **19**α (22 mg, 38%) and **19**β (9 mg, 15%). For **19**α, $R_f = 0.55$ (3:7 EtOAc/hexanes). ^{1}H NMR ($CDCl_3$): δ 3.38 (dd, 1H, J = 3, 9 Hz, H-2 glc), 3.47–3.72 (m, 9H), 3.74–4.03 (m, 13H), 4.13–4.66 (m, 20H), 4.78–4.92 (m, 5H), 4.98–5.33 (m, 5H), 5.77–5.89 (ddt, 1H, J = 10.4, 10.4, 15.3 Hz, allyl H-2), 7.08–7.32 (m, 55H, ArH). For **19**β, $R_f = 0.58$ in 3:7 EtOAc/hexanes. ^{1}H NMR ($CDCl_3$): δ 3.25 (ψt, 1H, J = 8 Hz, H-2 glc), 3.41–4.05 (m, 22H), 4.11–4.91 (m, 25H), 5.01–5.40 (m, 6H), 5.78–5.94 (ddt, 1H, J = 10.4, 10.4, 15.3 Hz, allyl H-2), 7.04–7.40 (m, 55H, ArH).

4.11. Synthesis of 2-*O*-allyl-3,4,6-tri-*O*-benzyl-D-mannopyranosyl-(1 → 6)-2,3,4-tri-*O*-benzyl-D- mannopyranosyl-(1 → 4)-2-azido-2-deoxy-3,6-di-*O*-benzyl-D-glucopyranosyl-(α and β1 → 6)-3,4,5-tri-*O*-benzyl-1-D-*myo*-inositol-1,2-cyclic carbonate (20α and 20β)

Trisaccharide **17** (15 mg, 0.01 mmol) was coupled with inositol **18** (10 mg, 0.02 mmol, respectively), following the procedures described for the synthesis of **12** and **13**. The products were purified by flash column chromatography (silica, 1:3 EtOAc/hexanes), providing **20**α (15 mg, 74%) and **20**β (4 mg, 18%). For **20**α, $R_f = 0.63$ (3:7 EtOAc/hexanes). ^{1}H NMR ($CDCl_3$): δ 3.37 (dd, 1H, J = 3.4, 9.8 Hz, H-2 glc), 3.48–4.02 (m, 22H), 4.16–4.65 (m, 20H), 4.78–5.12 (m, 10H), 5.61–5.86 (ddt, 1H, J = 10.4, 10.4, 15.3 Hz, allyl H-2), 7.08–7.31 (m, 55H, ArH). For **20**β, $R_f = 0.65$ in 3:7 EtOAc/hexanes. ^{1}H NMR ($CDCl_3$): δ .21 (ψt, 1H, J = 8.5 Hz, H-2 glc), 3.45–4.05 (m, 20H), 4.16–4.95 (m, 25H), 5.05–5.29 (m, 6H), 5.75–5.89 (ddt, 1H, J = 10.4, 10.4, 15.3 Hz, allyl H-2), 7.01–7.35 (m, 55H, ArH).

4.12. Synthesis of 2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 6)-2,3,4-tri- O-benzyl-D-mannopyranosyl-(α1 → 4)-2-azido-2-deoxy-3,6-di-*O*-benzyl-D-glucopyranosyl-(α1 → 6)-3,4,5-tri-*O*-benzyl-1-D-*myo*-inositol-1,2-cyclic carbonate (21α) or 3,4,6-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 6)-2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 4)-2-azido-2-deoxy-3,6-di-*O*-benzyl-D-glucopyranosyl-(α1 → 6)-3,4,5-tri-*O*-benzyl-1-D-*myo*-inositol-1,2-cyclic carbonate (22α)

To tetrasaccharide **19**α (5 mg, 0.003 mmol) or **20**α (5 mg, 0.003 mmol) and DABCO (1.3 mg, 0.012 mmol) in 9:1 EtOH:H_2O (0.3 mL) was added chlorotris(triphenylphosphine) rhodium(I) (0.4 mg) and the mixture was stirred at 20 °C for 20 min, followed by warming at reflux for 25 min. The reaction was allowed to cool to 20 °C, water (0.2 mL) and chloroform (0.5 mL) were added, and the layers were separated. The aqueous layer was extracted with chloroform (3× 0.5 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated. Chromatographic purifications (silica gel, 1:3 EtOAc/hexanes) yielded the intermediate enol ethers, which were dissolved in 80% acetic acid (100 μL) and heated at 95 °C for 30 min. The reaction was allowed to cool to 20 °C, diluted with water (100 μL), chloroform (100 μL), and neutralized by dropwise addition of sat. $NaHCO_3$. The aqueous layer was extracted with chloroform (4× 0.2 mL) and the combined organic layers were dried ($MgSO_4$) and concentrated. Purification with column chromatography (silica gel, 1:3 EtOAc/hexanes) yielded **21**α (3.7 mg, 75%) or **22**α (3.4 mg, 70%). For **21**α, R_f = 0.35 (3:7 EtOAc/hexanes). ^{1}H NMR ($CDCl_3$): δ 3.38 (dd, 1H, *J* = 3, 9 Hz, H-2 glc), 3.47–4.05 (m, 16H), 4.31–4.68 (m, 22H), 4.81–4.96 (m, 9H), 5.05 (ψs, 1H, H-1, man-2), 5.21 (d, 1H, *J* = 1.5 Hz, H-1 man-1), 5.35 (d, 1H, *J* = 3.5 Hz, H-1 glc), 7.11–7.49 (m, 55H, Ar*H*). For **22**α, R_f = 0.38 (3:7 EtOAc/hexanes). ^{1}H NMR ($CDCl_3$): δ 3.37 (dd, 1H, *J* = 3.4, 9.8 Hz, H-2 glc), 3.49–4.02 (m, 16H), 4.35–4.71 (m, 22H), 4.82–4.99 (m, 9H), 5.06 (ψs, 1H, H-1, man-2), 5.23 (d, 1H, *J* = 1.5 Hz, H-1 man-1), 5.38 (d, 1H, *J* = 3.5 Hz, H-1 glc), 7.15–7.51 (m, 55H, Ar*H*).

4.13. Synthesis of 2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 6)-2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 4)-2-azido-2-deoxy-3,6-di-*O*-benzyl-D-glucopyranosyl-(β1 → 6)-3,4,5-tri-*O*-benzyl-1-D-*myo*-inositol-1,2-cyclic carbonate (21β) or 3,4,6-tri-*O*-benzyl-D-mannopyranosyl-(α 1 → 6)-2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 4)-2-azido-2-deoxy-3,6-di-*O*-benzyl-D-glucopyranosyl-(β1 → 6)-3,4,5-tri-*O*-benzyl-1-D-*myo*-inositol-1,2-cyclic carbonate (22β)

Removal of the allyl groups from **19**β (3.2 mg, 0.0015 mmol) and **20**β (4.2 mg, 0.0024 mmol) was performed exactly as described for **21**α and **22**α, except that 0.65 mg (0.006 mmol) or 0.95 mg (0.009 mmol) of DABCO and 0.2 mg or 0.32 mg of chlorotris(triphenylphosphine) rhodium(I) were used in 0.3 mL of 9:1 EtOH/H_2O, respectively. Purification with column chromatography (silica gel, 1:3 EtOAc/hexanes) yielded **21**β (1.5 mg, 46%) or **22**β (1.3 mg, 31%). For **21**β, R_f = 0.35 (3:7 EtOAc/hexanes). ^{1}H NMR ($CDCl_3$): δ 3.25 (ψt, 1H, *J* = 8 Hz, H-2 glc), 3.41–3.96 (m, 16H), 4.25–4.65 (m, 22H), 4.76–4.91 (m, 10H), 5.07 (ψs, 1H, H-1, man-2), 5.25 (d, 1H, *J* = 1.5 Hz, H-1, man-1), 7.05–7.45 (m, 55H, Ar*H*). For **22**β, R_f = 0.35 (3:7 EtOAc/hexanes). ^{1}H NMR ($CDCl_3$): δ 3.21 (ψt, 1H, *J* = 8.5 Hz, H-2 glc), 3.48–4.05 (m, 16H), 4.29–4.65 (m, 22H), 4.78–4.95 (m, 10H), 5.10 (ψs, 1H, H-1, man-2), 5.25 (d, 1H, *J* = 1.5 Hz, H-1, man-1), 7.11-7.49 (m, 55H, Ar*H*).

4.14. Synthesis of 6-*O*-sulfato-2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 6)-2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 4)-2-azido-2-deoxy-3,6-di-*O*-benzyl-D-glucopyranosyl-(α1 → 6)-3,4,5-tri-*O*-benzyl-1-D-*myo*-inositol-1,2-cyclic carbonate (23α) or 2-*O*-sulfato-3,4,6-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 6)-2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 4)-2-azido-2-deoxy-3,6-di-*O*-benzyl-D-glucopyranosyl-(α1 → 6)-3,4,5-tri-*O*-benzyl-1-D-*myo*-inositol-1,2-cyclic carbonate (24α)

To the solution of **21**α (3.7 mg, 0.002 mmol) or **22**α (3.4 mg, 0.002 mmol) in dry pyridine (50 μL) was added the sulfur trioxide–pyridine complex (9.5 mg, 0.06 mmol) and the reaction mixture was stirred at 20 °C until TLC (silica gel, 1:2 EtOAc/hexanes) showed completion of reaction, approximately 2 h. The reaction was quenched with $NaHCO_3$ (1 M, 0.1 mL), diluted with water (1 mL), extracted with ethyl acetate (3× 2 mL), dried ($MgSO_4$), and concentrated to yield **23**α (2.4 mg, 61%) or **24**α (2 mg, 55%), respectively. For **23**α, R_f = 0.45 (silica gel, 3:1:0.05 toluene/EtOH/Et_3N). ^{1}H NMR ($CDCl_3$): δ 3.38 (dd, 1H, *J* = 3, 9 Hz, H-2 glc), 3.45–4.32 (m, 22H), 4.41–4.95 (m, 24H), 5.05 (ψs, 1H, H-1, man-2), 5.21 (d, 1H, *J* = 1.5 Hz, H-1 man-1), 5.35 (d, 1H, *J* = 3.5 Hz, H-1 glc), 7.08–7.49 (m, 55H, Ar*H*). For **24**α, R_f = 0.48 (silica gel, 3:1:0.05 toluene/EtOH/Et_3N). ^{1}H NMR ($CDCl_3$): δ 3.37 (dd, 1H, *J* = 3.4, 9.8 Hz, H-2 glc), 3.52–4.02 (m, 16H), 4.11–4.95 (m, 31H), 5.06 (ψs, 1H, H-1, man-2), 5.23 (d, 1H, *J* = 1.5 Hz, H-1 man-1), 5.38 (d, 1H, *J* = 3.5 Hz, H-1 glc), 7.15–7.45 (m, 55H, Ar*H*).

4.15. Synthesis of 6-*O*-sulfato-2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 6)-2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 4)-2-azido-2-deoxy-3,6-di-*O*-benzyl-D-glucopyranosyl-(β1 → 6)-3,4,5-tri-*O*-benzyl-1-D-*myo*-inositol-1,2-cyclic carbonate (23β) or 2-*O*-sulfato-3,4,6-tri-*O*-benzyl-D-mannopyranosyl-(α 1 → 6)-2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 4)-2-azido-2-deoxy-3,6-di-*O*-benzyl-D-glucopyranosyl-(β1 → 6)-3,4,5-tri-*O*-benzyl-1-D-*myo*-inositol-1,2-cyclic carbonate (24β)

The procedure for forming the sulfate esters of **21**β (1.5 mg, 0.88 μmol) and **22**β (1.3 mg, 0.76 μmol) was the same as for **23**α and **24**α, except that 4 mg (0.026 mmol) of sulfur trioxide–pyridine complex was used in 25 μL dry pyridine in each case. **23**β and **24**β were obtained in 60% (0.8 mg) and 58% (0.7 mg) yields, respectively. For **23**β, R_f = 0.45 (silica gel, 3:1:0.05 toluene/EtOH/Et_3N). ^{1}H NMR ($CDCl_3$): δ 3.25 (ψt, 1H, *J* = 8 Hz, H-2 glc), 3.45–4.15 (m, 20H), 4.28–4.91 (m, 27H), 5.07 (ψs, 1H, H-1, man-2), 5.25 (d, 1H, *J* = 1.5 Hz, H-1, man-1), 7.05–7.55 (m, 55H, Ar*H*). For **24**β, R_f = 0.47 (silica gel, 3:1:0.05 toluene/EtOH/Et_3N). ^{1}H NMR ($CDCl_3$): δ 3.21 (ψt, 1H, *J* = 8.5 Hz, H-2 glc), 3.45–4.18 (m, 18H), 4.25–4.96 (m, 29H), 5.10 (ψs, 1H, H-1, man-2), 5.25 (d, 1H, *J* = 1.5 Hz, H-1, man-1), 7.10–7.42 (m, 55H, Ar*H*).

4.16. Synthesis of 6-*O*-sulfato-2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 6)-2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 4)-2-azido-2-deoxy-3,6-di-*O*-benzyl-D-glucopyranosyl-(α1 → 6)-3,4,5-tri-*O*-benzyl-1-D-*myo*-inositol (25α) or 2-*O*-sulfato-3,4,6-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 6)-2,3,4-tri-*O*-benzyl-D-mannopyrano-syl-(α1 → 4)-2-azido-2-deoxy-3,6-di-*O*-benzyl-D-glucopyranosyl- (α1 → 6)-3,4,5-tri-*O*-benzyl-1-D-*myo*-inositol (26α)

To a stirred solution of **23**α (2.4 mg, 0.001 mmol) or **24**α (2 mg, 0.001 mmol) in THF (100 μL) at 0 °C was added LiOH (1 M, 20 μL). The reaction mixture was stirred at 0 °C for 1.5 h and quenched with NH_4Cl (1 M, 0.3 mL). The aqueous layer was extracted with ethyl acetate (4× 0.5 mL), and the combined organic layers were rinsed with brine, dried ($MgSO_4$), and concentrated. Purification via preparative TLC (silica gel, 2:1:0.05 toluene/EtOH/Et_3N) yielded **25**α (1.4 mg, 60%) or **26**α (1.2 mg, 60%). For **25**α, R_f = 0.3 (2:1:0.05 toluene/EtOH/Et_3N). ^{1}H NMR ($CDCl_3$): δ 2.52 (br s, 1H, O*H*), 3.21–4.05 (m, 22 H), 4.11–4.98 (m, 26H), 5.05 (ψs, 1H, H-1, man-2), 5.21 (d, 1H, *J* = 1.5 Hz, H-1 man-1), 5.35 (d, 1H, *J* = 3.5 Hz, H-1 glc), 7.11–7.48 (m, 55H, Ar*H*). For **26**α, R_f = 0.35 (2:1:0.05 toluene/EtOH/Et_3N). ^{1}H NMR ($CDCl_3$): δ 2.50 (br s, 1H, O*H*), 3.25–4.08 (m, 22H), 4.15–4.95 (m, 26H), 5.06 (ψs, 1H, H-1, man-2), 5.23 (d, 1H, *J* = 1.5 Hz, H-1 man-1), 5.38 (d, 1H, *J* = 3.5 Hz, H-1 glc), 7.11–7.50 (m, 55H, Ar*H*).

4.17. Synthesis of 6-*O*-sulfato-2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 6)-2,3,4-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 4)-2-azido-2-deoxy-3,6-di-*O*-benzyl-D-glucopyranosyl-(β1 → 6)-3,4,5-tri-*O*-benzyl-1-D-*myo*-inositol (25β) or 2-*O*-sulfato-3,4,6-tri-*O*-benzyl-D-mannopyranosyl-(α1 → 6)-2,3,4-tri-*O*-benzyl-D-mannopyrano-syl-(α1 → 4)-2-azido-2-deoxy-3,6-di-*O*-benzyl-D-glucopyranosyl-(β1 → 6)-3,4,5-tri-*O*-benzyl-1-D-*myo*-inositol (26β)

Syntheses were carried out from **23**β (0.8 mg, 0.44 μmol) and **24**β (0.7 mg, 0.38 μmol) exactly as described for **25**α and **26**α. **25**β and **26**β were obtained in 55% and 58% yields (0.4 mg of each), respectively. For **25**β, R_f = 0.3 (2:1:0.05 toluene/EtOH/Et_3N). ^{1}H NMR ($CDCl_3$): δ 2.55 (br s, 1H, O*H*), 3.25–4.15 (m, 22H), 4.25–4.93 (m, 27H), 5.07 (ψs, 1H, H-1, man-2), 5.25 (d, 1H, *J* = 1.5 Hz, H-1, man-1), 7.08–7.51 (m, 55H, Ar*H*). For **26**β, R_f = 0.35 (silica gel, 2:1:0.05 toluene/EtOH/Et_3N). ^{1}H NMR ($CDCl_3$): δ 2.55 (br s, 1H, O*H*), 3.20–4.11 (m, 22H), 4.20–4.98 (m, 27H), 5.10 (ψs, 1H, H-1, man-2), 5.25 (d, 1H, *J* = 1.5 Hz, H-1, man-1), 7.05–7.49 (m, 55H, Ar*H*).

4.18. Synthesis of 6-*O*-sulfato-D-mannopyranosyl-(α1 → 6)-D -mannopyranosyl-(α1 → 4)-2-amino-2-deoxy-D-glucopyranosyl-(α 1 → 6)-D-*myo*-inositol-1,2-cyclic phosphate (3α) or 2-*O*-sulfato-D-mannopyranosyl-(α1 → 6)-D-mannopyranosyl-(α1 → 4)-2-amino-2-deoxy-D-glucopyranosyl-(α1 → 6)-D-*myo*-inositol-1,2-cyclic phosphate (4α)

PCl_2O_2Me (500 μL) was slowly added to dry pyridine (5 mL). The reaction was stirred at 20 °C for 30 min. This solution (30 μL) was added to a stirred solution of **25**α (1.4 mg, 0.7 μmol) or **26**α (1.2 mg, 0.7 μmol) in dry pyridine (20μL) and stirring was continued at 20 °C until TLC (silica, 1:1:1 $CHCl_3$–diethyl ether–MeOH) showed completion of reaction. The reaction was quenched by addition of sat. $NaHCO_3$ (0.2 mL) and co-evaporation with heptane. The resulting solid was dissolved in water (0.75 mL) and adjusted to pH 1 by dropwise addition of 2 M HCl. The solution was extracted with EtOAc (5 × 0.5 mL) and the combined organic extracts were dried (Na_2SO_4), concentrated, dried by coevaporation with toluene, and dissolved in dry THF (0.5 mL). In a separate flask, NH_3 (1.5 mL) was condensed at −78 °C and sodium (4 mg, 0.17 mmol) was added. Once the blue color persisted, the THF solution of crude product from above was added dropwise, stirred at −78 °C for 15 min, and quenched with *solid* NH_4Cl (9 mg, 0.17 mmol) at −78 °C. Once the blue color had disappeared, methanol (1.25 mL) was added. The reaction mixture was allowed to warm to 20 °C and evaporated for 12 h. The resulting white powder was dissolved in deionized water (0.75 mL) and was desalted by passing through Sephadex G-10 (washed with water), yielding after evaporation **3**α (0.3 mg, 50%) or **4**α (0.3 mg, 50%). For **3**α,^{1}H NMR (D_2O): δ 2.55 (ψd, 1H, *J* = 7.5 Hz, H-2 glc), 3.20–4.15 (m, 15H), 4.35 (ddd, 1H, H-1 inos), 4.55 (ψt, 1H, H-2 inos), 4.85 (ψs, 1H, H-1 man), 5.02 (ψs, 1H, H-1 man), 5.15 (d, 1H, *J* = 3 Hz, H-1 glc). ^{31}P NMR (D_2O) δ 17.5. HRMS (ESI): *m*/*z* calcd for $C_{24}H_{43}NO_{25}PS$: 808.1577. Found: 808.1567. For **4**α, ^{1}H NMR (D_2O): δ 2.60 (ψd, 1H, *J* = 7.5 Hz, H-2 glc), 3.22–3.95 (m, 15H), 4.38 (ddd, 1H, H-1 inos), 4.52 (ψt, 1H, H-2 inos), 4.85 (ψs, 1H, H-1 man), 4.95 (ψs, 1H, H-1 man), 5.15 (d, 1H, *J* = 3 Hz, H-1 glc). ^{31}P NMR (D_2O) δ 16.85. HRMS (ESI): *m*/*z* calcd for $C_{24}H_{43}NO_{25}PS$: 808.1577. Found: 808.1571.

4.19. Synthesis of 6-*O*-sulfato-D-mannopyranosyl-(α1 → 6)-D-mannopyranosyl-(α1 → 4)-2-amino-2-deoxy-D- glucopyranosyl-(β1 → 6)-D-*myo*-inositol-1,2-cyclic phosphate (3β) or 2-*O*-sulfato-D-mannopyranosyl-(α1 → 6)-D-mannopyranosyl-(α1 → 4)-2-amino-2-deoxy-D-glucopyranosyl-(β1 → 6)-D-*myo*-inositol-1,2-cyclic phosphate (4β)

Syntheses of these compounds were carried out from **25**β and **26**β (0.4 mg, 0.22 μmol each) exactly as described for **3**α and **4**α, yielding **3**β and **4**β (40%, 0.07 mg each), respectively. For **3**β, ^{1}H NMR (D_2O): δ 3.01–4.05 (m, 22H), 4.31 (ddd, 1H, H-1 inos), 4.56–5.01 (m, 4H). ^{31}P NMR (D_2O) δ 16.65. HRMS (ESI): *m*/*z* calcd for C24H43NO25PS: 808.1577. Found: 808.1563. For **4**β,^{1}H NMR (D_2O): δ 3.05–4.20 (m, 22H), 4.29 (ddd, 1H, H-1 inos), 4.50–5.05 (m, 4H). ^{31}P NMR (D_2O) δ 17.01. HRMS (ESI): *m*/*z* calcd for C24H43NO25PS: 808.1577. Found: 808.1561.

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