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Synthesis of inositol phosphoglycans containing thiol-terminated spacers for efficient coupling to maleimide functionalized solid phases or proteins

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Abstract—The synthesis of inositol phosphoglycans (IPGs), analogous to second messengers of insulin, to provide a small targeted library of compounds is described. These derivatives contain the glucosamine(α1–6)*myo*-inositol 1,2-cyclic phosphate motif. A thiol-terminated spacer was introduced, for their immobilization, by a radical elongation of an allyl ether with benzyl mercaptane.

1. Introduction

Glycosylphosphatidylinositol (GPI)-anchors are anchoring proteins to the cell membrane and are widely distributed among eukaryotes. They are common in parasites but also occur in mammalian cells, although they are not as abundant as in lower eukaryotes. Mammalian GPIs usually anchor proteins with specialized functions.[1] The general structure of a GPI-anchor is depicted in Fig. 1. It contains an α-linked non-acetylated glucosamine coupled to phosphatidylinositol

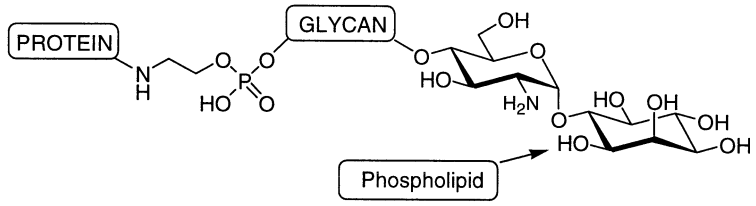
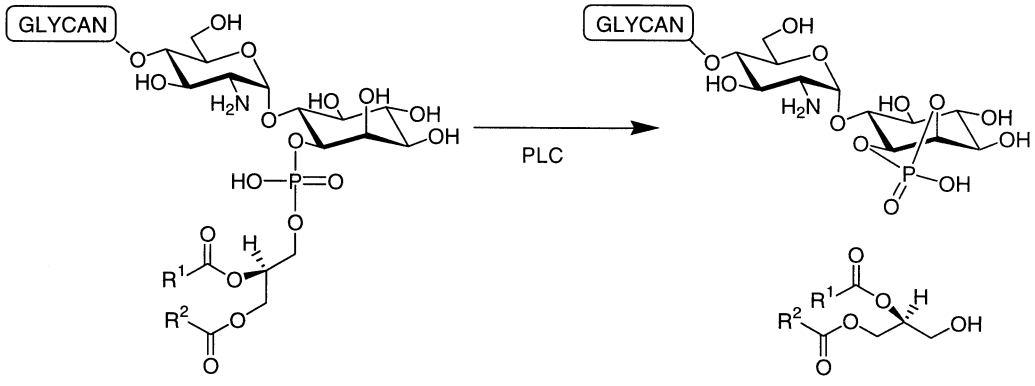


Figure 1. General structure for GPI-anchors.



Scheme 1. Proposed mechanism for generation of two second messengers.

Keywords: thiol-terminated spacer; inositol phosphoglycans; radical elongation.
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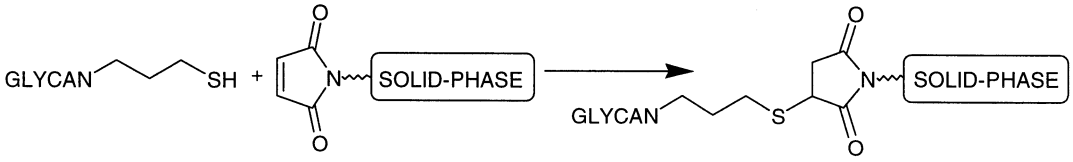


Scheme 2. Coupling of a thiol-functionalized glycan to a solid phase.

at the 6-OH. The glucosamine in all, thus far characterized, GPI-anchors is substituted at the 4-OH with an identical ethanolamine–PO_4-6Man*p*(α1–2)Man*p*(α1–6)Man*p*(α1–) core glycan, anchoring the protein at the C-terminus. This trimannoside is substituted with species-specific side chains.

Inositol phosphoglycans (IPGs) have been proposed as second messengers to insulin. They are supposed to be released from protein-free GPIs in response to insulin, by the action of a phosphatidylinositol-specific phospholipase C (PLC), together with diacylglycerol which is also acting as a second messenger (Scheme 1).[2–4] α-D-Glucosamine(1→6)*myo*-inositol-1,2-cyclic phosphate has been shown to be the minimum structural motif for the IPGs to possess insulin-mimetic activity.[5] The structure of the glycan part attached to glucosamine is unknown. However, insulin mimetic activity of IPGs has been demonstrated in several studies.[4,6] The IPGs were produced from GPI-anchors by protease digestion and cleavage of the phosphodiester bond using bacterial PLC, yielding an inositol 1,2-cyclic phosphate-containing IPG. This has been taken as an indication of a conserved glycan part, containing mannose building blocks. Presence of galactose but not mannose in the active substances has been shown by incorporation studies of radiolabeled monosaccharides, indicating a galactose[7,8]—but not a mannose—containing[8,9] oligosaccharide attached to the glucosamine. A major problem in several of these investigations is the poorly defined preparations of IPGs, acquired from natural sources.

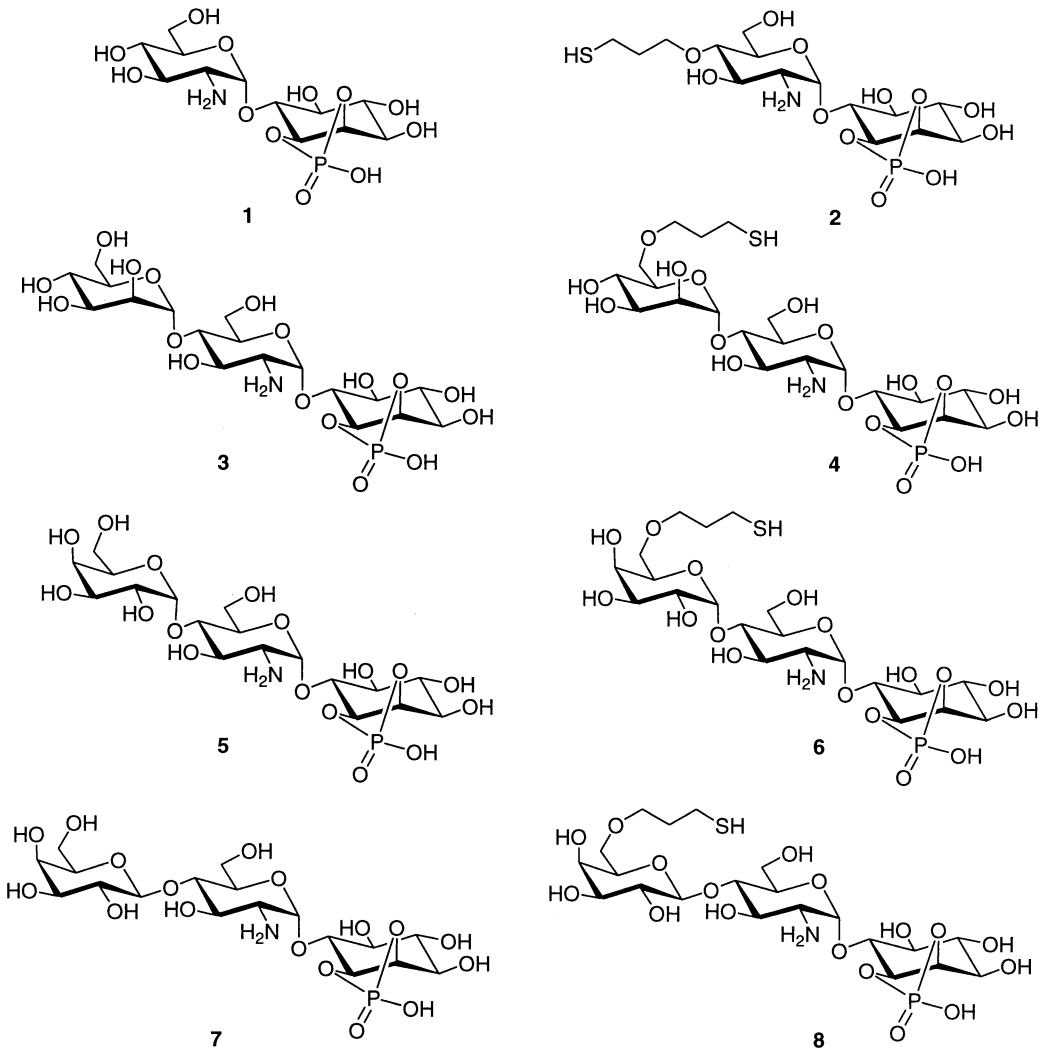
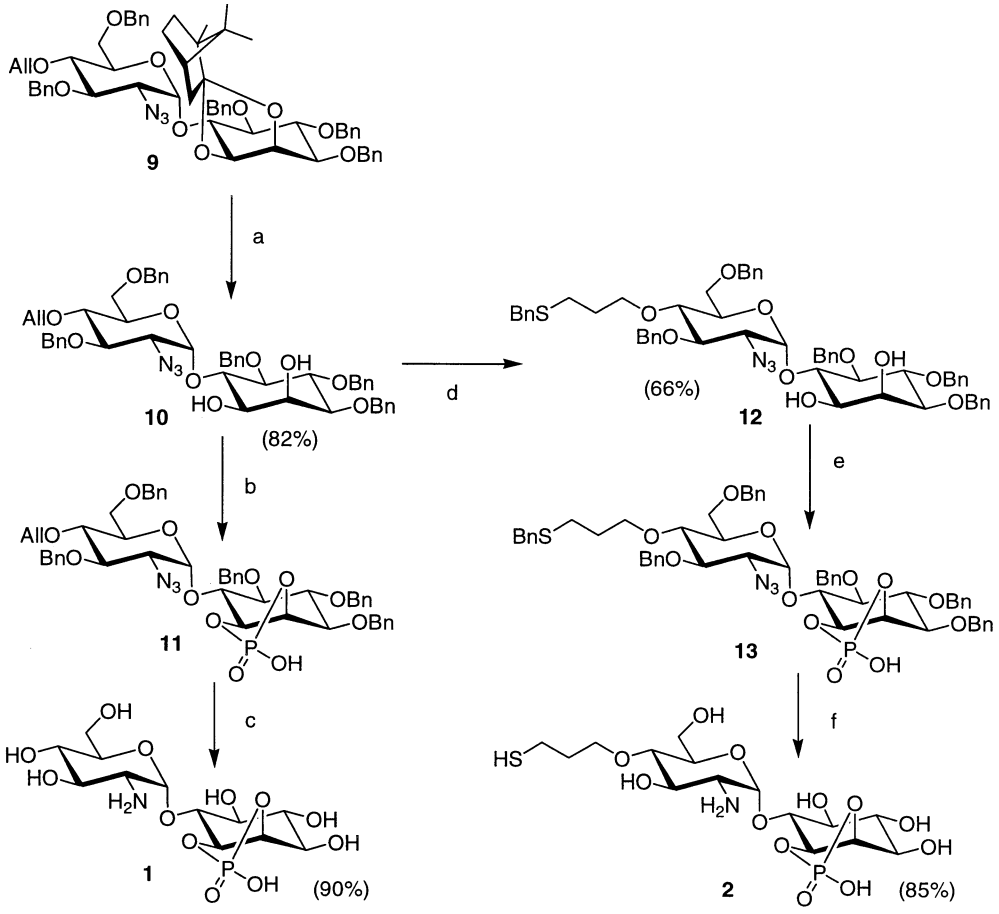


Figure 2. The targeted library of IPGs.



Scheme 3. (a) $CHCl_3$/TFA 10:1; (b) $MeOPOCl_2$, pyridine; (c) Na, NH_3 (l); (d) BnSH, AIBN, benzene, 70°C; (e) $MeOPOCl_2$, pyridine; (f) Na, NH_3 (l).

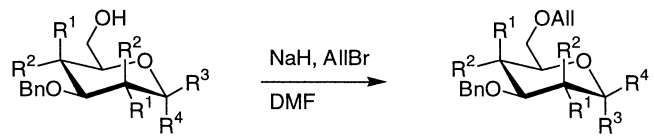
Therefore a well-defined library of synthetic IPGs is necessary in the search for the putative second messenger.

Furthermore, development of a spacer for coupling of the IPGs to a solid phase, proteins or various probes will open up new interesting possibilities, such as the isolation of antibodies raised against the IPGs and the identification of receptors by using photoaffinity or radiolabeling probes.

The presence of an amino group and a cyclic phosphate in the target molecules precludes the use of amino- or carboxylic acid-functionalized spacers. One convenient way to immobilize the IPGs is therefore to introduce a thiol-terminated spacer for coupling to a maleimide functionality as depicted in Scheme 2.

Alkenes are known to react with alkane thiols in radical reactions to produce dialkyl sulfides. Terminal alkenes usually give the terminal sulfide with high regioselectivity, and examples of acetyl or benzyl protected thiols generated by reaction of allyl ethers with thioacetic acid or benzyl mercaptane have been reported.[10–13] General protecting group considerations, such as the limited stability of thioacetates compared to benzyl sulfides, and ease of deprotection, makes the benzyl group more attractive as a protecting group for the thiol. Because of the presence of a sulfide, the use of catalytic hydrogenation in the deprotection step was not considered, leaving debenzylation using dissolving metal reduction in liquid ammonia as the only realistic alternative.

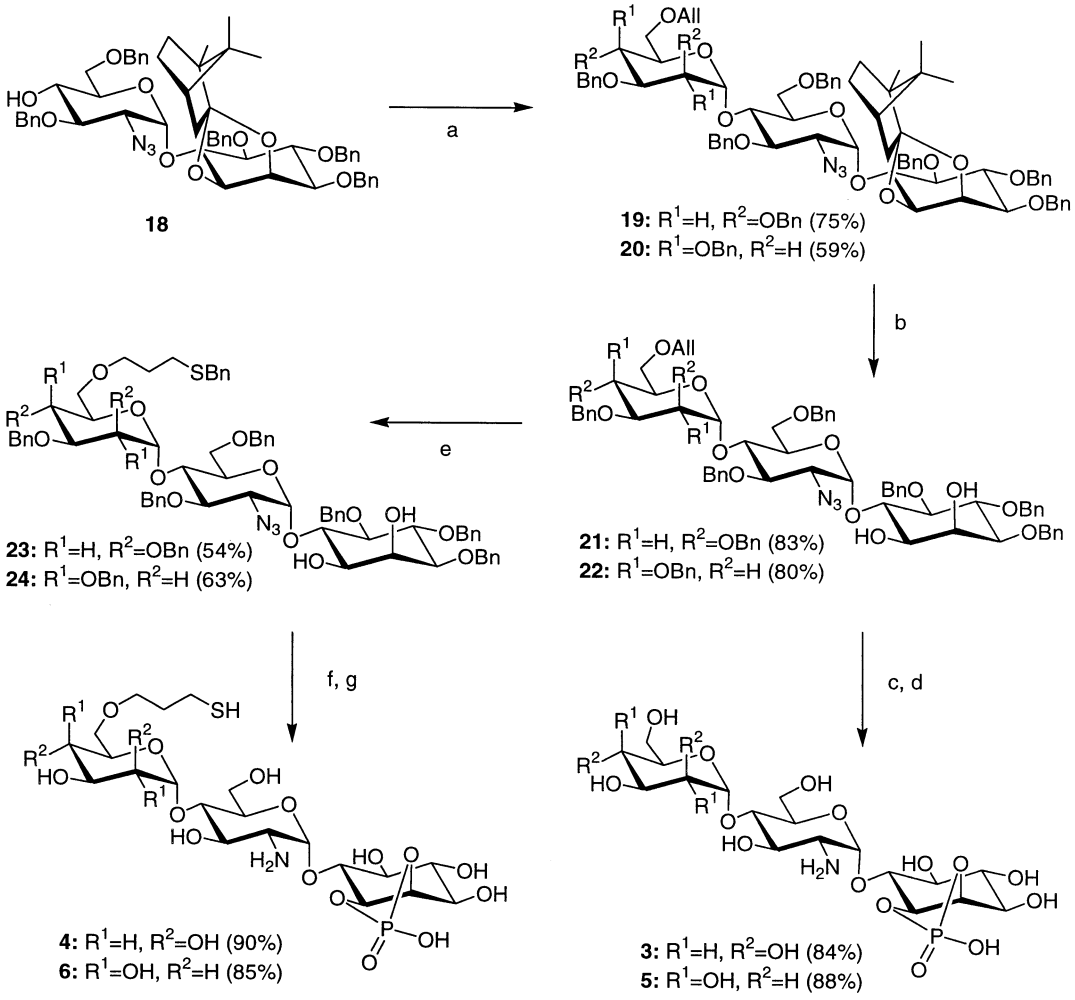
The disaccharide **1**, together with the three trisaccharides **3**, **5**, **7** and their spacer containing analogues **2**, **4**, **6** and **8** (Fig. 2) were chosen as synthetic targets. This small targeted library of IPGs, is based upon the indications of mannose or galactose residues in the putative second messenger.



14: R^1=H, R^2=OBn, R^3=H, R^4=SEt
16: R^1=OBn, R^2=H, R^3=SEt, R^4=H

15: R^1=H, R^2=OBn, R^3=H, R^4=SEt (97%)
17: R^1=OBn, R^2=H, R^3=SEt, R^4=H (98%)

Scheme 4.

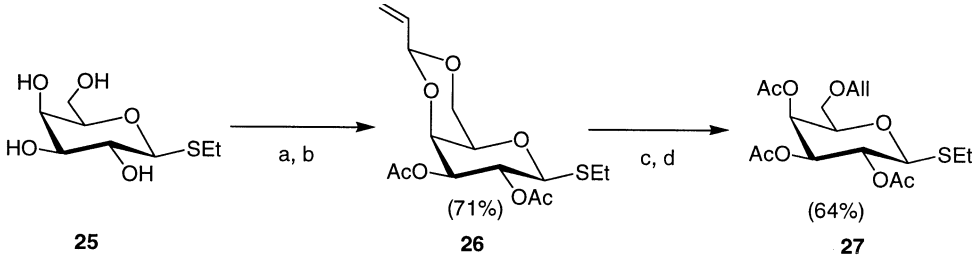


Scheme 5. (a) DMTST, diethyl ether, **15** or **17**; (b) $CHCl_3$/TFA 10:1; (c) $MeOPOCl_2$, pyridine; (d) Na, NH_3 (l); (e) BnSH, AIBN, benzene, 70°C; (f) $MeOPOCl_2$, pyridine; (g) Na, NH_3 (l).

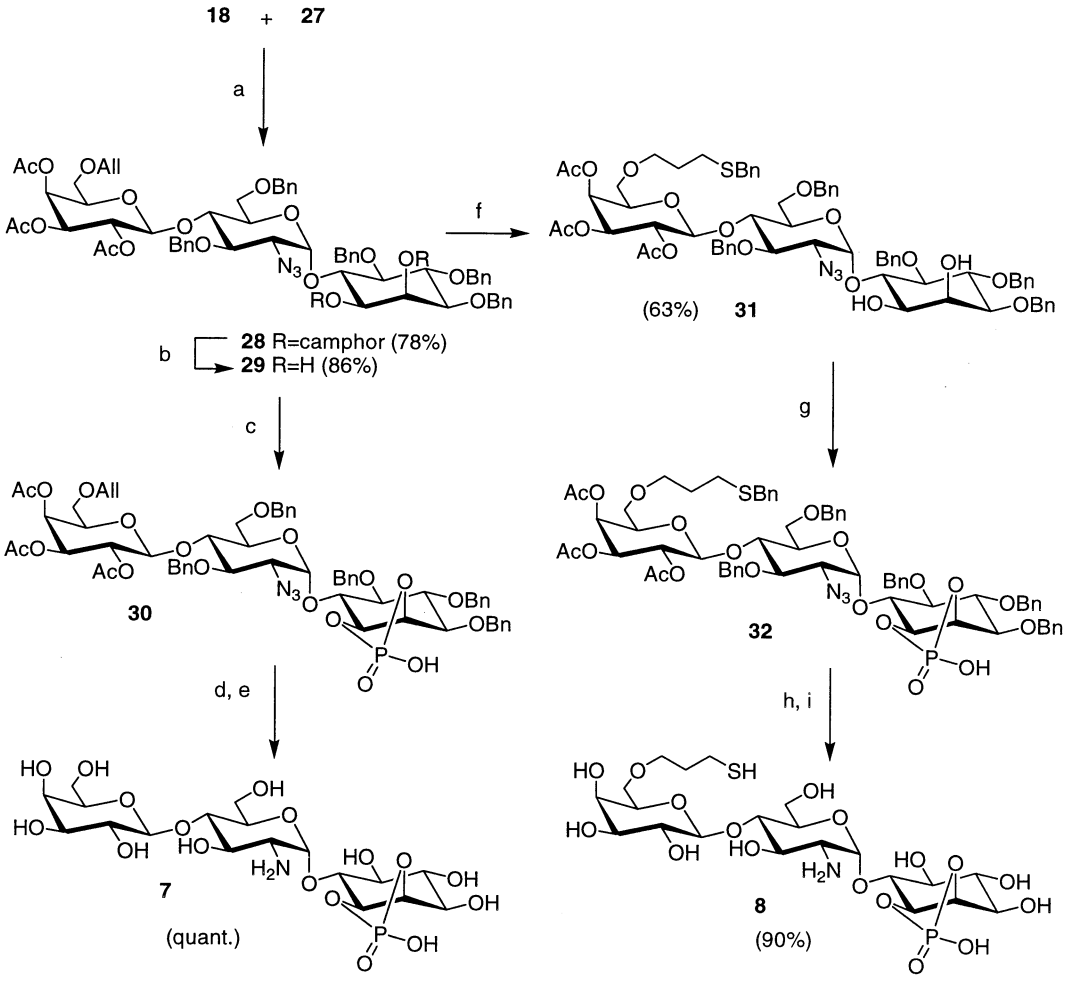
2. Results and discussion

The camphor acetal of compound **9**[14] was hydrolyzed in $CHCl_3$/TFA 10:1 to produce compound **10** in 82% yield. Phosphorylation using *N*-methylpyridinium dichlorophosphate, prepared in situ from methyl dichlorophosphate in pyridine, produced the cyclic phosphate-containing derivative **11**. Subsequent deprotection and reduction of the azide using sodium in liquid ammonia gave the target compound **1**. The radical reaction of **10** with benzyl mercaptane initiated by 2,2′-azobisisobutyronitrile (AIBN) in benzene at 70°C was very slow, and use of a large excess of both the thiol and AIBN combined with long reaction time at elevated temperature was needed for complete conversion of the allylic double bond to a benzyl sulfide. Use of smaller excess of the thiol and/or the initiator gave residual unreacted starting material, which could not be separated from the product. The resulting compound **12** containing a thiol terminated spacer, protected as a benzyl sulfide, was obtained in 65% yield. Subsequent phosphorylation and deprotection produced the target IPG **2** (Scheme 3).

The methods developed for the 'disaccharide' for introduction of the spacer and subsequent phospohorylation was then used to synthesize the IPGs **3**–**8**. The glycosyl donors



Scheme 6. (a) Acrolein dimethyl acetal, DMF; (b) Ac_2O, pyridine; (c) $NaBH_3CN$, THF, HCl; (d) Ac_2O, pyridine.



Scheme 7. (a) NIS, TfOH, CH_2Cl_2/diethyl ether 1:1; (b) $CHCl_3$/TFA 10:1; (c) $MeOPOCl_2$, pyridine; (d) MeOH, NH_3 (sat.); (e) Na, NH_3 (l); (f) BnSH, AIBN, benzene, 70°C; (g) $MeOPOCl_2$, pyridine; (h) MeOH, NH_3 (sat.); (i) Na, NH_3 (l).

15 and **17** were synthesized by allylation of **14** and **16** (Scheme 4), acquired from mannose or galactose in six steps.[15,16] Subsequent dimethyl(methylthio)sulfonium trifluorometheanesulfonate (DMTST)-promoted glycosylation of **18**[14] produced **19** and **20** in 75 and 59% yield, respectively. Hydrolysis of the camphor acetal gave compound **21** and **22**, which were subjected to the same transformations as the 'disaccharide' **10** to produce the target IPGs **3**–**6** (Scheme 5).

The β-galactose containing trisaccharides were produced in the same manner. For construction of the β-galactose-containing IPGs, an acetylated galactosyl donor was needed. Other neighbouring participating groups such as benzoates were ruled out because of the difficulties associated with deprotection of benzoates in the presence of a cyclic phosphate. An elegant method to introduce the allyl ether regioselectively at the 6-OH is to convert the galactose derivative **25** into the 4,6-*O*-acrolein acetal derivative **26**, followed by reductive opening of the acetal[17] by the same method as for the more familiar reductive opening of benzylidene acetals using $NaBH_3CN$. Thus, treatment of **25** with acrolein dimethyl acetal and TsOH, followed by acetylation produced **26** in 71% yield. Subsequent reaction with $NaBH_3CN$ and HCl in THF, followed by acetylation gave the donor **27** in 64% yield (Scheme 6).

The glycosylation of **18** promoted by *N*-iodosuccinimide (NIS) and TfOH acid produced trisaccharide **28** in 84% yield. Hydrolysis of the camphor acetal followed by phosphorylation or radical elongation and subsequent phosphorylation produced **30** and **32**. The following deacetylation had to be performed using mild conditions to preserve the cyclic phosphate. Methanol saturated with ammonia accomplished the deacetylation without affecting the cyclic phosphate. Subsequent debenzylation and reduction of the azide using sodium in liquid ammonia gave the target compounds **7** and **8** (Scheme 7).

3. Experimental

3.1. General methods

Organic phases were dried over ($MgSO_4$), filtered, and concentrated in vacuo at or below 40°C. TLC: 0.25 mm precoated silica-gel plates (MERCK silica-gel $60F_{254}$); detection by spraying the plates 1 M H_2SO_4 solution followed by heating at ~250°C. Flash Chromatography (FC): Silica gel MERCK 60 (0.040–0.063 mm). Gel filtrations were performed on a Pharmacia Sephadex G-15 column eluted with deoxygenated (Ar) H_2O containing 1% *n*-butanol. NMR spectra were recorded on Bruker AC-F 250

and Varian Mercury 300 spectrometers, temperature 25°C. Chemical shifts are given in ppm relative to TMS in $CDCl_3$ (δ=0.00) or acetone in D_2O (^{13}C: δ=31.00, ^1H: δ=2.22) as internal standards; ^{31}P, 85% phosphoric acid (δ=0.00) was used as external standard. pH* in D_2O is given as an uncorrected value calibrated against H_2O-buffer solutions. Optical rotations were recorded at room temperature with a Perkin–Elmer 241 polarimeter. Melting points were recorded with a Gallenkamp melting point apparatus. FAB-mass spectra were recorded on a JEOL SX 102 Mass Spectrometer using a 3-nitrobenzyl alcohol matrix. IR spectra were recorded as films on CaF_2 crystals (syrups) or as KBr pellets (solids) on a Perkin–Elmer SPECRUM 1000 FT-IR Spectrometer.

3.1.1. 6-*O*-(4-*O*-Allyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-3,4,5-tri-*O*-benzyl-D-*myo*-inositol (10). To **9**[14] (173 mg, 0.174 mmol) in $CHCl_3$ (10 mL) was added TFA (1 mL). After 16 h, the mixture was diluted with $CHCl_3$, washed with aqueous $NaHCO_3$ (sat.), dried, filtered and concentrated. FC (toluene/EtOAc 4:1) gave **10** (122 mg, 0.142 mmol, 82%) as a white solid. R_f=0.39 (toluene/EtOAc 2:1); mp 110–112°C (from ether); $[\alpha]_D$=+47 (*c* 1.4, $CHCl_3$); IR ν_{max} (cm^{-1}) 1052, 1453, 2107, 2867, 3441; NMR: ^1H (250 MHz, $CDCl_3$), δ 3.02 (1H, d, *J*=9.8 Hz), 3.20 (1H, dd, *J*=11.1, 2.0 Hz), 3.36 (1H, t, *J*=9.3 Hz), 3.43–3.61 (4H, m), 3.83–3.91 (3H, m), 3.95 (1H, t, *J*=9.5 Hz), 4.01 (1H, t, *J*=9.5 Hz), 4.09–4.19 (3H, m), 4.42 (1H, d, *J*=11.7 Hz), 4.66 (1H, d, *J*=11.0 Hz), 4.70 (2H, s), 4.78 (1H, d, *J*=10.6 Hz), 4.81 (1H, d, *J*=10.6 Hz), 4.88 (1H, d, *J*=11.3 Hz), 4.92 (1H, d, *J*=11.0 Hz), 5.01 (1H, d, *J*=11.0 Hz), 5.05–5.15 (2H, m), 5.35 (1H, d, *J*=3.7 Hz), 5.68–5.84 (1H, m), 7.12–7.42 (25H, m); ^{13}C (62.8 MHz, $CDCl_3$), δ 64.2 (CH), 67.4 (CH_2), 69.3 (CH), 71.1 (CH), 72.6 (CH_2), 72.8 (CH), 73.3 (CH_2), 73.5 (CH_2), 75.0 (CH_2), 75.4 (CH_2), 75.8 (CH_2), 77.9 (CH), 79.8 (CH), 80.6 (CH), 80.7 (CH), 80.9 (CH), 81.7 (CH), 98.8 (CH), 116.4 (CH_2=CH), 127.3–128.5 (Ph), 134.8 (CH$_2$=*C*H), 137.8 (Ph), 137.9 (Ph), 138.8 (Ph); Anal. Calcd for $C_{50}H_{55}N_3O_{10}$: C, 70.0; H, 6.5 Found: C, 70.2; H 6.3.

3.1.2. 6-*O*-(2-Amino-2-deoxy-α-D-glucopyranosyl)-D-*myo*-inositol 1,2-cyclic phosphate (1).[18] Methyl dichlorophosphate (50 μL, 0.50 mmol) was added to pyridine (1 mL) and the resulting suspension was stirred for 30 min. **10** (38 mg, 0.044 mmol) in pyridine (1 mL) was added and the mixture stirred for 2 h. $NaHCO_3$ (sat., 1 mL) was added and the mixture concentrated. The residue was dissolved in $CHCl_3$ (40 mL) and H_2O (10 mL). 1 M HCl (3 mL) was added and the organic layer separated. The aqueous layer was extracted with a second portion of $CHCl_3$ (40 mL) and the combined organic layers were dried and concentrated to give **11** quantitatively. A solution of **11** in THF (2 mL) was added to NH_3 (l) (~20 mL) at −33°C. To the stirred mixture was added a minimum amount of sodium for the mixture to turn deep blue. After 1 min, NH_4Cl was added until the color disappeared. The mixture was concentrated under a stream of argon and the residue dissolved in H_2O (10 mL), washed with ether (10 mL) and concentrated. Gel filtration of the residue afforded **1** (16 mg, 0.040 mmol, 90%) as a white solid. ^1H and ^{13}C NMR were in accordance with those previously published.[18]

3.1.3. 6-*O*-[2-Azido-4-*O*-{3-(benzylthio)propyl}-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl]- 3,4,5-tri-*O*-benzyl-D-*myo*-inositol (12). A deoxygenated (Ar) mixture of **10** (120 mg, 0.140 mmol), benzylmercaptane (0.53 mL, 4.5 mmol) and AIBN (130 mg, 0.79 mmol) in benzene (25 mL) was stirred under Ar at 70°C. After 11 h, the mixture was allowed to attain rt and after another 7 h, the mixture was concentrated. FC (toluene/EtOAc gradient 12:1→2:1) gave **12** (91 mg, 0.093 mmol, 66%) as a colorless syrup. R_f=0.39 (toluene/EtOAc 2:1); $[\alpha]_D$=+37 (*c* 0.52, $CHCl_3$); IR ν_{max} (cm^{-1}) 1051, 1453, 2107, 2917; NMR: ^1H (250 MHz, $CDCl_3$), δ 1.50–1.70 (2H, m), 2.18–2.38 (2H, m), 3.00 (1H, dd, *J*=11.1, 1.8 Hz), 3.15 (1H, dd, *J*=11.1, 2.2 Hz), 3.33–4.17 (15H, m), 4.40 (1H, d, *J*=11.3 Hz), 4.63–5.01 (8H, m), 5.32 (1H, d, *J*=3.7 Hz), 7.17–7.33 (30H, m); ^{13}C (62.8 MHz, $CDCl_3$), δ 27.9 (CH_2), 29.9 (CH_2), 36.2 (CH_2), 64.3 (CH), 67.4 (CH_2), 69.4 (CH), 71.1 (2C, CH, CH_2), 72.6 (CH_2), 72.8 (CH), 73.3 (CH_2), 75.0 (CH_2), 75.3 (CH_2), 75.9 (CH_2), 77.9 (CH), 79.8 (CH), 80.6 (CH), 80.7 (CH), 80.9 (CH), 81.7 (CH), 98.8 (CH), 126.9–128.8 (Ph), 137.7–138.5 (Ph); HRMS Calcd for $C_{57}H_{63}N_3O_{10}S$: $[M+Na]^+$ 1004.4132 Found: $[M+Na]^+$ 1004.4181.

3.1.4. 6-*O*-(2-Amino-4-*O*-{3-mercaptopropyl}-2-deoxy-α-D-glucopyranosyl)-D-*myo*-inositol 1,2-cyclic phosphate (2). 12 (17 mg, 0.017 mmol) was phosphorylated and deprotected by the same method as for **10** to give **2** (7 mg, 0.015 mmol, 85%) as a white solid. $[\alpha]_D$=+30 (*c* 0.43, H_2O); IR ν_{max} (cm^{-1}) 845, 1042, 1099, 1631, 3388; NMR: ^1H (250 MHz, D_2O, pH*=3.7), δ 1.82–1.96 (2H, m), 2.63 (2H, t, *J*=7.1 Hz), 3.36–3.46 (3H, m), 3.68 (1H, t, *J*=9.9 Hz), 3.72–4.04 (8H, m), 4.56 (1H, ddd, *J*=20.1, 8.0, 4.8 Hz), 4.71 (1H, t, *J*=3.8 Hz), 5.50 (1H, d, *J*=3.7 Hz); ^{13}C (62.8 MHz, D_2O, pH*=3.7), δ 21.1 (CH_2), 34.0 (CH_2), 55.0 (CH), 60.5 (CH_2), 70.2 (CH), 70.3 (CH, d, *J*=9 Hz). 71.3 (CH), 72.2 (3C, 2×CH, CH_2), 77.8 (CH), 78.2 (CH), 80.2 (CH), 81.5 (CH), 96.4 (CH); ^{31}P (121 MHz, D_2O, pH*=3.7), δ 16.0 (d, *J*=20.1 Hz); HRMS Calcd for $C_{15}H_{28}NO_{12}PS$: $[M-H]^-$ 476.0992 Found: $[M-H]^-$ 476.0974.

3.1.5. Ethyl 6-*O*-allyl-2,3,4-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (15). 14[14] (1.54 g, 3.11 mmol) and allyl bromide (0.40 mL, 4.7 mmol) dissolved in DMF (20 mL) was added dropwise over 5 min to 60% NaH (220 mg, 5.5 mmol) under N_2 at 0°C. The resulting mixture was stirred for 2 h at rt. MeOH (1.5 mL) was added and the mixture was diluted with toluene, washed with water, dried filtered and concentrated. FC (toluene/EtOAc 20:1) gave **15** (1.62 g, 3.03 mmol 97%) as a colorless syrup. R_f=0.42 (toluene/EtOAc 12:1); $[\alpha]_D$=+76 (*c* 1.1, $CHCl_3$); IR ν_{max} (cm^{-1}) 1097, 1454, 2869; NMR: ^1H (250 MHz, $CDCl_3$), δ 1.23 (3H, t, *J*=7.3 Hz), 2.46–2.69 (2H, m), 3.66 (1H, dd, *J*=11.0, 1.6 Hz), 3.75 (1H, dd, *J*=11.0, 4.8 Hz), 3.79–3.85 (2H, m), 3.93–4.13 (4H, m), 4.56–4.76 (5H, m), 4.93 (1H, d, *J*=11.0 Hz), 5.14 (1H, dd, *J*=10.2, 1.1 Hz), 5.25 (1H, dd, *J*=17.2, 1.5 Hz), 5.39 (1H, s), 5.84–5.99 (1H, m), 7.24–7.40 (15H, m); ^{13}C (62.8 MHz, $CDCl_3$), δ 15.0 (CH_3), 25.3 (CH_2), 69.2 (CH_2), 71.8 (CH), 71.9 (CH_2), 72.0 (CH_2), 72.3 (CH_2), 75.1 (CH), 75.2 (CH_2), 76.3 (CH), 80.3 (CH), 81.9 (CH), 116.8 (CH_2=CH), 127.6–128.3 (Ph), 134.9 (CH$_2$–*C*H), 138.1 (Ph), 138.3 (Ph), 138.7 (Ph);

Anal. Calcd for $C_{32}H_{38}O_5S$: C, 71.9; H, 7.2 Found: C, 71.8; H, 7.1.

3.1.6. Ethyl 6-*O*-allyl-2,3,4-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (17). 16[16] (1.23 g, 2.49 mmol) and allyl bromide (0.32 mL, 3.8 mmol) dissolved in DMF (20 mL) was added dropwise over 5 min to 60% NaH (160 mg, 4.0 mmol) under N_2 at 0°C. The resulting mixture was stirred for 4 h at rt. MeOH (0.5 mL) was added and the mixture was diluted with toluene, washed with water, dried filtered and concentrated. FC (toluene/EtOAc 20:1) gave **17** (1.31 g, 2.45 mmol, 98%) as white needles. R_f=0.73 (toluene/EtOAc 4:1); mp 65–66°C (from hexane); $[\alpha]_D$=−3.2 (*c* 1.2, $CHCl_3$); IR ν_{max} (cm^{-1}) 1094, 1360, 1452, 2920; NMR: ^1H (250 MHz, $CDCl_3$), δ 1.30 (3H, t, *J*=7.5 Hz), 2.66–2.82 (2H, m), 3.50–3.60 (4H, m), 3.83 (1H, t, *J*=9.5 Hz), 3.84–3.96 (3H, m), 4.43 (1H, d, *J*=9.9 Hz), 4.66 (1H, d, *J*=11.7 Hz), 4.73 (2H, s), 4.79 (1H, d, *J*=10.2 Hz), 4.88 (1H, d, *J*=10.2 Hz), 4.97 (1H, d, *J*=11.7 Hz), 5.14–5.27 (2H, m), 5.76–5.92 (1H, m), 7.25–7.41 (15H, m); ^{13}C (62.8 MHz, $CDCl_3$), δ 15.1 (CH_3),24.8 (CH_2), 68.7 (CH_2), 72.3 (CH_2), 72.8 (CH_2), 73.6 (CH), 74.4 (CH_2), 75.8 (CH_2), 77.3 (CH), 78.5 (CH), 84.1 (CH), 85.3 (CH), 117.4 (*C*H_2=CH), 127.5–128.5 (Ph), 134.4 (CH_2=*C*H), 138.3 (Ph), 138.4 (Ph), 138.8 (Ph); Anal. Calcd for $C_{32}H_{38}O_5S$: C, 71.9; H, 7.2 Found: C, 71.8; H 7.3.

3.1.7. 6-*O*-[4-*O*-(6-*O*-Allyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl]-3,4,5-tri-*O*-benzyl-1,2-*O*-(L-1,7,7-trimethyl-[2,2,1]bicyclohept-6-ylidene)-D-*myo*-inositol (19). To a stirred mixture of **18**[14] (216 mg, 0.227 mmol), **15** (172 mg, 0.322 mmol) and 4 Å molecular sieves in diethyl ether (20 mL) under argon was added DMTST (230 mg, 0.89 mmol). After 4 h, NEt_3 (0.3 mL) was added and after an additional 15 min, the mixture was filtered through Celite and concentrated. FC (toluene/EtOAc 25:1) gave **19** (242 mg, 0.170 mmol, 75%) as a colorless syrup. R_f=0.47 (toluene/EtOAc 12:1); $[\alpha]_D$=+47 (*c* 1.1, $CHCl_3$); IR ν_{max} (cm^{-1}) 1028, 1108, 1454, 2104, 2933; NMR: ^1H (250 MHz, $CDCl_3$), δ 0.83 (3H, s), 0.87 (3H, s), 1.06 (3H, s), 1.15–1.96 (7H, m), 3.35 (1H, dd, *J*=9.7, 3.5 Hz), 3.38–4.77 (33H, m), 4.88 (1H, d, *J*=11.3 Hz), 4.91 (1H, d, *J*=11.0 Hz), 5.06–5.26 (3H, m), 5.62 (1H, d, *J*=3.3 Hz), 5.77–5.92 (1H, m), 7.02–7.36 (40H, m); ^{13}C (62.8 MHz, $CDCl_3$), δ 9.7 (CH_3), 20.4 (CH_3), 20.6 (CH_3), 27.0 (CH_2), 29.8 (CH_2), 44.7 (CH_2), 45.1 (CH), 47.9 (C_q), 51.6 (C_q), 63.1 (CH), 68.4 (CH_2), 69.2 (CH_2), 70.1 (CH), 72.1 (CH_2), 72.2 (CH_2), 72.3 (CH_2), 72.5 (CH_2), 72.9 (CH), 73.0 (CH_2), 73.8 (CH), 73.9 (CH_2), 74.7 (CH_2), 74.8 (CH), 74.9 (CH_2), 75.0 (CH_2), 76.0 (CH), 76.3 (CH), 76.8 (CH), 77.6 (CH), 78.0 (CH), 79.6 (CH), 79.8 (CH), 80.5 (CH), 80.9 (CH), 95.4 (CH, J_{CH}=174 Hz), 100.9 (CH, J_{CH}=174 Hz), 116.6 (*C*H_2=CH), 118.1 (OCO), 126.9–128.5 (Ph), 134.9 (CH_2=*C*H), 137.8–138.6 (Ph); Anal. Calcd for $C_{87}H_{97}N_3O_{15}$: C, 73.3; H, 6.9 Found: C, 73.5; H 6.9.

3.1.8. 6-*O*-[4-*O*-(6-*O*-Allyl-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl]-3,4,5-tri-*O*-benzyl-1,2-*O*-(L-1,7,7-trimethyl[2,2,1]bicyclohept-6-ylidene)-D-*myo*-inositol (20). To a stirred mixture of **18** (195 mg, 0.205 mmol), **17** (200 mg, 0.374 mmol) and 4 Å molecular sieves in diethyl ether (10 mL) under argon was added DMTST (233 mg, 0.90 mmol). After 5 h, NEt_3 (0.3 mL) was added and after an additional 15 min, the mixture was filtered through Celite and concentrated. FC (toluene/EtOAc 25:1) gave **20** (172 mg, 0.121 mmol, 59%) as a colorless syrup. R_f=0.47 (toluene/EtOAc 12:1); $[\alpha]_D$=+72 (*c* 1.0, $CHCl_3$); IR ν_{max} (cm^{-1}) 1038, 1096, 1454, 2105, 2931; NMR: ^1H (250 MHz, $CDCl_3$), δ 0.83 (s, 3H), 0.86 (3H, s), 1.06 (3H, s), 1.15–1.96 (7H, m), 3.31–4.94 (36H, m), 5.11 (1H, dd, *J*=10.2, 1.8 Hz), 5.19 (1H, dd, *J*=17.2, 1.5 Hz), 5.58 (1H, d, *J*=3.3 Hz), 5.60 (1H, d, *J*=3.7 Hz), 5.71–5.86 (1H, m), 7.07–7.38 (40H, m); ^{13}C (62.8 MHz, $CDCl_3$), δ 9.7 (CH_3), 20.4 (CH_3), 20.6 (CH_3), 27.0 (CH_2), 29.8 (CH_2), 44.7 (CH_2), 45.1 (CH), 47.9 (C_q), 51.6 (C_q), 63.2 (CH), 68.4 (CH_2), 68.8 (CH_2), 69.5 (CH), 70.3 (CH), 72.1 (CH_2), 72.5 (2C, CH_2), 73.0 (CH_2), 73.4 (CH_2), 73.5 (CH_2), 73.8 (CH), 74.3 (CH), 74.5 (CH), 74.6 (CH_2), 74.7 (CH_2), 74.8 (CH_2), 75.9 (CH), 76.0 (CH), 76.8 (CH), 78.2 (CH), 79.0 (CH), 80.1 (CH), 80.5 (CH), 80.8 (CH), 95.5 (CH), 98.2 (CH), 116.9 (*C*H_2=CH), 118.0 (OCO), 127.2–128.4 (Ph), 134.5 (CH_2=*C*H), 138.2–138.7 (Ph); Anal. Calcd for $C_{87}H_{97}N_3O_{15}$: C, 73.3; H, 6.9 Found: C, 73.2; H, 6.8.

3.1.9. 6-*O*-[4-*O*-(6-*O*-Allyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl]-3,4,5-tri-*O*-benzyl-D-*myo*-inositol (21). To **19** (242 mg, 0.170 mmol) in $CHCl_3$ (20 mL) was added TFA (2 mL). After 16 h, the mixture was washed with aqueous $NaHCO_3$ (sat.), dried, filtered and concentrated. FC (toluene/EtOAc gradient 4:1→2:1) gave **21** (182 mg, 0.141 mmol, 83%) as a colorless syrup. R_f=0.46 (toluene/EtOAc 2:1); $[\alpha]_D$=+34 (*c* 0.8, $CHCl_3$); IR ν_{max} (cm^{-1}) 1046, 1454, 2106, 2867; NMR: ^1H (250 MHz, $CDCl_3$), δ 3.34–5.09 (37H, m), 5.18 (1H, dd, *J*=17.2, 1.5 Hz), 5.24 (1H, d, *J*=1.8 Hz), 5.53 (1H, d, *J*=3.3 Hz), 5.75–5.90 (1H, m), 7.10–7.35 (40H, m); ^{13}C (62.8 MHz, $CDCl_3$), δ 64.2 (CH), 68.6 (CH_2), 69.1 (CH_2), 69.6 (CH), 70.7 (CH), 72.0 (CH_2), 72.2 (CH_2), 72.3 (CH_2), 72.6 (CH_2), 72.7 (CH), 72.9 (CH), 73.0 (CH_2), 74.3 (CH_2), 74.7 (CH), 74.8 (CH_2), 75.2 (CH_2), 75.8 (CH_2), 76.1 (CH), 77.4 (CH), 79.5 (CH), 79.6 (CH), 79.7 (CH), 80.5 (CH), 81.0 (CH), 81.5 (CH), 98.0 (CH, J_{CH}=174 Hz), 100.6 (CH, J_{CH}=170 Hz), 116.6 (*C*H_2=CH), 126.8–129.0 (Ph), 134.9 (CH_2=*C*H), 137.6–139.0 (Ph); Anal. Calcd for $C_{77}H_{83}N_3O_{15}$: C, 71.7; H, 6.5 Found: C, 71.8; H 6.6.

3.1.10. 6-*O*-[4-*O*-(6-*O*-Allyl-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl]-3,4,5-tri-*O*-benzyl-D-*myo*-inositol (22). To **20** (154 mg, 0.108 mmol) in $CHCl_3$ (15 mL) was added TFA (1.5 mL). After 16 h, the mixture was washed with aqueous $NaHCO_3$ (sat.), dried, filtered and concentrated. FC (toluene/EtOAc 2:1) gave **22** (112 mg, 0.0868 mmol, 80%) as a colorless syrup. R_f=0.46 (toluene/EtOAc 2:1); $[\alpha]_D$=+43 (*c* 1.1, $CHCl_3$); IR ν_{max} (cm^{-1}) 1046, 1454, 2108, 2870; NMR: ^1H (250 MHz, $CDCl_3$), δ 3.24–5.32 (38H, m), 5.31 (1H, d, *J*=3.3 Hz), 5.54 (1H, d, *J*=3.7 Hz), 5.67–5.80 (1H, m), 7.18–7.36 (40H, m); ^{13}C (62.8 MHz, $CDCl_3$), δ 64.9 (CH), 68.3 (CH_2), 68.8 (CH_2), 69.2 (CH), 69.8 (CH), 71.4 (CH), 72.1 (CH_2), 72.4 (CH), 72.5 (2C, CH_2), 73.0 (CH_2), 73.7 (CH), 73.9 (CH_2), 74.2 (CH_2), 74.5 (CH), 74.6 (CH_2), 74.8 (CH_2), 75.7 (CH), 75.8 (CH_2), 79.1 (CH), 79.7 (CH), 81.0 (CH), 81.1 (CH), 81.7

(CH), 82.0 (CH), 98.0 (CH), 99.1 (CH), 117.1 (CH_2=CH), 125.3–129.0 (Ph), 134.5 (CH_2=CH), 137.8–138.7 (Ph); Anal. Calcd for $C_{77}H_{83}N_3O_{15}$: C, 71.7; H, 6.5 Found: C, 71.6; H 6.6.

3.1.11. 6-*O*-[4-*O*-(α-D-Mannopyranosyl)-2-amino-2-deoxy-α-D-glucopyranosyl]-D-*myo*-inositol 1,2-cyclic phosphate (3).[19,20] **21** (38 mg, 0.029 mmol) was phosphorylated and deprotected by the same method as for **10** to give **3** (14 mg, 0.025 mmol, 84%) as a white solid. $[\alpha]_D$=+75 (*c* 0.6, H_2O), Lit.[19] $[\alpha]_D$=+57 (*c* 1.0, H_2O); IR ν_{max} (cm^{-1}) 845, 1042, 1099, 1221, 1633, 3375; NMR: ^1H (250 MHz, D_2O, pH*=5.4), δ 3.37–3.44 (2H, m), 3.65–4.21 (14H, m), 4.56 (1H, ddd, *J*=20.1, 8.0, 4.8 Hz), 4.71 (1H, t, *J*=4.0 Hz), 5.28 (1H, d, *J*=1.5 Hz), 5.51 (1H, d, *J*=3.3 Hz); ^{13}C (62.8 MHz, D_2O, pH*=5.4), δ 55.0 (CH), 60.7 (CH_2), 61.6 (CH_2), 67.3 (CH), 70.2 (CH, d, *J*=11.1 Hz), 70.6 (CH), 71.0 (CH), 71.1 (CH), 71.4 (CH), 71.8 (CH), 72.2 (CH), 74.5 (CH), 76.5 (CH), 77.8 (CH), 80.2 (CH), 81.6 (CH), 96.4 (CH), 102.3 (CH); ^{31}P (121 MHz, D_2O, pH*=5.4), δ 16.0 (d, *J*=20.1 Hz); HRMS Calcd for $C_{18}H_{32}NO_{17}P$: $[M-H]^-$ 564.1330 Found: $[M-H]^-$ 564.1367.

3.1.12. 6-*O*-[4-*O*-(α-D-Galactopyranosyl)-2-amino-2-deoxy-α-D-glucopyranosyl]-D-*myo*-inositol 1,2-cyclic phosphate (5). 22 (31 mg, 0.024 mmol) was phosphorylated and deprotected by the same method as for **10** to give **5** (12 mg, 0.021 mmol, 88%) as a white solid. $[\alpha]_D$=+106 (*c* 0.8, H_2O); IR ν_{max} (cm^{-1}) 844, 1033, 1095, 1222, 1634, 3405; NMR: ^1H (250 MHz, D_2O, pH*=5.6), δ 3.38–3.45 (2H, m), 3.65–4.25 (14H, m), 4.57 (1H, ddd, *J*=20.1, 8.0, 4.8 Hz), 4.71 (1H, t, *J*=4.0 Hz), 5.45 (1H, d, *J*=2.6 Hz), 5.52 (1H, d, *J*=3.7 Hz); ^{13}C (62.8 MHz, D_2O, pH*=5.6), δ 54.8 (CH), 60.7 (CH_2), 61.9 (CH_2), 69.3 (CH), 69.9 (CH), 70.0 (CH), 70.3 (CH_2, d, *J*=9.2 Hz), 70.7 (CH), 71.4 (CH), 71.6 (CH), 72.2 (CH), 72.5 (CH), 76.6 (CH), 77.8 (CH), 80.3 (CH), 81.4 (CH), 96.3 (CH), 100.4 (CH); ^{31}P (121 MHz, D_2O, pH*=5.6), δ 16.0 (d, *J*=20.1 Hz); HRMS Calcd for $C_{18}H_{32}NO_{17}P$: $[M-H]^-$ 564.1330 Found: $[M-H]^-$ 564.1266.

3.1.13. 6-*O*-[4-*O*-(6-*O*-{3-(Benzylthio)propyl}-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl]-3,4,5-tri-*O*-benzyl-D-*myo*-inositol (23). 21 (88 mg, 0.068 mmol) was reacted with benzylmercaptane by the same method as for **10** to give **23** (52 mg, 0.037 mmol, 54%) as a colorless syrup. R_f=0.51 (toluene/EtOAc 2:1); $[\alpha]_D$=+39 (*c* 0.86, $CHCl_3$); IR ν_{max} (cm^{-1}) 1048, 1453, 2106, 2867; NMR: ^1H (250 MHz, $CDCl_3$), δ 1.60–1.80 (2H, m), 2.40 (2H, t, *J*=7.1 Hz), 3.29–4.96 (38H, m), 5.24 (1H, d, *J*=1.8 Hz), 5.48 (1H, d, *J*=3.6 Hz), 7.10–7.40 (45H, m); ^{13}C (62.8 MHz, $CDCl_3$), δ 28.1 (CH_2), 29.4 (CH_2), 36.2 (CH_2), 64.4 (CH), 68.6 (CH_2), 69.6 (CH), 69.7 (CH_2), 70.0 (CH_2), 70.9 (CH), 72.1 (CH_2), 72.3 (CH_2), 72.7 (CH_2), 72.8 (CH), 72.9 (CH), 73.2 (CH_2), 74.5 (CH_2), 74.7 (CH), 74.9 (CH_2), 75.1 (CH_2), 75.8 (CH_2), 76.1 (CH), 77.2 (CH), 79.6 (CH), 79.8 (CH), 80.1 (CH), 80.6 (CH), 81.0 (CH), 81.6 (CH), 98.3 (CH), 100.5 (CH), 126.9–129.0 (Ph), 137.6–138.9 (Ph); HRMS Calcd for $C_{84}H_{91}NO_{15}S$: $[M+Na]^+$ 1436.6069 Found: $[M+Na]^+$ 1436.6083.

3.1.14. 6-*O*-[4-*O*-(6-*O*-{3-(Benzylthio)propyl}-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl]-3,4,5-tri-*O*-benzyl-D-*myo*-inositol (24). 22 (116 mg, 0.090 mmol) was reacted with benzylmercaptane by the same method as for **10** to give **24** (80 mg, 0.057 mmol, 63%) as a colorless syrup. R_f=0.46 (toluene/EtOAc 2:1); $[\alpha]_D$=+37 (*c* 1.1, $CHCl_3$); IR ν_{max} (cm^{-1}) 1046, 1453, 1495, 2107, 2870, 2917; NMR: ^1H (250 MHz, $CDCl_3$), δ 1.63–1.68 (2H, m), 2.30–2.39 (2H, m), 3.14–5.00 (38H, m), 5.31 (1H, d, *J*=3.7 Hz), 5.54 (1H, d, *J*=3.7 Hz), 7.17–7.34 (45H, m); ^{13}C (62.8 MHz, $CDCl_3$), δ 28.0 (CH_2), 29.3 (CH_2), 36.3 (CH_2), 65.0 (CH), 68.7 (CH_2), 68.9 (CH_2), 69.2 (CH), 69.6 (CH_2), 69.7 (CH), 71.5 (CH), 72.3 (CH), 72.5 (CH_2), 72.6 (CH_2), 73.0 (CH_2), 73.5 (CH), 74.0 (CH_2), 74.3 (CH_2), 74.5 (2C, CH, CH_2), 74.8 (CH_2), 75.7 (CH) 75.9 (CH_2), 79.1 (CH), 79.8 (CH), 81.1 (CH), 81.2 (CH), 81.7 (CH), 82.3 (CH), 98.0 (CH), 99.2 (CH), 126.9–129.0 (Ph), 137.8–138.5 (Ph); HRMS Calcd for $C_{84}H_{91}N_3O_{15}S$: $[M+Na]^+$ 1436.6069 Found: $[M+Na]^+$ 1436.6064.

3.1.15. 6-*O*-[4-*O*-(6-*O*-{3-Mercaptopropyl}-α-D-mannopyranosyl)-2-amino-2-deoxy-α-D-glucopyranosyl]-D-*myo*-inositol 1,2-cyclic phosphate (4). 23 (37 mg, 0.026 mmol) was phosphorylated and deprotected by the same method as for **10** to give **4** (15 mg, 0.023 mmol, 90%) as a white solid. $[\alpha]_D$=+66 (*c* 0.7, H_2O); IR ν_{max} (cm^{-1}) 845, 1039, 1102, 1222, 1635, 3225; NMR: ^1H (250 MHz, D_2O, pH*=4.7), δ 1.85–1.97 (2H, m), 2.62 (2H, t, *J*=7.1 Hz), 3.37–3.44 (2H, m), 3.61–4.14 (16H, m), 4.56 (1H, ddd, *J*=20.1, 8.0, 4.8 Hz), 4.71 (1H, t, *J*=4.0 Hz), 5.26 (1H, s) 5.51 (1H, d, *J*=3.7 Hz); ^{13}C (62.8 MHz, D_2O, pH*=4.7), δ 21.2 (CH_2), 33.4 (CH_2), 55.0 (CH), 60.8 (CH_2), 67.4 (CH), 70.2 (CH, d, *J*=9 Hz), 70.3 (2C, CH_2), 70.6 (CH), 70.9 (CH), 71.0 (CH), 71.3 (CH), 71.9 (CH), 72.2 (CH), 73.3 (CH), 76.6 (CH), 77.8 (CH), 80.2 (CH), 81.5 (CH), 96.3 (CH), 102.3 (CH); ^{31}P (121 MHz, D_2O, pH*=4.7), δ 16.2 (d, *J*=20.1 Hz); HRMS Calcd for $C_{21}H_{38}NO_{17}PS$: $[M-H]^-$ 638.1520 Found: $[M-H]^-$ 638.1493.

3.1.16. 6-*O*-[4-*O*-(6-*O*-{3-Mercaptopropyl}-α-D-galactopyranosyl)-2-amino-2-deoxy-α-D-glucopyranosyl]-D-*myo*-inositol 1,2-cyclic phosphate (6). 24 (39 mg, 0.028 mmol) was phosphorylated and deprotected by the same method as for **10** to give **6** (15 mg, 0.024 mmol, 85%) as a white solid. $[\alpha]_D$=+76 (*c* 0.6, H_2O); IR ν_{max} (cm^{-1}) 845, 1036, 1102, 1224, 1634, 3225; NMR: ^1H (250 MHz, D_2O, pH*=4.1), δ 1.84–1.94 (2H, m), 2.61 (2H, t, *J*=7.1 Hz), 3.38–3.45 (2H, m), 3.60–4.24 (16H, m), 4.57 (1H, ddd, *J*=20.1, 8.0, 4.8 Hz), 4.71 (1H, t, *J*=4.0 Hz), 5.43 (1H, d, *J*=2.2 Hz), 5.52 (1H, d, *J*=3.7 Hz); ^{13}C (62.8 MHz, D_2O, pH*=4.1), δ 21.1 (CH_2), 33.3 (CH_2), 54.8 (CH), 60.8 (CH_2), 69.2 (CH), 69.9 (CH), 70.2 (CH), 70.2 (CH, d, *J*=9 Hz), 70.3 (CH_2), 70.6 (CH), 70.7 (CH_2), 70.9 (CH), 71.3 (CH), 71.7 (CH), 72.1 (CH), 76.9 (CH), 77.8 (CH), 80.2 (CH), 81.5 (CH), 96.3 (CH), 100.5 (CH); ^{31}P (121 MHz, D_2O, pH*=4.1), δ 16.2 (d, *J*=20.1 Hz); HRMS Calcd for $C_{21}H_{38}NO_{17}PS$: $[M-H]^-$ 638.1520 Found: $[M-H]^-$ 638.1613.

3.1.17. Ethyl 2,3,-di-*O*-acetyl-4,6-*O*-prop-2-enylidene-1-thio-β-D-galactopyranoside (26). A solution of **25** (1.50 g, 6.70 mmol), acrolein dimethylacetal (1.50 mL,

12.7 mmol) and $TsOH \cdot H_2O$ (100 mg) in DMF (5 mL), was stirred at 50°C. After 1.5 h, pyridine (10 mL) was added and the mixture allowed to attain rt. Acetic anhydride (5 mL) was added and after an additional 3 h, the mixture was diluted with CH_2Cl_2 and washed with H_2O, 1 M HCl, $NaHCO_3$ and H_2O, dried filtered and concentrated. FC (toluene/EtOAc 4:1) gave **26** (1.64 g, 4.73 mmol, 71%) as colorless needles. R_f=0.30 (toluene/EtOAc 4:1); mp 139–140°C (from ethanol); $[\alpha]_D$=−5.0 (*c* 1.4, $CHCl_3$); IR ν_{max} (cm^{-1}) 826, 927, 955, 991, 1057, 1093, 1171, 1223, 1243, 1376, 1742, 2985; NMR: ^1H (250 MHz, $CDCl_3$), δ 1.28 (3H, t, *J*=7.5 Hz), 2.07 (3H, s), 2.09 (3H, s), 2.63–2.91 (2H, m), 3.48 (1H, d, *J*=1.1 Hz), 3.87 (1H, dd, *J*=12.6, 1.6 Hz), 4.15–4.31 (2H, m), 4.43 (1H, d, *J*=9.9 Hz), 4.91–4.96 (2H, m) 5.33 (1H, d, *J*=10.6 Hz), 5.43 (1H, t, *J*=10.1 Hz), 5.48 (1H, d, *J*=17.5 Hz), 5.91 (1H, ddd, *J*=17.5, 10.6, 4.7 Hz); ^{13}C (62.8 MHz, $CDCl_3$), δ 14.8 (CH_3), 20.9 (2C, CH_3), 23.1 (CH_2), 66.7 (CH), 68.7 (CH_2), 69.8 (CH), 73.0 (CH), 73.2 (CH), 82.9 (CH), 100.5 (OCO), 119.3 (CH_2=CH), 133.9 (CH_2=CH), 169.5 (CH_3CO), 170.7 (CH_3CO); Anal. Calcd for $C_{15}H_{22}O_7S$: C, 52.0; H, 6.4 Found: C, 52.0; H 6.5.

3.1.18. Ethyl 6-*O*-allyl-2,3,4-tri-*O*-acetyl-1-thio-β-D-galactopyranoside (27). To a stirred mixture of **26** (268 mg, 0.774 mmol) and $NaBH_3CN$ (495 mg, 7.9 mmol) in THF (10 mL) ether saturated with HCl was added dropwise until all starting material was consumed. The mixture was neutralized with NEt_3 (2 mL) and passed through a short column of silica eluted with toluene/EtOAc 1:1. The crude mixture was concentrated and dissolved in pyridine (5 mL). Ac_2O (2 mL) was added to the stirred mixture. After 3 h, the mixture was concentrated. Purification by FC (petroleum ether 65–75/EtOAc 6:1) gave **27** (193 mg, 0.494 mmol, 64%) as a colorless syrup. R_f=0.34 (toluene/EtOAc 4:1); $[\alpha]_D$=−17 (*c* 1.0, $CHCl_3$); IR ν_{max} (cm^{-1}) 1222, 1749; NMR: ^1H (250 MHz, $CDCl_3$), δ 1.28 (3H, t, *J*=7.5 Hz), 1.98 (3H, s), 2.07 (3H, s), 2.15 (3H, s), 2.63–2.84 (2H, m), 3.4 (1H, dd, *J*=9.7, 6.4 Hz), 3.56 (1H, dd, *J*=9.8, 6.2 Hz), 3.86 (1H, dt, *J*=6.2, 0.7 Hz), 3.90 (1H, dddd, *J*=12.8, 5.8, 1.3, 1.3 Hz) 3.99 (1H, dddd, *J*=12.8, 6.5, 1.3, 1.3 Hz), 4.50 (1H, d, *J*=9.9 Hz), 5.06 (1H, dd, *J*=9.9, 3.3 Hz), 5.15–5.28 (3H, m), 5.49 (1H, d, *J*=2.6 Hz), 5.75–5.91 (1H, m); ^{13}C (62.8 MHz, $CDCl_3$), δ 14.9 (CH_3), 20.6 (CH_3), 20.7 (CH_3), 20.8 (CH_3), 24.4 (CH_2), 67.5 (CH), 67.8 (CH_2), 68.0 (CH), 72.2 (CH), 72.4 (CH_2), 76.0 (CH), 84.0 (CH), 117.6 (CH_2=CH), 134.2 (CH_2=CH), 169.6 (CH_3CO), 170.1 (CH_3CO), 170.2 (CH_3CO); Anal. Calcd for $C_{17}H_{26}O_8S$: C, 52.3; H, 6.7 Found: C, 52.4; H 6.9.

3.1.19. 6-*O*-[4-*O*-(6-*O*-Allyl-2,3,4-tri-*O*-acetyl-β-D-galactopyranosyl)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl]-3,4,5-tri-*O*-benzyl-1,2-*O*-(L-1,7,7-trimethyl-[2,2,1]bicyclohept-6-ylidene)-D-*myo*-inositol (28). To a stirred mixture of **27** (160 mg, 0.410 mmol), **18** (224 mg, 0.235 mmol) and 4 Å powdered molecular sieves in CH_2Cl_2/ether 1:1 (5 mL) at 0°C under argon was added NIS (98 mg, 0.44 mmol) and TfOH (0.20 mL, 1% in CH_2Cl_2). After 20 min, the mixture was diluted with CH_2Cl_2, filtered through celite, washed with $NaHCO_3$ (sat.) and 1 M NaS_2O_3, dried, filtered and concentrated. FC (toluene/EtOAc gradient 12:1→6:1) gave **28** (234 mg, 0.183 mmol, 78%) as a colorless syrup. R_f=0.57 (toluene/EtOAc 4:1); $[\alpha]_D$=+36 (*c* 0.8, $CHCl_3$); IR ν_{max} (cm^{-1}) 1057, 1220, 1753, 2107, 2936; NMR: ^1H (250 MHz, $CDCl_3$), δ 0.88 (6H, s), 1.06 (3H, s), 1.16–2.0 (7H, m), 1.72 (3H, s), 1.95 (3H, s), 2.11 (3H, s), 3.19–3.28 (2H, m), 3.32 (1H, dd, *J*=10.2, 3.7 Hz), 3.44–4.10 (13H, m), 4.30 (1H, m), 4.37 (1H, d, *J*=12.1 Hz), 4.50 (1H, d, *J*=8.1 Hz), 4.54 (1H, d, *J*=11.3 Hz), 4.57–4.80 (8H, m), 5.04–5.20 (4H, m), 5.34 (1H, d, *J*=2.9 Hz), 5.54 (1H, d, *J*=3.7 Hz), 5.66–5.81 (1H, m), 7.17–7.46 (25H, m); ^{13}C (62.8 MHz, $CDCl_3$), δ 9.9 (CH_3), 20.4 (CH_3), 20.5 (CH_3), 20.6 (CH_3), 20.7 (CH_3), 21.4 (CH_3), 27.0 (CH_2), 29.9 (CH_2), 44.4 (CH_2), 45.2 (CH), 47.9 (C_q), 51.6 (C_q), 63.0 (CH), 66.9 (CH_2), 67,3 (CH_2), 67.6 (CH), 69.8 (CH), 70.4 (CH), 71.4 (CH), 72.0 (CH), 72.3 (CH_2), 72.7 (CH_2), 73.7 (2C, CH, CH_2), 73.8 (CH_2), 74.1 (CH_2), 74.8 (CH_2), 75.8 (CH), 76.4 (2C, CH), 77.4 (CH), 78.6 (CH), 80.4 (CH), 80.5 (CH), 96.0 (CH), 100.0 (CH), 117.2 (CH_2=CH), 117.9(OCO), 127.4–129.0 (Ph), 134.3 (CH_2=CH), 137.6 (Ph), 137.8 (Ph), 138.2 (Ph), 138.3 (Ph), 138.4 (Ph), 138.7 (Ph), 169.1 (CH_3CO), 169.9 (CH_3CO), 170.0 (CH_3CO); Anal. Calcd for $C_{72}H_{85}N_3O_{18}$: C, 67.5; H, 6.7 Found: C, 67.3; H 6.9.

3.1.20. 6-*O*-[4-*O*-(6-*O*-Allyl-2,3,4-tri-*O*-acetyl-β-D-galactopyranosyl)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl]-3,4,5-tri-*O*-benzyl-D-*myo*-inositol (29). To **28** (160 mg, 0.125 mmol) in $CHCl_3$ (15 mL) was added TFA (1.5 mL). After 16 h, the mixture was diluted with $CHCl_3$, washed with aqueous $NaHCO_3$ (sat.), dried, filtered and concentrated. FC (toluene/EtOAc 2:1) gave **29** (124 mg, 0.108 mmol, 86%) as a colorless syrup. R_f=0.28 (toluene/EtOAc 2:1); $[\alpha]_D$=+11 (*c* 1.4, $CHCl_3$); IR ν_{max} (cm^{-1}) 1056, 1220, 1750, 2116, 2871; NMR: ^1H (250 MHz, $CDCl_3$), δ 1.77 (3H, s), 1.94 (3H, s), 2.09 (3H, s), 3.03–4.08 (17H, m), 4.23 (1H, br), 4.29 (1H, d, *J*=8.0 Hz), 4.55 (1H, d, *J*=11.8 Hz), 4.64–4.73 (6H, m), 4.89 (1H, d, *J*=10.6 Hz), 4.98–5.21 (m, 6H) 5.79 (1H, d, *J*=2.9 Hz), 5.65–5.80 (1H, m) 7.14–7.44 (25H, m); ^{13}C (62.8 MHz, $CDCl_3$), δ 20.6 (CH_3), 20.7 (2C, CH_3), 64.2 (CH), 66.6 (CH_2), 66.8 (CH_2), 67.4 (CH), 69.0 (CH), 69.7 (CH), 71.1 (CH), 71.2 (CH), 71.9 (CH), 72.2 (CH_2), 72.3 (CH), 72.5 (CH_2), 73.5 (CH_2), 73.6 (CH_2), 75.2 (CH_2), 75.7 (CH), 75.9 (CH_2), 78.8 (CH), 79.8 (CH), 80.6 (CH), 81.6 (CH), 83.1 (CH), 99.6 (CH), 99.8 (CH), 117.3 (CH_2=CH), 126.5 (Ph), 127.3–128.6 (Ph), 134.3 (CH_2=CH), 137.3 (Ph), 137.8 (Ph), 138.3 (2C, Ph), 138.8 (Ph), 169.2 (CH_3CO), 169.9 (CH_3CO), 170.0 (CH_3CO); Anal. Calcd for $C_{62}H_{71}N_3O_{18}$: C, 65.0; H, 6.2 Found: C, 64.8; H 6.2.

3.1.21. 6-*O*-[4-*O*-(β-D-Galactopyranosyl)-2-amino-2-deoxy-α-D-glucopyranosyl]-D-*myo*-inositol 1,2-cyclic phosphate (7). 29 (25 mg, 0.020 mmol) was phosphorylated by the same method as for **10** to give **30**. The product was dissolved in MeOH/NH_3 (sat., 2 mL). After 6 h, the mixture was concentrated and dissolved in THF (2 mL). NH_3 (l) (~20 mL) was condensed into the flask and a minimum amount of sodium for the mixture to turn deep blue was added to the stirred mixture at −33°C. After 1 min, NH_4Cl was added until the color disappeared. The mixture was concentrated under a stream of argon and the residue was dissolved in H_2O (10 mL), washed with ether (10 mL) and concentrated. Gel filtration of the residue on a

Pharmacia Sephadex G-15 column eluted with H_2O containing 1% *n*-butanol afforded **7** (11 mg, 0.020 mmol, quant.) as a white solid. $[\alpha]_D$=+49 (*c* 0.42, H_2O); IR ν_{max} (cm^{-1}) 848, 1038, 1095, 1223, 1634, 3188; NMR: ^1H (250 MHz, D_2O, pH^*=5.2), δ 3.38–4.16 (16H, m), 4.48 (1H, d, *J*=7.7 Hz) 4.56 (1H, ddd, *J*=20.1, 8.0, 4.8 Hz), 4.71 (1H, t, *J*=4.0 Hz), 5.51 (1H, d, *J*=3.7 Hz); ^{13}C (62.8 MHz, D_2O, pH^*=5.2), δ 54.7 (CH), 60.1 (CH_2), 61.9 (CH_2), 69.0 (CH), 69.3 (CH), 70.2 (CH, d, *J*=10.1 Hz), 71.3 (CH), 71.7 (2C, CH), 72.1 (CH), 73.3 (CH), 76.2 (CH), 77.8 (CH), 78.4 (CH), 80.2 (CH), 81.5 (CH), 96.2 (CH), 103.8 (CH); ^{31}P (121 MHz, D_2O, pH^*=5.2), δ 16.0 (d, *J*=20.1 Hz); HRMS Calcd for $C_{18}H_{32}NO_{17}P$: $[M-H]^-$ 564.1330 Found: $[M-H]^-$ 564.1282.

3.1.22. 6-*O*-[4-*O*-(6-*O*-{3-(Benzylthio)propyl}-2,3,4-tri-*O*-acetyl-β-D-galactopyranosyl)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl]-3,4,5-tri-*O*-benzyl-D-*myo*-inositol (31). 29 (69 mg, 0.060 mmol) was reacted with benzylmercaptane by the same method as for **10** to give **31** (48 mg, 0.038 mmol, 63%) as a colorless syrup. R_f=0.28 (toluene/EtOAc 2:1); $[\alpha]_D$=+11 (*c* 1.1, $CHCl_3$); IR ν_{max} (cm^{-1}) 1055, 1221, 1751, 2114, 2917; NMR: ^1H (250 MHz, $CDCl_3$), δ 1.54 (2H, m), 1.77 (3H, s), 1.94 (3H, s), 2.10 (3H, s), 2.34–2.40 (2H, m), 3.02–5.10 (32H, m), 5.19 (1H, d, *J*=3.7 Hz), 5.28 (1H, d, *J*=3.3 Hz), 7.14–7.43 (30H, m); ^{13}C (62.8 MHz, $CDCl_3$), δ 20.6 (3C, CH_3), 27.9 (CH_2), 29.0 (CH_2), 36.2 (CH_2), 64.3 (CH), 66.7 (CH_2), 67.4 (CH_2), 67.5 (CH), 69.0 (CH), 69.8 (2C, CH, CH_2), 71.1 (CH), 71.3 (CH), 71.8 (CH), 72.3 (CH), 72.6 (CH_2), 73.5 (CH_2), 73.7 (CH_2), 75.2 (CH_2), 75.8 (CH), 75.9 (CH_2), 78.8 (CH), 79.8 (CH), 80.6 (CH), 81.7 (CH), 83.2 (CH), 99.7 (CH), 99.8 (CH), 125.3 (Ph), 126.5–129.1 (Ph), 137.4–138.9 (Ph), 169.3 (CH_3CO), 169.9 (2C, CH_3CO); HRMS Calcd for $C_{69}H_{79}N_3O_{18}S$: $[M+Na]^+$ 1292.4977 Found: $[M+Na]^+$ 1292.4998.

3.1.23. 6-*O*-[4-*O*-(6-*O*-{3-Mercaptopropyl}-β-D-galactopyranosyl)-2-amino-2-deoxy-α-D-glucopyranosyl]-D-*myo*-inositol 1,2-cyclic phosphate (8). 31 (31 mg, 0.024 mmol) was phosphorylated and deprotected by the same method as for **29** to give **8** (14 mg, 0.022 mmol, 90%) as a white solid. $[\alpha]_D$=+40 (*c* 0.83, H_2O); IR ν_{max} (cm^{-1}) 845, 1039, 1097, 1221, 1634, 3152; NMR: ^1H (250 MHz, D_2O, pH^*=4.7), δ 1.84–1.94 (2H, m), 2.62 (2H, t, *J*=7.1 Hz), 3.38–3.45 (2H, m), 3.51–4.16 (16H, m), 4.48 (1H, d, *J*=7.7 Hz), 4.57 (1H, ddd, *J*=20.1, 8.0, 4.7 Hz), 4.71 (1H, t, *J*=4.0 Hz), 5.52 (1H, d, *J*=3.7 Hz); ^{13}C (62.8 MHz, D_2O, pH^*=4.7), δ 21.1 (CH_2), 33.2 (CH_2), 54.8 (CH), 60.1 (CH_2), 69.0 (CH), 69.5 (CH), 69.8 (CH_2), 70.2 (CH, d, *J*=9.2 Hz), 70.4 (CH_2), 71.3 (CH), 71.6 (CH), 71.7 (CH), 72.2 (CH), 73.2 (CH), 73.9 (CH), 77.8 (CH), 78.7 (CH), 80.2 (CH), 81.5 (CH), 96.2 (CH), 103.8 (CH); ^{31}P (121 MHz, D_2O, pH^*=4.7), δ 16.2 (d, *J*=20.1 Hz); HRMS Calcd for $C_{21}H_{38}NO_{17}PS$: $[M-H]^-$ 638.1520 Found: $[M-H]^-$ 638.1614.

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