Synthesis of a polyphosphorylated GPI-anchor core structure

Martina Lahmann, Per J. Garegg, Peter Konradsson, and Stefan Oscarson

Abstract: Using a linear assembly approach a highly differentially protected derivative of the common GPI-anchor core structure (α-D-Man-(1→6)-α-D-Man-(1→2)-α-D-Man-(1→4)-α-D-GlcNH$_2$-(1→6)-D-*myo*-inositol) has been synthesized. All mannose donors were prepared from a common thioglycoside precursor (**1**), and coupled to GlcN$_3$-*myo*-inositol acceptor **5** in a linear five-step glycosylation–deprotection sequence in 49% overall yield, to give the key intermediate **10**, with orthogonal temporary protecting groups at the 6″, 2″, 6′, and 2 positions of the trimannoside motif and at the 1 and 2 positions of the inositol part. Consecutive removal of the temporary protecting groups in the trimannoside moiety followed by phosphorylation, gave a tetraphosphate derivative in 60% overall yield. Removal of a camphor acetal afforded a 1,2-inositol diol, which was converted to a 1,2-cyclic phosphate using commercial methyl dichlorophosphate (→**17**, 95%). One-step deprotection using sodium in liquid ammonia afforded the target polyphosphorylated core structure **18** (60%), which will be tested for metabolic insulin action.

Key words: glycophosphatidylinositols, linear synthesis, glycosylations, inositolphosphoglycans, IPG.

Résumé : Faisant appel à une approche d'assemblage linéaire, on a réalisé la synthèse d'un dérivé, portant des groupes protecteurs fortement diversifiés, de la structure ancrée fondamentale du GPI, le α-D-Man-(1→6)-α-D-Man-(1→2)-α-D-Man-(1→4)-GlcNH$_2$-(1→6)-D-*myo*-inositol. Tous les groupes mannoses donneurs ont été préparés à partir d'un thioglycoside précurseur commun, **1**, et ils ont été couplés à un GlcN$_3$-*myo*-inositol accepteur, **5**, dans une séquence en cinq étapes de glycosylation-déprotection, avec un rendement global de 49%, pour conduire à l'intermédiaire clé **10** portant des groupes protecteurs orthogonaux temporaires dans les positions 6″, 2″, 6′ et 2 du motif trimannoside et dans les positions 1 et 2 de la portion inositol. Les éliminations consécutives des groupes protecteurs temporaires de la portion mannoside, suivies d'une phosphorylation, on conduit au dérivé tétraphosphate, avec un rendement global de 60%, à partir duquel on a enlevé un acétal du camphre pour obtenir un diol 1,2-inositol qui a été transformé en un phosphate 1,2-cyclique à l'aide de dichlorophosphate de méthyle commercial (→**17**, 95%). Une déprotection en une étape à l'aide de sodium dans l'ammoniac liquide fournit le structure fondamentale phosphorylée recherchée, **18**, avec un rendement de 60% qui a été évaluée pour son action d'insuline métabolique.

Mots clés : glycophosphatidylinositols, synthèse linéaire, glycosylations, inositolphosphoglycanes, IPG.

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Introduction

Ever since the discovery of a new way to anchor proteins to cell membranes through glycosylphosphatidylinositol structures (GPI-anchors, see refs. 1–3), these anchor structures have been of a main research interest, both analytically, synthetically, and biologically. Isolation and analysis have shown that these structures are present in almost all species, and that their structure is not only partly conserved but also species specific (2, 3). All GPI-anchors found and characterized so far contain a common pseudo-pentasaccharide backbone, α-D-Man-(1→6)-α-D-Man-(1→2)-α-D-Man-(1→4)-α-D-GlcNH$_2$-(1→6)-D-*myo*-inositol, but the substitution pattern varies between species (Fig. 1). Apart from functioning as protein anchors, a number of functions have been suggested for the GPI-structures (2, 3), such as they have been implicated as second messengers to insulin (4–6) and polyphosphorylated derivatives have been shown to give an insulin-type effect and have been considered as drug candidates towards diabetes (7).

The novelty and complexity of these structures in combination with the interest to investigate more in detail their biological functions have resulted in a number of syntheses of parts of the backbone, the complete backbone, and also the backbone with various substituents (8–10). The latter syntheses have generally been directed towards a species specific target molecule not allowing too much variation in the substitution pattern. Recently, Ley and co-workers (10) presented a synthesis of the backbone with orthogonal temporary protecting groups at relevant positions allowing the continued synthesis of a variant of species specific GPI-anchor structures. Another feature of their synthesis is that the orthogonal and one-pot glycosylations are used in the

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Dedicated to the memory of Professor Raymond U. Lemieux.

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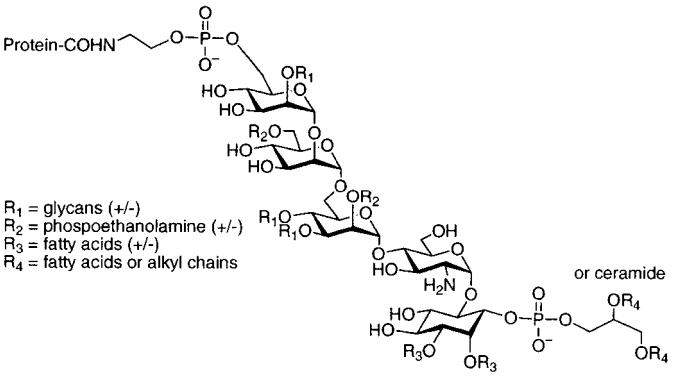
Fig. 1. Schematic structure of GPI anchors.



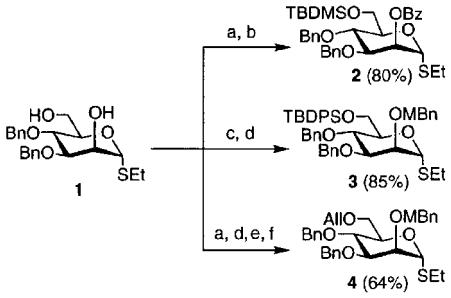
build-up of the target structures, utilizing the different reactivity of both the uniquely protected donors and the variety of donors. Although conceptually elegant, such an approach requires considerable fine-tuning of the reactivity to be successful. We herein describe a synthesis, using a linear synthetic approach, of a similar highly and differentially protected backbone structure that, will allow for the syntheses of most types of species-specific structures from the same intermediate. Furthermore, the temporary protecting groups were sequentially removed and the resulting hydroxyl derivatives were phosphorylated and deprotected to give a polyphosphorylated structure, which will be investigated for insulin activity.

Results and discussions

The linear approach can have considerable advantages, especially since the target structure is a rather small molecule containing no repeating units (at least no larger than a monosaccharide). Only monosaccharide donors have to be prepared and all mannose donors can be prepared from a common precursor. The protecting-group strategy can be planned to be optimal (selective and complete) in terms of protection and deprotection, since almost no considerations have to be taken to the reactivity or stereochemical influence of the protecting-group pattern in the various mannose donors. In the coupling reactions, self condensation and transglycosylation reactions, often found side reactions in orthogonal glycosylations, are not possible, which results in excellent glycosylation yields.

As donors we chose thioglycosides (11, 12). All the three different mannose donors could be obtained in a few high-yielding steps from the same precursor: ethyl 3,4-di-*O*-benzyl-1-thio-α-D-mannopyranoside (13) (**1**) (Scheme 1). Regioselective silylation at the primary position followed by benzoylation or *p*-methoxybenzylation gave donors **2** (80%) and **3** (85%), respectively. Subsequent desilylation and allylation afforded mannose donor **4** (64% overall yield). With these donors in hand the pseudo-pentasaccharide could then be assembled through three efficient glycosylations all promoted by DMTST in diethyl ether to ensure α-selectivity (Scheme 2). Acceptor **5** (14) and donor **2** were coupled to give pseudo-trisaccharide **6** in 98% yield. Removal of the TBDMS-protecting group afforded acceptor **7** (85%), which was condensed with donor **3** to yield the tetrasaccharide **8** in quantitative yield. Removal of the *p*-methoxybenzyl group

Scheme 1. Reagents and conditions: (*a*) TBDMSCl, pyridine; (*b*) BzCl, pyridine; (*c*) TBDPSCl, pyridine; (*d*) MBnCl, NaH, 0°C; (*e*) TBAF, THF; (*f*) AllBr, NaH, DMF, 0°C.



by DDQ treatment (→ **9**, 72%) and subsequent glycosylation with thioglycoside **4** gave the target intermediate **10** in 84% yield and a 49% overall yield over the five steps. The α-configuration of all glycosidic linkages was proven by the $^1J_{C,H}$ coupling constants of the anomeric carbons, which all were above 168 Hz.

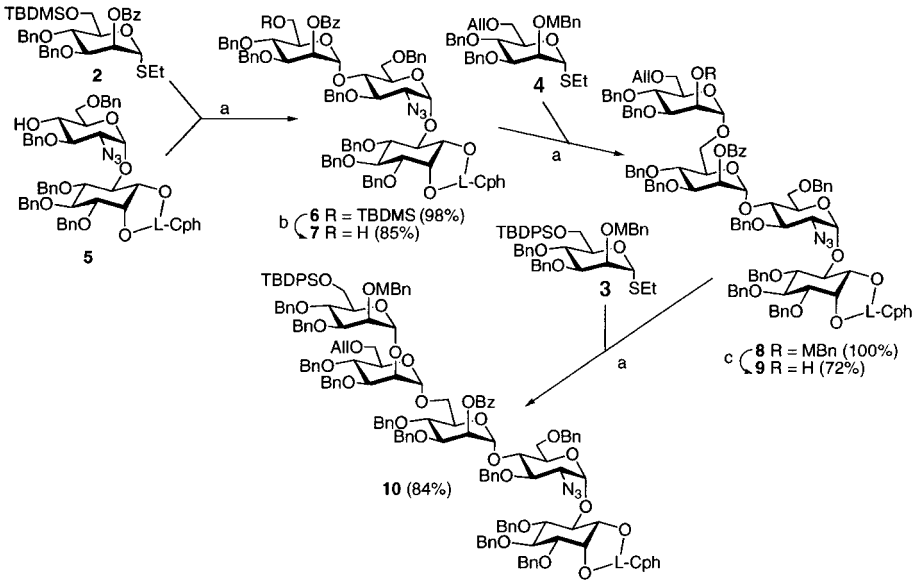
In compound **10** all the functionalities are now in place to allow continued syntheses of many different GPI anchor structures. To prove the orthogonality of the temporary protecting groups, the groups were removed one by one in sequence to produce a tetraol (Scheme 3). The allyl group was removed under neutral conditions using an iridium catalyst followed by hydrolysis of the obtained vinyl ether with NIS–H_2O (15) (→ **11**, 98%). Removal of the *p*-methoxybenzyl protecting group was accomplished by DDQ treatment to give diol **12** (84%). The benzoate was removed using Zemplén conditions (→ **13**, 85%) and finally the silyl group was cleaved by TBAF treatment to give the tetraol **14** (86%) in an overall deprotection yield of 60%.

As mentioned, GPI structures have been suggested as insulin second messengers (4, 5). A key structure is believed to be a 1,2-cyclic phosphate on the insulin moiety obtained through enzymatic cleavage of the native glycerol fragment (6). Additional phosphorylation has been shown to enhance the biological activity (7). Accordingly, the tetraol was phosphorylated using the amidite approach (16) to give benzyl protected 6‴,2‴,6″,2′-tetraphosphate derivative **15** in quantitative yield, which was subsequently processed to give the corresponding 1,2-cyclic phosphate **17** (Scheme 4). Acidic hydrolysis of the campher acetal (→ **16**, 46%) followed by treatment with commercial methyl dichlorophosphate afforded **17** in 95% yield after work-up. Catalytic hydrogenolysis to remove benzyl protecting group is often troublesome with phosphate-containing compounds; however, as found earlier (17, 18), Birch-type reaction conditions smoothly removed all benzyl groups without any indication (NMR, MALDI-TOF MS) of phosphate migration or hydrolysis or opening of the cyclic phosphate. Concomitantly, the azido function was reduced, in a one-pot reaction, to yield target structure **18** in 60% yield.

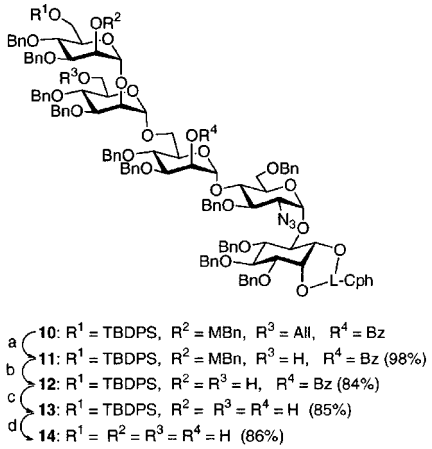
Conclusion

In summary, a most effective linear synthesis of a highly differentially protected backbone structure of GPI anchors has been performed. The temporary protecting groups can be removed in an orthogonal fashion to allow synthesis of

Scheme 2. Reagents and conditions: (*a*) DMTST, Et_2O, MS (4 Å); (*b*) TBAF, THF; (*c*) DDQ, CH_2Cl_2–H_2O.



Scheme 3. Reagents and conditions: (*a*) (*i*) Pd/C, iridium catalyst, H_2, (*ii*) NIS, H_2O; (*b*) DDQ, CH_2Cl_2–H_2O; (*c*) NaOMe, MeOH; (*d*) TBAF, THF.



various substituted anchor structures. The partly deprotected structures can be polyphosphorylated and deprotected using Birch-type reaction conditions in high yields, to give synthetic phosphoinositolglycans.

Experimental

All organic solvents were distilled before use, except Et_2O, which was stored over Na. Organic solutions were dried over $MgSO_4$ before concentration, which was performed under reduced pressure at <40°C (bath temperature). NMR spectra were recorded at 270 (JEOL), 300, 400, or 600 MHz (Varian) (^{1}H), or at 67.5, 75, or 100 MHz (^{13}C), unless otherwise stated, in $CDCl_3$. For ^{1}H NMR spectra, TMS was used as internal standard ($\delta = 0$). ^{13}C NMR spectra were referred to the chloroform signal ($\delta = 77.17$). ^{31}P NMR spectra were standardized by using an external H_3PO_4 sample ($\delta = 0$). TLC was performed on silica gel 60 F_{254} (Merck) plates with detection by UV-light and (or) charring with 8% sulfuric acid. Column chromatography was performed on silica gel (Matrix Silica Si 60A, 35–70m, Amicon). MALDI-TOF spectra were recorded on a Bruker Biflex III using PEG 1500 as calibration reference.

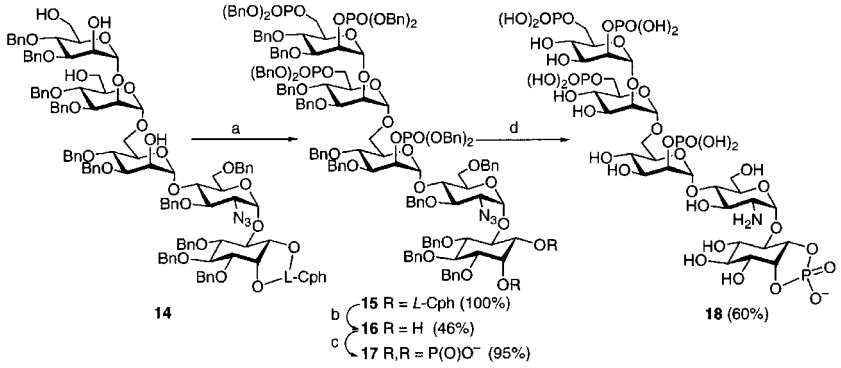
Ethyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-1-thio-α-D-mannopyranoside (2)

TBDMSCl (325 mg, 2.16 mmol) and a catalytic amount of DMAP were added to a solution of ethyl 3,4-di-*O*-benzyl-1-thio-α-D-mannopyranoside (13) (**1**, 550 mg, 1.36 mmol) in pyridine (2 mL). The reaction mixture was stirred at room temperature for about 3 h when benzoyl chloride (1 mL) was added and stirring was continued for an additional 45 min. For work up, the mixture was diluted with toluene, washed with water and brine, dried ($MgSO_4$), and concentrated. To remove the main part of pyridine, co-evaporation with toluene was performed twice before applying the residue to flash chromatography (light petroleum (bp 40–65°C) – Et_2O, 5:1) to give **2** (680 mg, 1.09 mmol, 80%). $[\alpha]_D$ +39 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 0.06 (s, 6H, Me_2Si), 0.92 (s, 9H, *t-Bu*Si), 1.30 (m, 3H, *Me*CH_2S), 2.65 (m, 2H, SCH_2), 3.80–4.10 (m, 5H), 4.54, 4.65, 4.76, 4.92 (4 × d, 4 × 1H, PhCH_2), 5.38 (s, 1H, H-1), 5.68 (br s, 1H, H-2) 7.18–8.12 (m, 13H), 8.12 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: – 4.9 (Me_2Si), 15.1 (*Me*CH_2S), 18.5 (*t-Bu*Si), 25.6 (SCH_2), 26.1 (*t-Bu*Si), 62.4, 71.2, 71.8, 73.2, 74.6, 75.3, 78.9 (C2–C6, CH_2Ph), 82.5 (C1), 127.8–138.8 (aromatic C), 165.9 (Ph*C*O). Anal. calcd. for $C_{35}H_{46}O_6SSi$: C 67.49, H 7.44; found: C 67.38, H 7.56.

Ethyl 3,4-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-2-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside (3)

TBDPSCl (1.3 mL, 4.9 mmol) and a catalytic amount of DMAP were added to a solution of **1** (1.35 g, 3.34 mmol) in pyridine (10 mL). The reaction mixture was stirred at room temperature for about 24 h, then diluted with toluene and washed with brine and water, dried ($MgSO_4$), and filtered. The filtrate was concentrated and the pyridine co-evaporated with toluene. The crude product was purified by flash-chromatography (toluene–EtOAc, 3:1) to give ethyl 3,4-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-1-thio-α-D-manno-

Scheme 4. Reagents and conditions: (*a*) (*i*) DBDIPPA, tetrazole, (*ii*) MCPBA; (*b*) TFA; (*c*) (*i*) $MeOPOCl_2$, pyridine, (*ii*) HCl; (*d*) Na, NH_3 (l).



pyranoside (1.98 g, 3.08 mmol, 92%). $[\alpha]_D$ +70 (*c* 1.0, CH_2Cl_2). ^{13}C NMR ($CDCl_3$) δ: 14.8 (*Me*CH_2S), 19.4 (*t-Bu*Si), 24.6 (S*CH*$_2$), 26.7, 26.9 (*t-Bu*Si), 63.2 (C6), 70.1, 72.3, 72.9, 74.7, 75.3, 80.7, (C2–C5, *CH*$_2$Ph), 82.8 (C1), 127.7–136.0 (aromatic C). A solution of this derivative (1.47 g, 2.29 mmol) in dry DMF (20 mL) was cooled to 0°C. NaH (190 mg, ~60% dispersed in oil) was added and the mixture was stirred for 30 min. Freshly prepared *p*-methoxybenzyl bromide (1.0 mL), dissolved in DMF (10 mL), was added dropwise. After 3 h the reaction was quenched by addition of MeOH (1 mL), diluted with toluene, washed with water and brine, dried ($MgSO_4$), and concentrated. Purification by flash chromatography (toluene–EtOAc, 18:1) gave **3** (1.60 g, 2.10 mmol, 92%). $[\alpha]_D$ +41 (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 1.06 (s, 9H, *t-Bu*Si), 1.22 (m, 3H, *Me*CH_2S), 2.56 (m, 2H, S*CH*$_2$), 3.68–4.10 (m, 8H), 4.46–4.76 (m, 5H, Ar*CH*$_2$), 4.92 (d, 1H, Ar*CH*$_2$), 5.38 (s, 1H, H-1), 5.68 (br s, 1H, H-2), 6.74–7.80 (m, 24H). ^{13}C NMR ($CDCl_3$) δ: 15.0 (*Me*CH_2S), 19.4 (*t-Bu*Si), 25.1 (S*CH*$_2$), 26.9 (*t-Bu*Si), 55.4 (*Me*OBn), 63.4 (C6), 71.8, 72.3, 73.5, 75.1, 75.3, 76.5, 80.6, 81.4 (C1–C5, *CH*$_2$Ph, MBn), 113.9 (MBn), 127.7–138.7 (aromatic C), 159.2 (MBn). Anal. calcd. for $C_{46}H_{54}O_6SSi$: C 72.40, H 7.13; found: C 72.27, H 7.28.

Ethyl 6-*O*-allyl-3,4-di-*O*-benzyl-2-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside (4)

TBDMSCl (920 mg, 6.1 mmol) and a catalytic amount of DMAP were added to a solution of **1** (1.6 g, 4.0 mmol) in pyridine (10 mL). The reaction mixture was stirred at room temperature for about 3 h and then diluted with toluene, washed with water and dried over $MgSO_4$. After filtration, the mixture was concentrated and co-evaporated from toluene. Subsequent purification by flash-chromatography (toluene–EtOAc, 9:1) of the crude product afforded ethyl 3,4-di-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-1-thio-α-D-mannopyranoside (1.7 g, 3.3 mmol, 83%) as a colourless syrup. A solution of this derivative (1.00 g, 1.93 mmol) in dry DMF (20 mL) was cooled to 0°C. NaH (120 mg, ~60% dispersed in oil) was added and the mixture was stirred for 30 min. Freshly prepared *p*-methoxybenzyl bromide (750 μL) dissolved in DMF (10 mL) was added dropwise. After 30 min the reaction was quenched by addition of MeOH (1 mL), diluted with toluene and washed with water and brine. Drying ($MgSO_4$), filtration, and evaporation gave a residue, which was purified by flash chromatography (toluene → toluene–EtOAc, 18:1) to yield ethyl 3,4-di-*O*-benzyl-2-O-*p*-methoxybenzyl-6-*O*-*tert*-butyldimethylsilyl-1-thio-α-D-mannopyranoside (1.16 g, 1.82 mmol, 94%). $[\alpha]_D$ +25 (*c* 1.0, CH_2Cl_2). ^{13}C NMR ($CDCl_3$) δ: 15.0 (*Me*CH_2S), 18.5 (*t-Bu*Si), 25.0 (S*CH*$_2$), 26.1 (*t-Bu*Si), 55.4 (*Me*OBn), 62.9 (C6), 71.6, 72.2, 73.6, 75.3, 76.1, 77.4, 80.5, 81.4 (C1–C5, *CH*$_2$Ph, MBn), 113.9 (MBn), 127.7–130.4, 138.7, 138.9 (aromatic C), 159.3 (MBn). This compound (1.10 g, 1.72 mmol) dissolved in a TBAF–THF solution (3.6 mL, 1 M) was stirred at room temperature overnight. After concentration, the residue was applied to flash chromatography (toluene → toluene–EtOAc, 12:1 → toluene–EtOAc, 3:1) to obtain ethyl 3,4-di-*O*-benzyl-2-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside (780 mg, 1.49 mmol, 87%). ^{13}C NMR ($CDCl_3$) δ: 15.0 (*Me*CH_2S), 25.5 (S*CH*$_2$), 55.4 (*Me*OBn), 62.5 (C6), 72.1, 72.2, 72.5, 75.2, 76.2, 80.5, 82.4 (C1–C5, *CH*$_2$Ph, MBn), 114.0 (MBn), 127.8–129.7, 138.4, 138.4 (aromatic C), 159.2 (MBn). This compound (780 mg, 1.49 mmol) was dissolved in dry DMF (5 mL) and cooled to 0°C. NaH (120 mg, ~60% dispersed in oil) and allyl bromide (300 μL, 3.55 mmol) were added and the reaction mixture was allowed to reach room temperature. After 2 h the reaction was quenched by addition of MeOH (1 mL). The mixture was diluted with toluene, washed with water, dried ($MgSO_4$), and concentrated. Purification by flash chromatography (toluene–EtOAc, 12:1) gave **4** (792 mg, 1.40 mmol, 94%). $[\alpha]_D$ +57 (*c* 1.0, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ: 1.24 (m, 3H, *Me*CH_2S), 2.58 (m, 2H, S*CH*$_2$), 3.60–3.84 (m, 7H), 3.90–4.18 (m, 4H), 4.48–4.74 (m, 6H), 4.92 (d, 1H, Ar*CH*$_2$), 5.14 (m, 1H), 5.26 (m, 1H), 5.36 (s, 1H, H-1), 5.94 (m, 1H), 6.80–6.84 (m, 2H), 7.16–7.40 (m, 12H). ^{13}C NMR ($CDCl_3$) δ: 15.2 (*Me*CH_2S), 25.5 (S*CH*$_2$), 55.4 (*Me*OBn), 69.4 (C6), 71.6, 71.9, 72.0, 72.5, 75.2, 75.3, 75.8, 80.4, 81.9 (C1–C5, *CH*$_2$Ph, MBn, allyl), 113.8 (MBn), 116.8 (allyl), 127.86–130.2 (aromatic C), 134.9 (allyl), 138.4, 138.7 (aromatic C), 159.2 (MBn). Anal. calcd. for $C_{33}H_{40}O_6S$: C 70.18, H 7.14; found: C 69.88, H 7.08.

(2-*O*-Benzoyl-3,4-di-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-1,2-*O*-(L-1,7,7-trimethyl[2.2.1]bicyclohept-6-yliden)-D-*myo*-inositol (6)

A solution of **5** (14) (46 mg, 48 μmol) and **2** (30 mg, 48 μmol) in dry Et_2O (10 mL) was stirred with powdered

molecular sieves (4 Å) under argon for 1 h when DMTST (41 mg, 158 μmol) was added. The reaction, followed by TLC (toluene–EtOAc, 12:1), was quenched after 3.5 h by addition of Et_3N (100 μL), and then the mixture was diluted with Et_2O (20 mL), filtered through Celite, and concentrated. The residue was subjected to silica gel column chromatography (toluene–EtOAc, 12:1) to obtain **6** (71 mg, 47 μmol, 98%). $[\alpha]_D$ +42 (*c* 1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ: 0.02 (s, 6H, *Me*$_2$Si), 0.74–2.10 (m, 27H, *t-Bu*Si, Cph), 3.24–4.98 (m, 29H), 5.50 (s, 1H), 5.61 (d, *J* = 1 Hz, 1H) 5.66 (s, 1H), 7.00–7.66 (m, 38H), 8.06 (d, 2H). ^{13}C NMR ($CDCl_3$) δ: –4.9 (*Me*$_2$Si), 10.1, 20.7, 20.9 (Cph), 26.2 (*t-Bu*Si), 27.2, 30.1, 44.9, 45.3, 48.2, 51.8 (Cph), 61.8, 63.4, 68.6–76.7, 78.3–80.8 ($C^{ino}1$–$C^{ino}6$, C2–C6, C2′–C6′, CH_2Bn), 95.7 (C1) 99.3 (C1′), 118.1 (Cph-acetal), 127.5–139.1 (aromatic C), 165.4 (Ph*C*O). Anal. calcd. for $C_{90}H_{105}N_3O_{16}Si$: C 71.45, H 7.00; found: C 71.28, H 6.84.

(6-*O*-Allyl-3,4-di-*O*-benzyl-2-*O*-*p*-methoxybenzyl-α-D-mannopyranosyl)-(1→6)-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-1,2-*O*-(L-1,7,7-trimethyl[2.2.1]bicyclo-hept-6-yliden)-D-*myo*-inositol (8)

TBAF (55 μL, 1 M solution in THF) was added to a solution of **6** (71 mg, 47 μmol) in freshly distilled THF (5mL). After 6 h additional TBAF (55 μL) was added and after 24 h 30 μL more. Although the reaction was not complete (as monitored by TLC (toluene–EtOAc, 12:1) after 48 h the mixture was diluted with toluene, washed with water, dried ($MgSO_4$), and concentrated. The residue was applied onto a silica gel column and eluted (toluene–EtOAc, 15:1) to give 2-*O*-benzoyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-1,2-*O*-(L-1,7,7-trimethyl[2.2.1]bicycloheptt-6-yliden)-D-*myo*-inositol (**7**, 56 mg, 40 μmol, 85%). $[\alpha]_D$ +35 (*c* 1.0, CH_2Cl_2). ^{13}C NMR ($CDCl_3$) δ: 10.2, 20.7, 20.9 (Cph), 27.2, 30.1, 45.0, 45.3, 48.2, 51.8 (Cph), 62.0, 63.6, 68.1–76.9, 78.3–80.7 ($C^{ino}1$–$C^{ino}6$, C2–C6, C2′–C6′, CH_2Bn), 95.6 (C1) 99.1 (C1′), 118.1 (Cph-acetal), 127.5–138.4 (aromatic C), 165.2 (Ph*C*O). A solution of **4** (28 mg, 50 μmol) and **7** (75 mg, 54 μmol) in dry Et_2O (10 mL) was stirred with powdered molecular sieves (4 Å) under argon for 1 h, when DMTST (40 mg, 155 μmol) was added. The reaction, monitored by TLC (light petroleum (bp 40–60°C) – Et_2O, 1:1), was quenched after 1.5 h by addition of Et_3N (100 μL) and stirred for a further 20 min. The mixture was diluted with Et_2O (20 mL), filtered through Celite, and concentrated. The residue was subjected to silica gel column chromatography (toluene–EtOAc, 12:1) to obtain **8** (95 mg, 50 μmol, quant.). $[\alpha]_D$ +41 (*c* 1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ: 0.78–1.96 (m, 16H, Cph), 3.30–5.20 (m, 52H), 5.50 (s, 1H), 5.61 (d, *J* = 1 Hz, 1H) 5.66 (s, 1H), 5.81 (m, 1H), 6.78 (d, 2H), 7.00–7.45 (m, 48H), 8.00 (d, 2H). ^{13}C NMR ($CDCl_3$) δ: 10.0 (Cph), 20.6, 20.9, 27.2, 30.0, 45.0, 45.4, 48.2, 51.8 (Cph), 55.4 (*MeO*Bn), 63.6–80.8 ($C^{ino}1$–$C^{ino}6$, C2–C6, C2′–C6′, C2″–C6″, CH_2Bn, MBn, allyl), 95.6 (C1), 98.4 (C1″), 99.1 (C1′), 113.7 (MBn), 116.7 (allyl), 118.2 (Cph-acetal), 125.3–138.7 (aromatic C, allyl), 159.0 (MBn), 165.4 (Ph*C*O). Anal. calcd. for $C_{115}H_{125}N_3O_{22}$: C 72.65, H 6.63; found: C 72.38, H 6.60.

(3,4-Di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-2-*O*-*p*-methoxybenzyl-α-D-mannopyranosyl)-(1→2)-(6-*O*-allyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-1,2-*O*-(L-1,7,7-trimethyl[2.2.1]bicycloheptt-6-yliden)-D-*myo*-inositol (10)

DDQ (17 mg, 75 μmol) was added to a solution of **8** (90 mg, 47 μmol) in water saturated CH_2Cl_2 (5% H_2O, 5 mL) and stirred overnight at room temperature. The mixture was diluted with CH_2Cl_2 (15 mL), washed with water and $Na_2S_2O_3$ (10% aq), dried ($MgSO_4$), and concentrated. The crude product was purified by flash chromatography (toluene–EtOAc, 6:1) to give 60 mg (34 μmol, 72%) of (6-*O*-allyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-1,2-*O*-(L-1,7,7-trimethyl[2.2.1]bicycloheptt-6-yliden)-D-*myo*-inositol (**9**). $[\alpha]_D$ +51 (*c* 1.0, CH_2Cl_2). ^{13}C NMR ($CDCl_3$) δ: 10.0, 20.6, 20.9, 27.2, 30.0, 45.0, 45.3, 48.2, 51.8 (Cph), 63.4–80.8 ($C^{ino}1$–$C^{ino}6$, C2–C6, C2′–C6′, C2″–C6″, CH_2Bn, allyl), 95.6 (C1), 98.4 (C1″), 99.1 (C1′), 116.9 (allyl), 118.2 (Cph-acetal), 125.3–138.7 (aromatic C, allyl), 165.3 (Ph*C*O). A solution of **9** (300 mg, 168 μmol) and **3** (130 mg, 170 μmol) in dry Et_2O (80 mL) was stirred with powdered molecular sieves (4 Å) under argon for 1 h. Then DMTST (150 mg, 0.58 mmol) was added. The reaction, monitored by TLC (light petroleum (bp 40–60°C) – Et_2O, 1:1), was quenched after 45 min by addition of Et_3N (500 μL) and stirred for another 20 min. The mixture was diluted with Et_2O (20 mL), filtered through Celite, and concentrated. The residue was subjected to silica gel column chromatography (toluene–EtOAc, 12:1) to obtain 350 mg (141 μmol, 84%) of **10**. $[\alpha]_D$ +21 (*c* 1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ: 0.78–1.96 (m, 25H, *t-Bu*Si, Cph), 3.30–5.20 (m, 61H), 5.28 (d, *J* = 2 Hz, 1H), 5.50 (s, *J* = 2 Hz, 1H), 5.58 (d, *J* = 4 Hz, 1H), 5.63 (m, 1H), 5.71 (m, 1H), 6.78 (d, 2H), 7.00–8.00 (m, 72H). ^{13}C NMR ($CDCl_3$) δ: 10.0 (Cph), 19.6 (*t-Bu*Si), 20.6, 20.9, 21.7 (Cph), 27.2 (*t-Bu*Si, Cph), 30.0, 45.0, 45.3, 48.2, 51.8 (Cph), 55.3 (*MeO*Bn), 63.4–80.8 ($C^{ino}1$–$C^{ino}6$, C2–C6, C2′–C6′, C2″–C6″, C2‴-C6‴, CH_2Bn, MBn, allyl), 95.5 (C1), 98.7 (C1″), 98.8 (C1‴), 99.2 (C1′), 113.6 (MBn), 116.3 (allyl), 118.2 (Cph-acetal), 125.4–139.0 (aromatic C, allyl), 158.9 (MBn), 165.3 (Ph*C*O). Anal. calcd. for $C_{151}H_{165}N_3O_{27}Si$: C 73.07, H 6.70; found: C 72.93, H 6.75.

(3,4-Di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-mannopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-1,2-*O*-(L-1,7,7-trimethyl[2.2.1]bicyclohept-6-yliden)-D-*myo*-inositol (12)

Compound **10** (700 mg, 282 μmol) was dissolved in freshly distilled THF (10 mL) and stirred 10 min with a catalytic amount of Pd/C. The filtered solution was degassed and set under argon. After addition of 1,5-cyclooctadienebis(methyldiphenylphosphine)iridium(I)·PF_6 (27 mg, 32 μmol), the solution was saturated with hydrogen until the intensive red colour of the mixture had disappeared (approx. after 20 min). The solution was stirred for 1 h under argon at

room temperature, then NIS (420 mg, 1.87 mmol) and H_2O (1.75 mL) were added. After 2.5 h the reaction mixture was diluted with EtOAc, washed consecutively with 10% aqueous $Na_2S_2O_3$, $NaHCO_3$, dried ($MgSO_4$), and concentrated. The crude product was purified by flash chromatography (toluene–EtOAc, 12:1) to give (3,4-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-2-*O*-*p*-methoxybenzyl-α-D-mannopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-1,2-*O*-(L-1,7,7-trimethyl[2.2.1]bicycloheptyliden)-D-*myo*-inositol (**11**, 674 mg, 276 μmol, 98%). $[\alpha]_D$ +30 (*c* 1.0, CH_2Cl_2). ^{13}C NMR ($CDCl_3$) δ: 10.0 (Cph), 19.6 (*t-Bu*Si), 20.6, 20.9 (Cph), 27.2 (*t-Bu*Si), 30.0, 45.0, 45.3, 48.2, 51.8 (Cph), 55.4 (*MeO*Bn), 61.9–80.8 ($C^{ino}1$–$C^{ino}6$, C2–C6, C2′–C6′, C2″–C6″, C2‴–C6‴ CH_2Bn, MBn), 95.5 (C1), 98.7 (C1″), 98.8 (C1‴), 99.1 (C1′), 113.6 (MBn), 118.2 (Cph-acetal), 125.4–138.7 (aromatic C), 158.9 (MBn), 165.3 (Ph*C*O). DDQ (80 mg, 350 μmol) dissolved in CH_2Cl_2 (2 mL) was added to an ice cooled solution of **11** (650 mg, 266 μmol) in water saturated CH_2Cl_2 (5% H_2O, 25 mL) and stirred for 4 h, an additional 30 mg of DDQ in portions of 10 mg were added until the conversion was complete. The mixture was diluted with CH_2Cl_2 (100 mL); washed with water, $Na_2S_2O_3$ (10% aq), and $NaHCO_3$; dried ($MgSO_4$); and concentrated. The crude product was purified by flash chromatography (toluene–EtOAc, 12:1) to give **12** (520 mg, 224 μmol, 84%). $[\alpha]_D$ +28 (*c* 1.0, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ: 0.80–1.96 (m, 25H, *t-Bu*Si, Cph), 3.30–4.90 (m, 52H), 5.19 (s, 1H), 5.52 (s, 1H), 5.59 (d, *J* = 3 Hz, 1H), 5.63 (m, 1H), 7.00–8.00 (m, 70H). ^{13}C NMR ($CDCl_3$) δ: 10.0 (Cph), 19.5 (*t-Bu*Si), 20.6, 20.9 (Cph), 27.1 (*t-Bu*Si), 30.1, 45.0, 45.3, 48.2, 51.8 (Cph), 61.9–80.8 ($C^{ino}1$–$C^{ino}6$, C2–C6, C2′–C6′, C2″–C6″, C2‴–C6‴, CH_2Bn), 95.5 (C1), 98.7 (C1″), 99.1 (C1′), 100.9 (C1‴), 118.2 (Cph-acetal), 125.4–138.7 (aromatic C), 165.3 (Ph*C*O).

(3,4-Di-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-1,2-*O*-(L-1,7,7-trimethyl[2.2.1]bicyclohept-6-yliden)-D-*myo*-inositol (14)

Compound **12** (490 mg, 211 μmol) was deacetylated under Zemplén conditions. To speed up the reaction, the mixture was warmed to 45°C. After 3 days the reaction mixture was neutralized with H^+ ion exchange resin, filtered, and concentrated. The residue was purified by flash-chromatography (toluene–EtOAc, 6:1→3:1) to obtain (3,4-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-mannopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-1,2-*O*-(L-1,7,7-trimethyl[2.2.1]bicyclohept-6-yliden)–D-*myo*-inositol (**13**, 400 mg, 180 μmol, 85%). $[\alpha]_D$ +40 (*c* 1.0, CH_2Cl_2). ^{13}C NMR ($CDCl_3$) δ: 10.0 (Cph), 19.6 (*t-Bu*Si), 20.6, 20.9 (Cph), 27.1 (*t-Bu*Si), 30.1, 45.0, 45.3, 48.2, 51.8 (Cph), 62.0–80.6 ($C^{ino}1$–$C^{ino}6$, C2–C6, C2′–C6′, C2″–C6″, C2‴–C6‴, CH_2Bn), 95.4 ($J_{C1,H1}$ = 173 Hz, C1), 99.1 ($J_{C1,H1}$ = 168 Hz, C1′), 100.2, 101.4 ($J_{C1,H1}$ = 176, 169 Hz, C1″, C1‴), 118.2 (Cph-acetal), 127.2–138.5 (aromatic C). Compound **13** (350 mg, 157 μmol) was dissolved in THF (10 mL) and TBAF (200 μL, 1 M in THF) was added. The mixture was stirred at room temperature overnight, then concentrated, and purified by flash chromatography (toluene–EtOAc, 3:1→2:1) to give 267 mg (135 μmol, 86%) of **14**. $[\alpha]_D$ +50 (*c* 1.0, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ: 0.80–2.04 (m, 16H, Cph), 3.34–4.90 (m, 52H), 5.03 (s, 1H), 5.06 (s, 1H), 5.28 (s, 1H), 5.58 (d, *J* = 2 Hz, 1H), 7.00–7.40 (m, 55H). ^{13}C NMR ($CDCl_3$) δ: 10.1, 20.7, 20.9, 27.3, 30.1, 45.0, 45.4, 48.2, 51.8 (Cph), 62.0–81.0 ($C^{ino}1$–$C^{ino}6$, C2–C6, C2′–C6′, C2″–C6″, C2‴–C6‴, CH_2Bn), 95.5 (C1), 99.5 (C1′), 100.8, 101.2 (C1″, C1‴), 118.2 (Cph-acetal), 127.5–128.6 and 137.5–138.6 (aromatic C). Anal. calcd. for $C_{117}H_{131}N_3O_{25}$: C 71.00, H 6.67; found: C 70.85, H 6.65.

(2,6-Di-[ammoniumphosphate]-α-D-mannopyranosyl)-(1→2)-(6-[ammoniumphosphate]-α-D-mannopyranosyl)-(1→6)-(2-[ammoniumphosphate]-α-D-mannopyranosyl)-(1→4)-(2-amino-2-deoxy-α-D-glucopyranosyl)-(1→6)-D-*myo*-inositol 1,2-cyclic phosphate ammonium salt (18)

1*H*-Tetrazole (115 mg, 1.64 mmol) was added to a solution of **14** (230 mg, 116 μmol) and dibenzyl diisopropylphosphoramidite (225 μL, 700 μmol) in CH_2Cl_2 (3.5 mL). After 20 min the reaction mixture was cooled to 0°C and 70% *m*-chloroperbenzoic acid (230 mg) was added. The mixture was stirred an additional 15 min and then diluted with CH_2Cl_2 and washed with $Na_2S_2O_3$ (10% aq), $NaHCO_3$ and, water. After drying ($MgSO_4$), filtration, and concentration, the crude product was subjected to flash chromatography (toluene → toluene–EtOAc, 20 → 25%) to yield (3,4-di-*O*-benzyl-2,6-bis-*O*-dibenzyloxyphosphoryl-α-D-mannopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-6-*O*-dibenzyloxyphosphoryl-α-D-mannopyranosyl)-(1→6)-(3,4-di-*O*-benzyl-2-*O*-dibenzyloxyphosphoryl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-1,2-*O*-(L-1,7,7-trimethyl[2.2.1]bicyclohept-6-yliden)-D-*myo*-inositol (**15**, 364 mg, 116 μmol, quant.). $[\alpha]_D$ +23 (*c* 1.0, CH_2Cl_2). ^{13}C NMR ($CDCl_3$) δ: 10.2, 20.8, 21.0, 27.4, 30.2, 45.2, 45.5, 48.3, 51.9 (Cph), 63.3–80.8 ($C^{ino}1$–$C^{ino}6$, C2–C6, C2′–C6′, C2″–C6″, C2‴–C6‴, CH_2Bn), 95.5 (C1), 98.9 (C1′), 99.4, 99.9 (C1″, C1‴), 118.3 (Cph-acetal), 127.2–138.6 (aromatic C). ^{31}P NMR ($CDCl_3$) δ: 4 signals between –0.5 and +0.5. TFA (1 mL) was added at 0°C to a solution of **15** (300 mg, 100 μmol) in CH_2Cl_2 (10 mL). The mixture was stirred for 18 h (room temperature), quenched by the addition of Et_3N (1 mL), and concentrated. The residue was purified by flash chromatography (toluene–EtOAc, 2:1) to give (3,4-di-*O*-benzyl-2,6-bis-*O*-dibenzyloxyphosphoryl-α-D-mannopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-6-*O*-dibenzyloxyphosphoryl-α-D-mannopyranosyl)-(1→6)-(3,4-di-*O*-benzyl-2-*O*-dibenzyloxyphosphoryl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-D-*myo*-inositol (**16**, 132 mg, 46 μmol, 46%). $[\alpha]_D$ +32 (*c* 1.0, CH_2Cl_2). ^{13}C NMR ($CDCl_3$) δ: 53.6, 64.2, 66.3–81.7 ($C^{ino}1$–$C^{ino}6$, C2–C6, C2′–C6′, C2″–C6″, C2‴–C6‴, CH_2Bn), 98.2, 98.7 (C1, C1″), 98.9, 99.0, 99.7, 99.7 (C1′, C1‴), 127.2–128.7, 135.6–137.8, 137.9–138.5 (aromatic C). ^{31}P NMR ($CDCl_3$) δ: 4 signals between –0.5 and +0.5. Methyl dichlorophosphate (50 μL, 50 mmol) was dissolved in dry pyridine (1 mL) at 15°C (water bath). The mixture was stirred for 20 min, after which a solution of **16**

(48 mg, 16.6 μmol) in dry pyridine (500 μL) was added. After 2 h the starting material was consumed (TLC: $CHCl_3$–MeOH, 9:1) and the reaction was quenched by addition of $NaHCO_3$. The mixture was transferred into a 100-mL round bottom flask (using approx. 10 mL pyridine) and concentrated. The residue was dissolved and partitioned between $CHCl_3$ (60 mL) and water (10 mL). HCl (1 M, aq.) was added until the solution was pH 1 (4 mL). The aqueous phase was extracted with $CHCl_3$, and the combined organic phases were dried ($MgSO_4$) and concentrated. After co-evaporation two times from EtOH, the crude product (3,4-di-*O*-benzyl-2,6-bis-*O*-dibenzyloxyphosphoryl-α-D-mannopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-6-*O*-dibenzyloxy-phosphoryl-α-D-mannopyranosyl)-(1→6)-(3,4-di-*O*-benzyl-2-*O*-dibenzyloxyphosphoryl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-D-*myo*-inositol 1,2-cyclic phosphate (**17**, 48 mg, 95%) was obtained, which was immediately used for the next reaction step. Raw material **17** dissolved in dry THF (2 mL) was dropped into liquid ammonia (20 mL). A catalytic amount of sodium was added to the white emulsion, which turned rapidly into a deep blue solution. After 2 min, NH_4Cl (s) was carefully added until the solution turned white again. The liquid ammonia was allowed to evaporate. The dry residue was dissolved in water (20 mL), and washed once with Et_2O (10 mL). After concentration, the crude product was purified by size exclusion chromatography (G15, eluent water containing 1% n-BuOH) to give, after freeze-drying, **18** (12 mg, 10 μmol, 60%). ^{1}H NMR (D_2O) δ: 3.38–4.60 (m, 31H), 5.14 (s, 1H), 5.25 (s, 1H), 5.49 (1H). ^{13}C NMR (D_2O) δ: 55.1, 60.9, 64.8, 64.9, 67.2, 67.3, 70.3–73.3, 74.8, 75.0, 76.6, 77.8, 79.5, 80.2, 82.2 ($C^{ino}1$–$C^{ino}6$, C2–C6, C2′–C6′, C2″–C6″, C2‴–C6‴), 96.8 (C1), 99.4 (C1″), 100.5, 101.5 (C1′, C1‴). ^{31}P NMR (D_2O) δ: 0.3, 0.9 ($PO(OH)_2$), 15.8 (cyclic phosphate). MALDI-TOF MS: 1209.2 $[M + H]^+$, 1227.2 $[M + H + NH_4]^+$, 1231.2 $[M + Na]^+$.

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References

1. M.A.J. Ferguson, S.W. Homans, R.A. Dwek, and T.W. Rademacher. Science (Washington, D.C.), **239**, 753 (1988).
2. M.J. McConville and M.A.J. Ferguson. Biochem. J. **294**, 305 (1993).
3. R.N. Cole and G.W. Hart. *In* Glycoproteins II. *Edited by* J. Montreuil, J.F.G. Vliegenthart, and H. Schachter. Elsevier, Amsterdam. 1997. pp. 69–88.
4. S. Alemany, J.M. Mato, and P. Strålfors. Nature (London), **330**, 77 (1987).
5. P. Strålfors. Nature (London), **335**, 554 (1988).
6. R. Plourde, M. d'Aarcao, and A.R. Saltiel. J. Org. Chem. **57**, 2606 (1992).
7. W. Frick, A. Bauer, J. Bauer, S. Wied, and G. Müller. Biochem. **37**, 13 421 (1998).
8. R. Madsen, U.E. Udodong, C. Roberts, D.R. Mootoo, P. Konradssson, and B. Fraser-Reid. J. Am. Chem. Soc. **117**, 1554 (1995).
9. T.G. Mayer and R.R. Schmidt. Eur. J. Org. Chem. **3**, 1153 (1999).
10. D.K. Baeschlin, L.G. Green, M.G. Hahn, B. Hinzen, S.J. Ince, and S.V. Ley. Tetrahedron: Asymmetry, **11**, 173 (2000).
11. S. Oscarson. *In* Carbohydrates in chemistry and biology: A comprehensive handbook. *Edited by* B. Ernst, G. Hart, and P. Sinaÿ. Wiley-VCH, Weinheim. 2000.
12. P.J. Garegg. Adv. Carbohydr. Chem. Biochem. **52**, 179 (1997).
13. R. Verduyn, J.J.A. Belien, C.M. Freef-Tromp, G.A. van der Marel, and J.H. van Boom. Tetrahedron Lett. **32**, 6637 (1991).
14. J. Lindberg, L. Öberg, P.J. Garegg, and P. Konradsson. Tetrahedron, **58**, 1387 (2002).
15. T. Nukada, H. Lucas, P. Konradsson, and C.A.A. van Boeckel. Synlett, 365 (1991).
16. K.-L. Yu and B. Fraser-Reid. Tetrahedron Lett. **29**, 979 (1988).
17. K. Ruda, J. Lindberg, P.J. Garegg, S. Oscarson, and P. Konradsson. Tetrahedron, **56**, 3969 (2000).
18. K. Ruda, J. Lindberg, P.J. Garegg, S. Oscarson, and P. Konradsson. J. Am. Chem. Soc. **122**, 11 067 (2000).