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# Synthesis of an Unnatural *N*-Glycan-linked Dolichyl Pyrophosphate Precursor

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**An unnatural α-D-mannopyranose-linked chitobiosyl dolichyl pyrophosphate, a stereoisomer of the *N*-glycan biosynthesis intermediate, was synthesized. The protected trisaccharide, α-D-Man-(1→4)-β-D-GlcNAc-(1→4)-D-GlcNAc, carrying a 4-methylbenzoyl group was prepared for the convenience of a TLC analysis. 1-*O*-Phosphorylation, condensation with dolichyl phosphate, and subsequent deprotection afforded the title compound.**

**Key words:** synthesis; glycosylation; *N*-glycan biosynthesis; unnatural *N*-glycan; dolichyl pyrophosphate precursor



Although it has been recognized that *N*-glycans of glycoprotein serve as recognition signals in a variety of biological phenomena such as cell-cell adhesion, receptor-ligand interaction, and cancer metastasis, the relationship between the glycan structure and glycopeptide function has not been well elucidated.[1] Transfer 'en bloc' of $Glc_3Man_9GlcNAc_2$ oligosaccharide from the dolichyl pyrophosphate (Dol-PP) precursor to asparagine residues on nascent proteins is a conserved biological process for the *N*-glycosylation of proteins in eukaryotic cells.[2] Subsequent processing in the endoplasmic reticulum and glycosyl transfer in the Golgi apparatus produce *N*-glycans of diverse structure. It is intriguing that the shorter oligosaccharides can also be transferred to protein acceptors by oligosaccharyl transferase (OT) owing to the broad substrate specificity of the enzyme.[3,4] Tai and Imperiali have recently reported a radiolabelling experiment aiming to clarify the minimal structural requirement for glycosyl donors in a yeast OT system by using chitobiosyl donor analogs with substitution at the C-2 acetamide sites.[5]

On the other hand, enzyme deficiency in the early process of *N*-glycan biosynthesis is known to be responsible for some severe genetic diseases.[6] More detailed studies on the specificity of the related enzymes as well as on the disordered mechanism of gene expression are required to establish a precise diagnosis and suitable therapeutic approach.

As part of our ongoing project designed to elucidate the nature of the functional importance of the *N*- and *O*-glycan structures in glycoproteins,[7] an unnatural trisaccharide-linked dolichyl pyrophosphate, a potent probe for substrate-specificity studies on *N*-glycan biosynthesis-associated enzymes such as mannosyl transferase and OT, was synthesized. In this paper, we describe synthesis of α-D-Man-(1→4)-β-D-GlcNAc-(1→4)-α-D-GlcNAc-PP-Dol **1**, a mannopyranoside-linkage stereoisomer of native glycan [β-D-Man-(1→4)-β-D-GlcNAc-]. Synthesis of the related unnatural pentasaccharides linked to the *N*-acetylasparagine amide has recently been reported by this group.[8]

Coupling reaction of known glycosyl donor **2**[8] and acceptor **3**[8] was promoted with $Cp_2ZrCl_2$–$AgClO_4$[9] to give trisaccharide **4** in an 85% yield. Ir-catalyzed isomerization of the allyl group[10] and subsequent hydrolysis with mercuric salt afforded **5** (92%), which was converted to corresponding acetamide derivative **6** (88%) by dephthaloylation and acetylation. The 3,6-hydroxyl groups on the mannose residue were protected with a 4-methylbenzoyl (MBz) group to afford **7** (91%), with the expectation that the presence of this UV-absorbing group would facilitate a TLC analysis of the protected glycosyl dolichyl pyrophosphate. The characteristic methyl proton signal of MBz, which appears apart from the huge signals of dolichols, would also provide an easy structural assignment of the compound by $^1$H-NMR. The silyl group was removed, and resulting hemiacetal **8** was acetylated (**9**: 81% in two steps) before hydrogenolytic cleavage of the benzyl ether, since an attempted conversion of the benzylated sugar into the acetylated one by hydrogenation of **7** and subsequent acetylation resulted in a complex mixture arising from hydrogenation of the TBDPS group and scission of the silyl ether linkage in part. Hydrogenolyzed product **10** was acetylated to give **11** (85% in two steps), which was treated with hydrazine acetate[11] to selectively split the anomeric acetyl group (85%). Resulting hemiacetal **12** was phosphorylated with tetrabenzyl pyrophosphate in the presence of

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*Abbreviations*: TBDPS, *tert*-butyldiphenylsilyl; TLC, thin-layer chromatography; LDA, lithium diisopropylamide

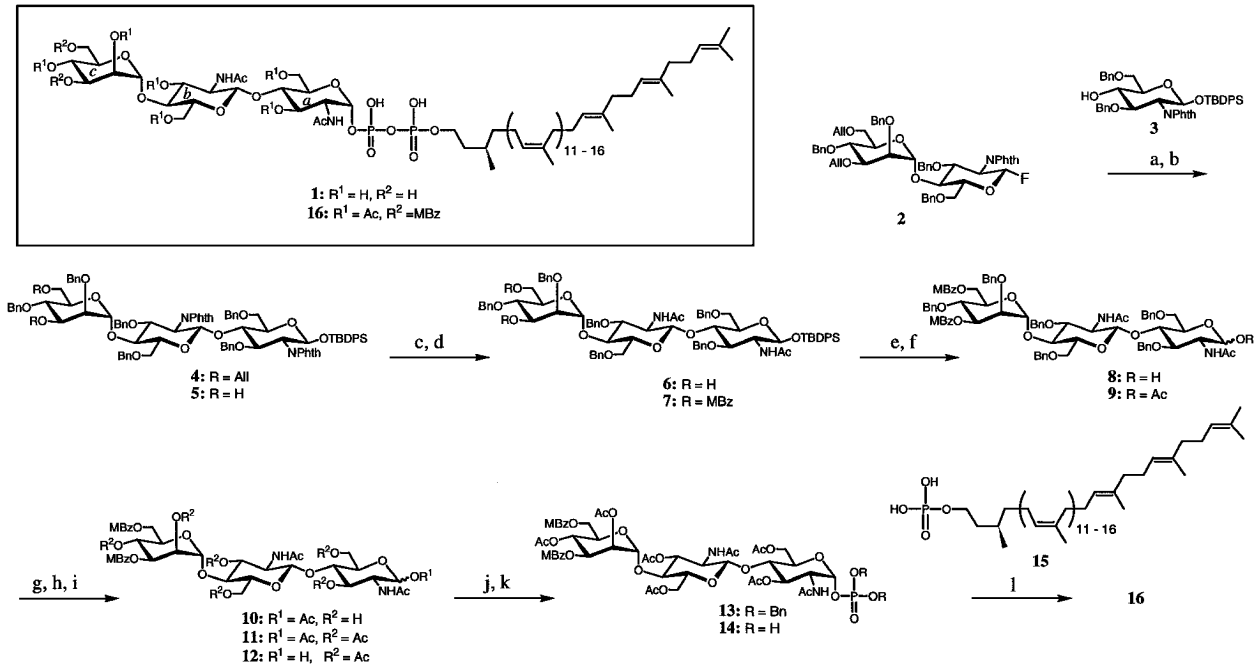


**Fig. 1.** Reagents and Conditions.

(a) $Cp_2ZrCl_2$, $AgClO_4$, $CH_2Cl_2$, $-10°C$, 2 h; (b) 1. Ir-complex, THF; 2. $HgCl_2$, HgO, aq. acetone, 5 h; (c) 1. $(CH_2NH_2)_2$, BuOH, 100°C, 48 h; 2. $Ac_2O$, MeOH, 24 h; (d) MBzCl, pyridine, 5 h; (e) TBAF, AcOH, THF, 16 h; (f) $Ac_2O$, pyridine, 4 h; (g) $H_2$, Pd–C, AcOH, 42 h; (h)) $Ac_2O$, pyridine, 48 h; (i) hydrazine acetate, DMF, 30 min; (j) LDA, $[(BnO)_2P(O)]_2O$, $-78°C$, 1.5 h; (k) $H_2$, Pd–C, MeOH, 3 h; (l) 1. $(C_3H_3N_2)_2CO$, DMF; 2. **15**, $CH_2Cl_2$, 48 h.



LDA according to the literature[3] to afford **13** in an 85% yield. Cleavage of the benzyl phosphate by hydrogenation exclusively gave key intermediate **14**. Compound **14** was activated with carbonyldiimidazole and reacted with dolichyl phosphate **15**[12] which had been prepared from semi-synthesized dolichol.[13] The coupling reaction was readily monitored by TLC and completed in 48 h. Pyrophosphate **16** was obtained in an 85% yield after purification by gel-permeation chromatography and subsequent chromatography on silica gel. The structure of **16** was proved by $^1$H- and $^{13}$C-NMR as well as by MALDI TOF-MS studies. Finally, the acetyl and 4-methylbenzoyl protecting groups on the trisaccharide were removed by a treatment with NaOMe in MeOH–$CH_2Cl_2$ to produce the target compound **1**. Successful deprotection was clearly demonstrated by the characteristic mass spectrum. A biological study of this synthesized unnatural precursor will be reported elsewhere.

## Experimental

Optical rotation data were determined with a Jasco DIP-370 polarimeter for solutions in $CHCl_3$, unless otherwise noted. Column chromatography was performed on PSQ 100B silica gel (Fuji Silysia). TLC and HPTLC were performed on 60 $F_{254}$ silica gel (E. Merck). $^1$H- and $^{13}$C-NMR spectra were recorded with a Jeol AL400 spectrometer [$^1$H at 400 MHz and $^{13}$C at 100 MHz. Chemical shifts are expressed in ppm downfield from the signal for internal $Me_4Si$ for solutions in $CDCl_3$. MALDI-TOF mass spectra were obtained with a Bruker AUTOFLEX-T spectrometer, 2,5-dihydroxybenzoic acid being used as a matrix. HPLC was performed with Mightysil RP-18 column (4.6 × 150 mm for analysis and 10 × 250 mm for preparation; Kanto Chemical Co.).

*tert-Butyldiphenylsilyl 3,6-di-O-allyl-2,4-di-O-benzyl-α-D-mannopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (4).* A mixture of $Cp_2ZrCl_2$ (0.72 g, 2.46 mmol), $AgClO_4$ (1.02 g, 4.92 mmol), and dried MS 4A (5 g) in anhydrous $CH_2Cl_2$ (20 ml) was stirred under Ar for 1 h at room temperature and then cooled to $-10°C$ in an ice-MeOH bath. To the stirred mixture was added a solution of **3** (1.07 g, 1.47 mmol) in $CH_2Cl_2$ (5 ml). The mixture was stirred for 1.5 h before adding a solution of **2** (1.12 g, 1.23 mmol) in $CH_2Cl_2$ (5 ml). The reaction mixture was stirred for 2 h, and the reaction was quenched by adding sat. $NaHCO_3$. The resulting mixture was filtered through Celite, and the filtrate was extracted with $CHCl_3$. The extract was successively washed with water and brine, dried over $Na_2SO_4$, and concentrated *in vacuo*. The crude product was chro-

matographed on silica gel with toluene-EtOAc (10:1) to give **4** (1.70 g, 85%). $R_f$ 0.51 (6:1 toluene-EtOAc). $[\alpha]_D$ +39.5° (c 1). $^1$H-NMR $\delta$: 5.29 (2H, m, $CH_2$=$CH$–), 5.28–5.21 (4H, m, H-1*a*, H-1*c*, $CH_2$=CH–), 5.14–5.09 (2H, m, $CH_2$=CH–), 5.02 (1H, d, $J$= 8.0 Hz, H-1*b*), 0.82 (9H, s, t-Bu). $^{13}$C-NMR $\delta$: 99.9 (C-1c), 96.6 (C-1*a*), 93.1 (C-1*b*). *Anal.* Calcd. for $C_{98}H_{100}O_{18}N_2Si$: C, 72.57; H, 6.21; N, 1.73%. Found: C, 72.47; H, 6.38; N, 1.62%.

*tert-Butyldiphenylsilyl 2,4-di-O-benzyl-α-D-mannopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (5).* A red suspension of the Ir complex {$[Ir(COD)(PMePh_2)]_2PF_6$, 30 mg, 36 $\mu$mol} in freshly distilled THF (5 ml) was stirred in an atmosphere of $H_2$ at room temperature for 30 min to give a colorless solution of the activated catalyst, and then the atmosphere was replaced with Ar. To the solution was added a carefully degassed solution of **4** (1.10 g, 0.68 mmol) in dry THF (10 ml). After stirring for 3 h, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in 90% aq. acetone (10 ml) and stirred with $HgCl_2$ (770 mg, 1.63 mmol) and HgO (60 mg, 0.28 mmol) at room temperature for 5 h. The mixture was concentrated *in vacuo* to remove the acetone, the residue was extracted with $CHCl_3$, and the extract was washed with 10% aq. KI and brine, dried ($Na_2SO_4$), and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel with toluene-EtOAc (5:1) to give **5** (0.96 g, 92%). $R_f$ 0.36 (3:1 toluene-EtOAc). $[\alpha]_D$ +37.8° (c 1). $^1$H-NMR $\delta$: 5.32 (1H, brs, H-1*c*), 5.26 (1H, d, $J$=8.3 Hz, H-1*a*), 5.04 (1H, d, $J$= 8.1 Hz, H-1*b*), 0.84 (9H, s, t-Bu). $^{13}$C-NMR $\delta$: 98.9 (C-1*c*), 96.5 (C-1*a*), 93.2(C-1*b*). *Anal.* Calcd. for $C_{92}H_{92}O_{18}N_2Si$ $0.5H_2O$: C, 71.25; H, 6.04; N, 1.81%. Found: C, 71.15; H, 6.01; N, 1.93%.

*tert-Butyldiphenylsilyl 2,4-di-O-benzyl-α-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (6).* A mixture of **5** (968 mg, 0.63 mmol) and ethylenediamine (0.84 ml, 12.6 mmol) in n-BuOH (20 ml) was heated at 100°C for 48 h and concentrated *in vacuo*. The residue was dissolved in MeOH (5 ml), stirred with $Ac_2O$ (2.4 ml) at 0°C-room temperature for 24 h, and then concentrated *in vacuo*. The residue was dissolved in $CHCl_3$, successively washed with sat. $NaHCO_3$, water and brine, dried ($Na_2SO_4$), and concentrated *in vacuo*. The crude product was chromatographed on silica gel with toluene-EtOAc (1:1) to give **6** (752 mg, 88%). $R_f$ 0.44 (2:1 toluene-EtOAc). $[\alpha]_D$ −1.7° (c 1). $^1$H-NMR $\delta$: 5.62 (1H, d, $J$ =9.0 Hz, N$H$), 5.23 (1H, d, $J$=1.5 Hz, H-1*c*), 5.15 (1H, d, $J$=8.5 Hz, N$H$), 4.67 (1H, d, $J$=6.4 Hz, H-1*a*), 4.47 (1H, d, $J$=7.8 Hz, H-1*b*), 1.79 (3H, s, Ac), 1.59 (3H, s, Ac), 1.04 (9H, s, t-Bu). $^{13}$C-NMR $\delta$: 99.4 (C-1*b*), 98.4 (C-1*c*), 95.5 (C-1*a*). *Anal.* Calcd. for $C_{80}H_{92}O_{16}N_2Si$ $0.5H_2O$: C, 69.90; H, 6.89; N, 2.04%. Found: C, 69.90; H, 6.72; N, 2.28%.

*tert-Butyldiphenylsilyl 2,4-di-O-benzyl-3,6-di-O-(4-methylbenzoyl)-α-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (7).* A mixture of **6** (1.52 g, 1.11 mmol) and 4-methylbenzoyl chloride (0.36 ml, 2.67 mmol) in $CH_3CN$-pyridine (1:4, 30 ml) was stirred for 5 h before being concentrated *in vacuo*. The residue was extracted with EtOAc, successively washed with sat. $NaHCO_3$, water and brine, dried ($Na_2SO_4$), and concentrated *in vacuo*. The crude product was chromatographed on silica gel with toluene-EtOAc (7:3) to give **7** (1.62 g, 91%). $R_f$ 0.41 (3:2 toluene-EtOAc). $[\alpha]_D$ −3.0° (c 1). $^1$H-NMR $\delta$: 5.61 (1H, d, $J$=9.0 Hz, N$H$), 5.57 (1H, dd, $J$= 2.9, 9.3 Hz, H-3*c*), 5.24 (1H, d, $J$=2.0 Hz, H-1*c*), 5.04 (1H, d, $J$=8.8 Hz, NH), 4.66 (1H, d, $J$= 6.4 Hz, H-1*a*), 4.44 (1H, dd, $J$=4.0, 11.5 Hz, H-6*c*), 4.42 (1H, d, $J$=7.6 Hz, H-1*b*), 4.38 (1H, dd, $J$=2.0, 11.5 Hz, H-6*c*′), 2.43 (3H, s, $CH_3C_6H_4$–), 2.35 (3H, s, $CH_3C_6H_4$–), 1.80 (3H, s, Ac), 1.59 (3H, s, Ac), 1.04 (9H, s, t-Bu). $^{13}$C-NMR $\delta$: 99.7 (C-1*b*), 99.4 (C-1*c*), 95.6 (C-1*a*). *Anal.* Calcd. for $C_{96}H_{104}O_{18}N_2Si$: C, 71.98; H, 6.54; N, 1.75%. Found: C, 71.71; H, 6.51; N, 1.73%.

*2,4-Di-O-benzyl-3,6-di-O-(4-methylbenzoyl)-α-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-1-O-acetyl-3,6-di-O-benzyl-2-deoxy-α,β-D-glucopyranose (9).* To a mixture of **7** (300 mg, 0.19 mmol) and AcOH (0.22 ml, 3.73 mmol) in freshly distilled THF (3 ml) was added 1M tetrabutylammonium fluoride-THF (0.76 ml, 0.76 mmol). The mixture was stirred at room temperature for 16 h and then concentrated *in vacuo*. The residue was extracted with EtOAc, successively washed with sat. $NaHCO_3$, water and brine, dried ($Na_2SO_4$), and concentrated *in vacuo*. The crude product was chromatographed on Bio-beads S X1 with $CHCl_3$ to give **8** (230 mg), which was dissolved in pyridine (2 ml) and stirred with $Ac_2O$ (0.5 ml) for 4 h. The mixture was concentrated *in vacuo*, and solution of the residue in $CHCl_3$ was washed with sat. $NaHCO_3$, water, and brine, dried ($Na_2SO_4$), and concentrated *in vacuo*. The product was purified by gel permeation chromatography on Bio-beads S X3 with EtOAc and then by silica gel chromatography with toluene-EtOAc (1:1) to afford **9** (215 mg, 81%). $R_f$ 0.60 and 0.67 (9:1 $CHCl_3$–MeOH). $^1$H-NMR (α-acetate) $\delta$: 6.16 (1H, d, $J$=3.4 Hz, H-1*a*), 5.62 (1H, dd, $J$=2.9, 9.3 Hz, H-3*c*), 5.22 (1H, d, $J$=2.0 Hz, H-1*c*), 2.44

(3H, s, C$H_3$C$_6$ H$_4$–), 2.35 (3H, s, C$H_3$C$_6$H$_4$–), 2.07 (3H, s, Ac), 1.75 (3H, s, Ac), 1.72 (3H, s, Ac). $^1$H-NMR ($\beta$-acetate) $\delta$: 5.60 (1H, d, $J$=6.4 Hz, H-1*a*), 5.58 (1H, dd, $J$=3.0, 9.5 Hz, H-3*c*), 5.29 (1H, d, $J$= 2.0 Hz, H-1*c*), 2.43 (3H, s, C$H_3$C$_6$H$_4$–), 2.37 (3H, s, C$H_3$C$_6$H$_4$–), 2.00 (3H, s, Ac), 1.99 (3H, s, Ac), 1.80 (3H, s, Ac). *Anal.* Calcd. for $C_{82}H_{88}O_{19}N_2$ 0.5$H_2O$: C, 69.62; H, 6.34; N, 1.98%. Found: C, 69.55; H, 6.34; N, 1.98%.

*2,4-Di-O-acetyl-3,6-di-O-(4-methylbenzoyl)-α-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-1,3,6-tri-O-acetyl-2-deoxy-α,β-D-glucopyranose (11).* Compound **9** (56 mg, 0.04 mmol) was hydrogenated with 10% Pd–C (50 mg) in AcOH (5 ml) for 72 h. The catalyst was filtered off through Celite, and the filtrate was concentrated *in vacuo*. The residue was dissolved in pyridine (2.5 ml), stirred with $Ac_2O$ (0.3 ml) for 48 h at room temperature, and concentrated *in vacuo*. The residue was extracted with EtOAc, successively washed with sat. $NaHCO_3$, water and brine, dried ($Na_2SO_4$), and concentrated *in vacuo*. The crude product was chromatographed on silica gel with hexane-acetone (1:2) to give **11** (38 mg, 85%). $R_f$ 0.66 (14:1 $CHCl_3$–MeOH). $^1$H-NMR ($\alpha$-acetate) $\delta$: 6.10 (1H, d, $J$=3.7 Hz, H-1*a*), 5.09 (1H, d, $J$=2.2 Hz, H-1*c*), 2.41 (3H, s, C$H_3$C$_6$H$_4$–), 2.40 (3H, s, C$H_3$C$_6$H$_4$–), 2.19 (3H, s, Ac), 2.17 (3H, s, Ac), 2.12 (3H, s Ac), 2.09 (3H, s, Ac), 2.05 (3H, s, Ac), 2.01 (3H, s, Ac), 1.96 (3H, s, Ac), 1.95 (3H, s, Ac), 1.93 (3H, s, Ac). $^{13}$C-NMR ($\alpha$-acetate) $\delta$: 101.9 (C-1*b*), 99.3 (C-1*c*), 90.5 (C-1*a*). *Anal.* Calcd. for $C_{52}H_{64}O_{25}N_2$ 1.5$H_2O$: C, 54.59; H, 5.90; N, 2.45%. Found: C, 54.17; H, 5.62; N, 2.38%. MALDI TOF-MS: $m/z$ 1138.90 $(M+Na)^+$; calcd. for $C_{52}H_{64}O_{25}N_2Na$, 1139.37.

*2,4-Di-O-acetyl-3,6-di-O-(4-methylbenzoyl)-α-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranose (12).* To a solution of **11** (483 mg, 0.43 mmol) in anhydrous DMF (20 ml) was added crystalline hydrazine acetate (171 mg, 1.86 mmol). The mixture was stirred for 30 min at room temperature. The mixture was diluted with EtOAc, successively washed with sat. $NaHCO_3$, water and brine, dried ($Na_2SO_4$), and concentrated *in vacuo*. The crude product was chromatographed on silica gel with $CHCl_3$–MeOH (39:1) to give **12** (394 mg, 85%). $R_f$ 0.30 and 0.40 (9:1 $CHCl_3$–MeOH). $^1$H-NMR $\delta$: 5.19 [1H, brt, $J$=4.0 Hz, H-1a(α-OH)], 5.06 (1H, brd, $J$=2.0 Hz, H-1*c*), 2.41 (3H, s, C$H_3$C$_6$H$_4$–), 2.40 (3H, s, C$H_3$C$_6$H$_4$–), 2.16 (3H, s, Ac), 2.12 (3H, s, Ac), 2.08 (3H, s Ac), 2.05 (6H, s, Ac), 1.97 (3H, s, Ac), 1.96 (3H, s, Ac), 1.94 (3H, s, Ac). $^{13}$C-NMR $\delta$: 101.7 (C-1*b*), 99.4 (C-1*c*), 91.5 [C-1*a*(α-OH)]. MALDI TOF-MS: $m/z$ 1097.56 $(M+Na)^+$; calcd for $C_{50}H_{62}O_{24}N_2Na$, 1097.36.

*2,4-Di-O-acetyl-3,6-di-O-(4-methylbenzoyl)-α-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl dibenzyl phosphate (13).* To a stirred solution of **12** (75 mg, 0.07 mmol) in anhydrous THF (3 ml) was added 0.76M LDA-hexane/THF (0.2 ml, 0.15 mmol) at −78°C under Ar. The mixture was stirred for 30 min. A solution of tetrabenzyl pyrophosphate (45 mg, 0.08 mmol) in anhydrous THF (1 ml) was added, and the mixture was stirred for 1.5 h before being concentrated *in vacuo*. The residue was dissolved in EtOAc, successively washed with sat. $NaHCO_3$, water and brine, dried ($Na_2SO_4$), and concentrated *in vacuo*. The crude product was purified by gel permeation chromatography on Biobeads S X3 with EtOAc to give **13** (79 mg, 85%). $R_f$ 0.60 (9:1 $CHCl_3$–MeOH). $[\alpha]_D$ +37.3° (c 1). $^1$H-NMR $\delta$: 7.98 (2H, d, $J$=8.0 Hz, Ar), 7.83 (2H, d, $J$ =8.0 Hz, Ar), 7.39–7.33 (10H, m, Ar), 7.30–7.23 (4H, m, Ar), 6.17 (1H, d, $J$=9.7 Hz, N*H*), 5.74 (1H, d, $J$=9.3 Hz, N*H*), 5.68 (1H, t, $J$=9.9 Hz, H-4*c*), 5.63 (1H, dd, $J$=3.1, 5.8 Hz, H-1*a*), 5.45 (1H, dd, $J$ =3.1, 9.9 Hz, H-3*c*), 5.15–5.03 (8H, m, H-3*a*, H-3*b*, H-1*c*, H-2*c*, 2 PhC$H_2$–), 4.55 (1H, dd, $J$=2.7, 12.7 Hz, H-6*c*), 4.44–4.15 (7H, m, H-2*a*, H-6*a*, H-1*b*, H-6*b*, H-6*b′*, H-5*c*, H-6*c′*), 4.06–3.97 (2H, m, H-6*a′*, H-2*b*), 3.93 (1H, m, H-5*a*), 3.87 (1H, t, $J$= 9.2 Hz, H-4*b*), 3.71 (1H, t, $J$=9.6 Hz, H-4*a*), 3.60 (1H, m, H-5*b*), 2.41 (3H, s, C$H_3$C$_6$H$_4$–), 2.40 (3H, s, C$H_3$C$_6$H$_4$–), 2.11 (3H, s, Ac), 2.09 (6H, s, Ac), 2.05 (3H, s, Ac), 1.98 (3H, s, Ac), 1.95 (3H, s, Ac), 1.93 (3H, s, Ac), 1.70 (3H, s, Ac). $^{13}$C-NMR $\delta$: 101.6 (C-1*b*), 99.3 (C-1*c*), 96.0 (d, $J_{CP}$=5.8 Hz, C-1*a*). *Anal.* Calcd. for $C_{64}H_{75}O_{27}N_2P$: C, 57.57; H, 5.66; N, 2.10%. Found: C, 57.94; H, 5.62; N, 2.08%.

*2,4-Di-O-acetyl-3,6-di-O-(4-methylbenzoyl)-α-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl phosphate (14).* Compound **13** (145 mg, 0.11 mmol) was hydrogenated with 10% Pd–C (27 mg) in MeOH (10 ml) for 3 h. The catalyst was fitered off, and the filtrate was concentrated with MeOH (5 ml) and pyridine (1 ml) *in vacuo*. The residual oil was dissolved in MeOH (5 ml) and stirred with tri-*n*-butylamine (80 μl, 2.6 eq). To the mixture was added distilled water (1.2 ml). The excess tri-*n*-butylamine was extracted three times with hexane (3 ml). The resulting aq. MeOH solution was concentrated *in vacuo*. The residual water in the product was co-evaporated with toluene *in vacuo* to give **14** as a tri-*n*-butylammonium salt (132 mg, 89%), which

was used for the next reaction without further purification.

*$P^1$-[2,4-Di-O-acetyl-3,6-di-O-(4-methylbenzoyl)-α-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl]-$P^2$-dolichyl pyrophosphate (16).* A mixture of the tri-*n*-butylammonium salt of **14** (66 mg, 49 μmol) and 1,1′-carbonyldiimidazole (60 mg, 370 μmol) in anhydrous DMF (2 ml) was stirred at room temperature for 4 h. The excess reagent was then decomposed by stirring with MeOH (25 μl) for 30 min. The mixture was diluted with MeOH and extracted with hexane. The methanolic layer was concentrated *in vacuo*. The residue was dissolved in anhydrous $CH_2Cl_2$ (1 ml) and stirred with a solution of dolichyl phosphate tri-*n*-butylammonium salt **15**[3] (150 mg, 2 equiv. based on $C_{90}$-dolichol) in anhydrous $CH_2Cl_2$ (1 ml) at room temperature for 48 h. The solvent was evaporated *in vacuo*, and the residue was chromatographed on Bio-beads S X3 with toluene-EtOH (9:1). The product was further purified by column chromatography on silica gel with $CHCl_3$–MeOH–$H_2O$ (80:20:1) to afford **16** (106 mg, 85% based on $C_{90}$-dolichol). $R_f$ 0.66 (70:30:3 $CHCl_3$–MeOH–$H_2O$). $^1$H-NMR $\delta$: 7.97 (2H, d, $J$= 8.0 Hz, Ar), 7.82 (2H, d, $J$=8.0 Hz, Ar), 7.27–7.20 (4H, m, Ar), 5.67 (1H, brt, $J$=9.3 Hz, H-4*c*), 5.45 (1H, brd, $J$=6.6 Hz, H-4*c*), 5.12 (ca. 34H, br), 2.40 (3H, s, $CH_3C_6H_4$–), 2.38 (3H, s, $CH_3C_6H_4$–), 2.04 (ca. 130H, br), 1.67 (ca. 78H, s), 0.85 [3H, m, –CH($CH_3$)–]. $^{13}$C-NMR $\delta$: 99.4 (C-1*a*), 99.2 (C-1*c*), 100.8 (C-1*b*), 143.8 and 144.2 (MBz C-4), 162.5, 165.2, 166.0, 169.5, 169.6, 170.7, 170.9, 171.4, 171.5, and 171.7 (CO). MALDI TOF-MS Found: *m/z* ($M^-$) 2393.93, 2461.98, 2530.04, 2598.10, 2666.01. Calcd.: 2393.38 ($C_{135}H_{202}N_2O_{30}P_2$), 2461.44 ($C_{140}H_{210}N_2O_{30}P_2$), 2529.51 ($C_{145}H_{218}N_2O_{30}P_2$), 2597.57 ($C_{150}H_{226}N_2O_{135}P_2$), 2665.63 ($C_{155}H_{234}N_2O_{135}P_2$).

*$P^1$-[α-D-Mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-α-D-glucopyranosyl]-$P^2$-dolichyl pyrophosphate (1).* To a stirred solution of **16** (28 mg) in anhydrous $CH_2Cl_2$ (1 ml) was added 0.2 M NaOMe/MeOH (1 ml). The mixture was stirred for 6 h at room temperature and neutralized with excess Dowex 8A (pyridine form). The resin was filtered off, and the filtrate was concentrated *in vacuo* to give **1** (18 mg, 80% based on the $C_{90}$-dolichyl congener). $R_f$ 0.19 (60:35:6 $CHCl_3$–MeOH–$H_2O$). MALDI TOF-MS Found: *m/z* ($M^-$) 1905.67, 1973.75, 2041.84, 2109.92, 2178.01. Calcd: 1905.23 ($C_{107}H_{178}N_2O_{22}P_2$), 1973.30 ($C_{112}H_{186}N_2O_{22}P_2$), 2041.36 ($C_{117}H_{194}N_2O_{22}P_2$), 2109.42 ($C_{122}H_{202}N_2O_{22}P_2$), 2177.49 ($C_{127}H_{210}N_2O_{22}P_2$).



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