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Synthesis of undecaprenyl pyrophosphate-linked glycans as donor substrates for bacterial protein *N*-glycosylation

Yong Joo Lee *, Akihiro Ishiwata, Yukishige Ito *

RIKEN Advanced Science Institute, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan

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ABSTRACT

Synthesis of undecaprenyl pyrophosphate (Und-PP)-linked glycans is described. Bacterial ($[E]_3$,$[Z]_7$)-undecaprenol was synthesized from *trans*-geranylgeranyl sulfone and isoprenoid building blocks, which was converted to undecaprenyl phosphate (Und-P). It was coupled with glycosyl phosphates to afford Und-PP-linked glycans, including core trisaccharide of *Campylobacter jejuni N*-glycan. Our synthetic method for Und-PP-linked glycan would provide various substrates as a useful tool for systematic analysis of bacterial protein *N*-glycosylation.

1. Introduction

A majority of eukaryotic proteins are glycosylated. Diversity of eukaryotic protein glycans is extremely high and their functions are numerous.[1] They are usually categorized into sub-groups such as Asn-linked (*N*-linked), Ser/Thr-linked (*O*-linked or mucin-type) and other minor groups. *N*-Linked glycans are particularly rich sources of biological functions. For instance, their roles in protein stabilization, transport and folding, cell–cell recognition, differentiation, malignant transformation, signal transduction, immune-responses and microbial infection have been well-documented. For many years, it had been perceived that protein glycosylation is limited to eukaryotes. However, recent study has revealed that certain prokaryotes such as Gram-negative bacterium *Campylobacter jejuni*[2] have *O*- as well as *N*-glycosylated proteins. Of particular interest, this protein *N*-glycosylation system is strikingly similar to that of eukaryotes.[1]

In eukaryotes, *N*-linked glycans are introduced by oligosaccharyl transferase (OST), a membrane-bound multiprotein complex; yeast OST, for instance, is composed of 9 different subunits. In most cases, eukaryotic OST transfers 14 sugar oligosaccharide, consisting of three α-linked glucose (Glc), eight α-linked mannose (Man), one β-linked Man and two β-linked *N*-acetylglucosamine (GlcNAc) residues ($Glc_3Man_9GlcNAc_2$), using dolychyl pyrophosphate (Dol-PP) as a carrier. Thus formed glycoproteins are processed in the ER, transported to Golgi apparatus, and converted to complex-type and hybrid-type structures before being secreted.

By contrast, *C. jejuni N*-linked glycan is composed of 7 sugar residues, consisting of five α-linked *N*-acetylgalactosamines (GalNAc), one β-linked Glc and a rare-sugar di-*N*-acetyl-bacillosamine (Bac), in which Bac constitutes the β-configured *N*-glycosidic linkage to the side chain of Asn. In the biosynthetic pathway, the heptasaccharide ($Glc_1GalNAc_5Bac_1$) is first assembled in an undecaprenyl-pyrophosphate (Und-PP) linked form, starting from Und-PP-Bac, through a series of chain-elongation steps catalyzed by glycosyltransferases. Then, Und-PP-$GalNAc_5Glc_1Bac_1$ flips to the periplasmic face of the plasma membrane, where OST transfers the heptasaccharide to Asn residues of nascent proteins (Fig. 1).[3] The presence of the *N*-linked glycan displayed on the surface of *C. jejuni* has proven to play a key role in enteric adhesion to host cells,[4]

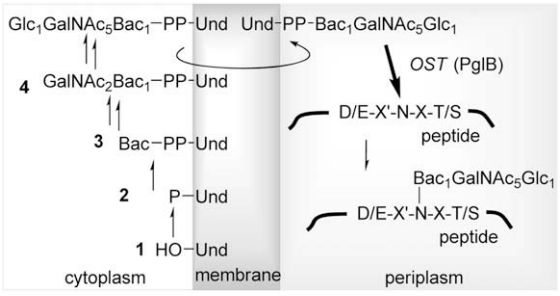

Figure 1. Biosynthesis of *N*-linked glycoprotein in *C. jejuni*.

* Corresponding authors. Tel.: +81 48 467 9430; fax: +81 48 462 4680.
E-mail addresses: yjlee@riken.jp (Y.J. Lee), yukito@riken.jp (Y. Ito).

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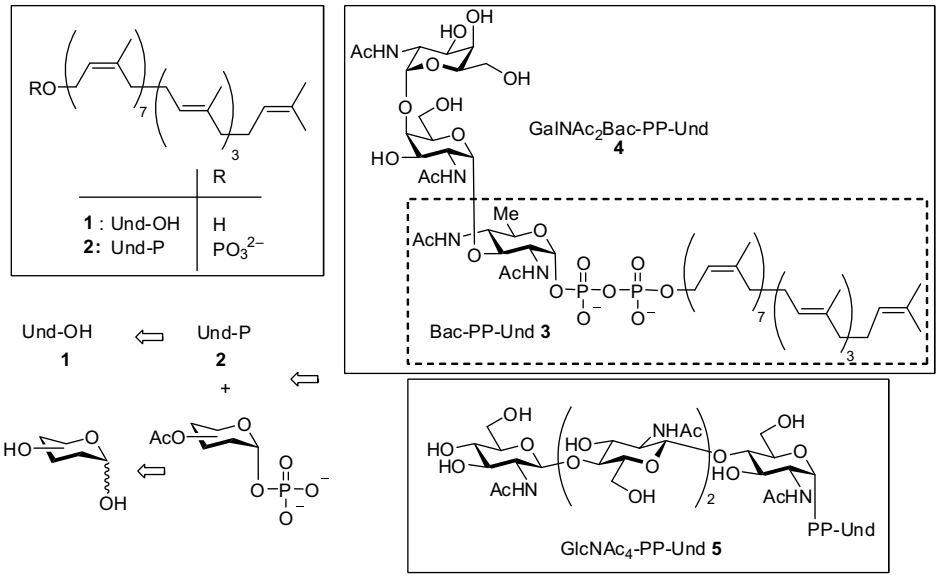


Scheme 1. Synthetic pathway toward Und-PP-linked glycans (**3**, **4**, **5**).

and this adhesion constitutes the first step of virulence.[5] Besides causing gastroenteric disorders, *C. jejuni* infection is suggested to be involved in neuromuscular paralysis, Guillian Barré syndrome (GBS).[6]

The composition of *C. jejuni* OST is remarkably simple; it consists of a single subunit PglB, which was revealed to be homologous to Stt3p, a putative catalytic subunit of yeast OST. Fascinatingly, catalytically active PglB was reconstituted in *E. coli*,[3a] making this enzyme amenable to biochemical studies. Subsequent studies have revealed that PglB preferentially accepts substrates having pentapeptide D/E-X′-N-X-T/S (X,X′ ≠ P),[7] indicating that it recognizes an upstream acidic amino acid in addition to well-known consensus sequence N-X-T/S of eukaryotic OST. Interestingly, PglB is characterized by its relaxed glycan specificity.[8] This property has allowed researchers to use chemo-enzymatically assembled Und-PP-linked glycans[9] for in vitro glycosylation of peptides[10] and proteins.[3a,11] Intriguingly, study by Aebi et al. has provided an indication that PglB is able to glycosylate even folded proteins.[11]

However, most of these studies have employed Und-PP-linked glycans derived from natural sources, which are available in limited amounts. In order to conduct more systematic studies to clarify the specificity of this enzyme and explore the possibility to exploit its activity for preparative purposes, larger quantities of highly purified Und-PP-linked glycan substrates will be required. Our previous study achieved the synthesis of the glycans that correspond to eukaryotic ($Glc_3Man_9GlcNAc_2$)[12]as well as *C. jejuni* ($Glc_1GalNAc_5$-Bac_1)[13] OST donor substrates. In this study, we first aimed to establish a practical synthetic route to the lipid part of the bacterial donor ($[E]_3$,$[Z]_7$)-undecaprenol **1**, which was to be converted into phosphate **2**. Subsequently, the latter was conjugated with glycan chains through pyrophosphate linkage is described. We selected Bac, $GalNAc_2Bac_1$, and chitotetraose as prototypical glycans, and converted them to **3**, **4**, and **5**, which will be tested in our future study toward the synthesis of glycoproteins using *C. jejuni* OST (Scheme 1).

2. Results and discussion

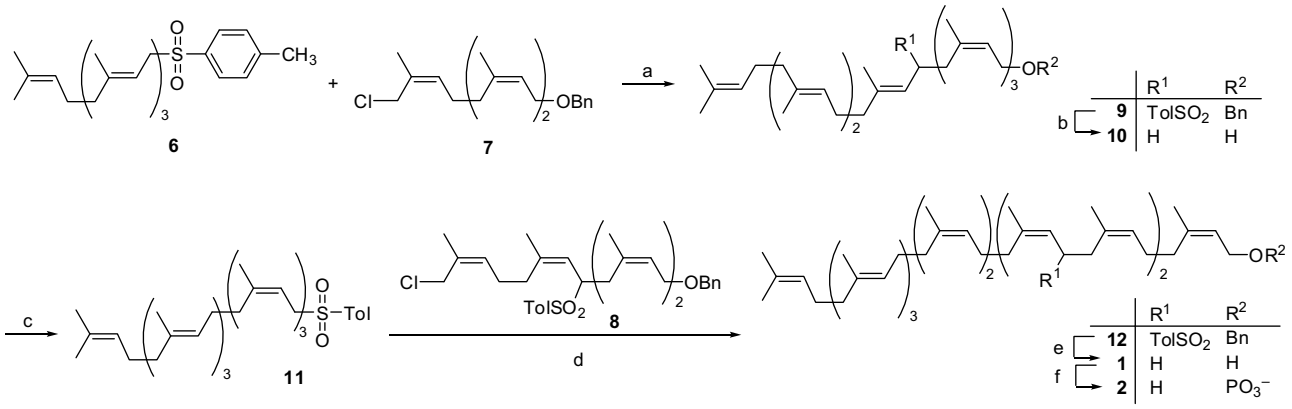
2.1. Synthesis of bacterial undecaprenol

Our research plan entailed establishment of rigorous and scalable synthetic route to the undecaprenol **1**, the key polyprenol anchor in the biosynthesis of bacterial cell surface polysaccharides.[14] Although it has been acquired by isolation from the natural sources[15] or through a biosynthetic machinery,[16] general and scalable synthetic method has been yet to be established.

Our study utilized all *trans*-geranylgeranyl *p*-tolyl sulfone (**6**)[17] and oligoprenyl chloride derivatives **7**[18] and **8**[19] as key components, which are available from *trans*-farnesol and nerol. To begin with, the compound **6** was lithionated by *n*-BuLi[20] and coupled with triprenyl chloride **7** to provide sulfone **9**. Treatment of sulfone **9** with Li in $EtNH_2$ at −78 °C,[20,21] caused reduction of the sulfone as well as deprotection of benzyl ether, giving heptaprenol **10** in 69% yield over two steps. The latter was then converted into ($[E]_3$,$[Z]_3$)-heptaprenyl *p*-tolyl sulfone (**11**), via corresponding heptaprenyl chloride, in 77% over two steps. Subsequent coupling with tetraprenyl chloride **8** provided disulfone **12**, which was subjected to Li–$EtNH_2$ reduction to give the desired ($[E]_3$,$[Z]_7$)-undecaprenol (**1**) in good yield. This route was easily amenable to scaling-up, securing sub-gram supply of compound **1**. Subsequent conversion into Und-P (**2**) was conducted by a standard procedure (Scheme 2).[22]

2.2. Synthesis of Und-PP-linked derivatives from unprotected glycan

In order to conjugate glycans with Und-P (**2**) through a pyrophosphate linkage, our strategy involved (1) conversion of unprotected glycans to peracetate, (2) selective deprotection of anomeric acetate, (3) transformation of hemiacetal to α-linked phosphates, and (4) coupling with Und phosphate. To begin with, preparation of monosaccharyl Und-PP (**3**) was attempted. Thus, synthetic bacillosamine (**13**)[23] was acetylated to afford peracetate **14**, which was subjected to chemoselective deacetylation in the presence of activated molecular sieves[24] in MeOH/CH_2Cl_2 (1:1, v/v) at room temperature to afford hemiacetal **15** in 64% yield over two steps. Phosphorylation of the hemiacetal **15** was conducted with tetrabenzyl pyrophosphate ($[(BnO)_2P(O)]_2O$)[25] and lithium hexamethyldisilazide (LiHMDS),[26] stereoselectively providing dibenzyl α-phosphate **16** in 46% yield. It was subjected to hydrogenolysis to give α-phosphate **17** in 98% yield, with no detectable anomerization. Finally, coupling with Und-P (**2**) in the presence of carbonyl diimidazole (CDI)[27] afforded acetyl protected compound **18** and subsequent deacetylation with 0.2 M methanolic NaOMe gave the



Scheme 2. Reagents and conditions: (a) **6**, *n*-BuLi, THF, HMPA, −40 °C, 30 min, then **7**, −78 °C, 3 h, 93%; (b) Li, $EtNH_2$, −78 °C, 20 min, 74%; (c) LiCl, MsCl, *s*-collidine, DMF, 0 °C, 5 h, then sodium *p*-toluenesulfinate, DMF, 6 h, 77%; (d) *n*-BuLi, THF, HMPA, −60 °C, 1 h, then **8**, −78 °C, 1 h, 86%; (e) Li, $EtNH_2$, −78 °C, 40 min, 65%; (f) Ref. 22.

desired Und-PP-linked bacillosamine (**3**) in 72% yield over two steps (Scheme 3).

As an oligosaccharide model substrate, conversion of tetra-*N*-acetylchitotetraose (**19**) into Und-PP-linked derivative **5** was first attempted (Scheme 3). Although poor solubility of **19** prevented smooth acetylation under standard conditions,[28] peracetate **20** was obtained in a satisfactory yield when the acetylation was initiated by sonication. Four step conversions, including chemoselective deacetylation of **20**, phosphorylation of **21**, hydrogenolysis of **22**, and coupling between α-phosphate **23** and Und-P (**2**) as described for **3**, gave the Und-PP-linked tetra-*N*-acetylchitotetraose **5**.

2.3. Synthesis of trisaccharyl Und-PP-linked glycan

The same procedure was then applied to the preparation of trisaccharyl Und-PP (**4**), which is known to be an excellent substrate of PglB (Scheme 4). Thus, the precursor **25** was prepared in a sterochemically homogeneous form through previously reported route,[29] Azide groups of trisaccharide **25**[13] were simultaneously reduced with $CoCl_2\cdot(H_2O)_6$ and $NaBH_4$,[30] and immediate acetylation gave tetraacetamide **26** in good yield. Desilylation of **26** with *n*-Bu_4NF in the presence of AcOH afforded hemiacetal **27** in 85% yield. Subsequent hydrogenolysis of benzyl ethers and peracetylation gave peracetate **28**, chemoselective deacetylation of which afforded hemiacetal **29** in 61% over three steps. Stereoselective phosphorylation of the hemiacetal **29** with tetrabenzyl pyrophosphate and hydrogenolysis afforded α-phosphate **30** in 37% over two steps. Finally, coupling with Und-P (**2**) activated by CDI afforded compound **31**, which was deacetylated to give the desired Und-PP-linked glycan **4**.

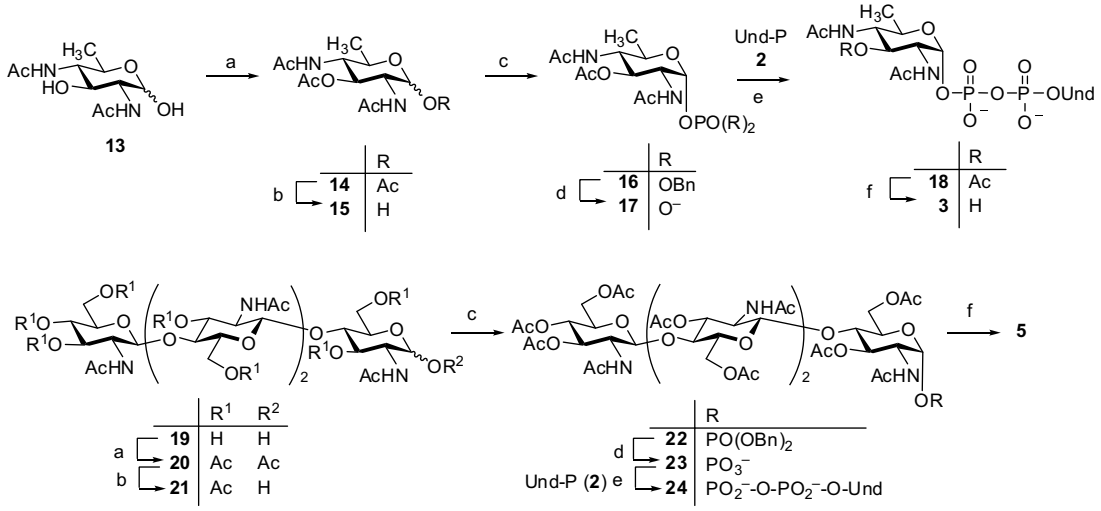
3. Conclusion

We established chemical synthesis of bacterial undecaprenol **1**. It was converted to Und-PP-linked derivatives possessing natural (**3**, **4**) as well as non-natural (**5**) glycans. Further synthetic and biological studies are now in progress. Our synthetic procedure would provide various substrates as a useful tool for the study of bacterial protein *N*-glycosylation.[31]

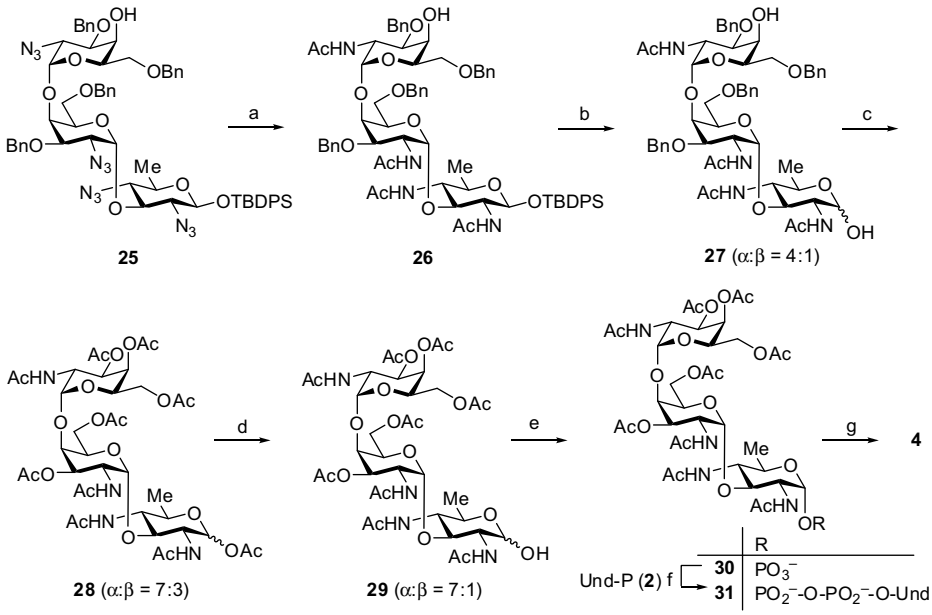
4. Experimental

4.1. General procedures

All reactions sensitive to air or moisture were carried out under nitrogen or argon atmosphere with anhydrous solvent. All reagents were purchased from commercial suppliers and used without further purification unless otherwise noted. Thin-layer chromatography was



Scheme 3. Reagents and conditions: (a) Ac_2O, pyridine, DMAP, for **14**: 3 h, 95%, α/β=9:1; for **20**: sonication, 4 h, then 48 h, 86%, α/β=2:1; (b) MS 4 Å, MeOH, CH_2Cl_2, for **15**: 2 h, 67%, α/β=4:1; for **21**: 5 h, 61%; (c) LiHMDS, DMF, −50 °C, 10 min, then $[(BnO)_2P(O)]_2O$, THF, −50 °C, 1 h, for **16**: 46%; for **22**: 87%; (d) H_2, Pd/C, MeOH, 3 h, for **17**: 98%; for **23**: 97%; (e) CDI, DMF, 6 h, then Und-P (**2**), $(CH_2Cl)_2$, 50 °C, 3 day, for **18**: 74%; for **24**: 82%; (f) NaOMe, MeOH, CH_2Cl_2, for **3**: 1 h, 97%; for **5**: 3 h, 80%.



Scheme 4. Reagents and conditions: (a) $NaBH_4$, $CoCl_2\cdot(H_2O)_6$, THF, H_2O, 0 °C, 12 h, then Ac_2O, 3 h, 66%; (b) *n*-Bu_4NF, AcOH, THF, 8 h, 85%; (c) H_2, $Pd(OH)_2$/C, AcOH, H_2O, 7 h, then Ac_2O, 10 h, 87%; (d) MS 4 Å, MeOH, CH_2Cl_2, 40 min, 70%; (e) (i) LiHMDS, DMF, −50 °C, 10 min, then $[(BnO)_2P(O)]_2O$, THF, −50 °C, (ii) H_2, Pd/C, MeOH, 3 h, 37%; (f) CDI, DMF, 6 h, then Und-P (**2**), $(CH_2Cl)_2$, 50 °C, 3 days, 67%; (g) NaOMe, MeOH, CH_2Cl_2, 2 h, 79%.

performed using silica gel 60 F_{254} precoated plates (0.25 mm thickness) with a fluorescent indicator. Visualization on TLC was achieved by UV light (254 nm) and a typical TLC indication solution (cerium sulfate/molybdic acid solution). Column chromatography was performed on silica gel 60N, 100–210 mesh (Kanto Kagaku Co., Ltd.) or Iatrobeads 6RS-8060 (Mitsubishi Kagaku Iatron, Inc.). ^{1}H NMR spectra were recorded at 400 MHz on a JEOL JNM-AL 400 spectrometer or JEOL ECX 400 spectometer and chemical shifts were referred to internal tetramethylsilane (0 ppm) or residual solvent peaks; $CDCl_3$ (7.26 ppm) or CD_3OD (3.31 ppm). ^{13}C NMR spectra were recorded at 100 MHz on the same instruments and chemical shifts were referred to internal $CDCl_3$ (77.16 ppm), CD_3OD (49.0 ppm). ^{31}P NMR spectra were recorded at 162 MHz on the same instruments and chemical shifts were referred to external standard H_3PO_4 (0.0 ppm). Melting points were determined with Büchi 510 melting point apparatus. Optical rotations were measured with a JASCO DIP 370 polarimeter. MALDI-TOF mass spectra were recorded on a SHIMADZU Kompact MALDI AXIMA-CFR spectrometer with 2,5-dihydroxybenzoic acid as the matrix. ESI-TOF mass spectra were recorded on a JEOL AccuTOF JMS-T700LCK with CF_3CO_2Na as the internal standard. Elemental analyses were performed with a Fisons EA1108 instrument.

4.2. 1-(2*Z*,6*Z*,10*Z*,14*E*,18*E*,22*E*)-1-Benzyloxy-(3,7,11,15,19,23,27-heptamethyloctacosa-2,6,10,14,18,22,26-heptaen-13-yl)sulfonyl-4-methylbenzene (9)

To a solution of all *trans*-geranylgeranyl sulfone (**6**)[17] (3.44 g, 8.02 mmol) in THF (40 mL) and HMPA (10 mL) was added *n*-BuLi (1.65 M, 4.86 mL, 8.02 mmol) at −40 °C, the reaction mixture was stirred at −40 °C for 30 min. To above solution was added dropwise a solution of allylic chloride **7**[18] (2.59 g, 7.47 mmol) in THF (20 mL) at −78 °C for 10 min. After stirring for 3 h at −78 °C, the reaction mixture was warmed up to room temperature, and then the reaction mixture was diluted with EtOAc (200 mL). The combined organic layer was washed with saturated aqueous NH_4Cl (2×50 mL) and brine (2×50 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by silica gel flash column chromatography (hexane/EtOAc, 10:1, v/v) to afford sulfone **9** (5.11 g, 6.91 mmol, 93%) as colorless oil.

R_f=0.28 (hexane/EtOAc, 10:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 (d, *J*=1.2 Hz, 3H), 1.59 (s, 3H), 1.60 (s, 9H), 1.65 (s, 3H), 1.68 (s, 3H), 1.75 (d, *J*=1.2 Hz, 3H), 1.95–2.06 (m, 20H), 2.43 (s, 3H), 2.51 (dd, *J*=13.2, 11.6 Hz, 1H), 2.77 (dd, *J*=13.2, 2.8 Hz, 1H), 3.85 (dt, *J*=10.8, 2.8 Hz, 1H), 4.01 (dd, *J*=6.8, 0.4 Hz, 2H), 4.49 (s, 2H), 4.93–4.96 (m, 1H), 5.03–5.19 (m, 5H), 5.42 (t, *J*=6.2 Hz, 1H), 7.27–7.34 (m, 7H), 7.72 (d, *J*=8.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.17, 16.2, 16.6, 17.9, 21.8, 23.5, 23.7, 23.74, 25.9, 26.4, 26.6, 26.62, 26.8, 27.0, 29.9, 32.0, 32.6, 39.9, 40.0, 63.7, 66.6, 72.2, 117.2, 122.0, 123.5, 124.2, 124.4, 125.0, 127.5, 127.8, 128.4, 128.6, 129.3, 129.4, 129.6, 130.4, 131.3, 135.1, 135.7, 138.6, 140.4, 144.3, 145.1. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{49}H_{70}NaO_3S$, 761.5, found 761.5; HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{49}H_{70}NaO_3S$, 761.4943, found 761.4964.

4.3. (2*Z*,6*Z*,10*Z*,14*E*,18*E*,22*E*)-3,7,11,15,19,23,27-Heptamethyloctacosa-2,6,10,14,18,22,26-heptaen-1-ol (10)

Li (944 mg, 136 mmol) was dissolved in $EtNH_2$ (200 mL) at −78 °C under N_2. After the solution became blue, a solution of sulfone **9** (5.02 g, 6.79 mmol) in ether (30 mL) was added dropwise for 20 min. The mixture was stirred for 20 min at −78 °C, while blue color was kept. Isoprene (10 mL) and MeOH (50 mL) was carefully added to quench. After addition of water (100 mL), mixture was extracted with EtOAc (2×100 mL). The combined organic layer was washed with brine (2×50 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by silica gel flash column chromatography (hexane/EtOAc, 6:1, v/v) to afford the compound **10** (2.48 g, 5.01 mmol, 74%) as colorless oil.

R_f=0.38 (hexane/EtOAc, 6:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.60 (s, 9H), 1.61 (d, *J*=0.8 Hz, 3H), 1.68 (d, *J*=0.8 Hz, 3H), 1.69 (s, 6H), 1.75 (d, *J*=0.8 Hz, 3H), 1.97–2.10 (m, 24H), 4.09 (dd, *J*=7.2, 0.8 Hz, 2H), 5.08–5.16 (m, 6H), 5.44 (t, *J*=6.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.2, 17.9, 23.6, 23.7, 25.9, 26.5, 26.9, 26.8, 26.9, 27.0, 32.2, 32.4, 39.9, 39.94, 59.1, 124.2, 124.3, 124.32, 124.5, 124.55, 124.6, 124.9, 131.3, 134.9, 135.0, 135.3, 135.5, 136.1, 139.8. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{35}H_{58}NaO$, 517.4, found 517.7. Anal. Calcd for $C_{35}H_{58}O$: C, 84.95; H, 11.81. Found C, 84.69; H, 11.98.

4.4. 1-((2*Z*,6*Z*,10*Z*,14*E*,18*E*,22*E*)-3,7,11,15,19,23,27-Heptamethyloctacosa-2,6,10,14,18,22,26-heptaen-1-yl)sulfonyl-4-methylbenzene (11)

To a solution of heptaprenol **10** (2.42 g, 4.89 mmol) and LiCl (518 mg, 12.22 mmol) in DMF (50 mL) were added *s*-collidine (1.62 mL, 12.26 mmol) and MsCl (946 μL, 12.22 mmol) at 0 °C. After stirring at 0 °C for 5 h, the reaction mixture was diluted with EtOAc (200 mL). The combined organic layer was washed with saturated aqueous NH_4Cl (2×50 mL) and brine (2×50 mL), dried ($MgSO_4$), and concentrated to afford the corresponding chloride (R_f=0.88, hexane/EtOAc, 7:1, v/v). Crude heptaprenyl chloride was treated with sodium *p*-toluenesulfinate (1.02 g, 5.72 mmol) in DMF (30 mL), reation mixture was stirred at room temperature for 6 h. The reaction mixture was diluted with EtOAc (200 mL). The combined organic layer was washed with saturated aqueous NH_4Cl (2×50 mL) and brine (2×50 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by silica gel flash column chromatography (hexane/EtOAc, 7:1, v/v) to afford the compound **11** (2.33 g, 3.68 mmol, 77%) as colorless oil.

R_f=0.4 (hexane/EtOAc, 7:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.60 (s, 12H), 1.64 (d, J=1.2 Hz, 3H), 1.68 (s, 6H), 1.72 (s, 3H), 1.75–1.79 (m, 2H), 1.85–1.90 (m, 2H), 1.94–2.10 (m, 20H), 2.43 (s, 3H), 3.77 (d, J=7.6 Hz, 2H), 4.94 (t, J=6.4 Hz, 1H), 5.06–5.14 (m, 5H), 5.19 (t, J=7.6 Hz, 1H), 7.31 (d, J=8.0 Hz, 2H), 7.73 (d, J=8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.2, 17.9, 21.8, 23.5, 23.65, 23.7, 25.9, 26.0, 26.5, 26.8, 26.84, 26.9, 32.1, 32.2, 32.3, 39.88, 39.9, 56.1, 111.1, 124.1, 124.12, 124.2, 124.3, 124.4, 124.8, 128.5, 129.6, 131.2, 134.9, 135.0, 135.3, 135.5, 135.95, 136.0, 133.4, 145.8. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{42}H_{64}NaO_2S$, 655.5, found 655.6; HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{42}H_{64}NaO_2S$, 655.4525, found 655.4545.

4.5. 1-(2*Z*,6*Z*,10*Z*,14*Z*,18*Z*,22*Z*,26*Z*,30*E*,34*E*,38*E*)-1-Benzyloxy-(17-tosyl-3,7,11,15,19,23,27,31,35,39,43-undecamethyl-tetratetraconta-2,6,10,14,18,22,26,30,34,38,42-undecaen-13-yl)sulfonyl-4-methylbenzene (12)

To a solution of heptaprenyl sulfone **11** (1.12 g, 1.77 mmol) in THF (40 mL) and HMPA (10 mL) was added *n*-BuLi (1.65 M, 1.08 mL, 1.78 mmol) at −60 °C, the reaction mixture was stirred at −60 °C for 1 h. To above solution was added dropwise a solution of allylic chloride **8**[19] (1.26 g, 2.21 mmol) in THF (20 mL) at −78 °C for 10 min. After stirring for 1 h at −78 °C, the reaction mixture was warmed up to room temperature, and then the reaction mixture was diluted with EtOAc (100 mL). The combined organic layer was washed with saturated aqueous NH_4Cl (2×50 mL) and brine (2×50 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by silica gel flash column chromatography (hexane/EtOAc, 5:1, v/v) to afford disulfone **12** (1.77 g, 1.52 mmol, 86%) as colorless oil.

R_f=0.3 (hexane/EtOAc, 5:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.57 (s, 3H), 1.59–1.65 (s, 24H), 1.68 (s, 6H), 1.72 (s, 3H), 1.41–1.80 (m, 8H), 1.92–2.10 (m, 24H), 2.41 (s, 3H), 2.44 (s, 3H), 2.41–2.47 (m, 2H), 2.66–2.69 (m, 2H), 3.79–3.87 (m, 2H), 3.97–4.00 (m, 2H), 4.49 (s, 2H), 4.83–4.88 (m, 1H), 4.93 (t, J=10.4 Hz, 2H), 4.99 (t, J=6.0 Hz, 1H), 5.05–5.19 (m, 6H), 5.41 (t, J=6.8 Hz, 1H), 7.27–7.34 (m, 9H), 7.68–7.72 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.2, 17.9, 21.8, 21.82, 23.5, 23.51, 23.6, 23.66, 23.7, 23.77, 23.79, 25.8, 25.9, 26.0, 26.1, 26.4, 26.75, 26.8, 26.9, 27.0, 30.2, 30.5, 32.1, 32.13, 32.2, 32.3, 32.33, 39.9, 39.93, 63.2, 63.4, 66.5, 72.3, 117.8, 117.83, 117.9, 122.3, 124.1, 124.14, 124.2, 124.3, 124.4, 124.7, 127.6, 127.75, 127.8, 128.2, 128.24, 128.4, 129.2, 129.24, 129.4, 129.5, 130.5, 130.7, 130.74, 131.3, 134.9, 135.0, 135.1, 135.3, 135.6, 135.7, 138.6, 140.0, 144.3, 144.4, 144.44, 144.6, 144.7, 144.9. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{76}H_{108}NaO_5S_2$, 1187.8, found 1187.3; HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{76}H_{108}NaO_5S_2$, 1187.7536, found 1187.7513.

4.6. (2*Z*,6*Z*,10*Z*,14*Z*,18*Z*,22*Z*,26*Z*,30*E*,34*E*,38*E*)-3,7,11,15,19,23,27,31,35,39,43-Undecamethyltetratetraconta-2,6,10,14,18,22,26,30,34,38,42-undecaen-1-ol (1)

Li (225 mg, 32.42 mmol) was dissolved in $EtNH_2$ (100 mL) at −78 °C under N_2. After the solution became blue, a solution of disulfone **12** (1.26 g, 1.081 mmol) in ether (20 mL) was added dropwise for 20 min. The mixture was stirred for 40 min at −78 °C, while blue color was kept. Isoprene (10 mL) and MeOH (50 mL) was carefully added to quench. After addition of water (100 mL), mixture was extracted with EtOAc (2×100 mL). The combined organic layer was washed with brine (2×50 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by silica gel flash column chromatography (hexane/EtOAc, 7:1, v/v) to afford the undecaprenol **1** (540 mg, 0.704 mmol, 65%) as colorless oil.

R_f=0.3 (hexane/EtOAc, 7:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.61 (s, 9H), 1.62 (s, 3H), 1.69 (s, 21H), 1.75 (s, 3H), 1.99–2.10 (m, 40H), 4.09 (d, J=6.8 Hz, 2H), 5.07–5.17 (m, 10H), 5.45 (t, J=6.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.2, 17.9, 23.6, 23.65, 23.66, 25.9, 26.5, 26.57, 26.6, 26.85, 26.9, 27.0, 32.2, 32.4, 39.9, 40.0, 59.2, 124.2, 124.3, 124.34, 124.5, 124.55, 124.6, 124.96, 125.0, 125.1, 126.0, 131.3, 134.9, 135.0, 135.25, 135.3, 135.33, 135.4, 136.1, 139.9; HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{55}H_{90}NaO$, 789.6889, found: 789.6876. Anal. Calcd for $C_{55}H_{90}O$: C, 86.09; H, 11.82. Found C, 85.82; H, 12.00.

4.7. Disodium undecaprenyl phosphate (2)

The title compound **2** was synthesized from undecaprenol **1** (52 mg, 0.068 mmol) according to known method:[22] (31 mg, 0.035 mmol, 51%) as colorless foam.

R_f=0.45 (CH_3Cl/MeOH/H_2O, 70:30:5, v/v); ^{1}H NMR (400 MHz, CD_3OD) δ 1.59 (s, 9H), 1.61 (s, 3H), 1.68 (s, 21H), 1.72 (d, J=0.9 Hz, 3H), 1.95–2.12 (m, 40H), 4.40 (br t, J=5.5 Hz, 2H), 5.07–5.17 (m, 10H), 5.45 (t, J=6.6 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 16.2, 17.9, 23.8, 23.84, 23.9, 26.0, 27.5, 27.6, 27.7, 27.8, 27.9, 32.9, 33.26, 33.3, 33.35, 33.37, 40.8, 40.9, 61.8 (d, J_{C-P}=3.8 Hz), 125.45, 125.5, 125.52, 125.8 (d, J_{C-P}=8.6 Hz), 126.1, 126.2, 126.22, 132.0, 135.8, 136.0, 136.1, 136.2, 136.3, 137.9. MALDI-TOF MS: $[M+2Na]^+$ calcd for $C_{55}H_{89}Na_2O_4P$, 891.6, found 891.6.

4.8. 2,4-Diacetamido-1,3-di-*O*-acetyl-2,4,6-trideoxy-D-glucopyranose (14)

To a solution of bacillosamine (**13**)[23] (200 mg, 0.812 mmol) in pyridine (2 ml) were added Ac_2O (500 μL) and DMAP (catalytic amount). After stirring at room temperature for 3 h, reaction mixture was concentrated in vacuo. The residure was purified by column chromatography on Iatrobeads ($CHCl_3$/MeOH, 9:1, v/v) to afford compound **14** (255 mg, 0.772 mmol, 95%, α/β=9:1).

R_f=0.38 ($CHCl_3$/MeOH, 9:1, v/v); **14α**: ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD, 5:1, v/v) δ 1.12 (d, J=6.0 Hz, 3H, H-6), 1.84 (s, 3H, Ac), 1.85 (s, 3H, Ac), 1.96 (s, 3H, Ac), 2.09 (s, 3H, Ac), 3.73–3.85 (m, 2H, H-4, H-5), 4.29 (dd, J=11.0, 3.6 Hz, 1H, H-2), 5.04 (t, J=11.0 Hz, 1H, H-3), 6.01 (d, J=3.6 Hz, 1H, H-1); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD, 5:1, v/v) δ 17.6, 20.6, 20.7, 22.4, 22.7, 51.2, 54.9, 68.7, 70.4, 91.0, 169.7, 171.2, 171.3, 172.2; **14β**: ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD, 5:1, v/v) δ 1.17 (d, J=6.4 Hz, 3H, H-6), 1.82 (s, 3H, Ac), 1.84 (s, 3H, Ac), 1.95 (s, 3H, Ac), 2.02 (s, 3H, Ac), 3.68–3.85 (m, 2H, H-4, H-5), 3.92 (dd, J=11.0, 9.2 Hz, 1H, H-2), 5.03 (t, J=11.0 Hz, 1H, H-3), 5.63 (d, J=9.2 Hz, 1H, H-1); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD, 5:1, v/v) δ 17.6, 20.5, 20.8, 22.5, 22.7, 51.2, 54.9, 71.7, 72.4, 92.2, 170.0, 171.4, 171.6, 172.2. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{14}H_{22}N_2NaO_7$, 353.1, found 353.3; HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{14}H_{22}N_2NaO_7$, 353.1325, found 353.1300.

4.9. 3-*O*-Acetyl-2,4-diacetamido-2,4,6-trideoxy-D-glucopyranose (15)

The mixture of acetylated bacillosamine **14** (255 mg, 0.772 mmol) and powdered, activated 4 Å molecular sieves (300 mg) in MeOH (3 mL) and CH_2Cl_2 (3 mL) was stirred at room temperature for 2 h. The reaction mixture was neutralized with Dowex CCR-3 (H^+ mode) resin, filtered through Celite®, and concentrated. The residue was purified by column chromatography on Iatrobeads ($CHCl_3$/MeOH, 7:1, v/v) to afford compound **15** (149 mg, 0.517 mmol, 67%, α/β=4:1).

15α: R_f=0.3 ($CHCl_3$/MeOH, 7:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD, 1:1, v/v) δ 0.89 (d, *J*=6.0 Hz, 3H, H-6), 1.63 (s, 3H, Ac), 1.66 (s, 3H, Ac), 1.72 (s, 3H, Ac), 3.54 (t, *J*=10.6 Hz, 1H, H-4), 3.72–3.79 (m, 1H, H-5), 3.89 (dd, *J*=10.6, 3.6 Hz, 1H, H-2), 4.81 (d, *J*=3.6 Hz, 1H, H-1), 4.84 (t, *J*=10.6 Hz, 1H, H-3); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD, 1:1, v/v) δ 17.2, 20.0, 21.8, 21.9, 52.4, 54.8, 65.5, 71.2, 90.9, 171.3, 171.4, 171.6; **15β**: R_f=0.23 ($CHCl_3$/MeOH, 7:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD, 1:1, v/v) δ 0.94 (d, *J*=6.4 Hz, 3H, H-6), 1.63 (s, 3H, Ac), 1.64 (s, 3H, Ac), 1.73 (s, 3H, Ac), 3.25–3.32 (m, 1H, H-5), 3.45 (t, *J*=10.6 Hz, 1H, H-4), 3.93 (dd, *J*=10.6, 8.7 Hz, 1H, H-2), 4.41 (d, *J*=8.7 Hz, 1H, H-1), 4.76 (t, *J*=10.6 Hz, 1H, H-3); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD, 1:1, v/v) δ 17.2, 19.9, 21.9, 22.0, 55.0, 55.9, 70.3, 72.6, 94.7, 171.1, 171.4, 171.5. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{12}H_{20}N_2NaO_6$, 311.1, found 311.1; HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{12}H_{20}N_2NaO_6$, 311.1219, found 311.1218.

4.10. Dibenzyl 3-*O*-acetyl-2,4-diacetamido-2,4,6-trideoxy-α-D-glucopyranosyl phosphate (16)

To a solution of hemiacetal **15** (55 mg, 0.19 mmol) in DMF (1 mL) was added dropwise lithium hexamethyldisilazide (1 M solution in THF, 248 μL, 0.248 mmol) at −50 °C. The mixture was stirred for 10 min and then a solution of tetrabenzyl pyrophosphate (154 mg, 0.286 mmol) in THF (0.3 mL) was added. After stirring at −50 °C for further 1 h, the reaction mixture was allowed to warm up to 0 °C over 30 min. The mixture was diluted with $CHCl_3$ (10 mL), washed with saturated aqueous $NaHCO_3$ (2×2 mL) and brine (2 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on Iatrobeads ($CHCl_3$/MeOH, 19:1, v/v) to afford compound **16** (48 mg, 0.088 mmol, 46%).

R_f=0.38 ($CHCl_3$/MeOH, 19:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.19 (d, *J*=6.4 Hz, 3H, H-6), 1.81 (s, 3H, Ac), 1.92 (s, 3H, Ac), 1.97 (s, 3H, Ac), 3.95–4.02 (m, 1H, H-5), 4.11 (q, *J*=10.6 Hz, 1H, H-4), 4.41–4.47 (m, 1H, H-2), 4.98–5.04 (m, 4H, 2×PhC*H*$_2$), 5.19 (t, *J*=10.6 Hz, 1H, H-3), 5.64 (dd, *J*=6.0, 3.2 Hz, 1H, H-1), 6.81 (d, *J*=9.2 Hz, 1H, N*H*-2), 7.24–7.36 (m, 10H, Ar–H), 7.42 (d, *J*=9.5 Hz, 1H, N*H*-4); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.8, 20.9, 22.6, 23.0, 52.3 (d, J_{C-P}=8.6 Hz, C-2), 54.0, 69.7, 69.8, 69.9, 70.1, 97.7 (d, J_{C-P}=8.6 Hz, C-1), 127.8, 128.0, 128.8, 128.9, 129.0, 129.1, 135.1 (d, J_{C-P}=6.7 Hz), 135.3 (d, J_{C-P}=7.6 Hz), 170.6, 170.8, 172.1; ^{31}P NMR (162 MHz, $CDCl_3$) δ −3.8. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{26}H_{33}N_2NaO_9P$, 571.2, found 571.3; HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{26}H_{33}N_2NaO_9P$, 571.1821, found 571.1845.

4.11. 3-*O*-Acetyl-2,4-diacetamido-2,4,6-trideoxy-α-D-glucopyranosyl phosphate (17)

The mixture of dibenzyl phosphate **16** (23 mg, 0.042 mmol) and Pd/C (10%, 23 mg) in MeOH (1 mL) under H_2 (1 atm) atmosphere was stirred at room temperature for 3 h. The reaction mixture was filtered through Celite® and concentrated in vacuo to afford compound **17** (15 mg, 0.041 mmol, 98%).

R_f=0.10 ($CHCl_3$/MeOH/H_2O, 70:30:5, v/v); ^{1}H NMR (400 MHz, CD_3OD) δ 1.17 (d, *J*=6.0 Hz, 3H, H-6), 1.91 (s, 3H, Ac), 1.94 (s, 3H, Ac), 1.97 (s, 3H, Ac), 3.83 (t, *J*=10.6 Hz, 1H, H-4), 4.07–4.14 (m, 1H, H-5), 4.19–4.22 (m, 1H, H-2), 5.17 (t, *J*=10.6 Hz, 1H, H-3), 5.45 (dd, *J*=6.4, 2.8 Hz, 1H, H-1); ^{13}C NMR (100 MHz, CD_3OD) δ 18.0, 20.7, 22.6, 22.7, 53.9 (d, J_{C-P}=8.6 Hz, C-2), 56.3, 68.4, 72.8, 95.3 (d, J_{C-P}=5.7 Hz, C-1), 172.4, 173.3, 173.5; ^{31}P NMR (162 MHz, CD_3OD) δ −0.6. MALDI-TOF MS: $[M-H]^-$ calcd for $C_{12}H_{20}N_2O_9P$, 367.1, found 367.1; HRMS ESI-TOF: $[M-H]^-$ calcd for $C_{12}H_{20}N_2O_9P$, 367.0906, found 367.0921.

4.12. Undecaprenyl 3-*O*-acetyl-2,4-diacetamido-2,4,6-trideoxy-α-D-glucopyranosyl diphosphate (18)

To a solution of bacillosamine phosphate **17** (4 mg, 10.9 μmol) in DMF (500 μL) was added carbonyl diimidazole (CDI) (4 mg, 24.7 μmol). After stirring at room temperature for 6 h, the excess CDI was quenched with MeOH (3 μL) and stirred for 1 h. The reaction mixture was concentrated in vacuo, then solution of undecaprenyl phosphate (**2**) (8 mg, 9.0 μmol) in DMF (0.5 mL) and $(CH_2Cl)_2$ (1 mL) was added to the reaction mixture. After stirring at 50 °C for 3 day, reaction mixture was concentrated in vacuo. The residure was purified by column chromatography on Iatrobeads ($CHCl_3$/MeOH/2 M NH_4OH, 80:20:4, v/v/v) to afford compound **18** (8 mg, 6.7 μmol, 74%).

R_f=0.45 ($CHCl_3$/MeOH/H_2O, 70:30:5, v/v/v); ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD, 1:1, v/v) δ 1.21 (m, 3H, H-6), 1.61 (s, 3H, C*H*$_3$), 1.62 (s, 9H, 3×C*H*$_3$), 1.69 (s, 21H, 7×C*H*$_3$), 1.74 (s, 3H, C*H*$_3$), 1.94–2.19 (m, 49H, 20×allylC*H*$_2$, 3×Ac), 3.88–3.90 (m, 1H, H-4), 4.24–4.28 (m, 2H, H-2, H-5), 4.48 (br s, 2H, =CHC*H*$_2$O), 5.02–5.19 (m, 11H, 10×=C*H*CH$_2$, H-3), 5.37 (br s, 1H, H-1), 5.56 (br s, 1H, =C*H*CH$_2$O); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD, 1:1, v/v) δ 15.7, 15.8, 17.4, 17.45, 17.5, 20.4, 23.1, 23.2, 23.3, 25.5, 25.52, 26.2, 26.3, 26.5, 26.6, 29.6, 31.8, 32.1, 39.6, 52.1 (d, J_{C-P}=2.9 Hz, C-2), 54.6, 62.8 (d, J_{C-P}=3.8 Hz, =CHCH$_2$O), 67.7, 70.9, 94.9 (d, J_{C-P}=5.7 Hz, C-1), 124.1, 124.2, 124.3, 124.7, 124.85, 124.9, 131.1, 134.77, 134.8, 135.0, 135.15, 135.2, 135.3, 140.8, 171.5, 171.6, 172.2; ^{31}P NMR (162 MHz, $CDCl_3$/CD_3OD, 1:1, v/v) δ −9.2, −12.0. MALDI-TOF MS: $[M-H]^-$ calcd for $C_{67}H_{109}N_2O_{12}P_2$, 1195.7, found 1195.4; HRMS ESI-TOF: $[M-H]^-$ calcd for $C_{67}H_{109}N_2O_{12}P_2$, 1195.7456, found 1195.7443.

4.13. Undecaprenyl 2,4-diacetamido-2,4,6-trideoxy-α-D-glucopyranosyl diphosphate (3)

To a stirred solution of compound **18** (6.0 mg, 5.0 μmol) in CH_2Cl_2 (1 mL) was added 0.2 M NaOMe/MeOH (1 mL). The mixture was stirred at room temperature for 1 h and neutralized with Dowex-50WX8-200 ion exchange resin (pyridinium form). The resin was filtered off and the filtrate was concentrated in vacuo to give compound **3** (5.6 mg, 4.9 μmol, 97%).

R_f=0.43 ($CHCl_3$/MeOH/H_2O, 70:30:5, v/v/v); ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD, 1:1, v/v) δ 1.18 (m, 3H, H-6), 1.60 (s, 12H, 4×C*H*$_3$), 1.68 (s, 21H, 7×C*H*$_3$), 1.74 (s, 3H, C*H*$_3$), 1.93–2.15 (m, 46H, 20×allylC*H*$_2$, 2×Ac), 3.73–3.81 (m, 1H, H-4), 3.98–4.14 (m, 2H, H-2, H-5), 4.50 (br s, 2H, =CHC*H*$_2$O), 5.02–5.19 (m, 11H, 10×=C*H*CH$_2$, H-3), 5.39 (br s, 1H, H-1), 5.54 (br s, 1H, =C*H*CH$_2$O); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD, 1:1, v/v) δ 15.5, 17.2, 17.4, 22.86, 22.9, 23.0, 25.2, 25.99, 26.0, 26.2, 26.3, 26.4, 29.3, 31.6, 31.9, 39.4, 54.4, 57.0, 62.6, 68.0, 69.8, 94.9, 123.9, 124.0, 124.1, 124.6, 124.68, 124.7, 130.9, 134.5, 134.6, 134.8, 134.9, 135.0, 135.1, 140.3, 172.3, 172.9; ^{31}P NMR (162 MHz, $CDCl_3$/CD_3OD, 1:1, v/v) δ −5.3, −7.8. MALDI-TOF MS: $[M-H]^-$ calcd for $C_{65}H_{107}N_2O_{11}P_2$, 1153.7, found 1153.9; HRMS ESI-TOF: $[M-H]^-$ calcd for $C_{65}H_{107}N_2O_{11}P_2$, 1153.7350, found 1153.7332.

4.14. Tetra-*N*-acetylchitotetraose peracetate (20)

To a solution of tetra-*N*-acetylchitotetraose (**19**) (50 mg, 0.060 mmol) in pyridine (1 ml) were added Ac_2O (300 μL) and DMAP (catalytic amount). The reaction mixture was stirred for 4 h under sonicated condition, then after stirring at room temperature

for 48 h, reaction mixture was concentrated in vacuo. The residue was purified by column chromatography on Iatrobeads ($CHCl_3$/MeOH, 9:1, v/v) to afford compound **20** (65 mg, 0.052 mmol, 86%, α/β=2:1).

R_f=0.38 ($CHCl_3$/MeOH, 9:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD, 3:1, v/v) δ 1.62 (s, 3H, Ac), 1.63 (s, 6H, 2×Ac), 1.64 (s, 3H, Ac), 1.71 (s, 3H, Ac), 1.72 (s, 3H, Ac), 1.73 (s, 3H, Ac), 1.74 (s, 3H, Ac), 1.75 (s, 3H, Ac), 1.79 (s, 3H, Ac), 1.83 (s, 3H, Ac), 1.85 (s, 3H, Ac), 1.86 (s, 3H, Ac), 1.91 (s, 3H, Ac), 3.28–3.36 (m, 2H), 3.38–3.55 (m, 7H), 3.65–3.87 (m, 5H), 4.03–4.14 (m, 5H), 4.22–4.27 (m, 2H), 4.38–4.41 (m, 1H), 4.71 (t, J=10.0 Hz, 1H), 4.78–4.85 (m, 2H), 4.90–4.95 (m, 1H), 4.98 (t, J=10.1 Hz, 1H), 5.35 (d, J=8.7 Hz, 0.33H, H-1$^{GlcN\beta}$), 5.75 (d, J=3.7 Hz, 0.67H, H-1$^{GlcN\alpha}$); **20α**: ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD, 3:1, v/v) δ 19.8, 19.9, 20.0 (2), 20.02 (2), 20.1 (2), 20.12 (2), 20.2 (2), 22.0 (2), 50.4, 54.0, 54.1, 54.5, 61.4, 61.7, 62.3, 62.4, 68.0, 70.2, 70.6, 71.1, 71.9, 72.15, 72.2, 72.5, 72.6, 75.36, 75.4, 75.5, 90.0, 100.2, 100.3, 100.5, 169.3, 169.6, 170.2, 170.37, 170.4, 170.6, 170.7, 171.0 (2), 171.2 (2), 171.5, 171.54, 171.6; **20β**: ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD, 3:1, v/v) δ 91.7, 100.0, 100.2, 100.3. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{52}H_{74}N_4NaO_{31}$, 1273.4, found 1273.3; HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{52}H_{74}N_4NaO_{31}$, 1273.4235, found 1273.4227.

4.15. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(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-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-D-glucopyranose (21)

The mixture of acetylated tetra-*N*-acetylchitotetraose **20** (216 mg, 0.173 mmol) and powdered, activated 4 Å molecular sieves (300 mg) in MeOH (3 mL) and CH_2Cl_2 (3 mL) was stirred at room temperature for 5 h. The reaction mixture was neutralized with Dowex CCR-3 (H^+ mode) resin, filtered through Celite®, and concentrated. The residue was purified by column chromatography on Iatrobeads ($CHCl_3$/MeOH, 9:1, v/v) to afford compound **21** (128 mg, 0.106 mmol, 61%).

R_f=0.25 ($CHCl_3$/MeOH, 9:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD, 3:1, v/v) δ 1.82 (s, 3H, Ac), 1.83 (s, 3H, Ac), 1.84 (s, 3H, Ac), 1.89 (s, 6H, 2×Ac), 1.90 (s, 3H, Ac), 1.92 (s, 6H, 2×Ac), 1.93 (s, 3H, Ac), 1.99 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.06 (s, 3H, Ac), 3.53–3.62 (m, 5H), 3.73 (t, J=9.6 Hz, 1H), 3.78–3.87 (m, 2H), 3.90–3.94 (m, 1H), 3.98–4.13 (m, 5H), 4.17–4.31 (m, 7H), 4.42 (d, J=8.7 Hz, 1H), 4.88–4.94 (m, 3H), 5.01 (d, J=3.7 Hz, H-1$^{GlcN\alpha}$), 5.07 (t, J=10.1 Hz, 1H), 5.30 (t, J=10.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD, 3:1, v/v) δ 20.1, 20.2 (3), 20.23 (2), 20.3, 20.4 (2), 20.43, 22.2, 22.3, 22.31, 51.9, 53.9 (2), 54.4, 61.5, 62.4, 62.5 (2), 67.7, 68.1, 71.2, 71.3, 72.0, 72.2, 72.3 (2), 72.5, 75.5, 75.8, 76.0, 90.9, 100.5, 100.6, 100.9, 169.6, 170.4, 170.5, 170.6, 170.7, 170.9, 171.1 (2), 171.3, 171.36, 171.4, 171.5, 172.0. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{50}H_{72}N_4NaO_{30}$, 1231.4, found 1231.4; HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{50}H_{72}N_4NaO_{30}$, 1231.4129, found 1231.4084.

4.16. Dibenzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(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-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl phosphate (22)

To a solution of hemiacetal **21** (38 mg, 0.031 mmol) in DMF (1 mL) was added dropwise lithium hexamethyldisilazide (1 M solution in THF, 40 μL, 0.040 mmol) at −50 °C. The mixture was stirred for 10 min and then a solution of tetrabenzyl pyrophosphate (25 mg, 0.046 mmol) in THF (0.3 mL) was added. After stirring at −50 °C for further 1 h, the reaction mixture was allowed to warm up to 0 °C over 30 min. The mixture was diluted with $CHCl_3$ (10 mL), washed with saturated aqueous $NaHCO_3$ (2×2 mL) and brine (2 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on Iatrobeads ($CHCl_3$/MeOH, 9:1, v/v) to afford compound **22** (39 mg, 0.027 mmol, 87%).

R_f=0.48 ($CHCl_3$/MeOH, 9:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD, 9:1, v/v) δ 1.68 (s, 3H, Ac), 1.82 (s, 3H, Ac), 1.83 (s, 3H, Ac), 1.84 (s, 3H, Ac), 1.91 (s, 3H, Ac), 1.93 (s, 9H, 3×Ac), 1.95 (s, 6H, 2×Ac), 2.00 (s, 3H, Ac), 2.07 (s, 6H, 2×Ac), 3.46–3.52 (m, 2H), 3.57–3.71 (m, 5H), 3.76–3.81 (m, 2H), 3.87–4.11 (m, 5H), 4.14–4.24 (m, 4H), 4.28–4.31 (m, 1H), 4.32 (d, J=8.2 Hz, 1H), 4.38 (d, J=8.2 Hz, 1H), 4.47 (d, J=8.7 Hz, 1H), 4.90–5.02 (m, 7H), 5.03–5.08 (m, 1H), 5.11 (t, J=10.1 Hz, 1H), 5.51 (dd, J=6.0, 3.2, H-1$^{GlcN\alpha}$), 7.23–7.32 (m, 10H, Ar–H); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD, 9:1, v/v) δ 20.3(4), 20.4, 20.47, 20.5, 20.6 (2), 22.2, 22.5 (3), 51.6 (d, J_{C-P}=8.6 Hz, C-2), 53.9, 53.94, 54.4, 61.7, 62.5, 62.6, 68.2, 69.6, 69.96, 70.0, 70.2, 70.3, 71.5, 72.3, 72.5, 72.6, 72.7, 72.9, 75.3, 75.6, 75.7, 96.0 (d, J_{C-P}=6.7 Hz, C-1), 100.8, 100.9, 100.91, 128.0, 128.7, 128.73, 128.9, 134.9 (d, J_{C-P}=7.6 Hz), 135.0 (d, J_{C-P}=6.7 Hz), 169.7, 170.5, 170.7, 170.8 (2), 170.9, 171.2, 171.3 (2), 171.4, 171.44 (3); ^{31}P NMR (162 MHz, $CDCl_3$/CD_3OD, 9:1, v/v) δ −2.1. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{64}H_{85}N_4NaO_{33}P$, 1491.5, found 1491.4; HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{64}H_{85}N_4NaO_{33}P$, 1491.4731, found 1491.4699.

4.17. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(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-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl phosphate (23)

The mixture of dibenzyl phosphate **22** (51 mg, 0.035 mmol) and Pd/C (10%, 50 mg) in MeOH (2 mL) and CH_2Cl_2 (1 mL) under H_2 (1 atm) atmosphere was stirred at room temperature for 3 h. The reaction mixture was filtered through Celite® and washed with MeOH. Et_3N (1 mL) was added followed by concentration in vacuo to give the mono (triethylammonium) salt of phosphate **23** (47 mg, 0.034 mmol, 97%).

R_f=0.18 ($CHCl_3$/MeOH/H_2O, 70:30:5, v/v/v); ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD, 1:1, v/v) δ 1.25 (t, J=7.3 Hz, 9H, $N(CH_2CH_3)_3$), 1.87 (s, 3H, Ac), 1.88 (s, 3H, Ac), 1.89 (s, 3H, Ac), 1.91 (s, 3H, Ac), 1.96 (s, 3H, Ac), 1.97 (s, 3H, Ac), 1.98 (s, 6H, 2×Ac), 2.02 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.09 (s, 3H, Ac), 2.11 (s, 3H, Ac), 2.12 (s, 3H, Ac), 3.01 (q, J=7.3 Hz, 6H, $N(CH_2CH_3)_3$), 3.59–3.76 (m, 8H), 3.81 (t, J=10.1 Hz, 1H), 3.98–4.16 (m, 6H), 4.33–4.42 (m, 3H), 4.49–4.52 (m, 1H), 4.57 (d, J=8.7 Hz, 1H), 4.65 (d, J=8.2 Hz, 1H), 4.73 (d, J=8.7 Hz, 1H), 4.95 (t, J=9.9 Hz, 1H), 5.12–5.19 (m, 2H), 5.21 (t, J=10.6 Hz, 1H), 5.30 (t, J=10.1 Hz, 1H), 5.40 (dd, J=6.8, 3.7 Hz, H-1$^{GlcN\alpha}$); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD, 1:1, v/v) δ 8.91, 20.6, 20.7, 20.8, 20.88, 20.9, 21.0 (4), 22.6, 22.8, 22.85, 22.9, 46.8, 52.7 (d, J_{C-P}=7.6 Hz, C-2), 55.3, 55.5, 55.6, 59.2, 62.4, 62.6, 63.3, 63.5, 69.1, 69.8, 72.1, 72.5, 72.8, 73.05, 73.1, 73.5, 76.3, 76.5, 76.7, 94.4 (d, J_{C-P}=5.7 Hz, C-1), 101.0, 101.1 (2), 170.7, 171.2, 171.3 (2), 171.5, 171.7, 171.9, 172.0, 172.1, 172.6, 172.7, 172.73, 127.8; ^{31}P NMR (162 MHz, $CDCl_3$/CD_3OD, 1:1, v/v) δ −0.9. MALDI-TOF MS: $[M-H]^-$ calcd for $C_{50}H_{72}N_4O_{33}P$, 1287.4, found 1287.4; HRMS ESI-TOF: $[M-H]^-$ calcd for $C_{50}H_{72}N_4O_{33}P$, 1287.3816, found 1287.3858.

4.18. Undecaprenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(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-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl diphosphate (24)

To a solution of phosphate **23** (12 mg, 8.6 μmol) in DMF (1 mL) was added carbonyl diimidazole (CDI) (4 mg, 24.7 μmol). After stirring at room temperature for 4 h, the excess CDI was quenched with MeOH (3 μL) and stirred for 1 h. The reaction mixture was

concentrated in vacuo, then solution of undecaprenyl phosphate (**2**) (8 mg, 9.0 μmol) in DMF (0.5 mL) and $(CH_2Cl)_2$ (1 mL) was added to the reaction mixture. After stirring at 50 °C for 3 day, reaction mixture was concentrated in vacuo. The residue was purified by column chromatography on Iatrobeads ($CHCl_3$/MeOH/H_2O, 70:30:5, v/v/v) to afford compound **24** (15 mg, 7.1 μmol, 82%).

R_f=0.48 ($CHCl_3$/MeOH/H_2O, 70:30:5, v/v/v); ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD, 1:1, v/v) δ 1.55 (s, 12H, 4×C*H*$_3$), 1.64 (s, 21H, 7×C*H*$_3$), 1.68 (s, 3H, C*H*$_3$), 1.86–2.14 (m, 79H, 20×allylC*H*$_2$, 13×Ac), 3.52–3.60 (m, 2H), 3.61–3.81 (m, 7H), 3.96–3.99 (m, 1H), 4.01–4.09 (m, 3H), 4.15–4.22 (m, 3H), 4.25–4.41 (m, 6H), 4.60–4.62 (m, 1H), 4.91–4.99 (m, 1H), 5.00–5.12 (m, 13H), 5.18–5.22 (m, 2H), 5.32 (br s, 1H), 5.48 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD, 1:1, v/v) δ 16.0, 17.6, 20.5, 20.6, 20.8, 22.7, 23.3, 23.4, 25.7, 26.2, 26.4, 26.6, 26.7, 29.7, 31.9, 32.2, 39.7, 53.9, 53.91, 54.2, 54.23, 54.4, 61.7, 61.8, 62.6, 62.7, 62.9, 68.2, 70.4, 71.6, 72.3, 72.4, 72.6, 72.62, 72.8, 75.6, 75.62, 76.8, 94.4, 101.0, 101.03, 101.1, 124.1, 124.2, 124.3, 124.9, 124.95, 125.0, 131.3, 134.9, 135.0, 135.3, 135.4, 135.5, 169.7, 170.6, 170.8, 170.9, 171.0 (3), 171.4 (3), 171.5, 171.9, 172.1; ^{31}P NMR (162 MHz, $CDCl_3$/CD_3OD, 1:1, v/v) δ −10.1, −12.5. MALDI-TOF MS: $[M-H]^-$ calcd for $C_{105}H_{161}N_4O_{36}P_2$, 2116.0, found 2116.5; HRMS ESI-TOF: $[M-H]^-$ calcd for $C_{105}H_{161}N_4O_{36}P_2$, 2116.0366, found 2116.0389.

4.19. Undecaprenyl tetra-*N*-acetylchitotetraosyl diphosphate (5)

To a stirred solution of compound **24** (9.0 mg, 4.3 μmol) in CH_2Cl_2 (1 mL) was added 0.2 M NaOMe/MeOH (1 mL). The mixture was stirred at room temperature for 3 h and neutralized with Dowex-50WX8-200 ion exchange resin (pyridinium form). The resin was filtered off and the filtrate was concentrated in vacuo to give compound **21** (5.9 mg, 3.4 μmol, 80%).

R_f=0.43 ($CHCl_3$/MeOH/2 M NH_4OH, 70:30:6, v/v/v); ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD/D_2O, 1:1:0.2, v/v/v) δ 1.51 (s, 12H, 4×C*H*$_3$), 1.59 (s, 21H, 7×C*H*$_3$), 1.63 (s, 3H, C*H*$_3$), 1.85–2.26 (m, 52H, 20×allylC*H*$_2$, 4×Ac), 3.32–3.40 (m, 4H), 3.42–3.54 (m, 10H), 3.62–3.92 (m, 9H), 3.98 (t, *J*=6.2 Hz, 1H), 4.04–4.15 (m, 2H), 4.98–5.08 (m, 10H), 5.25–5.32 (m, 2H), 5.38 (br s, 1H); ^{31}P NMR (162 MHz, $CDCl_3$/CD_3OD/D_2O, 1:1:0.2, v/v/v) δ −9.2, −11.8. MALDI-TOF MS: $[M-H]^-$ calcd for $C_{87}H_{143}N_4O_{27}P_2$, 1737.9, found 1737.8; HRMS ESI-TOF: $[M-H]^-$ calcd for $C_{87}H_{143}N_4O_{27}P_2$, 1737.9415, found 1737.9385.

4.20. *tert*-Butyldiphenylsilyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-galactopyranosyl-(1→3)-2,4-diacetamido-2,4,6-trideoxy-β-D-glucopyranoside (26)

To a solution of azido compound **25**[13] (150 mg, 0.126 mmol) in THF/H_2O (3:1, 8.0 mL) was added $CoCl_2\cdot(H_2O)_6$ (45 mg, 0.19 mmol) followed by slow addition of an aqueous solution of $NaBH_4$ (72 mg, 1.9 mmol in 1 mL H_2O) at 0 °C. The reaction mixture was stirred from ice bath to ambient temperature for 12 h. Monitoring of the reaction by MALDI-TOF MS revealed the reduction of four azides to amines. Then reaction mixture was again cooled to iced bath temperature followed by addition of Ac_2O (1 mL). After stirring for 3 h, reaction mixture was concentrated in vacuo. The residue was purified by column chromatography on Iatrobeads ($CHCl_3$/MeOH, 9:1, v/v) to afford compound **26** (104 mg, 0.083 mmol, 66%).

R_f=0.48 ($CHCl_3$/MeOH, 9:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (d, *J*=6.4 Hz, 3H), 1.03 (s, 9H), 1.73 (s, 3H), 1.79 (s, 3H), 1.86 (s, 3H), 1.95 (s, 3H), 3.03–3.10 (m, 1H), 3.16 (s, 1H, O*H*), 3.28 (dd, *J*=10.1, 5.0 Hz, 1H), 3.43 (dd, *J*=9.2, 5.0 Hz, 1H), 3.48–3.55 (m, 3H), 3.60–3.65 (m, 1H), 3.69–3.80 (m, 3H), 3.97 (t, *J*=6.2 Hz, 1H), 4.09–4.12 (m, 2H), 4.21 (d, *J*=11.9 Hz, 1H), 4.26 (d, *J*=12.4 Hz, 1H), 4.32–4.35 (m, 2H), 4.36–4.51 (m, 5H), 4.58–4.62 (m, 2H), 4.70 (d, *J*=12.4 Hz, 1H), 4.83 (d, *J*=3.2 Hz, 1H), 4.96 (d, *J*=3.2 Hz, 1H), 5.84 (d, *J*=9.6 Hz, 1H, N*H*), 5.91 (d, *J*=9.2 Hz, 1H, N*H*), 5.96 (d, *J*=8.7 Hz, 1H, N*H*), 6.38 (d, *J*=7.3 Hz, 1H, N*H*), 7.12–7.64 (m, 30H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.6, 19.2, 23.3, 23.4, 23.44, 26.8, 48.5, 48.6, 57.1, 58.3, 66.6, 68.8, 69.6, 69.8, 70.3, 71.0, 71.1, 71.4, 72.0, 73.6, 73.9, 75.7., 75.8, 77.4, 95.2, 97.8, 98.4, 127.4, 127.5, 127.8, 127.9, 128.3, 128.4, 128.5, 128.6, 129.1, 129.2, 133.3, 133.4, 135.8, 136.0, 136.6, 137,6, 137.9, 138.0, 170.1, 170.5 (2), 171.3. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{70}H_{86}N_4NaO_{15}Si$, 1273.6, found 1273.6; HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{70}H_{86}N_4NaO_{15}Si$, 1273.5757, found 1273.5713.

4.21. 2-Acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-galactopyranosyl-(1→3)-2,4-diacetamido-2,4,6-trideoxy-D-glucopyranose (27)

To a solution of TBDPS ether **26** (100 mg, 0.08 mmol) in THF (5 mL) were added AcOH (48 μL, 0.84 mmol) and *n*-Bu_4NF (400 μL, 0.4 mmol, 1.0 M solution in THF) at 0 °C. After stirring for 8 h at room temperature, reaction mixture was concentrated in vacuo. The residue was purified by column chromatography on Iatrobeads ($CHCl_3$/MeOH, 9:1, v/v) to afford compound **27** (69 mg, 0.068 mmol, 85%, α/β=4:1).

27α: R_f=0.35 ($CHCl_3$/MeOH, 9:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD, 9:1, v/v) δ 1.04 (d, *J*=4.1 Hz, 3H), 1.71 (s, 3H), 1.82 (s, 3H), 1.84 (s, 3H), 1.95 (s, 3H), 3.32–3.52 (m, 7H), 3.74–3.90 (m, 5H), 3.92–4.01 (m, 2H), 4.13–4.20 (m, 4H), 4.30–4.46 (m, 6H), 4.62–4.71 (m, 2H), 4.88 (br s, 1H), 4.99 (br s, 2H), 6.50 (d, *J*=9.2 Hz, 1H, N*H*), 6.72 (d, *J*=9.6 Hz, 1H, N*H*), 7.02–7.29 (m, 21H, Ar*H*, N*H*), 7.45 (d, *J*=8.7 Hz, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD, 9:1, v/v) δ 17.5, 22.5, 22.6, 22.7, 23.1, 48.4, 53.1, 56.9, 66.1, 66.6, 68.6, 69.2, 69.6, 70.4, 70.43, 71.1, 72.0, 73.0, 73.4, 73.7, 75.9, 76.0, 91.2, 97.1, 98.1, 126.5, 127.5, 127.6, 127.7, 128.0, 128.1, 128.2, 128.3, 128.4, 136.9, 137.7, 138.0, 138.1, 171.2, 171.3, 171.34, 172.4; **27β**: R_f=0.3 ($CHCl_3$/MeOH, 9:1, v/v); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD, 9:1, v/v) δ 91.6, 98.4, 99.1. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{54}H_{68}N_4NaO_{15}$, 1035.5, found 1035.5; HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{54}H_{68}N_4NaO_{15}$, 1035.4579, found 1035.4538.

4.22. 2-Acetamino-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-galactopyranosyl-(1→3)-1-*O*-acetyl-2,4-diacetamido-2,4,6-trideoxy-D-glucopyranose (28)

The mixture of benzyl ether compound **27** (69 mg, 0.068 mmol) and $Pd(OH)_2$/C (20%, 70 mg) in AcOH (4 mL) and H_2O (1 mL) under H_2 (1 atm) atmosphere was stirred at room temperature for 7 h. The reaction mixture was filtered through Celite®, washed with MeOH, and concentrated in vacuo. The residue was dissolved in pyridine (2 mL) and added Ac_2O (0.5 mL). After stirring at room temperature for 10 h, reaction mixture was concentrated in vacuo. The residue was purified by column chromatography on Iatrobeads ($CHCl_3$/MeOH, 9:1, v/v) to afford compound **28** (53 mg, 0.059 mmol, 87%, α/β=7:3).

R_f=0.35 ($CHCl_3$/MeOH, 9:1, v/v); **28α**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (d, *J*=6.9 Hz, 3H), 1.90–2.11 (m, 30H, 10×Ac), 3.81–4.25 (m, 9H), 4.32–4.40 (m, 1H), 4.42–4.52 (m, 3H), 4.61–4.68 (m, 1H), 4.93–5.02 (m, 2H), 5.18–5.23 (m, 2H), 5.43 (br s, 1H), 6.03 (br s, 1H, N*H*), 6.20 (br s, 1H, N*H*), 6.36 (br s, 1H, N*H*), 6.94 (br s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.7, 20.76, 20.8, 20.85, 20.9, 20.94, 21.1, 22.9, 23.18, 23.2, 23.5, 47.4, 48.7, 51.8, 60.8, 62.0, 66.86, 66.9, 67.0, 67.9, 68.3, 69.4, 72.1, 73.0, 73.9, 91.5, 98.1, 98.8, 170.28, 170.3, 170.4, 170.5, 170.6, 170.7, 170.8, 170.9, 171.0, 171.2; **28β**: ^{13}C NMR (100 MHz, $CDCl_3$) δ 92.1, 98.8, 99.1. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{38}H_{56}N_4NaO_{21}$, 927.3, found 927.3; HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{38}H_{56}N_4NaO_{21}$, 927.3335, found 927.3319.

4.23. 2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-3,6-di-*O*-acetyl-α-D-galactopyranosyl-(1→3)-2,4-diacetamido-2,4,6-trideoxy-α-D-glucopyranoside (29)

The mixture of acetylated compound **28** (138 mg, 0.153 mmol) and powdered, activated 4 Å molecular sieves (200 mg) in MeOH (15 mL) and CH_2Cl_2 (5 mL) was stirred at room temperature for 40 min. The reaction mixture was neutralized with Dowex CCR-3 (H^+ mode) resin, filtered through Celite®, and concentrated. The residue was purified by column chromatography on Iatrobeads ($CHCl_3$/MeOH, 7:1, v/v) to afford compound **29** (92 mg, 0.107 mmol, 70%, α/β=7:1).

29α: R_f=0.33 ($CHCl_3$/MeOH, 7:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD, 4:1, v/v) δ 1.11–1.13 (m, 3H), 1.76–2.00 (m, 27H, 9×Ac), 3.71–3.89 (m, 6H), 3.94–4.05 (m, 4H), 4.24–4.41 (m, 4H), 4.78–4.86 (m, 2H), 4.91–4.94 (m, 1H), 5.03–5.11 (m, 2H), 5.31 (br s, 1H), 6.45 (d, *J*=9.2 Hz, 1H, N*H*), 7.13 (d, *J*=9.6 Hz, 1H, N*H*), 7.41 (d, *J*=8.7 Hz, 1H, N*H*), 7.50 (br s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD, 4:1, v/v) δ 17.2, 20.3, 20.4 (3), 20.5, 22.2, 22.4, 22.6 (2), 47.2, 47.8, 52.9, 56.7, 61.0, 61.8, 66.5, 66.6, 66.8, 67.6, 68.1, 69.2, 72.3, 77.4, 91.3, 96.7, 97.5, 170.5 (4), 170.9, 171.1, 171.4, 171.7, 172.3; **29β**: R_f=0.25 ($CHCl_3$/MeOH, 7:1, v/v); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD, 4:1, v/v) δ 91.4, 97.2, 97.7. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{36}H_{54}N_4NaO_{20}$, 885.3, found 885.3; HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{36}H_{54}N_4NaO_{20}$, 885.3229, found 885.3221.

4.24. 2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-3,6-di-*O*-acetyl-α-D-galactopyranosyl-(1→3)-2,4-diacetamido-2,4,6-trideoxy-α-D-glucopyranosyl phosphate (30)

To a solution of hemiacetal **29** (10 mg, 11.6 μmol) in DMF (1 mL) was added dropwise lithium hexamethyldisilazide (1 M solution in THF, 14 μL, 14 μmol) at −50 °C. The mixture was stirred for 10 min and then a solution of tetrabenzyl pyrophosphate (8 mg, 14.9 μmol) in THF (0.1 mL) was added. After stirring at −50 °C for further 1 h, the reaction mixture was allowed to warm up to 0 °C over 30 min. The mixture was diluted with $CHCl_3$ (10 mL), washed with saturated aqueous $NaHCO_3$ (2×2 mL) and brine (2 mL), dried over $MgSO_4$, and concentrated in vacuo without further purification. The mixture of crude dibenzyl phosphate (R_f=0.55, $CHCl_3$/MeOH=9:1, v/v) and Pd/C (10%, 10 mg) in MeOH (1 mL) under H_2 (1 atm) atmosphere was stirred at room temperature for 3 h. The reaction mixture was filtered through Celite® and concentrated in vacuo. The residue was purified by column chromatography on Iatrobeads ($CHCl_3$/MeOH/H_2O, 6:4:1, v/v/v) to afford compound **30** (4 mg, 4.2 mmol, 37%).

R_f=0.45 ($CHCl_3$/MeOH/H_2O, 6:4:1, v/v/v); ^{1}H NMR (400 MHz, CD_3OD) δ 1.12 (d, *J*=5.0 Hz, 3H), 1.90 (s, 3H), 1.95 (s, 3H), 1.98 (s, 3H), 2.00 (s, 3H), 2.01 (s, 6H), 2.07 (s, 3H), 2.12 (s, 6H), 3.93–4.08 (m, 5H), 4.13–4.23 (m, 4H), 4.41–4.45 (m, 2H), 4.55–4.62 (m, 2H), 4.95–5.01 (m, 2H), 5.22–5.25 (m, 2H), 5.39 (br s, 1H), 5.50 (br s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 17.8, 20.5, 20.6, 20.7, 20.8, 21.1, 22.6, 22.9, 23.0, 23.1, 54.5, 54.6, 58.2, 62.0, 62.4, 67.9, 68.3, 68.8, 69.0, 69.8, 71.0, 74.9, 75.1, 95.4 (d, J_{C-P}=3.8 Hz, C-1), 98.8, 99.8, 171.7, 171.8, 171.9, 172.0, 172.1, 172.9, 173.8, 173.9, 174.1; ^{31}P NMR (162 MHz, CD_3OD) δ −0.3. MALDI-TOF MS: $[M-H]^-$ calcd for $C_{36}H_{54}N_4O_{23}P$, 941.3, found 941.3; HRMS ESI-TOF: $[M-H]^-$ calcd for $C_{36}H_{54}N_4O_{23}P$, 941.2916, found 941.2949.

4.25. Undecaprenyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-3,6-di-*O*-acetyl-α-D-galactopyranosyl-(1→3)-2,4-diacetamido-2,4,6-trideoxy-α-D-glucopyranosyl diphosphate (31)

To a solution of phosphate **30** (4 mg, 4.2 μmol) in DMF (500 μL) was added carbonyl diimidazole (CDI) (2 mg, 12.3 μmol). After stirring at room temperature for 6 h, the excess CDI was quenched with MeOH (2 μL) and stirred for 1 h. The reaction mixture was concentrated in vacuo, then solution of undecaprenyl phosphate (**2**) (4 mg, 4.5 μmol) in DMF (0.5 mL) and $(CH_2Cl)_2$ (1 mL) was added to the reaction mixture. After stirring at 50 °C for 3 day, reaction mixture was concentrated in vacuo. The residue was purified by column chromatography on Iatrobeads ($CHCl_3$/MeOH/H_2O, 70:30:5, v/v/v) to afford compound **31** (5 mg, 2.82 μmol, 67%).

R_f=0.55 ($CHCl_3$/MeOH/H_2O, 70:30:5, v/v/v); ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD, 2:1, v/v) δ 1.13 (s, 3H), 1.55 (s, 3H, C*H*$_3$), 1.60 (s, 18H, 6×C*H*$_3$), 1.68 (s, 12H, 4×C*H*$_3$), 1.72 (s, 3H, C*H*$_3$), 1.83–2.15 (m, 67H, 20×allylC*H*$_2$, 9×Ac), 3.65–3.88 (m, 9H), 3.99–4.04 (m, 1H), 4.09–4.15 (m, 2H), 4.29–4.33 (m, 2H), 4.41–4.55 (m, 4H), 4.97–5.12 (m, 11H), 5.20–5.27 (m, 1H), 5.33 (br s, 1H), 5.47 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD, 2:1, v/v) δ 15.9, 17.6, 20.5, 22.6, 23.2, 23.3, 25.6, 26.2, 26.4, 26.6, 26.7, 29.6, 31.9, 32.1, 32.2, 39.7, 43.3, 53.1, 60.9, 61.0, 66.6, 66.9, 67.0, 67.68, 67.7, 68.2, 68.3, 68.4, 69.1, 69.4, 70.3, 72.6, 97.7, 97.9, 98.0, 124.1, 124.2, 124.4, 124.8, 124.9, 125.0, 134.9, 135.2, 135.22, 135.2, 170.6, 170.7 (3), 170.8, 171.4, 171.42, 171.5, 171.6; ^{31}P NMR (162 MHz, $CDCl_3$/CD_3OD, 2:1, v/v) δ −10.3, −12.1. MALDI-TOF MS: $[M-H]^-$ calcd for $C_{91}H_{143}N_4O_{26}P_2$, 1769.9, found 1770.0; HRMS ESI-TOF: $[M-H]^-$ calcd for $C_{91}H_{143}N_4O_{26}P_2$, 1769.9466, found 1769.9492.

4.26. Undecaprenyl 2-acetamido-2-deoxy-α-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-α-D-galactopyranosyl-(1→3)-2,4-diacetamido-2,4,6-trideoxy-α-D-glucopyranosyl diphosphate (4)

To a stirred solution of compound **31** (5.0 mg, 2.8 μmol) in CH_2Cl_2 (1 mL) was added 0.2 M NaOMe/MeOH (1 mL). The mixture was stirred at room temperature for 2 h and neutralized with Dowex-50WX8-200 ion exchange resin (pyridinium form). The resin was filtered off and the filtrate was concentrated in vacuo to give compound **4** (3.5 mg, 2.2 μmol, 79%).

R_f=0.18 ($CHCl_3$/MeOH/H_2O, 70:30:5, v/v/v); ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD, 1:1, v/v) δ 1.26 (br s, 3H), 1.60 (s, 9H, 3×C*H*$_3$), 1.61 (s, 3H, C*H*$_3$), 1.68 (s, 21H, 7×C*H*$_3$), 1.73 (s, 3H, C*H*$_3$), 1.83–2.15 (m, 52H, 20×allylC*H*$_2$, 4×Ac), 3.61–3.69 (m, 4H), 3.76–3.91 (m, 5H), 4.09–4.24 (m, 5H), 4.26–4.34 (m, 2H), 4.42–4.50 (m, 2H), 5.01–5.22 (m, 12H), 5.36 (br s, 1H), 5.52 (br s, 1H); ^{31}P NMR (162 MHz, $CDCl_3$/CD_3OD, 1:1, v/v) δ −9.3, −12.1. MALDI-TOF MS: $[M-H]^-$ calcd for $C_{81}H_{133}N_4O_{21}P_2$, 1559.9, found 1559.4; HRMS ESI-TOF: $[M-H]^-$ calcd for $C_{81}H_{133}N_4O_{21}P_2$, 1559.8938, found 1559.8965.

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