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Synthesis of acceptor substrate analogs for the study of glycosyltransferases involved in the second step of the biosynthesis of O-antigen repeating units

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ABSTRACT

O-antigens of Gram negative bacteria are polysaccharides covalently attached to lipopolysaccharides (LPS) that have roles as virulence factors. Due to the lack of defined substrates for in vitro assays only a few of the enzymes involved in the biosynthesis of O-antigens have been studied. Many O-antigens have GlcNAc at the reducing end of the oligosaccharide chain linked to pyrophosphate-lipid. We therefore designed and synthesized a series of GlcNAc-pyrophosphate-lipid analogs of the natural GlcNAc-pyrophosphate-undecaprenol acceptor substrate for studies of the acceptor specificities of O-antigen biosynthetic enzymes. We synthesized analogs with modifications of the pyrophosphate bond as well as the lipid chain. These compounds will be useful for the specificity studies of many bacterial glycosyltransferases. Knowledge of the substrate specificities is the basis for the development of specific glycosyltransferase inhibitors that could block O-antigen biosynthesis.

1. Introduction

Lipopolysaccharides (LPS) of Gram negative bacteria are important components of the outer bacterial membrane where they function to support the structural integrity of the organism and protect the cell from external chemical attack.[1,2] LPS are antigenic, act as pro-inflammatory agents, and are considered to be important virulence factors. The outermost portion of the LPS is the O-antigenic polysaccharide (O-antigen) which is of crucial importance, since it determines the pathogenicity by controlling host–bacterial interactions.[3] The O-antigen is thought to be assembled as repeating units of shorter oligosaccharides linked to pyrophosphate-lipids.[1,2] The natural acceptor substrate for the first step of repeating unit biosynthesis is undecaprenol-phosphate which is enzymatically converted to sugar-pyrophosphate-undecaprenol by the reversible transfer of sugar-phosphate from nucleotide sugar. The biosynthetic enzymes required for the transfer of sugars to form the repeating unit are thought to reside on the cytosolic side of the inner bacterial membrane. Many of the O-antigens contain GlcNAc as the first sugar at the reducing end of the repeating unit which has been transferred as GlcNAcα-phosphate from UDP-GlcNAc. Subsequently, glycosyltransferases add a second sugar residue to the nonreducing end of GlcNAc, followed by other transferases that complete the synthesis of the repeating unit. In the biosynthetic pathway of heteropolymeric O-antigens, dependent on polymerase Wzy, the repeating unit is flipped to the periplasm, polymerized, and then transferred from undecaprenol-phosphate to the outer core oligosaccharide of lipid A to form LPS. The completed LPS is then translocated to the outer membrane.

A number of proteins are involved in the regulation of this process, and several different mechanisms of assembly have been proposed.[1] Many of the genes encoding biosynthetic enzymes have been identified in O-antigen gene clusters. The location of the genes and the structures of O-antigens suggest the pathways that are involved in the assembly of O-antigen repeating units. Genes that potentially encode glycosyltransferases have been assigned mainly by comparison of the sequences and overall folds of the gene products to those of other glycosyltransferases. However, very few of the glycosyltransferases have been assayed and biochemically characterized.[4–9]

We have recently synthesized GlcNAcα-PO_3-PO_3-$(CH_2)_{11}$-*O*-phenyl (GlcNAc-PP-PhU)[4] as a natural substrate analog and have shown that the GlcNAc derivative was an excellent acceptor substrate for enzymes from *Escherichia coli*, *Salmonella*, and *Shigella* that catalyze the second reaction of the repeating unit assembly by transferring a sugar residue to GlcNAc. This synthetic substrate allowed the characterization of these enzymes. In another synthetic method, UDP-GlcNAc which has GlcNAc in the α-configuration can be conveniently used as the starting material for the synthesis of GlcNAcα-pyrophosphate substrate analogs.[10]

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Table 1
O-antigen structures of different serotypes of Gram negative bacteria, terminating in GlcNAc at the reducing end of the O-antigen repeating unit (ECODAB), and the putative enzymes expected to add a second sugar to GlcNAc-PP-R in the synthesis of the repeating unit

Serotype	Linkage	Putative enzyme	Short name	Starting NTP	Donor
Escherichia coli					
O7	D-Galβ1-3	β3-Gal-T	WbbD	UDP-Glc	UDP-Gal
O55	D-Galβ1-3	β3-Gal-T	WbgM	UDP-Glc	UDP-Gal
O64; O114; O153	D-Galβ1-3	β3-Gal-T		UDP-Glc	UDP-Gal
O83; O136	D-Galβ1-4	β4-Gal-T		UDP-Glc	UDP-Gal
O1; O1B; O3; O10; O18A1; O18ac; O18B; O18B1; O18A, O18A1; O69; O113; O126	D-Galα1-3	α3-Gal-T		UDP-Glc	UDP-Gal
O111	D-Galα1-3	α3-Gal-T	WbbP	UDP-Glc	UDP-Gal
O116	D-GalAα1-3	α3-D-GalA-T		UDP-GlcA	UDP-GalA
O65	D-GalAβ1-3	β3-D-GalA-T		UDP-GlcA	UDP-GalA
O23A; O121	D-GalNAcα1-3	α3-GalNAc-T		UDP-GlcNAc	UDP-GalNAc
O138	D-GalNAcAα1-3	α3-GalNAcA-T		UDP-GlcNAc	UDP-GalNAcA
O56	D-Glcβ1-3	β3-Glc-T	WfaP	UDP-Glc	UDP-Glc
O152	D-Glcβ1-3	β3-Glc-T	WfgD	UDP-Glc	UDP-Glc
O173	D-Glcβ1-3	β3-Glc-T		UDP-Glc	UDP-Glc
O148	D-Glcα1-3	α3-Glc-T	WbbG	UDP-Glc	UDP-Glc
O126	D-GlcNAcα1-3	α3-GlcNAc-T		UDP-GlcNAc	UDP-GlcNAc
O98	L-QuiNAcα1-3	α3-L-QuiNAc-T	WbwW	UDP-D-GlcNAc	UDP-L-QuiNAc
O6; O44; O66; O77	D-Manβ1-3	β3-Man-T		GDP-Man	GDP-Man
O78; O88	D-Manα1-3	α3-Man-T		GDP-Man	GDP-Man
O58	D-Man2Acβ1-3	α3-Man2Ac-T		UDP-GlcNAc	UDP-Man2Ac
O141	D-Man6Acα1-3	α3-Man6Ac-T		UDP-GlcNAc	UDP-Man6Ac
O159	L-Fucα1-3	α3-Fuc-T		GDP-Fuc	GDP-L-Fuc
O4; O25; O26	L-FucNAcα1-3	α3-FucNAc-T		UDP-GlcNAc	UDP-L-FucNAc
O105	L-Rhaβ1-3	β3-L-Rha-T		dTDP-L-Rha	dTDP-L-Rha
O1A; O2; O149	L-Rhaβ1-4	β4-L-Rha-T		dTDP-L-Rha	dTDP-L-Rha
O16; O31; O75; O119; O139	L-Rhaα1-3	α3-L-Rha-T		dTDP-L-Rha	dTDP-L-Rha
O16	L-Rha2Acα1-3	α3-L-Rha2Ac-T		dTDP-L-Rha	dTDP-L-Rha
Salmonella					
O35 (23)	D-Galβ1-4	β4-D-Gal-T		UDP-Glc	UDP-Gal
O43	D-GalNAcα1-3	α3-D-GalNAc-T	WfbG	UDP-GlcNAc	UDP-GalNAc
O145 (105)	L-FucNAmα1-3	α3-L-FucNAm-T		UDP-GlcNAc	UDP-L-FucNAc
O111 (88)	D-Galα1-3	α3-D-Gal-T		UDP-Glc	UDP-Gal
Shigella					
O7 (83); O114 (85); O40	D-Galβ1-3	β3-D-Gal-T		UDP-Glc	UDP-Gal
D9	D-Galβ1-3	β3-D-Gal-T	WbdH	UDP-Glc	UDP-Gal
O136 (B14)	D-Galβ1-4	β4-D-Gal-T	WfeD	UDP-Glc	UDP-Gal
O167 (32)	D-Galfβ1-3	β3-D-Galf-T		UDP-Glc	UDP-Galf
O121 (78)	D-GalNAcAα1-3	α3-D-GalNAcA-T		UDP-GlcNAc	UDP-GalNAcA
O152 (D12)	D-Glcβ1-3	β3-D-Glc-T		UDP-Glc	UDP-Glc
O148	D-Glcα1-3	α3-D-Glc-T	WbbG	UDP-Glc	UDP-Glc
O143 (43)	D-GlcAβ1-3	β3-D-GlcA-T		UDP-Glc	UDP-GlcA
B5; B9	D-GlcAα1-3	α3-D-GlcA-T		UDP-GlcA	UDP-GlcA
B16	D-Manβ1-3	β3-D-Man-T		UDP-Man	UDP-Man
O58 (84)	D-Man2Acα1-3	α3-D-Man2Ac-T		UDP-GlcNAc	UDP-Man2Ac
O159 (15); O168(D4)	L-Fucα1-3	α3-L-Fuc-T		GDP-Fuc	GDP-L-Fuc
O29 (D11)	L-FucNAcβ1-3	β3-L-FucNAc-T		UDP-GlcNAc	UDP-L-FucNAc
O172 (4)	L-FucNAcα1-3	α3-L-FucNAc-T		UDP-GlcNAc	UDP-L-FucNAc
B11; O150(D13)	L-Rhaβ1-3	β3-L-Rha-T		dTDP-L-Rha	dTDP-L-Rha
O149 (B1); B4	L-Rhaβ1-4	β4-L-Rha-T		dTDP-L-Rha	dTDP-L-Rha
D10	L-Rhaα1-4	α4-L-Rha-T		dTDP-L-Rha	dTDP-L-Rha
B13	L-QuiNAcα1-3	α3-L-QuiNAc-T		UDP-GlcNAc	UDP-L-QuiNAc
Yersinia					
O98 (86)	L-QuiNAcα1-3	α3-L-QuiNAc-T		UDP-D-GlcNAc	UDP-L-QuiNAc

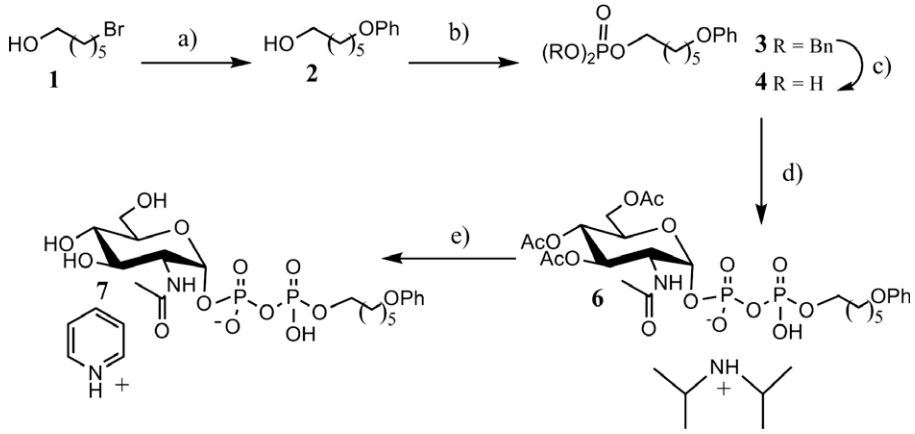
(*continued on next page*)

Table 1 (*continued*)

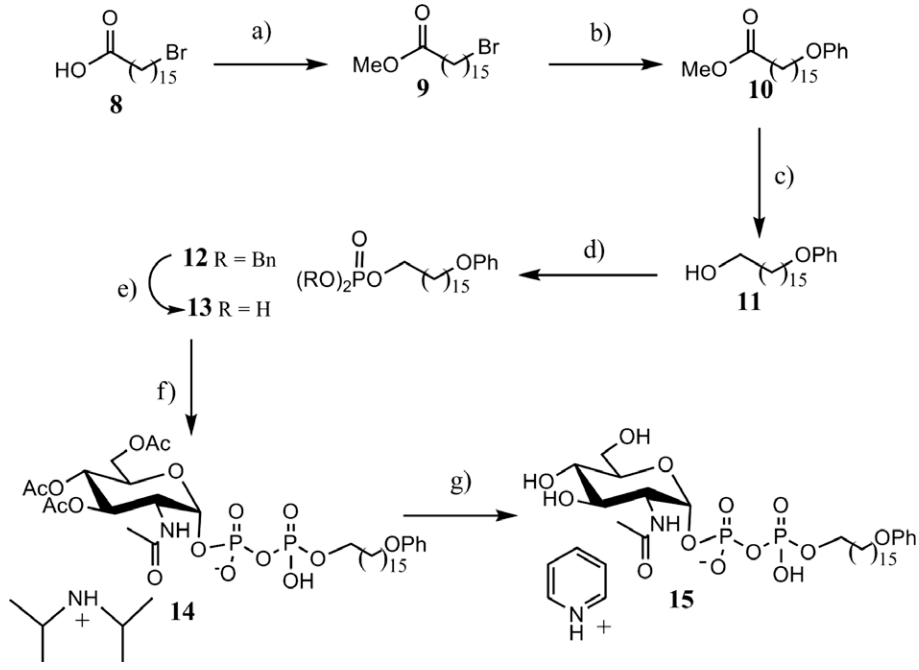
Serotype	Linkage	Putative enzyme	Short name	Starting NTP	Donor
O11, O23, O24	L-QuiNAcα1-3	α3-L-QuiNAc-T	WbwW	UDP-D-GlcNAc	UDP-L-QuiNAc
O139 (46)	L-Rhaα1-3	α3-L-Rha-T		dTDP-L-Rha	dTDP-L-Rha
Vibrio cholerae O136 (14)	D-Galα1-3	β4-D-Gal-T		UDP-Gal	UDP-Gal

Linkage between first sugar (GlcNAc-alpha or beta) and second sugar; starting NTP, sugar donor substrate precursor; –T, -transferase; donor, expected donor substrate for the transferase; L-FucNAm, 2-acetamidoylamino-2,6-dideoxy-L-galactose; L-FucNAc, 2-acetamidino-2,6-dideoxy-L-galactose; QuiNAc, 4-acetylamido-4,6-dideoxy-glucose; GalA, galacturonic acid; GalNAcA, *N*-acetylgalacturonic acid.

Table 1 shows examples of O-antigens containing a GlcNAc residue as the first sugar residue at the reducing end of the repeating unit but having different linkages between the second sugar and GlcNAc. Data banks of glycosyltransferases (CAZy) suggest many putative glycosyltransferases that are expected to transfer a sugar residue to GlcNAc, with examples from *E. coli, Salmonella, Yersinia, Vibrio cholerae*, and *Shigella*, as shown in Table 1. A few of the genes have been assigned to specific glycosyltransferases[4–6] but most of the enzyme activities have not been demonstrated or characterized. With the synthesis of substrate analogs reported here, it is



Scheme 1a. Synthesis of pyridinium 6-phenoxy-1-hexyloxydiphospho(1)-2-acetamido-2-deoxy-α-D-glucopyranose (**7**). Reagents and conditions: (a) PhOH, K_2CO_3/DMF, 60 °C, 95%; (b) HOP(O)$(OBn)_2$, PPh_3, DIAD/CH_2Cl_2, 53%; (c) H_2, Pd/C, MeOH, 95%; (d) (i) DIPA, 1,1′-carbonyldiimidazole, THF; (ii) MeOH; (iii) DIPA, **5**, THF, 62%; (e) (i) NaOMe/MeOH; (ii) pyridinium resin, 95%.



Scheme 1b. Synthesis of pyridinium 16-phenoxy-1-hexadecyloxydiphospho(1)-3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-glucopyranose (**15**). Reagents and conditions: (a) (i) $Cl_2(CO)_2$, DMF/CH_2Cl_2; (ii) MeOH, 100%; (b) PhOH, K_2CO_3/DMF, 60 °C, 81%; (c) LAH/ether, 95%; (d) HOP(O)$(OBn)_2$, PPh_3, DIAD/CH_2Cl_2, 53%; (e) H_2, Pd/C, MeOH, 95%; (f) (i) DIPA, 1,1′-carbonyldiimidazole, THF; (ii) MeOH; (iii) DIPA, **5**, THF, 62%; (g) (i) NaOMe/MeOH; (ii) pyridinium resin, 95%.

now possible to develop specific assays for a multitude of biosynthetic enzymes, allowing enzyme purification and studies of the enzyme mechanisms, specificities, properties, and regulation. The synthetic methods help to design additional substrate analogs for specificity studies, and allow the design of acceptor analog inhibitors that block the second step in the synthesis of the repeating unit and thus prevent the synthesis of a specific O-antigen.

2. Results and discussion

2.1. Synthesis of substrate analogs

Methods for the synthesis of GlcNAc-PP-lipid analogs were based on the synthesis of GlcNAc-PP-PhU[4] by linking GlcNAc-phosphate- or GlcNAc-derivative to lipid or lipid-phosphate. Phosphates were named according to IUPAC-IUB rules.[11]

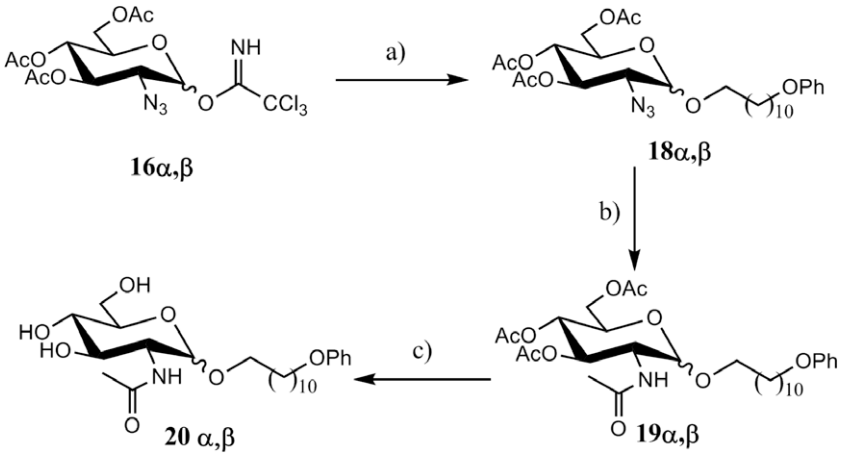
The synthesis of phospholipid glycosides **7** and **15** is shown in Scheme 1a and b, respectively.

The preparation of the tail fragment requires initial treatment of bromo alcohol **1** or bromo ester derivative **9**[12–15] giving the corresponding phenoxy alcohols **2** and **10**, respectively. The ethers are subsequently treated with dibenzyl phosphate and triphenylphosphine, followed by the addition of diisopropylazodicarboxylate (DIAD), giving the dibenzyl phosphate derivatives **3** and **12**.[16] The phosphate is then debenzylated by hydrogenolysis and the resulting dihydrogen phosphate (**4** and **13**, respectively) is activated by forming the phosphorimidazolidate which, by condensation with the acetylated sugar moiety **5**, gives the corresponding O-acetylated glycosides **6** and **14**, respectively.[17,18] At the end, the acetyl groups are removed using $NaOCH_3$ in MeOH; ion exchange gave the final product as a pyridinium salt (**7** and **15**, respectively).[19]

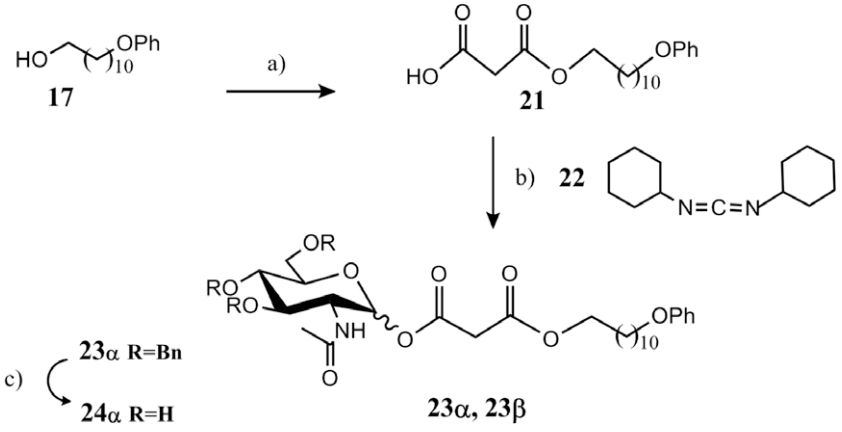
Scheme 2 shows the synthesis of the acetylamino glucopyranoside derivatives **20α** and **β**. The condensation of the trichloroacetimidate **16α** and **β**, with the corresponding tail fragment[4] 11-phenoxyundecanol **17**, was based on the methodology described.[20] The subsequent deprotection of the sugar moiety with $NaOCH_3$ in MeOH gave the target compounds. Further purification of compounds can be done using a C18 Sep-Pak cartridge.

The synthesis of the malonate derivative **24α** is shown in Scheme 3.

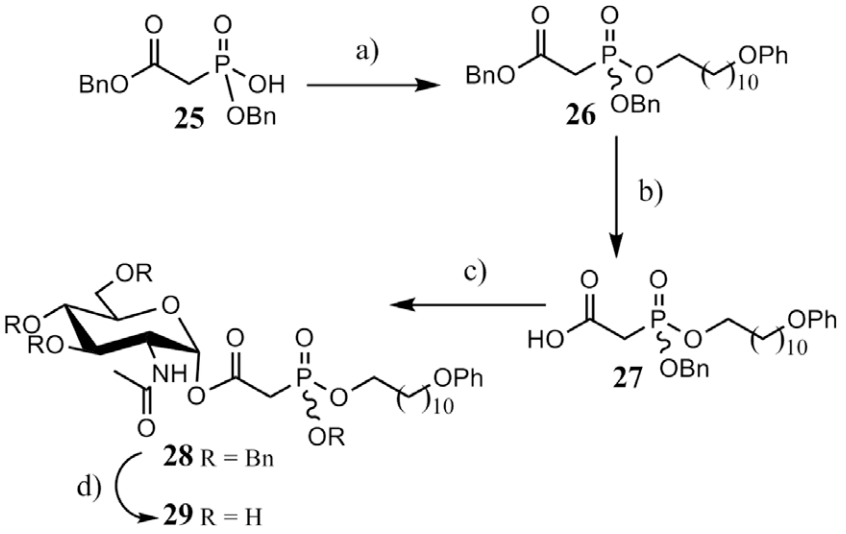
The tail fragment **21** was obtained by reacting Meldrum's acid (2,2-dimethyl-1,3-dioxane-4,6-dione) with the corresponding alcohol **17** in refluxing toluene.[21] The condensation with the sugar moiety **22a** (3,4,6-tri-*O*-benzyl-2-acetamido-2-deoxy-α/β-D-glucopyranoside[22]) was based on a Mitsunobu-type reaction[16] in the presence of dehydrating agent *N*,*N*′-dicyclohexylcarbodiimide (DCC) in tetrahydrofuran (THF). Debenzylation by hydrogenolysis gave the desired compound **24α**.



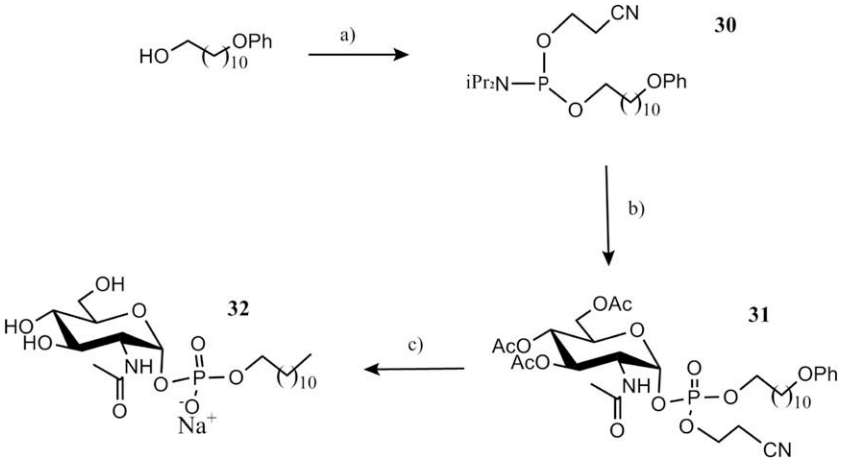
Scheme 2. Synthesis of 11-phenoxyundecyl 2-acetylamino-2-deoxy-α,β-D-glucopyranoside (**20α** and **20β**). Reagents and conditions: (a) **17**, TMSOTf/CH_2Cl_2, 53%; (b) (i) H_2, Pd/C, MeOH; (ii) Ac_2O/pyridine, α = 32%, β = 41%; (c) NaOMe/MeOH, α = 94%, β = 100%.



Scheme 3. Synthesis of 2-acetamido-2-deoxy-α-D-glucopyranosyl 11-phenoxyundecyl malonate (**24α**). Reagents and conditions: (a) Meldrum's acid/toluene, reflux, 98%; (b) **22a**, DCC, DMAP/THF/DMF, α = 62%, β = 9%; (c) H_2, Pd/C, MeOH, 92%.



Scheme 4. Synthesis of [hydroxy (11-phenoxyundecyloxy)phosphinyl]acetyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**29**). Reagents and conditions: (a) **17**, PPh_3, DIAD/CH_2Cl_2, 83%; (b) NaOH/H_2O/EtOH, 100%; (c) **22a**, DCC, DMAP/THF/DMF, 39%; (d) H_2, Pd/C, MeOH, 85%.



Scheme 5. Synthesis of 2-acetamido-2-deoxy-1-O-(11-phenoxyundecyloxy)phosphoryl-α-D-glucopyranose (**32**). Reagents and conditions: (a) (2-cyanoethoxy)bis(diisopropylamino)phosphine ([*i*Pr$_2$N]$_2$-POCH$_2$CH$_2$CN), tetrazole/DIPA (*i*Pr$_2$NH), CH_2Cl_2; (b) **22b** tetrazole, CH_2Cl_2 at 0 °C→rt, then mCPBA, CH_2Cl_2→0 °C; (c) NH_4OH, MeOH.

The synthesis of **29** is shown in Scheme 4. The coupling of the organophosphorus tail **27** and the sugar head group **22a** was done based on a Mitsunobu-type condensation reaction followed by deprotection by hydrogenolysis.

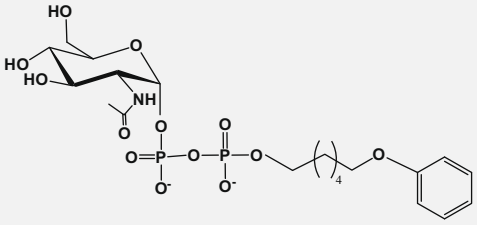
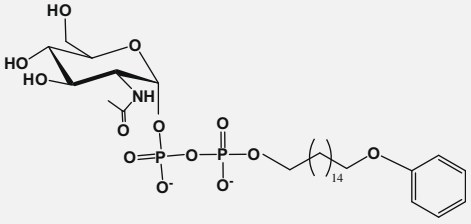
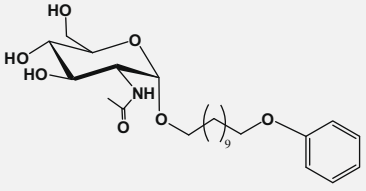
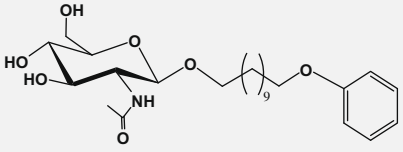
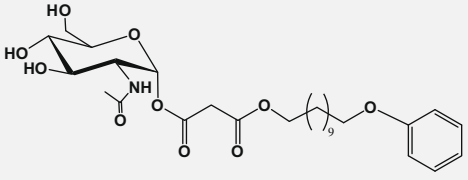
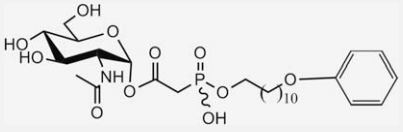
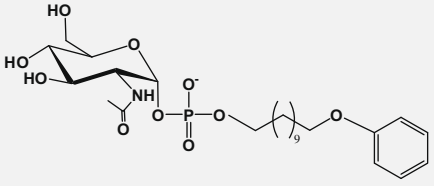
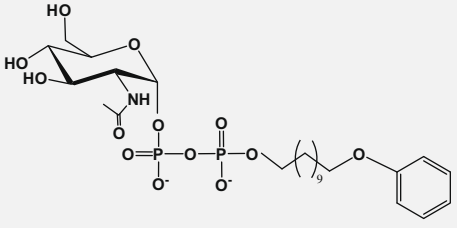
The synthesis of the monophosphate GlcNAcα-P-PhU (compound **32**) is shown in Scheme 5. A diisopropylaminophosphine **30** was synthesized first. The coupling with the sugar head group **22b** yielded **31** which was deprotected to yield the sugar-monophosphate-lipid **32**.

2.2. Use of synthetic derivatives as enzyme substrates

The compounds shown in Table 2 were synthesized and separated well by reverse-phase HPLC. After the enzymatic addition of a second sugar, the enzyme product was eluted earlier than the substrate and could be well separated. The synthesized compounds were tested as acceptor substrates with bacterial β3-Galtransferase WbbD from *E. coli* serotype O7,[4,7,8,10] β3-Glc-transferases WfaP from *E. coli* O56, WfgD from *E. coli* O152[5], and β4-Galtransferase WfeD from *Shigella* B14.[6] Thus far, all these enzymes that catalyze the second reaction of the repeating unit assembly were active using GlcNAc-PP-lipids with the aliphatic chain having 6–16 carbons, in addition to an *O*-phenyl group (compounds **7, 15, 33**). Compound **32**, having only one phosphate group linked to GlcNAc, was generally poor as a substrate, while compound **29**, having a phosphate group distant from GlcNAc, was not active. All other compounds, including GlcNAc-phenylundecyl **20**, compound **24**, and a series of GlcNAc-benzyl, -naphthyl and -quinolinyl derivatives[23] that lack phosphate were inactive as substrates for these bacterial glycosyltransferases (Table 2). Therefore, as a general rule, the enzymes (listed in Table 1) that add the second sugar of the repeating unit require GlcNAc linked to pyrophosphate for full activity. In contrast, the lipid moiety of the acceptors appears to play a minor role. Similar results were reported for an α1,3-GalNAc-transferase from *E. coli* O86 that adds GalNAc as the second sugar to GalNAc-PP-PhU.[9]

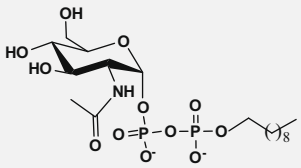
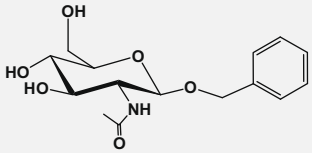
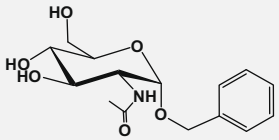
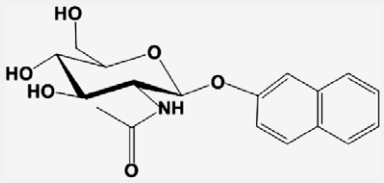
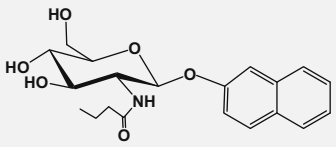
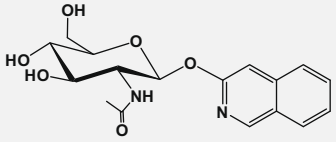
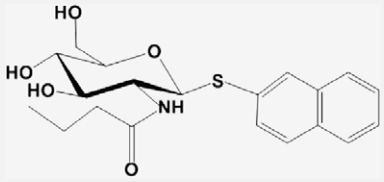
Based on these findings, we propose that assays are now available for the characterization of a large number of glycosyltransferases including those listed in Table 1 which are expected to act on GlcNAc-PP-lipid as acceptor substrates. The assays utilizing GlcNAc-PP-lipid-based substrates can serve to synthesize partial O-antigen repeating units which could be substrates for

Table 2
List of GlcNAc analogs synthesized and tested as a substrate for the second step of O-antigen synthesis

Compound	Structure	Activity (%)			
		WbbD	WfaP	WfgD	WfeD
7 GlcNAcα-PP-$(CH_2)_6$-O-Ph		47	60	183	39
15 GlcNAcα-PP-$(CH_2)_{16}$-O-Ph		94	156	174	92
20α GlcNAcα-$(CH_2)_{11}$-O-Ph		<1	<1	<1	<1
20β GlcNAcβ-$(CH_2)_{11}$-O-Ph		<1	<1	<1	<1
24α GlcNAcα-malonate-$(CH_2)_{11}$-O-Ph		<1	<1	<1	<1
29 GlcNAcα-hydroxy-phosphinyl-$(CH_2)_{11}$-O-Ph		<1	<1	<1	<1
32 GlcNAcα-P-$(CH_2)_{11}$-O-Ph		16	1	11	11
33 GlcNAcα-PP-$(CH_2)_{11}$-O-Ph		100	100	100	100

(*continued on next page*)

Table 2 (*continued*)

Compound	Structure	Activity (%)			
		WbbD	WfaP	WfgD	WfeD
34 GlcNAcα-PP-$(CH_2)_9$-CH3		70*	32	150	74
35 GlcNAcβ-Bn		<1	<1	<1	<1
36 GlcNAcα-Bn		<1	nd	nd	<1
37 GlcNAcβ-O-2-naphthyl		<1	nd	nd	<1
38 GlcNBuβ-O-2-naphthyl		<1	nd	nd	<1
39 GlcNAcβ-3-isoquinolinyl		<1	nd	nd	<1
40 GlcNBuβ-*S*-2-naphthyl		<1	<1	<1	<1

The enzymes tested were WbbD from *E. coli* O7, WfaP from *E. coli* O56, WfgD from *E. coli* O152, and WfeD from *Shigella* B14.[5–8,10] The activity is shown relative to that using the active substrate GlcNAc-PP-$(CH_2)_{11}$-O-Ph, compound **33**, at 0.2 mM (or *0.5 mM) concentration in the assay. nd, not determined.

the third and subsequent steps of the biosynthetic pathways of O-antigen repeating unit synthesis. However, the subsequently acting glycosyltransferases (beyond the second step) may not require the pyrophosphate bond for activity and may instead act on simpler oligosaccharide substrates.

A future goal is to synthesize pyrophosphate-sugar analogs containing a short lipid chain to produce water-soluble compounds. Although this may not necessarily lead to high enzyme activity, it can facilitate enzyme assays, as well as further enzyme–substrate-binding complex study by crystallization. The series of compounds described here could also be synthesized based on GalNAc-PP-PhU for studies of multiple enzymes that are expected to utilize GalNAc-pyrophosphate-lipids as acceptors.[9]

3. Experimental

3.1. Materials

The syntheses of GlcNAcα-pyrophosphate bound to a phenoxyhexyl moiety (**7**) and a phenoxyhexadecyl moiety (**15**), the phenoxyundecyl glycosides **20α** and **20β**, the phenoxyundecyl malonate glycoside **24α**, GlcNAcα-hydroxyphosphinyl bound to phenoxyundecyloxy moiety (**29**), and phenoxyundecylphosphoryl glycoside **32** are described below. Some of the compounds used in the synthesis were obtained as described in the corresponding references: compound **5**, 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-glucopyranose dihydrogen phosphate;[4] compounds **16α,β**, 3,4,6-tri-

O-acetyl-2-azido-2-deoxy-α,β-D-glucopyranosyl trichloroacetimidate,[24] compound **17**, 11-phenoxyundecanol;[4] compound **22a**, 3,4,6-tri-*O*-benzyl-2-acetamido-2-deoxy-α-D-glucopyranoside,[22] **22b**, 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-glucopyranoside,[4] compound **25**, (benzyloxyhydroxyphosphoryl)acetic acid benzyl ester.[25] Compound **33**, phenoxyundecylpyrophosphate-α-GlcNAc, was synthesized as described.[4] The benzyl-, naphthyl-, and quinolinyl derivatives were synthesized as described.[23] All other reagents were purchased from Sigma Chemical Co., St. Louis, MO, unless otherwise indicated. In most cases, the GlcNAc sugar head group and the fragment tail were assembled separately and then coupled using well-established methodologies. For use in enzyme assays, compounds were subjected to purification by C18 Sep-Pak columns. After purification, compounds were analyzed by HPLC for purity and to determine the best conditions for enzyme product separation.

3.2. General methods

The ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on Bruker Avance 100-, 200-, 300-, 400-, and 500-MHz spectrometers. Chemical shifts (δ) are reported in parts per million (ppm), and signals are described as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), quin (quintet), and m (multiplet). Proton chemical shifts are given relative to those of internal standards $CHCl_3$, CH_3OD, and dimethylsulfoxide (DMSO): $\delta = 7.26$, 3.30 or 2.25 ppm, respectively. Carbon chemical shifts are given relative to those of $CDCl_3$, CD_3OD, or DMSO: $d = 77$, 49 or 39.5 ppm, respectively. Phosphorus chemical shifts are given relative to that of 85% phosphoric acid: $\delta = 0$ ppm. High-resolution MALDI and electrospray mass spectra were recorded on an Applied Biosystems/MDS Sciex QSTAR XL spectrometer with an Agilent HP1100 CapLC system. Samples were run in 50% aqueous MeOH at a flow rate of 6 μL/min. Melting points were determined on a Mel-Temp II melting point apparatus and are uncorrected. Optical rotations were measured using an Autopol® II automatic polarimeter for solutions in a 1-dm cell at room temperature (rt). Thin-layer chromatography was performed using glass- or aluminum-backed Silicycle Silica Gel 60 F_{254} plates.

Compounds dissolved in MeOH were separated by reverse-phase HPLC using an analytical C18 column and acetonitrile/water mixtures as the mobile phase at 1 mL/min flow rate. The elution of compounds was monitored by measuring the absorbance at 195 nm.

3.3. Glycosyltransferase assays

For glycosyltransferase assays, bacteria were cultured and the homogenates prepared in 50 mM sucrose as described.[7,8] Diluted homogenates were used as the enzyme source. Standard assays were carried out in 10 min incubations utilizing radioactive sugar–nucleotides as donor substrates.[4–8] For Gal-transferases WbbD and WfeD, UDP-[^{3}H]Gal was used as the donor substrate and for Glc-transferases WfaP and WfgD, UDP-[^{14}C]Glc was used as the donor substrate. Under these conditions, initial rates were measured. Radioactive enzyme products having hydrophobic groups were separated using C18 Sep-Pak. Assay mixtures were loaded on small Sep-Pak cartridges in water, followed by washing with 4 mL water, eluting nucleotide sugars and free sugars. Enzyme products and substrates were then eluted with 3 mL MeOH as described.[4] Enzyme products from assays utilizing neutral substrates were also separated by anion exchange chromatography on AG1×8.[23] HPLC was used to separate substrates and products and to confirm that enzyme product was formed. Eluates were analyzed by measuring the absorbance at 195 nm and the radioactivity of collected fractions, in comparison to standard compounds.[7]

3.4. Synthesis of substrate analogs

3.4.1. Dibenzyl 6-phenoxyhexyl phosphate (3)

Compound **2** (6-phenoxy-1-hexanol) was synthesized from 6-bromo-1-hexanol **1** with phenol in dimethylformamide (DMF) and potassium carbonate according to Suzuki et al.[12,13] and Chang et al.[14] A solution of compound **2** (385 mg, 2.0 mmol), dibenzyl phosphate (828 mg, 3.0 mmol), and triphenylphosphine (PPh_3) (935 mg, 3.6 mmol) in CH_2Cl_2 (5 mL) was stirred under N_2 atmosphere for 30 min at rt. The solution was then cooled to 0 °C and treated with DIAD (0.690 mL, 3.6 mmol), stirred for 30 min, and then warmed up to rt. After 2 h the mixture was quenched with 1 mL MeOH, stirred for 5 min, and concentrated. The resulting residue was purified by reverse-phase preparative chromatography (C18 stationary phase) using acetonitrile/water (70:30) as the mobile phase to give the title compound as a colorless transparent solid (480 mg, 53%); ^{1}H NMR ($CDCl_3$) δ 1.38–1.47 (m, 4H), 1.66 (m, 2H), 1.76 (m, 2H), 3.94 (t, $J = 6.4$ Hz, 2H, PhOCH_2), 4.03 (q, $J = 6.6$ Hz, 2H, POCH_2), 5.04 (m, 4H, 2 × OCH_2Ph), 6.94 (m, 3H), 7.30–7.45 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 25.2, 25.6, 29.2, 30.1, 67.2 (d, $^{3}J_{CP} = 5.2$ Hz), 67.6 (PhOCH_2), 67.8 (d, $^{3}J_{CP} = 6.1$ Hz), 69.2 (d, $^{3}J_{CP} = 5.5$ Hz), 114.5, 120.6, 127.1, 127.5, 127.9, 127.9, 128.0, 128.4, 128.5, 128.6, 129.4, 129.8, 134.5, 135.2, 159.1 (Ar-C); ^{31}P NMR δ −0.60; electrospray ionization mass spectrometry (ESMS) *m/z* 455 $[M+H]^+$; high resolution MS (HRMS) $[M+H]^+$, Found: 455.1985. $C_{26}H_{32}O_5P$ requires *m/z*, 455.1987.

3.4.2. 6-Phenoxyhexyl dihydrogen phosphate (4)

A suspension of compound **3** (154 mg, 0.3 mmol) and 10% Pd/C (0.154 g) in MeOH (2.5 mL) was stirred under H_2 atmosphere for 16 h. The Pd/C was removed by filtration through a Celite plug, washed with MeOH, and the filtrate was concentrated under reduced pressure to give the title compound as a colorless solid (88 mg, 95%); ^{1}H NMR (CD_3OD) δ 1.52 (m, 4H), 1.72 (m, 2H), 1.79 (m, 2H), 3.97 (m, 4H, 2 × OCH_2), 6.90 (m, 3H), 7.25 (m, 2H); ^{13}C NMR δ 25.1, 25.4, 29.0, 30.1, 66.3 (d, $^{3}J_{CP} = 6.0$ Hz), 67.3, 114.1, 120.1, 129.0, 159.2; ^{31}P NMR δ 0.46; ESMS *m/z* 273 $[M-H]^-$; HRMS $[M-H]^-$, Found: 273.0890. $C_{12}H_{18}O_5P$ requires *m/z*, 273.0892.

3.4.3. Bis(diisopropylammonium) 6-phenoxy-1-hexyloxydiphospho(1)-3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-glucopyranose (6)

Both 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-glucopyranose dihydrogen phosphate **5** (101 mg, 0.2 mmol)[4] and compound **4** (71 mg, 0.3 mmol) were converted into the corresponding diisopropylamine (DIPA) salt by co-evaporation with toluene/DIPA (1 mL toluene/five drops DIPA, ×2). Compound **4** was then dissolved in dry THF (5 mL) and 1,1′-carbonyldiimidazole (186 mg, 1.1 mmol) was added to the solution. The reaction mixture was stirred at rt for 90 min, quenched with dry MeOH (50 μL), and stirred for an additional 30 min. The solvent was removed to give a colorless oil that was dried under vacuum for 30 min. Compound **5** was then dissolved in dry THF (15 mL) and the solution was added to the imidazolidate. The reaction was left stirring for 48 h and the solvent was removed under reduced pressure. The resulting residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 2:1 v/v) to give the title compound as a colorless oil (131 mg, 62%); ^{1}H NMR (CD_3OD) δ 1.34, 1.35 (2s, 24H, 8 × CH_3 DIPA), 1.44 (m, 2H, CH_2), 1.51 (m, 2H, CH_2), 1.69 (m, 2H, CH_2), 1.79 (m, 2H, CH_2), 2.00, 2.03, 2.06, 2.07 (4s, 12H, 4 × CH_3CO), 3.46 (m, 4H, 4 × C*H* DIPA), 3.97 (t, 2H, OCH_2, $J = 6.5$ Hz), 4.03 (br s, 2H, OCH_2), 4.19 (m, 1H), 4.33–4.45 (m, 3H), 5.12 (t, $J = 9.4$ Hz, 1H), 5.35 (t, $J = 9.8$ Hz, 1H), 5.58 (m, 1H, H-1), 6.89 (m, 3H), 7.25 (t, $J = 7.9$ Hz, 2H); ^{13}C NMR δ 19.0, 19.2, 19.3, 19.3 (4 × CH_3CO), 25.3, 25.5, 28.4, 29.0 (4 × CH_2), 47.0 (*C*H, DIPA), 51.8, 61.4, 65.8, 67.4, 67.7, 68.4, 71.7, 94.4 (C-1), 114.1, 120.0, 129.0, 159.1, 169.9,

170.4, 171.1, 172.7; ^{31}P NMR δ −13.4, −10.4; ESMS *m/z* 682 [M−H]$^-$; HRMS [M−H]$^-$, Found: 682.1699. $C_{26}H_{38}NO_{16}P_2$ requires *m/z*, 682.1665.

3.4.4. Pyridinium 6-phenoxy-1-hexyloxydiphospho(1)-2-acetamido-2-deoxy-α-D-glucopyranose (7)

Compound **6** (31 mg, 45 μmol) was dissolved in a solution of sodium methoxide (NaOMe)/MeOH (2 mL, 0.05 M) and stirred for 25 min at rt. The reaction mixture was then quenched with an excess of IR-120 ion exchange resin (pyridinium form) and stirred for an additional 20 min. The suspension was then filtered and concentrated under reduced pressure to give the title compound (21 mg, 95%) as a colorless oil; ^{1}H NMR (CD_3OD) δ 1.41 (m, 2H), 1.48 (m, 2H), 1.68 (m, 2H), 1.76 (m, 2H), 2.05 (s, 3H), 3.45 (m, 2H), 3.73 (m, 1H), 3.82 (m, 1H), 3.91–4.05 (m, 6H), 5.64 (br s, 1H, H-1), 6.89 (m, 3H), 7.25 (t, *J* = 8.1 Hz, 2H), 8.03 (br s, 3H), 8.55 (br s, 1H), 8.93 (br s, 2H); ^{13}C NMR δ 21.5, 25.1, 25.2, 28.3, 29.0, 53.7, 61.2, 67.3, 67.7, 70.5, 71.5, 73.8, 95.3 (C-1), 114.1, 120.0, 126.8, 129.0, 142.7, 145.1, 156.0, 159.2, 173.0. ^{31}P NMR δ −12.7, −10.6. ESMS *m/z* 556 [M−H]$^-$; HRMS [M−H]$^-$, Found: 556.1339. $C_{20}H_{32}NO_{13}P_2$ requires *m/z*, 556.1348.

3.4.5. Methyl 16-phenoxyhexadecanoate (10)

Methyl 16-bromohexadecanoate (**9**) was prepared from 16-bromohexadecanoic acid **8** as described.[15] To a solution of compound **9** (900 mg, 2.6 mmol) and phenol (364 mg, 3.9 mmol) in DMF (6 mL), potassium carbonate (1.07 g, 7.7 mmol) was added under stirring, at 60 °C. The suspension was stirred for 5 h, cooled to rt, and diluted with ethyl acetate (30 mL) and water (300 mL). The organic layer was separated and washed successively with water (2 × 30 mL), a solution of NaOH 1.0 M (2 × 30 mL), water (30 mL), and brine (30 mL). The organic layer was then dried (Na_2SO_4), filtered, and concentrated. The resulting residue was purified by column chromatography on silica gel (hexanes/ethyl acetate, 8:1 v/v) to give the title compound as a colorless solid (756 mg, 81%); ^{1}H NMR ($CDCl_3$) δ 1.23–1.39 (m, 22H), 1.48 (m, 2H), 1.65 (m, 2H), 1.81 (m, 2H), 2.33 (t, *J* = 7.6 Hz, C*H*$_2$CO), 3.69 (s, 3H, OC*H*$_3$), 3.97 (t, *J* = 6.6 Hz, 2H, PhOC*H*$_2$), 6.93 (m, 3H), 7.29 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 25.0, 26.1, 29.2, 29.3, 29.3, 29.5, 29.5, 29.6, 29.7, 34.1, 51.4 (*C*H$_2$CO), 67.9 (PhO*C*H$_2$), 114.5, 120.4, 129.4, 159.2 (Ar-C), 174.3 (*C*O); ESMS *m/z* 385 [M+Na]$^+$; HRMS [M+Na]$^+$, Found: 385.2714. $C_{23}H_{38}O_3Na$ requires *m/z*, 385.2719.

3.4.6. 16-Phenoxy-1-hexadecanol (11)

To a suspension of lithium aluminum hydride (LAH) (24 mg, 0.6 mmol) in ether (1.5 mL), a solution of compound **10** (114 mg, 0.3 mmol) in ether (1 mL) was added at 0 °C. The reaction mixture was then warmed up to rt, stirred for 2 h, and diluted with ethyl acetate (10 mL) and water (10 mL). The organic layer was then separated and the aqueous layer was extracted with ethyl acetate (3 × 10 mL). The combined organic extracts were then washed with brine (10 mL), dried (Na_2SO_4), filtered, and concentrated. The resulting residue was purified by column chromatography on silica gel (hexanes/ethyl acetate, 2:1 v/v) to give the title compound as a colorless solid (100 mg, 95%); ^{1}H NMR ($CDCl_3$) δ 1.23–1.39 (m, 22H), 1.34 (m, 2H), 1.48 (m, 2H), 1.80 (m, 2H), 3.66 (t, *J* = 6.6 Hz, C*H*$_2$OH), 3.97 (t, *J* = 6.6 Hz, 2H, PhOC*H*$_2$), 6.95 (m, 3H), 7.29 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 25.8, 26.1, 29.3, 29.4, 29.5, 29.6, 29.6, 29.6, 29.7, 32.8, 63.1 (*C*H$_2$OH), 67.9 (PhO*C*H$_2$), 114.5, 120.4, 129.4, 159.1 (Ar-C); ESMS *m/z* 335 [M+H]$^+$; HRMS [M+H]$^+$, Found: 335.2939. $C_{22}H_{39}O_2$ requires *m/z*, 335.2950.

3.4.7. Dibenzyl 16-phenoxyhexadecanyl phosphate (12)

A solution of compound **11** (603 mg, 1.8 mmol), dibenzyl phosphate (552 mg, 2.0 mmol), and triphenylphosphine (709 mg, 2.7 mmol) in THF (15 mL) was stirred under N_2 atmosphere for 30 min at rt. The solution was then cooled to 0 °C and treated with DIAD (0.523 mL, 2.7 mmol), stirred for 30 min, and then warmed up to rt. After 2 h, the mixture was treated with MeOH (3 mL), stirred for 5 min, and concentrated. The resulting residue was purified by column chromatography on silica gel (hexanes/ethyl acetate, 2:1 v/v) to give the title compound as a colorless oil (955 mg, 89%); ^{1}H NMR ($CDCl_3$) δ 1.23–1.39 (m, 22H), 1.47 (m, 2H), 1.62 (m, 2H), 1.81 (m, 2H), 4.00 (m, 4H, 2 × OC*H*$_2$CH$_2$), 5.06 (m, 4H, OC*H*$_2$Ph), 6.93 (m, 3H), 7.29 (m, 2H), 7.38 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 25.4, 26.1, 29.1, 29.3, 29.4, 29.5, 29.6, 29.6, 29.6, 29.7, 29.7, 30.2, 30.2, 67.9 (PhO*C*H$_2$), 68.1 (d, $^3J_{CP}$ = 6.1 Hz), 69.1 (d, $^3J_{CP}$ = 5.5 Hz), 114.5, 120.4, 127.9, 128.5, 128.6, 129.4, 136.0, 136.0, 159.1 (Ar-C); ^{31}P NMR δ −0.63; ESMS *m/z* 617 [M+Na]$^+$; HRMS [M+Na]$^+$, Found: 617.3393. $C_{36}H_{51}O_5NaP$ requires *m/z*, 617.3372.

3.4.8. 16-Phenoxyhexadecyl dihydrogen phosphate (13)

A suspension of compound **12** (153 mg, 0.3 mmol) and 10% Pd/C (200 mg) in MeOH (4 mL) was stirred under H_2 atmosphere for 1 h. The Pd/C was removed by filtration through a Celite plug, washed with MeOH (3 × 10 mL), and the filtrate was concentrated under reduced pressure to give the title compound as a colorless solid (90 mg, 84%); ^{1}H NMR (DMSO-d_6) δ 1.23–1.37 (m, 22H), 1.40 (m, 2H), 1.53 (m, 2H), 1.69 (m, 2H), 3.78 (m, 2H, POC*H*$_2$), 3.93 (t, *J* = 6.4 Hz, 2H, PhOC*H*$_2$), 6.90 (m, 3H), 7.27 (t, *J* = 7.8 Hz, 2H); ^{13}C NMR δ 25.4, 25.6, 29.1, 29.2, 29.2, 29.5, 30.4, 65.7 (POCH$_2$), 67.7 (PhO*C*H$_2$), 114.8, 120.8, 129.9, 159.1; ^{31}P NMR δ −0.99; ESMS *m/z* 413 [M−H]$^-$; HRMS [M−H]$^-$, Found: 413.2459. $C_{22}H_{38}O_5P$ requires *m/z*, 413.2457.

3.4.9. Bis(diisopropylammonium) 16-phenoxy-1-hexadecyloxydiphospho(1)-3,4,6-tri-*O*acetyl-2-acetamido-2-deoxy-α-D-glucopyranose (14)

Both compound **5** (63 mg, 0.1 mmol)[4] and compound **13** (67 mg, 0.2 mmol) were converted into the corresponding DIPA salt by co-evaporation with toluene/DIPA (1 mL toluene/five drops DIPA, ×2). Compound **13** was then dissolved in THF/toluene (2:1, 3 mL) and 1,1′-carbonyldiimidazole (105 mg, 0.6 mmol) was added to the solution. The reaction mixture was stirred at rt for 90 min, quenched with dry MeOH (40 μL), and stirred for an additional 30 min. The solvent was then removed to give a colorless oil that was dried under vacuum for 30 min. Compound **5** was then dissolved in dry THF (3 mL) and the solution was added to the imidazolidate. The reaction was left stirring for 48 h and then the solvent was removed under reduced pressure. The resulting residue was purified by column chromatography on silica gel (gradient of $CHCl_3$/MeOH, 2:1 to 3:2 v/v) to give the title compound as a colorless solid (70 mg, 53%); ^{1}H NMR (CD_3OD) δ 1.23–1.46 (m, 46H, 10 × OC*H*$_2$, 8 × C*H*$_3$ DIPA), 1.50 (m, 2H, C*H*$_2$), 1.67 (m, 2H, C*H*$_2$), 1.78 (m, 2H, C*H*$_2$), 1.95, 2.00, 2.02, 2.08 (4s, 12H, 4 × C*H*$_3$CO), 3.48 (m, 4H, 4 × C*H* DIPA), 3.96 (t, 2H, OC*H*$_2$, *J* = 6.5 Hz), 4.03 (br s, 2H, OC*H*$_2$), 4.16 (m, 1H), 4.31–4.42 (m, 3H), 5.12 (t, *J* = 10.0 Hz, 1H), 5.35 (t, *J* = 9.9 Hz, 1H), 5.61 (m, 1H, H-1), 6.90 (m, 3H), 7.26 (t, *J* = 8.1 Hz, 2H), 8.80 (d, *J* = 8.5 Hz, 1H); ^{13}C NMR δ 18.0, 18.0, 19.2, 21.4 (4 × *C*H$_3$CO), 25.6, 25.8, 29.0, 29.1, 29.2, 29.3, 29.4, 47.0 (*C*H, DIPA), 51.8, 61.4, 65.8, 67.4, 67.5, 68.3, 71.8, 94.4 (C-1), 114.1, 120.0, 129.0, 159.1, 170.0, 170.4, 171.1, 172.8; ^{31}P NMR δ −13.6, −10.6; ESMS *m/z* 822 [M−H]$^-$; HRMS [M−H]$^-$, Found: 822.3262. $C_{36}H_{58}NO_{16}P_2$ requires *m/z*, 822.3231.

3.4.10. Pyridinium 16-phenoxy-1-hexadecyloxydiphospho(1)-3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-glucopyranose (15)

Compound **14** (42 mg, 47 μmol) was dissolved in a solution of NaOMe/MeOH (4 mL, 0.05 M) and stirred for 40 min at rt. The reaction mixture was then quenched with an excess of IR-120 ion exchange resin (pyridinium form) and stirred for an additional 20 min. The suspension was filtered and concentrated under re-

duced pressure to give the title compound (35 mg, 97%) as a colorless solid; ^{1}H NMR (CD_3OD) δ 1.23–1.37 (m, 22H), 1.49 (m, 2H), 1.66 (m, 2H), 1.78 (m, 2H), 2.01 (s, 3H), 3.44 (m, 2H), 3.72 (m, 2H), 3.85 (m, 1H), 3.91–4.05 (m, 7H), 5.60 (br s, 1H, H-1), 6.89 (m, 3H), 7.25 (t, J = 7.7 Hz, 2H), 8.03 (br s, 3H), 8.55 (br s, 1H), 8.91 (br s, 2H); ^{13}C NMR δ 18.0, 21.6, 25.5, 25.8, 29.0, 29.1, 29.1, 29.3, 29.4, 29.4, 30.3, 54.0, 61.3, 66.5, 67.5, 70.5, 71.5, 73.8, 95.3 (C-1), 114.1, 120.1, 126.8, 129.0, 142.6, 145.2, 156.0, 159.2, 172.8.; ^{31}P NMR δ −12.7, −10.7; ESMS m/z 822 $[M-C_5H_6N]^-$; HRMS $[M-C_5H_6N]^-$, Found: 696.2930. $C_{30}H_{52}NO_{13}P_2$ requires m/z, 696.2914. For use in the assays, the compound was further purified by C18 Sep-Pak columns.

3.4.11. 11-Phenoxyundecyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α,β-D-glucopyranoside (18α,β)

A solution of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α,β-D-glucopyranosyl trichloroacetimidate **16α,β** (189 mg, 0.4 mmol)[19] and 11-phenoxyundecanol **17** (126 mg, 0.5 mmol)[4] in dichloromethane (2 mL) was stirred for 30 min under N_2 atmosphere over molecular sieves (4 Å). The solution was then cooled to 0 °C and trimethylsilyltrifluoromethanesulfonate (TMSOTf) (58 μL, 0.3 mmol) was added. The reaction mixture was warmed up to rt over a period of 15 min and was quenched with Et_3N (100 μL). The solution was then filtered and concentrated under reduced pressure. The resulting residue was purified by column chromatography on silica gel (CH_2Cl_2/acetone, 95:5 v/v) to give the title compounds as a mixture of anomers (α:β, 1:1.3 (122 mg, 53%); ^{1}H NMR ($CDCl_3$) δ 1.27–1.5 (2 m, 28H), 1.66 (m, 4H, OCH_2), 1.79 (m, 4H, OCH_2), 2.03, 2.06, 2.10 2.11 (4s, 18H, COCH_3), 3.29 (dd, J = 3.4, 7.2 Hz, 1H), 3.47–3.59 (m, 4H), 3.66 (br m, 1H), 3.72 (q, 1H), 3.95 (m, 5H), 4.06 (m, 1H), 4.12 (br d, 1H), 4.29 (br dd, 2H), 4.39 (d, J = 8.0 Hz, 1H, H-1β), 4.96–5.08 (m, 4H), 5.51 (t, J = 10.1 Hz, 1H), 6.92 (m, 6H), 7.30 (m, 4H); ^{13}C NMR δ 20.6, 20.7, 25.9, 26.1, 29.3, 29.3, 29.4, 29.5, 29.5, 60.8, 61.9, 62.0, 63.8, 67.5, 67.9, 68.5, 68.6, 69.1, 70.4, 70.8, 71.8, 72.5, 97.9 (C-1α), 102.1 (C-1β), 114.5, 120.4, 129.4, 159.1, 170.0; ESMS m/z 600 $[M+Na]^+$; HRMS $[M+Na]^+$, Found: 600.2873. $C_{29}H_{43}N_3O_9Na$ requires m/z, 600.2897.

3.4.12. 11-Phenoxyundecyl 3,4,6-tri-*O*-acetyl-2-acetylamino-2-deoxy-α,β-D-glucopyranoside (19α,β)

A suspension of compounds **18α,β** (106 mg, 0.2 mmol) and 10% Pd/C (100 mg) in MeOH (3 mL) was stirred under H_2 atmosphere for 2 h. The Pd/C was removed by filtration through a Celite plug, washed with MeOH, and the filtrate was concentrated under reduced pressure to give a colorless solid which was then dissolved in pyridine (1 mL) and Ac_2O (1 mL) and allowed to stir at rt for 16 h. The solvents were then removed under reduced pressure and the resulting residue was further purified by column chromatography on silica gel ($CHCl_3$/MeOH, 98:2 v/v) to give the title compounds, **19α** and **19β** as colorless solids (80 mg, 73%). Data for **19α** (35 mg, 32%); $[\alpha]_D$ = +48 (c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.27–1.40 (m, 12H), 1.47 (m, 2H, OCH_2), 1.63 (m, 2H, OCH_2), 1.80 (m, 2H, OCH_2), 1.97, 2.04, 2.05, 2.11 (4s, 12H, 4 × COCH_3), 3.44 (dt, J = 6.7, 3.0 Hz, 1H, one OCH_2), 3.70 (dt, J = 6.8, 2.7 Hz, 1H, one OCH_2), 3.97 (m, 3H), 4.11 (br d, J = 12.3 Hz, 1H), 4.26 (dd, J = 12.3, 4.5 Hz, 1H), 4.36 (dt, J = 9.8, 3.6 Hz, 1H), 4.84 (d, J = 3.6 Hz, 1H, H-1), 5.14 (t, J = 9.6 Hz, 1H), 5.23 (t, J = 9.8 Hz, 1H), 5.66 (d, J = 9.5 Hz, 1H), 6.94 (m, 3H), 7.29 (m, 2H); ^{13}C NMR δ 20.6, 20.7, 20.8, 23.2, 26.1, 26.2, 29.3, 29.4, 29.4, 29.5, 29.6, 51.9, 62.0, 67.7, 67.8, 68.2, 68.6, 71.5, 97.1 (C-1α), 114.5, 120.5, 129.4, 159.1, 169.3, 169.8, 170.7, 171.4 (4 × CO); ESMS m/z 594 $[M+H]^+$; HRMS $[M+H]^+$, Found: 594.3278. $C_{31}H_{48}NO_{10}$ requires m/z, 594.3278. Data for **19β** (45 mg, 41%); $[\alpha]_D$ = −4.0 (c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.27–1.40 (m, 12H), 1.44 (m, 2H, OCH_2), 1.58 (m, 2H, OCH_2), 1.80 (m, 2H, OCH_2), 1.96, 2.04, 2.05, 2.10 (4s, 12H, 4 × COCH_3), 3.47 (m, one OCH_2), 3.71 (br d, J = 8.4 Hz, 1H), 3.84 (q, J = 6.9 Hz, 1H), 3.88 (m, 1H, one OCH_2), 3.97 (t, J = 6.6, 2H PhOCH_2), 4.12 (br d, J = 12.2 Hz, 1H), 4.28 (dd, J = 12.1, 4.5 Hz, 1H), 4.70 (d, J = 8.3 Hz, 1H, H-1), 5.08 (t, J = 9.5 Hz, 1H), 5.33 (t, J = 9.8 Hz, 1H), 5.46 (d, J = 8.5 Hz, 1H), 6.95 (m, 3H), 7.29 (m, 2H); ^{13}C NMR δ 20.7, 20.7, 20.8, 23.4, 25.9, 26.1, 29.4, 29.4, 29.5, 29.5, 29.6, 29.6, 54.9, 62.0, 67.9, 68.7, 70.0, 71.8, 72.4, 100.7 (C-1β), 114.5, 120.5, 129.4, 159.1, 169.4, 170.1, 170.8, 170.9 (4 × CO); ESMS m/z 594 $[M+H]^+$; HRMS $[M+H]^+$, Found: 594.3278. $C_{31}H_{48}NO_{10}$ requires m/z, 594.3260.

3.4.13. 11-Phenoxyundecyl 2-acetylamino-2-deoxy-α-D-glucopyranoside (20α)

Compound **19α** (20 mg, 34 μmol) was dissolved in a solution of NaOMe/MeOH (1 mL, 0.05 M) and stirred for 20 min at rt. The reaction mixture was then quenched with an excess of IR-120 ion exchange resin and stirred for an additional 20 min. The suspension was then filtered and concentrated under reduced pressure to give the title compound (15 mg, 94%) as a colorless solid; $[\alpha]_D$ = +68.0 (c 1.0, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.31–1.40 (m, 12H), 1.47 (m, 2H, OCH_2), 1.61 (m, 2H, OCH_2), 1.78 (m, 2H, OCH_2), 2.00 (s, 3H, COCH_3), 3.39 (m, 2H), 3.60 (m, 1H), 3.65–3.72 (m, 3H), 3.82 (br d, J = 11.7 Hz, 1H), 3.89 (dd, J = 10.6, 3.3 Hz, 1H), 3.97 (t, J = 6.4 Hz, PhOCH_2), 4.80 (d, J = 3.4 Hz, 1H, H-1), 6.90 (m, 3H), 7.26 (t, J = 7.6 Hz, 2H); ^{13}C NMR δ 21.2, 25.8, 25.9, 29.0, 29.1, 29.2, 29.2, 29.3, 29.3, 29.4, 54.2, 61.4, 67.4, 67.5, 71.0, 71.4, 72.4, 97.0 (C-1α), 114.1, 120.1, 129.0, 159.2, 172.2 (CO); ESMS m/z 490 $[M+Na]^+$; HRMS $[M+Na]^+$, Found: 490.2766. $C_{25}H_{41}NO_7Na$ requires m/z, 490.2781.

3.4.14. 11-Phenoxyundecyl 2-acetylamino-2-deoxy-β-D-glucopyranoside (20β)

Compound **19β** (23 mg, 39 μmol) was dissolved in a solution of NaOMe/MeOH (1 mL, 0.05 M) and stirred for 20 min at rt. The reaction mixture was quenched with an excess of IR-120 ion exchange resin and stirred for an additional 20 min. The suspension was then filtered and concentrated under reduced pressure to give the title compound (18 mg, 100%) as a colorless solid. $[\alpha]_D$ = −6.0 (c 1.0, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.31–1.40 (m, 12H), 1.52 (m, 2H, OCH_2), 1.56 (m, 2H, OCH_2), 1.78 (m, 2H, OCH_2), 2.00 (s, 3H, COCH_3), 3.28–3.35 (m, 2H), 3.48 (m, 2H), 3.62–3.72 (m, 2H), 3.90 (m, 2H), 3.96 (t, J = 6.4 Hz, PhOCH_2), 4.40 (d, J = 8.4 Hz, 1H, H-1), 6.91 (m, 3H), 7.26 (t, J = 8.0 Hz, 2H); ^{13}C NMR δ 21.6, 25.8, 25.8, 29.0, 29.1, 29.3, 29.3, 29.4, 56.1, 61.4, 67.5, 69.2, 70.8, 74.7, 76.6, 101.3 (C-1β), 114.1, 120.1, 129.0, 159.2, 172.3 (CO); ESMS m/z 468 $[M+H]^+$; HRMS $[M+H]^+$, Found: 468.2954. $C_{25}H_{42}NO_7$ requires m/z, 468.2961.

3.4.15. 11-Phenoxyundecyl-malonic acid (21)

A stirred solution of 11-phenoxyundecanol **17** (650 mg, 2.5 mmol) and Meldrum's acid (354 mg, 2.5 mmol) in toluene (5 mL) was heated to reflux for 1 h. The reaction mixture was then cooled to rt, diluted with ethyl acetate (10 mL), and washed with water (10 mL) and brine (10 mL). The organic layer was then dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give the title compound as a colorless solid (840 mg, 98%); ^{1}H NMR ($CDCl_3$) δ 1.28–1.42 (m, 12H), 1.47 (m, 2H), 1.68 (m, 2H), 1.81 (m, 2H), 3.45 (s, 2H, COCH_2), 3.98 (t, J = 6.6 Hz, 2H, PhOCH_2), 4.18 (t, J = 6.7 Hz, 2H, OCH_2), 6.94 (m, 3H), 7.20 (m, 2H), 10.72 (br s, 1H, CO_2H); ^{13}C NMR ($CDCl_3$) δ 21.5, 25.8, 26.1, 28.4, 29.2, 29.3, 29.4, 29.5, 29.6, 41.0 (COCH_2), 66.0 (OCH_2), 67.9 (PhOCH_2), 114.5, 120.5, 129.4, 159.1 (Ar-C), 167.5, 170.8 (2 × CO); ESMS m/z 373 $[M+Na]^+$; HRMS $[M+Na]^+$, Found: 373.1974. $C_{20}H_{30}O_5Na$ requires m/z, 373.1991.

3.4.16. 3,4,6-Tri-*O*-benzyl-2-acetamido-2-deoxy-α-D-glucopyranosyl 11-phenoxyundecyl malonate (23α,β)

Compound **21** (190 mg, 0.5 mmol) and 3,4,6-tri-*O*-benzyl-2-acetamido-2-deoxy-α-D-glucopyranoside, **22a** (222 mg, 0.5 mmol)[22]

were dissolved in a mixture of THF (3 mL) and DMF (1 mL). DCC (201 mg, 1.0 mmol) and 4-dimethylamino)pyridine (DMAP) (12 mg, 0.1 mmol) were added to the solution and the reaction mixture was stirred at rt for 90 min. The reaction mixture was filtered and concentrated under reduced pressure. The residue was dissolved in ethyl acetate and washed with a solution of HCl 1.0 M (10 mL), satd aq $NaHCO_3$ (10 mL), brine (10 mL), and water (10 mL). The organic layer was then dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give a mixture of anomers (α:β, 7.5:1) that was purified by column chromatography on silica gel (ethyl acetate/hexanes, 1:1 v/v) to give both the α-anomer as a colorless oil (236 mg, 63%) and the β-anomer (32 mg, 9%) as a colorless oil. Data for **23α**; $[\alpha]_D$ = +37 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.27–1.40 (m, 12H), 1.47 (m, 2H, OC*H*$_2$), 1.67 (m, 2H, OC*H*$_2$), 1.80 (m, 2H, OC*H*$_2$), 1.89 (s, 3H, COC*H*$_3$), 3.46 (ABq, $J_{A,B}$ = 16.5 Hz, 2H, COC*H*$_2$), 3.71 (m, 2H), 3.78 (dd, *J* = 9.9, 3.2 Hz, 1H), 3.82–3.92 (br m, 2H), 3.97 (t, *J* = 6.5 Hz, 2H, PhOC*H*$_2$), 4.15 (br m, 2H, OC*H*$_2$) 4.48–4.59 (br m, 3H), 4.65 (d, *J* = 12.2 Hz, 1H, one OC*H*$_2$Ph), 4.72 (d, *J* = 11.4 Hz, 1H, one OC*H*$_2$Ph), 4.85 (br t, 2H, 2 × OC*H*$_2$Ph), 6.28 (d, 3.3 Hz, 1H, H-1), 6.39 (d, 9.6 Hz, 1H), 6.95 (m, 3H), 7.28 (m, 2H), 7.31–7.37 (m, 15H); ^{13}C NMR δ 23.1 (COC*H*$_3$), 25.8, 26.1, 28.4, 29.2, 29.3, 29.4, 29.5, 29.6, 41.4 (COC*H*$_2$), 51.8, 66.5, 67.8, 68.2, 73.6, 74.0, 74.5, 75.0, 75.3, 80.2, 93.2 (C-1α), 114.5, 120.5, 127.8, 127.8, 128.0, 128.1, 128.1, 128.4, 128.5, 129.4, 137.8, 137.9, 138.2, 159.1, 164.3, 168.2, 170.4 (3 × *C*O); ESMS *m/z* 824 $[M+H]^+$; HRMS $[M+H]^+$, Found: 824.4397. $C_{49}H_{62}NO_{10}$ requires *m/z*, 824.4374. Data for **23β**; $[\alpha]_D$ = +20 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.27–1.40 (m, 12H), 1.47 (m, 2H, OC*H*$_2$), 1.63 (m, 2H, OC*H*$_2$), 1.80 (m, 2H, OC*H*$_2$), 1.84 (s, 3H, COC*H*$_3$), 3.41 (ABq, $J_{A,B}$ = 16.2 Hz, 2H, COC*H*$_2$), 3.69 (m, 1H), 3.76–3.86 (m, 3H), 3.96–4.00 (m, 3H), 4.10–4.17 (m, 3H), 4.52 (d, *J* = 12.1 Hz, 1H, one OC*H*$_2$Ph), 4.59 (d, *J* = 10.9 Hz, 1H, one OC*H*$_2$Ph), 4.61 (d, *J* = 12.1 Hz, 1H, one OC*H*$_2$Ph), 4.69 (d, *J* = 11.6 Hz, 1H, one OC*H*$_2$Ph), 4.79 (d, *J* = 11.0 Hz, 1H, one OC*H*$_2$Ph), 4.82 (d, *J* = 10.9 Hz, 1H, one OC*H*$_2$Ph), 5.41 (d, *J* = 8.8 Hz, 1H, H-1), 5.79 (d, *J* = 7.9 Hz, 1H), 6.94 (m, 3H), 7.20 (m, 2H), 7.28–7.37 (m, 15H); ^{13}C NMR δ 21.1 (COC*H*$_3$), 23.4, 25.8, 26.1, 28.4, 29.3, 29.3, 29.4, 29.5, 29.6, 41.3 (COC*H*$_2$), 54.0, 60.4, 65.9, 67.9, 73.5, 74.3, 74.7, 75.7, 77.5, 80.3, 93.2 (C-1β), 114.5, 120.5, 127.7, 127.9, 127.9, 128.0, 128.2, 128.4, 128.5, 128.6, 129.4, 137.8, 137.9, 138.1, 159.1, 165.3, 166.3, 170.4 (3 × *C*O); ESMS *m/z* 846 $[M+Na]^+$; HRMS $[M+Na]^+$, Found: 846.4205. $C_{49}H_{61}NO_{10}Na$ requires *m/z*, 846.4193.

3.4.17. 2-Acetamido-2-deoxy-α-D-glucopyranosyl 11-phenoxyundecyl malonate (24α)

A suspension of compound **23α** (41 mg, 50 μmol) and 10% Pd/C (40 mg) in MeOH (4 mL) was stirred under H_2 atmosphere for 16 h. The Pd/C was removed by filtration through a Celite plug, washed with MeOH and the filtrate concentrated under reduced pressure to give the title compound as a colorless solid (25 mg, 92%); $[\alpha]_D$ = +61 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.03–1.42 (m, 12H), 1.50 (m, 2H, OC*H*$_2$), 1.68 (m, 2H, OC*H*$_2$), 1.77 (m, 2H, OC*H*$_2$), 1.99 (s, 3H, COC*H*$_3$), 3.50 (t, *J* = 9.3 Hz, 1H), 3.56 (br t, 1H), 3.66 to 3.82 (m, 4H), 3.97 (br t, 1H), 4.04 (dd, *J* = 3.2, 10.7 Hz, 1H), 4.17 (t, *J* = 6.6 Hz, 2H, PhOC*H*$_2$), 6.19 (d, *J* = 3.2 Hz, 1H, H-1), 6.89 (m, 3H), 7.25 (t, 2H); ^{13}C NMR δ 21.1 (COC*H*$_3$), 25.5, 25.6, 28.2, 28.9, 29.0, 29.1, 29.2, 29.3, 29.3, 53.0, 60.7, 61.6, 65.5, 67.5, 70.0, 70.9, 75.0, 91.8 (C-1α), 114.1, 120.1, 129.0, 158.2, 165.6, 167.2, 172.5 (3 × *C*O); ESMS *m/z* 554 $[M+H]^+$; HRMS $[M+H]^+$, Found: 554.2968. $C_{28}H_{44}NO_{10}$ requires *m/z*, 554.2965.

3.4.18. [Benzyloxy(11-phenoxyundecyloxy)phosphinyl]acetic acid benzyl ester (26)

A solution of (benzyloxyhydroxyphosphoryl)acetic acid benzyl ester **25** (95 mg, 0.3 mmol),[11] 11-phenoxyundecanol **17** (118 mg, 0.4 mmol)[4] and triphenylphosphine (116 mg. 0.4 mmol) in CH_2Cl_2 (1.5 mL) was stirred under N_2 atmosphere for 20 min at rt. The solution was then cooled to 0 °C and treated with DIAD (86 μL, 0.4 mmol), stirred for 30 min, and then warmed up to rt. After 4 h the mixture was treated with MeOH (250 μL), stirred for 5 min, and concentrated. The resulting residue was purified by column chromatography on silica gel (hexanes/ethyl acetate, 2:1 v/v) to give the title compound as a colorless oil (140 mg, 83%); ^{1}H NMR ($CDCl_3$) δ 1.23–1.42 (m, 12H), 1.48 (m, 2H), 1.60 (m, 2H), 1.79 (m, 2H), 3.05 (m, 2H, $J_{H,P}$ = 21.5 Hz), 3.96–4.03 (m, 4H, 2 × OC*H*$_2$), 5.11 (m, 2H, OC*H*$_2$Ph), 5.17 (s, 2H, CO_2C*H*$_2$Ph), 6.94 (m, 3H), 7.20 (m, 2H), 7.37 (s, 10H Ph-H); ^{13}C NMR ($CDCl_3$) δ 25.4, 26.1, 29.1, 29.3, 29.4, 29.5, 29.5, 29.6, 30.3, 30.4, 34.5 (d, $J_{C,P}$ = 134.8 Hz, *C*H$_2$P), 66.8 (d, $J_{C,P}$ = 6.8 Hz, *C*H$_2$OP), 67.3 (CO_2C*H*$_2$Ph), 67.9 (PhOC*H*$_2$), 66.8 (d, $J_{C,P}$ = 6.1 Hz, *C*H$_2$OP), 114.5, 120.5, 128.0, 128.4, 128.4, 128.5, 128.6, 128.6, 129.4, 135.3, 136.0, 136.1, 159.1 (Ar-C), 165.5 (d, $J_{C,P}$ = 6.0 Hz, *C*OCH$_2$P); ^{31}P NMR δ 20.1; ESMS *m/z* 589 $[M+Na]^+$; HRMS $[M+Na]^+$, Found: 589.2679. $C_{33}H_{43}O_6NaP$ requires *m/z*, 589.2695.

3.4.19. [Benzyloxy(11-phenoxyundecyloxy)phosphinyl]acetic acid (27)

To a solution of compound **26** (140 mg, 0.2 mmol) in ethanol (2 mL), 0.5 mL of 0.4 M NaOH were added under stirring. The reaction mixture was stirred for 1 h and was quenched with 0.3 mL 1.0 M HCl. The solvents were then removed and the residue was partitioned between CH_2Cl_2 (10 mL) and brine (10 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (10 mL). The combined organic extracts were then dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give the title compound as a colorless transparent solid (95 mg, 100%); ^{1}H NMR ($CDCl_3$) δ 1.22–1.42 (m, 12H), 1.46 (m, 2H), 1.63 (m, 2H), 1.81 (m, 2H), 3.04 (m, 2H, $J_{H,P}$ = 21.6 Hz), 3.97 (t, 2H, *J* = 6.6 Hz, PhOC*H*$_2$), 4.08 (m, 2H, POC*H*$_2$), 5.19 (m, 2H, OC*H*$_2$Ph), 6.94 (m, 3H), 7.28 (m, 2H), 7.32–7.38 (m, 5H Ph-H); ^{13}C NMR ($CDCl_3$) δ 24.5, 25.2, 28.2, 28.4, 28.5, 28.6, 28.6, 28.6, 33.5 (d, $J_{C,P}$ = 134.8 Hz, *C*H$_2$P), 66.4 (d, $J_{C,P}$ = 6.9 Hz, *C*H$_2$OP), 67.0 (PhOC*H*$_2$), 67.7 (d, $J_{C,P}$ = 6.1 Hz, *C*H$_2$OP), 113.6, 119.6, 127.2, 127.7, 128.1, 128.5, 128.6, 128.6, 129.4, 135.0, 135.0, 158.2, 166.9 (d, $J_{C,P}$ = 5.2 Hz, *C*OCH$_2$P); ^{31}P NMR δ 21.8; ESMS *m/z* 477 $[M+H]^+$; HRMS $[M+H]^+$, Found: 477.2413. $C_{26}H_{38}O_6P$ requires *m/z*, 477.2406.

3.4.20. [Benzyloxy(11-phenoxyundecyloxy)phosphinyl]acetyl 3,4,6-tri-*O*-benzyl-2-acetamido-2-deoxy-α-D-glucopyranoside (28)

Compound **27** (60 mg, 0.2 mmol) and 3,4,6-tri-*O*-benzyl-2-acetamido-2-deoxy-α-D-glucopyranoside **22a** (92 mg, 0.2 mmol)[4] were dissolved in a mixture of THF (1.5 mL) and DMF (0.75 mL). DCC (58 mg, 0.3 mmol) and DMAP (3 mg, 0.03 mmol) were added to the solution, and the reaction mixture was stirred at rt for 16 h. The reaction mixture was then filtered, concentrated under reduced pressure and purified by column chromatography on silica gel (ethyl acetate/hexanes, 4:1 v/v) to give the title compound as colorless oil (57 mg, 39%); ^{1}H NMR ($CDCl_3$) δ 1.24–1.40 (m, 12H), 1.47 (m, 2H, OC*H*$_2$), 1.66 (m, 2H, OC*H*$_2$), 1.81 (m, 2H, OC*H*$_2$), 1.94, 1.98 (2s, 3H, COC*H*$_3$), 2.90–3.04 (m, 2H, COC*H*$_2$P), 3.68 (dd, 1H), 3.774 (m, 1H), 3.82–3.90 (br m, 3H), 3.97 (t, *J* = 6.85 Hz, 2H, PhOC*H*$_2$), 4.04 (br m, 2H, OC*H*$_2$) 4.52–4.65 (br m, 4H), 4.80–4.86 (m, 3H), 5.09–5.15 (m, 2H), 6.22, 6.24 (2d, *J* = 3.4, 3.4 Hz, 1H, 2 × H-1), 6.94 (m, 3H), 7.18 (m, 2H), 7.28–7.42 (m, 20H), 7.48, 7.52 (2d, 1H); ^{13}C NMR δ 22.8, 22.9 (2 × COC*H*$_3$), 25.0, 25.4, 25.4, 25.6, 26.1, 29.1, 29.3, 29.4, 29.5, 29.6, 30.3, 30.3, 34.9 (d, $J_{C,P}$ = 110.6 Hz, COC*H*$_2$P), 52.0, 52.1, 67.2 (d, $J_{C,P}$ = 7.0 Hz), 67.5 (d, $J_{C,P}$ = 7.1 Hz), 67.8, 67.8 (2 × PhOC*H*$_2$), 68.4, 68.5 (d, $J_{C,P}$ = 6.4 Hz), 68.8 (d, $J_{C,P}$ = 6.3 Hz), 73.5, 74.0, 74.0, 75.2, 75.3, 75.4, 77.3, 80.0, 80.9, 93.5, 93.6 (C-1α), 114.5, 120.5, 120.5, 127.6, 127.7, 127.7, 127.8, 127.8, 127.9, 128.0, 128.2, 128.4, 128.4, 128.6, 128.8, 128.9,

129.0, 129.4, 137.9, 138.0, 138.1, 138.4, 159.1, 163.7 (d, $J_{C,P}$ = 6.4 Hz, $COCH_2P$), 170.8 (CONH); ^{31}P NMR δ 21.1; ESMS *m/z* 950 $[M+H]^+$; HRMS $[M+H]^+$, Found: 950.4595. $C_{55}H_{69}NO_{11}P$ requires *m/z*, 950.4608.

3.4.21. [Hydroxy (11-phenoxyundecyloxy)phosphinyl]acetyl 2-acetamido-2-deoxy-α-D-glucopyranoside (29)

A suspension of compound **23α** (42 mg, 44 μmol) and 10% Pd/C (40 mg) in MeOH (3 mL) was stirred under H_2 atmosphere for 16 h. The Pd/C was removed by filtration through a Celite plug, washed with MeOH, and the filtrate concentrated under reduced pressure to give the title compound as a colorless solid (22 mg, 85%); $[\alpha]_D$ = +46 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.24–1.40 (m, 12H), 1.47 (m, 2H, OCH_2), 1.66 (m, 2H, OCH_2), 1.81 (m, 2H, OCH_2), 1.94, 1.98 (2s, 3H, $COCH_3$), 2.90–3.04 (m, 2H, $COCH_2P$), 3.48 (m, 2H), 3.56 (t, *J* = 6.6 Hz, 1H), 3.73–3.79 (m, 4H), 3.96 (t, *J* = 6.7 Hz, 1H), 4.08 (m, 3H), 6.14 (d, *J* = 2.8 Hz, 1H, H-1), 6.90 (m, 3H), 7.24 (t, *J* = 7.7 Hz, 2H); ^{13}C NMR δ 21.3 ($COCH_3$), 24.7, 25.3, 25.4, 25.6, 25.8, 28.9, 29.0, 29.1, 29.2, 29.3, 30.3, 32.8 (d, $J_{C,P}$ = 108.8 Hz), 53.1, 60.8, 61.6, 66.0, 67.4, 70.0, 71.1, 75.0, 91.9 (C-1α), 114.1, 120.1, 129.0, 159.2, 165.6, 172.6 (2 × CO); ^{31}P NMR δ 19.3; ESMS *m/z* 590 $[M+H]^+$; HRMS $[M+H]^+$, Found: 590.2716. $C_{27}H_{45}NO_{11}P$ requires *m/z*, 590.2730.

3.4.22. (2-Cyano-ethoxy)(11-Phenoxyundecyloxy)diisopropylaminophosphine (30)

To a stirred solution of compound **17** (400 mg, 1.5 mmol) in dichloromethane (20 mL) were added (2-cyanoethoxy)bis(diisopropylamino)phosphine (503 mg, 1.7 mmol) and diisopropylammoniumtetrazolide (13 mg, 0.8 mmol). The mixture was allowed to stir at rt for 6 h. The reaction mixture was then washed with satd aq $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated to give crude compound **30**. The resulting crude material was purified by centrifugal chromatography (ether/hexanes, 3:1 v/v) to give compound **30** (488 mg, 70%); ^1H NMR ($CDCl_3$): δ 1.2–1.24 (m, 16H), 1.25 (1H), 1.32 (2H), 1.37 (m, 2H), 1.48 (m, 2H), 1.63 (m, 2H), 1.8 (m, 2H), 2.65 (m, 2H), 3.5 (m, 2H), 3.6–3.7 (m, 4H), 3.84 (m, 3H), 3.97 (m, 2H), 6.9 (m, 3H), 7.3 (m, 2H),; ^{13}C NMR: δ 25.1, 25.9, 26.3, 29.3, 29.5, 29.2, 30.5, 31.9, 43.0, 59.3, 68.1, 69.5, 114.3, 120.7, 129.4, 159.2; ^{31}P NMR: δ 140.5, 148.6; $C_{26}H_{45}N_2O_3P$ requires *m/z* 464.62.

3.4.23. 3,4,6-Tri-*O*-acetyl-2-acetamido-1-*O*-[(2-cyanoethoxy)(11-phenoxyundecyloxy)phosphoryl]-2-deoxy-α-D-glucopyranose (31)

To a stirred solution of compound **22b** (215 mg, 0.6 mmol) in dichloromethane (60 mL) at 0 °C was added compound **30** (488 mg, 1.1 mmol) and tetrazole (219 mg, 3.1 mmol). The mixture was warmed to rt and stirred for 6 h. The solution was then cooled to 0 °C, treated with *m*-chloroperoxybenzoic acid (*m*CPBA) (77%, 69 mg, 3.1 mmol), and allowed to stir overnight at rt The reaction mixture was then washed with a saturated solution of Na-sulfite, followed by satd aq $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$, and concentrated to give crude compound **31**. The material was purified by centrifugal chromatography (ethyl acetate/hexanes, 2:1 v/v) to give compound **31** (3.07 mg, 68%). ^1H NMR ($CDCl_3$): δ 1.20–1.26 (m, 5H), 1.37–1.43 (m, 1H), 1.63–1.77 (m, 2H), 1.93 (m, 3H), 1.98–2.0 (m, 12H), 2.05 (4H), 2.75 (2H), 3.9 (m, 2H), 4.04–4.14 (m, 5H), 4.2–4.3 (m, 5H), 4.38–4.42 (m, 1H), 5.12–5.25 (m, 2H), 5.66 (m, 1H), 6.34, 6.59 (2d, 1H), 6.86 (m, 3H), 7.2 (m, 2H); ^{13}C NMR ($COCH_3$), δ 20, 22.7, 25.5, 28.9, 29.2, 29.3, 51.7, 60.2, 61.2, 62.2, 67.4, 69, 77, 96.2, 114.3, 116.4, 120.3, 129.2, 158.9, 169, 170.4, 170.9; ^{31}P NMR δ −2.1, −2.2; $C_{34}H_{51}N_2O_{13}P$ requires *m/z* 726.75.

3.4.24. 2-Acetamido-2-deoxy-1-*O*-(11-phenoxyundecyloxy)phosphoryl-α-D-glucopyranose (32)

To a stirred solution of compound **31** (84 mg, 0.1 mmol) in MeOH (2 mL) was added concentrated NH_4OH (1.5 mL). The reaction mixture was stirred overnight at rt and the solvent was removed under reduced pressure. The resulting crude material was purified by Sephadex G15 size exclusion chromatography, using MeOH (0.1% NH_4CO_3) as the mobile phase to give compound **32** (572 mg, 91%). ^1H NMR ($CDCl_3$): δ 1.4–1.7 (m, 18H), 2.026 (3H, $COCH_3$), 3.4 (m, 3H), 3.72 (m, 2H), 3.89 (q, 5H), 3.97 (t, 4H), 5.46 (d, 1H-GlcNAcα, *J* = 2.4–3.2 Hz), 6.9 (3H), 7.23–7.28 (2H); ^{13}C NMR: δ ($COCH_3$) 22, 27.1, 31.9, 47.6, 55.5, 62.6, 66.8, 68.9, 71.9, 72.8, 74.9, 95.6, 115.5, 121.5, 130.4, 160.6, 171.8; ^{31}P NMR: δ 3.0; MS(-TOF): *m/z* 546 $[M-H]^-$ in MeOH; $C_{25}H_{41}NO_{10}P$ requires *m/z* 547.58.

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References

1. Raetz, R. H.; Whitfield, C. *Annu. Rev. Biochem.* **2002**, *71*, 635–700.
2. Samuel, G.; Reeves, P. *Carbohydr. Res.* **2003**, *338*, 2503–2519.
3. Allen, C.; Torres, A. *GI Microb. Regulat. Immune Syst.* **2008**, *635*, 93–98.
4. Montoya-Peleaz, P. J.; Riley, J. G.; Szarek, W. A.; Valvano, M. A.; Schutzbach, J. S.; Brockhausen, I. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1205–1211.
5. Brockhausen, I.; Liu, B.; Hu, B.; Lau, K.; Szarek, W. A.; Wang, L.; Feng, L. *J. Bacteriol.* **2008**, *190*, 4922–4932.
6. Xu, C.; Szarek, W. A.; Brockhausen, I.; Liu, B.; Hu, B.; Han, Y.; Feng, L.; Wang, L. *Glycoconj. J.* **2009**, *1*. abstract 148.
7. Riley, J. G.; Menggad, M.; Montoya-Peleaz, P.; Szarek, W. A.; Marolda, C. L.; Valvano, M. A.; Schutzbach, J. S.; Brockhausen, I. *Glycobiology* **2005**, *15*, 605–613.
8. Brockhausen, I.; Riley, J.; Joynt, M.; Yang, X.; Szarek, W. A. *Glycoconj. J.* **2008**, *25*, 663–673.
9. Yi, W.; Yao, Q.; Zhang, Y.; Motari, E.; Lin, S.; Wang, P. G. *Biochem. Biophys. Res. Commun.* **2006**, *344*, 631–639.
10. Brockhausen, I.; Larsson, E. A.; Hindsgaul, O. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 804–807.
11. IUPAC-IUB Commission on biochemical nomenclature *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 2222–2230.
12. Suzuki, T.; Kouketsu, A.; Matsuura, A.; Kohara, A.; Ninomiya, S.-I.; Kohda, K.; Miyata, N. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3313–3317.
13. Suzuki, T.; Nagano, Y.; Kouketsu, A.; Matsuura, A.; Maruyama, S.; Kurotaki, M.; Nakagawa, H.; Miyata, N. *J. Med. Chem.* **2005**, *48*, 1019–1032.
14. Chang, S. Y.; Choi, J. S.; Jeong, K. S. *Chemistry* **2001**, *7*, 2687–2697.
15. Savariar, E. N.; Aathimanikandan, S. V.; Thayumanavan, S. *J. Am. Chem. Soc.* **2006**, *128*, 16224–16230.
16. Wang, G.; Ella-Menye, J. R.; St. Martin, M.; Yang, H.; Williams, K. *Org. Lett.* **2008**, *10*, 4203–4206.
17. Loupy, A.; Sansoulet, J.; Vaziri-Zand, F. *Bull. Soc. Chim. Fr.* **1987**, *6*, 1027.
18. Chen, L.; Men, H.; Ha, S.; Ye, X.-Y.; Brunner, L.; Hu, Y.; Walker, S. *Biochemistry* **2002**, *41*, 6824–6833.
19. Hoard, D. E.; Ott, D. G. *J. Am. Chem. Soc.* **1965**, *87*, 1785–1788.
20. Iyer, S. S.; Rele, S. M.; Baskarana, S.; Chaikof, E. L. *Tetrahedron* **2003**, *59*, 631–638.
21. Ryu, Y.; Scott, A. I. *Tetrahedron Lett.* **2003**, *44*, 7499–7502.
22. Mbongo, A.; Frechou, C.; Beaupere, D.; Uzan, R.; Demailly, G. *Carbohydr. Res.* **1993**, *246*, 361–370.
23. Brockhausen, I.; Benn, M.; Bhat, S.; Marone, S.; Riley, J. G.; Montoya-Peleaz, P.; Vlahakis, J. Z.; Paulsen, H.; Schutzbach, J. S.; Szarek, W. *Glycoconj. J.* **2006**, *23*, 525–541.
24. Allen, J. R.; Harris, C. R.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 1890–1897.
25. Vincet, S.; Grenier, S.; Valleix, A.; Salesse, C.; Lebeau, L.; Mioskowsky, C. *J. Org. Chem.* **1998**, *63*, 7244–7257.