# Lipophilic sugar nucleotide synthesis by structure-based design of nucleotidylyltransferase substrates†

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Structure-based design of alkyl sugar-1-phosphates provides an efficient nucleotidylyltransferase-catalyzed synthesis of a series of new lipophilic sugar nucleotides possessing long or branched alkyl chains, thereby demonstrating the utility of nucleotidylyltransferases to catalyze the synthesis of sugar nucleotides with potential applications in lipopolysaccharide and lipoglycopeptide biosynthesis.

Sugar nucleotides play pivotal roles in glycobiology studies as substrates for glycosyltransferases and other sugar nucleotide processing enzymes involved in controlling a myriad of biological processes.[1–4] One key step in bacterial growth includes the processing of sugar nucleotides into the O-antigen[5] and lipid A[6] components of gram negative bacterial cells. Inhibition of the enzymes responsible for lipopolysaccharide biosynthesis offers a potential route for new antibiotic therapy distinct to current clinical antibiotics.[7] Some natural products contain lipophilic sugars, and in the case of teicoplanin, a lipoglycopeptide, the *N*-decanoyl glucosamine component may explain the improved activity relative to vancomycin, a glycopeptide, towards type B strains of vancomycin-resistant enterococci.[8] The glycosyltransferase responsible for attaching glucosamine is also able to transfer *N*-decanoyl glucosamine,[9] indicating that attachment of lipophilic sugars onto natural products using glycosyltransferases is feasible. Directed evolution has been used to significantly broaden the substrate specificity of glycosyltransferases and may improve catalytic efficiencies with lipophilic sugar nucleotides.[10,11] The synthetic approaches towards the prerequisite sugar nucleotides that are involved in lipopolysaccharide or natural product biosynthesis, or structurally related enzyme inhibitor synthesis, remain challenging due to a variety of reasons. Some difficulties arise from the lipophilic nature of the sugar nucleotides, the need to develop more efficient phosphate–phosphate coupling procedures to supersede classical morpholidate-mediated methodology[12,13] or the challenges associated with control of anomeric stereocontrol observed in general carbohydrate synthesis.[14–17]

One enzymatic approach to sugar nucleotide synthesis requires nucleotidylyltransferases to efficiently couple sugar-1-phosphates and nucleoside triphosphates (Scheme 1a).[18] As a result of high yields and lack of anomeric stereochemical control required using this enzymatic approach to sugar nucleotide synthesis, the substrate specificities of several homologous nucleotidylyltransferases have been explored with respect to both substrates.[19–25] The enzymes studied from primary metabolic pathways accept a variety of primarily α-D-sugar-1-phosphate analogues and a variety of nucleoside triphosphates. Site-specific mutagenesis has also been used to alter the sugar-1-phosphate specificity[26,27] and the base specificity[28] of the nucleoside triphosphate to provide enzymatic access to a variety of sugar nucleotides. Recently, three homologous wild-type thymidylyltransferases (RmlA, Cps2L and RmlA3) from different gram positive organisms (*Streptococcus mutans*, *Streptococcus pneumoniae* and *Aneurinibacillus thermoaerophilus*, respectively)[29] were described to have broad substrate specificity with a variety of nucleoside triphosphates, pyranose-1-phosphates[30] and furanose-1-phosphates.[31] Herein, we provide insight into a new aspect of the substrate specificity of these recombinant wild-type enzymes. Based on an analysis of the crystal structure of a homologous nucleotidylyltransferase, RmlA from *Pseudomonas aeruginosa*, bound to the product α-D-glucose thymidine diphosphate (Fig. 1),[32] we observed a pocket adjacent to

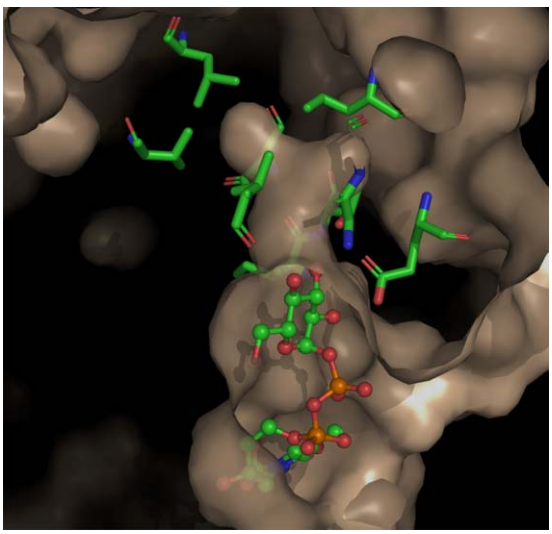

**Fig. 1** A cut-away view of the structure of RmlA from *Pseudomonas aeruginosa* (pdb: 1G1L) showing dTDP-α-D-glucose (ball-and-stick representation) bound to the solvent accessible enzyme surface. Only residues forming a pocket surrounding the glucose moiety are shown for clarity.

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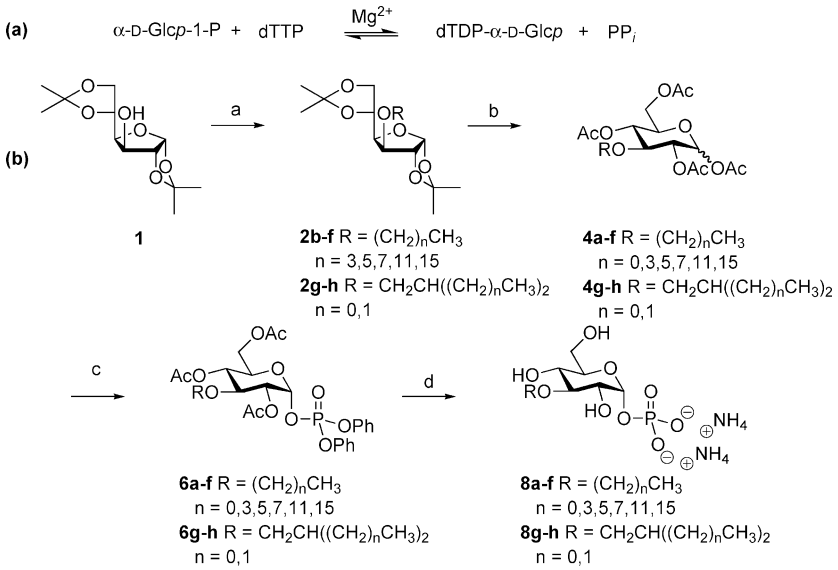


**Scheme 1** (a) Physiological reaction catalyzed by α-D-glucopyranosyl-1-phosphate thymidylyltransferases, and (b) synthesis of 3-*O*-alkyl-α-D-glucopyranosyl phosphates: (a) RBr, NaH, DMF, 17–76%; (b) (i) Amberlite 120 $H^+$, 5 : 1 MeOH–$H_2O$, 60 °C; (ii) $Ac_2O$, py, 32–100%; (c) (i) hydrazine acetate, DMF; (ii) $ClPO(OPh)_2$, DMAP, $CH_2Cl_2$, 23–61%; (d) (i) $H_2$, $PtO_2$, 1 : 1 EtOAc–EtOH; (ii) 2 : 2 : 1 MeOH–$H_2O$–$NEt_3$, 50 °C; (iii) Amberlite 120 $H^+$, $NH_4OH$, 42–100%.

the enzyme active site and anticipated binding of substrates with chemical functionality extending from the C3-OH of the glucose moiety into this pocket. A similar pocket was also observed in the crystal structure of the enzyme with the inhibitor β-L-rhamnose thymidine diphosphate (Fig. 2).

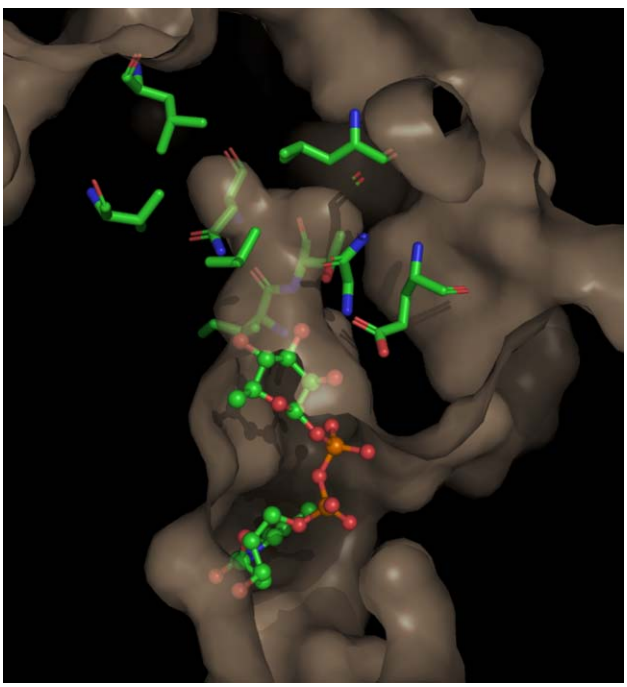

**Fig. 2** A cut-away view of the structure of RmlA from *Pseudomonas aeruginosa* (pdb: 1G3L) showing dTDP-β-L-rhamnose (ball-and-stick representation) bound to the solvent accessible enzyme surface. Only residues forming a pocket surrounding the rhamnose moiety are shown for clarity.

This pocket is directed away from the centre of catalysis where the new phosphoryl bond is formed and would, therefore, still enable formation of a productive ternary enzyme complex.[32] These results would provide the first support for nucleotidylyltransferases to affect the synthesis of lipophilic sugar nucleotides of relevance to lipopolysaccharide or lipoglycopeptide biosynthesis.

The synthetic strategy employed in the chemical preparation of the 3-*O*-alkyl-α-D-glucopyranosyl phosphates is outlined in Scheme 1b. 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**1**) was treated with sodium hydride and an appropriate alkyl bromide to furnish the alkylated furanoses.[33] Good yields were obtained for all products except the branched derivatives **2g** and **2h** where the steric bulk likely decreased reactivity. Acid hydrolysis of the diacetonides followed by acetylation of the 3-*O*-alkyl glucopyranoses afforded **4a–h**,[33] with the exception of compound **4a**, which was acetylated directly from commercially available 3-*O*-methyl-D-glucopyranose. The beta anomer was isolated in all cases except for that of compound **4a**. Selective deacetylation at the anomeric centre was accomplished using hydrazine acetate,[34] and after column chromatography, access to the fully protected 3-*O*-alkyl-α-D-glucopyranosyl-1-phosphates (**6a–h**) was provided by stereoselective phosphorylation using diphenyl chlorophosphate.[35] Deprotection of the phenyl phosphate esters was effected using platinum(IV) oxide-mediated catalytic hydrogenolysis,[35] followed by deacetylation in 2 : 2 : 1 MeOH–$H_2O$–$NEt_3$ resulting in the triethylammonium salt of the target sugar-1-phosphates (**7a–h**).[36] After cation ion exchange chromatography on Amberlite 120 $H^+$ resin, the ammonium salts (**8a–h**) were isolated by titration to pH 9–10 with ammonium hydroxide and lyophilized to remove any residual ammonium acetate. The $^1J_{C1,H1}$ coupling constants for **8a–8h** were in the range 171–174 Hz, confirming the configuration at the anomeric centre as alpha.[37]

Enzyme assays were performed as described previously[30] and results are presented in Fig. 3 for Cps2L (for RmlA3 see ESI†).

 

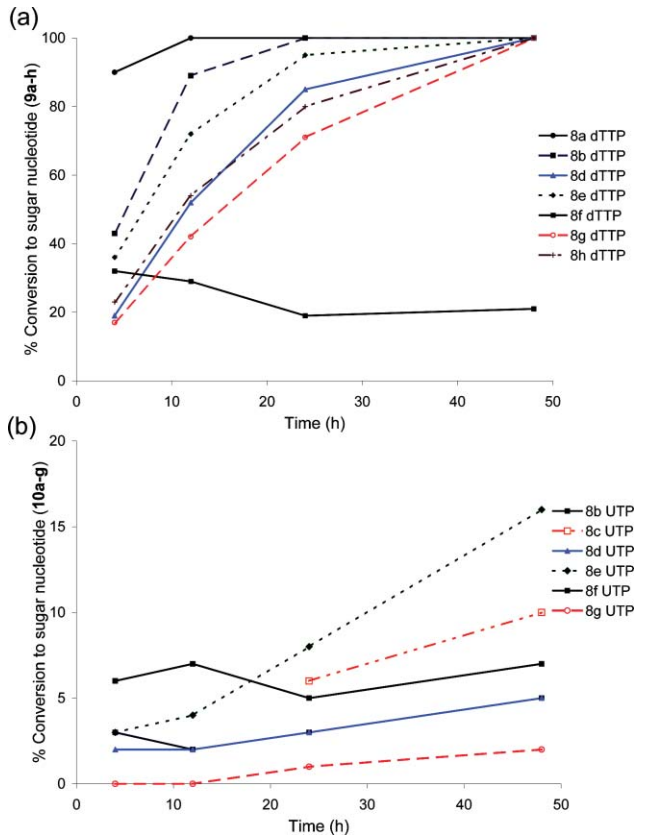


**Fig. 3** Nucleotidylyltransferase-catalyzed conversion of sugar-1-phosphates (**8a–h**) to (a) dTDP-sugars (**9a–h**) and (b) UDP-sugars (**10a–h**) by Cps2L. Products **9c** and **10a** co-eluted with dTTP and UDP, respectively.

Product formation was confirmed by ESI-MS/MS (ESI†).[38] Both Cps2L and RmlA3 afforded quantitative conversion of all eight synthesized sugar-1-phosphates to the corresponding dTDP-sugars over 48 h with the exception of **8f**. In general, the longer alkyl chain derivatives were converted to product more slowly than the shorter chain derivatives. The two branched chain analogues (**8g**, **8h**) were also converted readily, indicating that within the extended substrate binding pocket it is possible to accommodate more bulky substrate analogues. The significant surfactant-like properties and the limited solubility, in addition to the longer alkyl chain, may be contributing factors to explain why **8f** was a particularly poor substrate. In otherwise identical reaction conditions, neither enzyme was able to match the quantitative conversion observed with dTTP when using UTP as the nucleoside triphosphate substrate. RmlA3 obtained the highest conversion with UTP and **8b** (30% over the 48 h). This is in contrast to the high level of activity of these enzymes with various commercial sugar-1-phosphates,[30] but consistent with the catalysis observed with furanosyl-1-phosphates.[31] We observed no GDP-sugar formation on incubation of **8a–8h** with GTP. The second ionization constant of the phosphate ($pK_a2$) may influence the ability of the phosphate to act as a nucleophile in the enzymatic reaction. We observed an increase of + 0.6 units in the $pK_a2$ over the series **8b**, **8d**, **8g** to **8e**, consistent with an inductively donating effect of the alkyl substituent upon the sugar-1-phosphate. The decrease in the acidity of the phosphate as indicated by an increase in $pK_a2$ does not appear to significantly affect catalysis.

An alternative representation of the crystal structure (Fig. 4) indicated to us that the observed pocket described in Fig. 1 is formed primarily by the side-chains of the βc sheet and the α8 helix. Thus, the lipophilic functionality of the dodecyl- (**8e**) and branched analogues (**8g**, **8h**), may fit between the side-chains of these two secondary structural elements, since the size of the pocket is likely too small to accommodate these larger substrates without a significant steric clash. The acceptance of the alkyl sugar-1-phosphates by the nucleotidylyltransferases is potentially aided by an ordered Bi–Bi catalytic reaction mechanism where glucose-1-phosphate binds after dTTP, as has been observed for homologous enzymes.[26,39] Substrate binding and protein engineering studies are underway to substantiate these hypotheses.

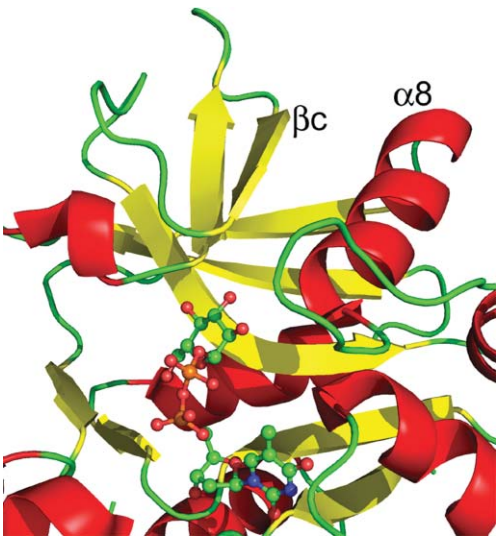


**Fig. 4** RmlA from *Pseudomonas aeruginosa* (pdb: 1G1L) as a cartoon representation showing dTDP-α-D-glucose (ball-and-stick representation) with the glucose C3 hydroxyl pointing between the side-chains of the βc sheet and the α8 helix.

In conclusion, structure-based substrate design of a series of alkyl sugar-1-phosphates has demonstrated a remarkably diverse new activity for thymidylyltransferases. This is the first report of the enzymatic preparation of lipophilic sugar nucleotides. Thus, these catalysts will likely be of significant importance in providing access to more elaborate sugar nucleotides for glycobiology studies involving lipopolysaccharide or lipoglycopeptide biosynthesis.

## Experimental

### General methods

Dichloromethane and THF were dried over alumina (Innovative Technology) and stored over 3 Å molecular sieves. All other reagents and solvents were purchased and used without further purification. All reactions were monitored by thin layer chromatography (TLC) using Silicycle precoated silica gel plates (250 μm thickness). The TLC plates were visualized with a potassium permanganate solution (3 g potassium permanganate, 20 g potassium carbonate, 5 mL 5% aqueous sodium hydroxide, 300 mL distilled water), a phenol solution (10 g phenol, 5 mL $H_2SO_4$, 95 mL ethanol), or ultraviolet light ($\lambda = 254$ nm). Unless otherwise specified, flash chromatographic purification was performed on a

Biotage SP1 HPFC. $^{1}H$, $^{13}C$ and $^{31}P$ NMR spectra were obtained on a Bruker AVANCE-500 NMR Spectrometer operating at frequencies of 500.13 MHz, 125.76 MHz, and 202.45 MHz, respectively. Assignments are based on COSY and HSQC 2D NMR experiments. For chloroform-*d*, $^{1}H$ NMR chemical shifts are reported as $\delta$ in units of parts per million (ppm) downfield from tetramethylsilane ($\delta$ 0.0) and $^{13}C$ NMR chemical shifts are reported as $\delta$ in units of parts per million (ppm) relative to the residual solvent signal of chloroform-*d* ($\delta$ 77.16). For methanol-$d_4$, $^{1}H$ and $^{13}C$ NMR chemical shifts are reported as $\delta$ in units of parts per million (ppm) relative to the residual solvent signal of methanol-$d_4$ ($\delta$ 3.31 and $\delta$ 49.00, respectively). $^{31}P$ NMR chemical shifts are reported as $\delta$ in units of parts per million (ppm) relative to 85% $H_3PO_4$. Enzymatic reactions were monitored by HPLC performed on a Hewlett Packard Series 1050 instrument with an Agilent Zorbax 5 μm Rx-C18 column (150 mm × 4.6 mm). Compounds with a nucleotide base chromophore were monitored using a UV detector ($\lambda = 254$ nm). The linear gradient used was 90 : 10 A–B to 40 : 60 A–B over 8.0 min, followed by a plateau at 40 : 60 A–B from 8.0 to 10.0 min at 1.0 mL $min^{-1}$ where A is an aqueous buffer containing 12 mM $NBu_4Br$, 10 mM $KH_2PO_4$, and 5% HPLC grade $CH_3CN$ (pH 4.9) and B is HPLC grade $CH_3CN$ except for assays containing compound **8f**, whereby the gradient was run to 80% $CH_3CN$ instead of 60%. High resolution mass spectra were recorded on a micrOTOF instrument (Bruker Dalton) running in negative ion mode (ESI). Low resolution mass spectra were recorded on an LCQDuo ion trap instrument (Thermo Finnigan) running in positive ion mode (ESI).

An Agilent 1100 LC system was coupled to an Applied Biosystems-MDS SCIEX hybrid triple quadrupole linear ion trap (4000QTRAP) mass spectrometer equipped with a Turbo V source for electrospray ionization for the ESI-MS/MS experiments. The sample was analyzed by flow-injection analysis in 75 : 25 (v/v) acetonitrile–de-ionized water using a flow-rate of 200 μL $min^{-1}$. Precursor ion scanning of *m/z* 323 ($[UMP - H]^-$) and *m/z* 383 ($[TDP - H]^-$) were used initially to selectively detect for uridine- and thymidine-linked sugar nucleotides, respectively, in the sample. ESI-MS/MS analysis in the enhanced product ion (EPI) mode was then performed to confirm the presence of the expected synthetic sugar nucleotide. The mass spectrometer settings for precursor ion scanning were: ionspray voltage 4.5 kV; mass range Q1 *m/z* 300–800; scan time 5 sec, Q1 and Q3 set to unit resolution. For the ESI-MS/MS experiments: ionspray voltage 4.5 kV; Q3 *m/z* 100–800; scan speed 1000 amu per sec; trap fill time was set to dynamic; Q1 and Q3 set to unit resolution. All acquisitions were made in the negative ion mode.

p$K_a$ values were measured in the following way: using an IQ Scientific Instruments IQ150 fitted with an ISFET probe, a 0.01 M solution of the diammonium salt was adjusted to pH 10 with 0.2 M NaOH. Titration was done with 5 μL aliquots of 0.2 M HCl until pH 2, and the p$K_a$ values were determined by plotting in GraFit 5.0.4 (Erithacus Software Limited).

**General procedure for selective deacetylation and phosphorylation (5a–h and 6a–h, respectively).** To a stirring solution of the acetylated glucopyranoside (**4a–h**) (2.41 mmol) in anhydrous DMF (10 mL) was added hydrazine acetate (0.33 g, 3.62 mmol) and the solution was subsequently heated to 60 °C for 15 min. The mixture was then stirred for 1 h at rt before being diluted with $H_2O$ (20 mL) and extracted with dichloromethane (2 × 20 mL). The combined organic layers were washed with brine (10 mL) and dried ($Na_2SO_4$), filtered and concentrated. The major product was purified by flash chromatography to forge the selectively deacetylated pyranose (**5a–h**). Under a nitrogen atmosphere, a solution of the selectively deacetylated product (**5a–h**) (1.49 mmol) and 4-dimethylaminopyridine (438 mg, 3.58 mmol) in anhydrous dichloromethane (8 mL) was stirred for 15 min at rt. The flask was cooled to −10 °C and charged with diphenyl chlorophosphate (464 μL, 2.24 mmol). After 1.5 h, the mixture was diluted with $H_2O$ (20 mL) and extracted with dichloromethane (2 × 15 mL). The combined organic extracts were washed with brine (10 mL) and dried ($Na_2SO_4$), filtered and concentrated. Purification by flash chromatography afforded the title compounds (**6a–g**).

**Diphenyl 2,4,6-tri-*O*-acetyl-3-*O*-methyl-α-D-glucopyranosyl-1-phosphate (6a).** Compound **5a** ($R_F = 0.28$ (hexanes–EtOAc, 60 : 40)), a colorless liquid, was provided by silica gel chromatography (hexanes–EtOAc, 60 : 40) (556 mg, 72%) and immediately carried onto the subsequent phosphorylation. Chromatographic purification (hexanes–EtOAc, 72 : 28) furnished compound **6a** as a colorless liquid (552 mg, 67%); $R_F = 0.36$ (hexanes–EtOAc, 67 : 33); $^{1}H$ NMR ($CDCl_3$) $\delta$ 7.38–7.20 (m, 10H, Ph × 2), 6.06 (dd, 1H, $^{1}J_{C,H} = 180$ Hz, $J_{1,P} = 6.5$ Hz, $J_{1,2} = 3.3$ Hz, H-1), 5.07 (dd, 1H, $J_{4,5} = 10.2$ Hz, $J_{3,4} = 9.6$ Hz, H-4), 4.90 (ddd, 1H, $J_{2,3} = 10.0$ Hz, $J_{1,2} = 3.1$ Hz, $^{4}J_{1,P} = 3.1$ Hz, H-2), 4.14 (dd, 1H, $^{2}J_{6a,6b} = 12.5$ Hz, $J_{5,6a} = 4.3$ Hz, H-6a), 4.02 (ddd, 1H, $J_{4,5} = 10.4$ Hz, $J_{5,6a} = 4.2$ Hz, $J_{5,6b} = 2.1$ Hz, H-5), 3.90 (dd, 1H, $J_{6a,6b} = 12.2$ Hz, $J_{5,6b} = 2.2$ Hz, H-6b), 3.73 (dd, 1H, $J_{2,3} = 9.7$ Hz, $J_{3,4} = 9.7$ Hz, H-3), 3.45 (s, 3H, $CH_3$), 2.10, 2.00, 1.94 (s, 9H, C(O)$CH_3$ × 3); $^{13}C$ NMR ($CDCl_3$) $\delta$ 170.8, 170.0, 169.4 ($C$(O)$CH_3$ × 3), 150.6–120.1 (12C, Ph × 2), 95.6 (d, $^{2}J_{1,P} = 6.3$ Hz, C-1), 77.9 (C-3), 71.9 (d, $^{3}J_{1,P} = 7.1$ Hz, C-2), 70.3 (C-5), 68.7 (C-4), 61.5 (C-6), 60.6 ($CH_3$), 20.9, 20.8, 20.7 (C(O)$CH_3$ × 3); $^{31}P$ NMR ($CDCl_3$) $\delta$ −13.98 (s, 1P, P-1); LRMS *m/z* calcd for $C_{25}H_{29}O_{12}P$ $[M + Na]^+$: 575.1. Found 575.0.

**Diphenyl 2,4,6-tri-*O*-acetyl-3-*O*-butyl-α-D-glucopyranosyl-1-phosphate (6b).** Compound **5b** ($R_F = 0.43$ (hexanes–EtOAc, 67 : 33)), a colorless liquid, was provided by silica gel chromatography (hexanes–EtOAc, 72 : 28) (690 mg, 79%) and immediately carried onto the subsequent phosphorylation. Chromatographic purification (hexanes–EtOAc, 78 : 22) furnished compound **6b** as a colorless liquid (602 mg, 68%); $R_F = 0.26$ (hexanes–EtOAc, 75 : 25); $^{1}H$ NMR ($CDCl_3$) $\delta$ 7.38–7.20 (m, 10H, Ph × 2), 6.06 (dd, 1H, $^{1}J_{C,H} = 183$ Hz, $J_{1,P} = 6.6$ Hz, $J_{1,2} = 3.3$ Hz, H-1), 5.08 (dd, 1H, $J_{4,5} = 10.0$ Hz, $J_{3,4} = 10.0$ Hz, H-4), 4.90 (ddd, 1H, $J_{2,3} = 10.0$ Hz, $J_{1,2} = 3.2$ Hz, $^{4}J_{1,P} = 3.2$ Hz, H-2), 4.14 (dd, 1H, $^{2}J_{6a,6b} = 12.5$ Hz, $J_{5,6a} = 4.2$ Hz, H-6a), 4.01 (ddd, 1H, $J_{4,5} = 10.3$ Hz, $J_{5,6a} = 4.1$ Hz, $J_{5,6b} = 2.1$ Hz, H-5), 3.89 (dd, 1H, $J_{6a,6b} = 12.5$ Hz, $J_{5,6b} = 2.2$ Hz, H-6b), 3.79 (dd, 1H, $J_{2,3} = 9.8$ Hz, $J_{3,4} = 9.8$ Hz, H-3), 3.56 (m, 2H, O$CH_2$), 2.08, 2.00, 1.92 (s, 9H, C(O)$CH_3$ × 3), 1.46 (m, 2H, $OCH_2CH_2$), 1.30 (m, 2H, $OCH_2CH_2CH_2$), 0.89 (t, 3H, $J = 7.5$ Hz, $(CH_2)_3CH_3$); $^{13}C$ NMR ($CDCl_3$) $\delta$ 170.7, 169.9, 169.3 ($C$(O)$CH_3$ × 3), 150.5–120.1 (12C, Ph × 2), 95.7 (d, $^{2}J_{1,P} = 6.0$ Hz, C-1), 76.5 (C-3), 73.0 (O$CH_2$), 72.1 (d, $^{3}J_{1,P} = 7.1$ Hz, C-2), 70.3 (C-5), 68.9 (C-4), 61.5 (C-6), 32.3 ($OCH_2CH_2$), 20.8, 20.7, 20.6 (C(O)$CH_3$ × 3), 19.1 ($OCH_2CH_2CH_2$), 13.9 ($(CH_2)_3CH_3$); $^{31}P$ NMR ($CDCl_3$) $\delta$ −14.00 (s, 1P, P-1); LRMS *m/z* calcd for $C_{28}H_{35}O_{12}P$ $[M + Na]^+$: 617.2. Found 617.2.

**Diphenyl 2,4,6-tri-*O*-acetyl-3-*O*-hexyl-α-D-glucopyranosyl-1-phosphate (6c).** Diethyl ether was used instead of dichloromethane as an extraction solvent *en route* to compound **5c** ($R_F$ = 0.30 (hexanes–EtOAc, 70 : 30)), a colorless liquid, which was provided by silica gel chromatography (hexanes–EtOAc, 63 : 37) (772 mg, 82%) and immediately carried onto the subsequent phosphorylation. Diethyl ether was again used instead of dichloromethane as an extraction solvent for compound **6c**, a colorless liquid, which was isolated by chromatographic purification (hexanes–EtOAc, 75 : 25) (538 mg, 58%); $R_F$ = 0.29 (hexanes–EtOAc, 75 : 25); $^1$H NMR ($CDCl_3$) $\delta$ 7.38–7.20 (m, 10H, Ph × 2), 6.06 (dd, 1H, $^1J_{C,H}$ = 180 Hz, $J_{1,P}$ = 6.5 Hz, $J_{1,2}$ = 3.4 Hz, H-1), 5.08 (dd, 1H, $J_{4,5}$ = 10.0 Hz, $J_{3,4}$ = 10.0 Hz, H-4), 4.90 (ddd, 1H, $J_{2,3}$ = 9.9 Hz, $J_{1,2}$ = 3.0 Hz, $^4J_{1,P}$ = 3.0 Hz, H-2), 4.14 (dd, 1H, $^2J_{6a,6b}$ = 12.5 Hz, $J_{5,6a}$ = 4.2 Hz, H-6a), 4.01 (ddd, 1H, $J_{4,5}$ = 10.4 Hz, $J_{5,6a}$ = 4.1 Hz, $J_{5,6b}$ = 2.0 Hz, H-5), 3.89 (dd, 1H, $J_{6a,6b}$ = 12.6 Hz, $J_{5,6b}$ = 2.1 Hz, H-6b), 3.79 (dd, 1H, $J_{2,3}$ = 9.7 Hz, $J_{3,4}$ = 9.7 Hz, H-3), 3.55 (m, 2H, OC$H_2$), 2.08, 2.00, 1.92 (s, 9H, C(O)C$H_3$ × 3), 1.47 (m, 2H, $OCH_2CH_2$), 1.28 (m, 6H, $OCH_2CH_2(CH_2)_3CH_3$), 0.88 (t, 3H, $J$ = 6.8 Hz, $O(CH_2)_5CH_3$); $^{13}$C NMR ($CDCl_3$) $\delta$ 170.8, 169.9, 169.3 ($C$(O)$CH_3$ × 3), 150.5–120.1 (12C, Ph × 2), 95.7 (d, $^2J_{1,P}$ = 6.0 Hz, C-1), 76.5 (C-3), 73.4 (O$CH_2$), 72.1 (d, $^3J_{1,P}$ = 7.3 Hz, C-2), 70.4 (C-5), 68.9 (C-4), 61.5 (C-6), 31.7, 25.8, 22.7 ($OCH_2CH_2(CH_2)_3CH_3$), 30.3 ($OCH_2CH_2$), 20.9, 20.8, 20.6 (C(O)$CH_3$ × 3), 14.2 ($O(CH_2)_5CH_3$); $^{31}$P NMR ($CDCl_3$) $\delta$ −14.00 (s, 1P, P-1); LRMS $m/z$ calcd for $C_{30}H_{39}O_{12}P$ [M + Na]$^+$: 645.2. Found 645.1.

**Diphenyl 2,4,6-tri-*O*-acetyl-3-*O*-octyl-α-D-glucopyranosyl-1-phosphate (6d).** Diethyl ether was used instead of dichloromethane as an extraction solvent *en route* to compound **5d** ($R_F$ = 0.27 (hexanes–EtOAc, 67 : 33)), a colorless liquid which was provided by silica gel chromatography (hexanes–EtOAc, 63 : 37) (625 mg, 62%) and immediately carried onto the subsequent phosphorylation. Chromatographic purification (hexanes–EtOAc, 81 : 19) furnished compound **6d** as a colorless liquid (717 mg, 74%); $R_F$ = 0.45 (hexanes–EtOAc, 67 : 33); $^1$H NMR ($CDCl_3$) $\delta$ 7.38–7.20 (m, 10H, Ph × 2), 6.06 (dd, 1H, $^1J_{C,H}$ = 181 Hz, $J_{1,P}$ = 6.6 Hz, $J_{1,2}$ = 3.4 Hz, H-1), 5.08 (dd, 1H, $J_{4,5}$ = 10.2 Hz, $J_{3,4}$ = 10.2 Hz, H-4), 4.90 (ddd, 1H, $J_{2,3}$ = 10.1 Hz, $J_{1,2}$ = 3.2 Hz, $^4J_{1,P}$ = 3.2 Hz, H-2), 4.14 (dd, 1H, $^2J_{6a,6b}$ = 12.5 Hz, $J_{5,6a}$ = 4.2 Hz, H-6a), 4.01 (ddd, 1H, $J_{4,5}$ = 10.2 Hz, $J_{5,6a}$ = 3.9 Hz, $J_{5,6b}$ = 2.0 Hz, H-5), 3.89 (dd, 1H, $J_{6a,6b}$ = 12.6 Hz, $J_{5,6b}$ = 2.1 Hz, H-6b), 3.78 (dd, 1H, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 9.8 Hz, H-3), 3.55 (m, 2H, OC$H_2$), 2.08, 2.00, 1.92 (s, 9H, C(O)C$H_3$ × 3), 1.47 (m, 2H, $OCH_2CH_2$), 1.34–1.19 (m, 10H, $OCH_2CH_2(CH_2)_5CH_3$), 0.88 (t, 3H, $J$ = 7.1 Hz, $(CH_2)_7CH_3$); $^{13}$C NMR ($CDCl_3$) $\delta$ 170.8, 169.9, 169.3 ($C$(O)$CH_3$ × 3), 150.6–120.1 (12C, Ph × 2), 95.7 (d, $^2J_{1,P}$ = 6.1 Hz, C-1), 76.5 (C-3), 73.5 (O$CH_2$), 72.1 (d, $^3J_{1,P}$ = 7.2 Hz, C-2), 70.4 (C-5), 68.9 (C-4), 61.5 (C-6), 32.0, 29.5, 29.4, 26.1, 22.8 ($OCH_2CH_2(CH_2)_5CH_3$), 30.3 ($OCH_2CH_2(CH_2)_5CH_3$), 20.9, 20.8, 20.6 (C(O)$CH_3$ × 3), 14.2 ($(CH_2)_7CH_3$); $^{31}$P NMR ($CDCl_3$) $\delta$ −14.00 (s, 1P, P-1); LRMS $m/z$ calcd for $C_{32}H_{43}O_{12}P$ [M + Na]$^+$: 673.2. Found 673.3.

**Diphenyl 2,4,6-tri-*O*-acetyl-3-*O*-dodecyl-α-D-glucopyranosyl-1-phosphate (6e).** Diethyl ether was used instead of dichloromethane as an extraction solvent *en route* to compound **5e** ($R_F$ = 0.29 (hexanes–EtOAc, 67 : 33)), a colorless liquid, which was not purified by silica gel chromatography (675 mg, 59%). For the phosphorylation, diethyl ether was again used instead of dichloromethane as an extraction solvent, which was isolated by chromatographic purification (hexanes–EtOAc, 80 : 20) to furnish compound **6e** as a colorless liquid (463 mg, 44%); $R_F$ = 0.38 (hexanes–EtOAc, 75 : 25); $^1$H NMR ($CDCl_3$) $\delta$ 7.38–7.20 (m, 10H, Ph × 2), 6.05 (dd, 1H, $^1J_{C,H}$ = 180 Hz, $J_{1,P}$ = 6.6 Hz, $J_{1,2}$ = 3.4 Hz, H-1), 5.08 (dd, 1H, $J_{4,5}$ = 10.3 Hz, $J_{3,4}$ = 9.7 Hz, H-4), 4.90 (ddd, 1H, $J_{2,3}$ = 10.0 Hz, $J_{1,2}$ = 3.1 Hz, $^4J_{1,P}$ = 3.1 Hz, H-2), 4.14 (dd, 1H, $^2J_{6a,6b}$ = 12.5 Hz, $J_{5,6a}$ = 4.2 Hz, H-6a), 4.01 (ddd, 1H, $J_{4,5}$ = 10.4 Hz, $J_{5,6a}$ = 4.1 Hz, $J_{5,6b}$ = 2.1 Hz, H-5), 3.89 (dd, 1H, $J_{6a,6b}$ = 12.5 Hz, $J_{5,6b}$ = 2.1 Hz, H-6b), 3.79 (dd, 1H, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 9.8 Hz, H-3), 3.55 (m, 2H, OC$H_2$), 2.08, 2.00, 1.92 (s, 9H, C(O)C$H_3$ × 3), 1.47 (m, 2H, $OCH_2CH_2$), 1.33–1.19 (m, 18H, $OCH_2CH_2(CH_2)_9CH_3$), 0.88 (t, 3H, $J$ = 6.7 Hz, $O(CH_2)_{11}CH_3$); $^{13}$C NMR ($CDCl_3$) $\delta$ 170.8, 169.9, 169.3 ($C$(O)$CH_3$ × 3), 150.6–120.1 (12C, Ph × 2), 95.7 (d, $^2J_{1,P}$ = 6.1 Hz, C-1), 76.6 (C-3), 73.5 (O$CH_2$), 72.1 (d, $^3J_{1,P}$ = 7.3 Hz, C-2), 70.4 (C-5), 68.9 (C-4), 61.5 (C-6), 32.1, 29.8, 29.8, 29.8, 29.8, 29.6, 29.5, 26.1, 22.8 ($OCH_2CH_2(CH_2)_9CH_3$), 30.4 ($OCH_2CH_2(CH_2)_9CH_3$), 20.9, 20.8, 20.7 (C(O)$CH_3$ × 3), 14.2 ($O(CH_2)_{11}CH_3$); $^{31}$P NMR ($CDCl_3$) $\delta$ −14.00 (s, 1P, P-1); LRMS $m/z$ calcd for $C_{36}H_{51}O_{12}P$ [M + Na]$^+$: 729.3. Found 729.1.

**Diphenyl 2,4,6-tri-*O*-acetyl-3-*O*-hexadecyl-α-D-glucopyranosyl-1-phosphate (6f).** Chloroform was used instead of dichloromethane as an extraction solvent *en route* to compound **5f** ($R_F$ = 0.21 (hexanes–EtOAc, 67 : 33)), a white solid, which was provided by silica gel chromatography (hexanes–EtOAc, 67 : 33) (524 mg, 41%) and immediately carried onto the subsequent phosphorylation. Chromatographic purification (hexanes–EtOAc, 80 : 20) furnished compound **6f** as a colorless liquid (625 mg, 55%); $R_F$ = 0.25 (hexanes–EtOAc, 80 : 20); $^1$H NMR ($CDCl_3$) $\delta$ 7.39–7.18 (m, 10H, Ph × 2), 6.06 (dd, 1H, $^1J_{C,H}$ = 180 Hz, $J_{1,P}$ = 6.6 Hz, $J_{1,2}$ = 3.3 Hz, H-1), 5.08 (dd, 1H, $J_{4,5}$ = 10.0 Hz, $J_{3,4}$ = 10.0 Hz, H-4), 4.90 (ddd, 1H, $J_{2,3}$ = 10.0 Hz, $J_{1,2}$ = 3.0 Hz, $^4J_{1,P}$ = 3.0 Hz, H-2), 4.14 (dd, 1H, $^2J_{6a,6b}$ = 12.6 Hz, $J_{5,6a}$ = 4.3 Hz, H-6a), 4.01 (ddd, 1H, $J_{4,5}$ = 10.3 Hz, $J_{5,6a}$ = 3.9 Hz, $J_{5,6b}$ = 2.1 Hz, H-5), 3.89 (dd, 1H, $J_{6a,6b}$ = 12.5 Hz, $J_{5,6b}$ = 2.2 Hz, H-6b), 3.79 (dd, 1H, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 9.8 Hz, H-3), 3.54 (m, 2H, OC$H_2$), 2.08, 2.00, 1.92 (s, 9H, C(O)C$H_3$ × 3), 1.47 (m, 2H, $OCH_2CH_2$), 1.33–1.19 (m, 26H, $OCH_2CH_2(CH_2)_{13}CH_3$), 0.88 (t, 3H, $J$ = 7.1 Hz, $O(CH_2)_{15}CH_3$); $^{13}$C NMR ($CDCl_3$) $\delta$ 170.8, 169.9, 169.3 ($C$(O)$CH_3$ × 3), 150.6–120.1 (12C, Ph × 2), 95.7 (d, $^2J_{1,P}$ = 6.2 Hz, C-1), 76.5 (C-3), 73.4 (O$CH_2$), 72.1 (d, $^3J_{1,P}$ = 7.2 Hz, C-2), 70.4 (C-5), 68.9 (C-4), 61.5 (C-6), 32.0, 29.8, 29.8, 29.8, 29.8, 29.8, 29.8, 29.8, 29.7, 29.6, 29.5, 26.1, 22.8 ($OCH_2CH_2(CH_2)_{13}CH_3$), 30.3 ($OCH_2CH_2(CH_2)_{13}CH_3$), 20.9, 20.7, 20.6 (C(O)$CH_3$ × 3), 14.2 ($O(CH_2)_{15}CH_3$); $^{31}$P NMR ($CDCl_3$) $\delta$ −14.00 (s, 1P, P-1); LRMS $m/z$ calcd for $C_{40}H_{59}O_{12}P$ [M + Na]$^+$: 785.4. Found 785.4.

**Diphenyl 2,4,6-tri-*O*-acetyl-3-*O*-(2-methylpropyl)-α-D-glucopyranosyl-1-phosphate (6g).** Compound **5g** ($R_F$ = 0.23 (hexanes–EtOAc, 67 : 33)), a colorless liquid, was provided by silica gel chromatography (hexanes–EtOAc, 60 : 40) (341 mg, 39%) and immediately carried onto the subsequent phosphorylation. Chromatographic purification (hexanes–EtOAc, 71 : 29) furnished compound **6g** as a colorless liquid (585 mg, 66%); $R_F$ = 0.36 (hexanes–EtOAc, 66 : 33); $^1$H NMR ($CDCl_3$) $\delta$ 7.39–7.18 (m, 10H, Ph × 2), 6.05 (dd, 1H, $^1J_{C,H}$ = 180 Hz, $J_{1,P}$ = 6.5 Hz, $J_{1,2}$ = 3.2 Hz, H-1), 5.10 (dd, 1H, $J_{4,5}$ = 10.0 Hz, $J_{3,4}$ = 10.0 Hz, H-4),

4.92 (ddd, 1H, $J_{2,3}$ = 9.9 Hz, $J_{1,2}$ = 3.0 Hz, $^4J_{1,P}$ = 3.0 Hz, H-2), 4.14 (dd, 1H, $^2J_{6a,6b}$ = 12.6 Hz, $J_{5,6a}$ = 4.2 Hz, H-6a), 4.01 (ddd, 1H, $J_{4,5}$ = 10.4 Hz, $J_{5,6a}$ = 4.2 Hz, $J_{5,6b}$ = 2.3 Hz, H-5), 3.90 (dd, 1H, $J_{6a,6b}$ = 12.5 Hz, $J_{5,6b}$ = 2.2 Hz, H-6b), 3.78 (dd, 1H, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 9.8 Hz, H-3), 3.37 (dd, 1H, $J$ = 6.2 Hz, $^2J$ = 8.4 Hz, OC$H_{2a}$), 3.28 (dd, 1H, $J$ = 6.4 Hz, $^2J$ = 8.8 Hz, OC$H_{2b}$), 2.08, 2.00, 1.92 (s, 9H, C(O)C$H_3$ × 3), 1.75 (nonet, 1H, $J$ = 6.6 Hz, $OCH_2CH$), 0.84 (d, 6H, $J$ = 6.6 Hz, $OCH_2CH(CH_3)_2$); $^{13}$C NMR ($CDCl_3$) $\delta$ 170.8, 170.0, 169.3 (*C*(O)$CH_3$ × 3), 150.6–120.2 (12C, Ph × 2), 95.8 (d, $^2J_{1,P}$ = 6.1 Hz, C-1), 80.3 (O$CH_2$), 76.7 (C-3), 72.1 (d, $^3J_{1,P}$ = 7.2 Hz, C-2), 70.4 (C-5), 68.9 (C-4), 61.5 (C-6), 29.0 ($OCH_2CH$), 20.9, 20.8, 20.6 (C(O)*C*$H_3$ × 3), 19.2, 19.2 ($OCH_2CH(CH_3)_2$); $^{31}$P NMR ($CDCl_3$) $\delta$ −14.00 (s, 1P, P-1); LRMS $m/z$ calcd for $C_{28}H_{35}O_{12}P$ [M + Na]$^+$: 617.2. Found 617.2.

**Diphenyl 2,4,6-tri-*O*-acetyl-3-*O*-(2-ethylbutyl)-α-D-glucopyranosyl-1-phosphate (6h).** Compound **5h** ($R_F$ = 0.23 (hexanes–EtOAc, 67 : 33)), a white solid, was provided by silica gel chromatography (hexanes–EtOAc, 61 : 39) (612 mg, 65%) and immediately carried onto the subsequent phosphorylation. Chromatographic purification (hexanes–EtOAc, 78 : 22) furnished compound **6h** as a colorless liquid (724 mg, 78%); $R_F$ = 0.24 (hexanes–EtOAc, 78 : 22); $^1$H NMR ($CDCl_3$) $\delta$ 7.39–7.17 (m, 10H, Ph × 2), 6.05 (dd, 1H, $^1J_{C,H}$ = 180 Hz, $J_{1,P}$ = 6.6 Hz, $J_{1,2}$ = 3.4 Hz, H-1), 5.09 (dd, 1H, $J_{4,5}$ = 10.2 Hz, $J_{3,4}$ = 9.5 Hz, H-4), 4.91 (ddd, 1H, $J_{2,3}$ = 10.0 Hz, $J_{1,2}$ = 3.2 Hz, $^4J_{1,P}$ = 3.2 Hz, H-2), 4.13 (dd, 1H, $^2J_{6a,6b}$ = 12.6 Hz, $J_{5,6a}$ = 4.4 Hz, H-6a), 4.01 (ddd, 1H, $J_{4,5}$ = 10.4 Hz, $J_{5,6a}$ = 4.2 Hz, $J_{5,6b}$ = 2.1 Hz, H-5), 3.89 (dd, 1H, $J_{6a,6b}$ = 12.5 Hz, $J_{5,6b}$ = 2.2 Hz, H-6b), 3.78 (dd, 1H, $J_{2,3}$ = 9.7 Hz, $J_{3,4}$ = 9.7 Hz, H-3), 3.52 (dd, 1H, $J$ = 4.6 Hz, $^2J$ = 9.0 Hz, OC$H_{2a}$), 3.42 (dd, 1H, $J$ = 4.4 Hz, $^2J$ = 8.8 Hz, OC$H_{2b}$), 2.08, 2.00, 1.92 (s, 9H, C(O)C$H_3$ × 3), 1.28 (m, 5H, $OCH_2CH(CH_2CH_3)_2$), 0.83 (t, 6H, $J$ = 7.0 Hz, $OCH_2CH(CH_3)_2$); $^{13}$C NMR ($CDCl_3$) $\delta$ 170.8, 170.0, 169.3 (*C*(O)$CH_3$ × 3), 150.5–120.2 (12C, Ph × 2), 95.7 (d, $^2J_{1,P}$ = 6.1 Hz, C-1), 76.6 (C-3), 75.4 (O$CH_2$), 72.3 (d, $^3J_{1,P}$ = 7.3 Hz, C-2), 70.4 (C-5), 69.0 (C-4), 61.5 (C-6), 42.0 ($OCH_2CH$), 23.2, 23.2 ($OCH_2CH(CH_2CH_3)_2$), 20.9, 20.8, 20.6 (C(O)*C*$H_3$ × 3), 11.3, 11.3 ($OCH_2CH(CH_2CH_3)_2$); $^{31}$P NMR ($CDCl_3$) $\delta$ −13.99 (s, 1P, P-1); LRMS $m/z$ calcd for $C_{30}H_{39}O_{12}P$ [M + Na]$^+$: 645.2. Found 645.2.

**General procedure for deprotection and ion exchange (7a–h and 8a–h, respectively).** A solution of phosphorylated pyranoside (0.384 mmol) and platinium(IV) oxide (0.242 mmol) in 1 : 1 EtOH–EtOAc (4 mL) was shaken under $H_2$ in a Parr apparatus at 54 PSI for 2 h. The reaction mixture was filtered through Celite and concentrated. The residue was taken up in 2 : 2 : 1 MeOH–$H_2O$–$NEt_3$ (10 mL) and stirred at 50 °C for 36 h. Again, the mixture was concentrated and the residue was partitioned between $H_2O$ (10 mL) and EtOAc (10 mL). The aqueous layer containing the triethyl ammonium salt (**7a–h**) was passed through Amberlite IR-120 PLUS(H) ion exchange resin. The resulting acidic aqueous fraction was immediately adjusted to pH 8 with $NH_4OH$ 0.1 M, concentrated to 5 mL, and lyophilized. Titration with $NH_4OH$ 0.1 M and lyophilization was repeated twice more to remove $NH_4OAc$ and isolate the ammonium salt (**8a–h**).

**3-*O*-Methyl-α-D-glucopyranosyl phosphate diammonium salt (8a)[40].** Deacetylation was performed at rt and compound **8a** was isolated as a colorless foam (50 mg, 42%); $^1$H NMR ($D_2O$) $\delta$ 5.35 (dd, 1H, $^1J_{C,H}$ = 176 Hz, $J_{1,P}$ = 7.2 Hz, $J_{1,2}$ = 3.0 Hz, H-1), 3.80 (ddd, 1H, $J_{4,5}$ = 9.6 Hz, $J_{5,6b}$ = 4.7 Hz, $J_{5,6a}$ = 2.0 Hz, H-5), 3.75 (dd, 1H, $^2J_{6a,6b}$ = 12.2 Hz, $J_{5,6a}$ = 2.0 Hz, H-6a), 3.65 (dd, 1H, $J_{6a,6b}$ = 12.4 Hz, $J_{5,6b}$ = 4.9 Hz, H-6b), 3.52 (s, 3H, C$H_3$), 3.47 (m, 1H, H-2), 3.44 (m, 1H, H-3), 3.39 (m, 1H, H-4); $^{13}$C NMR ($D_2O$) $\delta$ 94.2 (d, $^2J_{1,P}$ = 5.7 Hz, C-1), 82.8 (C-3), 72.3 (C-5), 71.4 (d, $^3J_{1,P}$ = 7.5 Hz, C-2), 69.0 (C-4), 60.5 (C-6), 60.0 (*C*$H_3$); $^{31}$P NMR ($D_2O$) $\delta$ 0.77 (s, 1P, P-1); HRMS $m/z$ calcd for $C_7H_{13}O_9P^{2-}$ [M + H]$^-$: 273.0386. Found 273.0390.

**3-*O*-Butyl-α-D-glucopyranosyl phosphate diammonium salt (8b).** Compound **8b** was obtained as a colorless foam (63 mg, 47%); $^1$H NMR ($D_2O$) $\delta$ 5.33 (dd, 1H, $^1J_{C,H}$ = 172 Hz, $J_{1,P}$ = 7.4 Hz, $J_{1,2}$ = 3.4 Hz, H-1), 3.79 (ddd, 1H, $J_{4,5}$ = 10.1 Hz, $J_{5,6b}$ = 5.0 Hz, $J_{5,6a}$ = 2.2 Hz, H-5), 3.75 (dd, 1H, $^2J_{6a,6b}$ = 12.5 Hz, $J_{5,6a}$ = 2.2 Hz, H-6a), 3.72 (t, 2H, $J$ = 6.7 Hz, OC$H_2$), 3.63 (dd, 1H, $J_{6a,6b}$ = 12.5 Hz, $J_{5,6b}$ = 5.2 Hz, H-6b), 3.50 (dd, 1H, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 9.2 Hz, H-3), 3.42 (ddd, 1H, $J_{2,3}$ = 9.6 Hz, $J_{1,2}$ = 3.3 Hz, $^4J_{2,P}$ = 2.0 Hz, H-2), 3.33 (dd, 1H, $J_{3,4}$ = 9.6 Hz, $J_{4,5}$ = 9.6 Hz, H-4), 1.49 (p, 2H, $J$ = 7.0 Hz, $OCH_2CH_2$), 1.26 (sextet, 2H, $J$ = 7.5 Hz, $O(CH_2)_2CH_2CH_3$), 0.79 (t, 3H, $J$ = 7.8 Hz, $O(CH_2)_3CH_3$); $^{13}$C NMR ($D_2O$) $\delta$ 94.1 (d, $^2J_{1,P}$ = 5.5 Hz, C-1), 81.5 (C-3), 73.1 (O$CH_2$), 72.3 (C-5), 71.8 (d, $^3J_{1,P}$ = 7.4 Hz, C-2), 69.3 (C-4), 60.7 (C-6), 31.5 ($OCH_2CH_2$), 18.5 ($OCH_2CH_2CH_2$), 13.1 ($O(CH_2)_3CH_3$); $^{31}$P NMR ($D_2O$) $\delta$ 1.32 (s, 1P, P-1); HRMS $m/z$ calcd for $C_{10}H_{19}O_9P^{2-}$ [M + H]$^-$: 315.0856. Found 315.0856.

**3-*O*-Hexyl-α-D-glucopyranosyl phosphate diammonium salt (8c).** Compound **8c** was obtained as a colorless foam (110 mg, 76%); $^1$H NMR ($D_2O$) $\delta$ 5.34 (dd, 1H, $^1J_{C,H}$ = 173 Hz, $J_{1,P}$ = 6.9 Hz, $J_{1,2}$ = 3.0 Hz, H-1), 3.78 (m, 1H, H-5), 3.74 (m, 1H, H-6a), 3.70 (t, 2H, $J$ = 6.6 Hz, OC$H_2$), 3.64 (dd, 1H, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6b}$ = 4.5 Hz, H-6b), 3.49 (dd, 1H, $J_{2,3}$ = 9.3 Hz, $J_{3,4}$ = 9.3 Hz, H-3), 3.44 (ddd, 1H, $J_{2,3}$ = 9.7 Hz, H-2), 3.35 (dd, 1H, $J_{3,4}$ = 9.5 Hz, $J_{4,5}$ = 9.5 Hz, H-4), 1.50 (p, 2H, $J$ = 7.0 Hz, $OCH_2CH_2$), 1.22 (m, 6H, $OCH_2CH_2(CH_2)_3CH_3$), 0.76 (m, 3H, $O(CH_2)_5CH_3$); $^{13}$C NMR ($D_2O$) $\delta$ 94.4 (d, $^2J_{1,P}$ = 5.7 Hz, C-1), 81.4 (C-3), 73.4 (O$CH_2$), 72.4 (C-5), 71.6 (d, $^3J_{1,P}$ = 7.7 Hz, C-2), 69.1 (C-4), 60.6 (C-6), 30.9, 24.8, 22.0 ($OCH_2CH_2(CH_2)_3CH_3$), 29.2 ($OCH_2CH_2$), 13.4 ($O(CH_2)_5CH_3$); $^{31}$P NMR ($D_2O$) $\delta$ 0.41 (s, 1P, P-1); HRMS $m/z$ calcd for $C_{12}H_{23}O_9P^{2-}$ [M + H]$^-$: 343.1169. Found 343.1176.

**3-*O*-Octyl-α-D-glucopyranosyl phosphate diammonium salt (8d).** Diethyl ether was used instead of ethyl acetate as an extraction solvent *en route* to compound **8d**, a colorless foam (66 mg, 42%); $^1$H NMR ($D_2O$) $\delta$ 5.36 (dd, 1H, $^1J_{C,H}$ = 173 Hz, $J_{1,P}$ = 7.2 Hz, $J_{1,2}$ = 3.2 Hz, H-1), 3.78 (ddd, 1H, $J_{4,5}$ = 9.8 Hz, $J_{5,6b}$ = 5.0 Hz, $J_{5,6a}$ = 2.1 Hz, H-5), 3.75 (m, 1H, H-6a), 3.72 (t, 2H, $J$ = 7.0 Hz, OC$H_2$), 3.66 (dd, 1H, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6b}$ = 4.9 Hz, H-6b), 3.50 (m, 1H, H-3), 3.45 (m, 1H, H-2), 3.37 (dd, 1H, $J_{3,4}$ = 9.4 Hz, $J_{4,5}$ = 9.4 Hz, H-4), 1.50 (p, 2H, $J$ = 7.2 Hz, $OCH_2CH_2$), 1.30–1.12 (m, 10H, $OCH_2CH_2(CH_2)_5CH_3$), 0.77 (t, 3H, $J$ = 7.0 Hz, $(CH_2)_7CH_3$); $^{13}$C NMR ($D_2O$) $\delta$ 94.6 (d, $^2J_{1,P}$ = 5.9 Hz, C-1), 81.4 (C-3), 73.5 (O$CH_2$), 72.5 (C-5), 71.5 (d, $^3J_{1,P}$ = 7.7 Hz, C-2), 69.1 (C-4), 60.5 (C-6), 31.1, 28.5, 28.4, 25.1, 22.0 ($OCH_2CH_2(CH_2)_5CH_3$), 29.3 ($OCH_2CH_2$), 13.4 ($O(CH_2)_7CH_3$); $^{31}$P NMR ($D_2O$) $\delta$ 0.12 (s, 1P, P-1); HRMS $m/z$ calcd for $C_{14}H_{28}O_9P^{2-}$ [M + H]$^-$: 371.1482. Found 371.1490.

**3-*O*-Dodecyl-α-D-glucopyranosyl phosphate diammonium salt (8e).** Deacetylation was performed at 70 °C instead of 50 °C

and diethyl ether was used instead of ethyl acetate as an extraction solvent. Triethylamine (1 mL) was added before rotary evaporation to suppress the surfactant-like behavior of the salt. After the third $NH_4OAc$ sublimation, the residue was dissolved in water and filtered through a short pad of Bio-Rad Chelex 100. The solution was then passed through Amberlite IR-120 PLUS(H) ion exchange resin, titrated with $NH_4OH$ 0.1 M, and lyophilized to forge compound **8e** as a colorless foam (103 mg, 58%); $^1$H NMR (MeOD) $\delta$ 5.48 (dd, 1H, $^1J_{C,H}$ = 173 Hz, $J_{1,P}$ = 6.9 Hz, $J_{1,2}$ = 3.3 Hz, H-1), 3.85 (ddd, 1H, $J_{4,5}$ = 9.8 Hz, $J_{5,6b}$ = 5.7 Hz, $J_{5,6a}$ = 2.1 Hz, H-5), 3.81 (m, 2H, $OCH_2$), 3.80 (m, 1H, H-6a), 3.63 (dd, 1H, $J_{6a,6b}$ = 11.6 Hz, $J_{5,6b}$ = 5.5 Hz, H-6b), 3.47 (dd, 1H, $J_{2,3}$ = 9.0 Hz, $J_{3,4}$ = 9.0 Hz, H-3), 3.42 (ddd, 1H, $J_{2,3}$ = 9.6 Hz, $J_{1,2}$ = 3.0 Hz, $^4J_{1,P}$ = 3.0 Hz, H-2), 3.32 (m, 1H, H-4), 1.62 (p, 2H, $J$ = 7.3 Hz, $OCH_2CH_2$), 1.41–1.23 (m, 18H, $OCH_2CH_2(CH_2)_9CH_3$), 0.90 (t, 3H, $J$ = 7.0 Hz, $(CH_2)_{11}CH_3$); $^{13}$C NMR (MeOD) $\delta$ 96.6 (d, $^2J_{1,P}$ = 6.2 Hz, C-1), 83.6 (C-3), 74.6 ($OCH_2$), 74.5 (C-5), 74.0 (d, $^3J_{1,P}$ = 7.5 Hz, C-2), 71.4 (C-4), 62.8 (C-6), 33.1, 30.8, 30.8, 30.8, 30.8, 30.7, 30.5, 27.1, 23.7 ($OCH_2CH_2(CH_2)_9CH_3$), 31.4 ($OCH_2CH_2$), 14.4 ($O(CH_2)_{11}CH_3$); $^{31}$P NMR (MeOD) $\delta$ −1.86 (s, 1P, P-1); HRMS $m/z$ calcd for $C_{18}H_{35}O_9P^{2-}$ [M + H]$^-$: 427.2108. Found 427.2115.

**3-*O*-Hexadecyl-α-D-glucopyranosyl phosphate diammonium salt (8f).** Deacetylation was performed at 70 °C instead of 50 °C and diethyl ether was used instead of ethyl acetate as an extraction solvent. Triethylamine (1 mL) was added before rotary evaporation to suppress the surfactant-like behavior of the salt. After the third $NH_4OAc$ sublimation, the residue was dissolved in water and filtered through a short pad of Bio-Rad Chelex 100. The solution was then passed through Amberlite IR-120 PLUS(H) ion exchange resin, titrated with $NH_4OH$ 0.1 M, and lyophilized to forge compound **8f** as a colorless foam (179 mg, 90%). Coupling constants are omitted from the $^1$H NMR spectrum since the compound's insolubility in a variety of NMR solvents led to significant line-broadening; $^1$H NMR ($D_2O$) $\delta$ 5.44 (d, 1H, $^1J_{C,H}$ = 174 Hz, H-1), 3.86 (m, 1H, H-5), 3.86 (m, 1H, H-6a), 3.74 (m, 2H, $OCH_2$), 3.69 (m, 1H, H-6b), 3.56 (m, 1H, H-2), 3.52 (m, 1H, H-3), 3.39 (m, 1H, H-4), 1.58 (m, 2H, $OCH_2CH_2$), 1.42–1.15 (m, 26H, $OCH_2CH_2(CH_2)_{13}CH_3$), 0.84 (m, 3H, $(CH_2)_{15}CH_3$); $^{13}$C NMR ($D_2O$) $\delta$ 94.9 (d, $^2J_{1,P}$ = 5.1 Hz, C-1), 81.0 (C-3), 72.7 (C-5), 72.2 ($OCH_2$), 71.1 (d, $^3J_{1,P}$ = 7.3 Hz, C-2), 69.4 (C-4), 61.0 (C-6), 32.1, 30.3, 30.3, 30.3, 30.3, 30.3, 30.3, 30.3, 30.3, 30.1, 30.1, 30.0, 29.7, 26.2, 22.7 ($OCH_2(CH_2)_{14}CH_3$), 13.8 ($O(CH_2)_{15}CH_3$); $^{31}$P NMR ($D_2O$) $\delta$ −0.57 (s, 1P, P-1); HRMS $m/z$ calcd for $C_{22}H_{43}O_9P^{2-}$ [M + H]$^-$: 483.2734. Found 483.2708.

**3-*O*-(2-Methylpropyl)-α-D-glucopyranosyl phosphate diammonium salt (8g).** Compound **8g** was obtained as a colorless foam (69 mg, 51%); $^1$H NMR ($D_2O$) $\delta$ 5.29 (dd, 1H, $^1J_{C,H}$ = 171 Hz, $J_{1,P}$ = 7.3 Hz, $J_{1,2}$ = 3.3 Hz, H-1), 3.76 (ddd, 1H, $J_{4,5}$ = 10.0 Hz, $J_{5,6b}$ = 4.8 Hz, $J_{5,6a}$ = 1.8 Hz, H-5), 3.72 (dd, 1H, $^2J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 1.9 Hz, H-6a), 3.60 (dd, 1H, $J_{6a,6b}$ = 12.1 Hz, $J_{5,6b}$ = 5.2 Hz, H-6b), 3.47 (dd, 1H, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 9.4 Hz, H-3), 3.46 (d, 2H, $J$ = 6.1 Hz, $OCH_2$), 3.41 (m, 1H, H-2), 3.31 (dd, 1H, $J_{3,4}$ = 9.5 Hz, $J_{4,5}$ = 9.5 Hz, H-4), 1.74 (nonet, 1H, $J$ = 6.5 Hz, $OCH_2CH$), 0.81, 0.81 (d, 3H, $J$ = 6.7 Hz, $OCH_2CH(CH_3)_2$); $^{13}$C NMR ($D_2O$) $\delta$ 94.0 (d, $^2J_{1,P}$ = 5.6 Hz, C-1), 81.7 (C-3), 80.1 ($OCH_2$), 72.2 (C-5), 71.8 (d, $^3J_{1,P}$ = 7.2 Hz, C-2), 69.3 (C-4), 60.7 (C-6), 28.2 ($OCH_2CH$), 18.6, 18.6 ($OCH_2CH(CH_3)_2$); $^{31}$P NMR ($D_2O$) $\delta$ 1.76 (s, 1P, P-1); HRMS $m/z$ calcd for $C_{10}H_{19}O_9P^{2-}$ [M + H]$^-$: 315.0856. Found 315.0869.

**3-*O*-(2-Ethylbutyl)-α-D-glucopyranosyl phosphate diammonium salt (8h).** Compound **8h** was obtained as a colorless foam (145 mg, 76%); $^1$H NMR ($D_2O$) $\delta$ 5.32 (dd, 1H, $^1J_{C,H}$ = 172 Hz, $J_{1,P}$ = 4.6 Hz, $J_{1,2}$ = 3.0 Hz, H-1), 3.79 (m, 1H, H-5), 3.75 (d, 1H, $^2J_{6a,6b}$ = 12.6 Hz, H-6a), 3.63 (m, 1H, H-6b), 3.61 (m, 2H, $OCH_2$), 3.49 (dd, 1H, $J_{2,3}$ = 9.2 Hz, $J_{3,4}$ = 9.2 Hz, H-3), 3.42 (d, 1H, $J_{2,3}$ = 9.6 Hz, H-2), 3.33 (dd, 1H, $J_{3,4}$ = 9.2 Hz, $J_{4,5}$ = 9.2 Hz, H-4), 1.39 (m, 1H, $OCH_2CH(CH_2CH_3)_2$), 1.25 (m, 4H, $OCH_2CH(CH_2CH_3)_2$), 0.76 (t, 6H, $J$ = 6.7 Hz, $OCH_2CH(CH_2CH_3)_2$); $^{13}$C NMR ($D_2O$) $\delta$ 94.1 (d, $^2J_{1,P}$ = 5.6 Hz, C-1), 81.7 (C-3), 75.7 ($OCH_2$), 72.3 (C-5), 71.8 (d, $^3J_{1,P}$ = 7.3 Hz, C-2), 69.3 (C-4), 60.7 (C-6), 41.0 ($OCH_2CH$), 22.5, 22.4 ($OCH_2CH(CH_2CH_3)_2$), 10.2, 10.2 ($OCH_2CH(CH_2CH_3)_2$); $^{31}$P NMR ($D_2O$) $\delta$ 1.62 (s, 1P, P-1); HRMS $m/z$ calcd for $C_{12}H_{23}O_9P^{2-}$ [M + H]$^-$: 343.1169. Found 343.1159.

**General procedure for nucleotidylyltransferase-catalyzed synthesis of sugar nucleotides (9a–h, 10a–h).** Enzymatic reactions were performed according to the method of Timmons ($MgCl_2$, 1.0 mM NTP, 2.0 mM S1P, 0.5EU inorganic pyrophosphatase, 2EU nucleotidylyltransferase in Tris-HCl buffer-total 50 μL)[30] except that $MgCl_2$ was used at a final concentration of 5.5 mM and incubations were performed at 41 °C. Sugar nucleotides were characterized by ESI-MS/MS (ESI†).

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