Improved synthesis of phytanyl α-D-cellobiosyldiphosphate as substrate for α-D-mannosyltransferase

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Dedicated to Professors Rita H. Rossi, Julio C. Podestá, Manuel González Sierra and Oscar S. Giordano

Abstract
Polyisoprenyl-pyrophosphate-linked cellobiose is the natural acceptor of the α-1,3-mannosyltransferase AceA from *Acetobacter xylinum,* which transfers mannose from GDP-mannose during the assembly of the heptasaccharide repeat unit of the exopolysaccharide acetan. Phytanyl α-D-cellobiosyldiphosphate **4** has been previously synthesized as an analogue acceptor by condensation of hepta-*O*-acetyl-α-D-cellobiosylphosphate **1** with phytanylphosphate **2**, but the procedure was briefly described. We report here a modified detailed synthesis of **4**. The complete NMR characterization of **4** is provided and also a selection of NMR signals of all the intermediate compounds which facilitate monitoring the synthesis.

Keywords: Mannosyltransferase, *Acetobacter xylinium*, glycosyl phosphate, phytanyl phosphate

Introduction

Glycosyltransferases are a group of enzymes catalyzing the most common and important reactions in nature. Yet, they are a group of enzymes that are relatively poorly understood. In bacteria, most glycosyltransferases are involved in the synthesis of the essential components of the cell envelope, *e.g.*, glycolipid intermediates for lipo-oligosaccharides, peptidoglycan, and polysaccharides[1,2] and may be rational targets for drug development against bacterial pathogens.[3] Glycosyltransferases are classified as inverting or retaining enzymes according to the anomeric configuration of the sugar residue transferred. So far, two structural superfamilies have been

described for nucleotide-diphospho-sugar-dependent glycosyltransferases, called GT-A and GT-B. These topologies are variations of Rossman-like domain.[4] The GT-A members display a core structure of a 7-8 *β*-strands with a DXD motif, where D is aspartic acid and X is any amino acid. This acidic motif coordinates the ribose and divalent cation in the catalytic center. In contrast GT-B proteins do not bind metals and are composed of two well defined Rossmann domains with a deep cleft between them, in which binding of substrates and catalytic activity occur.[5]

Despite the relative low structural variety, reproducible predictions of the reaction catalyzed by a proposed glycosyltransferase cannot yet be made. Currently, there are 92 glycosyltransferase families in the Carbohydrate-Active Enzyme (CAZy) database.[6,7] However, in December 2007, less than 10% of all proteins in CAZy were enzymatically characterized.[7] This is in part because most of these enzymes are integral membrane proteins, whose biochemical characterization is a difficult matter due to protein purification and stabilization, and in part because natural substrates for many of the glycosyltransferases are very difficult to produce and purify. These difficulties have slowed down the biochemical characterization of these enzymes. In addition, for those glycosyltransferases with solved x-ray crystal structure, the unavailability of substrates have delayed the identification of key amino acids involved in binding and catalytic mechanism.

Synthetic acceptor substrates have been developed for a number of glycosyltransferases and have been successfully used in the characterization of enzyme activity, proving that they are good substrate analogues.[8-10] AceA is a non-processive retaining *α*-1,3-mannosyltransferase from *Acetobacter xylinum* which transfers mannose from GDP-mannose to a polyisoprenyl-pyrophosphate-linked cellobiose acceptor during the assembly of the heptasaccharide repeat unit of the exopolysaccharide acetan (Fig. 1).[11-13] Although the allylic nature of the lipid portion of the natural acceptor has been confirmed,[14] the complete structure is not known yet. Lellouch *et al.*, synthesized phytanyl *α*-D-cellobiosyl diphosphate (Glc_2-PP-phytanyl, **4**) as an analogue acceptor for the reaction catalyzed by AceA.[15] The lipid portion of this synthetic substrate mimics the polyisoprene natural structure and its branched carbon backbone, but is relatively short (C_{20}) and saturated.

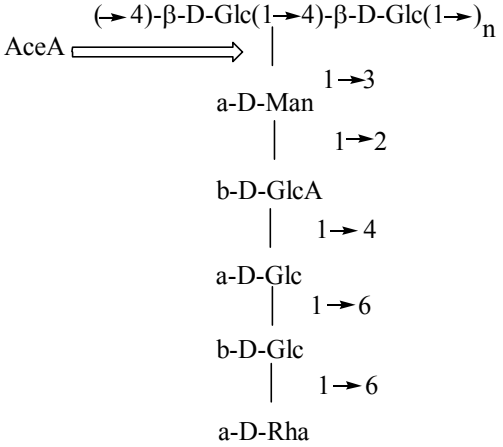
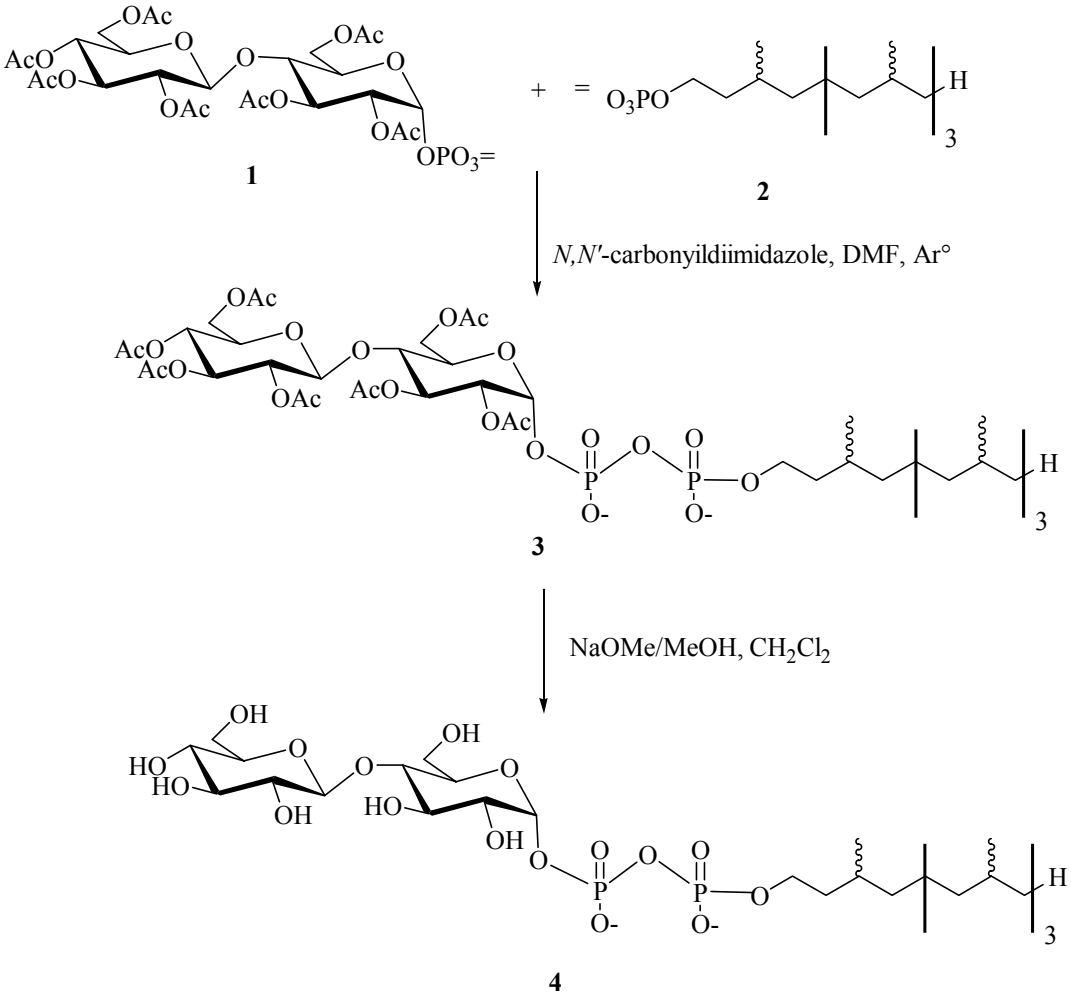


Figure 1. Acetan heptasaccharide repeat unit from *Acetobacter xylinium*. The formation of the α-1,3-glycosidic bond catalyzed by AceA is shown.

The shorter lipid chain makes the handling of the substrate easier and the saturated nature stabilizes it, as allylic pyrophosphates are unstable. They showed that AceA was able to catalyze the transfer of [^{14}C]Man from GDP-[^{14}C]Man to the synthetic acceptor.[15] The synthesis of phytanyl α-D-cellobiosyldiphosphate (Glc_2-PP-phytanyl, **4**), by condensation of hepta-*O*-acetyl-α-D-cellobiosylphosphate **1** with phytanylphosphate **2** was briefly described (Scheme 1) but much of the NMR data of the compounds were not provided. We encountered several problems associated with scale-up and reproducibility and therefore we introduced some modifications on the synthesis previously reported. We describe here in detail the preparation, purification and characterization of substrate **4** and intermediate products. Complete NMR characterization is now reported and diagnostic signals were tabulated to assist monitoring the synthesis.

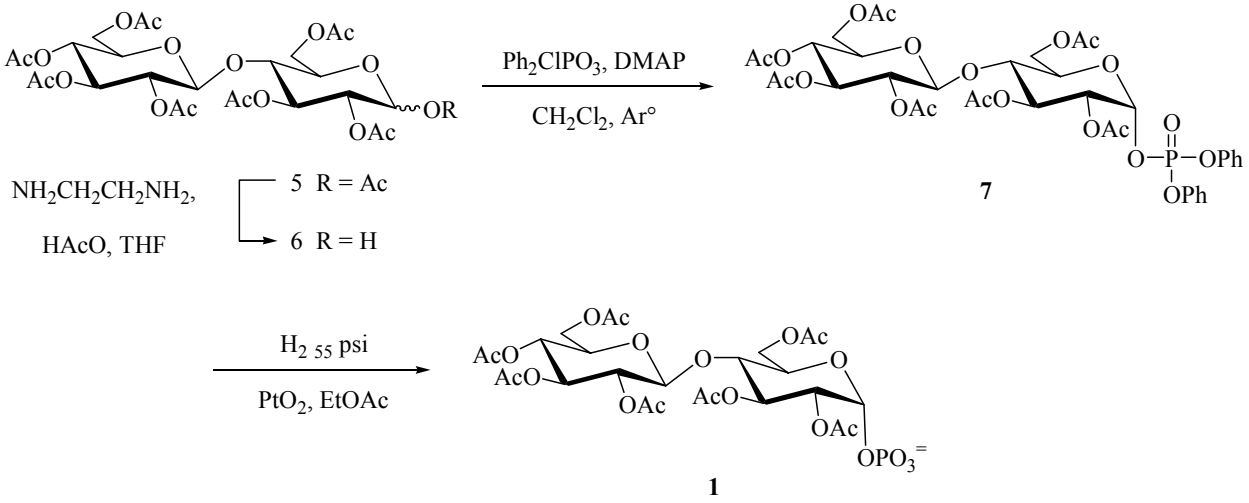


Scheme 1. Synthesis of Glc_2-PP-phytanyl **4**.

Results and Discussion

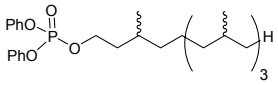
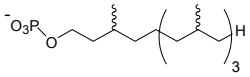
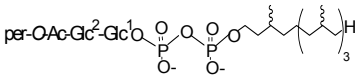
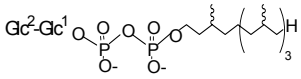
The synthesis of **4** began with the phosphorylation of a derivative of cellobiose. Selective anomeric deacylation of octa-*O*-acetyl-*α*-D-cellobiose **5** was performed with acetic acid/ethylenediamine[16] to afford **6** (Scheme 2). This one pot procedure is easier and high yielding with respect to the procedure previously used, involving silver carbonate catalyzed hydrolysis of the acetylated glycosyl halide.[17] The phosphate was introduced by treatment of **6** with diphenylchlorophosphate and 4-*N*,*N*-dimethylaminopyridine (DMAP) under the conditions established to favor *α*-anomers.[17] The ^{1}H NMR spectrum of **7** showed the H-1 signal as a doublet of doublets at δ 5.99 with $J_{1,2}$ 3.5 Hz indicating the *α* configuration, and $J_{H,P}$ 6.5 Hz, confirming that the phosphorylation occurred. The ^{13}C NMR spectrum showed the resonance at 100.7 ppm for C-1' and doublets at δ 94.9 (5.4 Hz) and 69.7 (3.8 Hz) for C-1 and C-2, respectively, also indicative of the coupling with phosphorous (Table 1). These values were similar to those observed for the monosaccharidic analogue per-*O*-acetyl-α-D-glucosyldiphenylphosphate.[17]

Cleavage of the phenyl groups from the anomeric phosphotriester of **7** was achieved by catalytic hydrogenation over PtO_2. Compound **1** was isolated as the triethylammonium salt and used directly without further purification. Also for this compound the ^{13}C NMR spectrum showed signals corresponding to C-1 and 2 as doublets due to $J_{C,P}$ (Table 1), confirming the presence of the phosphate group. The α-anomeric configuration was confirmed by the $J_{1,2}$ value (3.3 Hz), indicative of a 1,2-*cis* relationship.



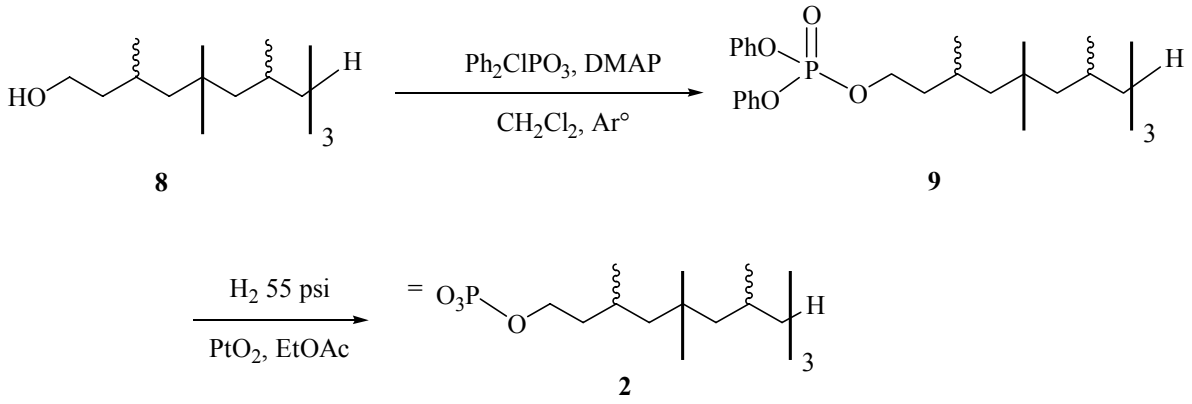
Scheme 2. Synthesis of hepta-*O*-acetyl-α-D-cellobiosylphosphate **1**.

Table 1. Chemical shifts for the diagnostic signals for compounds **1- 4, 7** and **9**

Compound	^{1}H NMR				^{13}C NMR		^{31}P NMR
	H-1		H-2		C-1	C-2	
	($J_{1,2}$)	($J_{H,P}$)	($J_{2,3}$)	($J_{H,P}$)	($J_{C,P}$)	($J_{C,P}$)	
7[a]							
Glc^1	5.99		-		94.9	69.7	-
	(3.5)	(7.1)			(5.4)	(3.8)	
Glc^2	-		-		100.7	-	
					-	-	
1[a]							– 1.18
Glc^1	5.65		-		91.0	70.3	
	(3.3)	(7.3)			(6.0)	(8.0)	
Glc^2	-		-		100.3	-	
					-	-	
	4.29		1.73		67.8	39.4	- 14.0
	m		m		(6.7)		
9[a]							
	4.20		1.84		63.4	39.4	- 2.5
	(5.6)	(1.6)	m		(5.4)		
2[a]							
							-
3[a]							
Glc^1	5.61				92.4	70.1	
	(3.5)	(8.2)			(8.0)	(8.0)	
Glc^2					100.5		
Phytanyl	-		-		64.7	39.2	
					(6.7)		
							- 13.7
							- 5.3
4[b]							
Glc^1	5.84		4.45		98.7	80.1	
	(5.5)	(5.5)	(6.0)	(12.7)	(6.4)	(6.5)	
Glc^2	4.57		3.39		105.8	75.8	
	(7.9)		(9.1)				
phytanyl	4.08		1.80		67.2	40.0	
					(<0.5)		

[a]$CDCl_3$. [b]$CDCl_3$-CD_3OD-D_2O (10:10:1).

The lipid phosphate **2** was previously prepared using the corresponding phosphoramidite chemistry, which involves three steps.[18] It was stored as the corresponding *t*-butyl ester and deprotected immediately before the coupling with **1**.[15] We found several problems using this strategy, so an alternative approach for the installation of this group was investigated. In view of the success in the phosphorylation of cellobiose with diphenylchlorophosphate, we tested the same method with phytanol and found it suitable. Thus, phytanol **8** was treated with diphenylchlorophosphate and DMAP under the same conditions described for the preparation of **7** (Scheme 3). Phosphate **9** was efficiently obtained in 85% yield and could be stored for several months at –20 °C. Hydrogenation of **9** over PtO_2 provided phosphate **2**. The characteristic coupling constant ^{13}C-^{31}P observed for C-1 at the ^{13}C NMR spectra of **9** ($J_{C,P}$ 6.7, Table 1) and **2** ($J_{C,P}$ 5.4, Table 1) confirmed that the phosphatation was efficient. The signal corresponding to geminal H-1 and H-1' of compound **9** was a complex multiplet, while the analogue signal in compound **2** was a well resolved doublet of doublets (Table 1).



Scheme 3. Synthesis of phytanylphosphate **2**.

With triethylammonium salts of **1** and **2** in hand the coupling was effected by activation with 1,1'-carbonyldiimidazole (Scheme 1). For the purification of condensed compound **3** HPLC was previously used.[15] As this is not an amenable procedure to preparative scale-up, we introduced a purification step by silica gel column chromatography instead, which afforded pure compound **3** in 79% yield, considerably higher than the reported procedure. The ^1H NMR spectrum of **3** was in agreement with the previously reported, but we now report the ^{13}C NMR data which helps the confirmation of the coupling. Thus, doublets at δ 92.4 ($J_{C,P}$ 8.0 Hz, C-1) and 70.1 ($J_{C,P}$ 8.0 Hz, C-2) are indicative that the cellobiose residue is phosphated and the doublet at 64.7 ppm ($J_{C,P}$ 6.7 Hz) corresponds to phosphorylated phytanyl C-1.

By *O*-deacylation of **3** with NaMeO/MeOH in CH_2Cl_2 solution, compound **4** was afforded. A purification step by anionic interchange chromatography was introduced. Thus, the crude *O*-deacetylated compound was fractionated on a DEAE-cellulose column in the acetate form by an ammonium formate gradient. Compound **4** was isolated as the triethylammonium salt in an excellent yield of 80%.

The ^{1}H NMR spectrum of **4** showed as diagnostic signal a doublet of doublets at δ 5.84 ($J_{1,2}$ 5.5 Hz, $J_{1,P}$ 5.5 Hz) corresponding to H-1 and doublets at δ 98.1 ($J_{C,P}$ 6.4 Hz, C-1), and 80.1 ($J_{C,P}$ 6.5 Hz, C-2) were observed in the ^{13}C NMR spectrum.

Conclusions

The synthesis of Glc_2-PP-phytanyl **4** was carried out, providing alternatives with respect to the synthesis and purification previously reported. The procedure introduced for the preparation of **2**, by phosphorylation of phytanol with diphenyl chlorophosphate and subsequent hydrogenation is simple and reproducible. The phosphorylated compounds were characterized by ^{1}H, ^{13}C and ^{31}P NMR spectra and the diagnostic signals are depicted in Table 1, which allows monitoring the sequence reaction. Scale-up the synthesis of **4** is thus easier and this fact could remove a significant obstacle in the study of the AceA.

Experimental Section

General. Analytical thin layer chromatography (TLC) was performed on silica gel 60 F_{254} (Merck) aluminum supported plates (layer thickness 0.2 mm) with solvent systems given in the text. Visualization of the spots was effected by exposure to UV light and charring with a solution of 10% (v/v) sulfuric acid in EtOH, containing 0.5% *p*-anisaldehyde. Column chromatography were carried out with silica gel 60 (230–400 mesh, Merck). The ^{31}P NMR spectra were recorded with a Bruker AMX 500 spectrometer, and the ^{1}H and ^{13}C NMR spectra with such equipment or with a Bruker AC 200, as indicated in each case. The chemical shift reference for ^{31}P was that of external phosphoric acid (85%) in D_2O set at 0.0 ppm. All the proton and carbon resonances for compound **4** were performed at 25 °C using a Bruker Avance 400 spectrometer and were assigned by 2D spectra: COSY, TOCSY (mixing time 80 ms) to identify the spin systems,[19] ^{1}H-^{13}C HSQC,[20] and ^{1}H-^{13}C HSQC-TOCSY (with a mixing time of 60 and 80 ms)[21] to assist with cross-peaks assignment. High resolution mass spectra (HRMS ESI) were recorded in a Bruker micrOTOF-Q II and a VG ZAB-HF mass spectrometers.

Triethylammonium per-*O*-acetyl-β-D-glucopyranosyl-(1-4)-α-D-glucopyranosylphosphate (triethylammonium hepta-*O*-acetyl-α-D-cellobiosylphosphate) (1). To a solution of per-*O*-acetyl-α-D-cellobiose **5** (13.56 g, 20.0 mmol) in THF (50 mL) acetic acid (0.8 mL, 14.0 mmol) and ethylendiamine (0.8 mL, 12.0 mmol) were added. The solution was stirred at room temperature during 20 h, when TLC examination showed total conversion to a lower migrating component (R_f 0.55, 3:1 EtOAc–hexane). The solution was diluted with CH_2Cl_2 and washed with HCl (5%), $NaHCO_3$ (ss), H_2O, dried (Na_2SO_4), and concentrated, to afford hepta-*O*-acetyl-α,β-cellobiose **6** (8.80 g, 69%). Compound **6** was used without further purification for the next step.

A solution of **6** (7.5 g, 11.7 mmol) with DMAP (3.36 g) in CH_2Cl_2 (50 mL) was stirred at room temperature during 15 min and then cooled to –10 °C. Then, diphenylchlorophosphate (5.74 mL) was added dropwise and the solution was left to reach room temperature and stirred overnight. The solution was poured over water and the organic layer was washed with ice cold water, ice cold 0.5 M HCl, and $NaHCO_3$ (ss), and dried. The syrup obtained after evaporation of the solvent was purified by column chromatography (3:1 EtOAc–hexane), and fractions of R_f 0.65 (EtOAc–hexane, 3:1) afforded per-*O*-acetyl-*α*-D-cellobiosyldiphenylphosphate **7 (**7.95 g, 78%), which was stored at –20 ºC several months. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.40–7.15 (H-aromatic), 5.99 (dd, $J_{1,2}$ 3.5 Hz, $J_{1,P}$ 7.1 Hz, 1H, H-1), 5.48 (dd, *J* 10.2, 9.9 Hz, 1H, H-3), 5.15–4.85 (m, 4H, H-2,2',3',4,4'), 4.48 (d, *J* 8.3 Hz, 1H, H-1'), 4.42–3.85 (m, 7H, H-4, 5,5',6ab,6'ab), 2.09, 2.06, 2.06, 2.04, 2.02, 2.01, 1.98 (7 s, 21H, C*H*$_3$CO). ^{13}C NMR (50.3 MHz, $CDCl_3$): δ 170.3, 170.1 × 2, 170.0, 169.6, 168.9 (CH_3*C*O), 129.9–120.0 (C-aromatic), 100.7 (C-1'), 94.9 ($J_{C,P}$ 5.4 Hz, C-1), 75.6, 72.9, 71.9, 71.5, 70.5, 69.7 ($J_{C,P}$ 3.6 Hz, C-2), 68.7, 67.6, 61.5, 60.9 (C-6,6'), 20.7, 20.6, 20.46, 20.40, 19.8, 19.5, 19.2 (*C*H_3CO).

Compound **7** (1.90, 2.15 mmol) dissolved in EtOAc (10 mL) was hydrogenated at 55 psi with PtO_2 (30 mg) during 16 h, and then, the solution was filtered through Celite. Triethylamine (0.3 mL) and toluene (10 mL) were added, and the solution was evaporated under reduced pressure, to yield 0.28 g (82%) of **1** (R_f 0.52, sv. $CHCl_3$-MeOH-TEA, 65:35:1). ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.65 (dd, $J_{1,2}$ 3.3 Hz, $J_{1,P}$ 7.3 Hz, 1H, H-1), 5.42 (apparent t, $J_{1,2}$ 9.7 Hz, 1H, H-3), 5.10-4.71 (m, 4H, H-2, 2',3',4'), 4.50-3.85 (m, 8H, H-1',4, 5,5',6ab,6'ab), 2.02, 1.98, 1.95, 1.92, 1.91 × 2, 1.88 (6 s, 21H, C*H*$_3$CO). ^{13}C NMR (50.3 MHz, $CDCl_3$): δ 170.2 × 2, 169.8, 169.5, 169.3, 169.1, 169.0 (CH_3*C*O), 100.3 (C-1'), 91.0 ($J_{C,P}$ 6.0 Hz, C-1), 76.0, 72.7, 71.5, 71.1, 70.4, 70.3 ($J_{C,P}$ 8.0 Hz, C-2), 69.5, 68.3, 67.7, 61.4, 20.7, 20.6, 20.4, 20.3, 20.2, 20.1, 19.9.

Diphenyl 3,7,11,15-tetramethylhexadecylphosphate (diphenylphytanylphosphate) (9). To a solution of phytanol **8** (2.0 g, 6.72 mmol), obtained by hydrogenation of phytol,[22] in anhydrous CH_2Cl_2 (18 mL), DMAP (1.9 g, 15.4 mmoL) and diphenylchlorophosphate (3.24 mL) were added. The solution was stirred overnight under Ar atmosphere, and then was poured over water. After extraction with CH_2Cl_2, the organic phase was washed with HCl (5%), $NaHCO_3$ (ss) and water. The organic extracts were dried over $MgSO_4$, filtered, and evaporated. The syrup obtained was purified by silica gel column chromatography, using 9:1 $CHCl_3$–MeOH as solvent. Fractions of R_f 0.51 ($CHCl_3$) were evaporated to afford compound **9** (3.02 g, 85%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.33–7.21 (H-aromatic), 4.29 (m, 1H, H-1), 1.73 (m, 2H, H-2,2'), 1.62–0.85 (phytanyl).^{13}C NMR (125.8 MHz, $CDCl_3$): δ 150.8, 129.7, 125.3, 120.1, 120.0, 67.8 (C-1, $J_{C,P}$ 6.7 Hz), 39.4, 37.5, 37.44, 37.43, 37.1, 32.83, 32.81, 29.2, 28.0, 24.8, 24.53, 24.51, 24.3, 22.7, 22.6, 19.8, 19.7, 19.6, 19.3, 19.2. HRMS (ESI) calcd for $C_{32}H_{51}O_4PNa^+$ $[M+Na]^+$: 553.3422; found: 553.3426.

Triethylammonium 3,7,11,15-tetramethylhexadecylphosphate (triethylammonium phytanyl phosphate) (2). A solution of diphenylphytanylphosphate **9** (0.75 g, 1.4 mmol) in EtOAc (5.0 mL) was hydrogenated at 55 psi with PtO_2 (30 mg) as catalyst. After 12 h the reaction mixture was filtered through Celite. Triethylamine (0.1 mL) and toluene (10 mL) were added, and the

solution was evaporated under reduced pressure, to yield 0.76 g (92%) of **2** (R_f 0.25, 65:35:1 $CHCl_3$–MeOH–TEA). ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.20 (dd, 1H, $J_{H,H}$ 5.6, $J_{H,P}$ 1.6 Hz, H-1), 1.66–0.82 (phytanyl). ^{13}C NMR (25.3 MHz, $CDCl_3$): δ 63.4 (C-1, $J_{C,P}$ 5.4 Hz), 39.6, 37.6, 37.5, 37.4, 37.3, 32.8, 29.5, 28.0, 24.8, 24.5, 24.48, 24.42, 22.7, 22.6, 19.8, 19.7, 19.6, 19.4, 19.3. HRMS (ESI) calcd for $C_{20}H_{42}O_4P^-$ $[M–H]^-$: 377.2826; found: 377.2822.

Phytanyl [hepta-*O*-acetyl-*β*-D-glucopyranosyl-(1→4)-*α*-D-glucopyranosyl]diphosphate (phytanyl hepta-*O*-acetyl-*α*-D-cellobiosyldiphosphate) (3). To a solution of triethylammonium hepta-*O*-acetyl-*α*-D-cellobiosylphosphate **1** (0.50 g, 0.54 mmol) in DMF (5 mL) under Ar atmosphere, 1,1'-carbonyldiimidazole (0.55 g, 3.4 mmol) was added, and the solution was stirred at room temperature. After 4 h, phytanylphosphate **2** (0.50 g, 0.86 mmol) in DMF (5 mL) was transferred with a syringe to the reaction flask and stirred during 48 h. TLC examination showed a main product (R_f 0.45, 65:35:1 $CHCl_3$–MeOH–TEA). After evaporation of the solvent under reduced pressure, triethylamine (0.1 mL) was added, and evaporated under vacuum with the aid of toluene. The syrup obtained was purified by silica gel column chromatography (95:5:1 $CHCl_3$–MeOH–TEA,) and fractions of R_f 0.45 (65:35:1 $CHCl_3$–MeOH–TEA) were evaporated to afford compound **3** (0.54 g, 79%) with ^{1}H NMR coincident with the previously reported.[15] ^{13}C NMR (50.3 MHz, $CDCl_3$): δ 170.3, 170.1 × 2, 169.9, 169.7, 169.2, 168.9 (CH_3CO), 100.5 (C-1'), 92.4 ($J_{C,P}$ 8.0 Hz, C-1), 75.7, 72.7, 71.7, 71.3, 70.1 ($J_{C,P}$ 8.0 Hz, C-2), 69.3, 69.2, 67.5, 64.7 ($J_{C,P}$ 6.7 Hz, C-1 phytanyl), 61.3, 60.8, 39.2, 37.3, 37.24, 37.20, 37.1, 36.8, 36.7, 34.2, 32.6, 29.4, 29.3, 27.8, 24.6, 24.3, 22.5, 22.4, 20.6, 20.4, 20.3, 19.6, 19.5, 19.1, 19.0.

Phytanyl [*β*-D-glucopyranosyl-(1→4)-*α*-D-glucopyranosyl]diphosphate (phytanyl *α*-D-cellobiosyldiphosphate) (4). Compounds **3** (0.1 g, 0.07 mmol) was dissolved in anhydrous CH_2Cl_2 (3 mL) and cooled to 0 °C. Then, a solution 0.35 M NaOMe/MeOH (1 mL) was added and after 30 min of stirring it was diluted with methanol and concentrated under reduced pressure to two thirds of the volume. The solution was deionized by elution with MeOH through a column with Amberlite IR-120 plus cation-exchange resin (H^+). The syrup obtained was purified by anion exchange column chromatography (DEAE-cellulose, acetate form, eluent: ammonium formate, linear gradient from 0.01 M to 0.20 M in 30:60:12 $CHCl_3$–MeOH–H_2O.

Fractions of R_f 0.31 (7:1:2 PrOH–EtOH–H_2O) eluted with 0.2 M ammonium formate. They were evaporated and lyophilized with water several times to afford **4** (0.06 g, 81%). ^{1}H NMR (400 MHz, 10:10:1 $CDCl_3$–CD_3OD–D_2O): δ 5.84 (1H, dd, $J_{1,2}$ 5.5 Hz, $J_{1,P}$ 5.5 Hz, H-1), 4.57 (1H, d, $J_{1',2'}$ 7.9 Hz, H-1'), 4.45 (1H, ddd, $J_{2,3}$ 6.0 Hz, $J_{2,P}$ 12.7 Hz, H-2), 4.29 (1H, dd, $J_{3,4}$ 6.2 Hz, H-3), 4.04 (1H, m, H-5), 4.04 (1H, H-1 phytanyl), 4.01 (1H, m, H-6'), 3.98 (1H, m, H-6), 3.82 (1H, dd, $J_{4,5}$ 9.2 Hz, H-4), 3.56 (1H, dd, $J_{3',4'}$ 9.0, H-3'), 3.49 (1H, m, H-5'), 3.48 (1H, dd, $J_{3',4'}$ 9.0 Hz, H-4'), 3.39 (1H, dd, $J_{2',3'}$ 9.1 Hz, H-2'), 1.54–1.18, 1.03, 1.01, 0.98 × 2, 0.95 (phytanyl). ^{13}C NMR (100 MHz, 10:10:1 $CDCl_3$–CD_3OD–D_2O): δ 105.8 (C1'), 98.7 ($J_{1,P}$ 6.4 Hz, C-1), 80.1 ($J_{2,P}$ 6.5 Hz, C-2), 79.5 (C-4), 79.0 (C-5'), 75.8 (C-2'), 78.5 (C-3'), 74.4 (C-5), 74.2 (C-3), 72.2 (C-4'), 67.2 (C-1''), 63.3 (C-6'), 62.8 (C-6), 40.0 (C-2''), 40.2–39.3 (C-4'', C-6'', C-8'', C-10'', C-12'', C-14''), 35.2 (C-3''), 31.7 (C-11''), 30.2 (C-10''), 26.8–26.7 (C-5'', C-9'', C-11", C-13''), 24.6

(C-16'', C-20''), 21.7 (C-17''), 21.6 (C-18''), 21.4 (C-19''). HRMS (ESI^-) calcd for $C_{32}H_{63}O_{17}P_2^-$ $[M–H]^-$: 781.3546; found: 781.3549.

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