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An undecaprenyl phosphate analog containing the phenoxy group at the ω-end of the oligoisoprene chain

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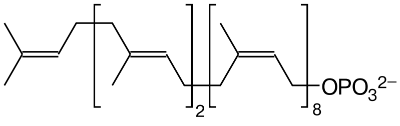
A simple method for the preparation of a biologically active analog of bacterial undecaprenyl phosphate bearing the phenoxy group at the ω-end of the chain was developed on the basis of accessible polyprenols from mulberry (*Morus nigra*) leaves. It includes the selective van Tamelen epoxidation of the ω-terminal isoprene unit of polyprenyl acetates, conversion of the epoxides into ω-terminal aldehydes, their hydride reduction into hydroxy acetates followed by the Mitsunobu condensation with phenol, and phosphorylation of the resulting phenoxy alcohols. The biological activity of the obtained phosphates was tested by the radiometry method in the initiation of O-antigenic polysaccharide assembly reaction using the cell membrane preparations from the Gram-negative bacteria *Salmonella arizona* O:59 and *Aeromonas hydrophila* AH-1.

Keywords: undecaprenyl phosphate analog, polyprenols, van Tamelen epoxidation, Mitsunobu reaction, phosphorylation, O-antigenic polysaccharide assembly, cell membranes, bacteria *Salmonella arizona* O:59, bacteria *Aeromonas hydrophila* AH-1.

Undecaprenyl phosphate **1** and undecaprenyl diphosphosugars are the key biosynthetic precursors of carbohydrate chains of glycopolymers of bacterial cells, including peptidoglycan, O-antigenic chains of lipopolysaccharides of Gram-negative bacteria, binding region of teichoic acids with peptidoglycan of Gram-positive bacteria, arabinan segments of mycobacterial cell walls and others.[1] In the initiation and growth processes of these carbohydrate chains of the most extensively studied bacteria, the above-mentioned intermediates serve as the substrate-donors and substrate-acceptor of the corresponding glycosyltransferases. The study of the processes controlled by these enzymes and especially the possibilities of their inhibition is of great importance for the search of the strategies to overcome the acquired bacterial resistance against the known antibiotics, as well as for the development of novel antibacterial agents. One of the most promising trends in this field is the design of model biochemical systems that include undecaprenyl phosphate analogs as the glycosyltransferase substrate-acceptors. It has earlier been shown that the hardly accessible bacterial undecaprenyl phosphate could effectively be replaced in such systems by synthetic phosphates of the available plant polyprenols with similar structures.[2—7] Recent studies demonstrated that 11-phenoxyundecyl phosphate could be used as the substrate-acceptor of several bacterial glycosyltransferases.[8—11] The presence of the phenoxy group increases the UV detection sensitivity of this compound and its derivatives in the isolation and analysis by HPLC and TLC, which opens additional potentialities in biochemical experiments. From this point of view, the preparation of compounds more close in structures to undecaprenyl phosphate and having the mentioned group is of obvious interest. In the present communication, this type of modification of accessible plant polyprenols is discussed.



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We used moraprenol (**2**), the natural mixture of C_{50}—C_{65}-polyprenols from mulberry (*Morus nigra*) leaves, where undecaprenol* is one of the major components (see Ref. 12), as the starting material. The strategy of the synthesis included the transformation of the ω-terminal unit of the oligoisoprene chain of prenols **2** with formation of the aldehyde group, its reduction into the alcohol group, the preparation of the phenyl ether followed by the phosphorylation of the thus modified polyprenols. The van

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* The plant undecaprenol WT_3C_7OH differs in the structure from the bacterial prenol WT_2C_8OH, where W is the ω-terminal isoprene unit of the oligoisoprene chain, T and C are the internal *trans*- and *cis*-isoprene units, respectively (see IUPAC rules[13]).

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Tamelen epoxidation was selected as the key step of synthesis. This method is characterized by a high chemoselectivity with respect to the trisubstituted double bond of the terminal isopropylidene unit of linear isoprenoid oligoolefins[14] and has successfully been used by us previously for similar purpose.[15]

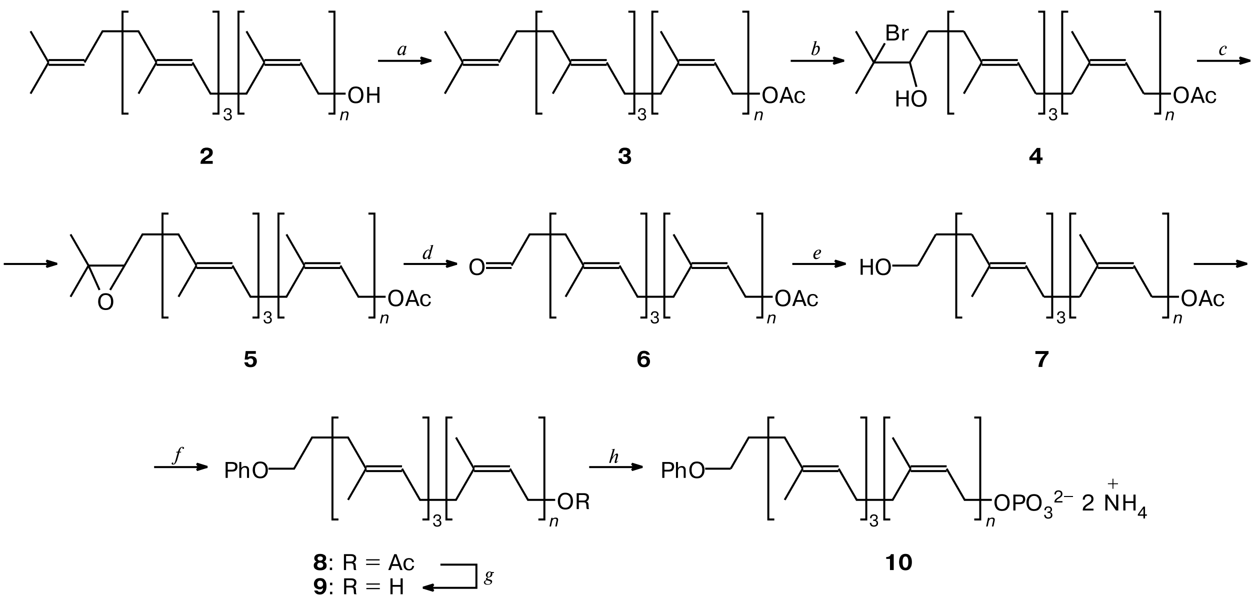
According to the chosen strategy, the starting polyprenols **2** were converted into acetates **3** (Scheme 1). Their two-step transformation through bromohydrins **4** into terminal epoxides **5** according to van Tamelen's method was preparatively acceptable and resulted in the desired product **5** in ~35% overall yield. The oxidative degradation of the latter with $HIO_4 \cdot 2H_2O$ smoothly led to acetoxy aldehydes **6**, which were selectively reduced to hydroxy acetates **7**. The introduction of the phenyl fragment into molecule **7** was performed by one of the versions of the Mitsunobu reaction with subsequent mild hydrolysis of the obtained mixture of phenoxy acetates **8**. Phenoxy alcohols **9*** prepared in eight steps in ~6% overall yield were then subjected to phosphorylation. For this purpose, the system $CCl_3CN—(Bu_4N)H_2PO_4$, which has been recommended for the phosphorylation of polyprenols,[2,16] was used. Phosphates **10** were isolated as the ammonium salts following cation exchange on a Dowex 50 W×8 (NH_4^+) resin and subsequent anion-exchange chromatography on DEAE-cellulose (AcO^-), a solution of $AcONH_4$ in MeOH being used for the elution and the excess of $AcONH_4$ being removed by precipitation with toluene.

The structures of hitherto unknown compounds **4**—**9** were confirmed by a combination of physicochemical data. Their ^1H and ^{13}C NMR spectra contained the signals typical of the linear oligoisoprenoids having (*E*)- and (*Z*)-isoprene fragments;[2,15,17] and also the signals corresponding to the functional groups at ω- and α-units of the chain. The IR spectroscopic data also agree with the structures of these compounds. The final products, *i.e.* phosphates **9**, were also characterized by the electrospray-ionisation (ESI) mass spectra, where the most abundant peaks correspond to the molecular ions of the respective oligomers.

The acceptor properties of phosphates **10** were studied in the reaction of initiation of assembly of a repeating unit of O-antigenic polysaccharides catalyzed by UDPGlcNAc : polyprenyl phosphate-GlcNAc-phosphotransferase of cytoplasmic membranes of the Gram-negative bacteria *Salmonella arizona* serogroup O:59 and *Aeromonas hydrophila* AH-1 using radioactive UDP[^{14}C]GlcNAc as the donor of monosaccharide [^{14}C]GlcNAc (Scheme 2). The control experiments were carried out using moraprenyl phosphate as the substrate-acceptor.[2]

The products of the enzymatic reactions were analyzed by TLC (SiO_2) using the liquid scintillation count-

Scheme 1



n = 6—9

Reagents and conditions: *a.* Ac_2O, Py, 20 °C, (~100%); *b.* NBS, aq. THF, 20 °C (55%); *c.* K_2CO_3, PhH—MeOH, 20 °C (62%); *d.* $HIO_4 \cdot 2H_2O$, Et_2O—THF, 20 °C (98%); *e.* $NaBH_4$, DME, 20 °C (60%); *f.* PhOH, DEAD, Ph_3P, THF, 0 → 20 °C (44%); *g.* K_2CO_3, PhH—MeOH, 20 °C (66%); *h.* 1) CCl_3CN, $Bu_4NH_2PO_4$, CH_2Cl_2, 20 °C, 2) Dowex 50W×8 (NH_4^+), Bu^{t}OMe—MeOH, 3) chromatography on DEAE-cellulose DE-52(OAc^-), NH_4OAc in MeOH (49%).

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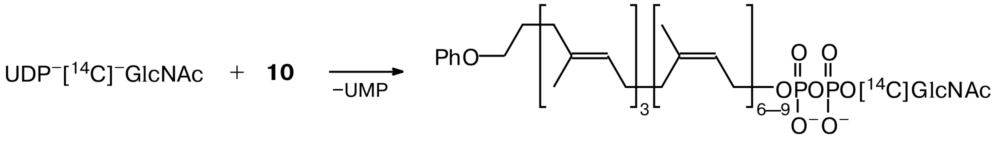
* According to the HPLC data, the ratio of homologs **9** (*n* = 6) : (*n* = 7) : (*n* = 8) : (*n* = 9) ≈ 5 : 25 : 20 : 2.

Scheme 2



ing of radioactivity. In the experiments with both abovementioned biological objects, it was found that the main radioactivity of the obtained products could be detected on the TLC plates in the zone with R_f 0.2 (Fig. 1). Virtually the same R_f value is characteristic of a synthetic sample of moraprenyl diphosphate α-D-glucose prepared by us earlier.[18] A comparison of the level of radioactivity of the products in this chromatographic zone in the reaction with moraprenyl phosphate and phosphates **10** as the substrate-acceptors shows that the relative efficacy in the latter was ~50% and ~66% for the enzymes from *S. arizonae* and *A. hydrophila* AH-1, respectively. Notably, the observed level of the activity of phosphates **10** is completely acceptable for their application in model systems in the study of such enzymatic processes, and the presence of the chromophore group (OPh) in their molecules will allow in future the use of a highly sensitive HPLC analysis of the products of the biochemical reactions.

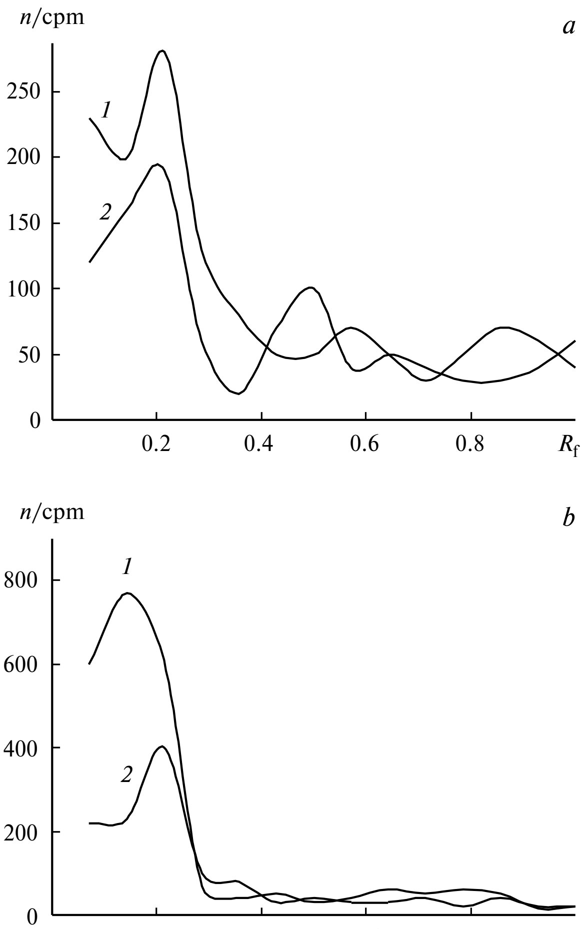


Fig. 1. The distribution of radioactivity n/cpm on the chromatogram of the reaction products involving moraprenyl phosphate (*1*) or phosphates **10** (*2*), UDP-[^{14}C]GlcNAc, and the cell membrane preparation of *A. hydrophila* AH-1 (*a*) or *S. arizonae* O:59 (*b*).

Experimental

The IR spectra were recorded on a Specord M-80 spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-200 spectrometer (unless otherwise stated) in $CDCl_3$. This spectrometer was also used to record the ^{31}P NMR spectra (the external standard was 85% H_3PO_4, the operating frequency for ^{31}P was 121.5 MHz). The ESI mass spectrum of phosphates **10** was recorded on a Bruker micrOTOF II spectrometer by the direct (by a syringe) inlet of a methanolic solution of a sample (3 μL min^{-1}), the potential on the capillary was 4500 V. The mass spectrum was obtained in the negative mode (scanning from m/z 300 to m/z 2000). The nitrogen flow rate was 4 L min^{-1} (180 °C).

Column anion-exchange chromatography of phosphates **10** was performed on DEAE-cellulose DE-52 (Whatman, England). The HPLC analysis was carried out on a column with Separon SGX C-18 (5 μm, 150×3.3 mm); the eluent was EtOH (0.5 mL min^{-1}), UV detector (210 nm). The isolation of the enzymatic reaction products was performed on mini-columns DIAPAK C18 (CC BioKhimMak ST, Moscow).

Radioactivity was measured in toluene scintillator using a Delta 300 (Tracor Analytic, Holland) liquid scintillation counter.

Silufol, silica gel 60 (Merck), and silica gel/TLC-cards DC-Alufolien-Kieselgel 254 nm (Fluka) plates with fixed SiO_2 layer were used for TLC.

A mixture of polyprenols **2** (WT_3C_6OH, WT_3C_7OH, WT_3C_8OH, WT_3C_9OH) was isolated by extraction from mulberry leaves (*M. nigra*) with petroleum ether followed by chromatography on SiO_2.

The cytoplasmic membrane preparation of *A. hydrophila* AH-1 cells (1 mg of protein per mL) was kindly provided by Professor Juan Tomas, the University of Barcelona (Spain). The membrane preparation of *S. arizonae* O:59 containing 1 mg of protein per mL was prepared according to the described method[19] from the cell culture of this microorganism grown at the L. A. Tarasevich State Institute of Standardization and Control of Medical Preparations.

The solvents including petroleum ether (b.p. 40—70 °C) were purified according to the standard methods.

The following Acros Organics reagents were used: NBS, $HIO_4 \cdot 2H_2O$, CCl_3CN, Ph_3P, $(Bu_4N)H_2PO_4$, DEAD, and

$NaBH_4$. UDP[^{14}C]GlcNAc, 285 mCu mmol^{-1} (Amersham, UK) was diluted with nonradioactive UDPGlcNAc (Sigma) to obtain 50 mCu mmol^{-1} radioactivity level.

Acetates 3. A solution of a mixture of alcohols **2** (3 g, ~4 mmol), DMAP (120 mg, 0.98 mmol), Ac_2O (0.61 g, 6.0 mmol), and Py (0.48 g, 6 mmol) in THF (12 mL) was kept for 20 min at 20 °C under argon. Then it was diluted with petroleum ether (80 mL), washed with saturated solution of $NaHCO_3$, water, brine, dried with Na_2SO_4, and concentrated *in vacuo*. The residue (4 g) was chromatographed on SiO_2 using a petroleum ether—MeOBu^t (9 : 1) mixture as the eluent to give 3.12 g (~99%) of acetates **3** as a colorless oil, R_f 0.51 (Silufol, petroleum ether—MeOBu^t, 6 : 1). ^{1}H NMR, δ: 1.61 (br.s, *cis*-Me); 1.70 (br.s, *trans*-Me); 1.78 (br.s, MeC(3)); 1.90—2.20 (m, CH_2); 2.12 (s, MeCO); 4.57 (d, H_2CO, J = 7.2 Hz); 5.14 (m, HC=); 5.37 (br.t, HC(2), J = 7.2 Hz), *cf.* Ref. 20.

Bromohydrins 4. *N*-Bromosuccinimide (0.96 g, 5.38 mmol) was gradually added over 20 min to a stirred solution of a mixture of acetates **3** (3.12 g, ~3.8 mmol) in a mixture of THF (54 mL) and H_2O (9 mL) at 10 °C under argon. The reaction mixture was kept for 3 h and then diluted with 100 mL of MeOBu^t. The organic layer was separated, washed with H_2O, brine, dried with Na_2SO_4, and concentrated *in vacuo*. The residue (~4 g) was chromatographed on SiO_2 using a petroleum ether—MeOBu^t (8 : 1) mixture as the eluent to give 1.95 g (~55%) of a mixture of bromohydrins **4** as a colorless oil, R_f 0.51 (Silufol, petroleum ether—MeOBu^t, 4 : 1). IR, ν/cm^{-1} (thin layer): 848, 1060, 1260, 1336, 1370, 1400, 1700, 2800—2900, 3550. ^{1}H NMR, δ: 1.34 and 1.36 (both s, Me_2CO); 1.61 (br.s, *cis*-Me); 1.69 (br.s, *trans*-Me); 1.77 (br.s, MeC(3)); 1.90—2.20 (m, CH_2); 2.11 (s, MeCO); 3.99 (dd, HCBr, J = 1.9 Hz, J = 11.1 Hz); 4.57 (d, H_2CO, J = 7.3 Hz); 5.05—5.26 (m, HC=); 5.38 (br.t, HC(2), J = 7.3 Hz). ^{13}C NMR, δ: 15.8; 16.0; 21.0; 23.4; 23.5; 25.8; 26.4; 26.6; 29.7; 32.0; 32.2; 32.3; 38.1; 39.6; 39.7; 61.1; 70.9, 72.4, 119.1; 124.1, 124.3; 124.4; 124.9; 125.0, 126.0, 132.9, 134.7, 135.2, 135.3, 135.8; 142.6; 171.0.

Epoxides 5. To a stirred solution of a mixture of bromohydrins **4** (3.5 g, ~3.9 mmol) in a mixture of PhH (30 mL) and MeOH (15 mL), K_2CO_3 (1.01 g, 7.3 mmol) was added at 20 °C under argon. The reaction mixture was kept for 4 h, then diluted with MeOBu^t (10 mL), washed with H_2O, brine, dried with Na_2SO_4, and concentrated *in vacuo*. The residue (~4 g) was chromatographed on SiO_2 (150 g) using a petroleum ether—MeOBu^t (gradient from 9 : 1 to 4 : 1) mixture as the eluent to give 1.99 g (~62%) of a mixture of epoxides **5** as a colorless oil, R_f 0.78 (Silufol, petroleum ether—MeOBu^t, 4 : 1). IR, ν/cm^{-1} (thin layer): 840, 930, 960, 1060, 1090, 1130, 1230, 1380, 1450, 1670, 1740, 2730, 2860, 2930, 2960. ^{1}H NMR, δ: 1.24 and 1.27 (both s, Me_2CO); 1.61 (br.s, *cis*-Me); 1.69 (br.s, *trans*-Me); 1.77 (br.s, MeC(3)); 1.92—2.20 (m, CH_2); 2.12 (s, MeCO); 2.70 (t, HCO, J = 6.6 Hz); 4.58 (d, H_2CO, J = 7.2 Hz); 5.10—5.27 (m, HC=); 5.39 (br.t, HC(2), J = 7.2 Hz). ^{13}C NMR, δ: 16.1; 18.8; 21.1; 23.4; 23.5; 25.0; 26.4; 26.7; 27.5; 32.0; 32.3; 32.4; 36.4; 39.7; 39.8; 58.3; 61.1; 64.2; 119.3; 124.2, 124.3; 124.4; 124.9; 125.0, 125.1; 134.0; 134.9, 135.2, 135.3, 135.4; 135.9; 142.6; 171.1.

Aldehydes 6. A solution of $HIO_4 \cdot 2H_2O$ (0.98 g, 4.8 mmol) in THF (25 mL) was gradually added in 5 min to a stirred solution of a mixture of epoxides **5** (1.99 g, ~2.4 mmol) in Et_2O (30 mL) at 20 °C under argon. The reaction mixture was kept for 2 h, then diluted with MeOBu^t (100 mL), washed with H_2O, brine, dried with Na_2SO_4, and concentrated *in vacuo*. The residue (~2 g) was chromatographed on SiO_2 (60 g) using a petroleum ether—MeOBu^t (gradient from 9 : 1 to 4 : 1) mixture as the eluent to give 1.85 g (~97%) of a mixture of aldehydes **6** as a colorless oil, R_f 0.36 (Silufol, petroleum ether—MeOBu^t, 5 : 1). IR, ν/cm^{-1} (thin layer): 840, 920, 1020, 1230, 1300, 1380, 1460, 1740, 2860—2960. ^{1}H NMR, δ: 1.62 (br.s, *cis*-Me); 1.68 (br.s, *trans*-Me); 1.76 (br.s, MeC(3)); 1.94—2.55 (m, CH_2); 2.11 (s, MeCO); 4.56 (d, H_2CO, J = 7.4 Hz); 5.13 (m, HC=); 5.37 (br.t, HC(2), J = 7.4 Hz), 9.75 (t, CHO, J = 1.7 Hz). ^{13}C NMR, δ: 15.9; 16.0; 21.0; 23.4; 23.5; 26.3; 26.4; 26.5; 26.6; 29.7; 31.8; 31.9; 32.1; 32.3; 39.4; 39.7; 42.1; 61.0; 119.2; 124.1; 124.2; 124.3; 124.4; 124.9; 125.0, 125.4; 134.5; 135.0, 135.1, 135.2; 135.7; 142.5; 171.2; 202.5.

Hydroxy acetates 7. To a stirred solution of aldehydes **6** (1.85 g, ~2.3 mmol) in dimethoxyethane (20 mL), $NaBH_4$ (70 mg, 1.85 mmol) was added in one portion at 20 °C under argon. The reaction mixture was kept for 50 min, then diluted with MeOBu^t (50 mL), washed with a saturated solution of NH_4Cl, water, brine, dried with Na_2SO_4, and concentrated *in vacuo*. The residue (~2 g) was chromatographed on SiO_2 (50 g) using a petroleum ether—MeOBu^t (gradient from 9 : 1 to 2 : 1) mixture as the eluent to give 1.1 g (~60%) of a mixture of hydroxy acetates **7** as a colorless oil, R_f 0.18 (Silufol, petroleum ether—MeOBu^t, 4 : 1). ^{1}H NMR, δ: 1.61 (br.s, *cis*-Me); 1.69 (br.s, *trans*-Me); 1.77 (br.s, MeC(3)); 1.90—2.60 (m, CH_2); 2.13 (s, MeCO); 3.53 (t, H_2COH, J = 6.4 Hz); 4.56 (d, H_2COAc, J = 7.3 Hz); 5.14 (m, HC=); 5.38 (br.t, HC(2), J = 7.3 Hz). ^{13}C NMR, δ: 15.8; 15.9; 21.0; 23.4; 23.5; 26.4; 26.6; 30.7; 31.9; 32.2; 32.3; 36.0; 39.6; 39.7; 42.0; 61.0; 62.8; 119.1; 124.1; 124.2; 124.3; 124.8; 124.9; 125.0; 134.5; 134.8; 135.2, 135.3, 135.8; 142.6; 171.1.

Phenoxy acetates 8. A solution of DEAD (0.09 g, 0.52 mmol) was added in 5 min to a stirred solution of a mixture of hydroxy acetates **7** (0.39 g, ~0.5 mmol), Ph_3P (0.17 g, 0.65 mmol), and PhOH (0.15 g, 1.59 mmol) in THF (5 mL) at 0 °C under argon. The reaction mixture was kept for 15 h at 0 °C, then diluted with MeOBu^t (20 mL), washed with H_2O, brine, dried with Na_2SO_4, and concentrated *in vacuo*. The residue (~0.5 g) was chromatographed on SiO_2 (20 g) using a petroleum ether—MeOBu^t (gradient from 9 : 1 to 4 : 1) mixture as the eluent to give 0.19 g (~44%) of a mixture of phenoxy acetates **8** as a colorless oil, R_f 0.77 (Silufol, petroleum ether—MeOBu^t, 4 : 1). IR, ν/cm^{-1} (thin layer): 680, 720, 780, 900, 1060, 1080, 1110, 1170, 1240, 1380, 1400. 1450, 1480, 1500, 1550, 1590, 1630, 1660, 1680, 1740, 2860, 2880, 2930, 2960. ^{1}H NMR, δ: 1.63 (br.s, *cis*-Me); 1.65 (s, $PhOCH_2CH_2(\underline{Me})C=$); 1.71 (br.s, *trans*-Me); 1.78 (br.s, MeC(3)); 1.98—2.22 (m, CH_2); 2.13 (s, MeCO); 3.95 (t, H_2COPh, J = 6.5 Hz); 4.58 (d, H_2COAc, J = 7.2 Hz); 5.15 (m, HC=); 5.39 (br.t, HC(2), J = 7.2 Hz); 6.93 and 7.29 (both m, HC_{arom}). ^{13}C NMR, δ: 16.0; 21.0; 23.4; 26.4; 26.6; 27.5; 32.0; 32.2; 32.4; 35.8; 39.6; 39.7; 61.1; 67.3; 114.5; 119.2; 120.4; 124.1; 124.3; 124.9; 125.0; 128.4; 128.5; 128.7; 129.4; 133.5; 133.9; 135.2, 135.3, 135.8; 142.3; 171.2.

Phenoxy alcohols 9. To a stirred solution of a mixture of acetates **8** (0.19 g, ~0.22 mmol) in a mixture of MeOH (5 mL) and PhH (5 mL), K_2CO_3 (0.15 g, 0.72 mmol) was added in one portion at 20 °C under argon. The reaction mixture was kept for 17 h at 20 °C, then diluted with MeOBu^t (20 mL), washed with H_2O, brine, dried with Na_2SO_4, and concentrated *in vacuo*. The residue (~0.2 g) was chromatographed on SiO_2 (20 g) using a petroleum ether—$MeOBu^t$ (gradient from 9 : 1 to 4 : 1) mixture as the eluent to give 0.12 g (~66%) of a mixture of phenoxy alcohols **9** as a colorless oil, R_f 0.23 (Silufol, petroleum ether—$MeOBu^t$, 6 : 1). The HPLC retention times of the main components of the mixture **9** are 4.7 ($C_{53}H_{82}O_2$), 5.7 ($C_{58}H_{90}O_2$); 7.0 ($C_{63}H_{98}O_2$), and 8.6 min ($C_{58}H_{106}O_2$) in the ratio ~5 : 25 : 20 : 2, respectively. ^{1}H NMR, δ: 1.62 (br.s, *cis*-Me); 1.65 (s, $PhOCH_2CH_2(\underline{Me})C=$); 1.70 (br.s, *trans*-Me); 1.77 (br.s, MeC(3)); 1.97—2.21 (m, CH_2); 3.94 (t, H_2COPh, J = 6.5 Hz); 4.12 (d, H_2COH, J = 7.2 Hz); 5.15 (m, HC=); 5.47 (br.t, HC(2), J = 7.2 Hz); 6.94 and 7.30 (both m, HC_{arom}). ^{13}C NMR, δ: 15.9; 23.3; 26.2; 26.3; 26.5; 27.4; 31.9; 32.1; 35.7; 39.5; 39.6; 58.9; 67.2; 114.4; 120.4; 124.0; 124.2; 124.3; 124.4; 124.8; 124.9; 129.3; 135.1; 135.2, 135.9; 139.7.

Phosphates 10. A 0.5 *M* solution of $Bu^n{}_4NH_2PO_4$ (0.64 mL, 0.32 mmol) in CH_2Cl_2 and 1 *M* solution of Cl_3CCN (0.5 mL, 0.5 mmol) in CH_2Cl_2 were sequentially added to a mixture of alcohols **9** (89 mg, ~0.1 mmol) at 20 °C under argon. The reaction mixture was kept for 48 h at 20 °C, then concentrated *in vacuo*. The residue was dissolved in the upper (organic) phase of an equilibrium mixture Bu^nOH—water. The solution was washed with the lower phase of this mixture (4×1 mL), concentrated *in vacuo*, the residue was dissolved in toluene (5 mL) and concentrated once again. A mixture $MeOBu^t$—MeOH (2 : 1) (6 mL), concentrated aqueous ammonia (50 μL) and a cation-exchange resin Dowex 50W×8 (NH_4^+) (1.5 g) were added to the residue and stirred for 1 h. The ion-exchange resin was filtered off, washed with a mixture $MeOBu^t$—MeOH (2 : 1) (100 mL). The obtained solution was passed through a column (8×1 cm) with DEAE-cellulose (OAc^-), the column was additionally washed with a mixture $MeOBu^t$—MeOH (2 : 1) (50 mL) and MeOH (50 mL) and phosphates **10** were eluted with a solution of $AcONH_4$ (150 mL 50 *mM*) in MeOH, the separation being monitored by TLC (Silica Gel 60 Merck) in the solvent system $CHCl_3$—MeOH—H_2O (60 : 25 : 4). The phosphate-containing fractions were concentrated *in vacuo*, the residue was dissolved in toluene (8 mL) and the solution was kept at 0 °C for 16 h. The supernatant was decanted from the precipitate and concentrated *in vacuo*. The precipitation procedure was repeated twice, then the product was dried under reduced pressure (40 °C, 2 Torr) for 2 h to obtain 49.2 mg (~50%) of an oily mixture of phosphates **10** with R_f 0.45 (Silica Gel 60, Merck, $CHCl_3$—MeOH—H_2O, 60 : 25 : 4). The ESI mass spectrum (m/z (I_{rel} (%), molecular formula, molecular weight of the ion $[M - 2NH_3 - H]^-$): 829.58 (19), $C_{53}H_{81}O_5P$, 829.19; 897.65 (100), $C_{58}H_{89}O_5P$, 897.31; 965.71 (82), $C_{63}H_{97}O_5P$, 965.43; 1033.77 (12), $C_{68}H_{105}O_5P$, 1033.55. ^{1}H NMR (500.13 MHz, $CDCl_3$—CD_3OD, 5 : 1), δ: 1.51 (br.s, *cis*-Me); 1.54 (br.s, $PhOCH_2CH_2(\underline{Me})C=$); 1.59 (br.s, *trans*-Me); 1.63 (br.s, MeC(3)); 1.83—2.07 (m, CH_2); 3.83 (t, H_2COPh, J = 6.4 Hz); 4.30 (m, H_2COP); 5.04 (m, HC=); 5.28 (br.t, HC(2), J = 7.0 Hz); 6.82 and 7.18 (both m, HC_{arom}). ^{13}C NMR (125.03 MHz, $CDCl_3$—CD_3OD, 5 : 1), δ: 15.7; 23.0; 23.1; 26.0; 26.2; 26.4; 27.3; 29.5; 31.7; 32.0; 35.6; 39.4; 39.5; 61.6 (CH_2OP); 67.2 ($\underline{C}H_2OPh$); 114.3; 120.3; 122.0; 124.0; 124.1; 124.2; 124.7; 124.8; 129.2; 135.0; 135.3; 139.2. ^{31}P NMR (δ, $CDCl_3$—CD_3OD, 5 : 1): 1.73.

Biochemical studies (general procedure). A mixture of phosphates **10** (40 nmol) (or moraprenyl phosphate), 0.1 *mM* Tris-HCl buffer (pH 8.0) (30 μL), 0.2 *M* $MgCl_2$ (4 μL), 0.5%-aqueous solution of Tween 85 (15 μL), and methanol (10 μL) was solubilized for 60 s on a Vortex 40 shaker. UDP-[^{14}C]GlcNAc (1—1.5 nmol, 85000—100000 cpm) followed by the respective membrane preparation (20 μL (~20 μg of protein) was added to the obtained emulsion. The samples were stirred for 45 min at 35 °C, then were diluted with cold distilled water to 400—500 μL and kept for 12—20 h at 0—4 °C. The coagulated protein was removed by centrifugation (8000 rpm, 10 min), the supernatant was applied onto a mini-column DIAPAK C18. The nonconsumed UDP-[^{14}C]GlcNAc was eluted with water, the completeness of elution being monitored by mesuaring radioactivity of the eluate. The reaction product, prenyldiphospho sugar, was eluted with methanol (5×1 mL), the methanol eluates were concentrated and analyzed by TLC on the plates (length 8 cm) Silica gel/TLC-cards DC-Alufolien-Kieselgel 254nm (Fluka).

The chromatographic mobility in the solvent system chloroform—methanol—water (60 : 25 : 4) of the UV absorbing products was determined by irradiation of plates with a UV-lamp, and radioactive zones were localized after cutting the plates into pieces 0.5×1 cm and measuring their radioactivity using the liquid scintillation counting method.

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