Chemistry of Natural Compounds, Bioorganic, and Biomolecular Chemistry

Fluorescent-labeled derivatives of dolichyl phosphate. Analogs of dolichyl phosphate with the 2-aminopyridine residue at the ω-end of the chain*

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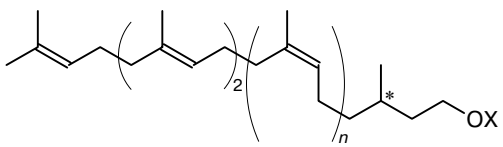
A simple approach to the synthesis of dolichyl phosphate derivatives with a fluorescent label, 2-aminopyridine residue, at the ω-end of the chain was developed. The method includes selective van Tamelen epoxidation of the ω-isoprene unit in dolichyl acetates, transformation of the epoxides to ω-terminal aldehydes, their reductive amination, and phosphorylation of the resulting amino alcohols.

Key words: dolichyl phosphate, (±)-dolichols, van Tamelen epoxidation, reductive amination of aldehydes, sodium triacetoxyborohydride, phosphorylation, fluorescent label.

(*S*)-Dolichyl phosphates (**1**, X = OPO_3^{2-}, $n > 6$) are intermediates in biosynthesis of glycoprotein carbohydrate chains in eukaryotic cells.[2]



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A specific localization of dolichyl phosphates in cellular membranes is assumed to be essential for the optimum activity of glycosyl transferases involved in biosynthetic reactions. Dolichols are highly lipophilic compounds and thus can play an important structuring role in biological membranes and participate in transmembrane transfer of activated carbohydrate residues. However, the details of interaction of dolichyl phosphates with the components of biological membranes currently remain unknown. Fluorescent methodology seems to be promising for compensation of gaps in understanding of these processes. This in turn requires synthesis of a series of fluorescent-labeled derivatives of dolichyl phosphates, the most simple variant, which includes introduction of the label into the phosphate

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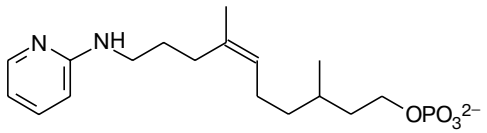
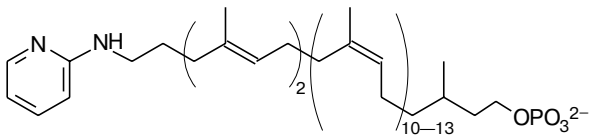
residue involved in the interaction with carbohydrates, being obviously unsuitable.

We report herein an approach, which allowed us for the first time to perform the synthesis of dolichyl phosphate derivatives with a fluorophore localized at the maximum distance from the phosphate group, namely, in the ω-unit of the oligoisoprene chain. The residue of 2-aminopyridine was used as the fluorophore. Carbohydrate derivatives containing this residue (λ_{ex} = 310—315 nm, λ_{em} = 380—400 nm), which are obtained by reductive amination of carbohydrates, are widely used in analysis of natural oligosaccharides.[3—5]

The projected synthetic sequence included introduction of the aldehyde group in the ω-unit of the isoprene chain, reductive amination of the resulting aldehydes, and subsequent phopsphorylation of amino alcohols generated. The van Tamelen epoxidation, which is known to be highly chemoselective towards the tri-substituted double bond in the terminal isopropylidene fragment of linear isoprenoid oligoolefins,[6] was chosen to be a key stage for functionalization of the ω-unit. First we performed the model synthesis of 2-aminopyridine derivative **2a** bearing a short-chain phosphate and then obtained the mixture **2b** of oligomers-homologs of racemic dolichyl phosphate derivatives starting from the available mixture of semisynthetic (±)-terpenols (**1**, X = H, n = 10—13).[7]



2a



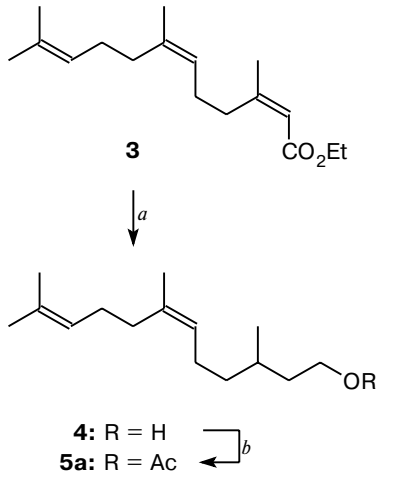
(*R*/*S*)-**2b**

The initial steps of the model synthesis (see Scheme 1) included reduction of ethyl farnesoate **3** [8] by Li/NH_3 as described previously[9] for related conjugated esters and transformation of sesquiterpenol **4** [10] into its acetate (**5a**).

Epoxidation of diolefin **5a** smoothly gives monoepoxide **7a** (see Scheme 2). The internal double bond remains intact as evidenced by the absence of the corresponding epoxy compound in the reaction mixture (^{1}H NMR data). It should be noted that treatment of intermediate bromhydrin **6a** with K_2CO_3 in MeOH results in partial deacetylation. This required additional acetylation of the crude product. The resulting acetoxy epoxide **7a** obtained in an ~40% yield was treated with $HIO_4 \cdot 2H_2O$ (*cf.* Ref. 11) to result in the formation of aldehydo acetate **8a** in an ~85% yield.

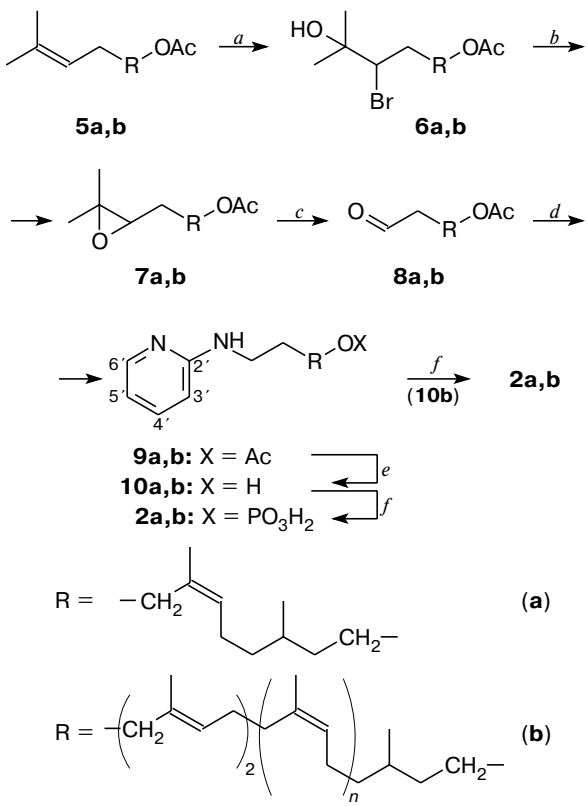
To obtain 2-aminopyridine derivative **9a**, we used reductive amination of aldehyde **8a** in the presence of

Scheme 1



Reagents and conditions: *a*. 1) Li/NH_3—Et_2O—dioxane, −40 °C, 2) EtOH; *b*. Ac_2O/Py, 20 °C.

Scheme 2



Reagents and conditions: *a*. NBS/THF—H_2O, 20 °C;
b. 1) K_2CO_3/MeOH, 20 °C, 2) Ac_2O/Py, 20 °C;
c. $HIO_4 \cdot 2H_2O$/THF, 20 °C;
d. 2-Aminopyridine/$NaBH(OAc)_3$/$(CH_2Cl)_2$, 20 °C;
e. K_2CO_3/MeOH, 20 °C;
f. $(Bu_4N)H_2PO_4/CCl_3CN/CH_2Cl_2$, 25 °C.

$NaBH(OAc)_3$ (*cf.* Ref. 12). The resulting acetoxy derivative **9a** was transformed to the target alcohol **10a**, which was used as a starting substrate for subsequent phosphorylation.

The reaction sequence mentioned above was successfully used for the similar transformation of long-chain (±)-alcohols **1** (X = H, n = 10—13). The selectivity of conversion of olefins **5b** to terminal monoepoxides **7b** appeared to be sufficiently high (the total yield was ~35%). Degradation of **7b** to aldehydes **8b**, amination of the latter compounds, and the subsequent transformation of the corresponding acetates **9b** to alcohols **10b** presented few problems and were characterized with sufficient yields.

Compounds **2—10** first synthesized were purified by chromatography on SiO_2, and their structure was confirmed by spectral data. In particular, their ^1H and ^{13}C NMR spectra contain the set of signals characteristic[13] for polyolefins of this type. The presence of the signals of the CHO group protons of the oxirane cycle in the region of δ ~2.6 (t, J ~ 7 Hz) and of the protons of the *gem*-dimethyl groups in the ^1H NMR spectra of epoxides **7** proves the terminal position of the epoxy group. The aldehyde group in acetoxy aldehydes **8** is characterized by the signals of the CHO proton at δ ~9.8. The ^1H NMR spectra of amino derivatives **9** and **10** are characterized by the presence of the signal of the CH_2N group (δ ~3.3) and of the corresponding signals of the protons of the aromatic fragment of these molecules. Their IR spectra contain characteristic absorption bands at 3440 and 3660 cm^{-1}.

Phosphorylation of 2-aminopyridine-labeled alcohols **10a,b** was performed by treatment with $(Bu_4N)H_2PO_4/CCl_3CN$ in CH_2Cl_2 as was previously described for a number of polyprenols and dolichols.[14] In the case of amino alcohols the reaction proceeded markedly slower than in the case of neutral alcohols even when using considerably larger excess of reagents (for **10a,** the reaction time was 48 h at a ratio of alcohol : phosphate : CCl_3CN equal to 1 : 1.8 : 2.0; in the case of **10b,** the reaction proceeded for 18 h at a reagent ratio of 1 : 2.5 : 2.8). The initial stages of purification of phosphates **2a,b** included distribution of the reaction products between Bu^nOH and water in the two-phase system and transformation of polyprenyl phosphates, which pass into the organic phase, to their ammonium salts by treatment with the excess of cationite. In the case of **2a**, further purification included anion-exchange chromatography on DEAE Cellulose (AcO^-) upon elution with the solution of $AcONH_4$ in MeOH, removal of the excess of $AcONH_4$ by precipitation with toluene, and additional chromatography on SiO_2. We succeeded in obtaining phosphate **2b** as a chromatographically homogeneous substance by distribution of polyprenol products between octane and MeOH. This treatment results in phosphate transition to the methanol phase. As phosphates **2a,b** are labile at storage when dry, we used their solutions in a heptane—Pr^iOH mixture for storage and characterization. The product concentration was determined from the content of inorganic phosphate after ashing with $HClO_4$.

The structure of phosphates **2a,b** was confirmed by physicochemical data. The UV spectra of compounds obtained match those expected for the 2-aminopyridine chromophore. The ^{31}P NMR spectra contain the only signal in the region close to 3 ppm corresponding to a phosphomonoester. The ^1H and ^{13}C NMR spectra of phosphates **2a,b** are close to those of amino alcohols **10a,b**, but the differences in the chemical shifts of the signals of H(1) and C(1) characteristic of polyprenyl phosphates as compared to those for polyprenols[14] are observed. The main peaks in the electrospray ionization (ESI) mass spectra of phosphates **2a,b** match the molecular ions of the corresponding acids.

Experimental

IR spectra were recorded on a Specord M-80 instrument. UV spectra were taken on a Specord UV VIS spectrophotometer. ^1H and ^{13}C NMR spectra were registered in $CDCl_3$ on a Bruker AC-200 spectrometer in the case of nonphosphorylated compounds and on a Bruker AM-300 spectrometer in the case of phosphates. The latter instrument was used for registration of ^{31}P NMR spectra (121.5 MHz) using 85% H_3PO_4 as the external reference. Mass spectra (EI, 70 eV) were taken on a Varian MAT 311A instrument and ESI mass spectra were measured on an API III (PE-Sciex, Canada) triple quadrupole mass spectrometer. The R_f values are given for plates precoated with SiO_2 (Silufol) in the case of nonphosphorylated compounds and with Silica Gel 60 (Merck, Germany) for phosphates. The spots on the plates were visualized under UV irradiation in the case of fluorescent derivatives, by treatment with iodine vapor in the case of unsaturated compounds, and by treatment with the Vaskovsky reagent[15] and subsequent heating of the plates in the case of phosphates.

Column anion-exchange chromatography of phosphates was performed on DEAE Cellulose DE-52 (Whatman, the United Kingdom). Colorimetric determination of phosphates was performed using the standard reagent[16] after heating a sample with 57% $HClO_4$ for 15 min at 200 °C.

All solvents were purified according to standard procedures. Reagents used in this study (NBS, DMAP, $HIO_4 \cdot 2H_2O$, $NaBH(OAc)_3$, $(Bu_4N)H_2PO_4$, and CCl_3CN) were purchased from Fluka. Column chromatography was performed on Silica gel 60 (Fluka).

The sample of ethyl farnesoate **3** was obtained by the method reported previously.[8]

A mixture of (±)-terpenols **1** (X = H) with a ratio of the main isoprenols $C_{70}H_{116}O : C_{75}H_{124}O : C_{80}H_{132}O : C_{85}H_{140}O$ ~ 6 : 13 : 14 : 7 was used (see Ref. 7).

(±)-3,7,11-Trimethyldodeca-6*Z*,10-dien-1-ol (4). Lithium (0.9 g, 130 mmol) was added in one portion to a solution of 4.53 g of ethyl farnesoate **3** (17.0 mmol) in a mixture of 200 mL of liquid NH_3, 30 mL of dioxane, and 30 mL of Et_2O with vigorous stirring at −40 °C (Ar). The reaction mixture was kept for 1.5 h at −40 °C and then quenched with an excess (10 mL) of 95% EtOH, NH_3 was evaporated, and the residue was treated with the saturated solution of NH_4Cl and $MeOBu^t$. The organic layer was separated, washed with water and brine, dried with $MgSO_4$, and concentrated *in vacuo*. The residue (4.5 g) was chromatographed on 150 g of SiO_2. Elution with a mixture of

CH_2Cl_2—Et_2O (4 : 1 v/v) yielded 2.15 g (56%) of alcohol **4** as a colorless oil. ^{1}H NMR, δ: 0.89 (d, 3 H, MeC(3), J = 6.75 Hz); 1.10—1.59 (m, 5 H, $H_2C(2)$, HC(3), $H_2C(4)$); 1.59 (br.s, 3 H, MeC(11)); 1.65 (br.s, 6 H, MeC(7), $H_3C(12)$); 1.81—2.11 (m, 6 H, 3 CH_2C=C); 3.64 (m, 2 H, $H_2C(1)$), 5.07 (m, 2 H, 2 HC=) (*cf.* Ref. 10).

(±)-3,7,11-Trimethyldodeca-6*Z*,10-dien-1-yl acetate (5a). A solution of 2.24 g (10.0 mmol) of alcohol **4**, 61 mg (0.5 mmol) of DMAP, and 1.33 g (13.0 mmol) of Ac_2O in 5 mL of Py was kept for 2 h at 20 °C (Ar); diluted with hexane (50 mL), washed with the saturated solution of $NaHCO_3$, water, and brine, dried with Na_2SO_4, and concentrated *in vacuo*. The residue (2.4 g) was chromatographed on 70 g of SiO_2. Elution with CH_2Cl_2 yielded 2.56 g (96%) of acetate **5a** as a colorless oil. ^{1}H NMR, δ: 0.90 (d, 3 H, MeC(3), J = 6.7 Hz); 1.09—1.61 (m, 5 H, $H_2C(2)$, HC(3), $H_2C(4)$); 1.55 (br.s, 3 H, MeC(11)); 1.65 (br.s, 6 H, MeC(7), $H_3C(12)$); 1.91—2.10 (m, 6 H, 3 CH_2C=C); 2.03 (s, 3 H, MeCO); 4.07 (m, 2 H, $H_2C(1)$), 5.06 (m, 2 H, 2 HC=) (*cf.* Ref. 10).

Acetates 5b. A solution of 9.5 g (~10 mmol) of the mixture of alcohols **1** (X = H, n = 10—13), 120 mg (1.0 mmol) of DMAP, 1.33 g (13.0 mmol) of Ac_2O, and 1.0 g (12.7 mmol) of Py in 40 mL of CH_2Cl_2 was kept for 6 h at 20 °C (Ar); diluted with hexane (100 mL), washed with the saturated solution of $NaHCO_3$, water, and then brine, dried with Na_2SO_4, and concentrated *in vacuo*. The residue (12 g) was chromatographed on 350 g of SiO_2. Elution with CH_2Cl_2 yielded 9.27 g (~93%) of a mixture of acetates **5b** as a colorless oil, R_f 0.67 (hexane—Et_2O, 4 : 1). ^{1}H NMR, δ: 0.92 (d, MeC(3), J = 6.6 Hz); 1.15—1.55 (m, $H_2C(2)$, HC(3), $H_2C(4)$); 1.62 (br.s, *cis*-Me); 1.71 (br.s, *trans*-Me); 1.95—2.15 (m, CH_2); 2.04 (s, MeCO); 4.12 (m, H_2CO); 5.16 (m, HC=).

12-Acetoxy-3-bromo-2,6,10-trimethyldodec-6*Z*-en-2-ols (6a). *N*-Bromosuccinimide (1.4 g, 7.87 mmol) was added portionwise to a solution of 2 g (7.5 mmol) of acetate **5a** in 40 mL of THF and 15 mL of H_2O during 1 h with stirring at 20 °C. The reaction mixture was kept for 30 min at 20 °C and then diluted with 100 mL of $CHCl_3$. The organic layer was separated, washed with water and brine, dried with $MgSO_4$, and concentrated *in vacuo*. The residue (~2 g) was chromatographed on 100 g of SiO_2. Elution with a mixture of hexane—Et_2O (3 : 2) yielded 1.42 g (52%) of a mixture of diastereomers **6a** as a colorless oil, R_f 0.43 (hexane—Et_2O, 1 : 1). IR (film), ν/cm^{-1}: 680, 780, 920, 970, 1050, 1130, 1250, 1370, 1460, 1740, 2250—3100, 3480. ^{1}H NMR, δ: 0.91 (d, 3 H, MeC(10), J = 6.5 Hz); 1.10—1.85 (m, 7 H, $H_2C(4)$, $H_2C(9)$, HC(10), $H_2C(11)$); 1.32 (s, 6 H, MeC(2), $H_3C(1)$); 1.67 (br.s, 3 H, MeC(6)); 1.95—2.30 (m, 4 H, 2 CH_2C=C); 2.01 (s, 3 H, MeCO); 3.92 (br.d, 1 H, HCBr, J = 12.0 Hz); 4.10 (br.t, 2 H, CH_2O, J = 7.1 Hz); 5.18 (br.t, 1 H, HC=, J = 7.4 Hz). MS, m/z (I_{rel} (%)): 265 [M − Br − OH]$^+$ (25), 205 (20), 203 (10), 163 (11), 148 (58), 137 (29), 135 (61), 125 (10), 123 (67), 121 (80), 109 (73), 107 (62), 95 (79), 93 (75), 82 (45), 81 (100), 79 (46), 71 (37), 69 (98), 67 (59).

Bromhydrins 6b. *N*-Bromosuccinimide (0.8 g, 4.49 mmol) was added portionwise in 20 min to a solution of 3.5 g (~3.2 mmol) of the mixture of acetates **5b** in 55 mL of THF and 9 mL of H_2O with stirring at 20 °C (Ar). The reaction mixture was kept for 3 h at 20 °C and then diluted with 100 mL of Et_2O. The organic layer was separated, washed with water and brine, dried with $MgSO_4$, and concentrated *in vacuo*. The residue (~4 g) was chromatographed on 100 g of SiO_2. Gradient elution (CH_2Cl_2 → CH_2Cl_2—Et_2O, 4 : 1) yielded 1.60 g (~42%) of a mixture of bromhydrins **6a** as a colorless oil, R_f 0.53 (CH_2Cl_2). IR ($CHCl_3$), ν/cm^{-1}: 840, 970, 1040, 1080, 1130, 1240, 1370, 1450, 1670, 1740, 2740—3040, 3480. ^{1}H NMR, δ: 0.91 (d, MeC(3), J = 6.3 Hz); 1.10—1.85 (m, HC(2), HC(3), HC(4), H_2CCBr); 1.31 (s, Me_2CO); 1.61 (br.s, *cis*-Me); 1.71 (br.s, *trans*-Me); 1.95—2.15 (m, CH_2C=C); 2.03 (s, MeCO); 3.90 (br.d, HCBr, J = 11.1 Hz); 4.10 (br.t, H_2CO, J = 6.7 Hz); 5.18 (m, HC=). ^{13}C NMR, δ: 15.8, 19.3, 20.8, 23.2, 24.2, 25.2, 25.9, 26.3, 26.6, 28.9, 29.6, 30.2, 31.9, 32.2, 35.5, 37.2, 38.1, 39.6, 62.9, 70.6, 72.3, 124.3, 125.0, 125.3, 125.9, 133.0, 134.8, 135.1, 135.2, 170.9.

3,7,11-Trimethyl-10,11-epoxydodec-6*Z*-en-1-yl acetates (7a). Potassium carbonate (1 g, 7.22 mmol) was added to a solution of 1.4 g (3.8 mmol) of the mixture of bromhydrins **6a** in 10 mL of MeOH with stirring at 20 °C. The reaction mixture was kept for 30 min at 20 °C, diluted with 100 mL of $CHCl_3$, washed with water, dried with $MgSO_4$, and concentrated *in vacuo*. The residue (~1 g) was dissolved in 5 mL of Py and 1 mL of Ac_2O and kept for 2 h at 20 °C. Then the reaction mixture was diluted with hexane (40 mL), washed with the saturated solution of $NaHCO_3$, water, and then brine, dried with Na_2SO_4, and concentrated *in vacuo*. The residue (1.2 g) was chromatographed on 50 g of SiO_2. Gradient elution (CH_2Cl_2 → CH_2Cl_2—Et_2O → Et_2O) yielded 1.02 g (95%) of a mixture of diastereomers **7a** as a colorless oil, R_f 0.63 (hexane—Et_2O, 1 : 1). IR (film), ν/cm^{-1}: 690, 870, 1050, 1060, 1130, 1250, 1370, 1460, 1740, 2250—3020. ^{1}H NMR, δ: 0.86 (d, 3 H, MeC(3), J = 6.6 Hz); 1.02—1.65 (m, 7 H, $H_2C(2)$, HC(3), $H_2C(4)$, $H_2C(9)$); 1.14 and 1.21 (both s, 6 H, MeC(11), $H_3C(12)$); 1.63 (br.s, 3 H, MeC(7)); 1.80—2.20 (m, 4 H, 2 CH_2C=C); 1.96 (s, 3 H, MeCO); 2.61 (t, 1 H, HCO, J = 6.3 Hz); 4.02 (br.t, 2 H, HC(1), J = 6.8 Hz); 5.07 (br.t, 1 H, HC=, J = 7.1 Hz). Found (%): C, 72.68; H, 10.78. $C_{17}H_{30}O_3$. Calculated (%): C, 72.30; H, 10.71.

Epoxides 7b. Potassium carbonate (0.5 g, 3.61 mmol) was added to a solution of 2.9 g (~2.4 mmol) of the mixture of bromhydrins **6b** in 20 mL of PhH and 10 mL of MeOH with stirring at 20 °C (Ar). The reaction mixture was kept for 20 min at 20 °C, diluted with 50 mL of Et_2O, washed with water and brine, dried with Na_2SO_4, and concentrated *in vacuo*. The residue (~2.5 g) was dissolved in 5 mL of Py and 1 mL of Ac_2O and kept for 2 h at 20 °C. The reaction mixture was treated as described above, and the resulting product was chromatographed on 50 g of SiO_2. Gradient elution (hexane → hexane—Et_2O, 6 : 4) yielded 2.20 g (~82%) of a mixture of epoxides **7b** as a colorless oil, R_f 0.36 (hexane—Et_2O, 9 : 1). IR ($CHCl_3$), ν/cm^{-1}: 840, 895, 915, 975, 1040, 1060, 1090, 1130, 1220, 1260, 1320, 1370, 1380, 1450, 1660, 1725, 2730, 2860, 2930, 2960, 3030. ^{1}H NMR, δ: 0.92 (d, MeC(3), J = 6.1 Hz); 1.10—1.70 (m, HC(2), HC(3), HC(4), C(O)CH—C$\underline{H}_2$); 1.24 and 1.27 (both s, Me_2CO); 1.62 (br.s, *cis*-MeC=C); 1.68 (br.s, *trans*-MeC=C); 1.90—2.20 (m, CH_2C=, MeCO); 2.70 (t, HCO, J = 6.6 Hz); 4.09 (br.t, H_2CO, J = 7.0) Hz; 5.15 (m, HC=). ^{13}C NMR, δ: 15.9, 18.7, 19.4, 20.9, 23.4, 23.8, 24.1, 24.9, 25.2, 25.4, 26.4, 26.6, 27.5, 29.6, 31.9, 32.2, 35.5, 36.3, 37.3, 39.6, 40.0, 58.2, 62.9, 64.1, 124.2, 125.0, 125.3, 125.7, 134.0, 135.0, 135.1, 135.2, 171.0.

10-Acetoxy-4,8-dimethyldec-4*Z*-en-1-al (8a). A solution of 0.8 g (3.51 mmol) of $HIO_4 \cdot 2H_2O$ in 8 mL of THF was added in 30 min to a solution of 0.6 g (2.5 mmol) of the mixture of epoxides **7a** in 8 mL of Et_2O with stirring at 20 °C. The reaction mixture was kept for 1 h, diluted with 30 mL of Et_2O, washed with water and brine, dried with $MgSO_4$, and concentrated *in vacuo*. The residue (0.6 g) was chromatographed on 50 g of SiO_2. Elution at 0 °C with a mixture of hexane—Et_2O (1 : 1) yielded 0.51 g (85%) of aldehyde **8a** as a colorless oil, R_f 0.51 (hexane—Et_2O, 1 : 1). IR (film), ν/cm^{-1}: 1050, 1250, 1370, 1450, 1730, 2740—3020. ^{1}H NMR, δ: 0.91 (d, 3 H, MeC(8), J = 6.7 Hz); 1.10—1.70 (m, 5 H, $H_2C(7)$, HC(8), $H_2C(9)$);

1.68 (br.s, 3 H, MeC(4)); 2.00 (m, 2 H, $H_2C(6)$); 2.03 (s, 3 H, MeCO); 2.27—2.55 (m, 4 H, $H_2C(2)$, $H_2C(3)$); 4.08 (br.t, 2 H, CH_2O, J = 7.2 Hz); 5.15 (br.t, 1 H, HC=, J = 7.3 Hz); 9.78 (t, 1 H, HCO, J = 1.6 Hz). ^{13}C NMR, δ: 19.3, 20.9, 23.0, 24.2, 25.1, 29.5, 35.3, 37.0, 42.2, 62.8, 126.6, 132.8, 171.1, 202.1. Found (%): C, 69.68; H, 10.03. $C_{14}H_{24}O_3$. Calculated (%): C, 69.96; H, 10.07.

Aldehydes 8b. A solution of 0.2 g (0.88 mmol) of $HIO_4 \cdot 2H_2O$ in 2 mL of THF was added in 5 min to a solution of 0.56 g (~0.5 mmol) of the mixture of epoxides **7b** in 6 mL of Et_2O with stirring at 20 °C (Ar). The reaction mixture was kept for 20 min at 20 °C, diluted with 10 mL of Et_2O, washed with water and brine, dried with Na_2SO_4, and concentrated *in vacuo*. The residue (0.55 g) was chromatographed on 30 g of SiO_2. Elution at 0 °C with a mixture of hexane—Et_2O (4 : 1) yielded 457 mg (~85%) of **8b** as a colorless oil, R_f 0.46 (hexane—Et_2O, 9 : 1). IR ($CHCl_3$), ν/cm^{-1}: 845, 895, 975, 1040, 1060, 1080, 1135, 1215, 1260, 1370, 1380, 1450, 1660, 1725, 2730, 2860, 2930, 2960, 3030. ^1H NMR, δ: 0.90 (d, MeC(3), J = 6.7 Hz); 1.10—1.70 (m, HC(2), HC(3), HC(4)); 1.60 (br.s, *cis*-MeC=C); 1.68 (br.s, *trans*-MeC=C); 1.90—2.20 (m, HC_2C=, MeCO); 2.25—2.53 (m, $C\underline{H}_2C\underline{H}_2CHO$); 4.13 (br.t, H_2CO, J = 7.1 Hz); 5.14 (m, HC=); 9.77 (m, HCO). ^{13}C NMR, δ: 19.7, 22.1, 22.2, 23.8, 24.5, 25.1, 26.3, 27.0, 28.2, 29.0, 29.5, 32.1, 34.7, 37.0, 37.9, 39.5, 40.0, 63.0, 123.4, 126.4, 135.1, 171.0, 201.0.

3,7-Dimethyl-10-(2-pyridyl)aminodec-6*Z*-en-1-yl acetate (9a). Sodium triacetoxyborohydride (283 mg, 1.32 mmol) was added in one portion to a solution of 0.21 g (0.88 mmol) of aldehyde **8a**, 83 mg (0.88 mmol) of 2-aminopyridine, and 106 mg (1.76 mmol) of AcOH in 3 mL of $(CH_2Cl)_2$ with stirring at 20 °C (Ar). The reaction mixture was kept for 1 h at 20 °C, diluted with 20 mL of Et_2O, washed with water and brine, dried with Na_2SO_4, and concentrated *in vacuo*. The residue (0.3 g) was chromatographed on 20 g of SiO_2. Elution with a mixture of hexane—Et_2O (1 : 4) saturated with aqueous NH_3 yielded 0.2 g (72%) of amine **9a** as a colorless oil, R_f 0.24 (hexane—Et_2O, 1 : 1, saturated with aqueous NH_3). IR ($CHCl_3$), ν/cm^{-1}: 840, 910, 980, 1050, 1150, 1260, 1370, 1450, 1510, 1570, 1605, 1730, 2740—3080, 3430 and 3660 (NH). ^1H NMR, δ: 0.89 (d, 3 H, MeC(3), J = 6.3 Hz); 1.08—1.75 (m, 7 H, $H_2C(2)$, HC(3), $H_2C(4)$, $H_2C(9)$); 1.70 (br.s, 3 H, MeC(7)); 1.91—2.19 (m, 4 H, CH_2C=C); 2.06 (s, 3 H, MeCO); 3.25 (m, 2 H, H_2CN); 4.07 (br.t, 2 H, $H_2C(1)$, J = 7.2 Hz); 4.65 (br.s, 1 H, HN); 5.14 (br.t, 1 H, HC=, J = 7.5 Hz); 6.36 (d, 1 H, HC(3′), J = 8.8 Hz); 6.55 and 7.41 (both m, 2 H, HC(4′), HC(5′)); 8.06 (br.d, 1 H, HC(6′), J = 5.6 Hz). MS, m/z (I_{rel} (%)): 318 $[M]^+$ (18), 260 (16), 204 (54), 190 (100), 176 (9), 161 (12), 149 (7), 134 (9), 121 (40), 119 (30), 108 (58), 107 (84), 95 (42), 81 (19), 79 (12), 67 (16). Found (%): C, 71.75; H, 9.43; N, 8.64. $C_{17}H_{30}N_2O_2$. Calculated (%): C, 71.66; H, 9.50; N, 8.80.

Acetoxy amines 9b. Sodium triacetoxyborohydride (128 mg, 0.6 mmol) was added in one portion to a solution of 436 mg (~0.4 mmol) of the mixture of aldehydes **8b**, 56 mg (0.6 mmol) of 2-aminopyridine, and 48 mg (0.8 mmol) of AcOH in 2 mL of $(CH_2Cl)_2$ with stirring at 20 °C (Ar). The reaction mixture was kept for 7 h at 20 °C and treated as described for acetoxy amine **9a**. The resulting product (0.49 g) was chromatographed on 30 g of SiO_2. Elution with a mixture of hexane—Et_2O (3 : 2) saturated with aqueous NH_3 yielded 327 mg (70%) of a mixture of aminoacetates **9b** as a colorless oil, R_f 0.31 (hexane—Et_2O, 3 : 2, saturated with aqueous NH_3). IR ($CHCl_3$), ν/cm^{-1}: 850, 980, 1040, 1090, 1160, 1250, 1370, 1450, 1510, 1580, 1600, 1660, 1730, 2740—3080, 3440 and 3660 (NH). ^1H NMR, δ: 0.92 (d, MeC(3), J = 6.2 Hz); 1.08—1.75 (m, HC(2), HC(3), HC(4), $NCH_2C\underline{H}_2$); 1.62 (br.s, *cis*-MeC=C); 1.70 (br.s, *trans*-MeC=C); 1.80—2.30 (m, H_2CC=, MeCO); 3.23 (m, H_2CN); 4.11 (m, H_2CO); 4.83 (m, HN); 5.15 (m, HC=); 6.37 (d, HC(3′), J = 8.9 Hz); 6.56 and 7.43 (both m, HC(4′), HC(5′)); 8.06 (br.d, HC(6′), J = 5.5 Hz).

3,7-Dimethyl-10-[(2-pyridyl)amino]dec-6*Z*-en-1-ol (10a). Potassium carbonate (138 mg, 1 mmol) was added in one portion to a solution of 0.16 g (0.5 mmol) of acetate **9a** in 4 mL of MeOH with stirring at 20 °C (Ar). The reaction mixture was kept for 40 min at 20 °C, diluted with 15 mL of Et_2O, washed with water and brine, dried with Na_2SO_4, and concentrated *in vacuo*. The residue (0.16 g) was chromatographed on 10 g of SiO_2. Elution with Et_2O saturated with aqueous NH_3 yielded 0.2 g (72%) of amine **9a** as a colorless oil, R_f 0.45 (hexane—Et_2O, 1 : 4, saturated with aqueous NH_3). IR ($CHCl_3$), ν/cm^{-1}: 980, 1050, 1160, 1280, 1330, 1370, 1460, 1510, 1570, 1610, 2740—3040, 3430 and 3660 (OH, NH). ^1H NMR, δ: 0.88 (d, 3 H, MeC(3), J = 6.4 Hz); 1.09—1.80 (m, 7 H, $H_2C(2)$, HC(3), $H_2C(4)$, $H_2C(9)$); 1.68 (br.s, 3 H, MeC(7)); 1.91—2.19 (m, 4 H, 2 CH_2C=C); 3.22 (m, 2 H, H_2CN); 3.64 (m, 2 H, $H_2C(1)$); 4.74 (br.s, 1 H, HN); 5.16 (br.t, 1 H, HC=, J = 7.5 Hz); 6.35 (d, 1 H, HC(3′), J = 8.6 Hz); 6.54 and 7.40 (both m, 2 H, HC(4′), HC(5′)); 8.04 (br.d, 1 H, HC(6′), J = 5.8 Hz). ^{13}C NMR, δ: 19.6, 23.2, 25.2, 27.6, 28.9, 29.2, 37.2, 39.6, 41.9, 60.6, 106.2, 112.5, 126.1, 134.0, 137.6, 147.8, 158.8. MS, m/z (I_{rel} (%)): 276 $[M]^+$ (16), 203 (18), 189 (16), 133 (8), 121 (36), 119 (32), 108 (81), 107 (100), 96 (58), 95 (69), 81 (18), 78 (24), 70 (16), 68 (24), 55 (17). Found (%): C, 73.79; H, 10.16. $C_{17}H_{28}N_2O$. Calculated (%): C, 73.87; H, 10.21.

Amino alcohols 10b. Potassium carbonate (100 mg, 0.72 mmol) was added in one portion to a solution of 304 mg (0.26 mmol) of the mixture of acetates **9b** in 4 mL of MeOH with stirring at 20 °C (Ar). The reaction mixture was kept for 2 h and treated as described for **10a**. The resulting product (0.3 g) was chromatographed on 15 g of SiO_2. Elution with a mixture of hexane—Et_2O (3 : 2) saturated with aqueous NH_3 yielded 281 mg (96%) of amines **9b** as a colorless oil, R_f 0.30 (hexane—Et_2O, 7 : 3, saturated with aqueous NH_3). IR ($CHCl_3$), ν/cm^{-1}: 850, 920, 980, 1070, 1130, 1160, 1240, 1330, 1380, 1450, 1510, 1530, 1600, 1660, 2740—3080, 3360, 3420, 3500, 3620. ^1H NMR, δ: 0.91 (d, MeC(3), J = 6.3 Hz); 1.08—1.68 (m, HC(2), HC(3), HC(4), $NCH_2C\underline{H}_2$); 1.59 (br.s, *cis*-MeC=C); 1.68 (br.s, *trans*-MeC=C); 1.80—2.30 (m, H_2CC=); 3.22 (m, H_2CN); 3.68 (m, H_2CO); 4.60 (m, HN); 5.14 (m, HC=); 6.37 (d, HC(3′), J = 9.2 Hz); 6.55 and 7.42 (both m, HC(4′), HC(5′)); 8.06 (br.d, HC(6′), J = 5.8 Hz).

3,7-Dimethyl-10-[(2-pyridyl)amino]dec-6*Z*-en-1-yl phosphate (2a). Trichloroacetonitrile (36 mg, 25 μL, 250 μmol) was added to a solution of 35.3 mg (128 μmol) of amino alcohol **10a** and 81.1 mg (227 μmol) of $(Bu_4N)H_2PO_4$ in 1 mL of CH_2Cl_2. After 48 h at 20 °C the solvent was evaporated, the residue was dissolved in 3 mL of the upper (organic) phase of an equilibrium Bu^nOH—water mixture, and the solution was washed with the lower phase of the same mixture (4×1 mL). Methanol (3 mL), the concentrated aqueous solution of NH_3 (50 μL), and Dowex 50Wx8 (NH_4^+) cation-exchange resin (1 mL) were added, and the mixture was stirred for 3 h. The cationite was filtered off and washed with 10 mL of MeOH. The combined filtrate was concentrated, and the residue was dissolved in 50 mL of MeOH. The solution was applied on a DEAE Cellulose DE-52 (AcO^-) (1.2 × 13 cm) column equilibrated with MeOH. The column was washed with MeOH (50 mL), and phosphates were eluted with 150 mL of 40 m*M* solution of $AcONH_4$ in MeOH. Separation was monitored by TLC in $CHCl_3$—MeOH—H_2O (60 : 25 : 4). The eluate was concentrated to ~3 mL, and 15 mL of toluene was added. The mixture

was concentrated to ~8 mL, 30 mL of toluene was added, and the mixture was kept for 16 h at 0 °C. The toluene solution was decanted, and the solvent was evaporated *in vacuo* to dryness. The residue was dissolved in 0.6 mL of a mixture of $CHCl_3$—MeOH (7 : 1) and chromatographed on a SiO_2 (1.1 × 6 cm) column. Elution was performed consecutively with $CHCl_3$—MeOH 7 : 1 and $CHCl_3$—MeOH—H_2O—AcOH 70 : 10 : 0.05 : 0.05 and 50 : 10 : 0.5 : 0.5 mixtures. Fractions containing phosphate **2a** with R_f 0.36 in 60 : 25 : 4 $CHCl_3$—MeOH—H_2O (the by-products had R_f 0.50 and 0.11) were combined and concentrated *in vacuo*. The residue was four times coevaporated with toluene (4×15 mL) to dryness and then dissolved in 10 mL of MeOH. The solution was filtered and concentrated *in vacuo*. The residue was dissolved in 5 mL of a mixture of heptane—Pr^{i}OH (4 : 1). Colorimetric determination showed the solution to contain 63.7 μmol of phosphate, which corresponds to a 50% yield of **2a**. UV (heptane—Pr^{i}OH, 4 : 1), λ_{max}/nm (ε): 246 (11400), 307 (4030). ^{1}H NMR, δ: 0.83 (d, 3 H, MeC(3), J = 7.0 Hz), 1.10—1.80 (m, 7 H, H_2C(2), HC(3), H_2C(4), H_2C(9)); 1.68 (br.s, 3 H, MeC(7)); 1.92, 2.18 (both m, each 2 H, H_2C(5) and H_2C(9)); 3.25 (m, 2 H, H_2CN); 3.93 (m, 2 H, H_2C(1)); 4.74 (br.s, 1 H, HN); 5.12 (br.t, 1 H, HC(6), J = 7.5 Hz); 6.60 (br.s, 1 H, HC(3′)); 6.70 (br.d, 1 H, HC(4′), J = 7.5 Hz); 7.64 (br.t, 1 H, HC(5′), J = 7.5 Hz); 7.86 (br.s, 1 H, HC(6′)). ^{13}C NMR, δ: 20.6 (MeC(3)), 23.3 (MeC(7)), 25.5 (C(5)), 26.8 (C(9)), 29.0, 29.7 (C(3), C(8)), 36.7 (C(4)), 37.1 (br, C(2)), 42.3 (C(10)), 63.7 (br, C(1)), 108.9 (C(3′)), 110.8 (C(5′)), 126.5 (C(6)), 133.7 (C(7)), 139.2 (C(4′)), 142.0 (C(6′)), 154.3 (C(2′)). ^{31}P NMR, δ: 3.06. MS (ESI, from a solution in MeCN—H_2O (1 : 1) containing 0.1% HCOOH, registration of negative ions), m/z: 355; calculated for [M (acid) − H]$^-$ ($C_{17}H_{28}N_2O_4P$): 355.

ω-[(2-Pyridyl)amino]-ω-trinordolichyl phosphates 2b. Trichloroacetonitrile (16.6 mg, 11.5 μL, 115 μmol) was added to a solution of 48.2 mg (~41 μmol) of amino alcohols **10b** and 37.4 mg (105 μmol) of $(Bu_4N)H_2PO_4$ in 1 mL of CH_2Cl_2. After 18 h at 20 °C, the solvent was evaporated, the residue was distributed between the phases of an equilibrium Bu^{n}OH—water mixture, and the solution was treated with the cation-exchange resin as described in the previous experiment. The cationite was filtered off and washed with 15 mL of a toluene—MeOH (2 : 1) mixture, and the combined filtrate was concentrated *in vacuo*. The residue was treated with a mixture of 6 mL of octane and 12 mL of MeOH. After phase separation, the octane layer was washed with 3 mL of MeOH. The combined methanol solution was extracted with octane (3×2 mL), and the solvent was evaporated *in vacuo*. Octane (5 mL) was added to the residue, and after 18 h at 0 °C the solvent was decanted. The residue was dried *in vacuo* and dissolved in 5 mL of a mixture of heptane—Pr^{i}OH (4 : 1). Colorimetric determination showed the solution to contain 31.8 μmol of phosphate, which corresponds to an ~82% yield of **2b**. UV (heptane—Pr^{i}OH, 4 : 1), λ_{max}/nm (ε): 246 (10600), 315 (3500). ^{1}H NMR, δ: 0.87 (d, MeC(3), J = 7.0 Hz); 1.00—1.80 (m, H_2C(2), HC(3), H_2C(4), C$\underline{H}_2$CH$_2$N); 1.60 (m, *cis*-Me); 1.68 (m, *trans*-Me); 2.04 (m, H_2CC=C); 3.20 (m, H_2CN); 3.91 (m, H_2C(1)); 5.12 (m, HC=); 6.41 (d, HC(3′)); 6.53 (t, HC(4′), J = 7.5 Hz); 7.46 (br.t, HC(5′), J = 7.5 Hz); 7.98 (d, HC(6′), J = 3.5 Hz). ^{13}C NMR, δ: 16.0 (*cis*-Me), 19.7 (MeC(3)), 23.4, 24.1 (*trans*-Me), 25.3, 26.4 (C$\underline{H}_2$CH=C), 26.7 (C$\underline{H}_2$CH$_2$NH), 27.3 (C$\underline{H}_2$CH$_2$CH$_2$NH), 29.5 (C(3)), 32.2 (CH$_2$C(Me)=C of (*Z*)-units), 36.9 (C(4)), 37.9 (br.s, C(2)), 39.7 (CH$_2$C(Me)=C of (*E*)-units), 41.9 (CH$_2$N), 63.4 (br.s, C(1)), 106.8 (C(3′)), 111.9 (C(5′)), 124.2, 125.0, 125.7 (H$\underline{C}$=C), 134.6, 135.1, 135.5 (Me$\underline{C}$=C), 138.7 (C(4′)), 145.0 (C(6′)), 163.6 (C(2′)). ^{31}P NMR, δ: 2.94. MS (ESI, from the solution in Pr^{i}OH containing 10 mmol of $AcONH_4$, registration of negative ions), the experimental m/z values of the peak of the isotope cluster with the least mass, its relative intensity (%), the empirical formula for the [M_{acid} − H]$^-$ ion, the corresponding m/z values of the monoisotope ion, and the n value in the molecule of **2b** are given: 1308 (8), $C_{87}H_{140}N_2O_4P$, 1308, 13; 1240 (45), $C_{82}H_{132}N_2O_4P$, 1240, 12; 1172 (100), $C_{77}H_{124}N_2O_4P$, 1172, 11; 1104 (60), $C_{72}H_{116}N_2O_4P$, 1104, 10; 1036 (15), $C_{67}H_{108}N_2O_4P$, 1036, 9; 968 (8), $C_{62}H_{100}N_2O_4P$, 968, 8.

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