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Dolichyl phosphate derivatives with a fluorescent label at an internal isoprene unit

Natalia Ya. Grigorieva,*[a] Olga A. Pinsker,[a] Sergei D. Maltsev,[a] Leonid L. Danilov,[a] Vladimir N. Shibaev[a] and Mark J. Jedrzejas[b]

[a] *N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 117913 Moscow, Russian Federation. Fax: + 7 095 135 5328; e-mail: ves@cacr.ioc.ac.ru*

[b] *Department of Microbiology, The University of Alabama at Birmingham, Birmingham, Alabama 35294, USA*

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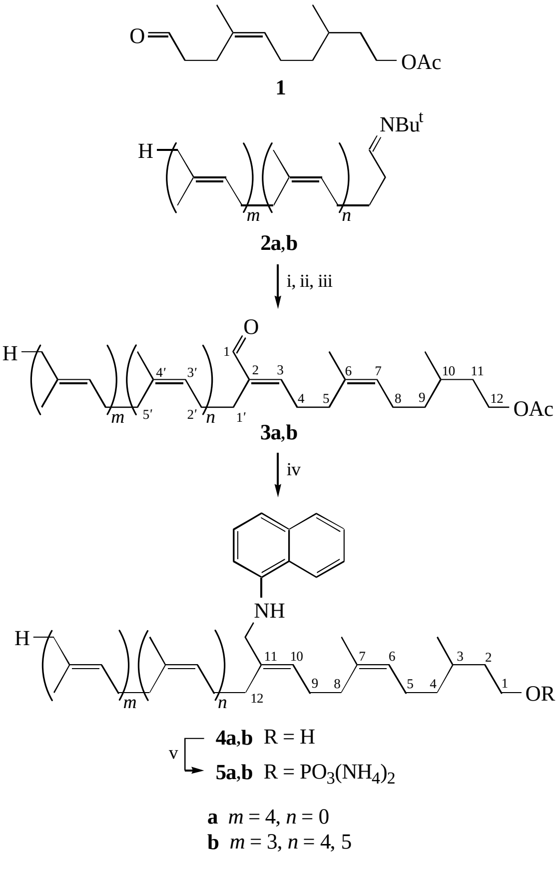
A general approach based on directed aldol condensation followed by reductive amination with fluorescent amines and phosphorylation was developed and illustrated by the synthesis of two dolichyl phosphate derivatives with the 1-aminonaphthalene fluorophore at the γ-isoprene unit of the chain.

The carbohydrate chains of glycoproteins are involved in many cellular activities[1] and are often structurally changed in diseases.[2] The biosynthesis of Asn-linked oligosaccharides on eukaryotic glycoproteins, the O-glycosylation of fungal glycoproteins and the formation of glycosylphosphatidylinositol anchors present in numerous membrane proteins require the participation of dolichyl phosphate (Dol-P), a derivative of a linear long-chain polyisoprene alcohol, and its glycosylated forms as key intermediates.[3] The fluorescence methodology seems to be promising for studies of molecular mechanisms of the interaction of Dol-P and its glycosylated derivatives with biological membrane components. The synthesis of Dol-P derivatives with fluorescent labels at the ω-end of the chain was reported recently.[4] Here, we describe a procedure for the incorporation of fluorophores in an internal isoprene unit of Dol-P.

The methodology is based on the preferential formation of thermodynamically more stable (*E*)-isomers of α,β-disubstituted acroleins in directed aldol condensation, which was found previously[5] and successfully used in the syntheses of dolichols, polyprenols and their analogues.[6,7] The key step in the synthetic strategy was the construction of dolichyl-like (*E*)-acroleins through the cross-condensation of two appropriate aldehydes, one of which was used as an aldimine. The resulting (*E*)-acrolein was subjected to reductive amination with a fluorescent amine, and the amino alcohol formed was phosphorylated.



Scheme 1 *Reagents and conditions*: i, LDA, Et_2O–hexane (1:1), –10 °C → → 0 °C, 30 min; ii, **1**, Et_2O, –70 °C (2 h) → –20 °C (2.5 h); iii, 3.5% aq. HCl, 20 °C, 3 h, then Ac_2O/Py–DMAP, 20 °C, 4 h; iv, 1-aminonaphthalene, $NaBH_3CN$/AcOH–MeOH (for **4a**) or MeOH–Et_2O (for **4b**), 20 °C, 92 h, then HCl to pH 2, 20 °C, 10 min, then NaOH/MeOH, 20 °C, 15 min; v, $Bu_4N{\cdot}H_2PO_4$, CCl_3CN/CH_2Cl_2, 20 °C, 48 h, chromatography on DE-52 (AcO^-).

This approach is illustrated by Scheme 1 where the synthesis of two Dol-P analogues (**5a**,**b**) with a 1-aminonaphthalene label at the γ-isoprene unit is shown. The aldehydoacetate **1**[4] was used as an aldehyde component to build the key intermediates, (*E*)-acroleins **3a**,**b**. Condensation of **1** with aldimine **2a**[8] deprotonated by treatment with LDA at –70 °C leads to acetoxyacrolein **3a** (40%, after flash chromatography on SiO_2) which contains ~2% of the (2*Z*)-isomer (^{1}H NMR data). In a similar manner, (*E*)-acetoxyacrolein **3b** [~3% of the (2*Z*)-isomer, according to ^{1}H NMR data] was obtained in ~21% yield through the condensation of **1** with aldimine **2b** prepared in five synthetic steps (*cf.* ref. 9) from a mixture of polyprenols WT_2C_n-OH isolated from birch tree[10] with C_{35}- and C_{40}-prenols (n = 4, 5) as main components.[†]

The reductive amination of **3a**,**b** with 1-aminonaphthalene and $NaBH_3CN$ (*cf.* ref. 11) led to amino alcohols **4a**,**b** in 37 and ~20% yields (after flash chromatography on SiO_2), respectively.[‡] To prepare fluorescent Dol-P derivatives, **4a**,**b** were subjected to phosphorylation with $Bu_4N{\cdot}H_2PO_4/CCl_3CN$[12] under conditions essentially similar to those described for the synthesis of ω-labeled analogues.[4] The resulting phosphates **5a**,**b**[§] were isolated as ammonium salts after purification by ion-exchange chromatography (DE-52, AcO^-) in 18 and 28% yields, respectively. As expected, they exhibited intense fluorescence with an excita-

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[†] **3a**: ^{1}H NMR ($CDCl_3$) δ: 0.92 (d, 3H, MeC-10, *J* 6.5 Hz), 1.05–1.50 (m, 5H, H_2C-9,11, HC-10), 1.62 (s, 12H, *cis*-Me), 1.71 (s, 6H, *trans*-MeC-6,16′), 2.0 (m, 16H, $CH_2C{=}C$), 2.01 (s, 3H, MeCO), 2.25 (m, 4H, H_2C-5,1′), 2.45 (td, 2H, H_2C-4, $J_1 = J_2$ = 7 Hz), 4.09 (td, 2H, H_2C-12, J_1 7 Hz, J_2 2.5 Hz), 5.12 (m, 5H, HC=C), 6.43 (t, 1H, HC-3, *J* 7 Hz), 9.34 (s, 1H, HC-1). ^{13}C NMR, δ: 15.9 (*cis*-Me), 17.5 (*cis*-MeC-16′), 19.3 (*Me*C-10), 20.9 (*Me*CO), 23.1 (*Me*C-6), 24.2 (C-1′), 25.5 (*trans-Me*C-16′), 25.2, 25.6, 26.0, 26.7, 27.0, 27.3 (*C*H_2CH=C), 29.5 (C-10), 30.6 (C-5), 35.4 (C-9), 37.1 (C-11), 39.6 [H_2*C*C(Me)=C of (*E*)-units], 62.8 (C-12), 123.2, 124.2, 124.35, 126.6 (H*C*=C), 131.1, (C-16′), 133.2, 134.8, 134.9, 136.0 (Me*C*=C), 143.3 (C-2), 154.3 (C-3), 171.0 (*C*OMe), 194.9 (C-1).

3b: ^{1}H NMR ($CDCl_3$) δ: 0.93 (d, 3H, MeC-10, *J* 6.5 Hz), 1.10–1. 60 (m, 5H, H_2C-9,11, HC-10), 1.59 (s, 9H, *cis*-Me), 1.68 (s, 19.5H, *trans*-MeC), 2.0 (m, 33H, $CH_2C{=}C$, MeCO), 2.20 (m, 4H, H_2C-5,1′), 2.45 (td, 2H, H_2C-4, $J_1 = J_2$ = 7 Hz), 4.11 (td, 2H, H_2C-12, J_1 7 Hz, J_2 2.5 Hz), 5.15 (m, 8.5H, HC=C), 6.45 (t, 1H, HC-3, *J* 7 Hz), 9.35 [s, 0.97H, HC-1 of (*E*)-isomer], 10.10 [s, 0.03H, HC-1 of (*Z*)-isomer]. ^{13}C NMR, δ: 15.9 (*cis*-Me), 17.6 (*cis*-Me of ω-terminal unit), 19.3 (*Me*C-10), 20.9 (*Me*CO), 23.1 (*Me*C-6), 23.4 (*trans*-Me), 24.4 (C-1′), 25.6 (*trans*-Me of ω-terminal unit), 25.2, 26.3, 26.6, 26.7, 26.8, 27.0, 27.3 (*C*H_2CH=C), 29.4 (C-10), 30.5, 31.9, 32.1 [H_2*C*C(Me)=C of (*Z*)-units], 35.3 (C-9), 37.1 (C-11), 39.7 [H_2*C*C(Me)=C of (*E*)-units], 62.8 (C-12), 124.1, 124.15, 124.3, 124.4, 124.95, 126.6 (H*C*=C), 131.15, (Me*C*=C of ω-terminal unit), 133.2, 134.8, 135.1, 135.3, 135.95 (Me*C*=C), 143.6 (C-2), 154.45 (C-3), 171.0 (*C*OMe), 194.9 (C-1).

tion/emission maximum at 340/410 nm (in *n*-heptane–2-propanol, 4:1) and are quite similar in this respect to the derivatives of Dol-P with a 1-naphthylamine residue at the ω-end of the chain[4] (see ref. 13 for the excitation and emission spectra).

In preliminary biochemical tests, phosphate **5a** was found to serve as a substrate for recombinant yeast Dol-P-mannose synthase (EC 2.4.1.83) isolated from *Escherichia coli*.[14]

It is likely that the described approach to the incorporation of a fluorescent label at an internal isoprene unit of Dol-P can be employed for the preparation of a wide range of related derivatives starting from the corresponding aldehydes and aldimines available from different terpenols.[7] The use of these Dol-P analogues is of paramount importance for studies of the interaction of Dol-P with biological membrane components.

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[‡] **4a**: ^{1}H NMR ($CDCl_3$) δ: 0.91 (d, 3H, MeC-3, J 6.6 Hz), 1.10–1.60 (m, 5H, H_2C-2,4, HC-3), 1.65 (s, 12H, *cis*-Me), 1.72 (s, 6H, *trans*-Me), 2.05 (m, 18H, $CH_2C{=}C$), 2.27 (m, 4H, H_2C-9,12), 3.20 (s, 1H, NH), 3.63 (dt, 2H, H_2C-1, J_1 1.5 Hz, J_2 6.6 Hz), 3.87 (s, 2H, CH_2N), 5.14 (m, 5H, HC=C), 5.45 (t, 1H, HC-10, J 6.7 Hz). ^{13}C NMR, δ: 16.0, 16.1 (*cis*-Me), 17.6 (*cis-Me*C-27), 19.5 (*Me*C-3), 23.4 (*Me*C-7), 25.1, 25.3, 26.2, 26.6, 26.7, 27.2 ($\mathit{C}H_2CH{=}C$), 25.7 (*trans-Me*C-27), 29.2, 29.4 (C-3,12), 31.8 (C-8), 37.4 (C-4), 39.7 [$\mathit{C}H_2C(Me){=}C$ of (*E*)-units], 39.8 (C-2), 50.0 (CN), 61.1 (C-1), 123.8, 124.2, 124.4, 125.5, 125.55, 127.1 (H*C*=C), 131.2 (C-27), 134.2, 134.6, 135.0, 135.4, 136.2 (Me*C*=C). Signals for 1-aminonaphthalene fragment are not shown.

4b: ^{1}H NMR ($CDCl_3$) δ: 0.90 (d, 3H, MeC-3, J 6.5 Hz), 0.95–1.55 (m, 5H, H_2C-2,4, HC-3), 1.57 (s, 9H, *cis*-Me), 1.65 (s, 19.5H, *trans*-Me), 2.01 (m, 32H, $CH_2C{=}C$), 2.20 (m, 4H, H_2C-9,12), 3.20 (s, 1H, NH), 3.70 (dt, 2H, H_2C-1, J_1 1.5 Hz, J_2 6.6 Hz), 3.87 (s, 2H, H_2CN), 5.13 (m, 8.5H, HC=C), 5.53 (t, 1H, HC-10, J 6.7 Hz). ^{13}C NMR, δ: 16.0 (*cis*-Me), 17.7 (*cis*-Me of ω-terminal unit), 19.5 (*Me*C-3), 23.4 (*trans*-Me), 25.7 (*trans*-Me of ω-terminal unit), 25.1, 26.2, 26.4, 26.6, 26.7, 27.0 ($\mathit{C}H_2CH{=}C$), 29.1 (C-3), 29.6 (C-12), 31.8, 31.9, 32.2 [$\mathit{C}H_2C(Me){=}C$ of (*Z*)-units], 37.4 (C-4), 39.7 [$\mathit{C}H_2C(Me){=}C$ of (*E*)-units], 39.8 (C-2), 50.0 (CN), 61.0 (C-1), 123.8, 124.1, 124.2, 124.3, 124.7, 124.9, 125.6, 125.7, 127.1, 128.8 (H*C*=C), 131.2 (Me*C*=C of ω-terminal unit), 134.2, 134.6, 135.15, 135.2, 135.3, 135.7, 136.0 (Me*C*=C). Signals for 1-aminonaphthalene fragment are not shown.

[§] **5a**: UV, λ_{max}/nm: 252, 335; ε_{max}: 18000, 5500. ESI–MS, *m*/*z*: 716 [M (free acid) – H]$^-$.

5b: UV, λ_{max}/nm: 252, 335; ε_{max}: 18500, 5000; ^{31}P NMR (CD_3OD–$CDCl_3$, 1:2) δ: 2.25.

^{1}H and ^{13}C NMR spectra of **5a**,**b** are similar to the spectra of **4a**,**b** except for signals of HC-1 (3.90 and 3.85 for **5a** and **5b**, respectively) and C-1 (64.0 and 63.8 for **5a** and **5b**, respectively).