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SYNTHESIS OF [^{32}P] LABELLED 1-O-ALKYL-2-DESOXY-2-AMINO-*SN*-GLYCERO-3-PHOSPHOCHOLINES

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SUMMARY

The syntheses of N-substituted 1-O-alkyl-2-desoxy-2-amino-*sn*-glycero-3-[^{32}P]phosphocholines were performed in four steps starting from [^{32}P] $POCl_3$ and the corresponding 1-O-alkyl-2-amino-propane-3-ols in 5-7 % total yield.

KEY WORDS

[^{32}P]etherphospholipids; 1-O-alkyl-2-desoxy-2-amino-sn-glycero-3-[^{32}P]phosphocholines

INTRODUCTION

Derivatives of 1-O-alkyl-2-desoxy-2-amino-*sn*-glycero-3-phosphocholine are potent inhibitors of phospholipase A_2 [1, 2] and potential ligands of the receptor of the platelet activating factor (PAF). Isotopically labelled, 2-amino-etherphospholipids therefore are of potential value in investigations involving interactions of phospholipids with cellular membranes as well as in metabolic studies.

In this report we describe the preparation of [^{32}P] labelled 2-desoxy-2-amino-*sn*-glycero-etherphospholipids utilizing [^{32}P] $POCl_3$ to introduce the radioactive label and 4-substituted 2-chloro-2-oxo-1,3,2-oxaza-[^{32}P]phospholanes as versatile intermediates.

RESULTS AND DISCUSSION

The synthetic route to labelled 1-O-alkyl-2-desoxy-2-amino-*sn*-glycero-3-phosphocholines is outlined in the scheme. Preparation of [^{32}P] labelled 2-desoxy-2-amino-lysophospholipids was carried out according to the synthetic sequence for unlabelled 2-amino-phospholipids published by us previously [3].

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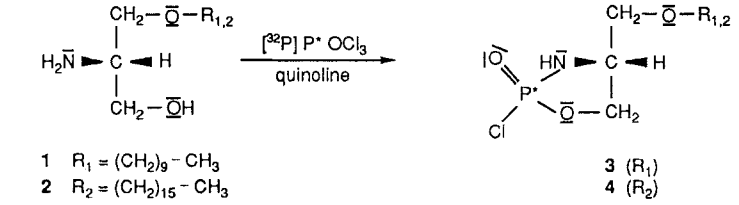

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2 $R_2 = (CH_2)_{15} - CH_3$

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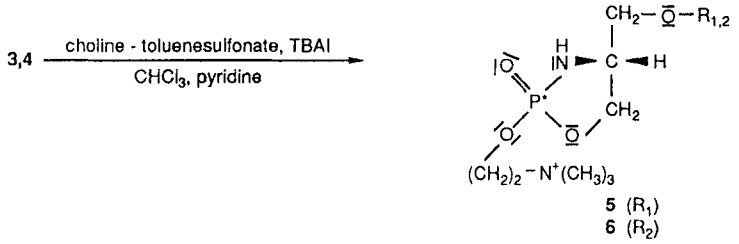
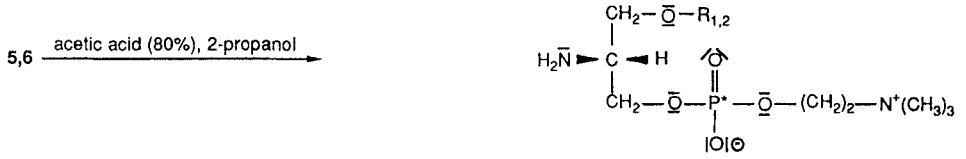
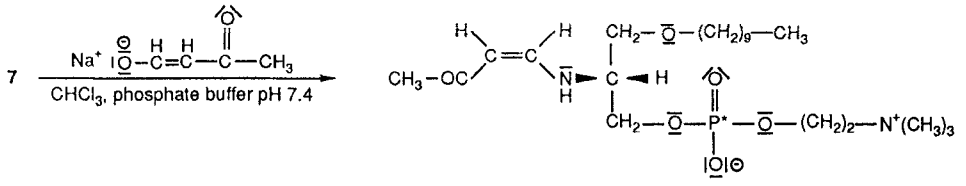
4 (R_2)



5 (R_1)
6 (R_2)



7 (R_1)
8 (R_2)



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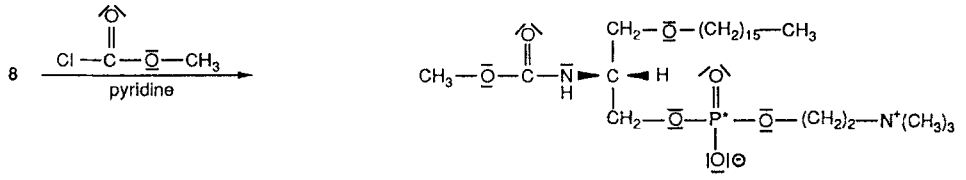

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SCHEME

Equilibration of [^{32}P] phosphoric acid with phosphorus oxychloride yielded isotopically labelled $POCl_3$; reaction with the corresponding 1-O-alkyl-2-amino-propane-3-ol (1, 2) gave the labelled oxazaphospholane intermediates (3, 4). The choline group was introduced by nucleophilic exchange of the chlorine, acid

hydrolysis of (5), (6) opened the ring to give the desired 1-O-alkyl-2-desoxy-2-amino-*sn*-glycero-[^{32}P]-phosphocholines (7) and (8). The total yield of the three consecutive steps was lower starting from the long-chain aminoalcohol (2) (12% vs. 32%) suggesting a decreased reactivity along with increasing carbon chain length. Conversion of the 2-amino-lysophospholipids (7) and (8) with the sodium salt of 1-hydroxy-but-1-en-3-on or with methylchloroformate and purification by chromatography on thin layer plates provided the labelled vinylogous amide (9) and the carbamic-acid-methylester (10) with a specific activity of approximately 720 MBq/mmol and a radiochemical purity of 95-96%. The specific radioactivity of the phospholipids was 0.78 of the theoretical value, a result which can be explained by incomplete exchange with [^{32}P]phosphoric acid .

MATERIALS

Tetrabutyl ammonium iodide was purchased from Fluka (Buchs, Switzerland), phosphorus oxychloride and p-toluenesulfonic acid from Aldrich (Steinheim, Germany). Chloroform and methanol were obtained from J. T. Baker (Deventer, Holland) and distilled from P_2O_5 and from Mg prior to use. Silica gel (grade 60, 70-230 mesh) for column chromatography and 2-propanol was from Machery-Nagel (Düren, Germany). TLC plates (0.5 mm, F 254) were from E. Merck (Darmstadt, Germany) and preeluted with methanol. [^{32}P]phosphoric acid (314-337 TBq/mmol) was from Du Pont de Nemours Deutschland GmbH (Bad Homburg).

METHODS

Thin-layer chromatography (TLC) and column chromatography were performed using a mixture of chloroform/methanol/water (65:45:8, v/v) as mobile phase. Phospholipids were detected with "Phospray" (Supelco, Bad Homburg, Germany). Mass spectra were obtained using a MAT 311 A mass spectrometer (Varian, Bremen, Germany) equipped with a FAB ion gun (Xe, 6 KV, ion current 1 mA) from Ion Tech (Teddington, U. K.) and glycerol as matrix. ^{1}H-NMR-spectra were recorded at 250 MHz (WM-250, Bruker Physik AG, Karlsruhe, Germany); tetramethyl silane was used as internal reference. Spectra were run in acetonitrile-D_3 or in $CDCl_3$/methanol-D_4, 2:1 (v/v). Multiplicities are reported as singlet (s), doublet (d), triplet (t) or multiplet (m).

[^{32}P] phosphorus oxychloride

Labelled phosphorus oxychloride was obtained applying the method of Keenan et al. [4].
[^{32}P] phosphoric acid (370 MBq, 314-337 TBq/mmol) was mixed with freshly distilled phosphorus oxychloride (30 μl, 0.32 mmol) to give a theoretical specific activity of 1.12 GBq/mmol (calculated for 100% conversion to $POCl_3$) and stirred for 24 h at 107°C in a screwed vial.

1-O-decyl-2-desoxy-2-amino-*sn*-glycero-[^{32}P]phosphocholine (7) and 1-O-hexadecyl-2-desoxy-2-amino-*sn*-glycero-3-[^{32}P]phosphocholine (8)

To 12 μl (129 μmol) of [^{32}P] phosphorus oxychloride dissolved in chloroform, a solution of 30 mg (130 μmol) of 1-O-decyl-2-amino-propane-3-ol (1) or 41 mg (130 μmol) 1-O-hexadecyl-2-amino-propane-3-ol (2) and 35 μl (290 μmol) quinoline was added dropwise at 4°C. The mixture was allowed to warm up at room temperature, then stirred at 55°C for 16 h.

The resulting solution of 1-O-decyl-2,3-(2'chloro-2'oxo-1',3',2')-oxaza[^{32}P]phospholane (3) or 1-O-hexadecyl-2,3-(2'chloro-2'oxo-1',3',2')-oxaza[^{32}P]phospholane (4) was cooled to 12°C and 55mg (0.2 mmol) choline tosylate in 0.3 ml pyridine was added. After stirring for 24 h at 55°C, the solvents were removed *in vacuo*, and the residue containing the crude oxazaphospholanes (5) or (6) was redissolved in 1 ml of 2-propanol/acetic acid (80%) 3:2 (v/v). Hydrolysis was carried out by stirring at 50°C for 30 min and at room temperature for additional 2 h. The solvents were removed by distillation *in vacuo* at under 40°C and silica gel chromatography afforded 16.3 mg (41 μmol, 31%) of (7) and 7.2 mg (15 μmol, 12%) of (8); MS (FAB; glycerol; pos. mode): m/z =397 $[M+H]^+$ (7) and m/z =481 $[M+H]^+$ (8).

1-O-decyl-2-desoxy-2-(1'-amino-but-1'-en-3'-on)-*sn*-glycero-3-phosphocholine (9)

To 16.3 mg (41 μmol) of (7), dissolved in 2 ml of a biphasic mixture (1:1, v/v) of chloroform/phosphate buffer (20 mM, pH 7.4) 200 mg (1.85 mmol) of the sodium salt of 1-hydroxy-but-1-en-3-on [5] were added and the solution stirred for 24 h at room temperature. The solvents were removed by distillation *in vacuo* and the residue purified by thin layer chromatography yielding 2.8 mg (6 μmol, 15%) of (9) (cis configuration, specific activity 724 MBq/mmol)

MS (FAB; glycerol; pos. mode): m/z = 465 $[M+H]^+$

^{1}H-NMR ($CDCl_3/D_4$-methanol, 2:1) δ ppm:

6.8 (1H, d, -C<u>H</u>=CH-C=O), 4.95 (1H, d, -CH=C<u>H</u>-C=O), 4.2 (1H, m, *sn*-2-C<u>H</u>), 3.95 (2H, m, C<u>H$_2$</u>-CH$_2$-N$^+$(CH$_3$)$_3$), 3.9-3.8 (2H, t, *sn*-3-C<u>H$_2$</u>), 3.6 (2H, m, -C<u>H$_2$</u>-N$^+$(CH$_3$)$_3$), 3.45 (2H, m, *sn*-1-C<u>H$_2$</u>-), 3.3 (3H, s, O=C-C<u>H$_3$</u>), 3.2 (9H, s, -N$^+$(C<u>H$_3$</u>)$_3$), 1.5 (2H, m, -C<u>H$_2$</u>-(CH$_2$)$_8$-CH$_3$), 1.25 (16H, m, -CH$_2$-(C<u>H$_2$</u>)$_8$-CH$_3$), 0.85 (3H, t, -(CH$_2$)$_9$-C<u>H$_3$</u>).

1-O-hexadecyl-2-desoxy-2-amino-carbamic-acid-methylester-*sn*-glycero-3-phosphocholine (10)

To a solution of 7.2 mg (15 μmol) (8) in chloroform/pyridine (3 ml, 5:1), 10 μl (130 μmol) of methylchloroformate were added dropwise at 0°C; the reaction mixture was allowed to warm up to room temperature and stirred for another 4 h. Evaporation *in vacuo* afforded crude (10) which was subjected to thin layer chromatography to give 4.8 mg (9 μmol, 60%) of (10) (specific activity 715 MBq/mmol)

MS (FAB; glycerol; pos. mode): m/z = 523 $[M+H]^+$.

^{1}H-NMR (CD_3-C$\equiv$N) δ ppm:

4.2 (1H, m, *sn*-2-C<u>H</u>), 3.9 (2H, m, C<u>H$_2$</u>-CH$_2$-N$^+$(CH$_3$)$_3$, 3.9-3.8 (2H, m, *sn*-3-C<u>H$_2$</u>), 3.6 (3H, s, -O-C<u>H$_3$</u>), 3.55 (2H, m, -C<u>H$_2$</u>-N$^+$(CH$_3$)$_3$, 3.45 (2H, m, *sn*-1-C<u>H$_2$</u>-), 3.2 (9H, s, -N$^+$C<u>H$_3$</u>)$_3$, 1.5 (2H, m, -C<u>H$_2$</u>-(CH$_2$)$_{14}$-CH$_3$), 1.25 (28H, m, -CH$_2$-(C<u>H$_2$</u>)$_{14}$-CH$_3$), 0.85 (3H, t, -(CH$_2$)$_{15}$-C<u>H$_3$</u>).

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