Three-Step Synthesis of Platelet-Activating Factor from Chiral Glycidol via Regioselective Monophosphitylation of 1-*O*-Hexadecyl-*sn*-glycerol

Ravi Kumar Erukulla, Hoe-Sup Byun, and Robert Bittman*

Department of Chemistry and Biochemistry, Queens College of The City University of New York, Flushing, New York 11367-1597

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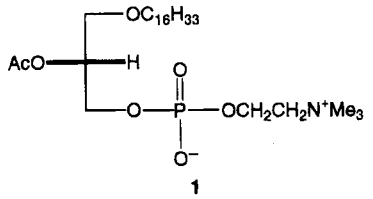
Ether phospholipids, such as 1-*O*-alkyl-2-acyl- and 1-*O*-alkenyl-2-acyl-*sn*-glycero-3-phosphocholines, are widely distributed in mammalian cell membranes.[1] Many ether phospholipids have a broad range of biological activities. For example, platelet-activating factor [1-*O*-hexadecyl- or 1-*O*-octadecyl-2-acetyl-*sn*-glycero-3-phosphocholine, PAF, (*R*)-**1**] causes aggregation of platelets and leukocytes, hypotension, smooth muscle contraction, vasopermeability, and many other physiological responses, including pathological processes such as inflammation, bronchial asthma, nephropathy, and gastric ulceration.[2] Unnatural ether phospholipids have been designed and used as membrane-directed antitumor[3] and antiviral agents,[4] as solid-phase supports for immobilized artificial membranes,[5] and as phospholipase inhibitors.[6] Intense interest in structure–function relationships of PAF analogs and in antagonists of PAF has led to the development of many useful routes to chiral glycerol lipids. A frequently used intermediate for preparing the natural enantiomer of PAF [(*R*)-**1**] is glycerol acetonide, which can be prepared from D-mannitol,[7] L-ascorbic acid,[8] L-serine,[9] D-tartaric acid,[10] and L-glyceric acid[11] via a multistep sequence involving several protection–deprotection reactions to avoid acetyl and/or phosphoryl migration within the glycerol moiety.[12] We have previously reported the use of chiral derivatives such as glycidyl tosylate as starting materials for ether phospholipid targets,[13] via $BF_3{\cdot}OEt_2$-catalyzed nucleophilic ring opening. Although an arenesulfonate is a convenient directing and protecting group, it must be removed after ring opening in order to insert the phosphorus functionality. We have developed a very short synthesis of **1** (Scheme 1) that starts with commercially available underivatized (*S*)-(−)- or (*R*)-(+)-glycidol (**2a,b**) that generates (*R*)- or (*S*)-**1** in only three steps, without use of any glycerol protecting group.



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Results and Discussion

In the first step, epoxide opening of **2** with 1-hexadecanol is carried out in the presence of stoichiometric amounts of DIBAL-H in methylene chloride at rt.[14] Ring opening was enantiospecific and regioselective.[15] In the second step, the phosphocholine moiety was introduced regioselectively[16] into diol **3** at the C-3 hydroxyl group via the following sequence of reactions in one pot:[17] phosphitylation (3.0 equiv of ethylene chlorophosphite at −20 °C for 15–20 min), quenching of excess chlorophosphite with dry methanol, oxidation of the cyclic phosphite

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(12) Previously, PAF (**1**) was prepared in six steps from 1-*O*-hexadecyl-*sn*-glycerol (**3a**) in about 50% overall yield by a sequence of reactions involving several protection and deprotection steps. For reviews of PAF syntheses, see: Mangold, H. K. *Prog. Biochem. Pharmacol.* **1988**, *22*, 1–16. Bittman, R. In *Phospholipids Handbook*; Cevc, G., Ed.; Marcel Dekker: New York, 1993; pp 97–140.

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(14) We have recently found that other long-chain alcohols, including secondary alcohols such as 2-hexadecanol, *dl*-menthol, and β-cholestanol, open **2** in the presence of DIBAL-H (Erukulla, R. K.; Byun, H.-S.; Locke, D. C.; Bittman, R. *J. Chem. Soc., Perkin Trans. 1* **1995**, 2219–2220.

(15) The reaction mixture was analyzed by GC/MS (Hewlett-Packard 5988A GC–quadrupole mass spectrometer equipped with a H-P 1000 data system). Gas chromatography was carried out on a 30 m × 0.25 mm i.d., 0.25 μm DB-5 bonded phase fused silica capillary column (J & N Scientific, Folsom, CA). The retention times of 1-*O*-hexadecyl-*sn*-glycerol (**3a**) and 2-hexadecylglycerol were 23.27 and 23.90 min, respectively (helium flow rate, 1 mL/min).

(16) The reaction product obtained from the phosphocholine insertion sequence was analyzed by HPLC. 2-Lyso-PAF (**5**) and authentic 3-lyso-PAF were analyzed on a normal-phase column (Prodigy, 5 μm, 150 × 4.6 mm) with monitoring at 206 nm. The mobile phase was acetonitrile–methanol–sulfuric acid (100:3:0.05 v/v), with a flow rate of 1 mL/min. The retention times of 2-lyso-PAF (obtained from Sigma Chemical Co.) and 3-lyso-PAF (prepared as described in ref 20) were 11.48 and 8.04 min, respectively. The retention time of product **5** obtained via Scheme 1 had t_R 11.50 min, with < 3-lyso-PAF detected at 8.0 min.

(17) To achieve the desired regioselectivity of monophosphitylation, the choice of temperature (−20 °C) and time (15–20 min) was critical, followed by quenching at −20 °C.

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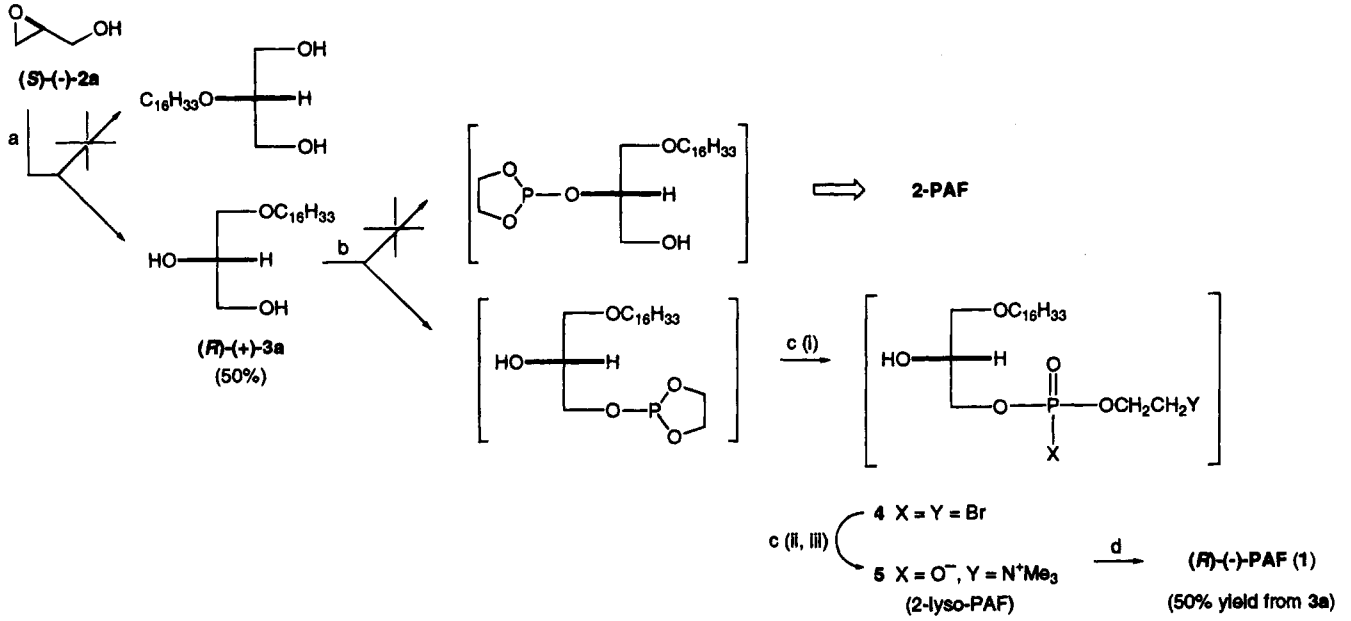
Scheme 1 [a] **Synthesis of 1-*O*-hexadecyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine (1)**



[a] Reagents: (a) DIBAL-H, hexadecyl alcohol, CH_2Cl_2, rt, 72 h; (b) (i) ethylene chlorophosphite, *N,N*-diisopropylethylamine, THF, −20 °C, 15−20 min; (ii) MeOH; (c) (i) Br_2, 1 min; (ii) H_2O, 15 min; (iii) aqueous Me_3N, CH_3CN/*i*-PrOH/$CHCl_3$ (3:3:1.8 v/v), 12 h; (d) Ac_2O, DMAP, $CHCl_3$, rt, 3 h.

and opening with Br_2 at −20 °C to give (2′-bromoethyl)-phosphate ester **4**, hydrolysis of the P−Br bond, and quaternization with aqueous trimethylamine to give 1-*O*-hexadecyl-*sn*-glycero-3-phosphocholine (2-lyso-PAF, **5**). To our knowledge, this is the first example of a regioselective introduction of a phosphocholine moiety at the primary hydroxyl group of a diol such as **3**. Previously, phosphitylation of a primary alcohol in a diol such as **3** was achieved by protecting the secondary hydroxyl group of a diol with a benzyloxymethyl[18] or tetrahydropyranyl group.[19] 2-Lyso-PAF **5** was used in the next step without further purification. The third step was acetylation of **5** (Ac_2O, DMAP) to give **1**, without any accompanying 1-*O*-hexadecyl-3-*O*-acetyl-*sn*-glycero-2-phosphocholine (2-PAF). The absence of 2-PAF was demonstrated by (a) finding (by HPLC)[16] that 2-lyso-PAF **5** was formed with less than 3% of the undesired 3-lyso-PAF (1-*O*-hexadecyl-*sn*-glycero-2-*O*-phosphocholine) and (b) comparing the specific rotation of (*R*)-**1** obtained via Scheme 1 with that of natural **1** and unnatural 2-PAF.[20]

The method outlined here is a highly efficient and convenient strategy to chiral **1** and related 1-ether 2-acyl-*sn*-glycero-3-phosphocholines. Glycidol (**2**) is converted into **1** in only three steps without using any protection−deprotection reactions.

Experimental Section

General Procedures. Chromatographic procedures, optical rotation measurements, drying of solvents, and spectral analyses were carried out as described previously.[13,21]

Chemicals. (*S*)-(−)-Glycidol of 91% ee was obtained from Aldrich Chemical Co. DIBAL-H and 1-hexadecanol were purchased from Aldrich. Ethylene chlorophosphite was purchased from Lancaster.

Evaluation of Enantiomeric Excess. The percent enantiomeric excess was determined by 400-MHz ^1H NMR of the crude bis-(*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid [(*R*)-(−)-MTPA] esters (prepared from **3** according to ref 21). Integration of two AB quartets of the (*R*)-MTPA esters derived from a racemic mixture of **3a** and **3b** on an expanded scale indicated a 1:1 ratio of the areas of the signals at δ 4.75−4.72 and 4.64−4.60. The individual diastereotopic protons of CH_2-OMTPA in each enantiomer showed baseline separation. The lower-field AB quartet at δ 4.73 and 4.62 is assigned to the protons of CH_2OMTPA of the 3-*O*-hexadecyl-*sn*-glycerol (**3b**) bis-MTPA ester and the higher-field AB quartet at δ 4.64 and 4.61 corresponds to the protons of CH_2OMTPA of the 1-*O*-hexadecyl-*sn*-glycerol (**3a**) bis-MTPA ester. Integration of the signal at δ 4.64 and 4.61 vs δ 4.75 and 4.72 indicated an ee of 92%.

1-*O*-Hexadecyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine [PAF, (*R*)-1]. To a solution of 1-hexadecanol (1.82 g, 7.5 mmol) in dichloromethane was added DIBAL-H in toluene (0.93 g, 6.5 mmol) at 0 °C, and the reaction mixture was warmed to rt and stirred for 0.5 h. (*S*)-(−)-Glycidol (**2a**, 0.37 g, 5.0 mmol) was added, and the reaction mixture was stirred at rt. After 70 h, potassium sodium tartrate (2.2 g, 7.5 mmol) was added in a minimum amount of water, and the mixture was stirred for 0.5 h. The product was extracted with ethyl acetate, washed with water, dried over sodium sulfate, and concentrated. The crude product was purified by flash chromatography, giving 0.8 g (51%) of **3a**. To a solution of 1-*O*-hexadecyl-*sn*-glycerol (**3a**) (115 mg, 0.36 mmol) in 10 mL of THF were added *N,N*-diisopropylethylamine (164 mg, 222 μL, 1.27 mmol) and ethylene chlorophosphite (138 mg, 97.5 μL, 1.09 mmol) at −20 °C. The mixture was stirred at −20 °C for 15 min. The reaction mixture was quenched with dry methanol (30 μL, 0.73 mmol) and stirred for 15 min at −20 °C. The oxidation and quaternization was carried

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(20) 2-PAF (1-*O*-hexadecyl-3-*O*-acetyl-*sn*-glycero-2-phosphocholine) was prepared by using the following sequence of reactions: (i) introduction of a hexadecyl group at the primary hydroxyl group of 3-*O*-benzyl-*sn*-glycerol by the reaction of its stannylidine derivative with hexadecyl bromide in the presence of cesium fluoride in DMF[21] and separation of the undesired regioisomer by column chromatography, providing 1-*O*-hexadecyl-2-*O*-hydroxy-3-*O*-benzyl-*sn*-glycerol; (ii) phosphorylation with phosphorus oxychloride, triethylamine, pyridine, and choline tosylate; and (iii) removal of the benzyl group at C-3 by catalytic hydrogenolysis provided 3-lyso-PAF, which was acetylated with acetic anhydride and DMAP in methylene chloride to give 2-PAF [$[\alpha]^{25}_D$ −4.48° (*c* 0.81, $CHCl_3$/CH_3OH, 1:1, v/v); HRMS [FAB, $(MH)^+$] calcd for $C_{26}H_{55}PNO_7$ 524.3716, found 524.2470].

(21) Byun, H.-S.; Erukulla, R. K.; Bittman, R. *J. Org. Chem.* **1994**, *59*, 2630−2633.

out as described previously.[22] The residue of 2-lyso-PAF **5** was lyophilized from benzene and acetylated with acetic anhydride (741 mg, 620 μL, 7.26 mmol) and DMAP (45 mg, 0.36 mmol) in 10 mL of freshly distilled alcohol-free chloroform with stirring for 3 h at rt under a nitrogen atmosphere. The residue was purified by column chromatography on silica (elution first with 25% methanol in chloroform and then with chloroform/methanol/water 65:25:4), giving 94 mg (50%) of **1**, which on lyophilization from benzene gave **1** as a white solid: $[\alpha]^{25}_D$ −3.40° (*c* 1.85, $CHCl_3/CH_3OH$, 1:1, v/v) [lit.[10,13] $[\alpha]^{25}_D$ −3.30° (*c* 0.53, $CHCl_3/CH_3OH$)]; HRMS [FAB, $(MH)^+$] calcd for $C_{26}H_{55}PNO_7$ 524.3716, found 524.3742.

(22) For insertion of the phosphocholine moiety into primary alcohols (1-hexadecanol, erucyl alcohol, and 1-*O*-hexadecyl-2-*O*-methyl-*sn*-glycerol) via phosphitylation, ring opening/oxidation with bromine, and quaternization, see: Erukulla, R. K.; Byun, H.-S.; Bittman, R. *Tetrahedron Lett.* **1994**, *35*, 5783−5784.

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