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A NEW METHOD FOR THE STEREOSPECIFIC SYNTHESIS OF ETHER PHOSPHOLIPIDS. PREPARATION OF THE AMIDE ANALOG OF PLATELET-ACTIVATING FACTOR AND RELATED DERIVATIVES

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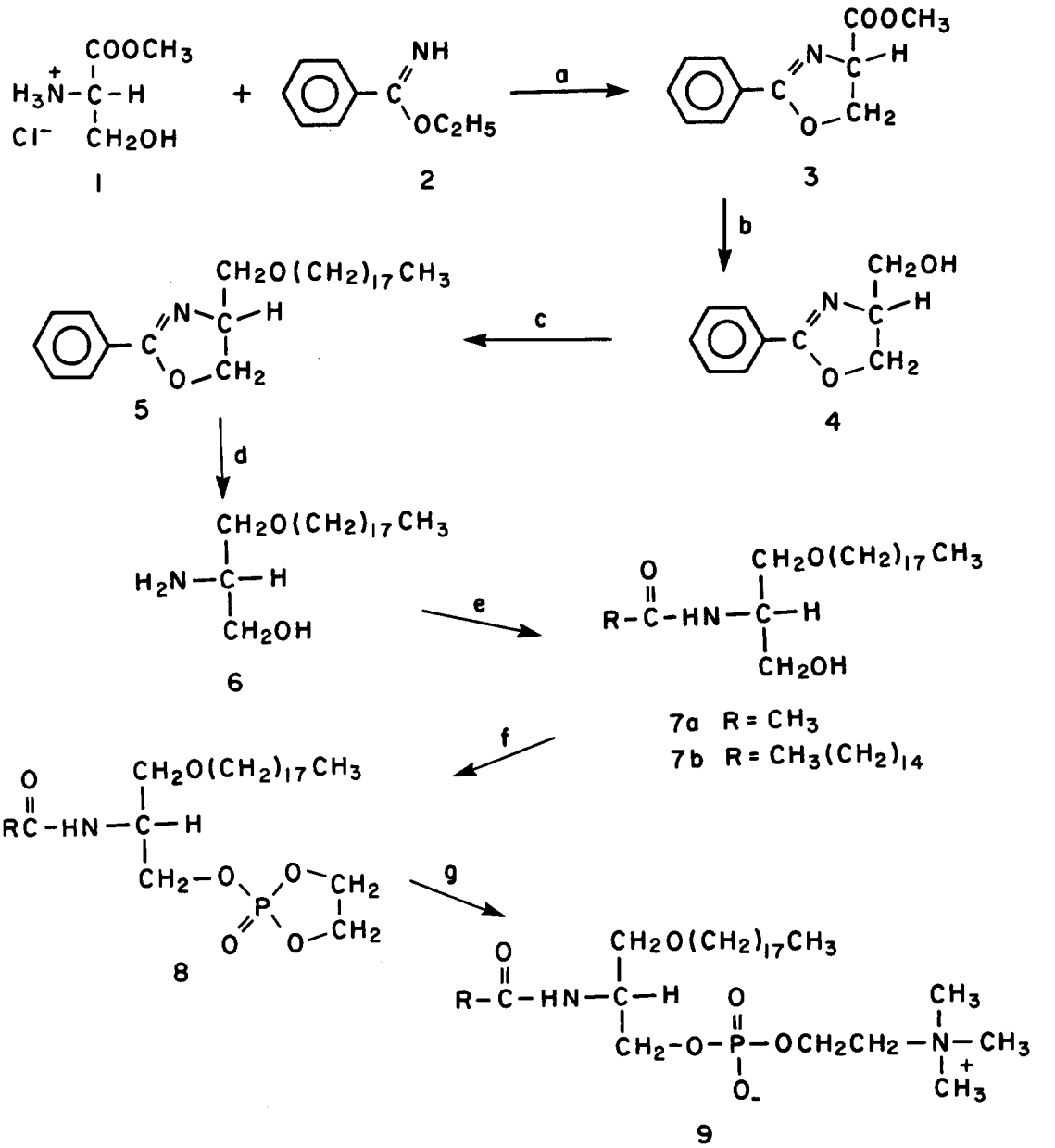
SUMMARY: A novel stereospecific synthesis of biologically active ether-phospholipids is reported.

Ether phospholipids are among the most potent biologically active phospholipid derivatives.[1,2] Naturally occuring as membrane-components, a number of 1-sn-alkoxyglycerophosphorylcholines have been shown to be required for a series of vitally important physiological processes.[2-4] Specifically, ether phospholipids are involved in platelet-activation,[2] they function as vasodilators[3] and as chemotactic agents.[4] Added importance has been attributed to these compounds as a result of recent studies demonstrating their selective tumor-cytotoxicity against a number of different cancer cells.[5] Elucidation of the specific mechanisms involved in the biological functioning of ether phospholipids is greatly hindered at the present by lack of efficient synthetic methods for the preparation of these compounds.[2] Development of stereospecific syntheses of 1-sn-alkoxyphospholipids that will allow systematic variation of the substituents, with particular emphasis on the critically important 2-sn-position[2,4,5] of the molecule, is a prerequisite for the establishment of structure-function correlations regarding the chemical, enzymological and physiological properties of the compounds. Better understanding of the structural requirements for their biological functioning should provide important clues as to how one might optimize the structure of ether phospholipids in order to achieve the desired biological activity and potency. We now describe a highly efficient stereospecific route to 1-sn-O-alkyl-2-sn-N-acylaminodeoxyglycero-3-sn-phosphorylcholines which renders a wide spectrum of substituted ether phospholipids available for structural, chemical and enzymological studies.

Our synthetic strategy is based upon the following elements: 1) the asymmetric α-carbon of the amino acid serine is used as the optically active nucleus for construction of the chiral phospholipid skeleton, 2) the nucleophilic amino-alcohol portion of the molecule is protected *via* formation of an oxazoline ring while introducing the incipient fatty-acid ether function, and 3) the phosphorylcholine residue is developed using the cyclic phosphochloridate (f)[6] followed by ring-opening of the phosphate triester (8) with anhydrous trimethylamine to produce the target molecule with the desired quaternary ammonium function.[7] Significantly, the sequence as outlined (Scheme I) has a great deal of flexibility, providing a convenient general method for the preparation of a wide scope of structurally related ether phospholipids.

L-serine methyl ester (1) was allowed to condense with ethyl benzimidate to form 2-phenyl-4-carbomethoxy-2-oxazoline (3).[8] Reduction of the carbomethoxy group with $LiAlH_4$ in ether[9] yielded the corresponding alcohol (4) (m.p. 99.5° from ether, 89%).[10] Alkylation

Scheme I


1
2
3
4
5
6
7a R = CH3
7b R = CH3(CH2)14
8
9
a) Et2O-water
b) LiAlH4;
c) CH3(CH2)17OSO2CH3, NaH, THF;
d) i. 6N H2SO4; ii. K2CO3;
e) RCOCl, DMAP, chloroform;
f) , DMAP-Et3N, benzene;
g) (CH3)3N, MeCN, 60°

of 2-phenyl-4-hydroxymethyl-2-oxazoline was carried out in tetrahydrofuran in presence of 1 equiv. NaH using stoichiometric amount of octadecyl methanesulfonate[11] to give the ether (5) as a white waxy solid (49 - 50° from methanol, 91%). Deprotection of the amino-alcohol function was accomplished with 6N H_2SO_4, at 100° for 10 hrs. The product was flash-chromatographed on silica gel to yield analytically pure 1-sn-octadecyl-2-sn-deoxyamino-glycerol (m.p. 95° from ether, 93%). N-acylation of the amino-alcohol was achieved using stoichiometric amounts of acetyl chloride / 4-dimethylaminopyridine in chloroform at r.t. for 48 hrs. The resulting acetamide (7a) (m.p. 84° from chloroform, 96%) was phosphorylated with 2-chloro-2-oxo-1,3,2-dioxaphospholane[6] in benzene using 4-dimethylaminopyridine / triethylamine as catalyst to form compound (8). Cleavage of the phosphate triester was carried out with 2 equiv. anhydrous trimethylamine in acetonitrile at 60° (in a pressure bottle) for 24 hrs., yielding the amide analog of platelet-activating-factor[1] (9a) as a white solid (70% from the alcohol (7a)). Chromatography of this product on silica gel ($CHCl_3$-MeOH-aq.NH_3 1:9:1) afforded analytically pure ether phospholipid. Calc. for $C_{28}H_{59}O_2N_6P \cdot H_2O$: C, 59.12; H, 10.81; N, 4.93; P, 5.45; found C, 59.15; H, 10.98; N, 4.92; P, 5.53. $[\alpha]_D^{25}$ = -9.87 (C = 1.15, $CHCl_3$-CH_3OH, 1:4). Similar procedure gave (9b) as a white crystalline solid (65 - 70% from the alcohol (7b)). Calc. for $C_{42}H_{87}N_2O_6P \cdot 2H_2O$: C, 64.41; H, 11.71; N, 3.58; P, 3.96; found C, 64.40; H, 11.55; N, 3.59; P, 4.15; $[\alpha]_D^{25}$ = -8.12 (C = 1.23, $CHCl_3$-CH_3OH, 1:4).

Preliminary results indicate that compound (9a) exhibits potent platelet activation while (9b) is inactive under similar conditions.[12]

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9. It must be pointed out that the reducing agent must be used in stoichiometric amounts (i.e., 1/2 mole $LiAlH_4$ per each mole of ester) in order to prevent reduction of the oxazoline ring.

10. The yields given throughout the synthesis refer to purified (crystallized/chromatographed) and isolated products. All compounds were checked on T.L.C. using precoated Whatman MK6F silica-gel plates. The spots were visualized by charring and the phosphate-containing compounds by molybdic acid spray. All products appeared as single spots and gave confirmatory NMR and i.r. spectra. For all new compounds satisfactory microanalytical data were obtained.

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12. We are grateful to Professor D.J. Hanahan for conducting the biochemical tests with compounds 9a and 9b.

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