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A NEW APPROACH TO THE SYNTHESIS OF ETHER PHOSPHOLIPIDS. PREPARATION OF 1,2-DIALKYLGLYCEROPHOSPHORYLCHOLINES FROM L-GLYCERIC ACID

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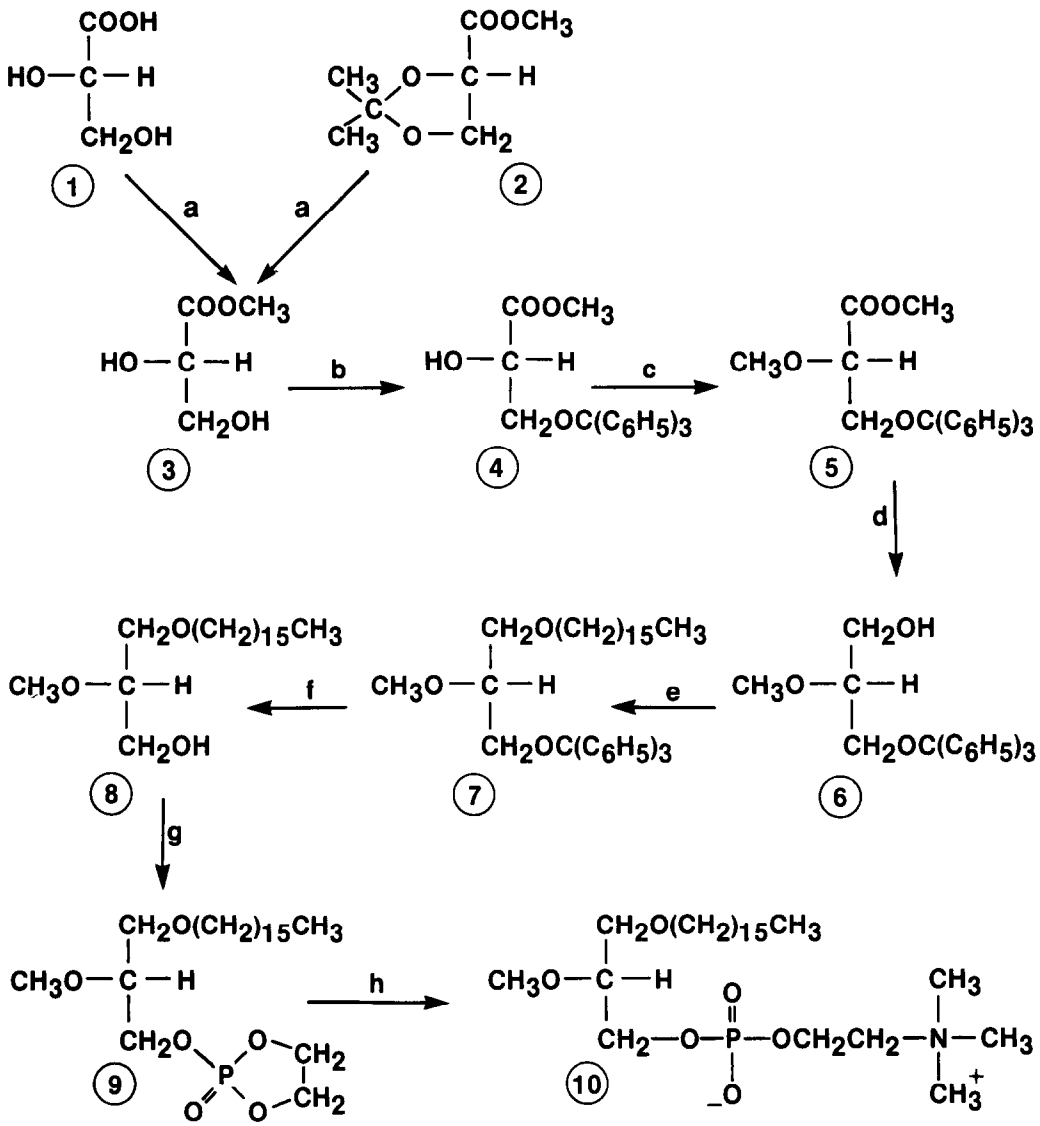
SUMMARY: A novel stereospecific synthesis of antitumor active ether phospholipids is reported.

Development of efficient methods for the synthesis of biologically active phospholipid derivatives is one of the most timely problems in membrane-chemistry and biochemistry today.[1] The compounds are required for structural as well as dynamic studies of biomembranes[2] and membrane-bound enzymes[3] with particular emphasis on establishing structure-function correlations with respect to phospholipid-phospholipid and phospholipid-protein interactions.[4] Specifically, ether-phospholipids are among the most potent biologically active phospholipid derivatives.[5] Naturally occurring as membrane components, a number of alkylglycerophosphorylcholines were shown to exhibit potent platelet activating, antihypertensive and immunomodulating properties.[1a] Added importance has been attributed to this class of compounds as recent evidence has been obtained demonstrating their selective tumor-cytoxicity against a wide spectrum of human cancers.[6] Elucidation of the specific mechanisms involved in the physiological functioning of ether phospholipids, however, remains to be accomplished, and it greatly depends on the availability of facile and efficient synthetic procedures leading to structurally variable phospholipid derivatives. Specifically, delineation of the structural requirements for the biological activities of phospholipids will not only advance the current level of understanding of the chemistry and biology of these compounds but also provide important insight into the design of new target molecules with the desired activity and potency.

We now describe a new stereospecific scheme focusing on the preparation of the antitumor active sn-1-palmityl-sn-2-methylglycero-sn-3-phosphorylcholine[6] that represents a highly efficient and flexible sequence applicable for the preparation of a wide range of substituted ether phospholipid compounds for structural, chemical and enzymological studies.

Our approach is based on the following elements: 1) L-glyceric acid is used as the chiral center for the construction of the optically active phospholipid molecule; 2) lipophilization of the three-carbon skeleton is

SCHEME 1



a) HCl / MeOH

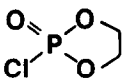
b) $(C_6H_5)_3C-\overset{+}{N}$◯ BF_4^-

c) CH_3I / $AgBF_4$ / Ag_2CO_3 / MeCN

d) $LiAlH_4$

e) $CH_3(CH_2)_{15}OSO_2CH_3$ / NaH / THF

f) HCl / MeOH—$CHCl_3$

g)  , Et_3N / BENZENE

h) $(CH_3)_3N$ / MeCN, 65°

accomplished in a two-step sequence, by first introducing the sn-2-short-chain substituent in an $AgBF_4$-catalyzed alkylation reaction that leaves the neighboring carbomethoxy group unaffected, then elaborating the long-chain sn-1-alkoxy moiety; and 3) the phosphorylcholine residue is developed using the cyclic phosphochloridate (g) that can be readily cleaved by anhydrous trimethylamine to yield the quaternary ammonium function of the target molecule directly.[7] Significantly, the sequence in which the substituents are introduced involves minimal use of protecting groups. Furthermore, the synthesis as outlined in Scheme I presents a general route to a wide spectrum of related derivatives as well.[8]

L-glyceric acid methyl ester (3) was prepared by esterification of the parent acid (1) in anhydrous HCl/MeOH, or by acid catalyzed deprotection of 2,3-isopropylidene-L-methyl glycerate (2) using HCl-anhydrous methanol at r.t. for 1 hr. (100%). The hygroscopic product, dried first over KOH in vacuo then in anhydrous acetonitrile solution on molecular sieves (Linde 3A), was allowed to react with 1.5 eqiv. triphenylmethyl pyridinium tetrafluoroborate at r.t. for 24 hrs. The product (4) was chromatographed on freshly activated silica gel ($CHCl_3$-hexane 75:25) yielding a semisolid (71%)[9], then methylated with 1.2 equiv. $CH_3I/AgBF_4$ (1:1) in acetonitrile using excess Ag_2CO_3 as a base. The resulting methyl ether (5) was obtained as a white crystalline solid (mp 103°, 80%).[9] The carbomethoxy group of compound (5) was reduced with $LiAlH_4$ in ether to give the corresponding alcohol (6) (85%). Treatment of (6) with hexadecylmethanesulfonate/NaH in THF gave triether (7) (70%) which was subsequently detritylated in anhydrous $CHCl_3$/MeOH (1:1) with HCl gas at r.t. for 1 hr (90%). Chromatography on silica gel ($CHCl_3$-EtOAc, 95:5) gave a colorless low melting solid[9] which was kept in vacuo over P_2O_5 then phosphorylated with 2-chloro-2-oxo-1,3,2-dioxaphospholane in benzene, in the presence of 1 equiv. triethylamine. The crude cyclic triester, obtained as a single phosphate positive product (9) was treated with anhydrous trimethylamine in acetonitrile at 65° (in a pressure bottle) for 24 hours to give the phospholipid (10) (92% from alcohol(8)) as a white hygroscopic solid. Chromatography on silica gel ($CHCl_3$:MeOH:aq.NH_3, 1:9:1) afforded analytically pure phospholipid (R_f = 0.17 $CHCl_3$-MeOH-H_2O 65:25:4). Calc. for $C_{25}H_{54}NO_6P \cdot 2H_2O$; C, 56.47; H, 10.99; N, 2.63; P, 5.82; found: C, 56.18; H, 11.07; N, 2.62; P, 6.06. $[\alpha]_D^{25} = -0.74°$[10].

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7. We have successfully employed a similar phosphorylation / ring opening sequence for the introduction of the phosphorylcholine moiety in the synthesis of phospholipase A_2 inhibitors and found it to be a great deal superior to the alternative method using bromethylphosphodichloridate. Chandrakumar, N.S., Hajdu, J., Tetrahedron Lett. (1981), 22, 2949-2952; J. Org. Chem. (1982) 47, 2144; Tetrahedron Lett. (1982) 23, 1043; and J. Org. Chem. (1983) 8, 1197.

8. The synthetic scheme also provides important intermediates such as compound (4) for construction of phospholipids incorporating additional types of functions, including heteroatom-carrying derivatives, either at the sn-1 or the sn-2 position of the glycerol moiety of the molecule. Along these lines we have already prepared a series of biologically active sn-2-substituted thiophospholipids. S.K. Bhatia and J. Hajdu, manuscript in preparation.

9. 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 spectra. For all new compounds satisfactory microanalytical data were obtained.

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