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SYNTHESIS OF ENZYME-INHIBITORY PHOSPHOLIPID ANALOGS II. PREPARATION OF CHIRAL 1-ACYL-2-ACYLAMIDO-2-DEOXYGLYCEROPHOSPHORYLCHOLINES FROM SERINE

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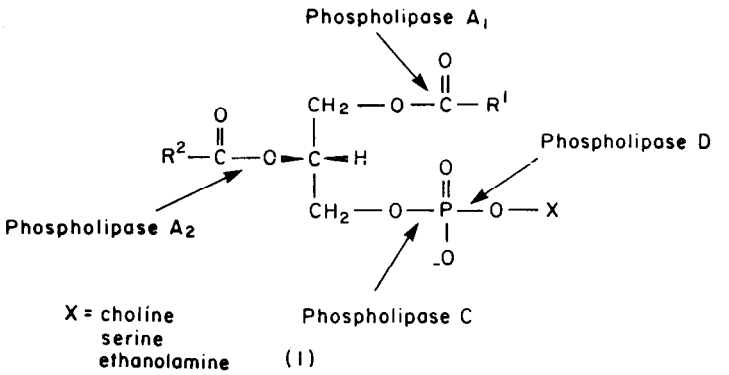
SUMMARY: A facile and efficient stereospecific route to the enzyme-inhibitory 2-amide analogs of phosphatidylcholine is reported.

Development of efficient methods leading to specific and potent phospholipase inhibitors is a key step toward understanding phospholipase biochemistry.[1] Specifically, these membrane-bound phospholipid-hydrolyzing enzymes are involved in a series of physiologically important regulatory processes.[2-5] Phospholipase A_2 for example is required for platelet aggregation,[2] cardiac contraction and excitation,[3] prostaglandin biosynthesis[4] and aldosterone-dependent

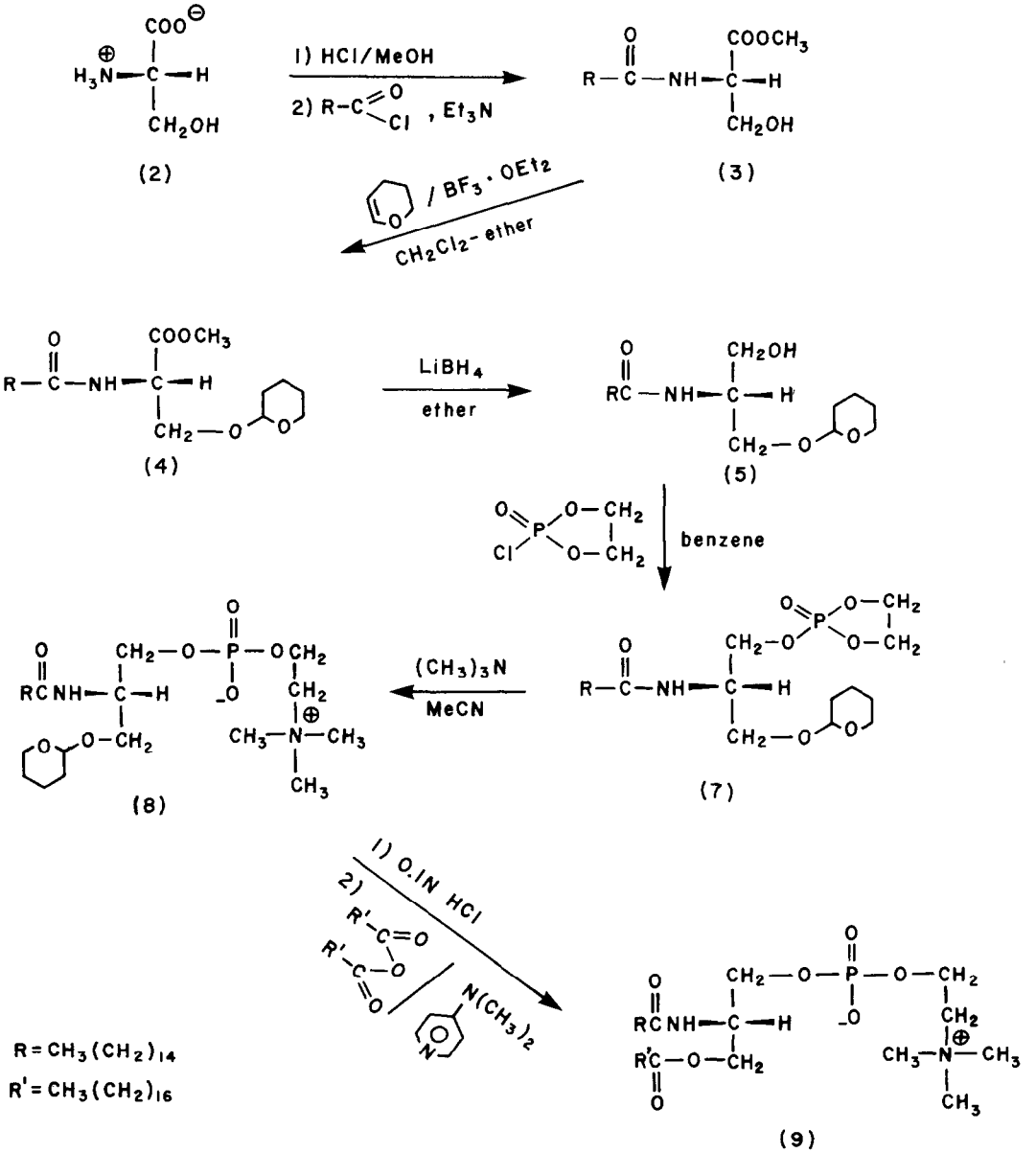


(I)

sodium transport.[5] Delineation of the precise mechanistic role of the enzyme in these events as well as elucidation of its catalytic mechanism of action requires highly specific and potent phospholipase A_2 inhibitors.

Well-recognized experimental difficulties involved in stereospecific derivatization of the required phosphate diesters with an adjacent chiral center at the glycerol 2-position[6] have long delayed the synthesis of non-scissile substrate analogs. Several years ago de Haas and his coworkers have demonstrated, using short-chain model compounds, that replacement of

Scheme I


1) HCl/MeOH
2) R-C(=O)Cl , Et3N
(2)
(3)
/ BF3 · OEt2
CH2Cl2 - ether
(4)
LiBH4
ether
(5)
benzene
(7)
(CH3)3N
MeCN
(8)
1) 0.1N HCl
2)
N(CH3)2
(9)
R = CH3(CH2)14
R' = CH3(CH2)16

the ester moiety by the corresponding amide function at the scissile 2-position abolishes the catalytic hydrolysis by the enzyme.[7] Their synthesis, however, proved to be rather lengthy and cumbersome, leading to a racemic product.[6] We now describe a facile and efficient method which renders the desired chiral compounds readily available.

Our approach to the problem is based on the following elements: (1) the chirality of the amino acid serine is utilized, and preserved throughout the synthesis, to provide the optically active center of the target molecule, (2) the sequence in which the substituents are introduced involves minimal use of protecting groups, and (3) the phosphorylation is carried out using the cyclic phosphochloridate (6)[8] whose nucleophilic ring-opening by trimethylamine yields the second portion of the phosphodiester directly. The synthesis here presented (Scheme I) has a great deal of flexibility in terms of the substituents that can be introduced, thus providing a general method applicable for the preparation of a wide spectrum of phospholipid analogs.

L-serine methyl ester was converted to the N-acyl derivative (3) with palmitoyl chloride/2 equiv. Et_3N in chloroform at r.t. for 24 hrs., (mp. 78-79°, 85%). The palmitoyl amide obtained was reacted with dihydropyrane in CH_2Cl_2-ether, using BF_3-etherate at room temperature for 6 hrs., yielding (4) (mp. 48-49°, 85%). Reduction of N-palmitoyl-O-tetrahydropyranyl serine methyl ester with $LiBH_4$ in ether gave the corresponding alcohol (5) (mp. 59-61°, 100%) which was phosphorylated using 2-chloro-2-oxo-1,3,2,-oxaphospholane[8] in benzene to give (7). Ring opening of the phosphate triester was accomplished using anhydrous trimethylamine in acetonitrile at 60-65° (pressure bottle) for 24 hrs. The phosphate diester (8) was isolated in 58% overall yield from the alcohol (R_f = 0.42, $CHCl_3$-MeOH-aqNH_3 1:9:9).[9] Deprotection of the hydroxymethyl group was carried out in 0.1N HCl at room temperature for 24 hrs. The 1-hydroxy-2-palmitoylamido-2-deoxy-3-phosphorylcholine obtained (100%, R_f = 0.28, $CHCl_3$-MeOH-aqNH_3 1:9:9) was acylated in chloroform with stearic anhydride/4-dimethylaminopyridine at r.t.[10] giving the final product in 65% yield (R_f = 0.29 $CHCl_3$-MeOH-H_2O 65:25:4). $C_{42}H_{85}N_2OP \cdot H_2O$, Calc. C,64.75; H,11.26; N, 3.60; P, 3.97, found C,64.56; H,11.49; N,3.51; P,3.93 $[\alpha]_D^{25}$ = +8.75 (c=1.025, $CHCl_3$).

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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 molibdic acid spray. All products appeared as single spots and gave confirmatory NMR and i.r. spectra.

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11. Obviously, utilizing the same synthetic scheme, with D-serine as the starting material, the amide analog of the enantiomeric L-phospholipid should be obtained. Since both amino acid starting materials are commercially available, both series of phospholipid analogs should now be readily accessible.

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