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STEREOSPECIFIC SYNTHESIS OF 2-THIOPHOSPHATIDYLCHOLINES; A NEW CLASS OF BIOLOGICALLY ACTIVE PHOSPHOLIPID ANALOGUES

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SUMMARY: A new stereospecific synthesis of biologically active sn-2-thiophosphatidylcholines is reported.

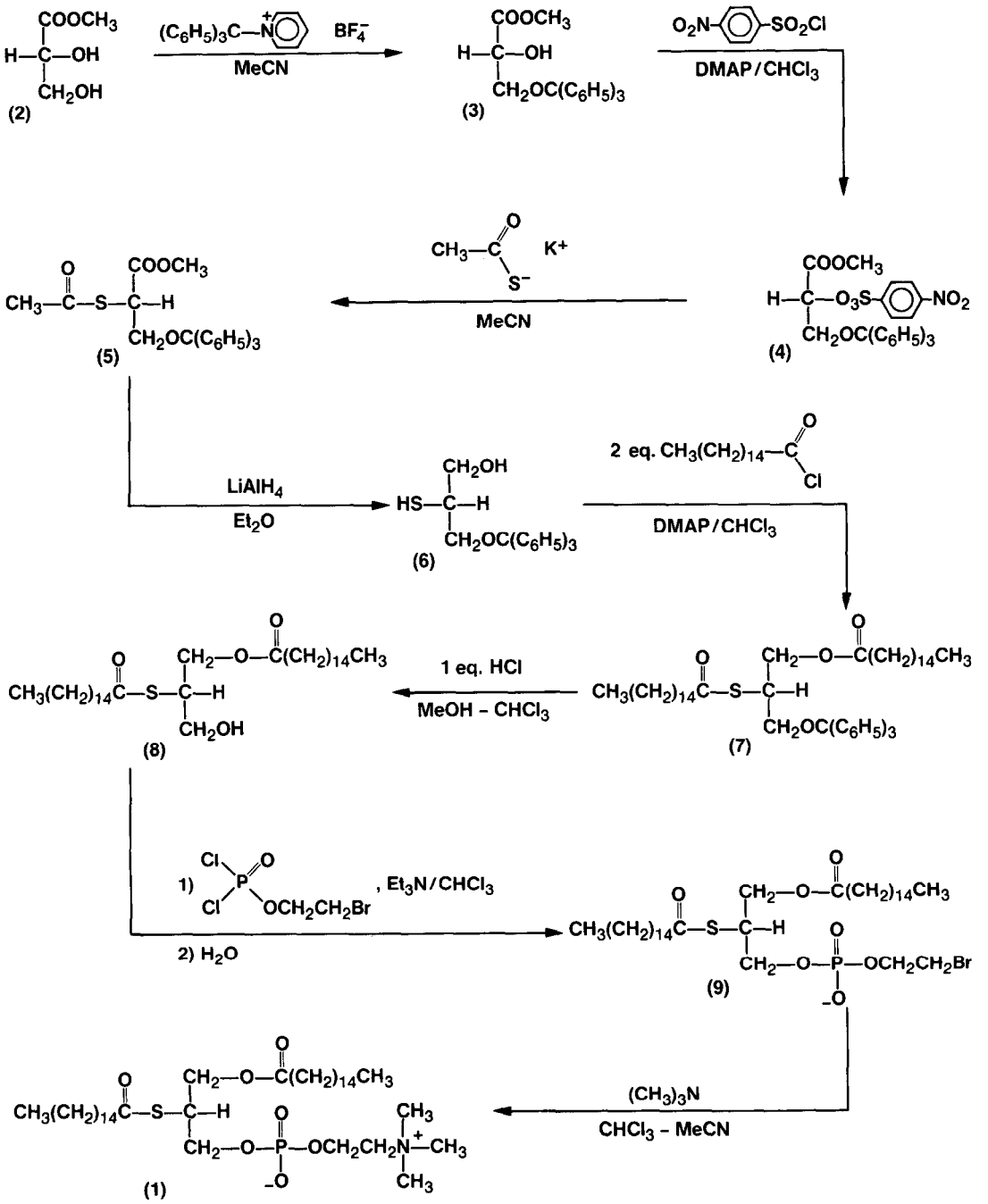
Development of new synthetic methods for the preparation of structurally variable phospholipid derivatives is one of the most timely problems of membrane chemistry today.[1] The compounds are required for physicochemical studies of phospholipid-phospholipid as well as phospholipid-protein interactions, and for elucidation of the physiological role of phospholipid metabolizing enzymes.[2-6] Phospholipase A_2 for example is required for platelet aggregation,[2] cardiac contraction and excitation,[3] prostaglandin biosynthesis and aldosterone-dependent sodium transport.[5]

Well-recognized experimental difficulties involved in stereospecific derivatization of phosphodiesters with an adjacent chiral center at the glycerol 2-position have long delayed the synthesis of thioesters as chromogenic substrates for the study of individual lipolytic enzymes. Early attempts resulted in racemic 1,2-dithiophospholipids[7] while more recent work led to sn-1,2-dithio analogues[8,9] which are 1) substrates for both phospholipase A_1 and phospholipase A_2 2) must be used under different assay-conditions[9] and 3) do not allow independent variation of the sn-1 and sn-2 substituents.[8]

We now describe a facile and efficient method which allows replacement of the scissile carboxylic ester moiety of the natural phospholipid compound by the corresponding thioester function in a site- and stereospecific manner, providing a series of chromogenic substrates for spectrophotometric studies of enzyme catalyzed lipolytic reactions. The sequence (Scheme I) allows synthesis of a wide range of related analogues through independent variation of the sn-1 vs. sn-2 substituents for delineation of the structural features involved in specific interactions at the catalytic vs. binding portions of the molecule (1).

Our synthetic approach is based on the following elements: 1) D-glyceric acid is used as the chiral center for construction of the optically active phospholipid molecule; 2) the thioester function at the 2-position is introduced by p-nitrobenzenesulfonyl activation of the hydroxyl group, followed by potassium thioacetate - displacement of the leaving-group (with inversion of the chirality of the 2-carbon); and 3) the phosphodiester

SCHEME 1


COOCH3
H—C—OH
CH2OH
(2)
(C6H5)3C—N
BF4−
MeCN
COOCH3
H—C—OH
CH2OC(C6H5)3
(3)
O2N—SO2Cl
DMAP/CHCl3
CH3—C(=O)—S−
K+
MeCN
COOCH3
H—C—O3S—NO2
CH2OC(C6H5)3
(4)
CH3—C(=O)—S—C—H
COOCH3
CH2OC(C6H5)3
(5)
LiAlH4
Et2O
CH2OH
HS—C—H
CH2OC(C6H5)3
(6)
2 eq. CH3(CH2)14—C(=O)—Cl
DMAP/CHCl3
CH3(CH2)14C(=O)—S—C—H
CH2—O—C(=O)(CH2)14CH3
CH2OC(C6H5)3
(7)
1 eq. HCl
MeOH - CHCl3
CH3(CH2)14C(=O)—S—C—H
CH2—O—C(=O)(CH2)14CH3
CH2OH
(8)
1) Cl2P(=O)OCH2CH2Br, Et3N/CHCl3
2) H2O
CH3(CH2)14C(=O)—S—C—H
CH2—O—C(=O)(CH2)14CH3
CH2—O—P(=O)(−O)—OCH2CH2Br
(9)
(CH3)3N
CHCl3 - MeCN
CH3(CH2)14C(=O)—S—C—H
CH2—O—C(=O)(CH2)14CH3
CH2—O—P(=O)(−O)—OCH2CH2N+(CH3)3
(1)

moiety is developed in a bromoethyl phosphodichloridate - trimethylamine sequence.[10]

D-glyceric acid methyl ester (2) obtained by acid catalyzed deprotection of 2,3-isopropylidene-D-methyl glycerate,[11] was converted to the trityl derivative (3)[11], then treated with p-nitrobenzenesulfonyl chloride/4-(dimethylamino)-pyridine in dry $CHCl_3$ to give a crystalline product ((4), 89%, mp 124-5°C) $[\alpha]_D^{23}$ = +19.94° (c 1.62, 1:4 CH_3OH-$CHCl_3$). Compound (4) in reaction with potassium thioacetate in dry acetonitrile afforded the corresponding 2-thioacetyl derivative ((5), 81%). The carbomethoxy and thioester groups of compound (5) were reduced with $LiAlH_4$ in ether to yield the alcohol-thiol ((6), 60%) $[\alpha]_D^{23}$ = +5.90° (c 1.1, 1:4 CH_3OH-$CHCl_3$) which was then acylated using 2 equiv. palmitoyl chloride to give (7) in 92% yield[12] $[\alpha]_D^{23}$ = +3.62° (c 1.82, 1:4 CH_3OH-$CHCl_3$). Detritylation of (7) to the corresponding alcohol was carried out with stoichiometric amounts of 12 M HCl in methanol-chloroform (1:1) at room temperature for 2.5 h. The product (8) isolated by Sephadex LH-20 chromatography was shown to be SH negative[13] (indicating that no S --> O acyl migration occurred in the procedure). It was dried over P_2O_5 in vacuo and phosphorylated with 2-bromoethyl phosphodichloridate[10] in dry chloroform in the presence of excess triethylamine at r.t. for 24 h. The crude bromoethyl phospholipid (9) was stirred with aq. KCl for 1 h, extracted at pH 3 with chloroform and dried in vacuo over P_2O_5. Treatment of (9) with anhydrous trimethylamine in chloroform-acetonitrile (in a pressure bottle) at 60° for 14 h yielded the target thiophosphatidylcholine (1) (52% after chromatography on silica gel[14] ($CHCl_3$-MeOH-H_2O 65:25:4). ^{1}H-NMR ($CDCl_3$) δ 0.88 (br t, 6H, $-CH_3$), 1.26 (s, 52H, $-CH_2$), 1.85-2.29 (m, 4H, $-COCH_2$), 3.46 (s, 9H, $-N(CH_3)_3$), 3.46-4.38 (m, 9H). Calc. for $C_{40}H_{80}NO_7PS \cdot 2\,H_2O$; C, 61.11; H, 10.77; N, 1.78; P, 3.94; S, 4.08; found C, 61.02; H, 10.75; N, 1.67; P, 3.93; S, 4.31. $[\alpha]_D^{23}$ = -8.61° (c 1.08, 1:4 CH_3OH-$CHCl_3$).

The stereochemistry of the product (1) was ascertained by enzymatic hydrolysis using bee-venom phospholipase A_2. Exhaustive hydrolysis of the thiophosphatidylcholine (1) in mixed micelles with Triton X-100 (1:8) at 40°C gave 98.3 ± 2% chiral purity.[15]

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14. Regarding the problem of recovery of phospholipids from silica-gel columns cf. Chandrakumar, N. S., Hajdu, J. J. Org. Chem. (1983), 48, 1197.

15. Enzymatic cleavage of compound (1) by bee-venom phospholipase A_2, acting on phospholipid-Triton X-100 mixed micelles (1 mM: 8 mM), was determined at pH 8.0 (0.025 M Tris HCl/0.1 M KCl) and 40°C in the presence of 0.01 M $CaCl_2$ and 1 mM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB). The liberated phospholipid-sulfhydryl was quantitated spectrophotometrically, from the absorption of 5-thio-2-nitrobenzoate 412 (13,000) generated by DTNB-trapping of the enzymatic reaction product.

16. The kinetic results obtained with the new thiophospholipid will be reported separately elsewhere.

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