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A NEW APPROACH TO THE SYNTHESIS OF THIOETHER PHOSPHOLIPIDS. PREPARATION OF 1-THIOHEXADECYL-2-N-ACYLAMINODEOXYGLYCEROPHOSPHOCHOLINES

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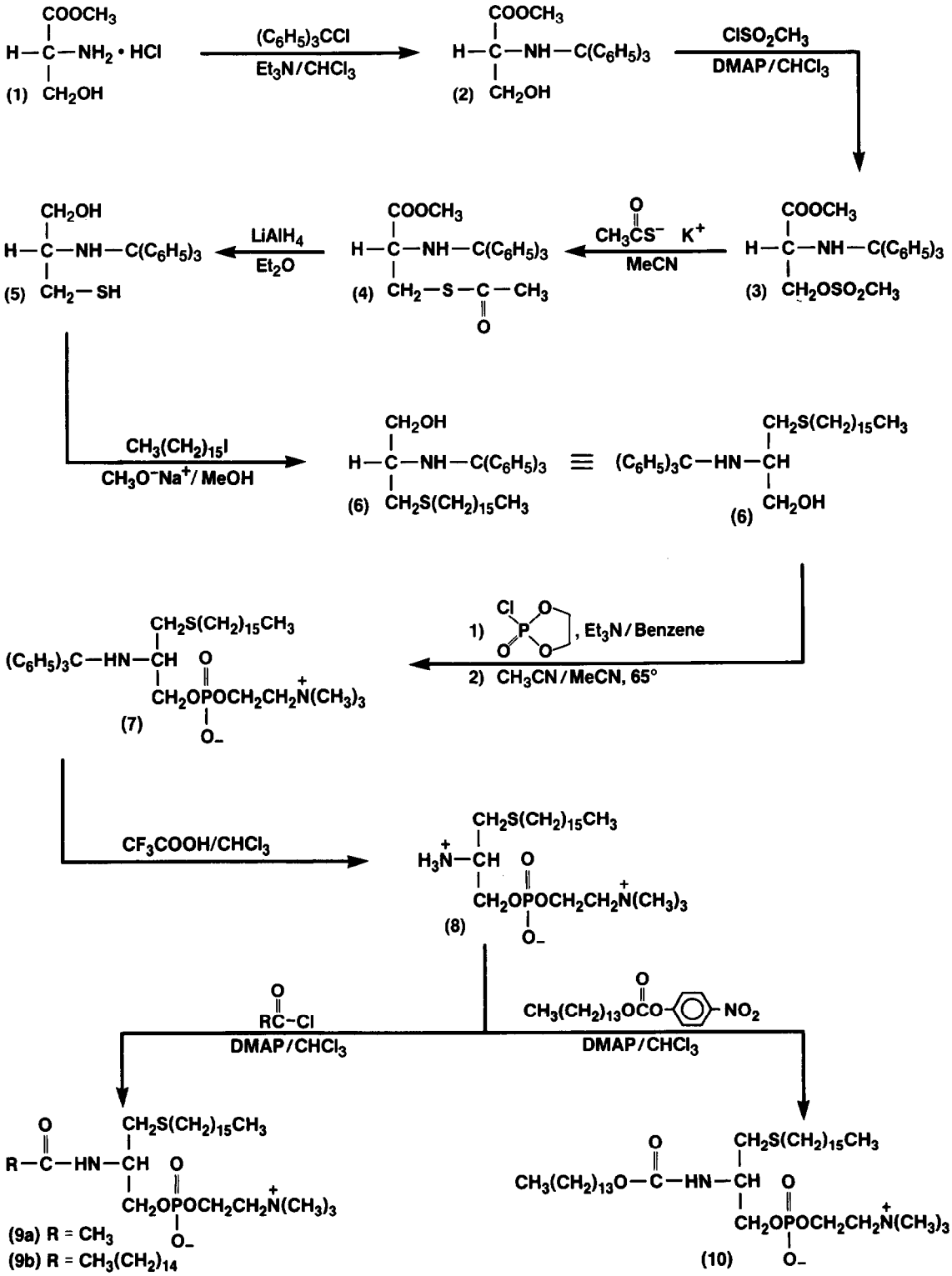
SUMMARY: A novel stereospecific route to biologically active thioether phospholipids is reported.

Thioether phospholipids are a new class of synthetic phospholipid analogues exhibiting high level of activity in a wide spectrum of biological systems.[1-4] Included among these are a series of antitumor active thioalkyl derivatives of platelet activating factor (PAF)[1-3] that have been shown to exceed the tumor-cytotoxicity of the corresponding oxygen compounds.[3] Furthermore, sulfur substitution at the sn-1 position appears to lower the platelet-aggregating potency of 1,2-dialkylphosphoglycerides,[1,3] alleviating an important side effect in antileukemic chemotherapy using PAF analogues. More recently thioether substituents have been introduced to enzyme inhibitory phospholipid derivatives to achieve enhanced protein binding at the sn-1 chain of the molecule.[4]

While these observations clearly indicate that thioether analogues are likely to become valuable probes for elucidation of the structural basis of biological functioning of phospholipids, relatively few synthetic methods have been developed for the preparation of the compounds.[3-5] We now describe a new stereospecific synthesis of enzyme inhibitory 1-thioalkyl-2-acylaminodeoxy-sn-glycero-3-phosphocholines. The sequence (Scheme I) also provides a flexible and efficient method for the synthesis of a wide range of related thioether phospholipids for structural, chemical and cell-biological studies.

Our approach is based on the following elements: 1) the chirality of the amino acid D-serine is used to provide the optically active center of the target molecule, 2) sulfur substitution is achieved via thioacetate-displacement at the methanesulfonate activated primary hydroxyl group, and 3) the phosphorylcholine moiety is elaborated using 2-chloro-2-oxo-1,3,2-dioxaphospholane which is subsequently cleaved by anhydrous trimethylamine to give the quaternary ammonium function directly. The sequence requires only a single protecting group and it provides a general route to other types of sulfur-substituted phospholipids as well.

SCHEME I


COOCH3
H—C—NH2 • HCl
CH2OH
(1)
(C6H5)3CCl
Et3N/CHCl3
COOCH3
H—C—NH—C(C6H5)3
CH2OH
(2)
ClSO2CH3
DMAP/CHCl3
CH2OH
H—C—NH—C(C6H5)3
CH2—SH
(5)
LiAlH4
Et2O
COOCH3
H—C—NH—C(C6H5)3
CH2—S—C—CH3
O
(4)
O
CH3CS⁻ K⁺
MeCN
COOCH3
H—C—NH—C(C6H5)3
CH2OSO2CH3
(3)
CH3(CH2)15I
CH3O⁻Na⁺/MeOH
CH2OH
H—C—NH—C(C6H5)3
CH2S(CH2)15CH3
(6)
≡
CH2S(CH2)15CH3
(C6H5)3C—HN—CH
CH2OH
(6)
1)
Cl
O
O
P
O
, Et3N/Benzene
2) CH3CN/MeCN, 65°
CH2S(CH2)15CH3
(C6H5)3C—HN—CH
O
CH2OPOCH2CH2N⁺(CH3)3
O⁻
(7)
CF3COOH/CHCl3
CH2S(CH2)15CH3
H3N⁺—CH
O
CH2OPOCH2CH2N⁺(CH3)3
O⁻
(8)
O
RC—Cl
DMAP/CHCl3
O
CH3(CH2)13OCO
NO2
DMAP/CHCl3
O
R—C—HN—CH
CH2S(CH2)15CH3
O
CH2OPOCH2CH2N⁺(CH3)3
O⁻
(9a) R = CH3
(9b) R = CH3(CH2)14
O
CH3(CH2)13O—C—HN—CH
CH2S(CH2)15CH3
O
CH2OPOCH2CH2N⁺(CH3)3
O⁻
(10)

D-Serine methyl ester (1) was treated with trityl chloride/triethylamine in dry $CHCl_3$ for 24 hrs. to afford the corresponding N-trityl derivative (2) in 92% yield (m.p. 140-41°). Compound (2) in reaction with methanesulfonyl chloride in the presence of triethylamine yielded mesylate (3) as viscous oil (91%). The product (3) was allowed to react with potassium thioacetate in anhydrous acetonitrile at r.t. for 2 days to give the thioester (4) (84%). Reduction of (4) with $LiAlH_4$ in ether gave alcohol-thiol (5) (68%), which was passed through silica gel with chloroform, and then alkylated with 1 equiv. hexadecyl iodide in MeOH/NaOMe to yield thioether ((6), 75% isolated by chromatography). $[\alpha]_D^{23}$ = -20.72° (c 1.53, 1:4 CH_3OH-$CHCl_3$). Anal. calcd. for $C_{38}H_{55}NOS$; C, 79.52; H, 9.66; N, 2.44; S, 5.59; found C, 79.64; H, 9.62; N, 2.29; S, 5.52. Alcohol (6) was dried over P_2O_5 at 40°, then phosphorylated with 2-chloro-2- oxo-1,3,2-dioxaphospholane[6] in benzene, in the presence of stoichiometric amount of triethylamine. The cyclic triester intermediate was directly treated with anhydrous trimethylamine in acetonitrile at 65° (in a pressure bottle) for 16 hrs. to give the phospholipid ((7), 61% isolated yield). $[\alpha]_D^{23}$ = -10.84° (c 1.31, 1:4 CH_3OH-$CHCl_3$). Anal. calcd. for $C_{43}H_{67}N_2O_4PS \cdot \frac{1}{2}H_2O$; C, 69.04; H, 9.16; N, 3.74; P, 4.14; S, 4.28; found C, 68.86; H, 9.33; N, 3.67; P, 3.86; S, 4.39. Detritylation of (7) was achieved using anhydrous trifluoroacetic acid in $CHCl_3$ at r.t. for 1 hr., the resulting amine (8) was treated <u>in situ</u> with acetyl chloride/4-(dimethylamino)pyridine (DMAP) for 20 hrs. at room temperature. The crude product was passed through Rexyn I-300 ion exchange resin ($CHCl_3$-MeOH-H_2O 4:5:1) followed by chromatography on activated silica-gel ($CHCl_3$-MeOH-H_2O 65:25:4) to give analytically pure phospholipid ((9a), 62%) as a hygroscopic solid. ^{1}H-NMR ($CDCl_3$) δ0.88, (t, 3H, $-CH_3$), 1.25 (br s, 28 H, $-CH_2$), 1.98 (s, 3H, $-COCH_3$), 2.52-2.64 (m, 4H, CH_2-S-CH_2), 3.34 (S, 9 H, $-N(CH_3)_3$), 3.74-4.27 (m, 7H). Anal calcd. for $C_{26}H_{55}N_2O_5PS \cdot H_2O$; C, 56.09; H, 10.32; N, 5.03; P, 5.56; S, 5.76; found C, 56.03; H, 10.22; N, 4.65; P, 5.50; S, 5.69. $[\alpha]_D^{23}$ = +7.57° (c 1.4, CH_3OH), -4.07° (c 1.08, 1:4 CH_3OH-$CHCl_3$). Similarly, compounds (9b) and (10) were prepared from phospholipid (8) by reaction with palmitoyl chloride/DMAP and tetradecyl p-nitrophenyl carbonate/DMAP in 51% and 57% yields respectively.[8] Compound (9b) Anal. calcd. for $C_{40}H_{83}N_2O_5PS \cdot 3H_2O$; C, 60.88; H, 11.37; N, 3.55; P, 3.92; S, 4.06; found C, 60.59; H, 11.53; N, 3.54; P, 3.78; S, 4.44. $[\alpha]_D^{23}$ = +9.62° (c 1.32, CH_3OH). Compound (10) Anal. calcd. for $C_{39}H_{81}N_2O_6PS \cdot 3H_2O$; C, 59.21; H, 11.08; N, 3.54; P, 3.91; S, 4.05; found C, 59.11; H, 10.63; N, 3.51; P, 3.87; S, 4.58. $[\alpha]_D^{23}$ = +6.36 (c 1.21, CH_3OH).

Preliminary results indicate that compound (11b) exhibits potent competitive-reversible inhibition of bee-venom phospholipase A_2.[9]

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7. Maffrand et. al. reported $[\alpha]_{578}^{20} = -4.8^{o}$ for the D-enantiomer, without specifying the solvent used. The experimental data in our hands clearly indicate that both the direction and magnitude of the optical rotation of thioether phospholipids vary significantly with the conditions, particularly with the solvent. Detailed studies regarding this point will be reported separately elsewhere.

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

9. The enzymological studies were carried out with bee-venom phospholipase A_2 acting on phospholipid-Triton X-100 mixed micelles. Synthetic dipalmitoyl phosphatidylcholine was used as substrate. For a closely related assay-system cf. Davidson, F. F., Hajdu, J., Dennis, E. A. Biochem. Biophys. Res. Commun. (1986) 137, 587. Detailed kinetic results obtained with the new thioether inhibitor will be reported separately.

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