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A facile asymmetric synthesis of glycerol phospholipids via tritylglycidol prepared by the asymmetric epoxidation of allyl alcohol. Thiolester and thioether analogs of phosphatidylcholine

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Trityl-glycidol was synthesized by in situ derivatization of glycidol, which was prepared by the catalytic asymmetric epoxidation of allyl alcohol. Depending on the enantiomer of diisopropyl tartrate used with the titanium catalyst, either (*R*)- or (*S*)-trityl-glycidol was obtained in a "one pot" synthesis in about 50% overall yield. The optical purity, determined by NMR spectroscopy of a Mosher ester, was greater than 98% ee. Nucleophilic opening of the chiral epoxide with dodecyl mercaptan gave optically active 1-*S*-dodecyl-3-*O*-trityl-1-thio-glycerol, which was used to synthesize 1-*S*-dodecyl-2-*O*-decanoyl-thio-*sn*-glycero-3-phosphocholine. Opening of the epoxide with methyl xanthate gave a 1,2-trithiocarbonate derivative of trityl glycerol which can be used to synthesize 1,2-bis(*S*-decanoyl)-1,2-dithio-*sn*-glycero-3-phosphocholine. Opening of the epoxide with thiodecanoic acid gave 1-*S*-decanoyl-3-*O*-trityl-1-thio-glycerol which was used to synthesize 1-*S*-decanoyl-2-*O*-decanoyl-1-thio-*sn*-glycero-3-phosphocholine.

Keywords: synthesis; trityl-glycidol; thiodecanoic acid; 1-*S*-dodecyl-2-*O*-decanoyl-1-thio-*sn*-glycero-3-phosphocholine; 1,2-bis(*S*-decanoyl)-1,2-dithio-*sn*-glycero-3-phosphocholine; 1-*S*-decanoyl-2-*O*-decanoyl-1-thio-*sn*-glycero-3-phosphocholine;

Introduction

The synthesis of optically active glycerol phospholipids involves the use of chiral C_3 intermediates. The use of 1,2-isopropylidene-*sn*-glycerol (from D-mannitol [1]), 2,3-isopropylidene-*sn*-glycerol (from L-serine [2]), 1-trityl-*sn*-glycerol (from D-mannitol [3]), and L-glyceric acid [4] involve several synthetic steps and a variety of protecting groups. Glycidol has been used in the synthesis of mixed acid diacyl glycerols [2,5,6], and dithiolester phospholipid analogs [7]. The method of asymmetric epoxidation developed by Sharpless et al., [8] affords a simple inexpensive route to chiral glycidol. The titanium-assisted opening of (*S*)-glycidol with stearic acid was used to synthesize 1-stearoyl-*sn*-glycerol, although in poor yield [9]. Arenesulfonate derivatives of optically active glycidol (tosyl- and 3-nitrobenzenesulfonyl-glycidol) are commercially available (Aldrich Chemical Co., Milwaukee, WI) and have been used to synthesize symmetric-chain glycerol phospholipids [10] and 1-alkyl-*sn*-glycerol [11]. The arenesulfonyl protecting groups, however, are not the most desirable since they cannot easily be removed in preference to acyl ester groups on glycerol and necessitate substitution by iodide and the use of expensive silver salts of phosphate esters to synthesize phospholipids [10]. The trityl group is preferable since it can easily be preferentially removed by treatment with BF_3/methanol [12]. We report here a simple in situ tritylation of chiral glycidol following the catalytic asymmetric

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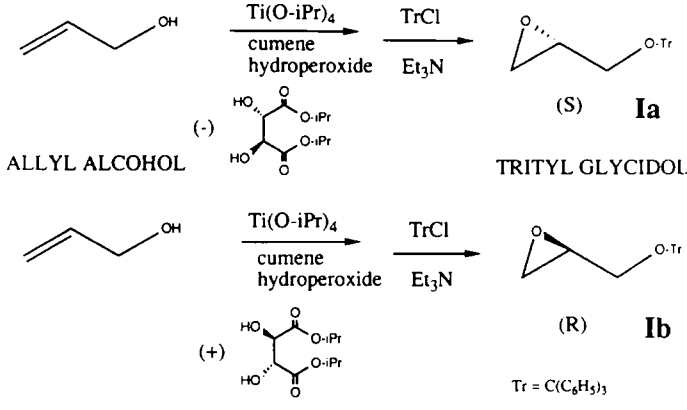

Fig. 1. Synthesis of chiral trityl-glycidol.

epoxidation of allyl alcohol (Fig. 1). Chiral trityl-glycidol, so produced, is used to synthesize a variety of phospholipids analogs, depending on the method used to open the epoxide (Fig. 2). Opening of the epoxide with (1) an alkyl mercaptan in the presence of a trace amount of butyl lithium, gives a 1-thioether derivative of trityl-glycerol (reported here); (2) a thioacid, gives a 1-thiolester derivative [13]; and (3) methyl xanthate, gives a 1,2-trithiocarbonate derivative

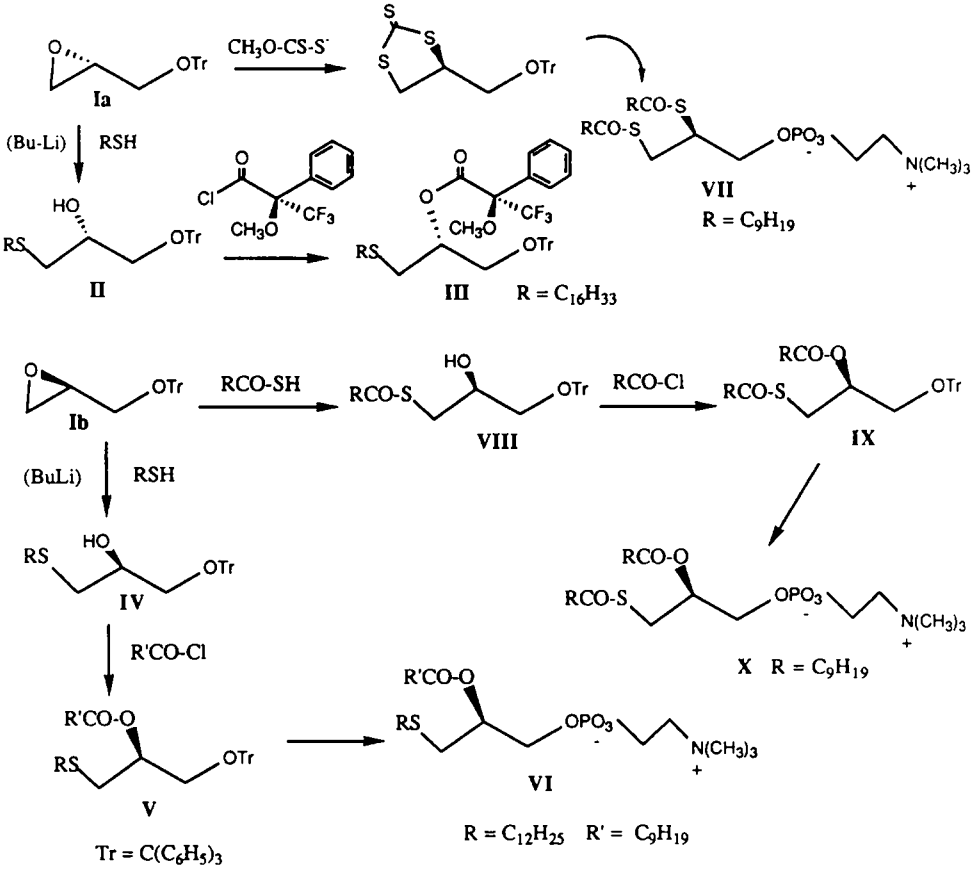

Fig. 2. Syntheses of chiral thioester and thioether analogs of phosphatidylcholine from trityl-glycidol.

which can be converted to a dithiolester phospholipid analog [7]. We report here the syntheses of chiral 1-thioether, 1-thiolester, and 1,2-dithiolester analogs of phosphatidylcholine.

Experimental

Materials

All organic chemicals were obtained from Aldrich Chemical Co., (Milwaukee, WI) and used as received. (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (Mosher acid chloride) was prepared from the free acid by treatment with oxalyl chloride (with a drop of dimethylformamide) at 80°C and was flash distilled. Dry tetrahydrofuran was prepared by distillation from sodium benzophenone ketyl. Benzene was dried by azeotropic distillation, and pyridine and triethylamine were distilled over barium oxide and stored over 4 Å molecular sieve. Silica gel for column chromatography (60 Å, 75—150 μm, cat. no. 13100), and TLC plates (cat. no. 01521) were obtained from Analtech, Inc. (Newark, DE).

Analyses

TLC was carried out as described by Hendrickson et al. [7]. Proton NMR spectra were recorded on a Varian VXR-300S, at 300 MHz in $CDCl_3$ (for phosphatidylcholines, 5—10% CD_3OD was added to reduce line-broadening due to inverted micelles), NMR peak assignments were confirmed by analysis of 2D COSY spectra. Elemental analyses (C,H,N,P,S) were performed by Galbraith Laboratories, Inc., (Knoxville, TN).

Chemical Syntheses

(S)-Trityl-glycidol (**Ia**)

Allyl alcohol (0.1 mol) was converted to (*R*)-glycidol using the procedure described by Gao et al. [8]. After the reaction with allyl alcohol, D-(−)-diisopropyl tartrate, $Ti(O\text{-}i\text{-}Pr)_4$, and cumene hydroperoxide had stirred for about 8 h at −3°C, the reaction mixture was cooled to −30°C and carefully neutralized with 21 ml of trimethylphosphite (tested for completion with starch iodide paper) while maintaining the temperature at −30°C. Triethylamine (17 ml, 0.12 mol) and 27.9 g (0.1 mol) of trityl chloride were added to the neutralized reaction mixture which was then stirred overnight at 2°C. The reaction mixture was filtered through a pad of Celite and the filtrate washed with 10% aqueous tartaric acid (2 × 30 ml), saturated $NaHCO_3$ (2 × 30 ml), and saturated NaCl (2 × 30 ml). The organic phase was dried over Na_2SO_4, filtered, and concentrated to an oil. The crude product was dissolved in a mixture of 6% ether in hexane and applied to a 100-g silica column. After eluting with 6% and 12% ether in hexane, 16.8 g (53% yield) of pure **Ia** was obtained (mp 99—100°C; TLC: single spot, $R_f = 0.5$, silica, hexane/acetone 7:1). NMR: δ 2.59, d of d, J = 14 and 8 Hz, and δ 2.74, d of d, J = 14 and 5 Hz (epoxide —CH_2); δ 3.2, d of d, J = 18 and 5 Hz, and δ 3.24, J = 18 and 6 Hz (—CH_2—O—Tr); δ 3.8—3.9, m (epoxide —CH); δ 7.2—7.6, m (trityl —CH). Anal. calc. for $C_{22}H_{20}O_2$: C, 83.5; H, 6.37. Found: C, 83.6; H, 6.36.

(R)-Trityl-glycidol (**Ib**)

This product was synthesized using the same procedure as for **Ia** above, but with L-(+)-diisopropyl tartrate.

3-S-hexadecyl-1-O-trityl-3-thio-sn-glycerol (**II**)

To a solution of 4.26 g (15.2 mmol) of hexadecyl mercaptan in 60 ml of dry tetrahydrofuran in an ice bath, 0.25 ml (2.5 mmol) of 10 M (in hexanes) butyl lithium was added with stirring. After 5 min, 4.0 g (12.6 mmol) of **Ia** was added and stirring was continued at room temperature for 0.5 h and 40°C for 2 h. TLC (silica, hexane/acetone 7:1) showed that the reaction was complete. The mixture was neutralized with 20% acetic acid in ethanol, concentrated to a small volume, taken up in 80 ml of $CHCl_3$, and washed twice with methanol/water (3:2). The chloroform layer was dried over Na_2SO_4 and then under vacuum. The crude product (9.3 g) was dissolved in hexane and applied to a 80-g silica column. The column was eluted with hexane and 2% ether in hexane. Pure (by TLC) **II** (5.97 g,

82% yield, 59—60°C) eluted with the latter solvent. NMR: δ 0.88, tr, J = 6 Hz (—CH_3); δ 1.2—1.4, m (—CH_2—); δ 1.5—1.6, m (—CH_2—C—S—); δ 2.46, tr, J = 7 Hz (—CH_2—S—); δ 2.59, d of d, J = 14 and 8 Hz, and δ 2.74, d of d, J = 14 and 5 Hz (*sn*-3 —CH_2); δ 3.16—3.28, m (*sn*-1 —CH_2); δ 3.78—3.86, m (*sn*-2 —CH); δ 7.2—7.5, m (trityl —CH).

1-S-Dodecyl-3-O-trityl-1-thio-sn-glycerol (**IV**)

This product (mp 47—48°C) was prepared in 71% yield by the same procedure as described above for **II**, but starting with **Ib** and using dodecyl mercaptan.

Mosher ester of 3-S-hexadecyl-1-O-trityl-3-thio-sn-glycerol (**III**)

Compound **II** was acylated with (*R*)-(+)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride (Mosher acid chloride) using triethylamine and dimethylaminopyridine in CH_2Cl_2. The product was purified (96% yield) by chromatography on a silica column (eluted with 2% ether in hexane). It gave a single spot upon TLC (silica, hexane/ether 9:1). NMR: δ 0.87, tr, J = 6 Hz (—CH_3); δ 1.2—1.4, m (alkyl —CH_2); δ 1.5—1.6, m (—CH_2—C—S—); δ 2.47, tr, J = 7 Hz (—CH_2—S—); δ 2.73, d of d, J = 11 and 6 Hz (*sn*-3 —CH_2); δ 3.27, d of d, J = 11 and 6 Hz, and δ 3.35, d of d, J = 11 and 4 Hz, (*sn*-1 —CH_2); δ 3.58, quartet (coupled to CF_3), J = 1 Hz (—O—CH_3); δ 5.33, m (*sn*-2 —CH); δ 7.3—7.6, m (trityl —CH).

1-S-Dodecyl-2-O-decanoyl-3-O-trityl-1-thio-sn-glycerol (**V**)

To a solution of 2.02 g (3.9 mmol) of **IV** in 6 ml of hexane and 3 ml of dry pyridine in an ice bath, 1.11 g (5.8 mmol) of decanoyl chloride in 6 ml of hexane was added dropwise with stirring. The reaction mixture was stirred for 10 min at 2°C and then at room temperature for 0.5 h. Benzene (30 ml) was added and the solution was washed three times with 0.5 M ammonia in methanol/water (3:1) and three times with methanol/water (3:1). The organic phase was dried over Na_2SO_4 and then under vacuum. The crude product (2.6 g) was purified on a 15-g silica column by elution with hexane and 2% ether in hexane. Pure **V** (2.46 g; TLC: silica, hexane/acetone 7:1) was obtained in 94% yield.

1-S-Dodecyl-2-O-decanoyl-1-thio-sn-glycero-3-phosphocholine (**VI**)

Compound **V** was detritylated with BF_3/methanol and the glycerol derivative converted to the choline phosphate with $POCl_3$ and choline tosylate using the procedures described previously for phosphatidylcholines [7,14]. NMR: δ 0.73, tr, J = 6 Hz (—CH_3); δ 1.0—1.2, m (—CH_2—); δ 1.35—1.5, m (—CH_2—C—S— and —CH_2—C—CO—); δ 2.16, tr, J = 7 Hz (—CH_2—S—); δ 2.41, tr, J = 7 Hz (—CH_2—CO—); δ 2.54, d of d, J = 14 Hz and 6 Hz, and δ 2.68, d of d, 14 and 7 Hz (*sn*-1 —CH_2); 3.07, s (—$N(CH_3)_3$); δ 3.4—3.5, m (—CH_2—N); δ 3.8—3.9, m (*sn*-3 —CH_2); δ 4.0—4.1, m (P—O—CH_2—); δ 4.9—5.0, m (*sn*-2 —CH). Anal. calcd. for $C_{30}H_{62}PSNO_6 \cdot H_2O$: C, 58.7; H, 10.5; N, 2.28; P, 5.05; S, 5.22. Found: C, 53.3; H, 9.85, N, 2.22; P, 5.15; S, 6.71.

1,2-Bis(S-decanoyl)1,2-dithio-sn-glycero-3-phosphocholine(**VII**)

Compound **Ia** was converted to 1,2-thiocarbonyl-3-trityl-1,2-dithio-*sn*-glycerol (trithiocarbonate) as described previously for the racemic compound [7]. The latter was then converted to **VII** by the procedure described previously for 1,2-bis(*S*-heptanoyl)-1,2-dithio-*sn*-glycero-3-phosphocholine [7]. This product gave exactly the same activity as that prepared previously (starting from D-mannitol [7,15]) when assayed with snake venom phospholipase A_2 [15].

Thiodecanoic acid

Sodium sulfide decahydrate was dried over P_2O_5 in a high vacuum (1 mmHg) at room temperature for several hours and then at 90°C overnight. About 90% of the hydrated water was removed as judged by the weight loss. The dried sodium sulfide (3.19 g, 41 mmol) was stirred in 30 ml of pyridine in an ice bath, and 7.8 g (41 mmol) of decanoyl chloride was added over 5 min. A deep-yellow color developed. The ice

bath was removed and stirring was continued at room temperature for 1 h. The mixture was cooled in an ice bath and 10 ml of water was added. It was then acidified with 6 N HCl, whereupon a yellow oil separated. The oil was extracted with 100 ml of ether, and the ether layer washed twice with water, dried over Na_2SO_4 and then under vacuum. Pure thiodecanoic acid (3.53 g, 46% yield) (single spot by TLC: silica, hexane/ether/acetic acid 90:10:1) was obtained by vacuum distillation (bp 103°C, 2 mmHg). IR: 2550 cm^{-1} (—S—H); 1705 cm^{-1} (—C=O).

1-S-Decanoyl-3-O-trityl-1-thio-sn-glycerol (**VIII**)

This procedure is based upon that of Ward [13]. To a solution of 1.58 g (5 mmol) of **Ib** in 20 ml of toluene and 20 ml of hexane, 1.94 g (10.3 mmol) of thiodecanoic acid was added dropwise. The reaction occurred immediately as monitored by TLC (silica, hexane/ether 7:3). The reaction mixture was washed with water, 0.5 M ammonia in methanol/water (3:1), and finally twice with methanol/water (3:1). The organic phase was dried over Na_2SO_4 and then under vacuum. The crude product (3.2 g) was purified on a 20-g silica column (eluted with hexane and then 2% ether in hexane). Pure **VIII** (2.37 g) was obtained in 94% yield. IR: 1690 cm^{-1} (thiolester). NMR: δ 0.88, tr, J = 7 Hz (—CH_3); δ 1.2—1.4, m (—CH_2—); δ 1.5—1.7, m (—CH_2—C—CO—S—); δ 2.2—2.4, m (—CH_2—CO—S—); δ 3.54, d of d, J = 12 and 5 Hz, and δ 3.64, d of d, J = 12 and 4 Hz (*sn*-1 —CH_2); δ 3.75—3.85, m (*sn*-2 —CH); δ 3.87, d of d, J = 12 Hz and 6 Hz, and δ 3.96, d of d, J = 12 and 4 Hz (*sn*-3 —CH_2); δ 7.2—7.4, m (trityl —CH).

1-S-Decanoyl-2-O-decanoyl-3-O-trityl-1-thio-sn-glycerol (**IX**)

Compound **VIII** was acylated with decanoyl chloride using the procedure described above for **VI**. Pure **IX** was obtained in 99% yield. IR: 1690 cm^{-1} (thiolester); 1740 cm^{-1} (oxyester). NMR: δ 0.87, tr, J = 7 Hz (—CH_3); δ 1.2—1.4, m (—CH_2—); δ 1.55—1.70, m (—CH_2—C—CO—S— and —CH_2—C—CO—O—); δ 2.32, tr, J = 8 Hz (—CH_2—CO—S—); δ 2.48, tr, J = 8 Hz (—CH_2—CO—O—); δ 3.10, d of d, J = 14 and 8 Hz, and δ 3.28, d of d, J = 14 and 5 Hz (*sn*-3 —CH_2); δ 3.20, d, J = 5 Hz (*sn*-1 —CH_2); δ 5.1—5.2, m (*sn*-2 —CH); δ 7.2—7.5, m (trityl —CH).

1-S-Decanoyl-2-O-decanoyl-1-thio-sn-glycero-3-phosphocholine (**X**)

Compound **IX** was detritylated with BF_3/methanol and the glycerol derivative converted to the choline phosphate (**X**) with $POCl_3$ and choline tosylate using the procedures described previously for phosphatidylcholines [7,14]. NMR: δ 0.79, tr, J = 6 Hz (—CH_3); δ 1.1—1.3, m (—CH_2—); δ 1.4—1.6, m (—CH_2—C—CO—S— and —CH_2—C—CO—O—); δ 2.20, tr, J = 8 Hz (—CH_2—CO—S—); δ 2.45, tr, J = 7 Hz (—CH_2—CO—O—); δ 2.93, d of d, J = 14 and 8 Hz (*sn*-1 —CH_2); δ 3.14, s (—$N(CH_3)_3$); δ 3.25, d of d, J = 14 and 4 Hz (*sn*-3 —CH_2); δ 3.4—3.7, m (—CH_2—N); δ 4.10—4.25, m (P—O—CH_2—); δ 4.95—5.05, m (*sn*-2 —CH). Anal. calc. for $C_{28}H_{56}PSNO_7 \cdot 3\ H_2O$: C, 52.9; H, 9.83; N, 2.20; P, 4.87. Found: C, 53.1; H, 9.97; N, 2.13; P, 4.89.

Results and discussion

Depending on the enantiomer of diisopropyl tartrate used in the Sharpless catalytic asymmetric epoxidation reaction [8], which was followed by in situ tritylation (Fig. 1), (*R*)- and (*S*)-trityl-glycidol were obtained in one-pot reactions in about 50% overall yield. The epoxide of trityl-glycidol was opened by reaction with an alkyl mercaptan, assisted by a trace amount of butyl lithium. TLC of the reaction mixture showed only one product which was identified by its NMR spectrum as the terminal *S*-alkyl derivative. Opening of the epoxide, thus, proceeds with good regiospecificity. In order to assess the optical purity of this product and also trityl-glycidol, the Mosher ester of **II** (**III**) was produced, and its NMR spectrum compared with that of the Mosher ester of racemic 3-*S*-hexadecyl-1-*O*-trityl-3-thio-glycerol. The NMR spectrum of the racemic compound showed two resolved peaks (δ 3.53 and 3.58) for the methoxy

protons, one for each diasteriomer. Optically active Mosher ester **III** showed a major peak at δ 3.58, and a very minor peak (less than 1% area) at δ 3.53. The optical purity of **III** is, thus, greater than 98% ee.

Figure 2 shows the syntheses of dithiolester, 1-thiolester and 1-thioether analogs of phosphatidylcholine, all starting with optically active trityl-glycidol. Opening of the epoxide **Ia** with methyl xanthate gave a trithiocarbonate derivative which could be converted to thio-phosphatidylcholine (**VII**) by the procedure of Hendrickson et al. [7]. This product gave exactly the same activity with phospholipase A_2 as the optically active thio-phosphatidylcholine previously synthesized from 1-trityl-*sn*-glycerol (prepared from D-mannitol [7,15]).

Opening of the epoxide **Ib** with thiodecanoic acid, following the procedure of Ward [13], gave a single product (by TLC), **VIII**, which was converted to the 1-thioester-2-oxyester analog of phosphatidylcholine (**X**). Opening of the same epoxide (**Ib**) with dodecyl mercaptan gave the thioether derivative (**IV**) which was acylated at the *sn*-2 hydroxyl group and then converted to the 1-thioether-2-oxyester analog of phosphatidylcholine (**VI**). Phospholipids **VI** and **X** were completely hydrolyzed by phospholipase A_2, each giving a single lyso-phosphatidylcholine (by TLC) with no free —SH evident (no reaction with 5,5′-dithiobis(2-nitrobenzoic acid).

The simple and inexpensive synthesis of chiral trityl-glycidol presented here provides starting material for the synthesis of both enantiomers of a variety of glycerol phospholipids, particularly thioelester and thioether phospholipid analogs. 1-Thiolester phospholipid analogs such as **X** can serve as substrates for the spectrophotometric assay of phospholipase A_1 [16]. The dithiolester analog of phosphatidylcholine, **VII**, is a good substrate for the assay of phospholipase A_2 [15]. 1-Thioether analogs of phosphatidylcholine such as **VI** and thioether analogs of platelet-activating-factor are of interest due to their reported antitumor activities [17]. The activities of these phospholipid analogs with phospholipases A_1 and A_2 will be reported in a subsequent publication.

Acknowledgements

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References

1 H. Eibl (1981) Chem. Phys. Lipids 28, 1—5.
2 C.M. Lok, J.P. Ward and D.A. van Dorp (1976) Chem. Phys. Lipids 16, 115—122.
3 J.A. Virtanen, J.R. Brotherus, O. Renkonen and M. Kates (1980) Chem. Phys. Lipids 27, 185—190.
4 S.K. Bhatia, and J. Hajdu (1988) J. Org. Chem. 53, 5034—5039.
5 C.M. Lok, A.P.J. Mank and J.P. Ward (1985) Chem. Phys. Lipids 36, 229—334.
6 W.T.M. De Groot, C.M. Lok and J.P. Ward (1988) Chem. Phys. Lipids 47, 75080.
7 H.S. Hendrickson, E.K. Hendrickson and R.H. Dybvig (1983) J. Lipid Res. 24, 1532—1537.
8 Y. Gao, R.M. Hanson, J.M. Klunder, S.Y. Ko, H. Masamune and K.B. Sharpless (1987) J. Am. Chem. Soc. 109, 5765—5780.
9 C.E. Burgos, D.E. Ayer and R.A. Johnson (1987) J. Org. Chem. 52, 4973—4977.
10 S. Ali and R. Bittman (1988) J. Org. Chem. 53, 5547—5549.
11 P.N. Guivisdalsky and R. Bittman (1989) J. Am. Chem. Soc. 111, 3077—3079.
12 A. Hermetter and F. Paltauf (1981) Chem. Phys. Lipids 29, 191—195.
13 J.P. Ward (1988) Chem. Phys. Lipids 47, 217—224.
14 H.S. Hendrickson, E.K. Hendrickson and T.J. Rustad (1987) J. Lipid Res. 28, 864—872.
15 H.S. Hendrickson, and E.A. Dennis (1984) J. Biol. Chem. 259, 5740—5744.
16 G.L. Kucera, C. Miller, P.J. Sisson, R.W. Wilcox, Z. Wiemer and M. Waite (1988) J. Biol. Chem. 263, 12964—12969.
17 E. Bosies, D.B.J. Herrmann, U. Bicker, R. Gall and W. Pahlke (1987) Lipids 22, 947—951.