Tetrahedron Letters, Vol. 34, No. 39, pp. 6193-6196, 1993
Printed in Great Britain

0040-4039/93 $6.00 + .00
Pergamon Press Ltd

# Synthesis of (R)-Lysothiophosphatidic Acid and (R)-Thiophosphatidic Acid

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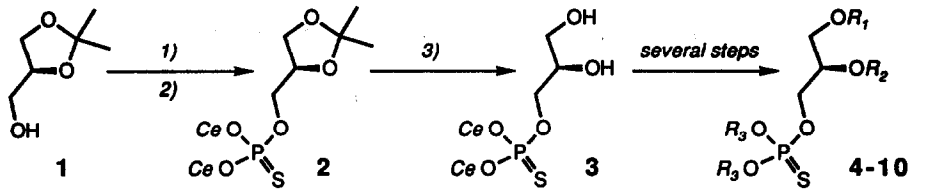
*Abstract*: (R)-Thiophosphatidic acid and (R)-lysothiophosphatidic acid were obtained in an efficient synthesis from a chiral glycerol precursor via sulfurization of a bis(2-cyanoethyl) phosphite triester.

Phospholipids are well known structural components of cell membranes. Recent studies have shown that phosphatidic and lysophosphatidic acids are not only important intermediates in phospholipid biosynthesis, but also play a significant role in signal transduction processes.[1] Phosphatidic and lysophosphatidic acids have been shown to stimulate DNA synthesis and calcium mobilization, and to have mitogenic properties.[2] These results have initiated a search for the identification of the respective receptors. Recently, two proteins were identified which act as receptors for lysophosphatidic acid.[3] To study the mechanism of action of these phospholipids, it is highly desirable to have hydrolytically stable analogs. Since thiophosphate groups are more stable to enzymatic hydrolysis, thiophosphatidic acid and lysothiophosphatidic acid are ideal candidates for mechanistic studies.[4] Eichberg *et al.* have reported the synthesis of thiophosphatidic acid in poor yield starting form 1,2-dioleoyl glycerol.[5] We observed that these conditions facilitate the migration of the acyl groups in the starting material and hence the optical purity of the final product is in doubt. Moreover, the synthesis of lysothiophosphatidic acid would not be possible by this route. Lysophosphatidic acids are usually obtained through selective enzymatic hydrolysis of natural phospholipids.[6] For example, phospholipase $A_2$ is used to hydrolyze the acyl group at C-2. Unfortunately, most phospholipases, including phospholipase $A_2$, are highly substrate specific and are often inhibited by thiophospholipids.[7] In this communication, we describe an efficient and general chemical synthesis of (R)-thiophosphatidic acid and (R)-lysothiophosphatidic acid. The reaction sequence is shown in scheme 1.

We chose (S)-2,2-dimethyl-1,3-dioxolane-4-methanol (**1**) as our precursor. With the phosphoramidite methodology, which is widely used in nucleic acid chemistry, alcohol (**1**) was efficiently phosphorylated.[8] The resulting phosphite triester was oxidized *in situ* with elemental sulfur to yield the corresponding thiophosphate triester (**2**, 92% from **1**).[9] This approach allows the introduction of $^{18}O$ or $^{35}S$ radioisotopes as needed. The acetonide (**2**) was readily cleaved in 99 % yield without racemization which was proven later by Mosher ester analysis (vide infra). Peracylation of diol (**3**) provides the protected thiophosphatidic acid (**4**) in 72 % yield. Good regioselectivity was observed when stoichiometric amount of acylating agent was used. The primary alcohol group of diol (**3**) was esterified preferentially to form lysothiophosphatidic acid (**5**) in 57 %. To verify the enantiomeric purity of (**5**), we investigated the synthesis of the corresponding Mosher esters. Interestingly, we observed migration of the oleoyl group to yield an approximately equimolar mixture of two regioisomers (**6a**) and (**7a**) starting from the optically active alcohol (**5**). Two pairs of diastereomers (**6a/b**) and (**7a/b**) were obtained from a racemic mixture of (**5**). Under basic aprotic conditions in presence of N,O-bis(trimethylsilyl)trifluoroacetamide, deprotection of cyanoethyl ester (**4**) was effected at r.t. without any side reactions to yield thiophosphatidic acid (**8**) in 84 %. The introduction of the tetrahydropyranyl group (THP) was necessary to overcome ester migration during the deprotection of lysothiophosphatidic acid (**5**). After

cleavage of the resulting cyanoethyl ester, the THP group was released under acidic conditions. Both the thiophosphatidic acid (**8**, 55 % in 5 steps from **1**) and lysothiophosphatidic acid (**10**, 28 % in 7 steps from **1**) were obtained in optically active form and in high purity after ion exchange chromatography. All the compounds were characterized by $^{1}H$-, $^{13}C$- and $^{31}P$-NMR spectroscopy and high resolution FAB-MS was obtained for key intermediates.[10]




| Compound | 3 | 4 | 5 | 6a | 7a | 8 | 9a/b | 10 |
|---|---|---|---|---|---|---|---|---|
| R1 | H | Oleoyl | Oleoyl | Oleoyl | Mosher | Oleoyl | Oleoyl | Oleoyl |
| R2 | H | Oleoyl | H | Mosher | Oleoyl | Oleoyl | THP | H |
| R3 | Ce | Ce | Ce | Ce | Ce | H | Ce | H |

***Scheme***: Thiophosphate ester synthesis via (R)-Glyceryl-thiophosphate-bis-(2-cyanoethyl)-ester (**3**). Oleoyl: (Z)-$CO(CH_2)_7HC{=}CH(CH_2)_7CH_3$, Mosher: (R)-(1-Methoxy-1-trifluoromethyl-1-phenyl)-acetyl, THP: Tetrahydropyranyl, Ce: 2-Cyanoethyl, 1) Tetrazole, $(i\text{-}Pr_2N)_2POCe$, $CH_2Cl_2$; 2) $S_8$, Pyridine-$CS_2$; 3) $CH_3OH$, Dowex $50W^R$ ($H^+$).

## EXPERIMENTAL DETAILS

3-Hydroxypropionitrile was allowed to react with 1.2 equiv. 2-cyanoethyl-N,N,N',N'-tetraisopropyl-phosphodiamidite in the presence of 1.2 equiv. of tetrazole in $CH_2Cl_2$ at r.t. for 1h. After the addition of 2 equiv. of tetrazole (S)-2,2-dimethyl-1,3-dioxolane-4-methanol (**1**) was added and the resulting phosphite triester was oxidized *in situ* with a solution of $S_8$ in $CS_2$-pyridine (1:1). The optically active product (**2**), readily purified by column chromatography ($SiO_2$, ether-hexane 8:2), was obtained in 92% yield[11] ($[\alpha]^D$: -0.4 (c=3.68, $CHCl_3$), FAB-MS calcd. for $C_{12}H_{20}N_2O_5PS$ ($M+H^+$): 335.0831, found 335.0850, $^{31}P$-NMR ($CDCl_3$) $\delta$=67.9). In $CH_3OH$ in the presence of Dowex $50W^R$ ($H^+$) the acetonide (**2**) was cleaved at r.t. overnight in 99% yield to the optically active diol[12] (**3**) ($[\alpha]^D$: -8.3 (c=0.52, $CH_3OH$), FAB-MS calcd. for $C_9H_{16}N_2O_5PS$ ($M+H^+$): 295.0518, found 295.0502, $^{31}P$-NMR ($CDCl_3$+$CD_3OD$ (5%)) $\delta$=68.2). A pyridine solution of diol (**3**) was acylated at r.t. for 48 h with 4 equiv. of oleic anhydride. The optically active product[13] (**4**), purified by column chromatography ($SiO_2$, ether-hexane 1:1), was isolated in 72% yield ($[\alpha]^D$: +3.5 (c=2.31, $CHCl_3$), $^{31}P$-NMR ($CDCl_3$) $\delta$=68.4). Regioselective monoacylation was accomplished as follows: a stoichiometric amount of an equimolar solution of N-methylimidazole and oleic anhydride in $CH_2Cl_2$ was added over a period of 2 h to a solution of diol (**3**) in $CH_2Cl_2$ at r.t. The optically active product[14] (**5**), purified by column chromatography ($SiO_2$, ether-hexane 4:1), was isolated in 57% yield ($[\alpha]^D$: -1.4 (c=2.41, $CHCl_3$), $^{31}P$-NMR ($CDCl_3$) $\delta$=68.3). The enantiomeric excess of alcohol (**5**) was determined to be >95% based on $^{1}H$-$^{19}F$- and $^{31}P$-NMR analysis of the Mosher ester derivatives.[15] Both the racemate and optically active alcohol (**5**) in $CDCl_3$ were reacted with (R)-Mosher acid chloride in presence of N-methylimidazole overnight. Approximately equimolar mixtures of two regioisomers (**6a**) and (**7a**) ($^{1}H$-NMR ($CDCl_3$) $\delta$=3.525 and 3.508, $^{19}F$-NMR ($CDCl_3$) $\delta$=107.20 and 107.07, $^{31}P$-NMR ($CDCl_3$) $\delta$=67.9 and 68.5) were formed starting from the optically active alcohol (**5**). Two diastereomeric pairs of regioisomers (**6a/b**) and (**7a/b**) ($^{1}H$-NMR

($CDCl_3$) δ=3.525 / 3.545 and 3.508 / 3.554, $^{19}$F-NMR ($CDCl_3$) δ=107.2 / 107.11 and 107.07 / 107.07, $^{31}$P-NMR ($CDCl_3$) δ=67.9 / 68.2 and 68.5 / 68.5) were obtained starting from the racemate (**5**). The $^{31}$P-NMR resonances of the two diastereomers (**6a**) δ=67.9 and (**6b**) δ=68.2 are baseline separated and with a signal-to-noise ratio of 100:1 the enantiomeric excess of (-) **5** was determined to be >95%. The cyanoethyl ester (**4**) was deprotected at r.t. in $CH_3CN$-$Et_3N$ (1:1) in presence of N,O-bis trimethylsilyl trifluoroacetamide in 48 h. The crude product was co-distilled with $CH_3OH$ (3x) and the resulting triethylammonium salt was converted to the free acid (**8**) by ion exchange column chromatography (Dowex 50W$^R$ ($H^+$), $CH_3OH$). The optically active product[16] (**8**) was isolated in 84% yield (($[\alpha]^D$: +5.6 (c=0.72, $CHCl_3$), FAB-MS calcd. for $C_{39}H_{72}O_7PS$ ($M-H^+$): 715.4736, found 715.4711, $^{31}$P-NMR ($CDCl_3$) δ=52.4). Direct deprotection of ester (**5**) under the same conditions was not successful. In $CH_2Cl_2$ the alcohol (**5**) was allowed to react with dihydropyran in the presence of Dowex 50W$^R$ ($H^+$) for 3 h and a mixture of two diastereomeric tetrahydropyranyl ethers[17] (**9a/b**) ($^{31}$P-NMR ($CDCl_3$) δ=68.6, 68.3) was obtained in 57% yield after column chromatography ($SiO_2$, ether-hexane 1:1). Deprotection of the cyanoethyl groups was achieved in $CH_3CN$-$Et_3N$ (1:1) in presence of N,O-bis(trimethylsilyl)trifluoroacetamide in 48 h. The crude product was co-distilled with $CH_3OH$ (3x) and the tetrahydropyranyl ether was cleaved in $CH_3OH$ with Dowex 50W$^R$ ($H^+$). Optically active lysothiophosphatidic acid[18] (**10**) was obtained after purification by ion exchange column chromatography (Dowex 50W$^R$ ($H^+$), $CH_3OH$) in 95% yield ($[\alpha]^D$: -1.2 (c=1.55, $CHCl_3$), FAB-MS calcd. for $C_{21}H_{40}O_6PS$ ($M-H^+$): 451.2283, found 451.2291, $^{31}$P-NMR ($CDCl_3$) δ=59.9).

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10 $^1$H- (300 MHz), $^{13}$C- (75 MHz), $^{19}$F- (282.8 MHz) and $^{31}$P- (121.7 MHz) NMR spectra were obtained on a General Electric GE-QE 300. Chemical shifts (δ) are reported in ppm, coupling constants (J) in Hz. $^1$H- and $^{13}$C-chemical shifts were determined with respect to $CHCl_3$ (7.24 ppm and 77.0 ppm) and 85% $H_3PO_4$ was used as an internal standard (0.0 ppm) for $^{31}$P-spectra.

11 **$^1$H-NMR** ($CDCl_3$): 1.37 (s, 3H, H-C(Me)); 1.45 (s, 3H, H-C(Me)); 2.78 (t, J=6.5 Hz, 4H, H-C(Ce)); 3.81 (dd, J=8.7, 5.4 Hz, 1H, H-C(3)); 4.08 (dd, J=8.7, 6.8 Hz, 1H, H-C(3)); 4.09-4.16 (m, 2H, H-C(1)); 4.25-4.37 (m, 5H, H-C(2) + H-C(Ce)). **$^{13}$C-NMR** ($CDCl_3$): 19.4 (td, $J_{P-C}$=7.5 Hz, C(Ce)); 25.2 (q); 26.7 (q); 62.6 (td, $J_{P-C}$=5.0 Hz, C(Ce)); 65.8 (t, C(3 )); 68.8 (td, J =6.0 Hz, C(1)); 73.8 (dd, $J_{P-C}$=8.3 Hz, C(2)); 110.0 (s); 116.6 (s, C(Ce)).

12 **$^1$H-NMR** ($CDCl_3$ + $CD_3OD$ (5%)): 2.79 (t, J=6.0 Hz, 4H, H-C(Ce)); 3.56 (dd, J=11.5, 5.9 Hz, 1H, H-C(3)); 3.64 (dd, J=11.5, 4.2 Hz, 1H, H-C(3)); 3.89 (tdd, J=5.9, 4.5, 4.2 Hz, 1H, H-C(2)); 4.09 (ddd, J=10.5, 5.9 Hz, $J_{P-H}$=9.2 Hz, 1H, H-C(1)); 4.15 (ddd, J=10.5, 4.5 Hz, $J_{P-H}$=8.5 Hz, 1H, H-C(1)); 4.29 (td, J=6.0 Hz, $J_{P-H}$=9.8 Hz, 4H, H-C(Ce)). **$^{13}$C-NMR** ($CDCl_3$): 19.4 (td, $J_{P-C}$=8.3 Hz, C(Ce)); 62.6 (t, C(3)); 62.6 (td, $J_{P-C}$=4.5 Hz, C(Ce)); 69.5 (td, J =6.0 Hz, C(1)); 70.2 (dd, $J_{P-C}$=7.5 Hz, C(2)); 116.6 (s, C(Ce)).

13 **$^1$H-NMR** ($CDCl_3$): 0.9 (t, J=7.0 Hz, 6H, H-C(Me)); 1.2-1.4 (m, 40H, H-C(Oleoyl)); 1.5-1.7 (m, 4H, H-C(Oleoyl)); 1.9-2.1 (m, 8H, H-C(Oleoyl)); 2.3-2.4 (m, 4H, H-C(Oleoyl)); 2.77 (t, J=6.1 Hz, 4H, H-C(Ce)); 4.1-4.35 (m, 8H, H-C(1) + H-C(3) + H-C(Ce)); 5.2-5.3 (m, 1H, H-C(2)); 5.3-5.4 (m, 4H, H-C(Oleoyl)). **$^{13}$C-NMR** ($CDCl_3$): 14.1(q); 19.4 (td, $J_{P-C}$=8.3 Hz, C(Ce)); 22.7 (t); 24.8 (t); 27.1 (t); 27.2 (t); 28.7 (t); 28.8 (t); 28.85 (t); 28.9 (t); 29.08 (t); 29.10 (t); 29.2 (t); 29.3 (t); 29.4 (t); 29.42 (t); 29.5 (t); 29.6 (t); 29.7 (t); 29.74 (t); 31.9 (t); 34.0 (t); 34.1 (t); 61.5 (t, C(3); 62.7 (td, $J_{P-C}$=3.8 Hz, C(Ce)); 66.5 (td, J =4.5 Hz, C(1)); 69.1 (dd, $J_{P-C}$=8.3 Hz, C(2)); 116.2 (s, C(Ce)); 129.7 (d); 130.0 (d); 172.8 (s).

14 **$^1$H-NMR** ($CDCl_3$): 0.9 (t, J=7.0 Hz, 3H, H-C(Me)); 1.2-1.4 (m, 20H, H-C(Oleoyl)); 1.6-1.7 (m, 2H, H-C(Oleoyl)); 2.0-2.1 (m, 4H, H-C(Oleoyl)); 2.39 (t, J=7.0 Hz, 2H, H-C(Oleoyl)); 2.80 (t, J=6.1 Hz, 4H, H-C(Ce)); 4.1-4.35 (m, 5H, H-C(1) + H-C(2) + H-C(3)); 4.29 (td, J=6.0 Hz, $J_{P-H}$=9.8 Hz, 4H, H-C(Ce)); 5.37 (m, 2H, H-C(Oleoyl)). **$^{13}$C-NMR** ($CDCl_3$): 14.1(q); 19.4 (td, $J_{P-C}$=7.5 Hz, C(Ce)); 22.7 (t); 24.8 (t); 27.1 (t); 27.2 (t); 29.1 (t); 29.2 (t); 29.3 (t); 29.5 (t); 29.7 (t); 29.74 (t); 31.9 (t); 34.1 (t); 62.7 (td, $J_{P-C}$=4.5 Hz, C(Ce)); 64.3 (t, C(3); 68.5 (dd, $J_{P-C}$=7.5 Hz, C(2)); 69.4 (td, J =5.3, C(1)); 116.6 (s, C(Ce)); 129.7 (d); 130.0 (d); 173.9 (s).

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16 **$^1$H-NMR** ($CDCl_3$): 0.9 (t, J=7.0 Hz, 6H, H-C(Me)); 1.2-1.4 (m, 40H, H-C(Oleoyl)); 1.5-1.7 (m, 4H, H-C(Oleoyl)); 1.9-2.1 (m, 8H, H-C(Oleoyl)); 2.3-2.5 (m, 4H, H-C(Oleoyl)); 4.1-4.3 (m, 3H, H-C(1) + H-C(3)); 4.34 (dd, J=4.0, 12.0 Hz, 1H, H-C(3)); 5.2-5.4 (m, 5H, H-C(2)+ H-C(Oleoyl)). **$^{13}$C-NMR** ($CDCl_3$): 14.1(q); 22.7 (t); 24.8 (t); 27.18 (t); 27.22 (t); 29.0 (t); 29.08 (t); 29.10 (t); 29.2 (t); 29.3 (t); 29.4 (t); 29.5 (t); 29.6 (t); 29.7 (t); 29.8 (t); 31.9 (t); 34.1 (t); 34.3 (t); 62.2 (t, C(3); 65.3 (td, J =3.8, C(1)); 69.7 (dd, $J_{P-C}$=8.3 Hz, C(2)); 129.7 (d); 130.0 (d); 173.9 (s); 174.2 (s).

17 Two diastereomers: **$^1$H-NMR** ($CDCl_3$): 0.9 (t, J=7.0 Hz, 3H); 1.2-1.4 (m, 20H, H-C(Oleoyl)); 1.45-1.8 (m, 8H, H-C(THP) + H-C(Oleoyl)); 2.0-2.1 (m, 4H, H-C(Oleoyl)); 2.30 (t, J=7.0 Hz, 1H, H-C(Oleoyl)); 2.32 (t, J=7.0 Hz, 1H, H-C(Oleoyl)); 2.75 (t, J=6.1 Hz, 2H, H-C(Ce)); 2.77 (t, J=6.1 Hz, 2H, H-C(Ce)); 3.70-3.95 (m, 2H, H-C(THP); 4.05-4.35 (m, 9H, H-C(1) + H-C(2) + H-C(3) + H-C(Ce)); 4.73 (t, J=3 Hz, 0.5H, H-C(THP)); 4.78 (t, J=3 Hz, 0.5H, H-C(THP)); 5.37 (m, 2H, H-C(Oleoyl)).

18 **$^1$H-NMR** ($CDCl_3$): 0.9 (t, J=7.0 Hz, 3H); 1.2-1.4 (m, 20H, H-C(Oleoyl)); 1.5-1.7 (m, 2H, H-C(Oleoyl)); 1.90-2.05 (m, 4H, H-C(Oleoyl)); 2.30 (t, J=7.0 Hz, 2H, H-C(Oleoyl)); 3.95-4.20 (m, 5H, H-C(1) + H-C(2) + H-C(3)); 5.37 (m, 2H, H-C(Oleoyl)). **$^{13}$C-NMR** ($CDCl_3$): 14.1(q); 22.6 (t); 24.8 (t); 27.2 (t); 29.10 (t); 29.13 (t); 29.2 (t); 29.3 (t); 29.5 (t); 29.65 (t); 29.72 (t); 31.9 (t); 34.0 (t); 64.4 (t, C(3); 67.7 (td, J =4.5 Hz, C(1)); 69.0 (dd, $J_{P-C}$=7.5 Hz, C(2)); 129.7 (d); 129.9 (d); 174.8 (s).

(Received in USA 10 June 1993; accepted 30 July 1993)