General and Efficient Route to Phosphorodithioate Analogues of Naturally Occurring Lipids

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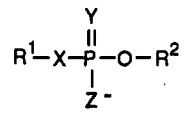
Summary: A general procedure has been developed for the efficient synthesis of lipid phosphorodithioate analogues **14a-f** from 2-alkoxy-2-thio-1,3,2-dithiaphospholanes **13a-c**, which are readily prepared from 2-chloro-1,3,2-dithiaphospholane (**11**) by sequential reaction with an alcohol and elemental sulfur.

The synthesis of nonhydrolyzable phosphate mimics has become an important adjunct to the study of those biological pathways and enzyme mechanisms that involve processing of mono- and diesters of phosphoric acid. Typically these investigations are executed using synthetic analogues of the natural phosphate in which a phosphonate **1**, difluorophosphonate **2**, phosphoramidate **3**, thiophosphate **4**, or phosphorothioate **5** group serves as a replacement of the normal phosphate moiety.[1] Although phosphorothioates have been exploited to gain important insights regarding the stereochemical aspects of nucleophilic displacements at phosphorous, the stereogenic center at phosphorous in **5** requires that the stereoisomeric phosphorothioates be first separated, a task that may prove laborious.[2] More recently, the phosphorodithioate group **6** has emerged as an efficacious phosphate replacement since it is generally nonhydrolyzable by normal enzymatic processing, and it is approximately isosteric and isopolar with the phosphate functional group. For example, when the phosphorodithioate moiety **6** is incorporated in oligonucleotides, the resulting linkage is resistant to cleavage by a wide variety of nucleases, and hence this moiety is an attractive phosphate replacement in studies involving the antisense modulation of gene expression and the inhibition of viral reverse transcriptases.[3-5]



1: X = CH_2; Y = Z = O
2: X = CF_2; Y = Z = O
3: X = NH; Y = Z = O
4: X = S; Y = Z = O
5: X = Z = O; Y = S
6: X = O; Y = Z = S

Stabilization of the phosphodiester function in phospholipids can also have significant biological consequences. For example, enzymes of the phospholipase C (PLC) class hydrolyze phospholipids to generate a diacylglycerol and the corresponding phosphorylated head group, which may be choline, ethanolamine, serine, or an inositol derivative.[6] In mammalian cells, PLC enzymes are intimately involved in the process of signal transduction, which is the cascade of metabolic events that eventuates in a cellular response to an extracellular stimulus. In this sequence, the PLC-mediated hydrolysis of phosphatidylinositol-4,5-bisphosphate releases two second messengers—inositol 1,4,5-trisphosphate, which then stimulates the release of calcium from the endoplasmic reticulum, and diacylglycerol, which activates protein kinase C. In order to study the mechanism of phospholipid hydrolysis by PLC isoenzymes and to identify the biological consequences of interfering with this process, we undertook the challenge of preparing modified phospholipids bearing the phosphate group replacements **1-6**. Several general and efficient methods for the synthesis of such phospholipid analogues have been disclosed,[7,8] and we have recently demonstrated that these substances are competitive inhibitors of the bacterial PLC from *Bacillus cereus*.[9] The crystal structure of one inhibitor bound to the active site of this enzyme has been solved, thereby revealing the key interactions between active site residues and the phospholipid ligand.[10]

When we attempted to prepare the requisite phosphorodithioate analogues of phospholipids, we discovered that many of the methods reported for such constructions, some of which we examined in detail, had significant limitations. The synthesis of phosphorodithioates **6** from *H*-phosphonothioates,[11] *H*-phosphonodithioates,[3,12] phosphorodiamidites,[3,12e,13] thiophosphoramidites,[3-5,12e,14] and phosphorodithioates[15] typically involves multistep processes that require protection of the PSH group, an operation which is sometimes problematic. Various side reactions

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(2) (a) Eckstein, F. *Acc. Chem. Res.* **1979**, *12*, 204-208. (b) Stec, W. J.; Grajkowski, A.; Koziolkiewicz, M.; Uznanski, B. *Nucleic Acids Res.* **1991**, *19*, 5883-5888. (c) Jiang, R.-T.; Shyy, Y.-J.; Tsai, M.-D. *Biochemistry* **1984**, *23*, 1661-1667. (d) Lin, G.; Bennett, F.; Tsai, M.-D. *Biochemistry* **1990**, *29*, 2747-2757.

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(4) Bjergarde, K.; Dahl, O. *Nucleic Acids Res.* **1991**, *19*, 5843-5850.

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(7) (a) Martin, S. F.; Josey, J. A. *Tetrahedron Lett.* **1988**, *29*, 3631-3634. (b) Martin, S. F.; Josey, J. A.; Dean, D. W.; Wong, Y.-L. Unpublished results.

(8) Martin, S. F.; Dean, D. W.; Wagman, A. S. *Tetrahedron Lett.* **1992**, *33*, 1839-1842.

(9) Martin, S. F.; Wong, Y.-L.; Wagman, A. S. Unpublished results.

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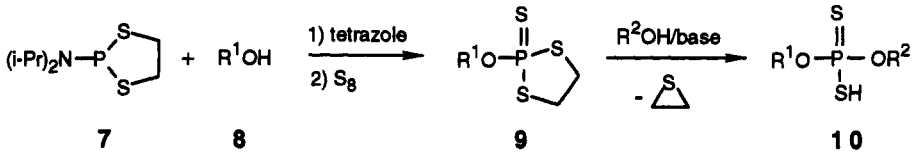
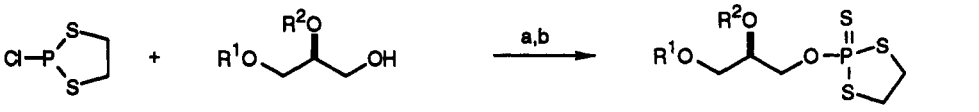
0022-3263/93/1958-5897$04.00/0

Scheme I



7 8 9 10

Scheme II[a]



11

12a: $R^1 = R^2 = n\text{-}C_5H_{11}CO$
12b: $R^1 = R^2 = n\text{-}C_{15}H_{31}CO$
12c: $R^1 = n\text{-}C_{16}H_{33}$, $R^2 = CH_3CO$

13a: $R^1 = R^2 = n\text{-}C_5H_{11}CO$
13b: $R^1 = R^2 = n\text{-}C_{15}H_{31}CO$
13c: $R^1 = n\text{-}C_{16}H_{33}$, $R^2 = CH_3CO$

[a] Key: (a) $EtN(i\text{-}Pr)_2$, CH_3CN or THF, −38 °C → rt, 3 h; (b) S_8, CS_2, rt, 6 h.

also attend many of these methods to further discourage their general application.[16] An attractive alternative to these procedures was recently reported by Stec,[17] who described a practical method for the preparation of di(deoxyribonucleoside) phosphorodithioates **10** (R^1 = 3′-*O*-deoxyribonucleoside; R^2 = 5′-*O*-deoxyribonucleoside) according to the general plan depicted in Scheme I. The key step in the sequence was inspired by the mechanistic work of Hamer[18] and involved the base-induced reaction of 5′-OH nucleosides with 2-thio-1,3,2-dithiaphospholanes **9** (R^1 = 5′-*O*-DMT-3′*O*-deoxyribonucleoside), which were generated by the reaction of 3′-OH nucleosides **8** with **7** in the presence of tetrazole. It occurred to us that a simple modification of this strategy might lead to an efficacious route to the phosphorodithioate analogues of phospholipids, and the successful realization of this objective constitutes the substance of this report.

On the basis of our earlier work,[7] we reasoned that the more reactive and readily available phosphatitylating reagent 2-chloro-1,3,2-dithiaphospholane (**11**)[19] might be superior to **7** for the synthesis of phospholipid derivatives. In accord with this expectation, we discovered that **11** underwent facile reaction with a variety of suitably substituted *sn*-glycerols **12a-c** to produce the 2-alkoxy-1,3,2-dithiaphospholanes **13a-c** in high yields (Scheme II).[20] This coupling was executed in the presence of a slight excess (1.1 equiv) of diisopropylethylamine, a non-nucleophilic base, and in dry, deoxygenated solvents to reduce the probability of undesired side reactions. The phosphite reagent **11**, unlike **7**, requires no activation using tetrazole, and it is sufficiently reactive that even bulky primary alcohols as **12b** react readily.[21] The sulfuration of the intermediate trivalent phosphite to give the product 2-alkoxy-2-thio-1,3,2-dithiaphospholanes **13a-c** was achieved in 74-95% yields simply by brief exposure to elemental sulfur dissolved in carbon disulfide.

The 2-alkoxy-2-thio-1,3,2-dithiaphospholanes **13a-c** were then transformed into a variety of phospholipid phosphorodithioates **14a-f** in 81–95% yields. The reaction, which proceeded with extrusion of ethylene sulfide, was simply induced by treating **13a-c** with a suitably protected head group in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (Schemes III and IV). Thus, reaction of **13a** with protected ethanolamine, serine, and *myo*-inositol derivatives gave the protected phosphorodithioates **14a-c**. In the cases of **14a,b**, the dithioate product was initially isolated as a DBU salt, but the free lipid could be liberated by elution through an Amberlyst-15 ion-exchange column (basic form) using ethanol. Alternatively, the DBU could be removed from the serine analogue **14b** by exposure to aqueous 2.8 M HF, followed by purification by flash chromatography. The phosphorodithioates **14a-c** were deprotected under acidic conditions to give **15a-c**, which are the phosphorodithioate derivatives of phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol, respectively. Base-induced reaction of **13a,b** with choline tosylate afforded the corresponding phospho-

(11) For leading references, see: (a) Stawinski, J.; Thelin, M.; Zain, R. *Tetrahedron Lett* **1989**, *30*, 2157–2160. (b) Dreef, K. E.; Dreef-Tromp, C. M.; van der Marel, G. A.; van Boom, J. H. *Synlett* **1990**, 481–483. (c) Wada, T.; Hata, T. *Tetrahedron Lett.* **1990**, *31*, 7461–7462. (d) Hosaka, H.; Nakamura, H.; Funakoshi, H.; Takaku, H. *Chem. Lett.* **1992**, 935–938.

(12) For leading references, see: (a) Poritt, G. M.; Reese, C. B. *Tetrahedron Lett.* **1989**, *30*, 4713–4716. (b) Brill, W. K.-D.; Yau, E. K.; Caruthers, M. H. *Tetrahedron Lett.* **1989**, *30*, 6621–6624. (c) Poritt, G. M.; Reese, C. B. *Tetrahedron Lett.* **1990**, *31*, 1319–1322. (d) Murata, T.; Iwai, S.; Ohtsuka, E. *Nucleic Acids Res.* **1990**, *18*, 7279–7286. (e) Brill, W. K.-D.; Nielsen, J.; Caruthers, M. H. *J. Am. Chem. Soc.* **1991**, *113*, 3972–3980.

(13) For example, see: (a) Grandas, A.; Marshall, W. S.; Bielsen, W. S.; Caruthers, M. H. *Tetrahedron Lett.* **1988**, *30*, 543–546. (b) Nielsen, J.; Brill, W. K.-D.; Caruthers, M. H. *Tetrahedron Lett.* **1988**, *29*, 2911–2914. (c) Dahl, B. H.; Bjergarde, K.; Sommer, V. B.; Dahl, O. *Nucleosides Nucleotides* **1989**, *8*, 1023-1027.

(14) For example, see: (a) Brill, W. K.-D.; Nielsen, J.; Caruthers, M. H. *Tetrahedron Lett.* **1988**, *29*, 5517–5520. (b) Farschtschi, N.; Gorenstein, D. G. *Tetrahedron Lett.* **1988**, *29*, 6843–6846. (c) Dahl, B. H.; Bjergarde, K.; Sommer, V. B.; Dahl, O. *Acta Chem. Scand.* **1989**, *43*, 896–901. (d) Piotto, M. E.; Granger, J. N.; Cho, Y.; Farschtschi, N.; Gorenstein, D. G. *Tetrahedron* **1991**, *47*, 2449–2461.

(15) (a) Yau, E. K.; Ma, Y. X.; Caruthers, M. H. *Tetrahedron Lett.* **1990**, *31*, 1953-1956. (b) Dahl, B. H.; Bjergarde, K.; Nielsen, J.; Dahl, O. *Tetrahedron Lett.* **1990**, *31*, 3489–3492.

(16) For example, in a number of procedures, an alkyl- or arylmercaptan is introduced at the trivalent phosphite stage. Trivalent phosphorous is not only prone to oxidation, it but can become resistant to nucleophilic substitution when first coupled with a thiol. A further complication of the thiophosphoramidite systems is that -SR groups attached to phosphorus become leaving groups in the acidic environment used to activate dialkylamines as leaving groups. This is known to lead to intermolecular rearrangement products as well as the desired additions. A number of these shortcomings are documented in refs 13c and 14c,d.

(17) Okruszek, A.; Sierzchala, A.; Sochacki, M.; Stec, W. J. *Tetrahedron Lett.* **1992**, *33*, 7585–7588.

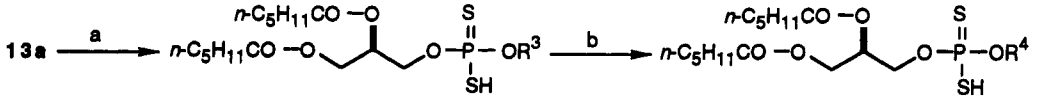
(18) (a) Hamer, N. K. *Chem. Commun.* **1968**, 1399. (b) Gay, D.; Hamer, N. K. *J. Chem. Soc. B* **1970**, 1123–1127.

(19) Boudjebel, H.; Goncalves, H.; Mathis, F. *Bull. Soc. Chim. Fr.* **1974**, *5*, 1671-1674.

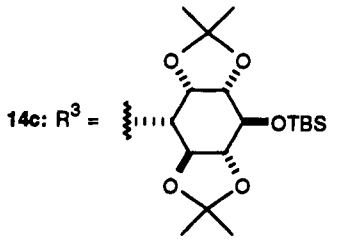
(20) The structure assigned to each compound was in full accord with its spectral (^1H and ^{13}C NMR, IR, MS) characteristics. Analytical samples of all new compounds were obtained by recrystallization, flash chromatography, or preparative HPLC and gave satisfactory identification by high-resolution mass spectrometry. All yields are based on isolated, purified materials.

(21) Stepanov, A. E.; Shvets, V. I. *Chem. Phys. Lipids* **1980**, *41*, 1–51.

Scheme III[a]

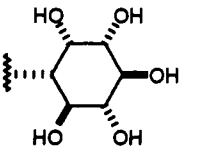


14a: $R^3 = CH_2CH_2NHBoc$
14b: $R^3 = CH_2CH(NHBoc)CO_2Bu^t$



14c: R^3 =

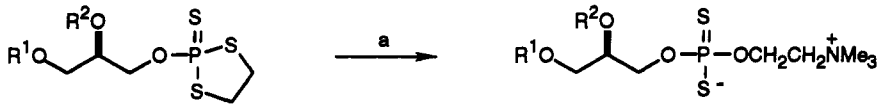
15a: $R^4 = CH_2CH_2NH_2$
15b: $R^4 = CH_2CH(NH_2)CO_2H$



15c: R^4 =

[a] Key: (a) R^3OH, DBU, MeCN, rt, 0.5 h; (b) aqueous HF or CF_3CO_2H, CH_2Cl_2, rt, 8 h.

Scheme IV[a]



13a: $R^1 = R^2 = n\text{-}C_5H_{11}CO$
13b: $R^1 = R^2 = n\text{-}C_{15}H_{31}CO$
13c: $R^1 = n\text{-}C_{16}H_{33}$, $R^2 = CH_3CO$

14d: $R^1 = R^2 = n\text{-}C_5H_{11}CO$
14e: $R^1 = R^2 = n\text{-}C_{15}H_{31}CO$
14f: $R^1 = n\text{-}C_{16}H_{33}$, $R^2 = CH_3CO$

[a] Key: (a) $HOCH_2CH_2N^+Me_3$, TsO^-, DBU, MeCN, rt, 0.5 h.

rodithioate analogues of phosphatidylcholine **14d,e**, whereas treatment of **13c** with choline tosylate in the presence of base gave **14f**, an analogue of platelet activating factor (PAF).[22]

General Experimental Procedure for the Conversion of 12a-c into 14a-f and 15a-c. To a stirred solution of alcohol **12a-c** (1.0 mmol) and diisopropylethylamine (1.1 mmol) in dry, oxygen-free MeCN (10 mL) at −38 °C (dry ice/1,2-dichloroethane slush) was added dropwise 2-chloro-1,3,2-dithiaphospholane (**11**) (0.16 g, 1.0 mmol) in MeCN (1 mL). After being stirred for 2 h, the reaction mixture was warmed to room temperature and stirring continued for an additional 1 h. A solution of S_8 (5 mmol) in CS_2 (5 mL) was then added, and the resultant light yellow heterogeneous mixture was stirred vigorously for 6 h. The reaction mixture was concentrated under reduced pressure and then dissolved in EtOAc (5 mL). The yellow flocculant solid that formed was removed by filtration through a plug of glasswool. The filtrate was concentrated under reduced pressure, and the residue was purified by flash chromatography using acetone/chloroform/water (6.7:3.2:0.1) as the solvent to deliver **13a-c**, which had a single peak in the ^{31}P NMR spectrum at approximately +124 ppm, as viscous oils. The 2-alkoxy-2-thio-1,3,2-dithiaphospholane intermediates **13a-c** were converted into phosphorodithioates **14a-f** by adding DBU (1.0 mmol) to a stirred solution of the appropriate alcohol (1.0 mmol) and **13a-c** (1.0 mmol) in MeCN (10 mL) at room temperature. When the reaction was complete, generally ca. 15–30 min as judged by TLC, the reaction mixture was concentrated under reduced pressure. The crude product was then purified by flash chromatography using acetone/chloroform/water (6.7:3.2:0.1) as the eluent.[23] The phosphorodithioates **14a-f** exhibited a single peak in the ^{31}P NMR spectrum at approximately +117 ppm. The phosphorodithioates **14a** and **14c** were deprotected by treatment with ca. 10 equiv of 2.8 M aqueous HF (4 mL) in MeCN (10 mL). The serine analogue **14b** was deprotected by stirring for 8 h in 30% TFA/CH_2Cl_2. Deprotected **14a-c** were then purified by flash chromatography eluting with either acetone/chloroform/water (6.7:3.2:0.1) or chloroform/methanol/water (6.5:3.2:0.3) to provide **15a-c** as yellow glasses.

The two-step procedure outlined herein for the synthesis of phosphorodithioate analogues of phospholipids appears general as well as being efficient and easy to execute. We anticipate that it may be readily extended to the preparation of a variety of phosphorodithioates and are currently exploring some of these possibilities.

Acknowledgment. We thank the National Institutes of Health and the Robert A. Welch Foundation for support of this work.

Supplementary Material Available: ^1H and ^{13}C NMR spectra of all new compounds (27 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(22) For some leading references to PAF and PAF antagonists, see: (a) Hanahan, D. J. *Ann. Rev. Biochem.* **1986**, *55*, 483–509. (b) Braquet, P. *CRC Handbook of PAF and PAF Antagonists*; CRC Press: Boca Raton, FL, 1991; pp 1–282.

(23) When the head group was choline, the excess choline tosylate was removed by extraction of the product oils from the insoluble amine salts with chloroform.