these conditions results in part from surface catalysis obtained with the partially stainless steel pressure bottles we employed. Thus, thermolysis of **2** in a sealed, base washed, pyrex tube (3 mg/mL in hexane) for 8 h at 140 °C resulted in only 20% conversion to **4**. The addition of stainless steel, iron powder, or powdered pyrex (less effective) to identical reaction mixtures produced significant rate accelerations and afforded **4** in good yield.

(16) Gompper, R.; Rühle, H. *Justus Liebigs Ann. Chem.* **1959,** 83.
(17) The stereochemistry of **10** has not been established. The indicated stereochemistry is that expected from an ene reaction.
(18) Blomquist, A. T.; Meador, J. D. *J. Org. Chem.* **1967,** *32,* 3986.
(19) A. P. Sloan Foundation Fellow, 1975–1977; Camille and Henry Dreyfus Teacher-Scholar Award Recipient, 1976–1981.
(20) Recipient of a Presidents Undergraduate Research Fellowship, 1977.

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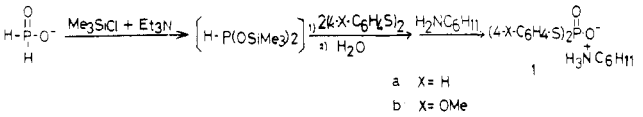
Synthesis and Properties of *S,S*-Diaryl Nucleoside Phosphorodithioates in Oligonucleotide Synthesis

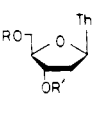
Summary: A new class of phosphorylating agents, *S,S*-diaryl phosphorodithioates, for the synthesis of oligothymidylates is described and properties of the bis(phenylthio) and bis(4-methoxyphenylthio) groups are also discussed.

Sir: In oligonucleotide synthesis, the so-called "triester approach" has recently been generalized and reported in a number of laboratories.[1-12] However, only a few examples are known of the synthesis of oligonucleotides bearing the 5′-phosphate end group utilizing this method.[13] Recently, we have investigated the chemical synthesis[14] of 5′-terminal regions of mRNAs from cytoplasmic polyhedrosis virus ($m^7G^{5'}$pppAmpGpUp... discovered by Furuichi and Miura[15]). For the large-scale synthesis of the terminal structure of mRNAs, an "activatable" protecting group for the 5′-terminal phosphate is required to construct the triphosphate structure.

In this paper we wish to report the synthesis of oligothymidylates bearing 5′-terminal phosphate by use of *S,S*-diaryl phosphorodithioates as the preliminary study for the chemical synthesis of defined mRNAs. Two kinds of cyclohexylammonium *S,S*-diaryl phosphorodithioates (**1a** and **1b**) were

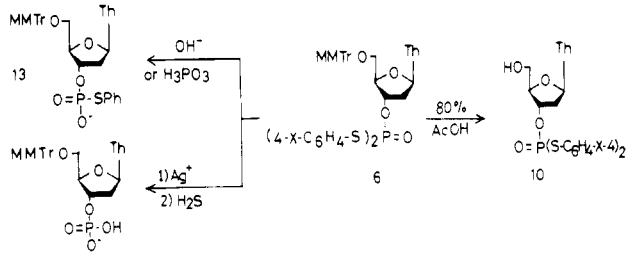


readily prepared by the reaction of bis(trimethylsilyl) hypophosphite, formed by the silylation of hypophosphorous acid, with 2.05 equiv of diaryl disulfide in 83 and 55% yields, respectively.[16] Compounds **1a** and **1b** (1.1–1.2 equiv) were condensed with appropriately protected thymidine derivatives (**2–4**) by the use of 2,4,6-triisopropylbenzenesulfonyl chloride



2 R = MMTr, R′ = H
3 R = Tr, R′ = H
4 R = H, R′ = Ac
5 R = H, R′ = H
6 R = MMTr, R′ = (4-X-C6H4-S)2P(O)
7 R = Tr, R′ = (4-X-C6H4-S)2P(O)
8 R = (4-X-C6H4-S)2P(O), R′ = Ac
9 R = (4-X-C6H4-S)2P(O), R′ = H
11 R = H, R′ = (HO)(PhS)P(O)
16 R, R′ = (4-X-C6H4S)2PTp(SC6H4-X-4)2

(TPS) (2.2–2.4 equiv)[17] in pyridine for 20–24 h. The phosphorylated products (**6–8**) were obtained in 88–96% yields. In a similar manner, unprotected thymidine was phosphorylated selectively on the 5′-hydroxyl group of the sugar moiety to afford *S,S*-diaryl thymidine 5′-phosphorodithioates (**9a** and **9b**) in 66 and 71% yields, respectively.[18] The products **9a** and **9b** were found to be quite stable in dry or aqueous pyridine and also in alcohols for several weeks. Selective removal of the monomethoxytrityl or trityl group from **6a, 6b, 7a,** and **7b** was performed without any loss of the arylthio group by treatment

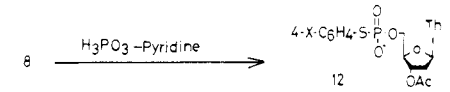


with 80% acetic acid at room temperature for 6 h or at 100 °C for 15 min. The corresponding detritylated products (**10a** and **10b**) were obtained in more than 94% yields in each case. Even when **6a** was heated in 80% acetic acid at 100 °C for 1 h, **10a** was isolated in 91% yield.[19]

Contrary to facile removal of the phenylthio group from *S*-phenyl nucleoside phosphorothioates (diester-type) by treatment with iodine in aqueous pyridine,[20] the bis(arylthio) groups of **6–9** were extremely stable toward oxidizing agents such as iodine, sodium periodate, hydrogen peroxide, iodosobenzene, and *N*-chlorosuccinimide. However, both phenylthio groups of **6a–9a** could be readily removed from **6a–9a** by treatment with 16 equiv of silver acetate or silver nitrate in aqueous pyridine at room temperature for 16 h to afford the corresponding thymidylates in quantitative yields.[21] For complete removal of the two 4-methoxyphenylthio groups from **6b–9b,** 20 equiv of silver acetate was required.[22]

S-Ethyl[23] and *S*-phenyl[20] nucleoside phosphorothioates are known to react with oxidizing agents and metal salts to generate metaphosphate intermediates, which react with nucleophiles to give the corresponding phosphorylated products. Accordingly, selective removal of one arylthio group from **6–9** is important in connection with the chemical synthesis of the 5′-terminus of mRNAs.

One of the arylthio groups could be removed from *S,S*-diaryl nucleoside phosphorodithioates (for example, **6a** and **6b**) by treatment with 0.2 N NaOH–dioxane (1:1, v/v) at room temperature for 15 min.[24] However, these conditions are similar to those for removal of acyl groups used often in oligonucleotide synthesis. Therefore, we have developed an alternative method for selective deprotection of one arylthio group from **6–9.** It was found that a solution of phosphorous



acid in pyridine was remarkably effective for this purpose. When **8a** was treated with 4 equiv of phosphorous acid in pyridine at room temperature for 24 h, *S*-phenyl 3′-*O*-acetylthymidine 5′-phosphorothioate (**12a**) was obtained quantitatively. On the other hand, on treatment of **6a** under the same conditions, the monomethoxytrityl group remained intact and *S*-phenyl 5′-*O*-monomethoxytritylthymidine 3′-phosphorothioate (**13**) was isolated in 86% yield.[25] *It is noteworthy that no deacylation and no detritylation occurred under these conditions.*[26] The specific effect of phosphorous acid should be emphasized because monoalkyl phosphates such as 2,2,2-trichloroethyl phosphate had only negligible effect.

Next, the synthesis of thymidylyl(3′→5′)thymidine 5′-phosphate (pTpT) was examined starting from **9a** and **9b.** For this purpose, the 2,2,2-trichloroethyl group[2] was chosen as the protecting group of the internucleotidic phosphate. First, **9a** was condensed with 1.2 equiv of 2,2,2-trichloroethyl phosphate at room temperature for 8 h and then the phosphorylated product (**14a**) was coupled with thymidine using two kinds of condensing agents, TPS and (4-nitrobenzenesulfonyl)triazole (NBST),[6] at room temperature for 18 h. TPS gave a better yield (92%) of the fully protected dinucleotide (**15a**) than

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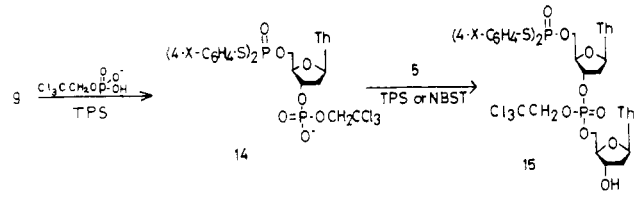

NBST (44%). The difference between TPS and NBST seems attributable to 1,2,4-triazole, which can accumulate as NBST reacts and attack the phosphoryl group of **14** and **15** to give phosphorotriazolides which hydrolyze during workup to water-soluble nucleotidic substances. This view is supported by the fact that treatment of **8a** with 1 equiv of 1,2,4-triazole in pyridine at room temperature for 70 h at the same concentration as that of the coupling reaction using NBST gave **12a** in 30% yield. On the other hand, when **9b** was employed in a similar coupling reaction, the corresponding protected thymidylate (**15b**) was obtained in 90% yield even in the case of NBST. In fact, an independent experiment in which **8b** was mixed with 1 equiv of 1,2,4-triazole in pyridine at room temperature for 70 h resulted in only 2.3% formation of **12b.**

Removal of all protecting groups from **15a** and **15b**[27] was carried out by treatment with 16–20 equiv of silver acetate in aqueous pyridine followed by treatment with zinc powder in the presence of acetylacetone in dimethylformamide–pyridine (2:1, v/v).[28] pTpT was isolated by paper chromatography in more than 99% yields from **15a** and **15b.** The following one-step removal of two phenylthio groups and 2,2,2-trichloroethyl group should also be noted. When **15a** was treated only with zinc–acetylacetone for 30 h, direct conversion to pTpT (65%) was realized. It appears that an active cation, $ZnCl^+$, formed as a result of removal of the 2,2,2-trichloroethyl group, attacked the phenylthio group. This deprotection reaction was accelerated by addition of 4 equiv of benzenethiol (84% of pTpT after 2 h). Mild treatment of **15a** and **15b** with 4–6 equiv of phosphorous acid in pyridine containing a small amount of water for 1–2 days gave PhSpTp(tc)T and 4-$MeOC_6H_4$SpTp(tc)T. The remaining arylthio groups were easily removed quantitatively by silver acetate (16–20 equiv) for 24 h or iodine (20 equiv) for 1 h in pyridine–water (2:1, v/v). All the dithymidylates obtained through several routes described above were completely degraded by snake venom phosphodiesterase to pT.[27] In a similar manner, (4-$MeOC_6H_4S)_2$pTp(tc)Tp(tc)T was synthesized in 82% yield. This trinucleotide derivative was also deprotected by the above methods and converted to pTpTpT in 75–97% yields.

References and Notes

(1) R. L. Letsinger and V. Mehadevan, *J. Am. Chem. Soc.*, **87,** 3526 (1965); R. L. Letsinger and K. K. Ogilvie, *ibid.*, **89,** 4801 (1967); **91,** 3350 (1969); R. L. Letsinger and W. B. Lunsford, *ibid.*, **98,** 3655 (1976).
(2) F. Eckstein and I. Rizk, *Angew. Chem.*, **79,** 939 (1967).
(3) C. B. Reese and R. Saffhill, *Chem. Commun.*, 767 (1968); C. B. Reese, *Tetrahedron Lett.*, 3785 (1974); C. B. Reese and Y. T. Y. Kui, *ibid.*, 802, (1977); C. B. Reese, R. C. Titmas, and L. Yau, *ibid.*, 2727 (1978).
(4) T. Neilson and E. S. Werstiuk, *Can. J. Chem.* **49,** 3004 (1971); R. J. Gregoire and T. Neilson, *ibid.*, **56,** 487 (1978).
(5) T. Catlin and F. Cramer, *J. Org. Chem.*, **38,** 245 (1973).
(6) K. Itakura, C. P. Bahl, N. Katagiri, J. J. Michniewicz, R. H. Wightman, and S. A. Narang, *Can. J. Chem.*, **51,** 3625 (1973); N. Katagiri, K. Itakura, S. A. Narang, *J. Am. Chem. Soc.*, **97,** 7332 (1975); A. K. Sood and Narang, *Nucleic Acids Res.*, 2757 (1977); R. Crea, T. Hirose, and K. Itakura, *Tetrahedron Lett.*, 395 (1979); R. Crea, A. Kraszewskin T. Hirose, and K. Itakura, *Proc. Natl. Acad. Sci. U.S.A.*, 75 (1978).
(7) J. Smrt, *Collect. Czech. Chem. Commun.*, **39,** 972 (1974).
(8) K. K. Ogilvie, E. A. Thompson, M. A. Quillian, and J. B. Westmore, *Tetrahedron Lett.*, 2865 (1974); K. K. Ogilvie, N. Theriault, and K. Sadana, *J. Am. Chem. Soc.*, **99,** 7741 (1977); K. K. Ogilvie, S. L. Beaucage, A. L. Schifman, and N. Y. Theriault, *Can. J. Chem.*, **56,** 2768 (1978).
(9) E. Ohtsuka, S. Tanaka, and M. Ikehara, *Chem. Pharm. Bull.*, **25,** 949 (1977); E. Ohtsuka, T. Tanaka, S. Tanaka, and M. Ikehara, *J. Am. Chem. Soc.*, **100,** 4580 (1978); E. Ohtsuka, S. Tanaka, and M. Ikehara, *ibid.*, **100,** 8210 (1978).
(10) M. Sekine and T. Hata, *Tetrahedron Lett.*, 1711 (1975); H. Takaku, M. Kato, and T. Hata, *J. Chem. Soc., Chem. Commun.*, 190 (1977).
(11) K. L. Sadana and P. C. Loewen, *Tetrahedron Lett.*, 5095 (1978).
(12) For recent reviews on oligonucleotide synthesis see V. Amarnath and A. D. Broom, *Chem. Rev.*, **77,** 183 (1977); C. B. Reese, *Tetrahedron*, 3143 (1978).
(13) J. H. van Boom, P. M. J. Burgers, and P. H. van Deursen, *ibid.*, 869 (1976); M. Hashimoto and T. Mukaiyama, *Chem. Lett.*, 513 (1973); D. G. Knorre, G. F. Mishenina, and T. N. Shubina, *Bioorg. Khim.*, **2,** 1189 (1976). In the later two cases, "diester method" was employed in the coupling reactions for chain elongation of oligonucleotides. On the other hand, there have been reported a number of "unit-type" triester approaches utilizing two kinds of protecting groups of 3′-terminal phosphate hydroxyl groups (see ref 12).
(14) I. Nakagawa and T. Hata, *Chem. Lett.*, 987 (1976); K. Yamaguchi, I. Nakagawa, T. Hata, K. Shimotohno, K. Shimotohno, M. Hiruta, and K. Miura, *Nucleic Acids Res., Spec. Publ.*, **No. 2,** 151 (1976).
(15) Y. Furuichi and K. Miura, *Nature (London)*, **253,** 374 (1975).
(16) Trimethylsilyl chloride (2.1 equiv) and triethylamine (2.1 equiv) were employed and the reaction was carried out at room temperature for 20 h.
(17) R. Lohrmann and H. G. Khorana, *J. Am. Chem. Soc.*, **86,** 1630 (1964).
(18) In each case, only trace amount of *S,S*-diaryl thymidine 3′-phosphorodithioate (**10a** or **10b**) was formed as a byproduct. Compound **10a** or **10b** could be distinguished from the corresponding 5′-isomer **9a** or **9b** on TLC. The major byproduct was 3′,5′-bisphosphorylated derivative **16a** or **16b.** They were also obtained in high yields from thymidine (1 equiv) and 2.4 equiv of **1a** or **1b** in the presence of 2.4 equiv of TPS.
(19) A small amount (2%) of *S*-phenyl thymidine 3′-phosphorothioate (**11**) was formed. On the contrary, the bis(4-methoxyphenylthio) group was completely stable under the same conditions.
(20) T. Hata and M. Sekine, *Chem. Lett.*, 837 (1974).
(21) Mercuric chloride can be used effectively as well as the silver salts. However, other transition metals such as mercuric acetate, cupric chloride, and cuprous chloride were not effective.
(22) Treatment of **6–10** with silver acetate gave the products as silver salts. The silver salts could be converted to metal-free pyridinium salts by bubbling hydrogen sulfide into their solution of aqueous pyridine.
(23) A. F. Cook, M. J. Holman, and A. L. Nussbaum, *J. Am. Chem. Soc.*, **91,** 1522 (1969).
(24) When **9a** was treated with 0.2 N NaOH–dioxane (1:1, v/v), the isomerization of the phosphoryl group from the 3′ position to the 5′ position occurred partially to give a mixture of PhSpT, cyclic pT, pT, and Tp.
(25) The reaction proceeded quantitatively because the TLC showed a single spot containing the trityl group.
(26) It is also noteworthy that **9a** was successfully converted to PhSpT quantitatively without isomerization of the phosphoryl group as described in ref 24.
(27) Before removal of all protecting groups, **15a** and **15b** were further purified by treatment with monomethoxytrityl chloride in pyridine followed by chromatographical separation on silica gel in order to take off traces of 3′→3′ isomers simultaneously formed in the second coupling reactions.[1]
(28) R. W. Adamiak, E. B. K. Grzeskowiak, R. Kierzek, A. Kraszewski, W. T. Markiewicz, J. Stawinski, and M. Wiewiorowski, *Nucleic Acids Res.*, **4,** 2321 (1977).

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Unsaturated Nitrosamines. Formation and Equilibration of Vinylic and Allylic Nitrosamines

Summary: Vinylic nitrosamines (*N*-nitrosoenamines), which heretofore have been difficult to prepare, can be formed in good yields by crown ether/potassium hydroxide elimination of the corresponding β-tosyloxy nitrosamines, by base-catalyzed equilibration of the corresponding allylic isomers, or by oxidative elimination of α-phenylselenyl nitrosamines.

Sir: Little is known about the chemistry and biological effects of α,β-unsaturated nitrosamines, partly due to a lack of generally useful synthetic methods. Only three members of the class have been reported. *N*-Nitrosomethylvinylamine[1] and *N*-nitrosoethylvinylamine[2] have been reported and characterized. The very unstable divinylnitrosamine has been reported[2] but its characterization is poor. We wish to report three methods of preparation of this interesting class of compounds, which should provide the basis for the study of their chemistry.

The aforementioned nitrosamines were prepared by simple dehydrohalogenation of alkyl(β-chloroethyl)nitrosamines using methanolic potassium hydroxide. However, with longer β-chloroalkyl chains, the elimination occurred to give the

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