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Synthesis and Properties of Thymidine Derivatives Bearing the $(PhS)_2P(O)$ and (PhS)(PhNH)P(O) groups

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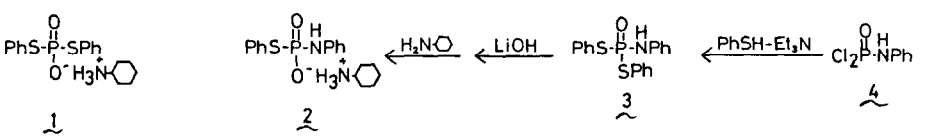
Summary: A new phosphorylating agent, cyclohexylammonium S-phenyl phosphoranilidothioate (2), was prepared in high yield. In this paper, the highly selective deprotection of the phenylthio or anilino group from thymidine 3',5'-diphosphate derivatives masked with the $(PhS)_2P(O)$ and (PhS)(PhNH)P(O) groups is also described.

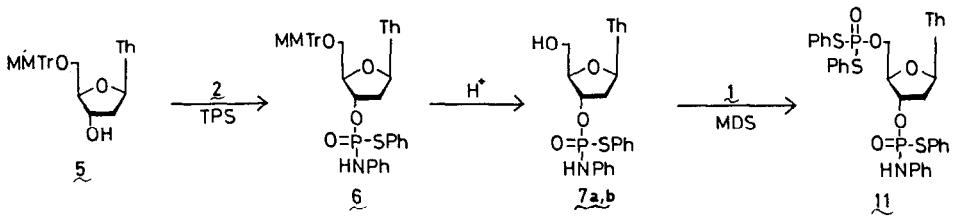
In previous papers,[1-9] we have demonstrated the utility of S,S-diphenyl nucleoside phosphorodithioates as the key intermediates in oligonucleotide synthesis. It has been proved that one of the two phenylthio groups can be removed from the phosphorodithioates under neutral conditions by use of pyridinium[1-3] or triethylammonium phosphinate[4-10] as well as weakly basic conditions.[1,7] The phenylthio group can also be deprotected from 3'-terminal or internal phosphate groups by the silver-ion catalyzed hydrolysis.[1,5-7,10] These characteristic features of the phenylthio group have been utilized for the chain elongation of DNA in the phosphotriester approach.[3,5-10]

Nucleoside hydroxyls have proved to be readily phosphorylated with cyclohexylammonium S,S-diphenyl phosphorodithioate (1) in the presence of condensing agents.[1-10] This type of phosphorylation seems to be convenient since all the reagents are nonhygroscopic and stable crystals and the reaction gives usually the desired product in high yield. Therefore, we have chosen cyclohexylammonium S-phenyl phosphoranilidothioate (2) as a phosphorylating agent to synthesize S-phenyl deoxyribonucleoside phosphoranilidothioates. Compound 2[11] was easily prepared as crystals in 83% yield by the alkaline hydrolysis of S,S-diphenyl phosphoranilidodithioate (3),[12] which was prepared in 88% yield by the reaction of phosphoranilidodichloridate (4) with two equiv each of thiophenol and triethylamine in dry ether for 2 h.

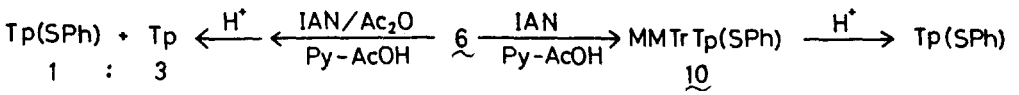


Phosphorylation of 5'-monomethoxytritylthymidine (5) with 2 (1.1 equiv) in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride[13] (TPS) in pyridine

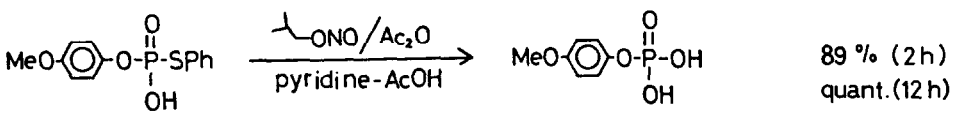
gave 6 in 91% yield. Treatment of 6 with 80% acetic acid gave the 5'-hydroxyl derivative (7)[14] in nearly quantitative yield, showing that the

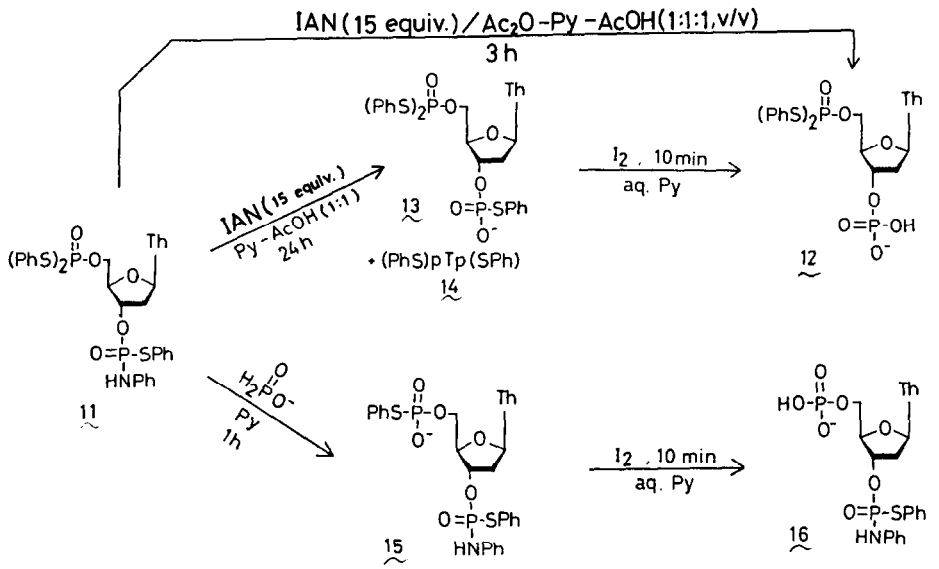


(PhS)(PhNH)P(O) group was stable under the usual acidic conditions. Ikehara and Ohtsuka have reported that the anilino group is removed by the action of isoamyl nitrite (IAN) under mild conditions.[15] Therefore, 6 was treated with IAN in pyridine-acetic acid at room temperature and after extraction with chloroform the MMTr group was deprotected by treatment with 80% acetic acid. After 24 h, S-phenyl thymidine 3'-phosphorothioate [Tp(SPh)] was obtained in 89% yield. Thymidine 3'-phosphate (Tp) was formed in less than 2% yield during this treatment. Virtually, the selective deprotection of the anilino group was achieved. On the other hand, we have known that nucleoside phosphorocyclicdiamidates are easily converted to the corresponding nucleotides more effectively and cleanly by addition of acetic anhydride to IAN-pyridine-acetic acid.[16] Treatment of 6 with IAN in the presence of acetic anhydride resulted in more rapid deprotection of the anilino group. The compound 6 disappeared in 3 h and three fourth of the phenylthio group was simultaneously eliminated to give Tp(SPh) and Tp in 25 and 75% yields, respectively.



In order to clarify the mechanism of the simultaneous removal of the phenylthio group, the following model experiments were conducted. When pyridinium S-phenyl 4-methoxyphenyl phosphorothioate (8)[17] was treated with IAN (15 equiv.) in Ac_2O-pyridine-acetic acid (1:1:1, v/v/v), 4-methoxyphenyl phosphate (9) was obtained as the sole product. No reaction occurred, when acetic anhydride or isoamyl nitrite was eliminated from the reaction mixture.





As shown in previous papers[18, 19] on the chemical synthesis of messenger RNA, the phenylthio group of diester-type can be activated by silver salts and iodine to generate metaphosphate derivatives that can be used for the construction of the so-called "cap" structure[18] or polyphosphates of nucleosides.[19] We next synthesized a thymidine 3',5'-diphosphate derivative (11) protected with the two phenylthio groups at the 5'-position and with the phenylthio and anilino groups at the 3'-position. Phosphorylation of 7a with 1 in the presence of mesitylenedisulfonyl chloride (MDS)[3] gave 11 in 94% yield. Treatment of 11 with IAN-acetic anhydride—pyridine-acetic acid (r.t., 3 h) gave predominantly $(PhS)_2$pTp (12) (95%) and a trace amount of $(PhS)_2$pTp(SPh) (13) was formed. It was found that the latter was converted to the former by treatment with iodine in aqueous pyridine without damage of the $(PhS)_2P(O)$ group. On the other hand, under the conventional conditions using IAN-pyridine-acetic acid (r.t., 24 h) 11 was converted to a 4:1 mixture of $(PhS)_2$pTp(SPh) 13 and PhSpTp(SPh) 14. In this case, partial deprotection of the phenylthio group at the 5'-phosphate was observed.

To examine the stability of the $(PhS)_2P(O)$ group under the above conditions, independently, $(PhS)_2$pTOAc[2] was treated with IAN in the presence or absence of acetic anhydride. As a consequence, the phenylthio group was lost gradually regardless of the presence or absence of acetic anhydride. However, the deprotected product was different from each other. In the absence of acetic anhydride PhSpTOAc was formed in 4, 9, and 25% yields after 2, 6.5, 28 h, respectively. On the contrary, the presence of the anhydride resulted in the simultaneous loss of the two phenylthio groups and pTOAc was formed in 2,

7, and 19% yields after 2, 6.5, 28 h, respectively. Since the IAN treatment of 11 in the presence of Ac_2O was very rapid, such deprotection at the 5'-phosphate was minimized to less than 2-3%.

The reaction of 11 with 3 M phosphinic acid in pyridine at room temperature for 30 min gave PhSpTp(SPh)(NHPh) (15) quantitatively. The latter was further converted to pTp(SPh)(NHPh) (16) as a sole product by oxidation with iodine in aqueous pyridine.

The selective deprotection of the phenylthio or anilino group attaching to the different phosphorus atoms would be useful for the synthetic strategy of nucleoside polyphosphates with more than two phosphate groups at the 3'- and 5'-positions.

Acknowledgement

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11) m.p. 177-178 °C (H_2O-THF): Calcd for $C_{18}H_{25}O_2SN_2P$: C, 59.32; H, 6.91; N, 7.69; S, 8.80. Found: C, 59.08; H, 6.94; N, 7.61; S, 8.99
12) m.p. 131-132 °C ($CHCl_3$): Calcd for $C_{18}H_{16}OS_2NP$: C, 60.49; H, 4.51; N, 3.92; S, 17.94. Found: C, 60.14; H. 4.52; N, 3.89; S, 18.14.
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14) In this case, a pair of diastereomers (7a,b) were obtained. The major isomer (7a, 50%) was separated as a solid insoluble in acetone and the soluble minor isomer (7b) was isolated in 35% yield by column chromatography on silica gel.
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17) Compound 8 was prepared as cyclohexylammonium salt by the reaction of 4-methoxyphenylphosphorodichloridate with one equiv each of thiophenol and triethylamine: m.p. 164-165 °C: UV(λmax) 277 nm (ε1650), (λmin) 261 nm: Calcd for $C_{19}H_{26}O_4NSP$: C, 58.60; H, 5.18; N, 3.60. Found; C, 57.44; H, 6.75; N, 3.64. The cyclohexylammonium salt of 8 was converted to the pyrinium salt by using Dowex 50W X 8 (pyridinium form).
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