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PHENYLTHIO GROUP AS A PROTECTING GROUP OF PHOSPHATES IN OLIGONUCLEOTIDE SYNTHESIS VIA PHOSPHOTRIESTER APPROACH

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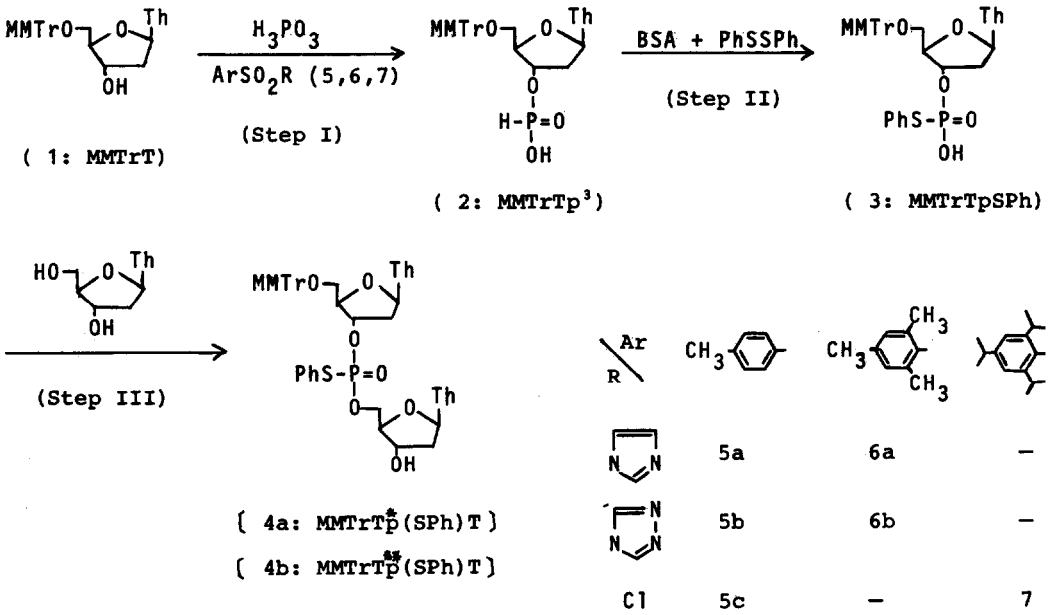
The so-called, "phosphotriester approach", in the synthesis of oligonucleotides has been utilized much more frequently[1] than the usual phosphodiester method. Recent developments in the phosphotriester approach seem to lie in how to introduce a protecting group into a phosphate function and how to remove it selectively and completely under mild conditions where no damage of internucleotidic bonds occurs. Consequently, it seems that a more ideal protecting group should be found and applied to the phosphotriester approach. More recently, useful methods for the synthesis of S-phenyl nucleoside phosphorothioates have been established in this laboratory.[2] We have been interested in a new method for the synthesis of oligonucleotides using the phenylthio group as the protecting group of internucleotidic phosphates.[3]

This communication describes the use of the phenylthio group as a protecting group for internucleotidic bonds by a phosphotriester approach via nucleoside silylphosphite intermediates.[2b] The present synthetic approach, which was used to prepare thymidylyl(5'-3')thymidine, is outlined in Scheme 1.

Scheme 1.



When 5'-O-(p-monomethoxytrityl)thymidine (1) was allowed to react with phosphorous acid in the presence of a coupling reagent in dry pyridine, 5'-O-(p-monomethoxytrityl)thymidine 3'-phosphite (2) was obtained (Step I). In this reaction, arylsulfonylamides[1g,4] were found to be the most suitable coupling reagents for the synthesis of 2 as shown in Table 1.

Table 1. Synthesis of 5'-O-(p-Monomethoxytrityl)thymidine 3'-phosphite

reagent	equiv.(mole)	time (hr)	yield (%)
5a	4	25	90
5b	4	21	94
5c	6	1.5	85
6b	4	25	94
7	4	24	90

In the above reactions, 1 equiv. of the compound 1 and 4 equiv. of phosphorous acid were used.

After the reaction, the compound 2 was extracted with chloroform and the chloroform layer was evaporated to dryness. The residue was treated with bis-(trimethylsilyl)acetamide (BSA) in the presence of a slight excess of diphenyl disulfide in dry pyridine to give S-phenyl 5'-O-(p-methoxytrityl)-thymidine 3'-phosphorothioate (3) (Step II). This was allowed to react with thymidine in the presence of a coupling reagent (one of the compounds from 5 to 7) to afford the corresponding dithymidine monophosphate derivative (4) (Step III). In this experiment, two major spots possessing Rf values of 0.49 and 0.40 (solvent system: CH_2Cl_2–tetrahydrofuran, 5:4 v/v) were observed on silica gel thin-layer chromatography. They were separated in the ratio of ca. 1:1 by silica gel column chromatography. Treatment of these products with 80% acetic acid gave the corresponding detritylated compounds (8a and 8b) which showed almost the same Rf values on silica gel thin-layer chromatography in several solvent systems. Both of these compounds were further treated with 0.1 N aqueous NaOH in dioxane for 15 min at room temperature to afford thymidylyl(5'-3')thymidine (9), which was degraded almost quantitatively (99%) with snake venom phosphodiesterase to thymidine (T) and thymidine 5'-phosphate (pT) in the ratio of 1.00 : 1.03.

Scheme 2.

$$\begin{array}{l} \text{4a} \xrightarrow{80\%\ \text{AcOH}} \text{8a} \\ \\ \text{4b} \xrightarrow{80\%\ \text{AcOH}} \text{8b} \end{array} \xrightarrow[15\ \text{min}]{0.1\ \text{N NaOH}} \text{TpT} \xrightarrow{\text{snake venom}} \begin{array}{l} \text{T(1.00) + pT(1.03)} \\ \\ \text{T(1.00) + pT(1.03)} \end{array}$$

The above enzymatic degradation shows that the natural internucleotidic bond was formed selectively even when unprotected thymidine was used. The structures of 4a and 4b were supported by elemental analyses and IR spectra. From these facts, it is concluded that the two products, 4a and 4b, might be diastereoisomers due to the asymmetric phosphorus atom. The reactions and the results are summarized in Table 2.

Further, a trithymidine diphosphate derivative, MMTrTp(SPh)Tp(SPh)T (10) was obtained in 40% yield as diastereoisomers starting from 4a. Both of the diastereoisomers, MMTrT$\overset{*}{\text{p}}$(SPh)T$\overset{*}{\text{p}}$(SPh)T (10a) and MMTrT$\overset{*}{\text{p}}$(SPh)T$\overset{**}{\text{p}}$(SPh)T (10b),

could be easily separated and isolated. After removal of the protecting groups, both of the unprotected trithymidine diphosphates were almost completely degraded with snake venom phosphodiesterase to T and pT in the ratios of 1.00 : 2.01 (from 10a) and 1.00 : 1.88 (from 10b).

Table 2. Synthesis of Thymidylyl(5'-3')thymidine Derivatives (4a and 4b)

Step I		Step II		Step III			Yields of 4*		
coupling reagent	time (hr)	$(PhS)_2$ (equiv.)	time (hr)	coupling reagent	(equiv.)	time (hr)	total (%)	4a (%)	4b (%)
5a	20	1.1	2.5	7	1.5	19	61	28	30
5a	25	1.1	2.0	6b	1.5	19	65	26	30
5b	24	1.1	2.0	7	1.5	18	73	32	36
6b	18	1.0	2.0	6b	1.5	25	63	28	32

In the above reactions, 4 equiv. of coupling reagent and phosphorous acid were used (in Step I), and 2.5 equiv. of BSA (in Step II) and 1.5 equiv. of thymidine (in Step III) were employed. * Isolated yields are recorded.

In conclusion, it may be noted that the phenylthio group could be introduced smoothly via a nucleoside silylphosphite and that it was completely removed under mildly alkaline conditions. It was also found that diastereoisomers of phenylthio derivatives of oligothymidylates were successfully separated as the first example among various phosphotriesters.

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