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Acylphosphonates. 5.[1] A new method for Stereospecific Generation of Phosphorothioate via Aroylphosphonate Intermediate

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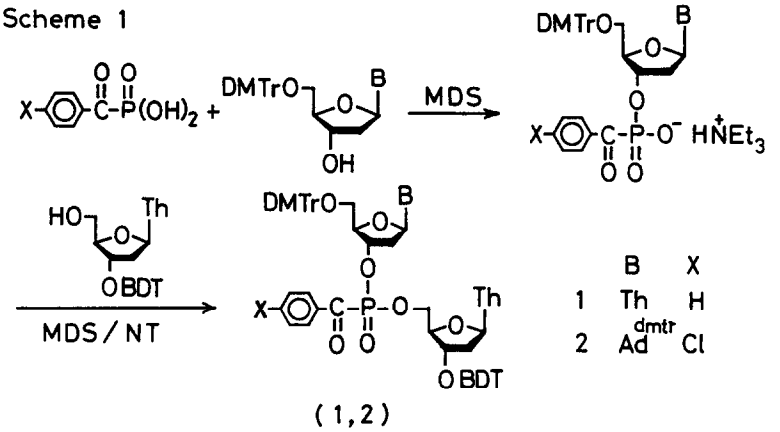
Summary: Dideoxynucleoside phosphorothioates were synthesized by a new method via dideoxynucleoside aroylphosphonates. The aroyl groups were easily removed from 1 and 2 by the action of n-$BuNH_2$ and in situ converted to only one diastereomer (Rp-configuration) of dideoxynucleoside phosphorothioates by treatment with elemental sulfur.

In recent years, a number of oligonucleotide analogues which involved phosphorothioate functions have been synthesized and their physical properties and biological activities have been studied.[2-7] Generally, these approaches involve chromatographic separation of a diastereomeric mixture of protected dinucleoside phosphorothioates followed by removal of the protecting groups. In these cases, the ratios of Rp/Sp-diastereomers are known to be nearly 1:1.[5-7] No specific methods for the synthesis of Rp- or Sp-dinucleoside phosphorothioates have been reported. In this paper, we wish to report a highly stereospecific synthesis of Rp-dinucleoside phosphorothioates.

Previously we reported the synthesis of dinucleoside phosphonates by using aroylphosphonates. It was also shown that 3'-thymidine 5'-thymidine phosphonate (5) could be converted to 3'-thymidine 5'-thymidine phosphorothioates (7) via trimethylsilylation followed by addition of elemental sulfur. Now, we found that the sulfurization could occur upon treatment of 5 with elemental sulfur in the presence of triethylamine without the silylation procedure. In this study, dinucleoside aroylphosphonates (1 and 2), protected with the 4,4'-dimethoxytrityl (DMTr) group at the 5'- and N^6-positions and 1,3-benzodithiol-2-yl (BDT)[8] group at the 3'-position, were synthesized by the published procedure (Scheme 1).[1,9]

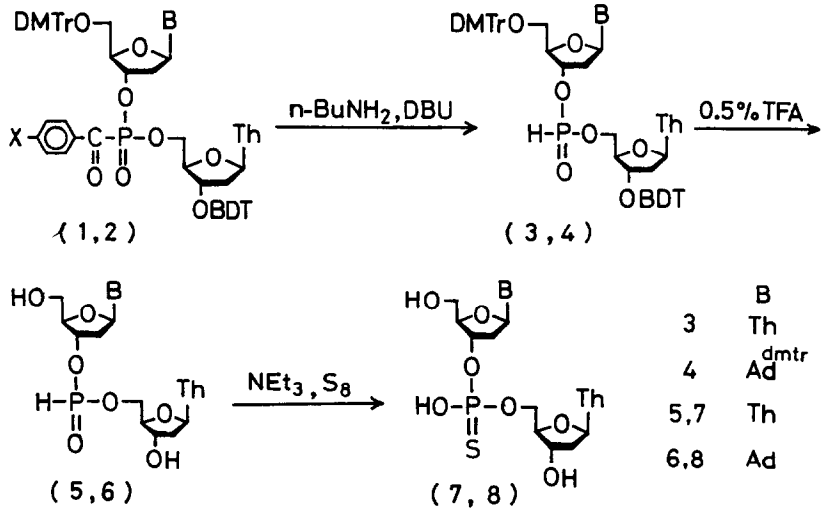
Removal of the aroyl groups from 1 and 2 by the action of n-$BuNH_2$ (10 equiv.) in the presence of DBU (0.5 equiv.)[10] at room temperature for 1h and 1.5h gave 3 and 4, respectively, in 72% yield each. Treatment of 3 and 4 with 0.5% TFA at 0°C for 1h gave compounds 5 and 6 in 53% and 51% yields, respectively.

Scheme 1



Sulfurization of 5 and 6 in the presence of triethylamine in pyridine at room temperature for 1h resulted in the direct formation of phosphorothioates 7 and 8 in 75% and 71% yields, respectively. The ^{31}P nmr analysis showed that the ratios of the Sp/Rp-isomers were 2.0:1 and 2.5:1 for compounds 7 and 8, respectively (Figure 1a). The deprotection procedure described above provided predominantly the Sp-isomers over the Rp-isomers (Scheme 2).

Scheme 2



Contrary to this fact, it was found that treatment of 1 with n-$BuNH_2$ (10

equiv.) in the presence of DBU (0.5 equiv.) and elemental sulfur (15 equiv.) for 1h prior to removal of the DMTr and BDT groups gave exclusively the Rp-isomer (9b). In a similar manner, compound 2 was converted stereospecifically to the Rp-isomer (10b, Figure 1b). On subsequent treatment of 9b and 10b with 0.5% TFA in $CHCl_3$ at 0°C for 1h, dinucleoside phosphorothioates 7b and 8b were isolated in 61% and 56% yields, respectively, by paper chromatography (Scheme 3).[11]

Scheme 3

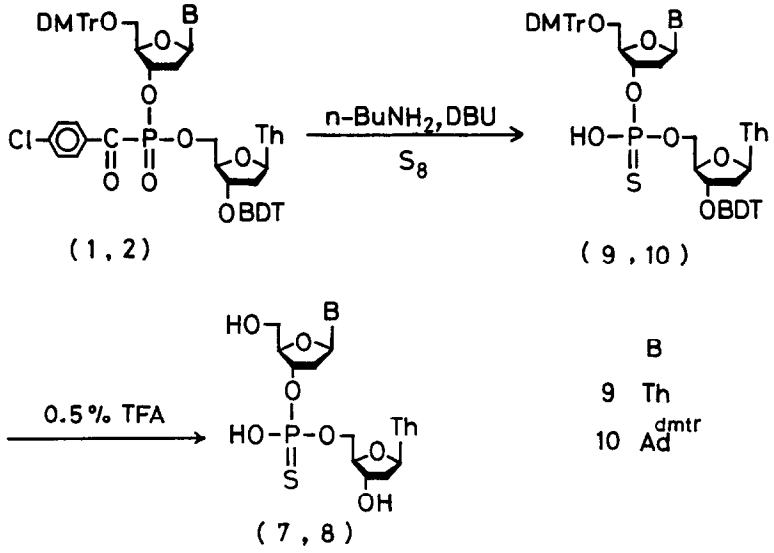


Table 1 ^{31}P nmr spectra of compounds 1,2, and 7-10.

compound	^{31}P(ppm)	compound	^{31}P(ppm)
1a,b	- 7.31,- 8.45	8a,b	-56.62,-57.31*
2a,b	- 7.25,- 8.21	9a,b	-56.22,-56.98
7a,b	-59.51,-57.17*	10a,b	-56.73,-57.38

The chemical shifts of compounds in $CDCl_3$/Py (3:1,v/v) were relative to internal standard of 85% H_3PO_4 (aq.).
*The chemical shifts were measured in D_2O/Py (3:1,v/v).

It is clearly shown from the ^{31}P nmr analysis that the configuration at phosphorus was maintained in the conversion of 9 or 10 to 7 or 8 (Table 1).

The stereochemistry of 7 and 8 obtained in these experiments was also confirmed by enzyme assay reported by Eckstein and Stec.[4-6] Nuclease P1 digested 7b and 8b but did not interact with 7a and 8a. On the contrary, snake venom phosphodiesterase lead to digestion of 7a and 8a but did not digest 7b and 8b.

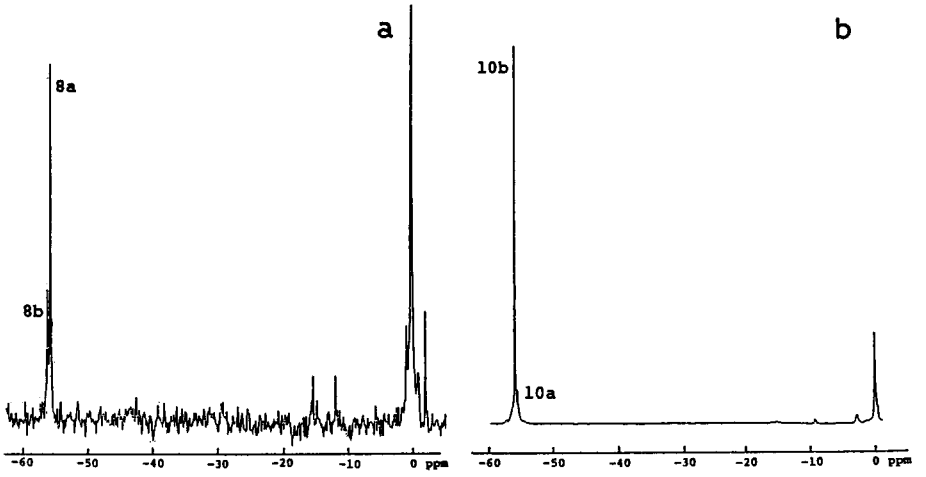


Figure 1 ^{31}P nmr spectra of compounds 8,10

The exclusive formation of the Rp-isomer in the latter process may be explained in terms of a strong interaction between the two nucleoside residues having the DMTr and BDT groups which is capable of the stereospecific fixation of a triethylamine-catalyzed reaction intermediate, i.e., a tervalent dinucleoside phosphonate.

In conclusion, the present method provides the first successful route to the stereo-controlled synthesis of dinucleoside phosphorothioates.

Application of the present method to deoxyguanosine- or deoxycytidine-contaning deoxydinucleoside phosphorthioates is now under investigation.

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9. Compound 2 was synthesized in 61% yield.
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11. During this deprotection process, naturally occurring dinucleoside phosphates were found to an unnegligible extent (~20%) probably owing to the TFA-mediated dehydrosulfurization.

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