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Transformation of Dinucleoside *S*-Aryl Phosphorothioates into the Corresponding *O*-Alkyl Phosphates by Use of Tributyltin Alkoxides

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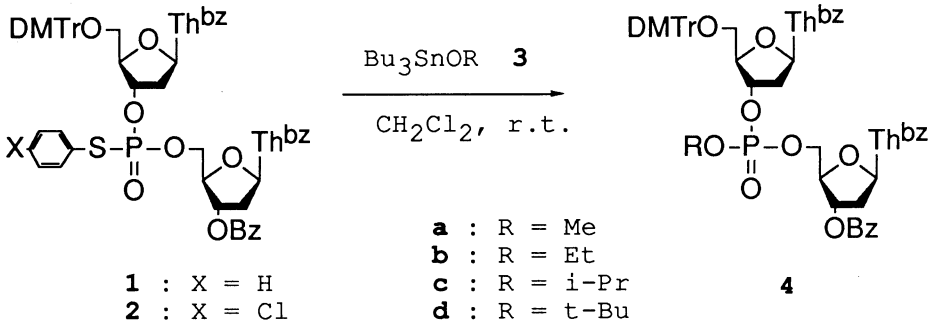
Several alkyl dinucleoside phosphotriesters were easily obtained by use of the reaction of *S*-aryl dinucleoside phosphorothioates with tributyltin alkoxides. This reaction proceeds at room temperature under neutral conditions.

Letsinger[1] founded so-called the phosphotriester method where internucleotidic bond of the synthetic intermediate bore protecting groups. The phosphotriesters are neutral compounds which can be easily handled by means of general synthetic techniques such as silica gel chromatography. In this laboratory, phenylthio group[2] was frequently used as the protecting group and found to be the most suitable for this purpose, because during the coupling reaction the corresponding tetrasubstituted pyrophosphorodithioate intermediate was highly reactive than that of the pyrophosphate. The phenylthio groups could be removed easily from the fully protected oligonucleotide by treatment with bis(tributyltin) oxide to convert into the tin salt.[3] The driving force of the reaction seems to be the affinity between tin and sulfur. On the other hand, Watanabe[4] described the transformation of *O*-alkyl *S,S'*-diaryl phosphorodithioates into *S*-aryl *O,O'*-dialkyl phosphorothioates by means of tributyltin alkoxides. This result prompted us to examine the conversion of the protected oligonucleotide having phenylthio group into the corresponding nucleoside phosphotriester by using tributyltin alkoxide. This type of reaction may be applied to the modification of the internucleotidic bond consisting of marker or reporter molecules.[5,6]

In this paper, we wish to report the reaction of appropriately protected *S*-aryl phosphorothioate derivatives of thymidylyl(3'-5')thymidine (**1** and **2**) with tributyltin alkoxides.

The fully protected thymidylyl(3'-5')thymidine (**1**) synthesized by the phosphotriester method[2] was allowed to react with 2 equiv of tributyltin methoxide[7] in $CDCl_3$ at room temperature and the reaction was monitored by means of ^{31}P-NMR.



In this case, the reaction proceeded very sluggishly. For example, after 12 h, the desired phosphotriester was obtained in only 8% yield. When a large excess amount of the tributyltin methoxide was employed, the yield was improved. Especially, when **2** having 4-chlorophenyl group[8] was employed, the result was successful: To a solution of **2** (160 mg, 0.12 mmol) in CH_2Cl_2 (2 ml) was added **3a** (1.22 g, 3.80 mmol), and the mixture was stirred at room temperature for 24 h. After the usual work-up, the reaction mixture was applied to a silica gel column chromatography. Elution was performed by CH_2Cl_2 - MeOH. Compound **4a** was obtained in 80% (117 mg) yield. The reaction conditions and the results are shown in Table 1.

Table 1. The reaction of tributyltin alkoxides with **1** and **2**

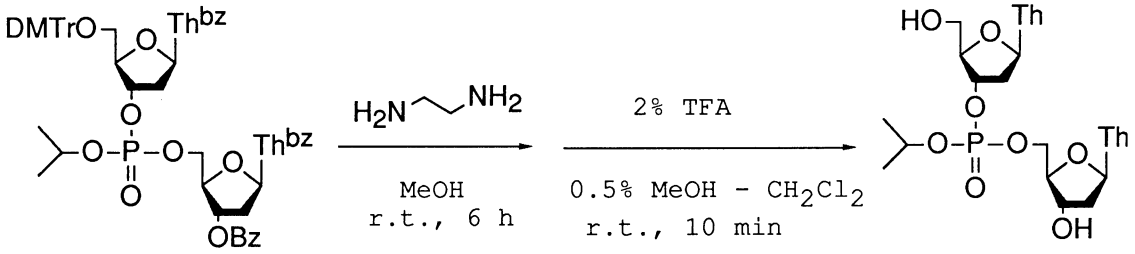
X	R	equiv. of **3**	Time / h	Yield / %	^{31}P-NMR[a] / ppm	(ratio)
H	Me	40	12	76	-0.68, -1.02	(39 : 61)
	Et	40	12	41	-1.84, -2.13	(65 : 35)
	i-Pr	40	12	trace[b]	-1.94	
Cl	Me	20	20	80	-0.68, -1.11	(45 : 55)
	Et	40	24	61	-1.89, -2.28	(66 : 34)
	i-Pr	40	48	40	-2.27, -2.65	(56 : 44)
	t-Bu	40	36	34	-0.68, -1.07	(65 : 35)

a) ^{31}P-NMR was mesured in $CDCl_3$ using 85% H_3PO_4 as external standard.

b) Trace amount of the product was assigned as **4c** by ^{31}P-NMR analysis.

Compound **2** gave much better results than those of **1**, even when the bulky tributyltin t-butoxide was employed. ^{31}P-NMR spectra of **4** in $CDCl_3$ showed two signals which corresponded to the diastereoisomers due to the chiral phosphorus atom.

To obtain the unprotected phosphotriester derivative from **4**, the benzoyl group could be removed with concentrated aqueous ammonia at 55 °C.[9] However, under the conditions the phosphotriester bond was also cleaved at the same time. To avoid the cleavage of the phosphotriester bond, ethylenediamine was chosen.[10] When ethylenediamine was added to **4** in MeOH, the corresponding phosphotriester of thymidylyl(3'-5')thymidine was obtained in good yield. Typical procedure is described as follows: Compound **4c** was treated with 10 equiv of ethylenediamine in MeOH at room temperature for 6 h and the reaction mixture was concentrated. The residual oil was treated with a solution of 2% trifluoroacetic acid in 0.5% MeOH - CH_2Cl_2 for removal of the DMTr group. Resulting alkyl dinucleoside phosphotriester (**5**) was isolated by silica gel column chromatography in 48% yield. Two diastereoisomers could be separated by reversed phase HPLC and each was determined to be the R_p or S_p configuration.[11]



4c **5**, 48%

Here, we have described introduction of simple alkoxy groups into the internucleotidic bond. The reaction may be applied to the introduction of reporter or marker molecules[6] since the reaction proceeded at room temperature under neutral conditions.

References

1) R. L. Letsinger and K. K. Ogilvie, *J. Am. Chem. Soc.*, **89**, 4801 (1967).
2) M. Sekine and T. Hata, *Tetrahedron Lett.*, **16**, 1711 (1975); M. Sekine, J. Matsuzaki and T. Hata, *Tetrahedron Lett.*, **22**, 3209 (1981); S. Honda, K.

Terada, Y. Satoh, M. Sekine, and T. Hata, *Chem. Lett.*, **1982**, 15.
3) M. Sekine, H. Tanimura, and T. Hata, *Tetrahedron Lett.*, **26**, 4621 (1985); H. Tanimura, M. Sekine, and T. Hata, *Tetrahedron*, **42**, 4179 (1986).
4) Y. Watanabe and T. Mukaiyama, *Chem. Lett.*, **1979**, 389.
5) E. Uhlmann and A. Peyman, *Chem. Rev.*, **90**, 543 (1990).
6) M. Durand, J. C. Maurizot, U. Asseline, C. Barbier, N. T. Thuong, and C. Helene, *Nucleic Acids Res.*, **17**, 1823 (1989).
7) **5a-d** was prepared from tributyltin chloride and corresponding sodium alkoxide.
8) 3'-Phosphorylation of N^3-benzoyl-5'-*O*-DMTr-thymidine was performed by the use of cyclohexylammonium *S,S'*-bis(4-chlorophenyl) phosphorodithioate with 2,4,6-triisopropylbenzenesulfonyl chloride as a coupling reagent in pyridine. However, after usual work up, the phosphorothioate derivative was obtained instead of the 3'-*O*-nucleoside *S,S'*-bis(4-chlorophenyl) phosphorodithioate.
9) P. Guga, M. Koziolkiewicz, A. Okruszek, B. Uznanski, and W. J. Stec, *Nucleosides & Nucleotides*, **6**, 111 (1987).
10) P. S. Miller, M. P. Reddy, A. Murakami, K. R. Blake, S.-B. Lin, and C. H. Agris, *Biochemistry*, **25**, 5092 (1986).
11) W. J. Stec and G. Zon, *J. Chromatogr.*, **326**, 263 (1985).

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