Alkylation of Phosphate Group of Nucleotides *via* Tributylstannyl Phosphate Derivatives

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(Received September 30, 1994)

Alkylation reaction of phosphates *via* tributylstannyl salt was applied to dinucleotide derivatives. Some alkyl groups were successfully introduced into the internucleotidic phosphate by use of alkyl bromides in the presence of tetraethylammonium bromide. In case of thymidylyl(3'-5')thymidine, the phosphate moiety was alkylated selectively.

Syntheses of phosphate esters are usually performed by phosphorylation reaction. Alkylation reaction is another route to obtain phosphate esters. When alkylation reaction can be applied to the modification of phosphate of nucleic acids, this method has an advantage that the modification does not interfere to form base-pairs. Because the phosphates are located outside in double-stranded DNA. In a previous paper, we have reported the model alkylation reaction of phosphoric acid derivatives *via* tributylstannyl phosphates.[1] There is always the problem of the solubility of nucleic acids in aprotic organic solvents. In order to overcome the problem, we have employed tributylstannyl salts whose solubility remarkably increase in organic solvents, such as DMF, acetonitrile, and dichloromethane. The tributylstannyl salts were also advantageous over the tetraalkylammonium salts because of their low hygroscopicity and were easily prepared from the phosphate and stoichiometric amount of tributyltin methoxide. Therefore, we have applied the method to the dinucleotide derivatives.

First of all, the alkylation was carried out with tributylstannyl salts of the adequately protected dinucleoside monophosphates. Alkyl bromide was added to a mixture of the dinucleoside phosphate and tetraethylammonium bromide in dry acetonitrile and stirred under refluxing. The yields after purification by silica-gel column chromatography are shown in Table 1. ^{31}P-NMR spectra of the obtained triesters showed two peaks corresponding to diastereoisomers and the ratios were about 1 : 1. In the case of the pentyl triester, deprotection of benzoyl groups was easily carried out according to the method described previously.[2]

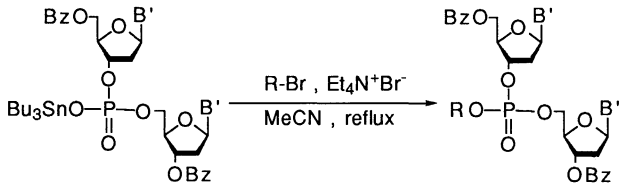

Table 1. Reaction of Tributylstannyl Salts of Protected Dinucleoside Phosphates and Alkyl Bromides

B'	R-Br	Equiv.	Time /h	Yield /%
Th^{bz}	4-$O_2NC_6H_4CH_2$-Br	10	2	59
	n-C_5H_{11}-Br	40	18	90
Ad^{bz_2}	4-$O_2NC_6H_4CH_2$-Br	10	6	68
	$C_6H_5CH_2$-Br	20	15	43
	n-C_5H_{11}-Br	40	11	40

Next, the alkylation reaction was applied to the unprotected dinucleoside phosphate, TpT. In this case, a small amount of methanol was added to solve TpT (Table 2).

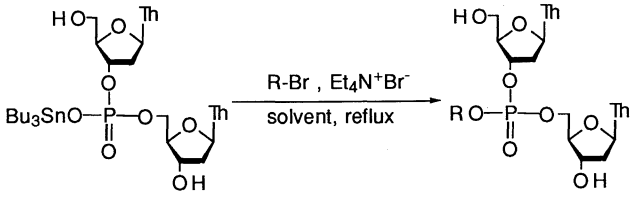

Table 2. Reaction of Tributylstannyl Salt of TpT and Alkyl Bromides

R-Br	Equiv.	Solvent (ratio, v/v)	Time /h	Yield /%
$C_6H_5CH_2$-Br	20	MeCN / MeOH (3:1)	10	35
$C_6H_5CH_2CH_2$-Br	20	MeCN / MeOH (20:1)	10	48
4-$O_2NC_6H_4CH_2$-Br	10	MeCN / MeOH (20:1)	6	75
$C_6H_5COCH_2$-Br	20	MeCN / MeOH (20:1)	4	53
n-C_5H_{11}-Br	40	MeCN / MeOH (20:1)	18	74[a]

[a] NMR data is shown in Note 3.

It is surprising that the yield of triester using 1-bromopentane was very high. The triester involving benzyl group was very unstable and the yield was low. This result suggests that the alkylation reaction is applicable to modification of internucleotidic bond by use of a long chain alkyl group employed as a linker of genetic markers. It is emphasized that the alkylation reaction of TpT took place regio-selectively on the phosphate function without undesirable alkylation at N-3 position on thymine moiety.

Other monodeoxynucleotides, such as pdA, pdC, pdG, were alkylated with less chemo-selectivity under the conditions described above. Selective alkylation reactions of phosphate moieties of these nucleotides are now in progress.

References and Notes

1 S. Ohuchi, T. Imada, and T. Hata, *Nucleosides and Nucleotides*, **11**, 749 (1992).
2 S. Ohuchi, H. Ayukawa, and T. Hata, *Chem. Lett.*, **1992**, 1501.
3 NMR data of the pentyl triester: ^{1}H-NMR (CD_3OD, 60 MHz); δ 0.30-1.25 (m, 9H, 2,3,4,5-H of pentyl), 1.48-1.60 (m, 6H, 5-CH_3), 1.80-2.34 (m, 4H, 2'-H), 2.65-3.55 (m, 2H, 5'-H), 3.60-4.18 (m, 5H, 4',5'-H and 1-CH_2 of pentyl), 4.20-4.95 (m, 3H, 3' and 4'-H), 5.74-6.10 (m, 2H, 1'-H), 7.15, 7.38 (s, 2H, 6-H). ^{31}P-NMR (CD_3OD, 40.5 MHz); δ -2.62, -2.76, ^{31}P- chemical shifts were given relative to 85% H_3PO_4 as an external standard.