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The Synthesis of Partially-protected 2′-Deoxyribonucleotide Dimers by the Selective Phosphorylation of Stannylated Nucleosides[1)]

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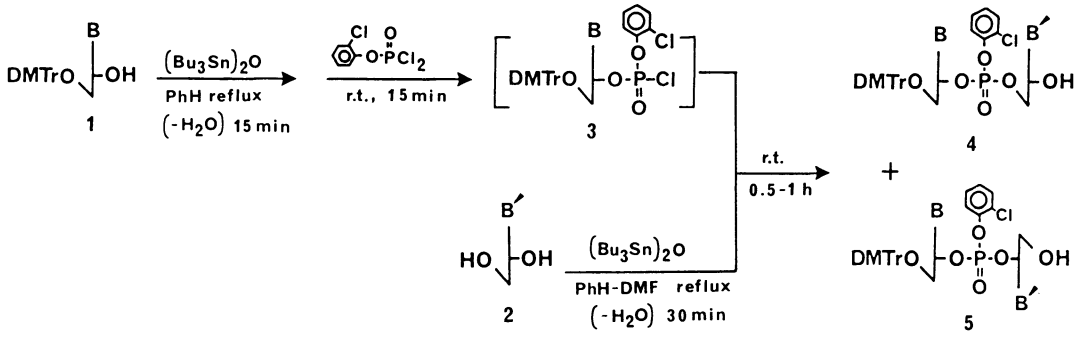
A new method for the synthesis of the title compounds is described. The activation of the hydroxyl moieties of 5′-*O*-dimethoxytrityl and 3′,5′-*unprotected* 2′-deoxyribonucleosides, which were appropriately protected on their base residues, was done with bis(tributyltin) oxide in separate flasks. The resulting stannylated nucleosides were coupled with 2-chlorophenyl phosphorodichloridate to form the dimers with 3′→5′ internucleotide linkages in yields of more than 71%, along with the corresponding 3′→3′ isomers in yields of less than 10%. The method of Cashion *et al.* was also evaluated, in which up-to-date protecting groups were employed.

The formation of internucleotide linkages by the coupling of two nucleoside components which have minimum protecting groups on their hydroxyl functions is, in principle, very attractive for the rapid synthesis of the nucleotide dimers that have been useful for the preparation of oligonucleotides of defined length and sequence.[2a,b)] Cashion *et al.*[3)] have reported on such method. Thus, 3′→5′ linked 2′-deoxyribonucleotide dimers were synthesized in a one-pot procedure by phosphorylation of 5′-*O*-methoxytritylated nucleosides with 4-chlorophenyl phosphorodi-1,2,4-triazolidate, followed by condensation with 3′-terminal units of 3′,5′-*unprotected* nucleosides: The starting nucleosides were appropriately protected on their base residues. A phosphomonotriazolide intermediate produced in this reaction was activated by the addition of a large amount of 1-methyimidazole. Later, Agarwal *et al.*[4)] reported that triethylammonium benzenesulfonate was also effective for this activation. Even though the 5′-(primary hydroxyl) group of the incoming nucleoside in this coupling reaction is more reactive than its 3′-(secondary hydroxyl) one, there is the possibility that an undesired isomeric dimer with a 3′→3′ internucleotide linkage is produced.

We wish to report herein the evaluation of Cashion's method using up-to-date protecting groups for the synthesis of oligodeoxynucleotides, and our alternative approach to the dimers by the activation of hydroxyl functions with bis(tributyltin) oxide. Recently, metal-mediated phosphorylation by activating the hydroxyl groups in nucleosides has been shown to be useful for the synthesis of deoxyribonucleotides.[5)] The reaction proceeded smoothly without the activation of a phosphotriester intermediate. It is well known that organic tin compounds have been used effectively for the selective activation of hydroxyl groups in ribofuranosides[6)] and glycopyranosides.[7)]

Results and Discussion

We first examined the synthetic reaction of the partially-protected thymidylyl(3′→5′)thymidine (**4a**) (Scheme 1). A mixture of 1 mol equiv of 5′-*O*-dimethoxytritylthymidine (**1a**) and 0.6 mol equiv of bis(tributyltin) oxide in benzene was refluxed for 15 min with an azeotropic removal of water; a clear solution was obtained and no decomposition of the starting nucleoside was observed. Similarly, a mixture of 1.5 mol equiv of thymidine (**2a**) and 0.9 mol equiv of bis(tributyltin) oxide in benzene containing a small amount of *N*,*N*-dimethylformamide (DMF) was treated for 30 min. The stannylated derivative prepared in the first solution was then phosphorylated with 1.4 mol equiv of 2-chlorophenyl phosphorodichloridate at room temperature. The reaction was completed within 15 min, as judged by its TLC analysis; we could detect no formation of a 3′→3′ dimer at this step. To this solution was added the second solution containing the incoming nucleoside. The coupling reaction was over



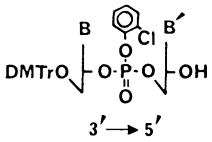
BB′=TT (**a** series), CT, AT, TC, AC, or TA.
T=thymin-1-yl; C=4-*N*-benzoylcytosin-1-yl; A=6-*N*-benzoyladenin-9-yl; DMTr=4,4′-dimethoxytrityl.

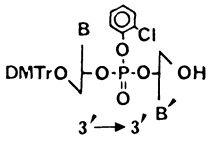
Scheme 1.

at room temperature in 30 min. Evaporation of the benzene followed by chromatography gave **4a** in a 94% yield (93% purity), while the 3′→3′ dimer (**5a**) was formed in a yield of less than 3%. These yields were not appreciably changed, when the amount of the tin compound required for the activation of **2a** was increased to 1.5 mol equiv, which was enough to derivatize its 3′ and 5′-hydroxyl groups into their stannylated functions (Table 2, the second line).

On the other hand, according to the slightly modified procedure of Cashion *et al.* using the same protecting groups as ours, **4a** was prepared in a 73% yield, while a large amount (15%) of **5a** was also isolated. The ^{1}H NMR spectrum of **4a** prepared by this procedure was identical with that from ours except difference in the intensity of signals between two diastereoisomers due to the chirality of the phosphorous triester. The 3′→5′ dimer could easily be distinguished from its 3′→3′ isomer by means of their TLC and ^{1}H NMR spectroscopic analyses (Tables 2 and 3). These analyses also showed that **4a** from our procedure contained a very small amount (less than 3 mol%) of an unidentified tin compound,[8] which could not be excluded without loss of the desired dimer. However, it seemed that this contamination did not interfere with further reaction.[9]

Table 1. The yields(%) of the partially-protected nucleotide dimers





BB′ [a]	Tin method[b] 3′→5′	Tin method[b] 3′→3′	Triazolide method[b] 3′→5′	Triazolide method[b] 3′→3′
TT	94	<3	73 (92) [c]	15
CT	81	6	59	9
AT	76	<3	59	12
TC	77	7	57 (90) [c]	20
AC	71	10	50	16
TA	75	<3	66 (90) [c]	7

a) T=thymin-1-yl; C=4-*N*-benzoylcytosin-1-yl; A=6-*N*-benzoyladenin-9-yl; DMTr=4,4′dimethoxytrityl. b) The dimers were obtained as foams, which were dried at room temperature over diphosphorus pentaoxide *in vacuo* for 4 h; the purity of the 3′→5′ dimers was grater than 90%, as judged by their ^{1}H NMR spectroscopic assay. c) The value in the parentheses is the original data of Cashion *et al.* (Ref. 3) with the following protecting groups: 4-Chlorophenyl for the phosphate; 4-methoxytrityl for the 5′-hydroxyl group; anisoyl for the cytosine residue; benzoyl for the adenine residue. No data for the corresponding 3′→3′ isomers are described in their paper.

Our method was successfully applied to the synthesis of five partially-protected dinucleotides of d(CT), d(AT), d(TC), d(AC), and d(TA) in yields of more than 71%. Both results from the procedure of Cashion *et al.* and ours are summarized in Table 1. The formation of a relatively large amount of the 3′→3′ dimers was observed in the former procedure. Originally, they have used the 4-chlorophenyl protecting group for the phosphate moiety, instead of the present 2-chlorophenyl group, and have obtained the partially-protected 3′→5′ dimers of d(TT), d(TC), d(TA), and d(AG) in about 90% yields (Table 1). It is considered that subtle changes in the protecting groups at the 5′-position and the base residue (from the original nucleoside to the present one) did not exert an important effect upon the reactivity of the coupling reaction, since the changed moieties are located far from the reaction center. Therefore, it seemed likely that the 2-chlorophenyl group was not suitable for the original procedure, presumably because of its steric reason.

Our method with bis(tributyltin) oxide has improved not only regarding yields of the desired 3′→5′ dimers, but also regarding selectivity of the coupling reaction with the 3′,5′-unprotected nucleosides as far as the six dimers are concerned. However, the synthetic reactions for ten other possible dimers still have problems to be solved. There are mainly due to the lower reactivity of the phosphorochloridate intermediates (**3**) with incoming stannylated nucleosides.

Table 2. The synthesis of the partially-protected nucleotide dimers with $(Bu_3Sn)_2O$

BB′ [a]	5′-Protected component mmol (equiv)	$(Bu_3Sn)_2O$[b] mmol (equiv)	2-Cl-Phenyl phosphorodichloridate mmol (equiv)	3,5′-Unprotected component mmol (equiv)	$(Bu_3Sn)_2O$[c] mmol (equiv)	DMF[d] ml	Yield/% (R_f value)[e] 3′→5′	Yield/% (R_f value)[e] 3′→3′
TT	0.7 (1.0)	0.42 (1.2)	0.98 (1.4)	1.05 (1.5)	0.63 (1.8)	1	94 (0.46)	<3 (0.56)
TT	0.7 (1.0)	0.7 (2.0)	0.98 (1.4)	1.05 (1.5)	1.05 (3.0)	0	92	<3
CT	0.5 (1.0)	0.3 (1.2)	0.7 (1.4)	0.75 (1.5)	0.75 (3.0)	1	81 (0.54)	6 (0.63)
AT	0.5 (1.0)	0.5 (2.0)	0.7 (1.4)	0.75 (1.5)	0.75 (3.0)	1	76 (0.51)	<3 (0.59)
TC	0.5 (1.0)	0.3 (1.2)	0.6 (1.2)	0.70 (1.4)	0.53 (2.1)	1.5	73 (0.51)	10 (0.65)
TC	0.5 (1.0)	0.3 (1.2)	0.7 (1.4)	0.75 (1.5)	0.45 (1.8)	2	77	7
AC	0.5 (1.0)	0.5 (2.0)	0.7 (1.4)	0.75 (1.5)	0.75 (3.0)	1.5	71 (0.54)	10 (0.68)
TA	0.5 (1.0)	0.3 (1.2)	0.7 (1.4)	0.75 (1.5)	0.75 (3.0)	1.5	75 (0.45)	<3 (0.64)

a) For the abbreviations and protecting groups of the dimers, see the footnote in the Table 1. b) Used for the 5′-protected component. c) Used for the 3′,5′-unprotected component. d) See the Experimental section. e) TLC on silica gel with chloroform-methanol (9:1). Broad spots due to the diastereoisomers were observed.

Experimental

The ^{1}H NMR spectra were recorded on a JEOL JNM-GX 400 spectrometer, with tetramethylsilane as the internal standard. Merck silica gel GF_{254} was used for TLC, and the compounds were detected by heating after spraying them with a methanol-sulfuric acid-4-methoxybenzaldehyde mixture (85:15:5, v/v) or 5% ethanolic solution of molybdophosphoric acid. Merck silica gel 60 (0.063—0.29 mm) was utilized for column chromatography.

The protected nucleosides were prepared according to known procedures.[10] Bis(tributyltin) oxide and 1-methylimidazole, as well as 4-chlorophenyl phosphorodichloridate and 1*H*-1,2,4-triazole, were purchased from Tokyokasei Co., Ltd. (Tokyo, Japan) and Dojindo Lab. (Kumamoto, Japan), respectively.

The General Procedure for the Synthesis of Partially-protected 2′-Deoxyribonucleotide Dimers. Some of the results and reaction conditions are described in Tables 1 and 2, while the ^{1}H NMR spectral data are summarized in Table 3.

(A) The Method with Bis(tributyltin) Oxide. A mixture of 5′-*O*-(4,4′-dimethoxytrityl)nucleoside (0.5 mmol) and bis(tributyltin) oxide (0.3—0.5 mmol) in benzene (20 ml) was refluxed at 110 °C (bath temperature) for 15 min with azeotropic removal of water by means of a Dean-Stark apparatus. The resulting solution was concentrated to *ca.* 5 ml (solution A). Similarly, a mixture of 3′,5′-unprotected nucleoside (0.75 mmol) and bis(tributyltin) oxide (0.45—0.75 mmol) in benzene (20 ml) and dry DMF (1—2 ml) was refluxed at 110 °C (bath temperature) for 30 min with a water trap. The resulting solution was concentrated to *ca.* 5 ml (solution B). To solution A was added a solution of 2-chlorophenyl phosphorodichloridate[11] (0.6—0.7 mmol) in dry benzene (1 ml) at room temperature under an atmosphere of dry nitrogen. The solution was stirred for *ca.* 15 min. Solution B was then added at room temperature, and the mixture was stirred for 0.5—1 h. Dry pyridine (0.2 ml) was added, and the benzene was then removed at room temperature *in vacuo*. The residue was chromatographed on a silica-gel (65 ml) column made up in benzene-ethyl acetate-pyridine (20:20:1). The tin compounds were first eluted successively with the same solvent system (150 ml) and benzene-ethyl acetate-methanol (20:20:1, 130 ml), and the dimers were then eluted with the latter solvent system (5:5:0.8—5:5:1). Under these conditions for the chromatography, the isolated 3′→5′ dimers of d(AT) and d(AC) were still contaminated with a small amount of the starting 3′,5′-unprotected nucleosides. The thymidine in the former was removed by washing a chloroform solution of the dimer with 0.5 M† phosphate buffer (KH_2PO_4-K_2HPO_4, pH 5.0), and 4-*N*-benzoyl-2′-deoxycytidine in the latter was taken off by means of rechromatography on a silica-gel (20 ml) column with chloroform-methanol (95:5).

(B) The Slightly Modified Method of Cashion *et al.*[3] The nucleosides used in this procedure had been dried by means of azeotropic distillation with dry pyridine.

To a solution of 1*H*-1,2,4-triazole (2.8 mmol, 4 equiv) and triethylamine (2.8 mmol, 4 equiv) in dry pyridine (4 ml) there was added 2-chlorophenyl phosphorodichloridate (0.84 mmol, 1.2 equiv) at room temperature with stirring under an atmosphere of dry nitrogen. After 3 min, 1-methylimidazole (11.2 mmol, 16 equiv) was added, and the stirring was continued for another 5 min. To this mixture was added a solution of 5′-*O*-(4,4′-dimethoxytrityl)nucleoside (0.7 mmol, 1 equiv) in dry pyridine (2.5 ml) at room temperature. After the mixture had been stirred for 30 min, a solution of the 3′,5′-unprotected nucleoside (1.4 mmol, 2 equiv) in dry pyridine (3 ml) was added. In the case of the deoxycytidine derivative, a mixture of dry pyridine (3 ml) and dry DMF (2 ml) was used. The mixture was stirred at room temperature for 4—5 h, quenched with 50% aqueous pyridine, and diluted with chloroform (100 ml). The mixture was washed, successively, with 0.5M phosphate buffer (KH_2PO_4-K_2HPO_4, pH 5.0, 30 ml×2) and brine (30 ml), dried over magnesium sulfate, and concentrated. The pyridine was removed below 45 °C *in vacuo* by co-evaporation with toluene. The residue was chromatographed on a silica-gel (100 ml) column with chloroform-methanol (97:3→9:1) to give the dimers.

† 1 M=1 mol dm^{-3}.

Table 3. The ^{1}H NMR spectral data (δ) of the partially-protected nucleotide dimers[a]

BB′[b]	3′→5′ Dimer		3′→3′ Dimer	
	$H_{1'}$,$_{1''}$(2H)	$H_{3'}$(1H)[c]	$H_{1'}$,$_{1''}$(2H)	$H_{3'}$,$_{3''}$(2H)[c]
TT	6.31 (t)	5.27 (br. t)	6.25 (q)	5.31 (br. s)
	6.44 (m)	5.35 (br. t)	6.47 (m)	
CT	6.30 (m)	5.27 (br. t)	6.21 (t)	5.3 (m)
		5.33 (br. t)	6.26 (t)	5.4 (br. s)
			6.31 (t)	
AT	6.23 (t)	5.50 (br. q)	6.21 (q)	5.35 (br. q)
	6.27 (t)		6.52 (q)	5.47 (br. q)
	6.47 (m)			
TC	6.26 (q)	5.33 (br. t)	6.23 (q)	5.33 (br. s)
	6.40 (m)	5.27 (br. t)	6.45 (m)	
AC	6.27 (t)	5.48 (br. t)	6.17 (q)	5.36 (br. s)
	6.52 (q)		6.55 (q)	5.46 (br. q)
TA	6.47 (m)	5.24 (br. t)	6.45 (m)	5.31 (br. t)
		5.31 (br. t)		5.49 (br. t)

a) Measured in $CDCl_3$. The clear signals were not obtained because of the presence of the diastereoisomers. b) For the abbreviations and protecting groups of the dimers, see the footnote in the Table 1. c) Deshielded by the phosphate moiety.

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8) The signal due to the methyl group of this tin compound appeared at δ 0.92 as a triplet (J=7.3 Hz). This compound was also visualized on a TLC plate by treating it with the ethanolic solution of molybdophosphoric acid.

9) We have succeeded in the conversion of **4a** into a 2-chlophenyl ester of thymidylyl(3′→5′)-3′-*O*-benzoylthymidine (78% yield from **1a**) in a one-pot procedure by the conventional benzoylation followed by detritylation without isolation of any intermediates starting from **1a** and **2a**.

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11) This reagent which had been repeatedly exposed to moisture colored the reaction mixture in red for an instant. Such occasion gave unsatisfactory results.