Synthesis of Bis(deoxyribonucleoside) Phosphoromorpholidate Derivatives for Oligodeoxyribonucleotide Preparation by Use of a Selective Phosphitylating Reagent

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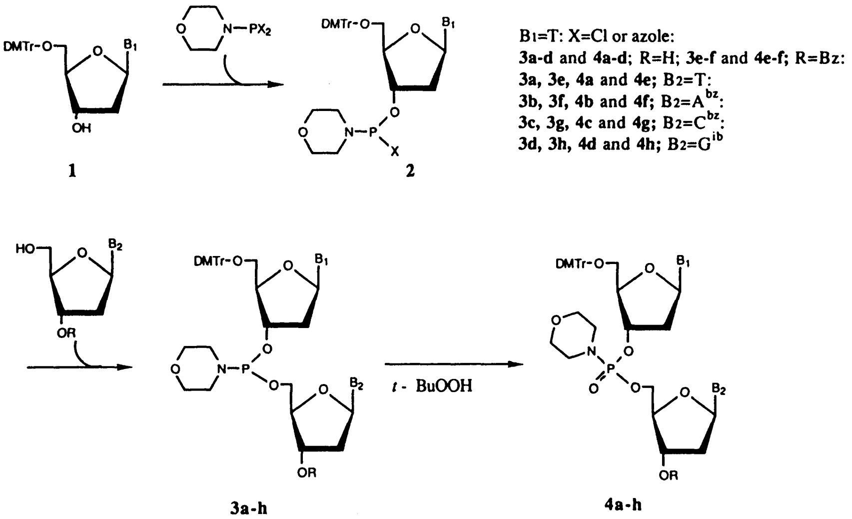
Sequence-defined bis(deoxyribonucleoside) phosphoromorpholidate derivatives as stable intermediates to prepare oligodeoxyribonucleotides have been synthesized using morpholinodi(1-tetrazolyl)phosphine as a phosphitylating reagent. The general procedure is composed of following steps (in situ); a) a reaction of 5′-O-protected deoxyribonucleoside with morpholinodi(1-tetrazolyl)phosphine (phosphitylation), b) a reaction of the resulting 5′-O-protected deoxyribonucleoside phosphorobisamidite with the second deoxyribonucleoside (coupling), and c) a nonaqueous oxidation with *t*-BuOOH (oxidation). In the coupling step, the 5′-O-protected deoxyribonucleoside phosphorobisamidite was selectively reacted with 5′-OH group of the second deoxyribonucleoside, and therefore, a protection of hydroxyl groups of the second deoxyribonucleoside is not necessary. As the resulting P-N bond in the phosphoramidate linkage after oxidation is stable both in acidic and basic conditions, the morpholino function as a phosphoryl protecting group may have many potential use in the synthetic chemistry of oligonucleotide. A thymidine trimer can be obtained in a high yield by extending the present synthetic method.

Since Letsinger¹⁾ and Caruthers²⁾ introduced phosphite and phosphoramidite approaches to the synthesis of oligonucleotides, extensive research has been directed toward developments of more promising methodologies by using the efficient phosphitylating reagents.³,⁴⁾ Moreover, it is of particular importance to develop a method for a large scale synthesis of sequence-defined oligonucleotides in order to solve many genetic phenomena by physical chemical studies and to use them for new medicines. The purpose of our study has been focused on a rapid and large scale synthesis of sequence-defined oligonucleotides by using the selective phosphitylating reagents. Previously, we have developed tris(azolyl)phosphines as phosphitylating reagents which react selectively with the cis-glycol of ribonucleosides. Based on the selective phosphitylation, homo-oligoribonucleotides can be obtained directly from unprotected ribonucleosides by the chemical polymerization approach.⁵,⁶⁾ A new class of phosphitylating reagents, (dialkylamino)dichlorophosphine possessing the same selectivity to the ribonucleoside hydroxyl groups, were synthesized and adapted to the solution phase synthesis of oligoribonucleotides having defined sequences.⁷⁾

The next objective of our research is to use the phosphitylating reagent for synthesizing sequence-defined oligodeoxyribonucleotides and their analogs. We have developed the convenient procedure for the synthesis of sequence-defined bis(deoxyribonucleoside) derivatives having phosphoromorpholidate linkage, which contains the following steps (Scheme 1); i) a reaction of 5′-O-protected deoxyribonucleoside with morpholinodi(1-tetrazolyl)phosphine (phosphitylation), ii) a reaction of the resulting 5′-O-protected deoxyribonucleoside phosphorobisamidite with the second deoxyribonucleoside (coupling), and



B1=T: X=Cl or azole:
3a-d and 4a-d; R=H; 3e-f and 4e-f; R=Bz:
3a, 3e, 4a and 4e; B2=T:
3b, 3f, 4b and 4f; B2=A^{bz}:
3c, 3g, 4c and 4g; B2=C^{bz}:
3d, 3h, 4d and 4h; B2=G^{ib}

Scheme 1.

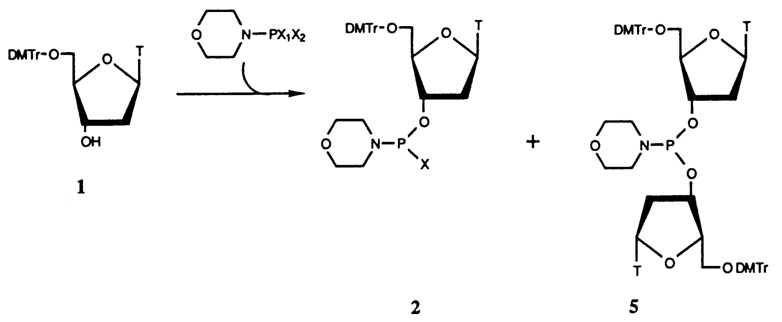
iii) a nonaqueous oxidation of resulting phosphoromorpholidite linkage by using *t*-butyl hydroperoxide (*t*-BuOOH) (oxidation).[8,9] In order to demonstrate the possibility of a chain elongation, the synthesis of a trimer was performed by repeating the procedure described above.

Additionally, our present approach may provide a useful method for the preparation of biologically interesting oligonucleotide analogs having phosphoramidate linkages. In the literature, several oligonucleotide analogs having various internucleotide bonds were synthesized and it was shown that they had resistance in cleavage by a variety of nucleases.[10,11] Also, nonionic oligonucleotide analogs having methylphosphonates have been synthesized,[12] and shown to form complexes with complementary DNA or RNA with different stabilities and to exert inhibitory effects on a protein synthesis.[13] The oligodeoxyribonucleotide analogs having phosphoramidate linkages could be also expected to have biochemical and biological effects and the analogs were synthesized by H-phosphonate method.[14] The present synthetic method is a simple and convenient procedure for the synthesis of oligonucleotide analogs having phosphoramidate linkage.

In this paper, we describe the synthesis of the sequence-defined bis(deoxyribonucleoside) phosphoromorpholidate derivatives, as the stable intermediates for the synthesis of oligodeoxyribonucleotides by use of a selective phosphitylating reagent.

Results and Discussion

Phosphitylation of 5′-O-Protected Deoxyribonucleoside (Scheme 2). Several (dialkylamino)dichlorophosphines have been prepared and their reactivity and selectivity have been examined in the reaction with ribonucleosides.[7] Among them, dichloromorpholinophosphine was found to be highly reactive toward the secondary hydroxyl groups of ribonucleosides. We therefore tried to use the reagent for the phosphitylation of 5′-O-protected deoxyribonucleoside. However, it was found that the reaction of 5′-*O*-[bis(4-methoxyphenyl)phenylmethyl]thymidine (5′-*O*-dimethoxytritylthymidine, d-DMTrT) with the phosphitylating reagent is non-selective and gave a considerable amount of an undesired 3′,3′-linked dithymidylate analog, **5**. On the contrary, the use of its azolide instead of the chloride for the phosphitylation improved the selectivity. Results are summarized in Table 1. In case of using monoazolide derivatives as the phosphitylating reagent, a significant amount of side products were still yielded. A good selectivity in the reaction was obtained by using di(triazolide) and di(tetrazolide) derivatives. From the practical point of view, morpholinodi(1-tetrazolyl)phosphine was found to give the phosphitylated product **2** in a yield of over 80% within 30 min under the present condition.

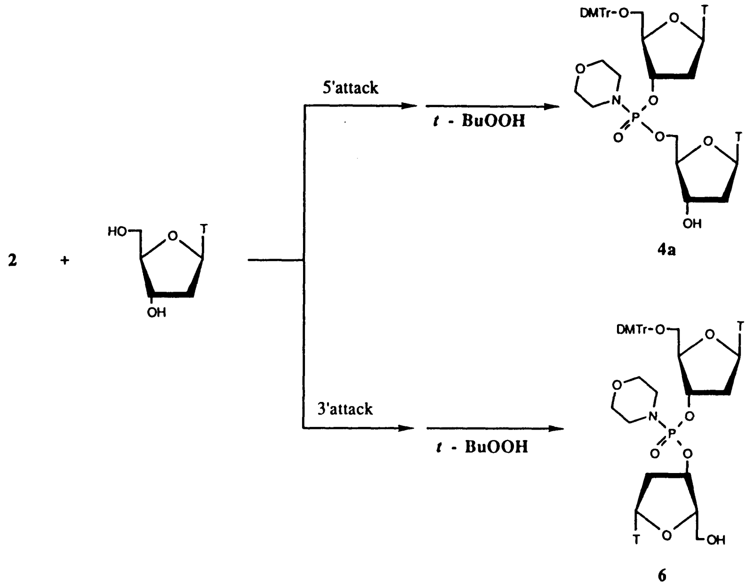


Scheme 2.

Table 1. Selectivity in the Phosphitylation of d-DMTrT with $X_1X_2PN(CH_2CH_2)_2O$

$X_1X_2PN(CH_2CH_2)_2O$		Yield[b)]		
X_1[a)]	X_2[a)]	**1**	**2**	**5**
Im	Cl	30.6	20.4	49.0
Im	Im	92.2	7.8	—
Tri	Cl	35.2	22.9	41.9
Tri	Tri	41.1	58.6	—
Te	Cl	16.9	60.3	22.8
Te	Te	9.3	82.1	8.6

a) Im, imidazolyl; Tri, triazolyl; Te, tetrazolyl. b) d-DMTrT was phosphitylated by $X_1X_2PN(CH_2CH_2)_2O$ in CH_2Cl_2-pyridine at 0 °C under N_2 for 30 min. After the reaction mixture was treated by absolute CH_3OH, TLC analysis was carried out.



Scheme 3.

Selectivity in the Coupling Reaction (Scheme 3). d-DMTrT phosphorobisamidite **2** (X=tetrazolyl) thus obtained was allowed to react with thymidine. The reaction was completed within 30 min which is indicated by TLC and HPLC. After the in situ oxidation using *t*-BuOOH, 3′-5′ linked dithymidine phosphoromorpholidate **4a** was obtained as a main product together with only a little amount of an undesired 3′-3′ linked product **6** (**4a**/**6**=96.2/3.8). The result clearly indicates that compound **2** reacts predominantly with the 5′-OH of the second deoxyribonucleoside in the coupling reaction. Therefore, this highly selective reaction provides a simple and convenient route to the synthesis of bis(deoxyribonucleoside) derivatives without any protection of 3′-OH of the second deoxyribonucleoside.

Oxidation of Phosphoromorpholidite Linkage. Since an intermediate **3a** having a phosphoromorpholidite linkage is sensitive to atmospheric oxygen and moisture, its isolation by recrystallization or silica-gel column was not successful. Preliminary experiments showed that the in situ oxidation of **3a** with I_2–H_2O and *m*-chloroperbenzoic acid gave a large number of side products. We have solved this problem by using *t*-BuOOH.[8,9] The reaction product was also analyzed by ^{31}P NMR (Fig. 1). ^{31}P NMR shows that after the oxidation of **3a** with 3 M *t*-BuOOH in toluene (1 M=1 mol dm^{-3}) for 10 min at 0 °C, **3a** (138.4 ppm) completely disappeared to give the single peak (7.08 ppm) due to the phosphoromorpholidate derivative **4a**. This result indicated that the phosphoromorpholidite linkage in **3a** was smoothly converted to the corresponding phosphoromorpholidate linkage without any side reactions.

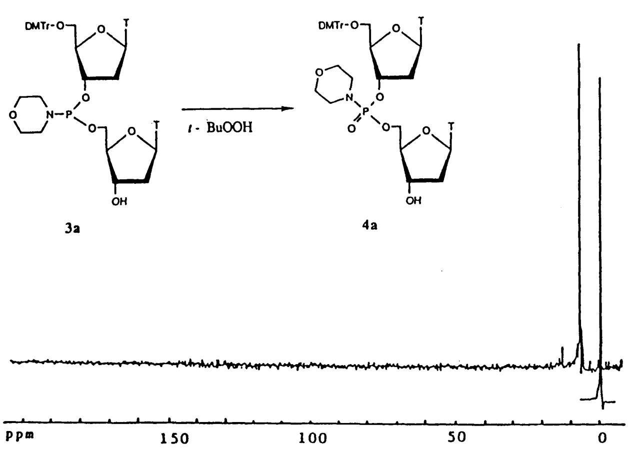


Fig. 1. ^{31}P NMR of bis(deoxyribonucleoside) derivative (**4a**) obtained by use of *t*-BuOOH (in $CDCl_3$, relative to external 85% H_3PO_4).

Effect of (*i*-Pr)$_2$NEt. We examined the effect of ethyldiisopropylamine ((*i*-Pr)$_2$NEt) on coupling reaction. When the synthesis of **4a** was carried out by using (*i*-Pr)$_2$NEt of 2 equiv for the phosphitylating reagent, namely equimolar amounts of hydrochloride released to the system, a lot of unknown products were formed in the reaction (Fig. 2). On the other hand, when it was carried out by using (*i*-Pr)$_2$NEt of 4 equiv for the phosphitylating reagent, unknown products decreased and desired products (**4a**) increased. This result suggests that it is required to trap the released tetrazolium ion by using base in order to conserve the phosphoromorpholidite linkage and to increase efficiency of the coupling reaction.

Synthesis of Bis(deoxyribonucleoside) Phosphoromorpholidate Derivatives (4a—d). The applicability of the synthetic route described above was tested in the syntheses of bis(deoxyribonucleoside) phosphoromorpholidate derivatives having different sequences. The yields and physical chemical data of the obtained bis(deoxyribonucleoside) phosphoromorpholidate derivatives (**4a**—**d**) were summarized in Table 2. **4a**—**d** agreed with 3′-5′ linked bis(deoxyribonucleoside) phosphoromorpholidates derived from **4e**—**h** (Scheme 1) on HPLC, which were synthesized by use of 3′-O-protected deoxyribonucleosides as the second deoxyribonucleosides in the similar procedure. In the every case, the desired 3′,5′-linked bis(deoxyribonucleoside) phosphoromorpholidate derivatives were obtained with good yields by the convenient method without the protection of the hydroxyl groups of the second deoxyribonucleosides.

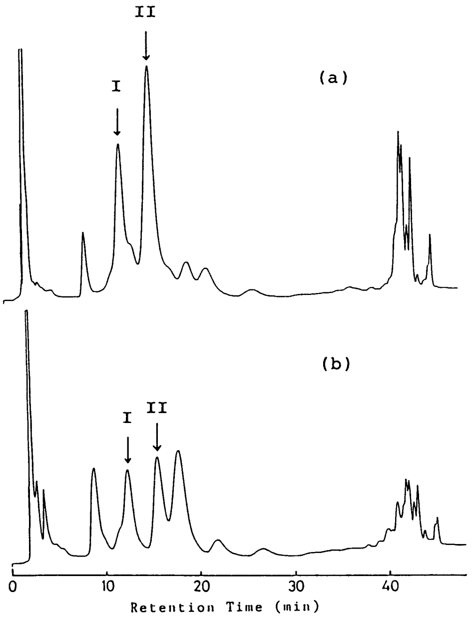
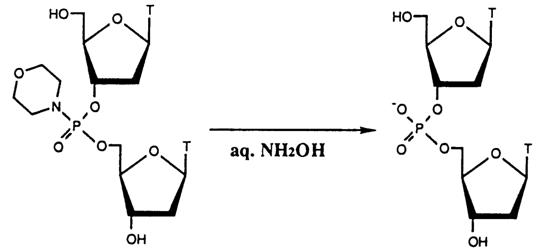


Fig. 2. Effect of (i-Pr)$_2$NEt on dithymidine derivative (**4a**) synthesis. (a) 4 equiv of (i-Pr)$_2$NEt for phosphitylating reagent. (b) 2 equiv of (i-Pr)$_2$NEt for phosphitylating reagent. HPLC condition: column, Cosmosil 5C$_{18}$ (4.6 mm i.d. ×150 mm length); eluent, 70—100% CH$_3$OH gradient in H$_2$O; flow rate, 1 mL min^{-1}; detect: UV at 254 nm. The peaks of **4a** are indicated by the arrows.

Stability of Phosphoromorpholidates. Table 3 shows the stability of the P-N bond in **4a** under various conditions. The P-N bond was stable in 80% acetic acid, concentrated aq. NH$_3$, and 0.5 M NaOH. Since the phosphoromorpholidate linkage is stable for acid and alkaline, the fully protected dimer block, **4e**, can be used to elongate the nucleotide chain both toward 3′- and 5′-direction from 3′-free derivatives obtained by alkaline treatment and 5′-free derivatives obtained by acid treatment, respectively. Moreover, it is possible to introduce phosphoramidate at all or any specific sites within oligodeoxyribonucleotides and these molecules are expected to have several interesting biological effect.

Removal of Phosphoryl Protecting Group (Scheme 4). Since hydroxylamine is known to hydrolyze phosphoramidates,[15,16] it appeared a potential deprotection agent for a phosphoramidate link. The P-N bond underwent hydrolysis to phosphodiester by a treatment with hydroxylamine. In the case of treat-



Scheme 4.

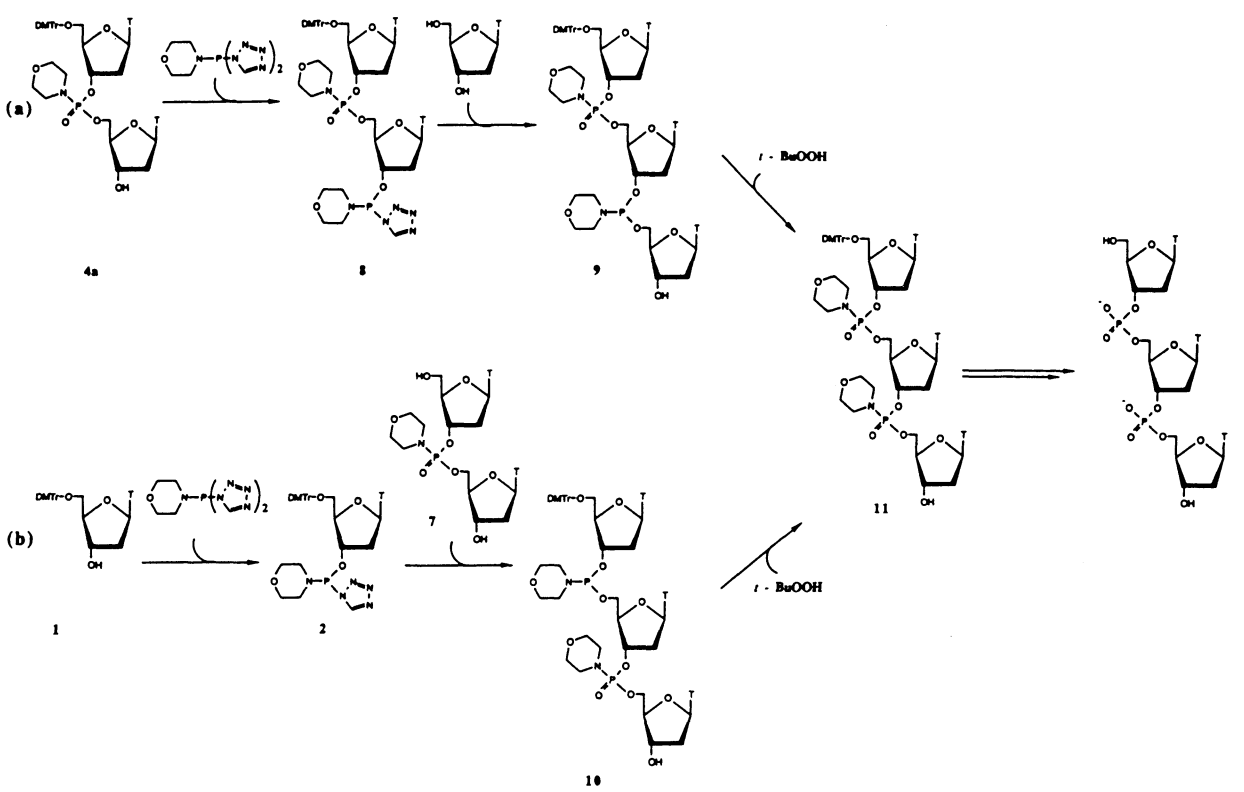
Table 3. Stability of Phosphoromorpholidate in **4a** under Several Conditions

Reagent	Solvent	pH	Temp/°C	Time/h
80% AcOH	H$_2$O	0.4	r.t.	48
Isopentyl nitrite	Py-AcOH(1 : 1)	4.6	r.t.	24
28% NH$_3$ aq.	H$_2$O	12.5	r.t.	12
0.5 M NaOH	EtO-H$_2$O(1 : 1)	13.0	30	29

Table 2. Yields and Physical Chemical Data of Sequence Defined Bis(deoxyribonucleoside) Phosphoromorpholidate Derivatives (**4a**—**d**)

Compound	Yield[a]	δ-^{31}P[b]	$\lambda_{\max}$	R_f[c]	T_r[d]	Isomer[e] ratio I/II
	%	ppm	nm		min	
4a	74 67[f]	7.08	266, 235	0.39	11.8 14.5	36/64
4b	62	7.01, 7.08	275, 234	0.42	21.2 25.1	33/67
4c	55	6.98, 7.22	304, 261, 236	0.43, 0.44	30.2 32.7	31/69
4d	70	6.04	261, 237	0.27, 0.28	18.8 22.2	30/70

a) Based on HPLC. b) Relative to external 85% H$_3$PO$_4$. c) TLC on silica using CHCl$_3$: CH$_3$OH (8 : 1, v/v). d) Reversed-phase HPLC on Cosmosil 5C$_{18}$ using eluent A. e) Diastereomer. Isomer I was earlier eluted isomer and isomer II was later eluted isomer on the reversed-phase HPLC. Absolute configuration was not assigned. f) Isolated yield.



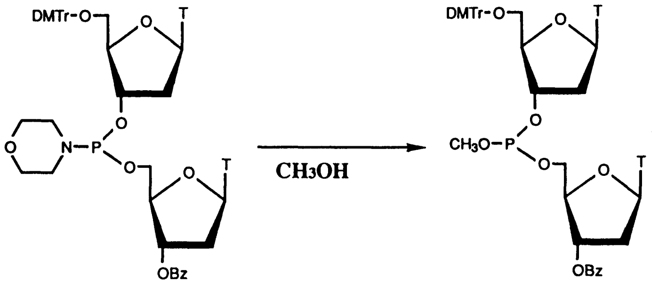
Scheme 5.

ing compound **7** with 4.0 M aqueous hydroxylamine, a small amount of unknown product formed. Compound **7** was treated with 2.0 M aqueous hydroxylamine at 50 °C. Based on HPLC analysis, the reaction resulted in complete deprotection within one week to give thymidylyl(3′-5′)thymidine (d(T-T)). The structure of this product was confirmed by an authentic sample, and a degradation with snake venom phosphodiesterase and spleen phosphodiesterase.

Chain Elongations of Bis(deoxyribonucleoside) Derivatives Both toward 3′- and 5′-Direction. In order to demonstrate chain elongations of bis(deoxyribonucleoside) derivative toward 3′- and 5′-direction, syntheses of $(dT)_3$ derivatives, trimer, were attempted by the coupling of the dithymidine derivatives such as **4a** or **7**, and thymidine or d-DMTrT **1**, respectively. First, the trimer synthesis was carried out in the similar synthetic method of bis(deoxyribonucleoside) phosphoromorpholidate derivatives by use of compound **4a** (3′-OH free derivative) as a starting material and unprotected thymidine as a coupling deoxyribonucleoside (Scheme 5a). After oxidation the total products were analyzed by HPLC. Yield of compound **11** was 77% (based on HPLC). ^{31}P NMR (in $CHCl_3$) δ=6.47 ppm (relative to external 85% H_3PO_4). The structure was confirmed by the following method. The product isolated by HPLC was treated with 80% acetic acid for 30 min at room temperature to remove the 5′-O-protecting group. After the reaction mixture was evaporated, 2.0 M aqueous hydroxylamine was added to the residue, and then the mixture was stirred at 50 °C for one week to remove phosphoryl protecting group. The resulting thymidine trimer $(dT)_3$ was confirmed by an agreement with an authentic sample (Sigma) on HPLC and a degradation method with snake venom phosphodiesterase and spleen phosphodiesterase.

Also, d-DMTrT **1** as a starting material and **7** (3′,5′-OH free dithymidine derivative) as a second deoxyribonucleoside derivative were used (Scheme 5b). The other conditions were similar to those of the abovementioned trimer synthesis. The structure of the obtained product **11** was confirmed in the similar manner as the identification of **11** obtained above. Yield of compound **11** in this procedure was 67% based on HPLC analysis. This results indicate that chain elongations both toward 3′- and 5′-direction from dithymidine derivatives were carried out with as high yield as dithymidine derivative synthesis (74%, see Table 2).

Conversion of 3e into Phosphorous Triester. The phosphoramidite in **3e** can easily be converted to phosphorous triester by reaction with alcohol (Scheme 6). Accordingly, **3e** was readily converted to **12** by a reaction with methanol, and this intermediate **12** is widely used in phosphite chemistry. This experiment was examined by ^{31}P NMR. The addition of absolute CH_3OH (3.0 equiv) to a solution of **3e** resulted in the complete disappearance of the signal at 138.4 ppm (in $CDCl_3$) and its replacement by two new singlets at 141.7 and 142.0 ppm, which were in agree-



3e **12**

Scheme 6.

ment with the structure of **12**, as expected for two diastereomers. This result suggests that bis(deoxyribonucleoside) phosphoromorpholidite derivatives can be adapted as a key intermediate which can easily be converted to compounds bearing other phosphite linkages by the reaction of nucleophilic reagents.

Conclusion

The present approach using morpholinodi(1-tetrazolyl)phosphine provides a simple and convenient procedure for synthesizing bis(deoxyribonucleoside) derivatives, which does not require any protection of hydroxyl group of the second deoxyribonucleoside. The phosphoromorpholidite linkage can be used as a key intermediate capable of converting to other phosphite compounds and the stability of the P-N bond in the phosphoromorpholidate linkage provides a phosphoryl-protecting group in the oligodeoxyribonucleotide synthesis. In addition, it is expected that the present method to synthesize the bis(deoxyribonucleoside) derivatives having phosphoramidate linkages is applied to the syntheses of several functional oligonucleotide derivatives whose phosphorus is linked with fluorescent, intercalating and cross-linking moiety, through the reaction of nucleoside and phosphorus derivatives bearing those moieties.

Experimental

HPLC was carried out on a Shimadzu LC 6A apparatus using Cosmosil 5C$_{18}$ analytical column (4.6 mm i.d. × 150 mm length, Nacalai Tesque Co.). The eluents were 70—100% CH_3OH gradient in water (eluent A) or 8—25% CH_3CN gradient in 0.1 M ammonium acetate solution (eluent B). TLC was carried out on Merck silica-gel plates (No. 5554 for analysis or No. 5717, 13793 for preparative work). Preparative silica-gel column chromatography was performed on Wako gel C-200 (Wako Pure Chemicals Co.). Middle pressure liquid chromatography was carried out on a Yamazen 540-3 apparatus using LC-SORB SP-B-SI preparative column (30 mm i.d. × 300 mm length, Chemco Scientific Co., Ltd.). ^{31}P NMR were recorded on a Hitachi R-900FT NMR (^{31}P 36.43 MHz) or a JEOL JNM-EX90 (^{31}P 36.23 MHz) relative to external 85% H_3PO_4.

Thymidine, deoxyadenosine, deoxycytidine, and deoxyguanosine were supplied from Yamasa Shoyu Co., Ltd. d-DMTrT was synthesized according to published procedures.[17] N^6-benzoyldeoxyadenosine (d-bzA),[18] N^4-benzoyldeoxycytidine (d-bzC),[18] N^2-isobutyryldeoxyguanosine (d-ibG),[18] 3′-*O*-benzoylthymidine (d-TBz),[19] N^6, 3′-*O*-dibenzoyldeoxyadenosine (d-bzA(Bz)),[20] N^4, 3′-*O*-dibenzoyldeoxycytidine (d-bzC(Bz)),[20] and 3′-*O*-benzoyl-N^2-isobutyryldeoxyguanosine (d-ibG(Bz))[20] were also synthesized according to published procedures. These compounds were coevaporated with pyridine prior to use. CH_2Cl_2 was refluxed over CaH_2 and distilled immediately prior to use. Pyridine was refluxed over CaH_2, distilled and stored over 4A Molecular Sieves. Ethyldiisopropylamine ((i-Pr)$_2$NEt) was refluxed over CaH_2, distilled over CaH_2, and then stored over CaH_2. *t*-Butyl hydroperoxide (*t*-BuOOH) in toluene (3 M solution) was purchased from Aldrich Chemicals Co. and stored over 4A Molecular Sieves. Other reagents were used without further purification.

Preparation of Dichloromorpholinophosphine. A solution of morpholine (86.5 mL, 1.0 mol) in 100 mL of dry ether was added dropwise over 2 h to a vigorously stirred solution of phosphorus trichloride (43.6 mL, 0.5 mol) in 300 mL of dry ether at 0 °C. After stirring for 1 h, the amine hydrochloride was removed by filtration and ether was distilled at atmospheric pressure. The residue was distilled from cesium fluoride under reduced pressure and the desired material was isolated in a pure form (Yield: 56.9 g (60.5%), 58.0 °C/1.0 mmHg; 1 mmHg=133.322 Pa). ^{31}P NMR ($CDCl_3$) δ=156.5. Found: C, 25.48; H, 4.33; N, 7.66; P, 16.63; Cl, 37.45%. Calcd for $C_4H_8NOPCl_2$: C, 25.55; H, 4.29; N, 7.45; P, 16.47; Cl, 37.71%.

Preparation of Azolylchloromorpholinophosphine. A solution of azole (0.55 mmol) in CH_2Cl_2-pyridine (1:1 v/v, 1 mL) was added dropwise to dichloromorpholinophosphine (77.5 mL, 0.55 mmol) in CH_2Cl_2 (1 mL) containing (i-Pr)$_2$NEt (0.2 μL) with stirring at 0 °C under N_2 and the solution was stirred for 20 min. These phosphitylating reagents prepared in situ were then used in the phosphitylation of d-DMTrT.

Preparation of Bis(azolyl)morpholinophosphine. A solution of azole (1.1 mmol) in CH_2Cl_2-pyridine (1 : 1 v/v, 1 mL) was added dropwise to dichloromorpholinophosphine (77.5 μL, 0.55 mmol) in CH_2Cl_2 (1 mL) containing (i-Pr)$_2$NEt (0.2 mL) with stirring at 0 °C under N_2 and the solution was stirred for 20 min. These phophitylating reagents prepared in situ were then used in the phosphitylation of d-DMTrT.

Phosphitylation of d-DMTrT. A solution of d-DMTrT (272.5 mg, 0.5 mmol) in CH_2Cl_2 (1.5 mL) was added dropwise over a period of 10 min to azolylchloromorpholinophosphine (0.55 mmol) or bis(azolyl)morpholinophosphine (0.55 mmol) in CH_2Cl_2 (1.0 mL) containing (i-Pr)$_2$NEt (0.2 mL) with stirring at 0 °C under N_2. After the appropriate time intervals, the aliquot of the reaction mixture was treated with absolute CH_3OH. The resulting thymidine phosphoramidite derivatives were analyzed by TLC (on siica gel, CH_2Cl_2-EtOAc-Et_3N, 40/50/10, v/v/v).

General Procedures for the Preparation of Bis(deoxyribonucleoside) Phosphoromorpholidate Derivatives (4a—h). A solution of tetrazole (77.1 mg, 1.1 mmol) in CH_2Cl_2-pyridine (1 : 1 v/v, 1.0 mL) was added dropwise to dichloromorpholinophosphine (77.6 μL, 0.55 mmol) in CH_2Cl_2 (1 mL) containing (i-Pr)$_2$NEt (0.2 mL). After the reaction mixture was stirred at 0 °C for 20 min under N_2, a solution of d-DMTrT (272.5 mg, 0.5 mmol) in CH_2Cl_2 (1.5 mL) was added dropwise over a period of 10 min to the solution with stirring at 0 °C. After 30 min, the solution was added dropwise to thymidine (243 mg, 1.0 mmol) in CH_2Cl_2-pyridine (1 : 2 v/v, 3 mL) containing (*i*-Pr)$_2$NEt (0.2 mL) with stirring and the reaction mixture was stirred for an additional 30 min. Further 3 M *t*-BuOOH in toluene (0.2 mL) was added to the reaction mixture and the solution was stirred for 10 min at 0 °C. The mixture was evaporated and coevaporated with toluene. Analysis of HPLC (in eluent A) revealed two major peaks corresponding to a diastereomeric mixture of compound **4a**. The mixture was applied to a silica-gel column and eluted with $CHCl_3$-CH_3OH (95 : 5 v/v). The appropriate fractions were collected, evaporated, and precipitated with CH_2Cl_2 (5 mL) into hexane (300 mL). The white precipitate was collected by filtration, washed and dried over P_2O_5 in vacuo. Isolated yield of **4a** was 67% (307.2 mg)(for d-DMTrT) and the purity was analyzed by HPLC (>90%). Isomer I, Found: C, 57.06; H, 5.61; N, 7.65%. Calcd for $C_{45}H_{52}O_{14}N_5P$: C, 58.88; H, 5.71; N, 7.63%, mp 137—139 °C. Isomer II, Found: C, 56.91; H, 5.62; N, 7.50%, mp 135—138 °C.

Compound **4b** was prepared by the similar procedure, except that the second deoxyribonucleoside was d-bzA (355.4 mg, 1.0 mmol) in pyridine (3 mL). Yield was 62% (based on HPLC). Isomer I, Found: C, 59.10; H, 5.37; N, 10.25%. Calcd for $C_{52}H_{55}O_{13}N_8P$: C, 60.58; H, 5.38; N, 10.87%, mp 144—148 °C. Isomer II, Found: C, 59.58; H, 5.12; N, 10.67%, mp 142—144 °C.

Compound **4c** was prepared by the similar procedure, except that the second deoxyribonucleoside was d-bzC (331.4 mg, 1.0 mmol) in pyridine (5 mL). Yield was 55% (based on HPLC). Isomer I, Found: C, 59.15; H, 5.26; N, 7.99%. Calcd for $C_{51}H_{55}O_{14}N_6P$: C, 60.83; H, 5.50; N, 8.35%, mp 146—149 °C. Isomer II, Found: C, 59.43; H, 5.41; N, 8.17%, mp 146—147 °C.

Compound **4d** was prepared by the similar procedure, except that the second deoxyribonucleoside was d-ibG (337.3 mg, 1.0 mmol) in pyridine (3 mL). Yield was 70% (based on HPLC). Isomer I, Found: C, 55.29; H, 5.44; N, 10.90%. Calcd for $C_{49}H_{57}O_{14}N_8P$: C, 58.10; H, 5.67; N, 11.06%, mp>159 °C. Isomer II, Found: C, 56.43; H, 5.51; N, 10.41%, mp>155 °C.

Compound **4e** was prepared by the similar procedure, except that the second deoxyribonucleoside was d-Tbz (173.2 mg, 0.5 mmol) in CH_2Cl_2-pyridine (1 : 2 v/v, 3 mL). Yield was 62% (based on HPLC, isomer I/isomer II=33/67). Isomer I, ^{31}P NMR ($CDCl_3$) δ=7.03, UV (CH_3OH): λ_{max} 266 and 231 nm, λ_{min} 250 nm. R_f (on silica gel, $CHCl_3$: CH_3OH=8 : 1)=0.57. Found: C, 59.57; H, 5.42; N, 6.77%. Calcd for $C_{52}H_{56}O_{15}N_5P$: C, 61.11; H, 5.52; N, 6.85%, mp 137—139 °C. Isomer II, ^{31}P NMR ($CDCl_3$) δ=6.96, UV (CH_3OH): λ_{max} 266 and 231 nm, λ_{min} 250 nm, R_f (on silica gel, $CHCl_3$: CH_3OH= 8 : 1)=0.57. Found: C, 59.24; H, 5.46; N, 6.75%, mp 140—141 °C.

Compound **4f** was prepared by the similar procedure, except that the second deoxyribonucleoside was d-bzA(Bz) (229.7 mg, 0.5 mmol) in pyridine (3 mL). Estimated yield from ^{31}P NMR was 72%. ^{31}P NMR ($CHCl_3$) δ=6.87.

Compound **4g** was prepared by the similar procedure, except that the second deoxyribonucleoside was d-bzC(Bz) (217.2 mg, 0.5 mmol) in pyridine (3 mL). Estimated yield from ^{31}P NMR was 64%. ^{31}P NMR($CHCl_3$) δ=6.33.

Compound **4h** was prepared by the similar procedure, except that the second deoxyribonucleoside was d-ibG(Bz) (220.7 mg, 0.5 mmol) in CH_2Cl_2-pyridine (1:2 v/v, 3 mL). Estimated yield from ^{31}P NMR was 41%. ^{31}P NMR ($CHCl_3$) δ=6.06.

Conversion of 4e to 7. Compound **4e** was treated with methanolic ammonia at room temperature overnight and extracted with $CHCl_3$ three times. The organic layer was evaporated to dryness and the residue was treated with 80% acetic acid at room temperature for 30 min. The mixture was evaporated to dryness and freed from acetic acid by three coevaporation with hexane. The residue was dissolved in H_2O and washed with EtOAc three times. Compound **4e** was quantitatively converted into **7** on the basis of HPLC analysis. ^{31}P NMR (H_2O) δ=7.92.

Removal of Phophoryl Protecting Group. Compound **7** was treated with 2.0 M aqueous hydroxylamine (pH 4.75) at 50 °C. Analysis by HPLC in eluent B revealed that **7** was completely converted to d(T-T) in one week. The resulting unprotected d(T-T) was confirmed by a degradation of snake venom phosphodiesterase and spleen phosphodiesterase.

Chain Elongation of Dithymidine Derivative. Route a: (dT)$_3$ derivative was synthesized from compound **4a** as starting material and unprotected thymidine as a coupling deoxyribonucleoside. The synthesis was carried out in the similar procedure as the preparation of bis(deoxyribonucleoside)phosphoromorpholidate derivatives. Yield of compound **11** was 77% (based on HPLC). ^{31}P NMR ($CHCl_3$) δ=6.47 (relative to external 85% H_3PO_4). The structure was confirmed by the following method. The product isolated by HPLC was treated with 80% acetic acid for 30 min at room temperature to remove the 5′-O-protecting group. After the reaction mixture was evaporated, 2.0 M aqueous hydroxylamine was added to the residue, and then the mixture was stirred at 50 °C for one week to remove phosphoryl protecting group. The resulting thymidine trimer (dT)$_3$ was confirmed by an agreement with an authentic sample (Sigma) on HPLC and a degradation method with snake venom phosphodiesterase and spleen phosphodiesterase.

Route b: d-DMTrT **1** as a starting material and compound **7** as a second deoxyribonucleoside derivative were used. The synthetic procedures were similar to those of the synthetic route a. The structure of the obtained product **11** was confirmed in the same manner as the identification of **11** obtained by the above-mentioned synthetic procedure. Yield of compound **11** in this procedure was 67% based on HPLC analysis.

Preparation of 5′-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-3′-thymidine 3′-*O*-Benzoyl-5′-thymidine Methylphosphite (12). The preparation of **3e** was repeated in preparation of **4e**. At the stage before oxidation an absolute CH_3OH was added to the reaction mixture. The mixture was washed with $NaHCO_3$ (5% aq.). The organic layer was dried over Na_2SO_4. Analysis by TLC on silica gel using 10% benzene in EtOAc containing 1% Et_3N revealed two spots (R_f=0.44 and 0.57). The product was dissolved in 1 mL of a mixture of 40% benzene in EtOAc containing 1% Et_3N, applied to a silica-gel column and eluted with 30% benzene in EtOAc containing 1% Et_3N. The appropriate fractions were collected and evaporated to dryness. The residue was taken up in 2 mL $CHCl_3$ and added dropwise to 200 mL hexane at

−20 °C (47.6 mg, 50%). UV ($CHCl_3$): λ_{max} 246 and 268 nm; λ_{min} 252 nm. ^{31}P NMR ($CDCl_3$) $\delta=141.7$ and 142.0. R_f (10% benzene in EtOAc containing 1% Et_3N)=0.44 and 0.57.

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