Unique Participation of Unprotected Internucleotidic Phosphodiester Residues on Unexpected Cleavage Reaction of the Si–O Bond of the Diisopropylsilandiyl Group Used as a Linker for the Solid-Phase Synthesis of 5′-Terminal Guanylated Oligodeoxynucleotides

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Dedicated to Prof. Dr. *Wolfgang Pfleiderer* on the occasion of his 75th birthday

In connection with the synthesis of guanosine-capped oligodeoxynucleotides on polymer supports, we found an unprecedented Si–O bond cleavage reaction, which occurred when polymer-linked oligodeoxynucleotides having unprotected internucleotidic phosphate groups were allowed to react with the guanosine 5′-phosphorimidazolide derivative **18** in the presence of 4-nitro-6-(trifluoromethyl)-1*H*-benzotriazol-1-ol (Ntbt-OH) as an effective activator in pyridine. This side reaction was confirmed by the fact that the liquid-phase reaction of DMTrTpT–O–Si(iPr_2)OEt **42** with a simpler model compound, methyl phosphorimidazolide **34**, in the presence of Ntbt-OH gave DMTrTpT **43**. It turned out that the side reaction hardly occurs without unprotected internucleotidic phosphate groups on oligodeoxynucleotides. The detailed study of this side reaction disclosed that Ntbt-OH directly attacks the Si-atom to release oligonucleotides from the resin. It is likely that Ntbt-OH serves as a very strong nucleophile in pyridine, especially to the Si-atom of the linker.

1. Introduction. – It is well-known that mature mRNAs having a 7-methylguanosine cap structure ($m^7G^{5'}$ppp-) move from the nucleus to the cytoplasm through the nuclear membrane [1]. On the other hand, U1 snRNA, which is a small nuclear RNA having a unique 5′-terminal 2,2,7-trimethylguanosine cap structure ($m_3^{2,2,7}G^{5'}$ppp-) has proven to be transported in the reverse direction [2]. This unique behavior derived from the two different types of cap structures might be utilized for the selective transportation of antisense DNA derivatives into the nucleus or the cytoplasm upon addition of these structural motifs at the 5′-terminal site. Therefore, we designed a simpler molecule represented by $m^7G^{5'}$pp-DNA or $m_3^{2,2,7}G^{5'}$pp-DNA, which lacks a phosphate group and has a DNA chain in place of the RNA chain. In connection with this idea, we also focused our attention on the synthesis of $G^{5'}$ pp-DNA, since this nonmethylated G-capped oligodeoxynucleotide might be used as a precursor of $m^7G^{5'}$- or $m_3^{2,2,7}G^{5'}$-ppDNA. With these possibilities in mind, we studied the solid-phase synthesis of $X^{5'}$pp-d(ATA) (X = G, m^7G or $m_3^{2,2,7}$G) as the simplest model. To realize this synthesis, we used a Bu_4NF (TBAF)-labile silanediyl-type linker. However, we found an unexpected cleavage of the Si–O bond at the final stage of pyrophosphorylation when we tried to synthesize $G^{5'}$ppd(ATA).

In this paper, we report the details of this unprecedented side reaction, which has proved to result from the substitution of the silanediyl group with a strong nucleophile, 4-nitro-6-(trifluoromethyl)-1*H*-benzotriazol-1-ol (Ntbt-OH), used for activation of the capping reagent.

2. Results and Discussion. – 2.1. *Synthesis and Application of Diisopropylsilanediyl Linker.* To synthesize $G^{5'}$ppd(ATA) in the solid phase, we used a new silanediyl type linker **1** and a highly cross-linked (aminomethyl)polystyrene (HCP) [3] resin, as shown in the *Figure*. This is because we ultimately aimed to synthesize $m^7G^{5'}$- or $m_3^{2,2,7}G^{5'}$-ppDNA, and, therefore, we required a base-resistant but fluoride-ion-labile linker. *Fraser et al.* [4] originally reported the solid-phase synthesis of oligonucleotides using a silanediyl-type linker **2** attached to CPG, as depicted in the Figure, which can be cleaved by treatment with TBAF under neutral conditions. However, this linker contains a base-labile succinate moiety. It is also known that CPG supports are fragile upon heating under weakly basic conditions such as aq. NH_3 [5]. Therefore, we developed a new silanediyl linker without the succinate moiety on the HCP support, which is resistant to ammonia and, thereby, allows the general synthesis of oligonucleotides having G-cap, m^7G-cap, or $m_3^{2,2,7}$G-cap structures.

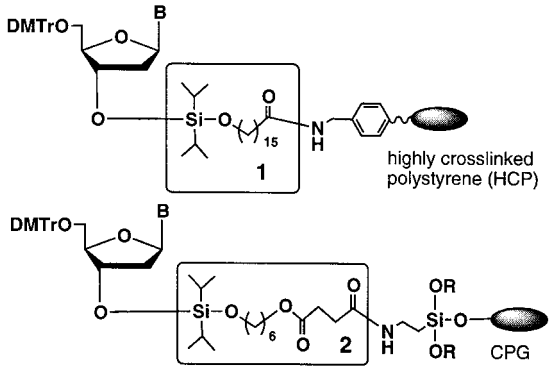
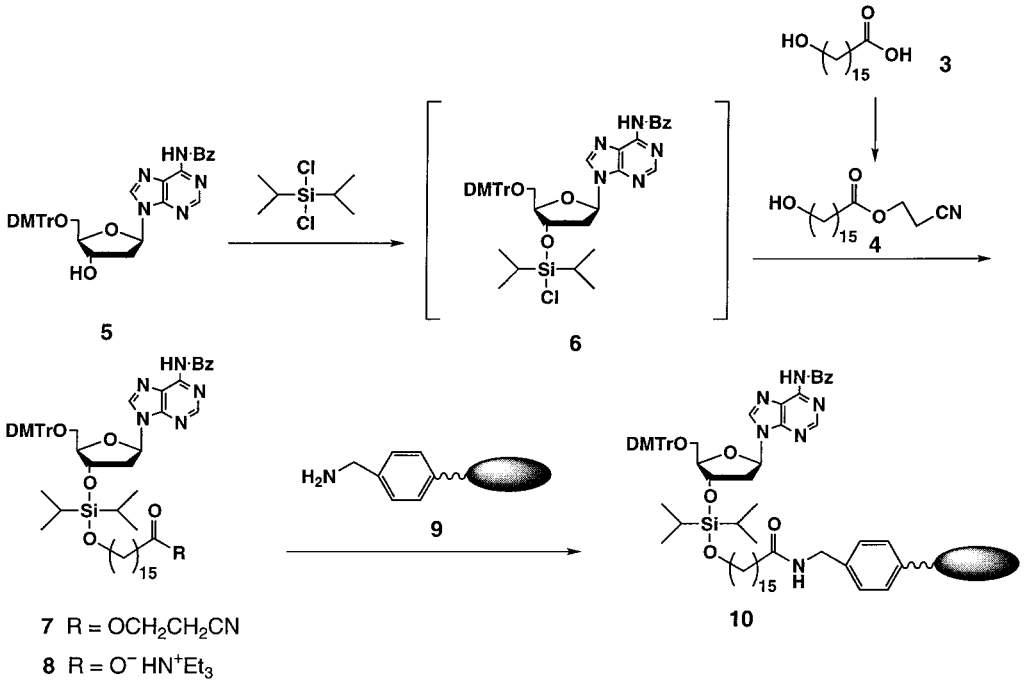


Figure. *The new linker* **1** *developed in this study and the linker* **2** *reported by* Fraser *and co-workers*

2-Cyanoethyl 16-hydroxyhexadecanate (**4**) was prepared in 67% yield by reaction of 16-hydroxyhexadecanoic acid (**3**) with 2-hydroxyethanenitrile in the presence of 1,3-dicyclohexylcarbodiimide (DCC). This backbone structure was used as part of the spacer between the resin and a 3′-terminal deoxynucleoside (*Scheme 1*). Reaction of 6-*N*-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)deoxyadenosine (**5**) with $(i\text{-}Pr)_2SiCl_2$ in the presence of 1*H*-imidazole in DMF gave the 3′-*O*-silylated species **6**, which was allowed to react *in situ* with **4** to give the diisopropylsilanediyl ether derivative **7** in 61% yield from **5**. Treatment of **7** with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) gave the carboxylic acid derivative **8** in 80% yield. Condensation of the HCP resin **9** with **8** in the presence of DCC gave the solid-supported material **10**. The DMTr cation assay indicated that 18.0–28.5 μmol/g of deoxyadenosine could be loaded on the support.

To investigate the efficiency of cleavage of the silyl linkage under neutral conditions, a portion of the derivatized resin **10** was treated with a 1M solution of TBAF in the presence of 1M AcOH in THF for 1 h at room temperature. The DMTr cation assay indicated that quantitative cleavage of the diisopropylsilanediyl linker was achieved. In addition, the stability of the linker under usual deprotection conditions was studied. The derivatized resin **10** was treated with aq. NH_3/EtOH 4 : 1 (*v*/*v*) for 4 h at 55°. The amounts of the nucleoside derivative released and the remaining nucleoside

Scheme 1

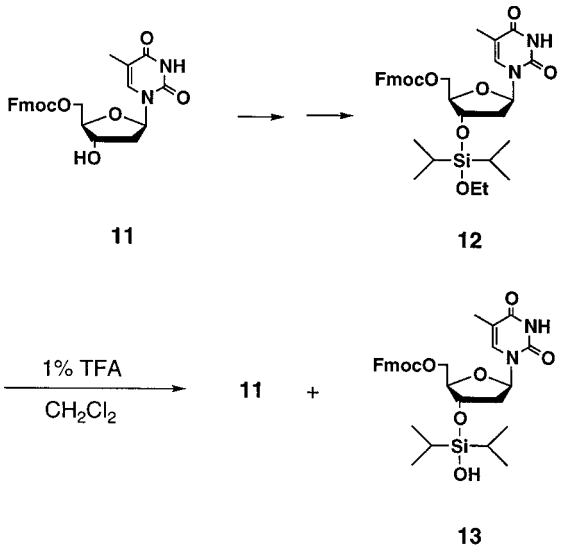


residue on the solid support were estimated by the DMTr cation analysis. Consequently, it was confirmed that more than 99% of the 5′-*O*-DMTr-deoxyadenosine residue remained intact on the solid support. This result means that the silanediyl linker was essentially stable under the conditions prescribed for removal of *N*-protecting groups.

Generally, deprotection of trialkylsilyl groups can be carried out not only by treatment with TBAF but also under acidic conditions [6]. Therefore, we examined the stability of the diisopropylsilandiyl group under acidic conditions prescribed for removal of the DMTr group in the solid-support synthesis, with a model compound **12**, which was synthesized from 5′-*O*-[(9*H*-fluoren-9-yl)-methoxycarbonyl]thymidine (**11**) [7] (*Scheme 2*). Treatment of **12** with 1% CF_3COOH (TFA) in CH_2Cl_2 for 6 h at room temperature gave two new products along with recovery (66%) of **12**. The desilylated compound **11** and the silanol product **13** were isolated in 19 and 7% yields, respectively. This instability of the silanediyl group might become more serious when the chain elongation is repeated for the synthesis of longer oligonucleotides. Upon cleavage of the linker, OH groups emerge on solid support so that they might react directly with the phosphoramidite units. Therefore, we searched for better reagents capable of selective removal of the DMTr group. As the result, 1M $ZnBr_2$ in CH_2Cl_2/i-PrOH 85 : 15 (*v*/*v*) [8] was found to be as favorable as the reagent. The model compound **12** was completely stable under these conditions.

2.2. *Synthesis of a Guanosine 5′-*O*-Phosphorimidazolide Unit.* For the solid-phase synthesis of G-capped oligonucleotides with our silanediyl linker, we designed the

Scheme 2

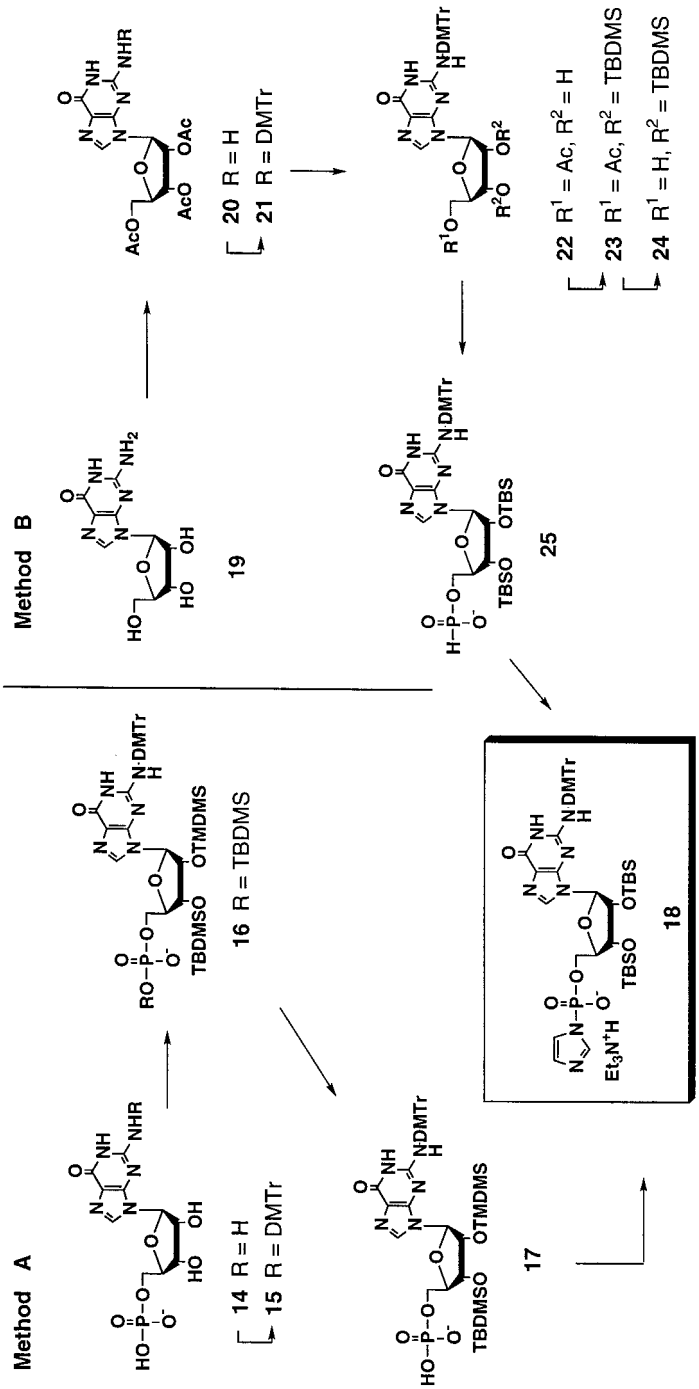


guanosine 5′-phosphorimidazolide unit **18**, which has highly liphophilic groups for protection of the 2′- and 3′-OH groups, as well as the *exo*-amino group, as shown in *Scheme 3*.

First, we attempted to synthesize **18** from guanosine 5′-*O*-monophosphate (**14**) to reduce the number of reaction steps: transient protection of **14** with Me_3SiCl (TMSCl) in pyridine followed by tritylation with DMTrCl in the presence of DMAP gave 2-*N*-(4,4′-dimethoxytrityl)guanosine 5′-monophosphate (**15**). However, it was very difficult to isolate **15** from the reaction mixture because of its high polarity. The crude product was silylated *in situ* with a large excess of (*t*-Bu) Me_2SiCl (TBDMSCl) in the presence of imidazole in pyridine. The reaction was not complete, even when 10 equiv. of TBDMSCl were added. The main product was isolated in an overall yield of 43% from **14** by silica-gel column chromatography. Based on the ^1H- and ^{31}P-NMR analysis, this product was identified as compound **16**, which has three TBDMS groups involving a silyl phosphate moiety. Treatment of **16** with aq. NH_3/EtOH 1:3 (*v*/*v*) at 60° for 12 h gave the guanosine monophosphate derivative **17** in 94% yield. Although we tried to synthesize **17** by silylation of guanosine 5′-*O*-monophosphate with TBDMSCl, followed by tritylation, this straightforward synthesis failed. The reaction gave a mixture of many products. The 5′-terminal phosphate of **17** was allowed to react with 1,1'-carbonyldiimidazole to give the phosphorimidazolide **18**.

Although we established the synthesis of **18** by the above-mentioned method (*Method A*), the inherent high polarity of guanylic acid derivatives hampered purification of the product at each step. It is rather difficult to isolate pure materials by *Method A*. Therefore, we explored an alternative route to compound **18** *via* a more reliable pathway, as shown in *Method B* in *Scheme 3*. 2′,3′,5′-Tri-*O*-acetyl-2-*N*-(4,4′-dimethoxytrityl)guanosine (**21**) [9a], which was obtained from guanosine (**19**) by a two-step reaction, was converted to the 5′-*O*-acetyl derivative **22** [9b] in 61% yield by a

Scheme 3


Method A
Method B
14 R = H
15 R = DMTr
16 R = TBDMS
17
18
19
20 R = H
21 R = DMTr
22 R1 = Ac, R2 = H
23 R1 = Ac, R2 = TBDMS
24 R1 = H, R2 = TBDMS
25

modification of the procedure of *Ishido et al.* [9c]. Silylation of **22** gave compound **23** in 87% yield. Treatment of **23** with NaOH gave the 5′-free guanosine derivative **24**. Phosphitylation of **24** according to the previously reported method gave the 5′-*O*-phosphonylated product **25** in 90% yield. Further treatment of **25** with *N*-(trimethylsilyl)-1*H*-imidazole in the presence of CCl_4 gave the phosphorimidazolide derivative **18** in 94% yield.

It was concluded that *Method B* is superior to *Method A*, since the former could provide a sufficiently pure phosphoramidate derivative **18**.

2.3. *Solid-Phase Synthesis of 5′-Guanylated Trinucleotides by Means of the Diisopropylsilandiyl Linker.* The resin **10** containing 5′-*O*-(4,4′-dimethoxytrityl)-6-*N*-benzoyldeoxyadenosine (22.9 μmol/g) was used for the synthesis of $G^{5'}$pp-d(ATA). The conditions of the detritylation step in the general phosphoramidite method [10] were changed from 3% DCA in CH_2Cl_2 to $ZnBr_2$ in CH_2Cl_2/i-PrOH 85 : 15 (*v*/*v*) [7]. The 5′-terminal site of the synthesized trinucleotide (dApTpdA) on the solid support was phosphorylated in the usual way by using a commercially available phospha-linker to give the fully protected trimer **26** (*Scheme 4*). The partially deblocked trinucleotide **27** was obtained by reaction of **26** with aq. NH_3/EtOH 4 : 1 (*v*/*v*) at 55° for 4 h. Under these conditions, the silanediyl group was found to be stable, since pd(ATA) could be recovered in high yield by treatment with TABF.

The coupling reaction of **27** with **18** in the presence of 4-nitro-6-(trifluoromethyl)-1*H*-benzotriazol-1-ol (**28**; Ntbt-OH) [11] was carried out three times at room temperature for 2 h. These conditions have proved to be effective at the level of the liquid-phase synthesis of unsymmetrical dinucleoside pyrophosphate derivatives [12]. However, it was surprisingly observed that the total coupling yield calculated by the DMTr cation assay was almost 0%. Furthermore, nucleotidic derivatives of pdApTpdA, which should be cleaved from the solid support by treatment with TBAF, could not be detected. When the resin **27** was treated with TBAF before the coupling reaction, pdApTpdA was obtained in sufficient quantity, as mentioned above. These results strongly suggested that an unexpected cleavage reaction occurred simultaneously with the coupling reaction. Therefore, we have investigated this interesting side reaction in great detail and were able to learn what happened.

2.4. *Detailed Studies of the Unexpected Cleavage.* To clarify the side reaction, several experiments were performed, as summarized in the *Table*. A partially deprotected trimer **30** with the DMTr group at the 5′-terminal site was prepared on HCP. This resin was treated with the guanosine 5′-phosphorimidazolide unit **18** in the presence of Ntbt-OH, as described in the case of **27**. The loss of the DMTr group on the resin was estimated to be 76% by the DMTr assay (*Entry 1*). When the phosphoramidate **18** was added in the absence of Ntbt-OH, 9% of the DMTr group was released from the resin (*Entry 2*). To our surprise, the reaction of **30** with Ntbt-OH in the absence of **18** resulted in more loss (81%) of the DMTr group (*Entry 3*). From these results, it is likely that Ntbt-OH reacted with the Si-atom *via* nucleophilic substitution to cleave the Si–O bond. It is also noted that the phosphoramidate **18** induced Si–O bond cleavage, although its rate is very slow compared with that with Ntbt-OH. However, simultaneous addition of **18** and Ntbt-OH did not accelerate the cleavage reaction. This is probably because Ntbt-OH is consumed by reaction with **18**, giving rise to an active phosphodiester derivative so that the concentration of Ntbt-OH decreases.

Scheme 4

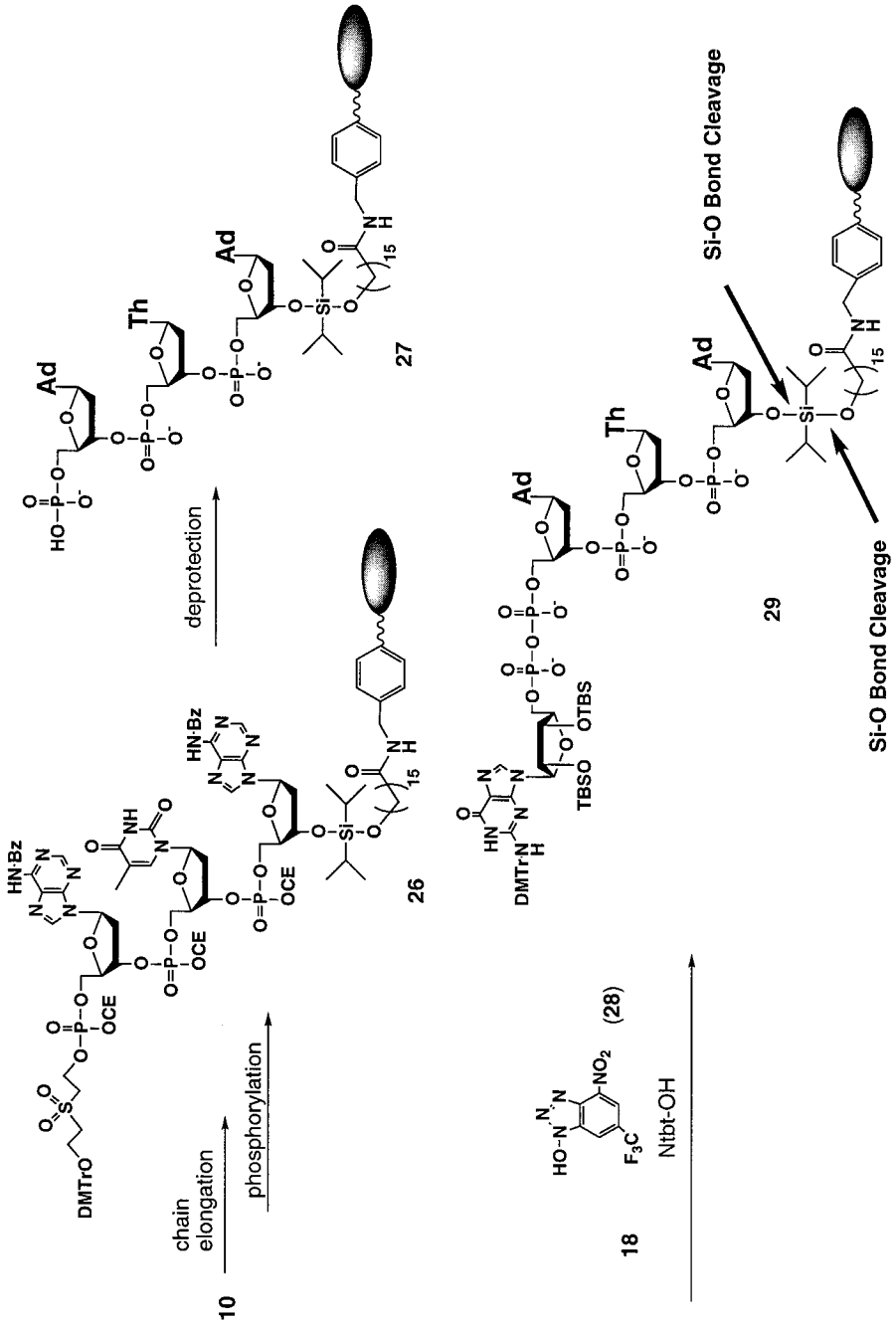
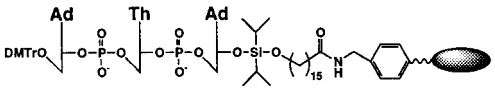
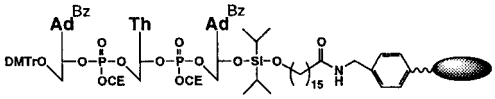
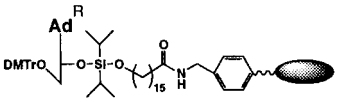
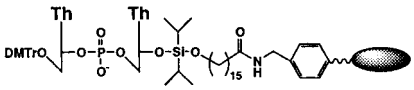
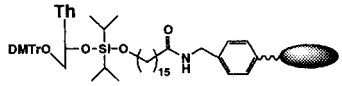

chain elongation
phosphorylation
deprotection
Ntbt-OH
Si-O Bond Cleavage
Si-O Bond Cleavage
10
18
(28)
26
27
29
Ad
Th
Ad
HN·Bz
DMTrO
OCE
TBSO
OTBS
DMTr-N
H
HO
F_3C
NO_2
Si
NH
15

Table. *The Si–O Bond Cleavage of Oligodeoxynucleotides Attached to Polystyrene Resins* via *a Silanediyl-Type Linker*

Entry	Oligo-loaded resin	Phosphoro-amidate	Ntbt-OH	Loss of the DMTr group from the resin[a]) 2 h
1		**18**	Ntbt-OH	76%
2		**18**	–	9%
3		–	Ntbt-OH	81%
	30			
4		**18**	Ntbt-OH	2%
5		**18**	–	0%
6		–	Ntbt-OH	4%
	31			
7		**18**	Ntbt-OH	0%
8		**18**	–	0%
9		–	Ntbt-OH	0%
	32a R = Bz **32b** R = NH_2			
10		**34**	Ntbt-OH	75%
11		**34**	–	43%, 87% (24 h)
12		–	Ntbt-OH	71%
	35			
13		**34**	Ntbt-OH	0%
	36			

[a]) This value was calculated by the DMT-cation assay estimated by UV before and after treatment of **30**, **31**, **32**, **35**, or **36** with reactants listed in this *Table*.

Further experiments were performed with protected substrates. A protected trimer **31** and dA monomer derivatives **32a**,**b** were prepared as substrates on HCP (*Table*). Reaction of **31** with Ntbt-OH in the presence or absence of **18** resulted in loss of trace amounts of the DMTr-protected materials from the resin, as shown in *Entries 4* and *6*. The use of only **18** did not release the DMTr group at all (*Entry 5*). Furthermore, no

loss of the DMTr group on dA monomer derivatives was observed in all cases (*Entries 7–9*). These results suggest that phosphate anions of internucleotidic phosphodiester bonds might be responsible for the significant cleavage reaction observed in the case of **30**.

To see whether this unexpected reaction, observed only when internucleotidic phosphate anions exist, is independent of the nucleobases of substrates, the guanosine moiety of **18**, and solid resin beads, more simplified experiments were performed.

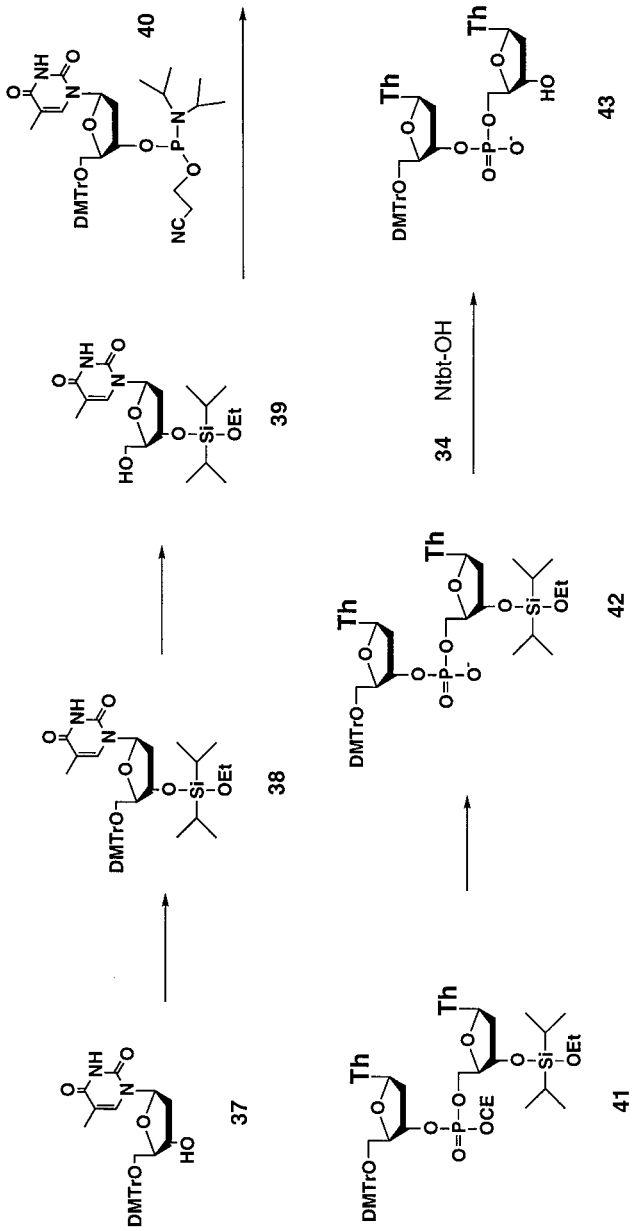
2.5. *Confirmation of the Cleavage Reaction with Methyl Phosphorimidazolide in the Solid Phase and the Liquid Phase.* In view of the results mentioned above, we synthesized a smaller reactant to see whether the guanosine moiety is associated with the side reaction. Thus, methyl phosphorimidazolide (**34**) was synthesized by reaction of methyl phosphonate (**33**) with *N*-(trimethylsilyl)-1*H*-imidazole in the presence of CCl_4 [13] (*Scheme 5*). The simplified reactant was allowed to react with TpT-dimer-loaded resin **35** and T-loaded resin **36**. These results are shown in *Entries 10–13* of the *Table*. Similar results were obtained compared with the previous examples. These results implied that the base sequence on the resin is not important. It was also found that the loss of the DMTr group increased significantly in *Entry 11* compared with the result of *Entry 2*. The prolonged reaction led to up to 87% cleavage of the Si–O bond after 24 h, as shown in *Entry 11*.

Scheme 5

33 **34**

More straightforward confirmation of the above-mentioned cleavage reaction was achieved by using a model compound **34** in the liquid-phase system. The model compound **42** was synthesized from 5′-*O*-(4,4′-dimethoxytrityl)thymidine (**37**) by a four-step reaction, as shown in *Scheme 6*. The compound **42** has a diisopropylsilanediyl group at the 3′-terminal site and an internucleotidic dissociated phosphate structure. The cleavage reaction was carried out under the conditions of *Entry 10*. As the result, actually DMTrOTpT (**43**) could be isolated in 52% yield. Characterization of this product was performed by ^{1}H-,^{13}C-, and ^{31}P-NMR spectroscopy, ESI mass spectrometry, and enzyme analysis.

3. Conclusions. – In conclusion, we synthesized a useful guanosine phosphorimidazolide unit for the synthesis of guanosine-capped oligodeoxynucleotides on polymer supports. In connection with the coupling reaction on solid supports, we found an intriguing side reaction associated with polymer-linked oligodeoxynucleotides having unprotected internucleotidic phosphate groups. It should be noted that no other study has been reported about such remote group participation of the 5′-phosphodiester group affected the stability of the 3′-*O*-silyl group. To clarify the unique effect of internucleotidic phosphate residues, further research is needed. It is concluded that the capping reaction should be designed without use of strong nucleophilic activators such



Scheme 6

as Ntbt-OH in consideration of this type of side reaction, when specific linkers containing Si–O linkages are used.

Experimental Part

General. Pyridine was distilled two times from TsCl and from CaH_2 and then stored over molecular sieves (4 Å). Column chromatography (CC) was performed with silica gel *C-200* purchased from *Wako Co. Ltd.*, and a minipump for a goldfish bowl was conveniently used to attain sufficient pressure for rapid chromatographic separation. Reverse-phase (RP) HPLC was performed on a combination of *Waters 2690* and *996* systems and *SHIMADZU 6A* system with the *mBondasphere* and *mBondapak C-18* columns (*Waters*, 3.9 × 150 mm and 7.8 × 300 mm, resp.) with a linear gradient starting from 0.1M NH_4OAc (pH 7.0) and increasing MeCN at flow rates of 1.0 ml/min and 3.0 ml/min, resp. Anion-exchange HPLC was performed on *a Gen-Pak™ FAX* column (*Waters*, 4.6 × 100 mm) on *Waters 2690* and *996* systems with a 10–30% linear gradient of 0.5M phosphate, 20% MeCN buffer (pH 6.0) in 5.0 mM phosphate, 20% MeCN buffer (pH 6.0) at 50° at a flow rate of 1.0 ml/min for 30 min. UV Spectra were recorded on a *U-2000* spectrometer. ^{1}H-,^{13}C-, and ^{31}P-NMR spectra were recorded at 270, 68, and 109 MHz, resp,. the chemical shifts were measured from TMS or DSS for ^{1}H-NMR spectra, $CDCl_3$ (77 ppm) or DSS (0 ppm) for ^{13}C-NMR spectra, and 85% phosphoric acid (0 ppm) for ^{31}P-NMR spectra. ESI mass spectra were measured on *Mariner™* (*PerSeptive Biosystems Inc.*). Elemental analyses were performed by the Microanalytical Laboratory, Tokyo Institute of Technology at Nagatsuta.

2-Cyanoethyl 16-Hydroxyhexadecanate (**4**). To a soln. of *16-hydroxyhexadecanoic acid* (**3**; 6.73 g, 23.38 mmol) and 2-hydroxyethanenitrile (32 ml, 46.76 mmol) in pyridine (200 ml), 1,3-dicyclohexylcarbodiimide (DCC, 5.30 mg, 25.72 mmol) was added. The mixture was stirred at 0° for 30 min, and stirred at r.t. for 24 h. The mixture was filtered. The filtrate was diluted with AcOEt, and washed two times each with 0.1M aq. HCl and with H_2O. The org. phase was collected, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was dissolved again in AcOEt (200 ml) and filtered. Crystallization from cooled AcOEt (200 ml) gave **4** (5.09 g, 67%): M.p. 81.5°. ^{1}H-NMR (270 MHz, $CDCl_3$): 1.25–1.66 (*m*, CH_2(3–15)); 2.36 (*t*, *J*(2,3) = *J* (2,3′) = 7.6, H–C(2), H′–C(2)); 2.70 (*t*, J_{vic} = 6.3, OCH_2CH_2CN); 3.63 (*t*, *J*(15,16) = *J*(15′,16) = 6.6, H–C(16), H′–C(16)); 4.28 (*t*, J_{vic} = 6.3, OCH_2CH_2CN). ^{13}C-NMR (67.8 MHz, $CDCl_3$): 18.11; 24.84; 25.79; 29.12; 29.26; 29.46; 29.62; 29.65; 29.67; 32.85; 34.00; 58.44; 63.05; 116.67; 173.09. ESI-MS: 326.2690 ($[M+H]^+$; calc. 326.2695).

*2-Cyanoethyl 16-{[6-*N*-Benzoyl-5′-*O*-(4,4′-dimethoxytrityl)deoxyadenosin-3′-*O*-yl](diisopropyl)silyloxy}hexadecanate* (**7**). To a soln. of 1*H*-imidazole (817 mg, 12.0 mmol) and $(i\text{-}Pr)_2SiCl_2$ (794 µl, 4.4 mmol) in DMF (30 ml), **5** (2.63 g, 4.0 mmol) was added dropwise at −60° under Ar. After stirring at r.t. for 1 h, **4** (1.30 g, 4.0 mmol) was added to the mixture. The resulting mixture was stirred at r.t. for 4 h. The mixture was diluted with $CHCl_3$, and washed with aq. sat. $NaHCO_3$ soln. The org. phase was dried (Na_2SO_4), filtered, and dried *in vacuo*. The crude product was chromatographed on a column of silica gel (120 g) with hexane/AcOEt 60:40 (*v*/*v*) containing 0.5% pyridine to give **7** (2.67 g, 61%). ^{1}H-NMR (270 MHz, $CDCl_3$): 0.86–1.52 (*m*, 2 $(Me)_2CH$, 2 Me_2CH, CH_2(3–15)); 2.24 (*t*, *J*(2,3) = *J* (2,3′) = 7.1, H–C(2), H′–C(2)); 2.45–2.78 (*m*, OCH_2CH_2CN, H–C(2′), H′–C(2′)); 3.26–3.34 (*m*, H–C(5′), H′–C(5′)); 3.57–3.65 (*m*, H–C(15), H′–C(15), 2 MeO); 4.11–4.15 (*m*, OCH_2CH_2CN, H–C(4′)); 4.73 (br., H–C(3′)); 6.23 (*dd*, *J*(1′,2′) = 5.9, *J*(1′,2″) = 6.3, H–C(1′)); 6.67–7.94 (*m*, 18 arom. H); 8.12 (*s*, H–C(2) of A); 8.61 (*s*, H–C(8) of A); 9.41 (*s*, NH). ^{13}C-NMR (67.8 MHz, $CDCl_3$): 11.97; 11.98; 12.84; 13.29; 13.31; 16.90; 17.08; 17.22; 17.87; 24.66; 25.66; 28.94; 29.09; 29.30; 29.46; 29.51; 32.65; 33.80; 40.73; 55.01; 58.30; 63.07; 63.40; 72.46; 76.52; 84.75; 86.29; 87.06; 112.86; 116.59; 123.23; 123.48; 126.60; 127.54; 127.71; 127.81; 128.41; 129.70; 132.34; 133.41; 135.32; 135.34; 135.37; 135.79; 141.27; 144.19; 149.25; 149.29; 151.18; 158.17; 172.87. ESI-MS: 1095.5084 ($[M+H]^+$; calc. 1095.5991).

*16-{[6-*N*-Benzoyl-5′-*O*-(4,4′-dimethoxytrityl)deoxyadenosin-3′-*O*-yl](diisopropyl)silyloxy}hexadecanoic Acid Triethylammonium Salt* (**8**). To a soln. of **7** (1.10 g, 1.0 mmol) in pyridine (10 ml), DBU (1.49 ml, 10.0 mmol) was added at r.t. After 30 min of stirring, the mixture was poured into $CHCl_3$ (50 ml), washed with 0.1M TEAB buffer, and dried (Na_2SO_4). The resulting soln. was concentrated and purified on a column of silica gel (40 g) eluted with $CHCl_3$/MeOH 100:1 (*v*/*v*) containing 0.5% Et_3N to give **8** as an oil (0.70 g, 80%). ^{1}H-NMR (270 MHz, $CDCl_3$): 1.01–1.05 (*m*, Me_2CH, 2 Me_2CH); 1.24–1.59 (*m*, CH_2(3–15), Et_3N); 2.29 (*t*, *J* (2,3) = *J*(2,3′) = 7.4, H–C(2), H′–C(2)); 2.56–2.84 (*m*, H–C(2′), H′–C(2′)); 3.00–3.08 (br., Et_3N); 3.31–3.44 (*m*, H–C(5′), H′–C(5′)); 3.69 (*t*, *J*(15,16) = *J*(15′,16) = 6.4, H–C(16), H′–C(16)); 3.76 (*s*, 2 MeO); 4.25 (*d*, *J* (3′,4′) = 2.6, H–C(4′)); 4.80 (br., H–C(3′)); 6.53 (*dd*, *J*(1′,2′) = 6.6, *J*(1′,2″) = 6.9, H–C(1′)); 6.77–8.08 (*m*, 18 arom. H); 8.24 (*s*, H–C(2) of A); 8.70 (*s*, H–C(8) of A); 9.81 (br., NH). ^{13}C-NMR (67.8 MHz, $CDCl_3$): 8.55;

12.08; 12.11; 17.33; 25.04; 25.79; 29.19; 29.30; 29.45; 29.59; 29.65; 32.78; 34.64; 40.96; 44.90; 55.14; 63.20; 63.53; 72.55; 84.91; 86.44; 87.23; 113.00; 123.51; 126.71; 127.67; 127.93; 128.08; 128.46; 129.81; 132.48; 133.33; 135.46; 135.50; 141.45; 144.29; 149.59; 151.48; 151.87; 158.30; 178.31. ESI-MS: 1042.5427 ($[M + H]^+$; calc. 1042.5725).

Preparation of Nucleoside–Diisopropylsilanediyl–HCP. The soln. of appropriate nucleoside unit **8** (193 mg, 169 mmol) in CH_2Cl_2 (5 ml) was drawn into a syringe containing a highly cross-linked (aminomethyl)polystyrene (HCP; 1.0 g, 33.7 mmol) and DCC (174 mg, 843 mmol). This mixture was allowed to stand for 6 h; then, the liquid was ejected, and the solid in the syringe was treated successively with pyridine (wash), CH_2Cl_2 (wash), and dried under reduced pressure. An equivolume mixture of pyridine/Ac_2O 1:9 (*v*/*v*) was added into the syringe to cap any residual amino groups. After standing for 12 h, the solid was treated with pyridine (wash) and CH_2Cl_2 (wash). The nucleoside loading averaged *ca.* 18.0–28.5 mmol/gram, as determined by absorbance of the DMTr cation liberated on treatment of an aliquot with 60% $HClO_4$/EtOH 3:2 (*v*/*v*).

*3′-*O*-[Ethoxy(diisopropyl)silyl]-5′-*O*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]thymidine* (**12**). To a soln. of 1*H*-imidazole (544 mg, 8.0 mmol) and $(i\text{-}Pr)_2SiCl_2$ (540 µl, 3.0 mmol) in DMF (10 ml) were dropwise added **11** [10] (929 mg, 2.0 mmol) at −60° under Ar. After stirring at r.t. for 1 h, the mixture was quenched by adding EtOH (5 ml). The resulting mixture was stirred at r.t. for 4 h. The mixture was diluted with AcOEt and washed with aq. sat. $NaHCO_3$ soln. The org. phase was dried (Na_2SO_4), filtered, and dried *in vacuo*. The crude product was chromatographed on a column of silica gel (25 g) eluted with hexane/AcOEt 80:20 (*v*/*v*) containing 0.5% pyridine to give **12** (319 mg, 26%). ^{1}H-NMR (270 MHz, $CDCl_3$): 1.00 (br., 2 Me_2CH); 1.17 (*t*, $J_{vic} = 6.9$, $MeCH_2O$); 1.77 (*s*, Me–C(5)); 2.02–2.32 (*m*, H–C(2′), H′-C(2′)); 3.76 (*q*, $J_{vic} = 6.9$, $MeCH_2O$); 4.10–4.54 (*m*, $CHCH_2$ of Fmoc, H–C(3′), H–C(4′), H–C(5′), H′–C(5′)); 6.33 (*dd*, *J* (1′,2′) = 6.3, *J* (1′,2″) = 6.6, H–C(1′)); 7.22–7.71 (*m*, 9 arom. H); 9.99 (*s*, NH). ^{13}C-NMR (67.8 MHz, $CDCl_3$): 11.84; 11.96; 12.48; 17.05; 17.07; 17.09; 17.10; 18.44; 40.68; 46.57; 58.64; 66.73; 69.82; 71.25; 84.41; 84.69; 110.96; 119.85; 124.56; 126.83; 126.87; 127.70; 134.94; 140.94; 140.99; 142.68; 142.74; 150.29; 154.54; 163.88. ESI-MS: 623.2780 ($[M+H]^+$; calc. 623.2789).

The Stability of the Diisopropylsilanediyl Group under Acidic Conditions. Compound **12** (232 mg, 0.37 mmol) was dissolved with 1% TFA in CH_2Cl_2 (5 ml). After stirring at r.t. for 6 h, the mixture was diluted with $CHCl_3$/pyridine 9:1 (*v*/*v*) and washed with H_2O. The org. phase was dried (Na_2SO_4), filtered, and dried *in vacuo*. The crude product was chromatographed on a column of silica gel (20 g) eluted with hexane/AcOEt 80:20, 65:35, 50:50 (*v*/*v*) containing 0.5% pyridine to give **12**, **11**, and **13** (153 mg, 15 mg, 32 mg, 66%, 7 %, 19%) respectively.

Data of **11**: ^{1}H- and ^{13}C-NMR: identical with those of the authentic sample. ESI-MS: 463.1621 ($[M+H]^+$; calc. 465.1662).

Data of **13**: ^{1}H-NMR (270 MHz, $CDCl_3$): 1.03 (br., 2 $Me_2(CH)$); 1.80 (*s*, Me–C(5)); 2.14–2.48 (*m*, H–C(2′), H′–C(2′)); 4.13–4.65 (*m*, $CHCH_2$ of Fmoc, H–C(3′), H–C(4′), H–C(5′), H′–C(5′)); 6.27 (*dd*, *J* (1′,2′) = *J* (1′,2″) = 6.3, H–C(1′)); 7.24–7.72 (*m*, 9 arom. H); 9.90 (*s*, NH). ^{13}C-NMR (67.8 MHz, $CDCl_3$): 12.28; 12.47; 12.50; 17.02; 31.45; 36.48; 40.82; 46.58; 66.77; 69.98; 70.78; 84.42; 85.19; 110.85; 119.90; 124.65; 124.69; 126.91; 126.93; 127.74; 135.43; 140.99; 141.02; 142.79; 142.81; 150.37; 154.74; 162.45; 163.83. ESI-MS: 595.2493 ($[M+H]^+$; calc. 595.2476).

*Triethylammonium 2′,3′-*O*-Bis[(tert-butyl)dimethylsilyl]-6-*N*-(4,4′-dimethoxytrityl)guanosine 5′-*O*-[(tert-butyl)dimethylsilyl]phosphate* (**16**). Guanosine 5′-*O*-monophosphate (**14**; 1.00 g, 2.75 mmol) was rendered anh. by coevaporations three times with dry DMF, and finally dissolved in dry DMF (27 mL). To the soln., pyridine (5.56 ml, 68.8 mmol) and Me_3SiCl (3.5 ml, 27.5 mmol) were added. The mixture was stirred at r.t. for 1 h. To the mixture, DMTr-Cl (1.86 g, 5.5 mmol), Et_3N (0.77 ml, 5.5 mmol) and 4-(dimethylamino)pyridine (DMAP; 13 mg, 0.11 mmol) were added. After stirring at r.t. for 30 min, the soln. was treated with aq. NH_3 (5 ml) at r.t. for 30 min. After removal of NH_3 by aspirator, the soln. was diluted with H_2O and washed with Et_2O. The aq. phase was collected, evaporated under reduced pressure, and diluted with 1M TEAB buffer. The soln. was washed 20 times each with $CHCl_3$/BuOH 1:1 (*v*/*v*). The org. phase was collected and co-evaporated with pyridine. The residue was reprecipitated with Et_2O to give the crude mixture. The mixture was rendered anh. by co-evaporations three times with dry pyridine, and finally dissolved in dry pyridine (27 ml). To the soln. was added 1*H*-imidazole (3.74 g, 55 mmol) and (t-Bu)Me_2SiCl (4.13 g, 27.5 mmol). After stirring at r.t. for 12 h, the soln. was diluted with $CHCl_3$/pyridine 2:1 (*v*/*v*) and washed with sat. $NaHCO_3$ soln. The org. phase was collected, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (170 g) eluted with $CHCl_3$/MeOH 94:6 (*v*/*v*) containing 0.5% Et_3N to give **16** (1.31 g, 43%). ^{1}H-NMR (270 MHz, $CDCl_3$): −0.50–0.73 (*m*, 3 (*t*-Bu)$SiMe_2$); 0.90–0.95 (br., Et_3N); 2.64–2.71 (br., Et_3N); 3.49–4.01 (*m*, 2 MeO, H–C(3′), H–C(4′), H–C(5′), H′–C(5′)); 4.41 (br, H–C(2′), H′–C(2′)); 5.31 (*d*, *J* (1′,2′) = 7.6, H–C(1′)); 6.51–7.92 (*m*, 13 arom. H). ^{13}C-NMR (67.8 MHz, $CDCl_3$): −4.91; −4.59; −4.56; −4.49; −3.72; −3.44; 8.18; 9.00; 17.91; 17.94; 18.00; 18.04; 25.61; 25.77; 45.36; 54.91; 69.90; 112.74; 112.80;

116.61; 127.47; 128.49; 129.96; 151.27; 151.62; 157.74; 157.78. ^{31}P-NMR (109 MHz, $CDCl_3$): −8.36. ESI-MS: 1008.4988 ($[M+H]^+$; calc. 1008.4559).

*Triethylammonium 2′,3′-*O*-Bis[(*tert*-butyl)dimethylsilyl]-6-*N*-(4,4′-dimethoxytrityl)guanosine 5′-*O*-Phosphate* (**17**). A soln. of **16** (111 mg, 0.1 mmol) in EtOH (0.75 ml) and aq. NH_3 (0.25 ml) was stirred at 60° for 12 h. The mixture was concentrated under reduced pressure, and the residue was purified by silica-gel chromatography ($CHCl_3$/MeOH 85:15) containing 0.5% Et_3N to give **17** (94 mg, 94%). ^{1}H-NMR (270 MHz, $CDCl_3$): −0.37–0.01 (*m*, 2 (*t*-Bu)$SiMe_2$); 0.62–0.88 (*m*, 2 *Me*$_3$$CSiMe_2$); 0.98 (br., Et_3N); 2.81 (br., Et_3N); 3.61–3.84 (*m*, 2 MeO, H−C(4′), H−C(5′), H′−C(5′)); 4.16 (*s*, H−C(3′)); 4.56 (br., H−C(2′)); 5.45 (br., H−C(1′)); 6.64–7.28 (*m*, 13 arom. H); 8.11 (*s*, H−C(8)). ^{13}C-NMR (67.8 MHz, $CDCl_3$): −4.97; −4.50; −4.45; 0.09; 8.48; 17.95; 18.02; 21.50; 25.87; 29.72; 45.54; 55.03; 55.23; 69.98; 73.65; 75.89; 85.32; 113.04; 125.16; 126.60; 127.69; 128.08; 128.47; 128.88; 128.98; 129.92; 136.81; 137.69; 151.37; 151.67; 158.01. ^{31}P-NMR (109 MHz, $CDCl_3$): 1.06. ESI-MS: 894.3732 ($[M+H]^+$; calc. 894.3695).

*Triethylammonium 2′,3′-*O*-Bis[(*tert*-butyl)dimethylsilyl]-6-*N*-(4,4′-dimethoxytrityl)guanosine 5′-*O*-Phosphorimidazolide* (**18**). *Method A*. A soln. of **17** (20 mg, 20 μmol) in DMF (0.2 ml) was added *N,N′*-carbonyldiimidazole (4.0 mg, 24 μmol). The mixture was stirred at r.t. for 1 h, and MeOH (0.1 ml) was then added to the mixture. After stirring for 10 min, the soln. was evaporated under reduced pressure, and the residue was chromatographed on a column of silica gel (10 g) eluted with $CHCl_3$/MeOH 96:4 (*v*/*v*) containing 0.5% Et_3N to give **18** as a foam (16 mg, 77%). ^{1}H-NMR (270 MHz, $CDCl_3$): −0.36–0.04 (*m*, 2 *t*-Bu)Si*Me*$_2$); 0.67–0.91 (*m*, 2 Me_3CSiMe_2); 1.04 (*t*, *J* = 7.3, Et_3N); 2.79 (*q*, *J* = 7.3, Et_3N); 3.60–3.97 (*m*, 2 MeO, H−C(4′), H−C(5′), H′−C(5′), H−C(3′)); 4.48 (br., H−C(2′)); 5.47 (*d*, *J* (1′,2′) = 6.9, H−C(1′)); 6.66–7.29 (*m*, 13 arom. H); 7.82–8.10 (*m*, H−C(8), 3 H of imidazole). ^{13}C-NMR (67.8 MHz, $CDCl_3$): −4.88; −4.66; −4.54; −4.45; 9.42; 17.94; 17.98; 25.76; 25.82; 45.91; 55.01; 55.02; 70.10; 75.66; 113.00; 116.25; 120.61; 127.66; 128.44; 129.91; 151.33; 151.64; 158.02; 190.23. ^{31}P-NMR (109 MHz, $CDCl_3$): −9.81. ESI-MS: 944.3995 ($[M+H]^+$; calc. 944.3963).

Method B. A soln. of **25** in MeCN/CCl_4 (10 ml) 1:1 (*v*/*v*) was added (trimethylsilyl)-1*H*-imidazole (738 μl, 5 mmol) and Et_3N (702 μl, 5 mmol). The mixture was stirred at r.t. for 40 min, and MeOH (1.0 ml) was then added to the mixture. After stirring for 20 min, the soln. was evaporated under reduced pressure, and the residue was chromatographed on a column of silica gel (50 g) eluted with $CHCl_3$/MeOH 96:4 (*v*/*v*) containing 0.5% Et_3N to give **18** as a foam (982 mg, 94%). ^{1}H-, ^{13}C-, and ^{31}P-NMR: identical with those of the authentic sample. ESI-MS: 944.3995 ($[M+H]^+$; calc. 944.3963).

*5′-*O*-Acetyl-2′,3′-*O*-bis[(*tert*-butyl)dimethylsilyl]-6-*N*-(4,4′-dimethoxytrityl)guanosine* (**23**). To a soln. of **22** (6.28 g, 10.0 mmol) in DMF (100 ml), imidazole (4.08 g, 60.0 mmol) and (*t*-Bu)Me_2SiCl (4.50 g, 30.0 mmol) were added. The resulting mixture was vigorously stirred for 12 h. The clear soln. was evaporated, treated with sat. $NaHCO_3$ soln., extracted with AcOEt, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (150 g) with hexane/$CHCl_3$ 0:100 (*v*/*v*) containing 0.5% pyridine to give **23** as a foam (7.44 g, 87%). ^{1}H-NMR (270 MHz, $CDCl_3$): −0.23–0.01 (*m*, 2 (*t*-Bu)Si*Me*$_2$); 0.76–0.84 (*m*, 2 *Me*$_3$$CSiMe_2$); 2.01 (*s*, MeC(O)); 3.70 (*s*, 2 MeO); 4.07–4.31 (*m*, H−C(4′), H−C(5′), H′−C(5′), H−C(3′)); 4.46 (*dd*, *J*(1′,2′) = 4.6, *J*(2′,3′) = 4.3, H−C(2′)); 6.74–7.27 (*m*, 13 arom. H); 7.51 (*s*, H−C(8)); 8.12 (br., NH). ^{13}C-NMR (67.8 MHz, $CDCl_3$): −4.85; −4.82; −4.40; −4.26; 18.03; 18.11; 20.90; 25.80; 25.82; 55.25; 63.28; 70.12; 72.06; 74.96; 81.80; 113.88; 127.61; 127.99; 128.07; 128.51; 129.54; 134.10; 135.97; 151.49; 158.80. ESI-MS: 856.4634 ($[M+H]^+$; calc. 856.4137).

*2′,3′-*O*-Bis[(tert-butyl)dimethylsilyl]-6-*N*-(4,4′-dimethoxytrityl)guanosine* (**24**). Compound **23** (4.30 g, 5.00 mmol) was dissolved with 1N aq. NaOH (10 ml) in pyridine (50 ml). After stirring at r.t. for 10 min, and the mixture was applied to a resin of *Dowex 50W* × *8* (pyridinium form, 100 ml). The eluate was evaporated under reduced pressure. The mixture was diluted with $CHCl_3$/pyridine 9:1 (*v*/*v*) and washed with H_2O. The org. phase was dried (Na_2SO_4), filtered, and dried *in vacuo*. The crude product was chromatographed on a column of silica gel (50 g) eluted with $CHCl_3$ containing 0.5% pyridine to give **24** (3.38 g, 83%). ^{1}H-NMR (270 MHz, $CDCl_3$): −0.52–0.00 (*m*, 2 (*t*-Bu)$SiMe_2$); 0.69–0.84 (*m*, 2 Me_3CSiMe_2); 3.55–3.88 (*m*, 2 MeO, H−C(5′), H′−C(5′)); 4.04 (*s*, H−C(4′)); 4.19 (*d*, *J*(2′,3′) = 4.6, H−C(3′)); 4.76 (*dd*, *J*(1′,2′) = 7.9, *J* (2′,3′) = 4.6, H−C(2′)); 5.53 (*d*, *J* (1′,2′) = 7.9, H−C(1′)); 6.75–7.63 (*m*, 13 arom. H, H−C(8)); 8.55 (br., NH). ^{13}C-NMR (67.8 MHz, $CDCl_3$): −5.66; −4.51; −4.45; −4.32; 17.91; 18.11; 21.49; 25.77; 25.83; 55.26; 63.09; 70.31; 73.82; 74.96; 88.79; 90.28; 114.20; 125.14; 127.72; 127.93; 128.67; 128.81; 128.87; 129.30; 134.49; 134.51; 138.01; 143.18; 148.59; 151.68; 154.98; 159.04. ESI-MS: 814.4045 ($[M+H]^+$; calc. 814.4031).

*Triethylammonium 2′,3′-*O*-Bis[(*tert*-butyl)dimethylsilyl]-6-*N*-(4,4′-dimethoxytrityl)guanosine 5′-*O*-Phosphonate* (**25**). Compound **24** (3.26 g, 4.00 mmol) was rendered anh. by co-evaporations three times with dry pyridine, and finally dissolved in dry pyridine (15 ml). To a soln. of diphenyl phosphonate (5.40 ml, 28.0 mmol)

in dry pyridine (20 ml), the soln. of **24** at r.t. under Ar was added dropwise. After stirring at r.t. for 2 h, the soln. was treated with Et_3N/H_2O (5 ml, 1:1 (*v*/*v*)) at r.t. for 30 min. The soln. was diluted with $CHCl_3$/pyridine 1:1 (*v*/*v*) and washed with 1M TEAB buffer. The org. phase was dried (Na_2SO_4), filtered, and dried *in vacuo*. The residue was chromatographed on a column of silica gel (100 g) eluted with $CHCl_3$/MeOH 96:4 (*v*/*v*) containing 0.5% Et_3N to give **25** (3.52 g, 90%). ^{1}H-NMR (270 MHz, $CDCl_3$): −0.40–0.02 (*m*, 2 (*t*-Bu)Si*Me*$_2$); 0.62–0.90 (*m*, 2 Me_3CSiMe_2); 1.11 (*t*, Et_3N); 2.92 (*q*, Et_3N); 3.58–3.89 (*m*, 2 MeO, H−C(4′), H−C(5′), H′−C(5′)); 4.09 (*d*, *J* (2′,3′) = 3.6, H−C(3′)); 4.47 (*dd*, *J* (1′,2′) = 6.9, *J* (2′,3′) = 3.6, H−C(2′)); 5.48 (*d*, *J* (1′,2′) = 6.9, H−C(1′)); 5.58 (*s*, H_aP); 6.66–7.47 (*m*, 13 arom. H); 7.87 (*s*, H_bP); 8.00 (br., H−C(8)); 11.95 (br., NH). ^{13}C-NMR (67.8 MHz, $CDCl_3$): −4.86; −4.49; −4.40; 8.57; 17.62; 18.00; 18.03; 25.87; 45.55; 55.08; 113.12; 127.81; 128.46; 129.89. ^{31}P-NMR (109 MHz, $CDCl_3$): 3.81. ESI-MS: 878.3863 ($[M+H]^+$; calc. 878.3746).

Synthesis of pd(ATA) by Using Resin **10**. In a dried syringe (5 ml, equipped with a glass-wool filter at the base), the resin **10** (0.5 μmol, 22.9 μmol/g, 21.9 mg) containing 6-*N*-benzoyl-2′-deoxy-5-*O*′-(4,4′-dimethoxytrityl)-adenosine was treated with the soln. of $ZnBr_2$ in CH_2Cl_2/i-PrOH 85:15 (*v*/*v*) (1 ml) for 1 min three times. The soln. was expelled, and the solid was washed with pyridine, EtOH, pyridine and CH_2Cl_2. A soln. containing the phosphoramidite unit (15 μmol) and tetrazole (60 μmol) in MeCN (150 μl) was poured into the syringe. After 3 min, the soln. was expelled, and solid was washed with pyridine. The contents of syringe were then successively treated with 0.15M I_2 in THF/pyridine/H_2O 10:10:1 (*v*/*v*/*v*) for 15 s three times, pyridine (wash), 0.12M DMAP in pyridine/Ac_2O 9:1 (*v*/*v*) for 2 min, pyridine, CH_2Cl_2 (wash). Repeating the procedure mentioned above, trinucleotide (dApTpdA) was synthesized on the solid support. After detritylation of the 5′-terminal site, the phosphorylation, in the usual way with a commercially available phospha-linker, gave the fully protected trimer **26**. After treatment of the resin with aq. NH_3/EtOH 4:1 (*v*/*v*) at 55° for 4 h, EtOH (wash) and CH_2Cl_2 (wash), a soln. of 1M TBAF-AcOH in THF (0.5 ml) was added into the syringe. After shaking for 1 h at r.t., the soln. was collected, the solid was washed with THF, and the combined org. solns. were evaporated under reduced pressure. The residue was eluted with H_2O (1 ml). The eluate was purified by using a *C-18* cartridge and lyophilized to give pdApTpdA (12.6 A_{260} units, 71%). The product was characterized by MS (ESI-MS: 949.1823 ($[M+H]^+$; calc. 949.1796) and enzymatic treatment.

Enzymatic Treatment of pd(ATA) with Snake Venom Phosphodiesterase (SVPD) and Alkaline Phosphatase (AP). The compound pdApTpdA (0.5 A_{260} units) was dissolved in 0.05M *Tris*·HCl (pH 8.0, 50 μl) containing 0.02M $ZnCl_2$ and 4 μl of SVPD (4 units, 1.0 unit/μl) in glycerin/H_2O 1:1 (*v*/*v*) were added. The resulting mixture was incubated at 37° for 4 h and heated at 90° for 2 min. AP (4 μl, 4 unit, 1.0 unit/μl) was added to the resulting mixture. After incubation for 3 h, the mixture was heated at 90° for 2 min. The mixture was analyzed by RP-HPLC.

5′-O-(4,4′-Dimethoxytrityl)-3′-O-[(ethoxy)diisopropylsilyl]thymidine (**38**). *5′-O-(4,4′-Dimethoxytrityl)thymidine* (**37**, 3.26 g, 6.0 mmol) was rendered anh. by co-evaporation three times with dry pyridine, and finally dissolved in dry DMF (20 ml). To a soln. of 1*H*-imidazole (1.634 g, 24.0 mmol) and $(i\text{-}Pr)_2SiCl_2$ (1.62 ml, 9.0 mmol) in DMF (30 ml) was added dropwise **37** at 0° under Ar. After stirring at r.t. for 1 h, the mixture was added to EtOH (10 ml). The resulting mixture was stirred at r.t. for 10 min. The mixture was diluted with $CHCl_3$, and washed with aq. sat. $NaHCO_3$ soln. The org. phase was dried (Na_2SO_4), filtered, and dried *in vacuo*. The crude product was chromatographed on a column of silica gel (200 g) eluted with hexane/AcOEt 70:30 (*v*/*v*) containing 0.5% pyridine to give **38** (2.62 g, 62%). ^{1}H-NMR (270 MHz, $CDCl_3$): 1.00–1.01 (*m*, 2 Me_2CH); 1.13 (*t*, J_{vic} = 6.9, $MeOCH_2$); 1.47 (*s*, Me−C(5) of T); 2.21–2.45 (*m*, H−C(2), H′−C(2)); 3.28–3.50 (*m*, H−C(5′), H′−C(5′)); 3.67–3.74 (*q*, J_{vic} = 6.9, $MeCH_2O$); 3.79 (*s*, 2 MeO); 4.07–4.08 (*d*, *J* (3′,4′) = 2.3, H−C(4′)); 4.70 (br., H−C(3′)); 6.42 (*dd*, *J*(1′,2′) = 5.9, *J*(1′,2″) = 7.9, H−C(1′)); 6.81–7.65 (*m*, 13 arom. H, H−C(6) of T); 8.94 (*s*, NH). ^{13}C-NMR (67.8 MHz, $CDCl_3$): 11.81; 11.95; 12.04; 17.17; 17.20; 18.48; 21.26; 30.86; 41.49; 55.14; 58.56; 63.33; 72.29; 84.73; 86.73; 86.91; 110.92; 113.02; 113.05; 126.89; 127.73; 127.90; 129.86; 135.15; 135.22; 135.47; 144.09; 150.10; 158.43; 163.59. ESI-MS: 725.3200 ($[M+Na]^+$; calc. 725.3234).

3′-O-[(Ethoxy)diisopropylsilyl]thymidine (**39**). Compound **38** (860 mg, 1.22 mmol) was dissolved with CH_2Cl_2/i-PrOH 85:15 (*v*/*v*) (49 ml). To the soln., $ZnBr_2$ (8.28 g, 36.7 mmol) was added. After stirring at r.t. for 1 h, the mixture was diluted with $CHCl_3$ and washed with H_2O. The org. phase was dried (Na_2SO_4), filtered, and dried *in vacuo*. The crude product was chromatographed on a column of silica gel (20 g) eluted with hexane/AcOEt 60:40 (*v*/*v*) to give **39** (370 mg, 76%). ^{1}H-NMR (270 MHz, $CDCl_3$): 0.97–1.44 (*m*, 2$CH(Me)_2$, 2$CH(CH_3)_2$); 1.13 (*t*, J_{vic} = 6.9, *Me*CH_2O); 1.89 (*s*, Me−C(5) of T); 2.30–2.34 (*m*, H−C(2), H′−C(2)); 3.77–3.86 (q, J_{vic} = 6.9, $MeCH_2O$); 3.90–4.00 (*m*, H−C(4′), H−C(5′), H′−C(5′)); 4.65 (br., H−C(3′)); 6.24 (*dd*, *J* (1′,2′) = 6.9, *J*(1′,2″) = 6.6, H−C(1′)); 7.52 (*s*, H−C(6) of T); 9.84 (*s*, NH). ^{13}C-NMR (67.8 MHz, $CDCl_3$): 11.89; 11.99; 12.38; 17.09; 17.10; 17.13; 18.40; 40.60; 58.63; 61.91; 71.44; 85.90; 87.51; 110.62; 136.67; 150.35; 164.13. ESI-MS: 401.2190 ($[M+H]^+$; calc. 401.2108).

P-*(2-Cyanoethyl)-5′*-O-*(4,4′-dimethoxytrityl)thymidyl-(3′ → 5′)-3′*-O-*[(ethoxy)diisopropylsilyl]thymidine* (**41**). Compound **39** (200 mg, 0.5 mmol) was rendered anh. by co-evaporation three times with dry pyridine, dry toluene, dry MeCN and finally dissolved in dry MeCN (5 ml). To a soln. of **39** and 1*H*-tetrazole (105 mg, 1.5 mmol) in MeCN, 5′-*O*-(4,4′-dimethoxytrityl)thymidine 3′-*O*-phosphoramidite (447 mg, 0.6 mmol). After stirring at r.t. for 5 min, the mixture was added to *t*-BuOOH (1 ml). The resulting mixture was stirred at r.t. for 5 min. The mixture was diluted with $CHCl_3$, and washed with aq. sat. $NaHCO_3$ soln. The org. phase was dried (Na_2SO_4), filtered, and dried *in vacuo*. The crude product was chromatographed on a column of silica gel (25 g) eluted with $CHCl_3$/MeOH 98 : 2 (*v*/*v*) containing 0.5% pyridine to give **41** (494 mg, 93%). ^{1}H-NMR (270 MHz, $CDCl_3$): 1.02 – 1.04 (*m*, 2 Me_2CH); 1.20 (*ddd*, $J_{vic} = 6.9$, $J_{vic} = 3.6$, $MeCH_2O$); 1.47 (*s*, Me – C(5) of T_a, Me – C(5) of T_b); 2.27 – 2.35 (*m*, H_a – C(2), H'_a – C(2), H_b – C(2), H'_b – C(2)); 2.74 (t, $J_{vic} = 5.9$, OCH_2CH_2CN) 3.41 – 3.76 (*m*, H_a – C(5′), H'_a – C(5′)); 3.79 – 4.56 (*m*, 2 MeO, $MeCH_2O$, OCH_2CH_2CN, H_a – C(3′), H_a – C(4′), H_b – C(4′), H_b – C(5′), H'_b – C(5′)); 5.17 (br., H_b – C(3′)); 6.22 – 6.43 (*m*, H_a – C(1′), H_b – C(1′)); 6.82 – 7.54 (*m*, 13 arom. H, H – C(6) of T_a, H – C(6) of T_b); 9.00 (*s*, NH_a); 9.06 (*s*, NH_b). ^{13}C-NMR (67.8 MHz, $CDCl_3$): 11.67; 11.92; 12.00; 12.02, 12.40; 16.95; 17.12; 17.15; 18.56; 19.50; 19.61; 19.69; 39.89; 40.18; 55.19; 58.76; 62.13; 62.21; 63.19; 71.13; 84.18, 84.70; 84.84; 85.33; 85.74; 87.16; 111.06; 111.24; 111.62; 113.15; 115.89; 127.10; 127.84. 127.92; 129.89; 134.69; 134.75; 134.87; 135.54; 135.77; 143.70; 143.73; 150.02; 150.22; 158.55; 163.45. ^{31}P-NMR (109 MHz, $CDCl_3$): −1.94. ESI-MS: 1082.3856 ($[M + Na]^+$; calc. 1082.3960).

5′-O-*(4,4′-Dimethoxytrityl)thymidylyl-(3′ → 5′)-3′*-O-*[(ethoxy)diisopropylsilyl]thymidine* (**42**). Compound **41** (265 mg, 0.25 mmol) was dissolved with dry pyridine (3 ml). To the soln., DBU (135 µl, 0.9 mmol) was added. After stirring at r.t. for 10 min, the mixture was diluted with $CHCl_3$ and washed with 2M TEAB buffer. The org. phase was dried (Na_2SO_4), filtered, and dried *in vacuo*. The crude product was chromatographed on a column of silica gel (13 g) eluted with $CHCl_3$/MeOH 96 : 4 (*v*/*v*) containing 0.5% Et_3N to give **42** (260 mg, 94%). ^{1}H-NMR (270 MHz, $CDCl_3$): 1.00 – 1.32 (*m*, 2 Me_2CH, $MeCH_2O$, $(MeCH_2)N$); 1.91 – 1.97 (*s*, Me – C(5) of T_a, Me – C(5) of T_b); 2.07 – 2.66 (*m*, H_a – C(2), H_a' – C(2), H_b – C(2), H_b' – C(2)); 3.00 (*q*, $J_{vic} = 7.3$, $N(MeCH_2)_3N$); 3.35 – 3.48 (*m*, H_a – C(5′), H'_a – C(5′)); 3.73 – 3.81 (*m*, 2 MeO, H_b – C(4′)); 4.03 – 4.05(*m*, H_b – C(5′), H_b' – C(5′)); 4.31 (br., H_a – C(4′)); 4.58 (br., H_a – C(3′)); 5.04 (br., H_b – C(3′)); 6.39 – 6.49 (*m*, H_a – C(1′), H_b – C(1′)); 6.79 – 7.38 (*m*, 13 arom. H); 7.60 (*s*, H – C(6) of T_a or T_b); 7.78 (*s*, H – C(6) of T_a or T_b). ^{13}C-NMR (67.8 MHz, $CDCl_3$): 8.71; 11.54; 11.97; 12.03; 12.41; 17.23; 18.55; 30.88; 39.52; 40.77; 45.53; 55.15; 58.59; 63.91; 65.62; 72.86; 84.49; 84.72; 85.17; 85.26; 86.57; 86.68; 86.90; 110.95; 111.12; 113.05; 126.92; 127.72; 128.01; 129.94; 134.96; 135.13; 135.50; 136.24; 143.97; 150.48; 150.53; 158.43; 163.82; 163.95. ^{31}P-NMR (109 MHz, $CDCl_3$): −1.05. ESI-MS: 1029.3734 ($[M + Na]^+$; calc. 1029.3695.)

This work was supported by a grant from the '*Research for the Future'* program of the *Japan Society for the Promotion of Science* (JSPS-RFTF 97I00301) and a Grant-in-Aid for Scientific Research from the *Ministry of Education, Culture, Sports, Science and Technology*, Japan.

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Received May 30, 2002