New Protected Protecting Groups for the 5′-Hydroxy Group of Deoxynucleosides by Use of 2-(Hydroxymethyl)- and 2-[(Methylamino)methyl]benzoyl Skeletons and Oxidatively Cleavable Tritylthio and (4-Methoxytrityl)thio Groups

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The new protecting groups **1a**,**b** and **2a**,**b** were developed for the 5′-OH group of deoxynucleosides by utilizing the unique characters of the sulfenate and sulfenamide linkage. These new protecting groups have a 2-(hydroxymethyl)benzoyl or 2-[(methylamino)methyl]benzoyl skeleton whose hydroxy O-atom or amino N-atom was blocked with a tritylthio-type substituent. They are removable by intramolecular cyclization following the oxidative hydrolysis of the tritylthio-type substituents under mildly oxidative conditions (*Schemes 3* and *6*). Among them, 2-{{[(4-methoxytrityl)sulfenyl]oxy}methyl}benzoyl (MOB; **2b**) was found to be the most preferable for protection of the 5′-OH function of deoxynucleosides. MOB can be introduced at the 5′-OH groups of various deoxynucleosides without the protection of the 3′-OH functions (*Scheme 5*). The applicability of the MOB group to a new oligodeoxynucleotide synthesis protocol without acid treatment was demonstrated by the solid-phase synthesis of a tetrathymidylate (*Scheme 8*).

Introduction. – Oxidatively cleavable protecting groups in place of the acid-labile 4,4′-dimethoxytrityl ($(MeO)_2Tr$) group for the 5′-hydroxy function [1][2] have been developed aiming at new oligonucleotide-synthesis protocols without acid treatment. These new protocols suppress the unfavorable depurination promoted by the acid treatment during the detritylation step in the current DNA synthesis [3–6]. Moreover, if appropriately designed, use of such protecting groups can reduce the chemical steps included in a single-chain-elongation cycle from the conventional four (coupling, capping, oxidation, and detritylation) to three (coupling, capping, and oxidation/deprotection) steps [1][2]. To date, there have been reported only two classes of protecting groups that can be used for this strategy. One is phenoxycarbonyl-type protecting groups reported by *Caruthers*'s group [1], and the other is the (4-methoxytrityl)thio (MeOTrS) group [2] reported by us.

We have been interested in the MeOTrS group because of the unique property of the single bond between the O- and divalent S-atom, which are cleavable under mild oxidation conditions such as I_2 solution in H_2O/pyridine [2][7]. Moreover, the MeOTrS-protected nucleoside could be converted to the corresponding nucleoside phosphoramidite unit, which was successfully applied to the three-step oligodeoxynucleotide synthesis without acid treatment [2]. The MeOTrS group was superior in this respect to the (2,4-dinitrophenyl)thio [8][9] group (DNPS) reported previously as a

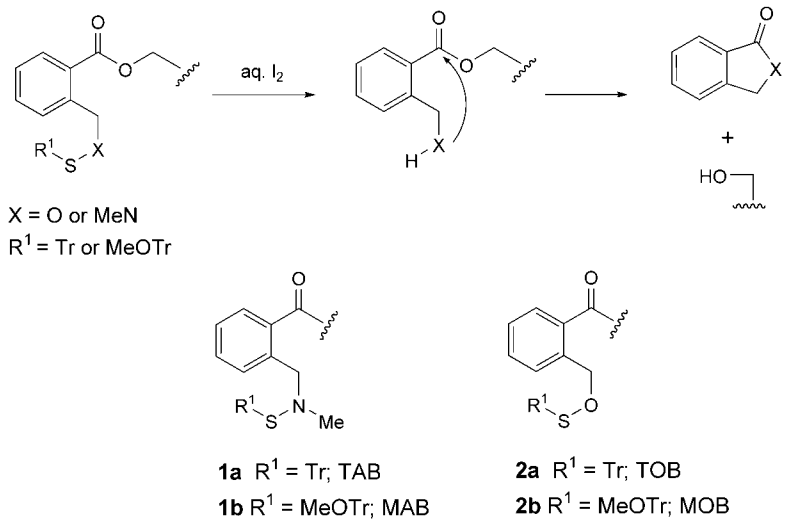
sulfenate-type protecting group, because the 5′-*O*-DNPS-nucleoside phosphoramidite units were reported to be unstable due to the inter- or intramolecular nucleophilic attack of the trivalent P-atom of the 3′-phosphoramidite to the electron-deficient S-atom [9].

In spite of these advantages of the MeOTrS group in the oligodeoxynucleotide synthesis, the following problems still remained unsolved, impeding full benefit from the fascinating nature of the sulfenate-type protecting group in the oligonucleotide synthesis [2]. The first problem is that the introduction of the MeOTrS group at the 5′-OH group requires the abstraction of the OH proton by a strong base such as lithium hexamethyldisilazide. The second is that the selective activation of the 5′-OH group requires the protection of the 3′-OH group by a base-stable protecting group such as ^{t}BuMe$_2$Si. Because of the former problem, it is difficult to introduce the MeOTrS group at the 5′-position of nucleoside derivatives having a base-labile acyl-type protecting group at their amino group. The latter leads to the time-consuming preparation of the phosphoramidite *via* a multi-step procedure. Therefore, it is necessary to design new protecting groups that can keep the favorable nature of the MeOTrS group unchanged and allow its easy and selective introduction at the 5′-OH group.

To overcome these problems, we designed new protecting groups, 2-{[methyl(tritylthio)amino]methyl}benzoyl (TAB); (**1a**), 2-{{[(4-methoxytrityl)thio]methylamino}-methyl}benzoyl (MAB) (**1b**), 2-{[(tritylthio)oxy]methyl}benzoyl (TOB) (**2a**), and 2-{{[(4-methoxytrityl)thio]oxy}methyl}benzoyl (MOB) (**2b**), in which a tritylthio (TrS) or MeOTrS group was introduced to their amino N-atom or alcoholic O-atom.

These protecting groups were expected to be easily introduced at the 5′-OH function as carboxylic acid esters and removable under mild conditions triggered by the oxidative cleavage of the TrS or MeOTrS group with aqueous I_2 solution followed by the intramolecular cyclization, as shown in *Scheme 1*.

Scheme 1



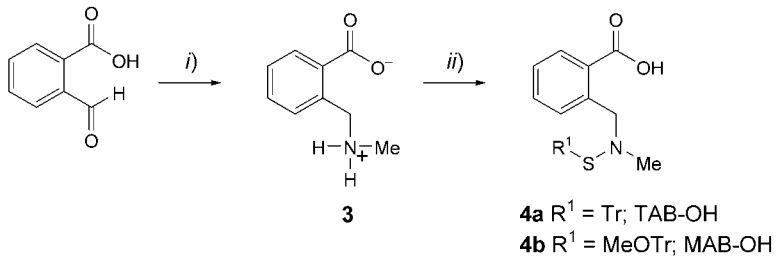
Such protecting groups cleavable by a two-step mechanism are called 'protected protecting groups [10][11] and the mechanisms are called 'assisted cleavage' [12]. Some examples of protected protecting groups have been reported for the protection of hydroxy or amino functions of nucleosides [10][11][13–15].

In this paper, we report the preparation and the properties of **1a**,**b** and **2a**,**b** as new 'protected protecting groups', which can be cleaved by the intramolecular cyclization triggered by the oxidative hydrolysis of the sulfenate or the sulfenamide linkage. Their application to the oligoDNA synthesis without acid treatment was also examined by the solid-phase synthesis of tetrathymidylate.

To the best of our knowledge, TAB (**1a**) is the first example of a protected protecting group with a 2-[(methylamino)methyl]benzoyl skeleton that can be successfully introduced at the 5′-OH group of nucleosides. Therefore, it is also interesting to study the intrinsic properties of TAB as a model of 2-[(methylamino)-methyl]benzoyl-containing protecting groups.

Results and Discussion. – 1. *Synthesis of TAB-OH* (**4a**) *and MAB-OH* (**4b**). For the synthesis of **4a** and **4b**, *o*-phthalaldehydic acid (=2-formylbenzoic acid) was quantitatively converted to the amino acid **3** (*Scheme 2*). This product had a zwitterionic structure (IR: 1373 and 1553 cm^{-1} (COO^-) and 2295–2818 cm^{-1} (NH_2Me^+). Compound **3** was further converted to TAB-OH (**4a**) in 69% yield by the reaction with TrSCl [7][16–22]. Since **3** was highly polar and insoluble in organic solvents, this conversion was only successful when the reaction was carried out in aqueous solvent systems such as acetone/THF/H_2O 1:1:1 (*v*/*v*/*v*), which were slightly modified systems compared to those reported by *Branchaud* [16][17].

Scheme 2



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4a R^1 = Tr; TAB-OH

4b R^1 = MeOTr; MAB-OH

i) 1. $MeNH_2$, MeOH/H_2O, r.t., 1 h; 2. $NaBH_4$, r.t., 30 min. *ii*) R^1SCl, Na_2CO_3, acetone/THF/H_2O 1:1:1.

Interestingly, TAB-OH (**4a**) had not a zwitterionic but a neutral structure (IR: 1690 cm^{-1} (C=O, characteristic of COOH), no ammonium-ion absorption). This difference in the structures of **3** and **4a** suggested that the basicity of the N-atom of the sulfenamide moiety in **4a** is lower than that of the corresponding amine moiety in **3**. *Bayfield* and *Cole* [23] reported a pK_a value of 2.82 for *N*-(phenylthio)aniline, which is smaller by two orders than that for aniline (pK_a = 4.69).

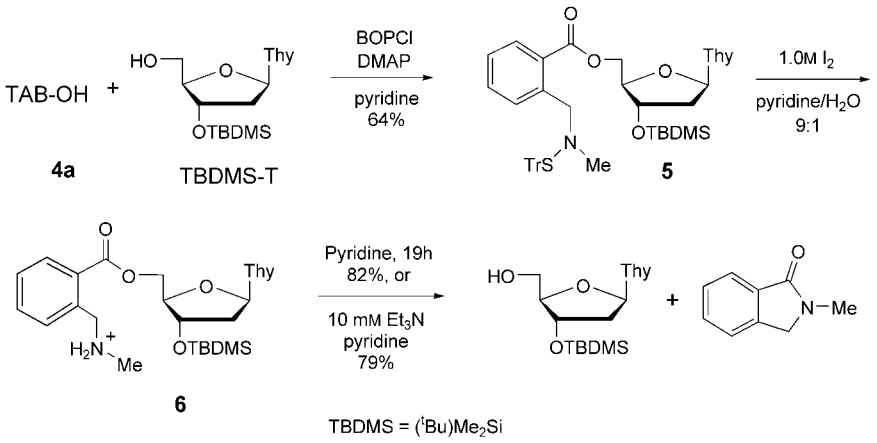
The lower basicity could be due to the back-donation effect arising from the molecular-orbital interaction between the vacant d-orbital of the S-atom and the lone-pair of the amino N-atom. The effect of the tritylthio-type substituents (see **4a**) to

reduce the basicity of the N-atom might be weaker than that of the phenylthio groups because of the absence of the delocalization of π-electrons over the aromatic ring. However, it is possible that the steric hindrance of the trityl group provides an additional contribution to lower the basicity of **4a** to such an extent as to prohibit the intramolecular salt formation at least in the solid state.

Next, we attempted to convert compound **3** to MAB-OH (**4b**) by treatment with MeOTrSCl [2] according to a procedure similar to that described in the case of TAB-OH (**4a**). Although the presence of **4b** was detected in the reaction mixture by TLC, it could not be isolated, probably because of the decomposition during the evaporation and purification by column chromatography (CC; silica gel). These observations indicated that the more acid-labile MeOTrS group was incompatible with the neighboring carboxy group in MAB-OH (**4b**). Therefore, the chemically stable TAB-OH (**4a**) was chosen as a reagent for the protection of the 5′-OH function of nucleosides.

2. *TAB Group for Protection of the 5′-Hydroxy Function*. The introduction of TAB-OH (**4a**) at the 5′-OH group of nucleosides was examined with 3′-*O*-[(*tert*-butyl)dimethylsilyl]thymidine (TBDMS-T) as a model compound. Various combinations of condensing agents, nucleophilic catalysts, and solvents were examined. As the result, compound **5** was obtained in 64% yield by reaction of TBDMS-T with **4a** in pyridine in the presence of BOPCl (= bis(2-oxooxazolidin-3-yl)phosphinic chloride) and DMAP (= *N*,*N*-dimethylpyridin-4-amine) (*Scheme 3*). It should be noted that our protecting group TAB is the first example having a 2-[(methylamino)methyl]benzoyl skeleton which could be successfully introduced at the 5′-OH group of nucleosides. Previously, *Christodoulou et al.* [15] proposed two 2-(aminomethyl)benzoyl-type protecting groups in which the reactivity of the N-atom was suppressed by the introduction of a (2,4-dinitrophenyl)thio group, but the successful introduction at the 5′-OH groups of nucleosides has not been reported.

Scheme 3



In the preparation of **5**, we observed the competitive self-cyclization of TAB-OH (**4a**) during the reaction. Actually, **4a** underwent cyclization when treated with BOPCl

and DMAP in pyridine to give 2,3-dihydro-2-methyl-1*H*-isoindol-1-one in 63% yield after 5 h. Although the basicity of the amino N-atom of **4a** was lowered significantly by the introduction of the TrS group as described above, the suppression of the nucleophilicity of the N-atom of **4a** was not efficient enough to prevent its intramolecular cyclization when the neighboring carbonyl group was strongly activated by the condensing agent. However, in contrast to the instability of **4a** under the coupling conditions, the condensation product **5** was stable during the purification. Therefore, the deprotection of TAB was examined with **5**.

First, removal of the TrS group of **5** was examined. When **5** was treated with 1.0M I_2 in pyridine/H_2O 9:1 [2][7], compound **5** was immediately converted to a more-polar material (TLC). Stirring the polar material in pyridine gave TBDMS-T in 82% yield after 19 h (*Scheme 3*; see *Exper. Part* for details). This result suggested that TAB has a potential as a new protecting group for the OH group removable under mild oxidation conditions. The detailed mechanism of the oxidative cleavage of the sulfenamide linkage in **5** is not evaluated yet. However, the generation of a trityl cation might be involved as suggested by the fact that the MeOTrS group, which can generate the more-stable MeOTr cation, was more labile than the TrS group towards I_2 in MeCN/pyridine/H_2O [2]. The rather slow kinetics of the second-step reaction to liberate the 5′-OH group suggested that the polar material detected as an intermediate was the ammonium species **6**, which is weakly nucleophilic toward the neighboring carbonyl group. If so, the nucleophilic attack must be accelerated by neutralization of this ammonium intermediate by addition of a base. As expected, when 1.0 equiv. of Et_3N was added to intermediate **6**, the second-step cyclization reaction yielding TBDMS-T was completed within a minute.

Next, the stability of TAB was examined with **5** as a model compound. Compound **5** was stable for hours when dissolved and stirred at room temperature in MeCN. However, **5** gradually decomposed to give several materials when dissolved in pyridine so that the intact **5** was recovered in only 39% yield after 17 h; the formation of TBDMS-T, 2,3-dihydro-2-methyl-1*H*-isoindol-1-one and trityl alcohol was detected by TLC.

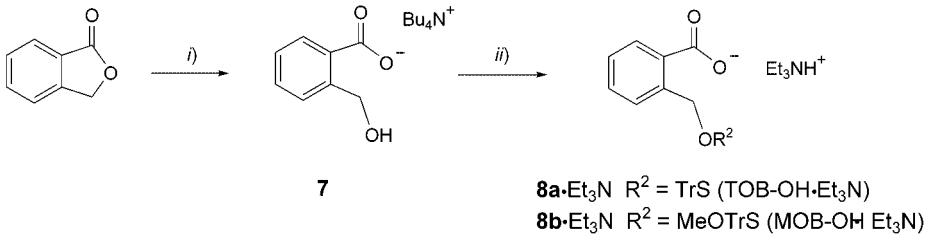
On the basis of these observations, we concluded that a protecting group having a 2-[(methylamino)methyl]benzoyl skeleton like TAB has interesting characteristics as a new protecting group for an OH group that can be deprotected with aqueous I_2 solution and Et_3N. However, TAB itself was too labile for further applications because it was unstable when dissolved in pyridine.

3. *Synthesis of TOB-OH* (**8a**) *and MOB-OH* (**8b**). To improve the above-mentioned instability of TAB, we designed new protecting groups, 2-{[(tritylthio)oxy]-methyl}benzoyl (**2a**; TOB) and 2-{{[(4-methoxytrityl)thio]oxy}methyl}benzoyl (**2b**; MOB). These protecting groups have a hydroxymethyl substituent in place of the (methylamino)methyl group of TAB at the position *ortho* to the carbonyl group. The lower nucleophilicity of the hydroxy O-atom than that of the amino N-atom would make TOB and MOB chemically more stable than TAB. Several 5′-OH protecting groups with a 2-(hydroxymethyl)benzoyl skeleton have been reported, and the possibility to use these protecting groups for the oligonucleotide synthesis without acid treatment has also been suggested [9][13][14].

MOB-OH (**8b**) was synthesized by hydrolysis of phthalide with Bu_4NOH according to a known procedure [13], and the resulting Bu_4N^+ salt **7** of 2-(hydroxymethyl)ben-

zoic acid was treated with NaH and then MeOTrSCl to give **8b** as the Et_3NH^+ salt in 70% yield after CC (silica gel, eluent containing Et_3N (*Scheme 4*). As expected, MOB-OH (**8b**) was much more stable during the purification by CC (silica gel) than TAB-OH, probably because of the lower nucleophilicity of the sulfenate-type O-atom than the sulfenamide-type N-atom. Similarly, compound TOB-OH (**8a**) having a TrS group instead of the MeOTrS group of MOB-OH (**8b**) was synthesized in 54% yield (*Scheme 4*).

Scheme 4



i) Bu_4NOH (1.0 equiv.), 30 min, 98%. *ii*) 1. NaH (1.5 equiv.), TrSCl or MeOTrSCl (1.5 equiv.), THF, 20 min; 2. column chromatography ($CHCl_3$/MeOH/Et_3N 50 : 1 : 5); 54% of **8a** · Et_3N, 70% of **8b** · Et_3N.

In contrast to the clear difference in stability between the sulfenamide derivatives TAB-OH (**4a**) and MAB-OH (**4b**), it turned out that both **8a** and **8b** were chemically stable enough during CC (silica gel) purification and on storage, independent of the presence of a MeO group at the trityl moiety. Since the MeOTrS group was superior to the TrS group as a OH-protecting group in terms of the ease of deprotection by dilute aqueous I_2 solution, we chose MOB for the further development as a new protecting group.

Next, the condensation of MOB-OH (**8b**) with the 5′-OH function of TBDMS-T was examined. In the case of **8b**, the use of 4-methoxypyridine 1-oxide (MPO) [24–26], in place of DMAP, in combination with BOPCl proved to be the best and gave the desired product **9a** in 89% yield (*Scheme 5*, *Table 1*).

Scheme 5

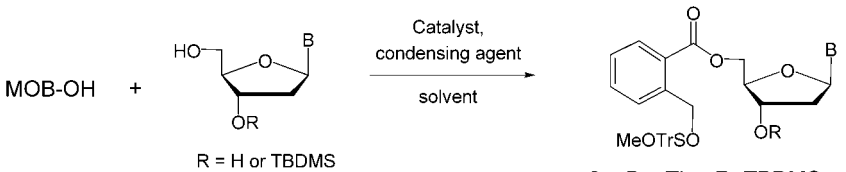


Table 1. *Incorporation of MOB-OH* (**8b**) *at the 5′-Hydroxy Group of Nucleosides in the Presence of Condensing Agents and Nucleophilic Catalysts*

Product	Condensing agent	Catalyst (equiv.)	Time [h]	Yield [%]
9a	BOPCl	MPO (0.75)	15	89
9b	HBTU	DMAP (1.0)	36	39
9b	HBTU	DABCO (1.0)	48	31
9b	BOPCl	MPO (0.75)	15	71
9c	BOPCl	MPO (0.75)	13	76
9d	BOPCl	MPO (0.75)	13	66
9e	BOPCl	MPO (0.75)	13	69

We also examined the selective reaction of **8b** with the nucleoside derivatives where both the 5′- and 3′-OH groups were unprotected. The selectivity for the primary over the secondary OH group is a quite important property of a protecting group for OH functions. As expected, the condensation of unprotected thymidine with **8b** in the presence of BOPCl and MPO gave the desired product **9b** in 71% yield with good selectivity for the 5′-OH group (*Table 1*). The attempts to improve the selectivity and reactivity by using other condensing agents such as HBTU [27–30] or other nucleophilic catalysts such as DMAP or 1,4-diazabicyclo[2.2.2]octane (DABCO) [31] gave less-satisfactory results because of the formation of an acid anhydride intermediate having poor reactivity. Therefore, the combination of MPO and BOPCl was applied to other deoxynucleoside derivatives such as N^6-benzoyl-2′-deoxyadenosine, N^4-benzoyl-2′-deoxycytidine, and 2′-deoxy-N^2-isobutyrylguanosine to give **9c** (76%), **9d**, (66%), and **9e** (69%), respectively (*Scheme 5*)

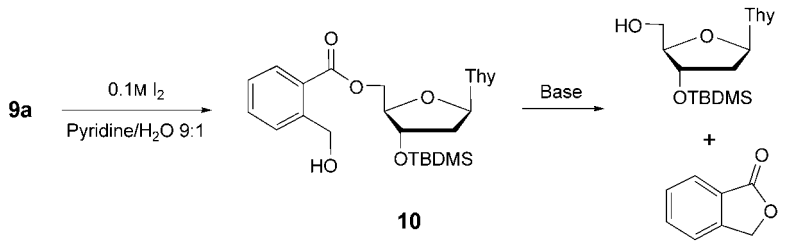
4. *Stability and Deprotection Conditions of the MOB Group.* The stability and deprotection conditions of the MOB group were examined with **9a** as a model compound (*Table 2*). Treatment of **9a** with 3% CCl_3COOH in CH_2Cl_2 resulted in the facile removal of the MeOTrS group and the formation of 5′-*O*-[2-(hydroxymethyl)-benzoyl]thymidine. On the other hand, the protecting group showed considerable stability to other reagents listed in *Table 2*. For example, Ac_2O acetylated the base moiety of **9a** within 2.5 h; the intact starting material **9a** was recovered quantitatively after the hydrolysis of the acetyl group followed by CC (silica gel) purification. The protecting group was also stable for hours towards several oxidizing agents, 0.5–0.6M ^{t}BuOOH [32] and sulfurizing agents [33][34] (*Table 2*).

Table 2. *Stability of the MOB Group under Various Conditions*

Reagents	3% CCl_3CO_2H in CH_2Cl_2	Ac_2O/pyridine 1:9, 0.1M DMAP	0.5–0.6M ^{t}BuOOH/MeCN	0.2M 1,2-benzodithiol-3-one 1,1-dioxide	0.5M *N,N,N′,N′*-tetramethylthiouram disulfide
Stability	dec. < 1 min	stable (> 2.5 h)	stable (11 h)	stable (2.5 h)	stable (> 18 h)

In contrast, the MeOTrS group of **9a** could be removed quite rapidly (< 1 min) by treatment with 0.1M I_2 in pyridine/H_2O 9:1 (*v*/*v*) (*Scheme 6*). An attempt to purify the partially deprotected product **10** failed because further cyclization proceeded on CC (silica gel) purification to give a 7:2 mixture **10/**TBDMS-T.

Scheme 6



The crude mixture **10**/TBDMS-T thus obtained was still useful to study the second-step cyclization of **10** releasing TBDMS-T because the presence of TBDMS-T does not affect the kinetics. The second-step deprotection was studied by the reaction of crude **10**/TBDMS-T with 30 equiv. of various bases as catalysts (*Table 3*).

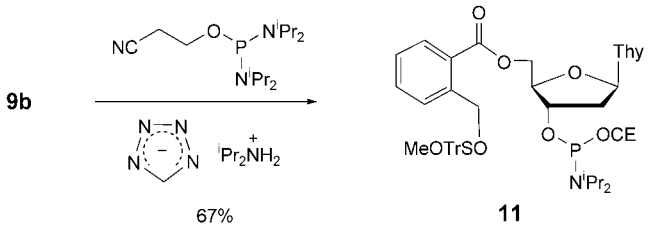
Previously, *Kamaike et al.* reported the cyclization of 2-(hydroxymethyl)-5-nitrobenzoate derivatives, which were formed by the deprotection of the 5′-*O*-[2-{(levulinyloxy)methyl]-5-nitrobenzoyl}ribonucleoside derivatives [14] by the action of 0.5M 1*H*-imidazole in MeCN. Therefore, these conditions were tested first (*Table 3*). When the crude product **10** was dissolved in 0.5M 1*H*-imidazole/MeCN, the cyclization was accelerated, and **10** was hydrolyzed completely to TBDMS-T and phthalide within 3 h. Interestingly, the cyclization was accelerated significantly by adding a small amount (10%, *v*/*v*) of H_2O to this reaction mixture (T_{comp} 40 min). A similar acceleration was also observed when more-basic amines were used instead of 1*H*-imidazole. Interestingly, while Et_3N was a poorer catalyst than 1*H*-imidazole in MeCN, Et_3N was more efficient than 1*H*-imidazole when H_2O was added (see *Table 3*). Among the amines tested in this study, $^{i}Pr_2NEt$ was efficient and comparable to a much stronger base, *N*,*N*,*N*′,*N*′-tetramethylnaphthalene-1,8-diamine which is known as a proton sponge [35] (*Table 3*).

Table 3. *Kinetics of the Cyclization of Intermediate* **10**

Base	0.5M 1*H*-imidazole	0.5M 1*H*-imidazole	0.2M Et_3N	0.2M Et_3N	0.2M $^{i}Pr_2NEt$	0.2M *N*,*N*,*N*′,*N*′-tetramethylnaphthalene-1,8-diamine
Solvent	MeCN	MeCN/H_2O 9 : 1	MeCN	MeCN/H_2O 9 : 1	MeCN/H_2O 9 : 1	MeCN
T_{comp}	3 h	40 min	6.5 h	< 2 min	< 1 min	< 1 min

5. *MOB in Solid-Phase DNA Synthesis.* As described above, the rate of the deprotection achieved by $^{i}Pr_2NEt$ was so fast and comparable to the deprotection kinetics of the $(MeO)_2Tr$ group which is the most-popular protecting group in current DNA synthesis and removable within a minute by the acid treatment. Therefore, it was important to test the possibility to use the MOB group in the solid-phase synthesis of DNA. For this purpose, the synthesis of tetrathymidylate (T_4) on CPG supports was carried out with MOB phosphoramidite **11**, which was synthesized from **9b** (*Scheme 7*).

Scheme 7



Initially, the time required for the cyclization (*Step 3b* in *Scheme* 8) on CPG solid supports was checked by the T_4 synthesis in which the thymidine residues at the second and the last positions from the 3′-end were incorporated by using the conventional $(MeO)_2Tr$ phosphoramidite building block and acidic deprotection of the $(MeO)_2Tr$ group, and only the third residue was incorporated by using the MOB phosphoramidite. In the chain elongation with the conventional $(MeO)_2Tr$ phosphoramidite building block, the times for the coupling, the capping with Ac_2O, and the treatment with aqueous I_2 solution for the oxidation of the trivalent P-intermediates and the removal of the MeOTrS group, were set to 1, 2, and 2 min, respectively.

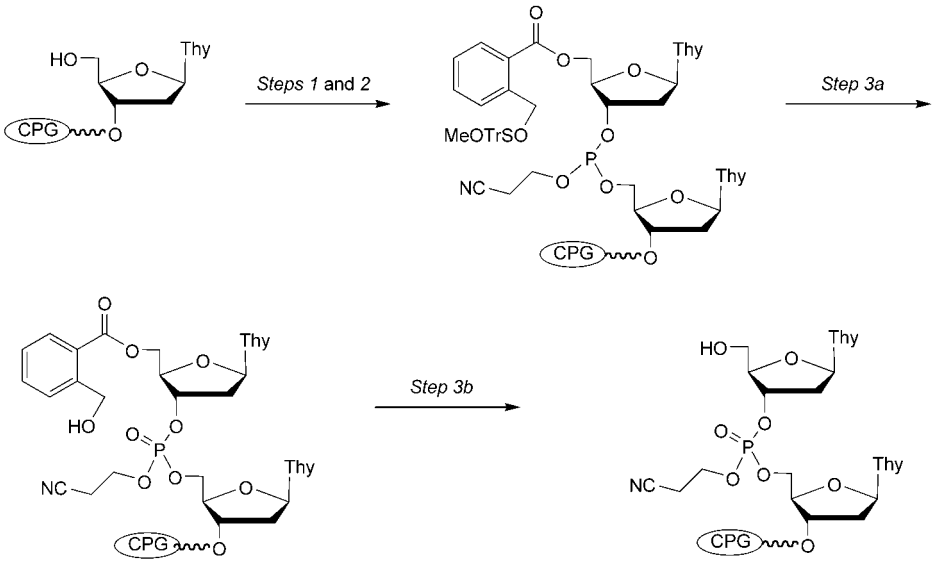
On the other hand, the reaction time for *Step 3b* in *Scheme 8* was optimized by performing the synthesis under several conditions. At first, on the basis of the liquid-phase data shown in *Table 3*, the reaction time for *Step 3b* was set to 1 min. The time periods for coupling, capping, and oxidation were identical to those for the $(MeO)_2Tr$ phosphoramidite described above. The T_4 thus synthesized on the CPG support was deprotected and cleaved from the solid support by treatment with aqueous ammonia. Unexpectedly, the reversed-phase HPLC analysis of the products revealed considerable amounts of trithymidylate (T_3), T_4, and an unidentified product with a slower retention time than T_4 (*Fig. 1*,*a*). The unidentified product gave T_3 after an additional treatment with aqueous ammonia for 21 h (data not shown). This observation indicated that the unidentified product was a modified tetrathymidylate **12** in which the 5′-OH group of T_3 and the phosphate group of Tp were linked *via* a 2-(hydroxymethyl)benzoyl linker (see *Fig. 1*,*a*). When the time period for *Step 3b* was prolonged to 5 min, no trace of **12** could be detected by reversed-phase HPLC (*Fig. 1*,*b*).

However, the same HPLC profile also revealed that the crude product contained 95% (HPLC) of T_4 and 5% of T_3. Assuming that the coupling yield of the conventional $(MeO)_2Tr$ phophoramidite building block was usually more than 99%, this result suggested that the coupling yield of the MOB phosphoramidite was *ca.* 95%, which is too low when DNA is synthesized by use of only the MOB phosphoramidite.

Therefore, we prolonged the coupling time to 2 min, which was long enough to achieve a coupling yield of more than 99%, as judged from the HPLC profiles of thymidylyl(3′-5′)thymidine (TpT) synthesized by the reaction with CPG-bounded thymidine and MOB phosphoramidite (data not shown), and carried out the chemical synthesis of T_4 on the CPG support with the MOB phosphoramidite without using the (MeO)Tr phosphoramidite unit and the acid detritylation step.

Fig. 2 shows the HPLC profile of T_4 which was synthesized by using only the MOB phosphoramidite. The desired product T_4 was successfully obtained as the main

Scheme 8



Step 1: **11**, 1*H*-tetrazole/MeCN, 2 min. *Step 2:* Ac_2O/pyridine 1:9, 0.1M DMAP, 2 min. *Step 3a:* 0.1M I_2, pyridine/H_2O 9:1, 2 min. *Step 3b:* 0.2M $^iPr_2NEt$, MeCN/H_2O 9:1

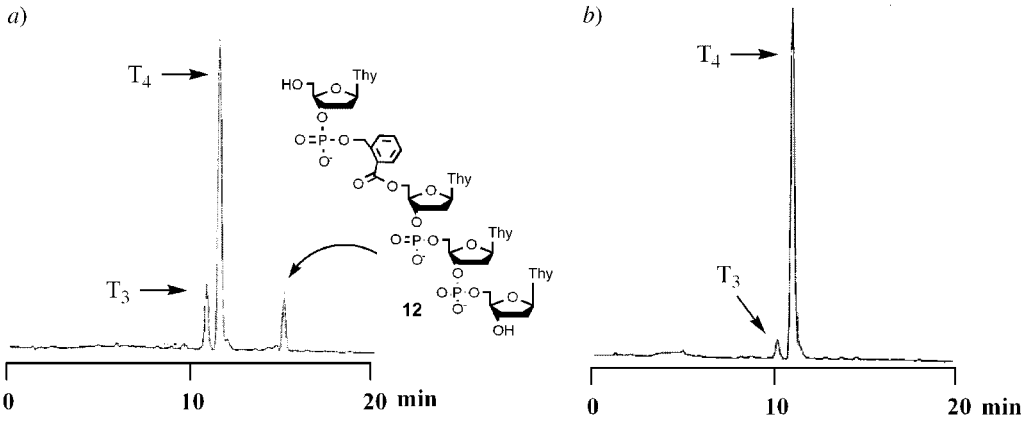


Fig. 1. *Reversed-phase HPLC profiles of T_4 synthesized by a combination of conventional $(MeO)_2$Tr-phosphoramidite and MOB-phosphoramidite* **11** *for a time period of* a) *1 min and* b) *5 min for* Step 3b *in* Scheme 8, *resp.*

product. No detectable by-products like **12** were observed so that the complete removal of the MOB group and chain elongation without acid treatment could be performed on the solid support.

The coupling yield of the MOB phosphoramidite was estimated to be 96%. The yield was comparable to the coupling yields reported by *Guzaev* and *Manoharan* [36] for the elongation of the nucleotide chain in the presence of unprotected internucleo-

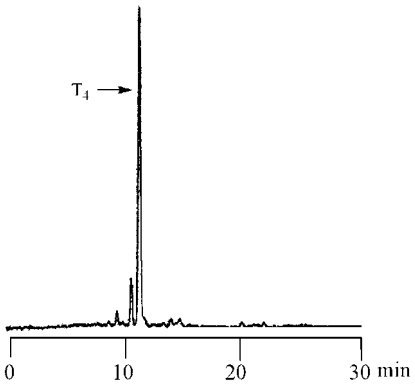


Fig. 2. *Reversed-Phase HPLC profiles of T_4 synthesized without acid treatment by use of MOB-phosphoramidite* **11**

tidic phosphates. Although tertiary amines such as $^{i}Pr_2NEt$ have been recognized to be less reactive towards the 2-cyanoethyl group at the phosphate moiety than primary amines [37], use of excess $^{i}Pr_2NEt$ in the solid-phase synthesis might cause partial or full deprotection of the 2-cyanoethyl group.

Conclusions. – The new protecting groups TAB (**1a**) and MOB (**2b**) reported in this study possess several unique characteristics that are potentially useful for oligonucleotide synthesis and other organic synthesis.

To the best of our knowledge, TAB is the first example with a 2-[(methylamino)-methyl]benzoyl skeleton and can be introduced successfully at the 5′-OH group of thymidine. The use of the 2-[(methylamino)methyl]benzoyl group became possible due to the inactivation of the nucleophilicity of the amino group by the introduction of a tritylthio group at its N-atom. Although the suppression of the nucleophilicity was not sufficient for the general application as described above, further structural modification on either the substituent at the N-atom or the tritylthio group might overcome this problem.

MOB (**2b**) was more promising because of the stability and the facile removal by the 0.1M I_2/pyridine/H_2O treatment followed by the cyclization catalyzed by weak base. These properties must be preferable for a protecting group in the synthesis of organic molecules having OH functions such as sugar and nucleic acid derivatives. As an example, we applied MOB protection to oligonucleotide synthesis without acid treatment and achieved the synthesis of tetrathymidylate avoiding any acid treatment.

Currently, complete and facile removal of the MOB group requires treatment with trialkylamines such as $^{i}Pr_2NEt$ in the presence of a small amount of H_2O. The conditions are somewhat problematic when MOB protection is applied to longer oligodeoxynucleotides, probably because of the partial elimination of the 2-cyanoethyl groups from the internucleotidic phosphate units.

We previously observed the different rate of the acid-catalyzed hydrolysis of the $(MeO)_2Tr$ groups on oligoDNA terminal depending on the surface conditions and chemicals of the solid support [38]. If this is also the case for the elimination of 2-

cyanoethyl groups under basic conditions, the use of another solid support such as highly cross-linked polystyrene [39][40] might suppress the unfavorable side reactions.

More-straightforward approaches to avoid the above-mentioned problem are to design new protecting groups which can be removed under milder nonaqueous basic or neutral conditions. For example, *Kamaike et al.* [14] reported the 2-(hydroxymethyl)-5-nitrobenzoyl skeleton as a component of their 5′-OH protecting group for RNA synthesis, and *Shafer et al.* [41] reported the 2-(1-hydroxy-1-phenylethyl)benzoyl skeleton which cyclized 2700 times faster than the unsubstituted 2-(hydroxymethyl)-benzoyl skeleton under certain conditions. These substituted 2-(hydroxymethyl)benzoyl groups are easily combined with our tritylthio-type protecting groups. Such molecular designs considering both the electronic and steric factors should be pursued in the future. Further studies in this direction are now under way and will be reported elsewhere.

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Experimental Part

General. Abbreviations: BOPCl, bis(2-oxooxazolidin-3-yl)phosphinic chloride; DMAP, *N*,*N*-dimethylpyridin-4-amine; MPO, 4-methoxypyridine 1-oxide. Pyridine was distilled after being refluxed for several hours, redistilled from CaH_2, and stored over 4-Å molecular sieves. HPLC: μ*Bondapak* column (*Waters*, *C18-100*), linear gradient of 0–30% MeCN in 0.1M NH_4OAc (pH 7.0) for 30 min at 50°C, flow rate 1.0 ml/min. Column chromatography (CC): *NH* silica gel from *Fuji Silysia Chemical Ltd*. ^{1}H- and ^{13}C-NMR Spectra: at 500 and 67.8 MHz, resp.; $SiMe_4$ as an internal standard in $CDCl_3$; δ in ppm, J in Hz. ESI-TOF-MS: positive-ion mode; calibration with reserpine and dioctyl phthalate.

2-[(Methylamino)methyl]benzoic Acid (**3**). To a soln. of *O*-phthalaldehydic acid (50 g, 0.33 mol) in MeOH (670 ml) was added 40% $MeNH_2$ in H_2O (67 ml, 0.67 mol), and the resulting soln. was stirred at r.t. After 1 h, $NaBH_4$ (5.0 g, 0.18 mol) was added portionwise, and the soln. was stirred for an additional 30 min. The reaction was quenched by addition of acetone (30 ml), and the solvent was evaporated. The residue was trituated with acetone, and the precipitation was collected by filtration to give **3** (54 g, 98%). ^{1}H-NMR (500 MHz, D_2O): 2.48 (*s*, Me), 3.99 (*s*, 1 H, CH_s); 7.37–7.28 (*m*, 3 arom. H); 7.57 (*m*, 1 arom. H). ESI-MS: 166.0581 ($C_9H_{12}NO_2^+$, $[M+H]^+$; calc. 166.0868).

2-{[Methyl(tritylthio)amino]methyl}benzoic Acid (**4a**). To a soln. of **3** (165 mg, 1 mmol) and Na_2CO_3 (212 mg, 2 mmol) in H_2O acetone 2:1 (6 ml) was added tritylsulfenyl chloride (311 mg, 1 mmol) in THF/acetone 2:1 (6 ml) within 10 min, and the resulting mixture was stirred at r.t. for an additional 10 min. Na_2CO_3 was removed by filtration, and the filtrate was diluted with $CHCl_3$ (50 ml). The $CHCl_3$ soln. was washed twice with sat. Na_2HPO_4 soln. (50 ml) and evaporated, the residual H_2O was removed by repeated co-evaporation with acetone and $CHCl_3$. and the residue subjected to CC (silica gel): **4a** (300 mg, 69%). ^{1}H-NMR (500 MHz, $CDCl_3$): 2.46 (*s*, Me); 4.03 (*s*, CH_2); 7.46–7.13 (*m*, 18 arom. H); 7.94 (*m*, 1 arom. H (Bz)). ^{13}C-NMR (500 MHz, $CDCl_3$): 46.6; 63.0; 66.9; 72.0; 126.4–131.8; 140.6; 144.1; 146.9; 146.9; 172.1. Anal. calc. for $C_{28}H_{25}NO_2S \cdot 7/5\,H_2O$: C 72.36, H 6.03, N 3.01, S 6.90; found: C 72.23, H 5.59, N 3.08, S, 6.58.

*3′-O-[(*tert*-Butyl)dimethylsilyl]-5′-O-{2-{[methyl(tritylthio)amino]methyl}benzoyl}thymidine* (**5**). 3′-*O*-[(*tert*-Butyl)dimethylsilyl]thymidine (356 mg, 1.0 mmol) and **4a** (440 mg, 1.0 mmol) were rendered anhydrous by repeated co-evaporation with dry pyridine. To the nucleoside soln. in 1,4-dioxane (2 ml) were added successively the anh. **4a**, DMAP (122 mg, 2.0 mmol), and BOPCl (255 mg, 1.0 mmol). After 2 h stirring at r.t., the mixture was quenched by addition of sat. $NaHCO_3$ soln. (5 ml). The mixture was extracted with AcOEt (15 ml), the org. layer washed with sat. aq. $NaHCO_3$ soln. (3 × 15 ml), the combined org. extract evaporated, and the residue subjected to CC (silica gel, hexane/AcOEt 8:2): **5** (500 mg, 64%). ^{1}H-NMR (500 MHz, $CDCl_3$): 0.07 (*s*, Me_2Si); 0.88 (*s*, ^{t}BuSi); 1.60 (*s*, Me–C(5)); 2.07 (*m*, 1 H–C(2′)); 2.31 (*m*, 1 H–C(2″)); 4.13 (*m*, H–C(3′)); 4.23 (*s*, CH_2N); 4.36–4.46 (*m*, H–C(4′), 2 H–C(5′)); 6.26 (*t*, J = 6.5, H–C(1′)); 7.18–7.43 (*m*, 18 arom. H, H–C(6)); 7.58 (*d*, J = 6.5, 1 arom. H (Bz)); 8.38 (*s*, NH(3)). ^{13}C-NMR (500 MHz, $CDCl_3$): −4.9;

– 4.7; 12.3; 17.9; 25.7; 41.0; 46.5; 62.6; 63.7; 71.9; 72.1; 84.7; 85.1; 111.1; 126.7; 130.3; 132.4; 135.0; 141.0; 144.1; 150.0; 163.3; 166.7. Anal. calc. for $C_{44}H_{51}N_3O_6SSi \cdot H_2O$: C 66.39, H 6.71, N 5.27, S 4.02; found: C 66.57, H 6.42, N 5.33, S 4.00.

Deprotection of **5** *without Adding* Et_3N. Compound **5** (155 mg, 0.2 mmol) was dissolved in 2 ml of 1M I_2 in pyridine/H_2O 9:1 (*v*/*v*). After 1 min, the reaction was quenched by addition of 1M aq. $Na_2S_2O_3$ (2.0 ml). The soln. was diluted with H_2O (10 ml) and extracted with CH_2Cl_2 (15 ml), the org. layer concentrated at r.t., and the resulting pyridine soln. stirred at r.t. for 19 h. Then the remaining pyridine was evaporated and the residue subjected to CC (silica gel, hexane/AcOEt 1:1): TBDMS-T (61.8 mg, 82%).

Deprotection of **5** *in the Presence of* Et_3N. Compound **5** (163 mg, 0.21 mmol) was dissolved in 2.1 ml of 1M I_2 in pyridine/H_2O 9:1. After 1 min, the reaction was quenched with 1M aq. $Na_2S_2O_3$ (4.0 ml). The soln. was diluted with H_2O (10 ml) and extracted with CH_2Cl_2 (15 ml) and the org. layer concentrated at r.t.. To the resulting pyridine soln. was added Et_3N (0.21 mmol), and the mixture was stirred at r.t. for 1 min. After evaporation, the residue was subjected to CC (silica gel, hexane/AcOEt 1:1): TBDMS-T (59 mg, 79%).

Triethylammonium 2-{[(Tritylthio)oxy]methyl}benzoate (**8a**). Tetrabutylammonium 2-(hydroxymethyl)-benzoate (394 mg, 1.0 mmol) was suspended in THF (5 ml), and the mixture was heated to 47° to give a clear soln. NaH (60 mg, 1.5 mmol; 60% in oil) was added, and the resulting suspension was stirred at 47° for 15 min. The mixture was cooled to r.t., and to this soln. was added tritylsulfenyl chloride (443 mg, 1.5 mmol). After 20 min stirring, the mixture was quenched by addition of conc. ammonia (2 ml), and the soln. was partitioned between CH_2Cl_2 (100 ml) and aq. $NaHCO_3$ soln. (100 ml). The org. layer was washed twice with aq. $NaHCO_3$ soln. (100 ml), dried ($MgSO_4$), and evaporated, and the residue purified by CC with a two-layer column (silica gel (30 g, upper layer), *NH* silica gel (15 g, lower layer), CHCl/MeOH/Et_3N 100:2:10): **8a** (268.4 mg, 54%). ^{1}H-NMR (500 MHz, $CDCl_3$): 1.27 (*t*, *J* = 7.0, 3 *Me*CH_2); 3.03 (*m*, 3 MeC*H*$_2$); 4.78 (*s*, CH_2O); 7.14–7.38 (*m*, 18 arom. H); 7.80 (*m*, 1 arom. H (Bz)). ^{13}C-NMR (126 MHz, $CDCl_3$): 8.4; 44.9; 71.9; 78.6; 127.0; 127.1; 127.7; 127.7; 129.8; 129.9; 130.0; 133.4; 138.2; 142.8; 172.1. Anal. calc. for $C_{27}H_{22}O_3S \cdot Et_3N \cdot H_2O$: C 72.63, H 7.20, N 2.57, S 5.88; found: C 72.69, H 6.66, N 2.35, S 6.13.

Triethylammonium 2-{[(4-Methoxytrityl)thio]oxy}methyl}benzoate (**8b**). As described for **8a**, with (4-methoxytrityl)sulfenyl chloride (510 mg, 1.5 mmol) instead of tritylsulfenyl chloride: **8b** (391 mg, 70%). ^{1}H-NMR (500 MHz, $CDCl_3$): 1.27 (*t*, *J* = 7.0, 3 *Me*CH_2); 3.05 (*q*, *J* = 7.0, 3 MeC*H*$_2$); 3.72 (*s*, MeO); 4.76 (*s*, CH_2); 6.77 (*dd*, ^3J(H,H) = 2.5, 3.8, 2 H, MeO*Tr*); 7.20–7.37 (*m*, 15 arom. H); 7.84 (*dd*, 1 arom. H (Bz)). ^{13}C-NMR (500 MHz, $CDCl_3$): 8.5; 45.3; 55.1; 71.6; 78.6; 113.2; 125.2–131.2; 134.4; 139.4; 143.3; 158.6; 171.8. Anal. calc. for $C_{28}H_{24}O_4S \cdot Et_3N \cdot 4\,H_2O$: C 66.08, H 7.56, N 2.34, S 5.35; found: C 65.91, H 6.03, N 2.29, S 5.16.

General Procedure for **9a**–**e**. *3′-*O*-[(*tert*-Butyl)dimethylsilyl]-5′-*O*-{2-{[(4-methoxytrityl)thio]oxy}methyl}benzoyl}thymidine* (**9a**). The 3′-*O*-[(*tert*-butyl)dimethylsilyl]thymidine (254 mg, 0.71 mmol), MOB-OH (**8b**; 596 mg, 1.07 mmol), and MPO hydrate (100.2 mg, 0.801 mmol) were rendered anhydrous by repeated co-evaporation with dry pyridine, and each material was dissolved in 2, 5, and 5 ml of pyridine, resp. To the soln. of 3′-*O*-[(*tert*-butyl)dimethylsilyl]thymidine were added successively the pyridine soln. of **8b** and MPO, and finally BOPCl (227 mg, 1.07 mmol) was added. After 15 h stirring at r.t., the mixture was quenched by addition of H_2O (5 ml). The soln. was diluted with AcOEt (50 ml) and washed with aq. $NaHCO_3$ soln. (3×). The org. layer was dried ($MgSO_4$) and evaporated and the residue subjected to CC (*NH* silica gel, hexane/AcOEt 3:7): **9a** (366 mg, 89%). ^{1}H-NMR (500 MHz, $CDCl_3$): 0.069 (*s*, Me_2Si); 0.88 (*s*, *t*BuSi); 1.57 (*s*, Me–C(5)); 2.08 (*m*, 1 H–C(2′)); 2.31 (*m*, 1 H–C(2′)); 3.77 (*s*, MeO); 4.15 (*dd*, *J* = 3.5, 7.5, H–C(3′)); 4.39–4.47 (*m*, H–C(4′) 2 H–C(5′)); 4.66 (*s*, CH_2); 6.27 (*t*, *J* = 6.5, H–C(1′)); 6.80, (*m*, 2 H MeO*Tr*); 7.16–7.45 (16 H, *m*, 15 arom. H–C(6)); 7.76 (*d*, *J* = 7.0, 1 arom. H (Bz)); 8.21 (*s*, NH(3)). ^{13}C-NMR (500 MHz, $CDCl_3$): –4.9; –4.7; 12.2; 17.9; 25.7; 41.0; 55.2; 63.8; 71.8; 72.0; 77.9; 84.7; 85.1; 111.1; 113.2; 127.2–135.0; 140.3; 143.1; 149.9; 158.7; 163.2; 166.1. Anal. calc. for $C_{44}H_{50}N_2O_8SSi \cdot H_2O$: C 65.00, H 6.45, N 3.45, S 3.94; found: C 65.10, H 6.35, N 3.58, S 3.76.

*5′-*O*-{2-{[(4-Methoxytrityl)thio]oxy}methyl}benzoyl}thymidine* (**9b**). With thymidine (259 mg, 1.07 mmol), **8b** (900 mg, 1.61 mmol), and MPO hydrate (100.7 mg, 0.81 mmol): **9b** (516 mg, 71%). ^{1}H-NMR (500 MHz, $CDCl_3$): 1.53 (*s*, Me–C(5)); 2.09 (*m*, 1 H–C(2′)); 2.44 (*m*, 1 H–C(2′)); 3.74 (*s*, MeO); 4.22 (*dd*, *J* = 3.5, 7.0 H–C(3′)); 4.57 (*m*, H–C(4′), 2 H–C(5′), CH_2); 6.3 (*t*, *J* = 6.5 Hz, H–C(1′)); 6.79 (*d*, *J* = 8.5, 2 H, MeO*Tr*); 7.18–7.4 (*m*, 15 arom. H, H–C(6)); 7.74 (*d*, *J* = 26.5, 1 arom. H (Bz)); 10.00 (*s*, NH(3)). ^{13}C-NMR (500 MHz, $CDCl_3$): 12.0; 40.2; 55.1; 64.0; 71.2; 71.6; 77.8; 84.3; 84.9; 111.2; 113.2; 127.2–135.1; 139.9; 143.0; 150.6; 164.0; 166.3. Anal. calc. for $C_{38}H_{36}N_2O_8S \cdot H_2O$: C 65.32, H 5.48, N 4.01, S 4.59; found: C 65.62, H 5.60, N 3.70, S 4.47.

N^6*-Benzoyl-2′-deoxy-5′-*O*-{2-{[(4-methoxytrityl)thio]oxy}methyl}benzoyl}adenosine* (**9c**): With-*N*^{6}-benzoyl-2-deoxyadenosine (710 mg, 2.0 mmol), **8b** (1.7 g, 3.0 mmol), and MPO hydrate (188 mg, 1.5 mmol): **9c** (1.3 g, 76%). ^{1}H-NMR (500 MHz, $CDCl_3$): 2.57 (*m*, 1 H–C(2′)); 2.93 (*m*, 1 H–C(2′)); 3.76 (*s*, MeO); 4.28 (*m*,

H–C(3′)); 4.49–4.79 (*m*, H–C(4′), 2 H–C(5′), CH_2); 6.42 (*t*, *J* = 6.0, H–C(1′); 6.80 (*d*, *J* = 9.0, 2 arom. H); 7.21–7.59 (*m*, 18 arom. H, 2 H); 7.71 (*d*, *J* = 8, 1 arom. H); 7.99 (*d*, *J* = 9.5, 2 arom. H); 8.91 (*s*, H–C(2)); 8.64 (*s*, H–C(8)). ^{13}C-NMR): 14.2; 21.0; 39.6; 55.2; 60.4; 63.8; 71.5; 71.7; 78.2; 84.7; 84.8; 113.3; 123.4; 127.3–134.3; 139.8; 141.7; 143.1; 143.1; 149.5; 151.2; 152.5; 158.7; 166.5. Anal. calc. for $C_{45}H_{39}N_5O_7S \cdot 2\ H_2O$: C 65.13, H 5.22, N 8.44, S 3.86; found: C 64.74, H 4.99, N 8.04, S 3.74.

N^4*-Benzoyl-2′-deoxy-5′*-O*-{2-{[[(4-methoxytrityl)thio]oxy}methyl]benzoyl}cytidine* (**9d**). With *N*^{4}-benzoyl-2′-deoxycytidine (662 mg, 2.0 mmol), **8b** (1.7 g, 3.0 mmol), and MPO hydrate (188 mg, 1.5 mmol): **9d** (1.0 g, 66%). ^{1}H-NMR (500 MHz, $CDCl_3$): 2.16 (*m*, 1 H–C(2′)); 2.72 (*m*, 1 H–C(2′)); 3.76 (*s*, MeO); 4.29–4.71 (*m*, H–C(3′), H–C(4′), 2 H–C(5′), CH_2); 6.246 (*t*, *J* = 6.0, H–C(1′)); 6.794 (*d*, *J* = 9.0, 2 arom. H); 7.21–7.58 (19 H, *m*, 17 arom. H, H–C(5), H–C(6)); 7.76 (*d*, *J* = 7.0, 1 arom. H (Bz)); 7.87 (*d*, *J* = 7.5, 2 arom. H (Bz)); 8.06 (*d*, *J* = 7.0, 1 arom. H (Bz)). ^{13}C-NMR (500 MHz, $CDCl_3$): 14.2; 21.0; 41.4; 55.2; 60.4; 63.5; 70.5; 71.7; 78.1; 84.9; 87.2; 96.7; 113.3; 127.3–134.2; 139.9; 143.1; 143.1; 144.0; 158.7; 162.3; 166.5. Anal. calc. for $C_{44}H_{39}N_3O_8S \cdot 1.5\ H_2O$: C 66.32, H 5.31, N 5.27, S 4.02; found: C 66.41, H 5.07, N 5.17, S 3.95.

*2′-Deoxy-*N^2*-isobutyryl-5′*-O*-{2-{[[(4-methoxytrityl)thio]oxy}methyl]benzoyl}guanosine* (**9e**). With 2′-deoxy-*N*^{2}-isobutyrylguanosine (675 mg, 2.0 mmol), **8b** (1.7 g, 3.0 mmol), and MPO hydrate (188 mg, 1.5 mmol): **9e** (1.1 g, 69%). ^{1}H-NMR (500 MHz, $CDCl_3$): 1.24 (*d*, *J* = 6.3, 3 H, *Me*$_2$CHCO); 1.27 (3 H, *d*, *J* = 6.3, 3 H, *Me*$_2$CHCO); 2.52 (*m*, 1 H–C(2′)); 2.79 (*m*, 1 H–C(2′)); 2.86 (*m*, Me_2CHCO); 3.76 (*s*, MeO); 4.35 (*m*, H–C(4′)); 4.45 (*m*, 1 H–C(5′)); 4.62–4.69 (*m*, 1 H–C(5′), CH_2); 4.96 (*m*, 1 H–C(3′)); 6.21 (t, *J* = 6.5, H–C(1′)); 6.79 (*d*, *J* = 9.0, 2 H, MeO*Tr*); 7.18–7.40 (*m*, 15 arom. H); 7.72 (*d*, *J* = 8.0, 1 arom. H (Bz)); 7.82 (*s*, H–C(8)); 10.14 (*s*, 1 H, NH_2–C(2)); 12.34 (*s*, NH(1)). ^{13}C (500 MHz, $CDCl_3$): 14.1; 18.9; 19.0; 21.0; 36.1; 39.5; 55.2; 60.4; 64.4; 71.3; 71.7; 78.0; 84.7; 85.2; 113.2; 121.5; 127.2–134.1; 138.6; 139.8; 143.1; 147.8; 148.0; 155.80; 158.6; 166.8; 171.2; 180.0. Anal. calc. for $C_{42}H_{41}N_5O_8S \cdot 3/5\ H_2O$: C 64.12, H 5.41, N 8.90, S 4.08; found: C 64.42, H 5.58, N 8.45, S 3.92.

Crude Mixture of 3′-O*-[(*tert*-Butyl)dimethylsilyl]-5′*-O*-[2-(hydroxymethyl)benzoyl]thymidine* (**10**) *and 3′*-O*-[(*tert*-Butyl)dimethylsilyl]thymidine.* Compound **9a** (163 mg, 0.2 mmol) was dissolved in 10 ml of 0.6M I_2 in pyridine/H_2O 9:1 (*v*/*v*). After 1 min, the reaction was quenched by adding 30 ml of 5% $Na_2S_2O_3$ soln. The mixture was extracted with CH_2Cl_2 (50 ml), the org. phase dried ($MgSO_4$) and evaporated, and the residue subjected to CC (silica gel, hexane/AcOEt 4:6): **10** TBDMS-T 7:2 (97% yield). ^{1}H-NMR (500 MHz, $CDCl_3$): 0.10 (*s*, Me_2Si); 0.91 (*s*, ^{t}BuSi); 1.69 (*s*, Me–C(5)); 2.15–2.21 (*m*, 1 H–C(2′)); 2.34–2.39 (*m*, 1 H–C(2′)); 4.19 (*m*, 1 H–C(3′)); 4.50–4.59 (*m*, 1 H–C(3′), 1 H–C(4′), CH_2); 4.82 (*m*, 2 H–C(5′)); 6.27 (*d*, *J* = 7, H–C(1′)); 7.19 (*s*, 6 H); 7.36–7.60 (*m*, 3 arom. H (Bz)); 7.97 (*d*, 1 arom. H (Bz)); 8.53 (*s*, H–C(6)). ^{13}C-NMR (126 MHz, $CDCl_3$): –4.6; 12.3; 25.7; 40.9; 64.1; 64.6; 71.8; 84.5; 85.3; 128.0; 128.2; 130.6; 130.7; 133.6; 135.1; 143.5; 167.4. Anal. calc. for a 7:2 mixture of $C_{24}H_{34}N_2O_7Si$ and $C_{18}H_{28}N_2O_5Si$: C 57.92, H 7.15, N 6.08; found: C 57.55, H 6.87, N 5.77.

5′-O*-{2-{[[(4-Methoxytrityl)thio]oxy}methyl}benzoyl}thymidine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (= *MOB Phosphoramidite;* **11**). Compound **9b** (480 mg, 0.71 mmol) was rendered anhydrous by repeated co-evaporation with dry pyridine, toluene, and CH_2Cl_2, and finally dissolved in dry CH_2Cl_2. To this soln. was added 2-cyanoethyl tetraisopropylphosphoramidite (269 µl, 0.85 mmol) and diisopropylammonium tetrazolide (48 mg, 0.28 mmol), and the resulting soln. was stirred at r.t. for 15 h. The reaction was quenched by adding H_2O (5 ml). The mixure was diluted with AcOEt/Et_2O/^{i}Pr_2O 1:1:1 (30 ml), washed with 5% Na_2CO_3 soln. (7 × 30 ml), dried ($MgSO_4$), and evaporated and the residue subjected to CC (*NH* silica gel (15 g), hexane/AcOEt 1:3 containing 2% of Et_3N): **11** (416 mg, 67%). ^{1}H-NMR (500 MHz, $CDCl_3$): 1.19 (*m*, 2 *Me*$_2$CH); 2.29 (*m*, 1 H–C(2′)); 2.55 (*m*, 1 H–C(2′)); 2.66 (*m*, $NCOH_2CH_2O$); 3.61 (*m*, 2 Me_2CH); 3.71–3.79 (*m*, 1 H of $NCCH_2CH_2O$, MeO); 3.88 (*m*, 1 H, $NCCH_2CH_2O$); 4.32 (*m*, H–C(4′)); 4.56 (*m*, H–C(3′), 2 H–C(5′)); 4.81–4.82 (*m*, CH_2); 6.27 (*t*, *J* = 6.75 H–C(1′)); 6.80 (*d*, *J* = 8.5, 2 H, MeO*Tr*); 7.13–7.37 (*m*, 13 arom. H); 7.57 (*m*, 1 arom. H (Bz)); 8.24 (*m*, 1 arom. H (Bz)); 8.67 (*m*, 1 arom. H (Bz)); 9.14 (*s*, NH(3)). ^{13}C-NMR (500 MHz, $CDCl_3$): 12.3; 20.4; 20.5; 24.5; 24.6; 39.1; 39.2; 43.3; 43.3; 43.4; 43.4; 55.2; 57.9; 57.9; 58.1; 64.5; 64.7; 72.1; 72.8; 72.9; 73.1; 73.2; 77.6; 82.7; 82.8; 83.2; 85.4; 111.4; 113.3; 117.6; 125.0; 126.7; 127.4–131.2; 133.8; 135.2; 135.3; 142.8; 146.5; 148.0; 150.0; 158.8; 163.3; 164.0. Anal. calc. for $C_{47}H_{53}N_4O_9PS \cdot H_2O$: C 62.79, H 6.17, N 6.23, S 3.57; found: C 62.35; H 6.03; N 6.21; S 3.35.

Solid-Phase Synthesis. The chain-elongation cycle with commercially available 5′-*O*-(4,4′-dimethoxytrityl)thymidine (2-cyanoethyl diisopropylphosphoramidite) $(MeO)_2$Tr phosphoramidite), consists of four chemical steps, 1) coupling, 2) capping, 3) oxidation, 4) detritylation. The conditions for each step are the following.

1) Coupling: Chain elongation was performed by the reaction of the free OH group on the solid support and 40 equiv. of $(MeO)_2$Tr phosphoramidite in the presence of 80 equiv. of 1*H*-tetrazole in dry MeCN (125 µl) for

2 min. 2) Capping: Failure sequence was capped by the reaction with 50 µl of Ac_2O for 2 min in pyridine (450 µl) in the presence of 0.1M DMAP. 3) Oxidation: The oxidation of the phosphite intermediate was performed by the reaction with 0.1M I_2 for 2 min in pyridine/H_2O 9:1 (250 µl). 4) Detritylation: The MeO_2Tr group was removed by treating several times with 3% CCl_3COOH in CH_2Cl_2.

In the case of the chain elongation with MOB phosphoramidite **11**, the chain elongation cycle was changed to 1) coupling, 2) capping, 3) oxidation/deprotection. The conditions for coupling and capping were identical to those in the case of conventional phosphoramidite. The conditions for the oxidation/deprotection are the following.

Oxidation/deprotection: The oxidation of the phosphite intermediates and oxidative cleavage of the MeOTrS groups were performed by the reaction with 0.1M I_2 for 2 min in pyridine/H_2O 9:1 (250 µl). After the oxidation, the 5′-OH group of the oligonucleotides were liberated by the treatment with 0.2M $^iPr_2EtN$ in MeCN/H_2O 9:1 for 5 min.

After the chain elongation was completed, the oligoDNA was treated with 28% ammonia for 30 min to perform the deprotection of the 2-cyanoethyl group and cleavage from the solid support.

The whole experiment was carried out on a 0.5-µmol scale commercially available CPG solid support (11.36 mg, 44 µmol/g) bearing thymidine residues.

The structure of the tetrathymidylate was confirmed by the comparison of the retention time and UV with those of an authentic sample by means of reversed-phase HPLC.

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