Synthesis of oligodeoxynucleotides using the oxidatively cleavable 4-methoxytritylthio (MMTrS) group for protection of the 5′-hydroxyl group†‡

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Received (in Montpellier, France) 16th November 2009, Accepted 28th January 2010
First published as an Advance Article on the web 4th March 2010
DOI: 10.1039/b9nj00678h

We examined the synthesis of oligodeoxynucleotides containing all four nucleobases using the 4-methoxytritylthio (MMTrS) group for protection of the 5′-hydroxyl group. The MMTrS group could be introduced into 3′-*O*-TBDMS-deoxycytidine, -deoxyadenosine and -deoxyguanosine with the appropriate base protecting groups using strong bases such as *n*-butyl lithium and lithium hexamethyldisilazide. Because the MMTrS group could be removed by oxidation with an aqueous I_2 solution, the oxidation of internucleotidic phosphite intermediates could be performed simultaneously. Thus, the nucleotide chain could be extended in a three-step protocol that comprised coupling, capping and oxidation/deprotection. Oligodeoxynucleotides with 10 and 20 mixed-base sequences could be synthesized using this protocol.

Introduction

Chemically synthesized oligodeoxynucleotides (ODNs) have been used in various techniques in life science, such as real-time PCR,[1] DNA microarrays,[2] nucleic acid drugs[3–4] and genome synthesis.[5] Most ODNs are synthesized on automated DNA synthesizers that are customized for phosphoramidite chemistry.[6] Usual phosphoramidite chemistry consists of four chemical steps (A) the reaction of phosphoramidite building blocks with the 5′-hydroxyl group of the growing DNA chain on polymer supports (coupling), (B) protection of the unreacted 5′-hydroxyl group by acylation (capping), (C) I_2 oxidation of internucleotidic phosphite intermediates (oxidation) and (D) removal of the 5′-*O*-(4,4′-dimethoxytrityl) (DMTr) group by acid treatment. Several alternative protocols have been reported that eliminate the use of the DMTr group. Previously, oxidatively-cleavable 5′-protecting groups such as 3-(trifluoromethyl)phenoxycarbonyl[7] and 2,2,5,5-tetramethylpyrrolidin-3-one-1-sulfinyl[8] have been described. Using these protecting groups, steps (C) and (D) can be performed in a one-step reaction so that the total chemical steps required for the chain extension are reduced to three. Independently, we also reported 4-methoxytritylthio (MMTrS) as such a protecting group for the 5′-protection of the thymidine phosphoramidite unit and demonstrated pentathymidylate synthesis using this MMTrS group.[9] The MMTrS group could be designed as a sterically hindered group capable of protecting the 5′-hydroxyl group as a sulfenic acid ester, and could be removed by treatment with aqueous I_2. In this study, we attempted to apply this original MMTrS strategy to the synthesis of ODNs containing all four nucleobases to clarify the applicability of this unique sulfenate-type protecting group. Because the MMTrS group can be removed by treatment with aqueous I_2, the chain extension protocol using MMTrS could be readily implemented in widely-distributed automated DNA synthesizers. In the present paper, we report our detailed results of the three-step synthesis of ODNs up to a 20-mer based on the MMTrS strategy using a DNA synthesizer.

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† This article is part of a themed issue on Biophosphates.
‡ Electronic supplementary information (ESI) available: Further experimental data. See DOI: 10.1039/b9nj00678h

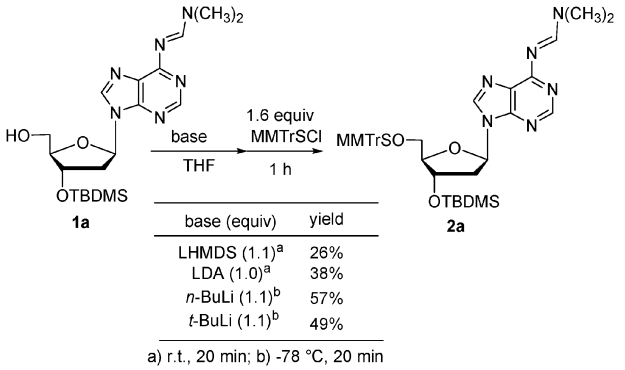
Results and discussion

Preparation of 5′-*O*-MMTrS-deoxynucleoside derivatives

We have previously reported the introduction of the MMTrS group to the 5′-hydroxyl group of 3′-*O*-TBDMS-thymidine by its metallation with lithium hexamethyldisilazide (LHMDS) followed by the reaction with MMTrSCl.[9] In this study, we first examined the conditions for introducing the MMTrS group to the 5′-hydroxyl group of 3′-*O*-TBDMS-deoxyadenosine derivatives.

As shown in Scheme 1, we chose a dimethylaminomethylene (dmf) group[10] as the protecting moiety for the amino group. The reaction of **1a** with MMTrSCl, using LHMDS as the base, gave the desired compound, **2a**, in 26% yield. Using lithium diisopropylamide (LDA) in place of LHMDS resulted in a somewhat better yield (38%) of **2a**.

On the other hand, the use of a stronger base, *n*-BuLi, yielded the best result (57%), while the use of *t*-BuLi slightly reduced the yield to 49%. Thus, it was shown that when the amino group of adenosine was protected by a dmf group, deprotonation of the 5′-hydroxyl group by *n*-BuLi gave the best result. We also examined acyl-type protecting groups, such as benzoyl and phthaloyl,[11] for protection of the amino group. However, these protected adenine moieties were unstable toward strong bases like LHMDS and *n*-BuLi, and



Scheme 1 Preparation of **2a**.

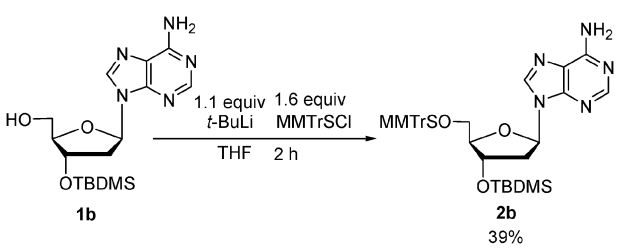
the reactions gave complex mixtures with dark purple colors (data not shown).

Previously, Hayakawa and co-workers reported the 5′-*O*-selective phosphorylation of base-unprotected adenosine derivatives by selective deprotonation of the 5′-hydroxyl group with strong bases.[12] Therefore, we tested the introduction of the MMTrS group to the 5′-hydroxyl group without protection of the amino group of the adenine ring. Although the 5′-*O*-MMTrS derivative **2b** was obtained in 39% yield from 3′-*O*-TBDMS-deoxyadenosine (**1b**)[13] when *t*-BuLi was used as the base, the yield was lower than that obtained using the starting material **1a** protected with the dmf group (Scheme 2).

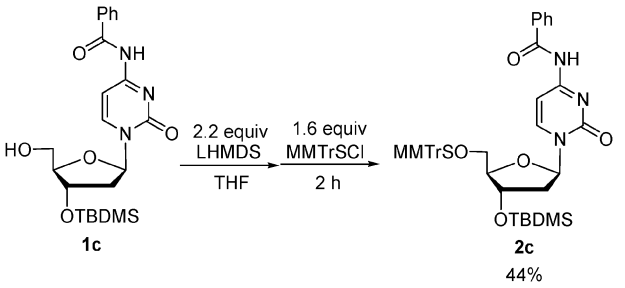
For the synthesis of the deoxycytidine derivative, we first used 3′-*O*-TBDMS-4-*N*-benzoyl-deoxycytidine (**1c**)[14] as the starting material (Scheme 3). In contrast to the case of the deoxyadenosine derivative, the base moiety protected with the benzoyl group was stable to LHMDS, and the reaction gave the 5′-*O*-MMTrS derivative **2c** in 44% yield.

Taking into account the results of the preparation of the deoxyadenosine derivative **2a**, we next used deoxycytidine derivative **1d** protected with a dibutylaminomethylene (dbf) group at the amino group. We used the dbf group instead of the dmf group because of the instability of the dmf group on the cytosine base moiety toward the acidic conditions of the detritylation and of silica gel column chromatography.[10] Unfortunately, the dbf group was unstable to the LHMDS conditions (Scheme 4) and the starting material decomposed. In contrast, the use of *n*-BuLi gave 5′-*O*-MMTrS derivative **2d** in the best yield (58%).

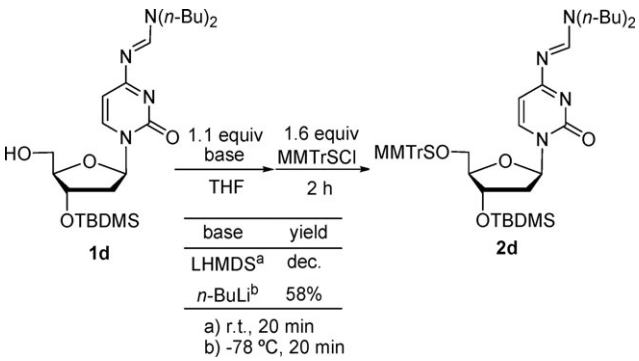
We next examined the synthesis of 5′-*O*-MMTrS-deoxyguanosine derivatives. We used 2-*N*-isobutyryl derivative **1e**[15] as the substrate and reacted it with MMTrSCl, using LHMDS as the base (Scheme 5). Although the reaction gave the desired product, **2e**, as the main product, pure **2e** could not be isolated



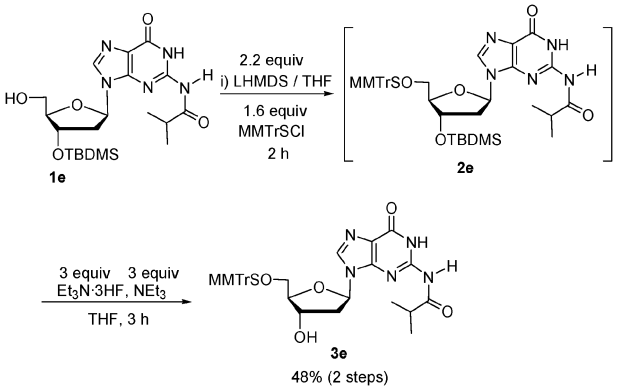
Scheme 2 Preparation of 6-*N*-unprotected **2b**.



Scheme 3 Preparation of the deoxycytidine derivative **2c**.



Scheme 4 Preparation of the deoxycytidine derivative **2d**.



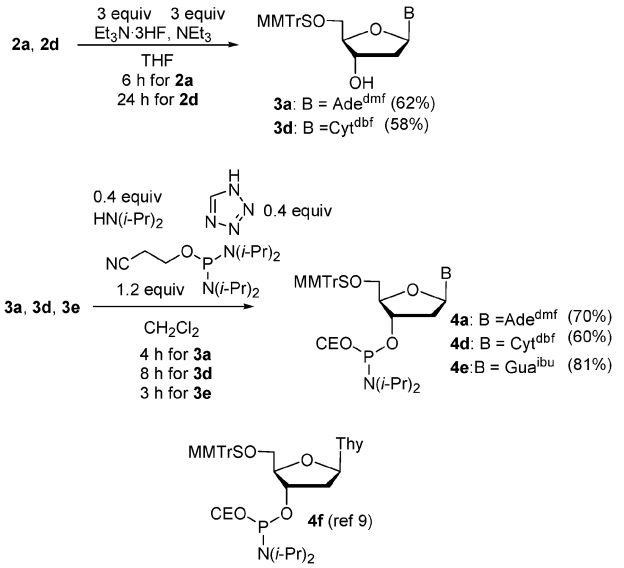
Scheme 5 Preparation of the deoxyguanosine derivative **3e**.

from some by-products. Therefore, crude **2e** was converted to **3e** by treatment with $Et_3N{\cdot}3HF$,[16] and the pure compound was isolated in 48% yield. Thus, the attachment of the MMTrS group to the 5′-hydroxyl group was achieved in 57% yield for deoxycytidine, 58% for deoxyadenosine and 48% for deoxyguanosine.

5′-*O*-MMTrS derivatives **2a** and **2d** were converted to their 3′-*O*-free derivatives **3a** and **3d**, respectively, using $Et_3N{\cdot}3HF$, and compounds **3a**, **3d** and **3e** were converted to phosphoramidite units **4a**, **4d** and **4e** (Scheme 6).[17] Using phosphoramidites **4a**, **4d**, **4e** and the phosphoramidite of thymidine (**4f**), as previously reported,[9] we further studied the ODN synthesis on an automated DNA synthesizer.

Re-investigation of the oxidative cleavage of the MMTrS group on solid supports

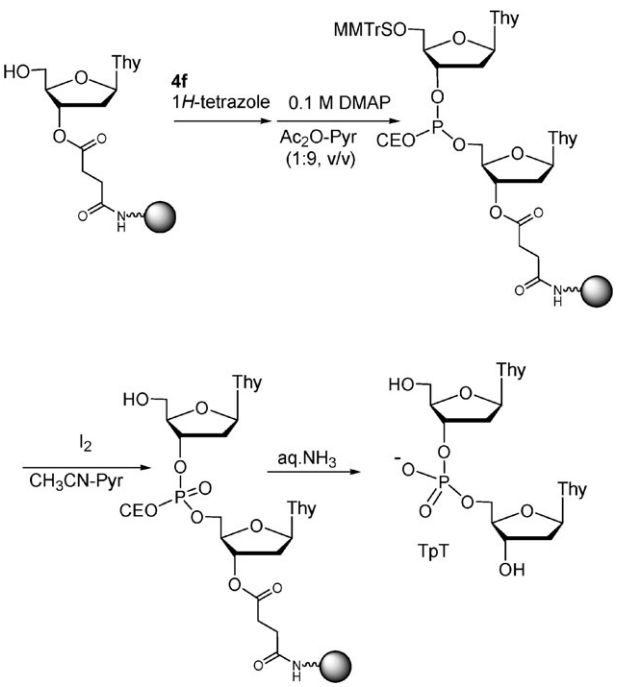
Previously, we reported the use of 0.1 M I_2 in pyridine–H_2O–CH_3CN (45 : 5 : 50, v/v/v) for oxidation of the internucleotidic



Scheme 6 Synthesis of phosphoramidite units.

linkage and removal of the MMTrS group.[9] We again investigated the effectiveness of these conditions in the solid support synthesis of ODNs by the manual synthesis of thymidylyl-(3′,5′)thymidine (TpT) on solid supports (Scheme 7) and compared the results with those obtained in the oxidation/deprotection using 0.1 M I_2 in pyridine–H_2O (90 : 10, v/v).

The thymidine residues attached to the solid supports were coupled with phosphoramidite **4f** in the presence of 1*H*-tetrazole. After the capping reaction with acetic anhydride, removal of the MMTrS group and oxidation of the internucleotidic phosphite intermediate to the phosphotriester linkage were performed using the previously mentioned conditions. The fully protected thymidylyl(3′,5′)thymidine



Scheme 7 Synthetic scheme leading to TpT.

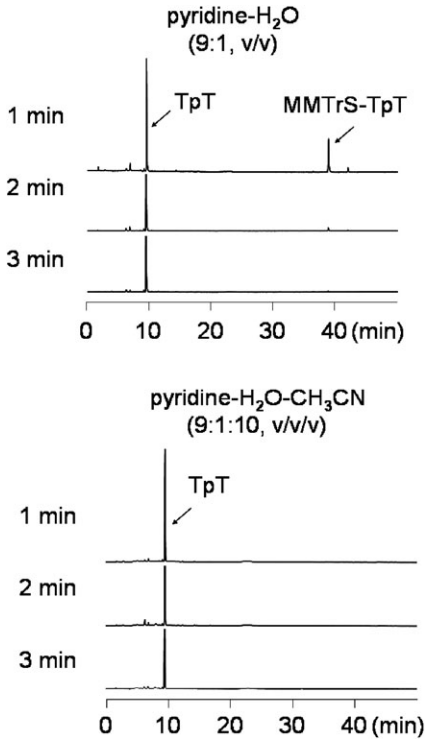


Fig. 1 Reverse-phase HPLC profiles of the oxidative deprotection.

derivative thus synthesized was cleaved by treatment with aqueous NH_3 to give TpT, having a normal phosphodiester linkage, and the products were analyzed by reverse-phase HPLC; the results of which are shown in Fig. 1. The completion of the oxidation/deprotection using 0.1 M I_2 in pyridine–H_2O (90 : 10, v/v) required at least 3 min, and the 5′-MMTrS derivatives of TpT were also observed at 1 and 2 min. Interestingly, when the composition of the solvent was changed to pyridine–H_2O–CH_3CN (45 : 5 : 50, v/v/v), the oxidation/deprotection was completed more quickly (within 1 min).

Synthesis of decathymidylate (T10-mer)

We first carried out the automated synthesis of decathymidylate (T10-mer) using thymidine phosphoramidite building block **4f**, 0.45 M 1*H*-tetrazole, capping reagents containing phenoxyacetic anhydride[18,19] and the 0.1 M I_2 solution in pyridine–H_2O–CH_3CN (45 : 5 : 50, v/v/v) described above. The coupling time was set to 38 s, which was identical to that of the conventional DNA synthesis protocol using DMTr phosphoramidites. After cleavage of the products from the solid support by treatment with aqueous NH_3, the crude products were analyzed on an anion-exchange HPLC; the result is shown in Fig. 2. The desired T10-mer was observed as the major peak at 26.5 min. The presence of by-products were also observed in the eluate: hexathymidylate at 15.2 min, heptathymidylate at 18.8 min, octathymidylate at 21.8 min and nonathymitylate at 24.4 min, together with surrounding smaller peaks. At first, we assumed that these by-products were formed because the reactivity of the 5′-*O*-MMTrS-phosphoramidite **4f** is lower than that of the conventional 5′-*O*-DMTr-phosphoramidite. However, the formation of the by-products could not be suppressed by carrying out the coupling process twice

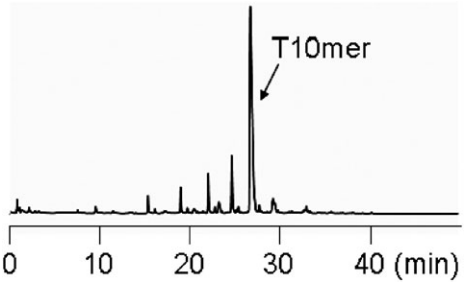


Fig. 2 Anion-exchange HPLC profile for the T10-mer.

(double coupling) in a single chain extension. This result suggests that the by-products are generated from side reactions attributable to the reactivity, or conversely the stability, of the MMTrS group. Another possible side reaction is the formation of the H-phosphonate linkage due to the incomplete oxidation of the internucleotidic linkage. In order to check this possibility, we measured the MALDI-TOF mass spectrum of the crude T10-mer. However, the major peaks observed were those of T9-, T8-, T7-, T6- and T5-mers, and derivatives having H-phosphonate termini or H-phoshonate diester linkages were not observed. This result suggests that incomplete oxidation is not the major reason.

To suppress the side reactions, we checked the effect of the activators, capping reagents and I_2 oxidation conditions by monitoring the ratio of the HPLC peak of the T10-mer to the sum of all peak areas; the results are summarized in Table 1. As described previously, the use of 1*H*-tetrazole as an activator gave the desired T10-mer in 72% purity, and the double coupling using 1*H*-tetrazole yielded an almost identical purity (71%). More nucleophilic 4,5-dicyanoimidazole[20] and more acidic activator 42[21] yielded similar results. We also checked the capping reaction using acetic anhydride, but there was no significant difference in the T10-mer synthesis between the conditions with and without the capping reaction. These results indicated that neither the coupling conditions nor the capping conditions are the origin of the side reactions.

Next, we checked the solvent composition used in the I_2 oxidation, focusing particularly on the water content. In the oxidation/deprotection, the I_2 reagent first oxidized both the sulfur atom of the MMTrS group and the internucleotidic phosphite part to yield the sulfonium and phosphonium intermediates. The successive nucleophilic attack of a water molecule on the phosphorus center gave the phosphotriester moiety, and a similar attack of a water molecule on the sulfonium intermediate led to generation of the free 5′-hydroxyl group, possibly after elimination of the MMTr group. In these mechanisms, not only I_2 but also water molecules might play an important role. Therefore, we changed the water content of the oxidant solution and analyzed the purity of the T10-mer.

When the water concentration was raised to 10%, the purity of the T10-mer increased slightly to 76% (Table 1). Changing the ratio of pyridine and acetonitrile to 40 : 50 had no effect. Interestingly, the increase of the water concentration to 15, 20 and 30% improved the purity of the T10-mer to 78, 81 and 80%, respectively. Although the improvement achieved was not perfect, these results suggest that the concentration of water is an important factor that determines the purity of the products.

Table 1 Fine tuning of the reaction conditions

Conditions tested	Yield of T10-mer (%)
(a) Activator	
1*H*-Tetrazole	72
1*H*-Tetrazole (double coupling)	71
4,5-Dicyanoimidazole	71
Activator 42	69
(b) Capping agents	
Acetic anhydride	71
(no capping)	68
(c) Solvents for the I_2 oxidation pyridine–H_2O–CH_3CN (v/v/v)	
20 : 10 : 70	76
40 : 10 : 50	76
15 : 15 : 70	78
20 : 20 : 60	81
20 : 30 : 50	80

Unfortunately, increasing the water concentration reduced the solubility of I_2 in these solvents, meaning that solvents containing more than 20% water could not be used conveniently in the DNA synthesizer. Therefore, we used pyridine–H_2O–CH_3CN (15 : 15 : 70, v/v/v) for later experiments to clarify the applicability of these conditions to the synthesis of longer ODNs (see supporting information).

Synthesis of DNA 10-mer and 20-mer

We attempted to synthesize the sequence-mixed 20- and 10-mer ODNs 5′-d[GTTACACATGTTACAGCTAT]-3′ (**ODN1**) and 5′-d[TTACAGCTAT]-3′ (**ODN2**), respectively, using 5′-*O*-MMTrS-phosphoramidites **4a** and **4d–f**.

The anion-exchange profiles of the crude products and the purified product are shown in Fig. 3. The purities of **ODN1** and **ODN2** in each crude material were calculated to be 39 and 61%, respectively, on the basis of HPLC peak areas. In all the anion-exchange HPLC profiles, the target ODNs were obtained as single peaks after purification by anion-exchange HPLC. The pure **ODN1** and **ODN2** materials were obtained after anion-exchange HPLC purifications in 3 and 7% yields, respectively. The molecular weights were confirmed by MALDI-TOF mass analyses (shown in the Experimental section).

The relatively low yields were due to the by-products eluted before and after the target peaks, as shown in the left column of Fig. 3. These by-products must be generated in a process similar to those described for the T10-mer synthesis shown in Fig. 2. Despite these yet to be overcome problems, our experiments revealed that the 5′-*O*-MMTrS-phosphoramidites described herein are applicable to the three-step ODN synthesis, and could be used to obtain up to 20-mer chromatographically-pure oligodeoxynucleotides.

Conclusions

We examined the synthesis of ODNs containing all four nucleobases using the 4-methoxytritylthio (MMTrS) group for protection of the 5′-hydroxyl group. Because the MMTrS group could be removed by oxidation with an aqueous I_2 solution, the chain extension cycle could be reduced to a three-step procedure, consisting of coupling,

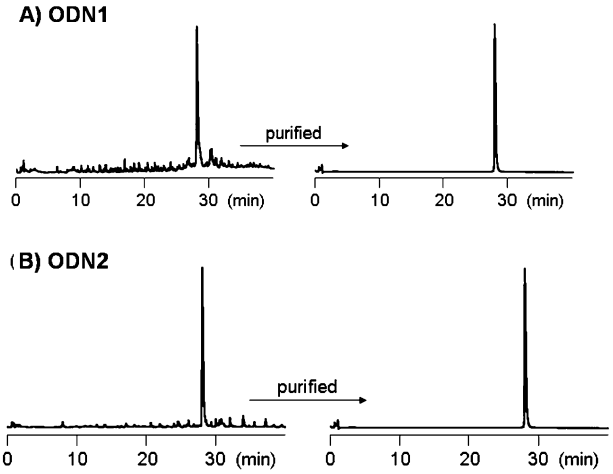


Fig. 3 Anion-exchange HPLC profiles of **ODN1** and **ODN2**.

capping and oxidation/deprotection. The MMTrS group could be introduced to the 5′-hydroxyl group of 3′-*O*-TBDMS-deoxycytidine, -deoxyadenosine and -deoxyguanosine, having appropriate base-protecting groups. We also carried out the synthesis of ODNs with mixed 10- and 20-base sequences and succeeded in obtaining chromatographically-pure products.

The results shown in this paper suggest several aspects of the procedure that need to be improved to make the MMTrS protection protocol more useful. For example, the MMTrS group was introduced to the 5′-hydroxyl group of 3′-protected nucleosides using strong bases like LHMDS and *n*-BuLi. New methods to form the S–O bond without such strong bases would enable the introduction of MMTrS to the 3′-*O*-protected or unprotected nucleoside with greater ease. As previously reported, other sulfenyl ester-type protecting groups, such as 2,4-dinitrophenylsulfenyl, could be introduced to the 5′-hydroxyl group by simply mixing the corresponding sulfenyl chloride and 5′-free nucleosides in pyridine.[22]

Although the introduction of the TrS group to the 5′-hydroxyl has been reported using similar reaction conditions,[23] MMTrS could not be introduced because of the instability of MMTrSCl in pyridine. Therefore, a new methodology for the formation of the S–O bond needs to be developed to allow the introduction of the MMTrS group to the 5′-hydroxyl group under mild conditions.

In addition, the formation of shorter strand by-products must be suppressed to improve the yield and purity of the ODNs. As described in this study, the by-products could not be reduced by simple repetition of the coupling step. Although the mechanism of formation of the by-products has not been clarified, we have already obtained preliminary but useful information that the by-products could be reduced by increasing the water content in the solvents used for oxidation. Unfortunately, this approach could not be developed further because of the low solubility of I_2 in water.

However, this observation suggests that the use of more water-soluble oxidizing agents might be favorable if they are applicable for oligonucleotide synthesis. Unfortunately, because the TrS-type sulfenates were rather stable to several peroxide reagents, *e.g.* *t*-BuOOH and CSO,[24] the use of other oxidizing agents capable of removing the MMTrS group and oxidation of the phosphite intermediates have to be investigated. Studies in this direction are now under way to improve the oxidation/deprotection protocol using the MMTrS group, and these will be reported later.

Experimental

General remarks

Dry solvents were purchased and stored over molecular sieves 4A. Methoxytritylsulfenyl chloride was synthesized according to a published procedure.[9] ^{1}H NMR spectra were obtained at 500 MHz with tetramethylsilane (TMS) as an internal standard in $CDCl_3$. ^{13}C NMR spectra were obtained at 125 MHz with the solvents' peak as the internal standard. ^{31}P NMR spectra were obtained at 202 MHz with 85% H_3PO_4 as an external standard. ESI-TOF mass spectra were obtained in the positive ion mode.

Reverse-phase HPLC was performed at 50 °C with a linear gradient (0–50% in 50 min) of CH_3CN in 0.1 M NH_4OAc, pH 7.0, at a flow rate of 1.0 cm^3 min^{-1} for 50 min.

Anion-exchange HPLC was carried out at 50 °C with solvent A: 1 M NaCl in 25 mM phosphate buffer (pH 6.0) and solvent B: 25 mM phosphate buffer (pH 6.0) at a flow rate of 1.0 cm^3 min^{-1}. Analyses were performed with a linear gradient (0–30% in 15 min, 30–55% over the next 25 min and 55%–75% over the next 10 min) of solvent A.

Synthesis

3′-*O*-(*tert*-Butyldimethylsilyl)-6-*N*-[(dimethylamino)methylene]-2′-deoxyadenosine (1a). 5′-*O*-(4,4′-Dimethoxytrityl)-6-*N*-[(dimethylamino)methylene]-2′-deoxyadenosine[10] (22 g, 36 mmol) was rendered anhydrous by repeated co-evaporation with dry pyridine, toluene and DMF, and finally dissolved in dry DMF (36 cm^3). To the solution were added imidazole (108 mmol, 7.3 g) and *tert*-butyldimethylchlorosilane (54 mmol, 8.1 g). After being stirred at room temperature for 1 h, the reaction was quenched by the addition of water (10 cm^3). The solution was diluted with ethyl acetate (400 cm^3) and washed three times with water (300 cm^3). The organic layer was collected, dried over $MgSO_4$, filtered and concentrated under reduced pressure. On the basis of a TLC analysis of this residue, it was confirmed that the starting material had completely changed to 3′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(4,4′-dimethoxytrityl)-6-*N*-[(dimethylamino)methylene]-2′-deoxyadenosine. The residue was dissolved in CH_2Cl_2 (100 cm^3). A 20 cm^3 aliquot was separated and the DMTr group was removed by addition of 1% trifluoroacetic acid (10 equiv., 530 cm^3, CH_2Cl_2) for 1 min. The reaction mixture was immediately decanted to a solution of pyridine–methanol (1 : 2, v/v, 150 cm^3) and then stirred for 5 min. The detritylation was repeated 5 times (each 20 cm^3 aliquot ×5). Finally, all the pyridine–methanol solutions were collected and evaporated. The residue was diluted with ethyl acetate (400 cm^3), washed twice with water (400 cm^3) and twice with sat. $NaHCO_3$ (300 cm^3). The organic layer was collected, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was crystallized from hexane–ethyl acetate (7 : 3, v/v), and the crystals were collected

by filtration to give 3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (7.9 g). This material was rendered anhydrous by repeated co-evaporation with dry methanol and finally dissolved in dry methanol (30 cm^3). To the solution was added *N*,*N*-dimethylacetamide dimethyl acetal (14 cm^3, 108 mmol). After being stirred at room temperature for 3 h, the reaction mixture was concentrated under reduced pressure. The residue was diluted with ethyl acetate (200 cm^3) and washed three times with water (200 cm^3). The organic layer was collected, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was crystallized from hexane and the crystals collected by filtration to give **1a** (8.4 g, 92%). δ_H(500 MHz; $CDCl_3$; TMS) 0.12 (6H, s × 2), 0.92 (9H, s), 2.16–2.20 (1H, m), 3.03–3.09 (1H, m), 3.21 (3H, s), 3.26 (3H, s), 3.72–3.78 (1H, m), 3.95–3.99 (1H, m), 4.15 (1H, s), 4.70 (1H, d, J = 4.6), 6.30 (1H, dd, J = 5.5 and 9.6), 6.59–6.62 (1H, m), 7.92 (1H, s), 8.49 (1H, s), 8.95 (1H, s); δ_C(126 MHz; $CDCl_3$; $CDCl_3$) −4.65, −4.61, 18.14, 25.91, 35.36, 35.39, 41.37, 41.48, 41.52, 63.62, 74.39, 74.43, 88.22, 88.25, 90.61, 90.68, 128.01, 141.49, 141.52, 150.39, 151.96, 158.35, 158.33, 160.42; m/z (ESI) 421.2368 $[M + H]^+$. $C_{19}H_{33}N_6O_3Si^+$ requires 421.2378.

3′-*O*-(*tert*-Butyldimethylsilyl)-6-*N*-[(dimethylamino)methylene]-5′-*O*-[(4-methoxytrityl)sulfenyl]-2′-deoxyadenosine (2a) by use of *n*-BuLi as a base. Compound **1a** (3.0 g, 7.1 mmol) was rendered anhydrous by repeated co-evaporation with dry THF and finally dissolved in dry THF (36 cm^3). The solution was cooled to −78 °C and then, *n*-BuLi (1.59 M in THF, 7.8 mmol, 4.9 cm^3) was added to the solution. After being stirred at −78 °C for 20 min, the reaction was warmed to ambient temperature. Subsequently, MMTrSCl (11.4 mmol, 14 g) was added and the resulting solution stirred for 1 h. The reaction was quenched by the addition of concentrated NH_3 (5 cm^3). The solution was diluted with ethyl acetate (100 cm^3), washed with sat. $NaHCO_3$ (150 cm^3) and three times with water (50 cm^3 × 3). The organic layer was collected, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was chromatographed on a silica gel column (30 g) with hexane–ethyl acetate (1 : 6, v/v) to yield **2a** (3.0 g, 57%). δ_H(500 MHz; $CDCl_3$; TMS) 0.04 (6H, s × 2), 0.87 (9H, s), 2.28 (1H, m), 2.45 (1H, m), 3.21 (3H, s), 3.27 (3H, s), 3.37–3.45 (2H, m), 3.79–3.83 (4H, m), 4.25 (1H, m), 6.39 (1H, dd, J = 7.3 and 6.1), 6.81–6.84 (2H, m), 7.24–7.37 (12H, m), 8.05 (1H, s), 8.54 (1H, s), 8.97 (1H, s);δ_C(126 MHz; $CDCl_3$; $CDCl_3$) −4.7, −4.7, −4.6, 18.0, 25.8, 35.0, 42.2, 55.4, 71.1, 72.2, 86.9, 87.3, 96.5, 113.6, 127.7, 128.3, 129.2, 130.0, 131.2, 133.3, 134.0, 142.9, 144.6, 159.0, 162.2; m/z (ESI) 725.3266 $[M + H]^+$. $C_{39}H_{48}N_6O_4SSi$ requires 725.3300.

3′-*O*-(*tert*-Butyldimethylsilyl)-5′-*O*-[(4-methoxytrityl)sulfenyl]-2′-deoxyadenosine (2b). 3′-*O*-(*tert*-Butyldimethylsilyl-2′-deoxyadenosine (200 mg, 0.5 mmol) was rendered anhydrous by repeated co-evaporation with dry THF and finally dissolved in dry THF (2.3 cm^3). MMTrSCl (300 mg 0.9 mmol) was added to the solution. Then, the resultant solution was cooled to −78 °C. In addition, *t*-BuLi (1.46 M in hexane, 0.6 mmol, 0.4 cm^3) was added to the solution. After being stirred at −78 °C for 30 min, the reaction was warmed to ambient temperature. The resulting solution was stirred for 2 h. The reaction was quenched by the addition of concentrated NH_3 (1 cm^3). The solution was diluted with ethyl acetate (40 cm^3), washed with sat. $NaHCO_3$ (100 cm^3) and three times with water (30 cm^3 × 4). The organic layer was collected, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was chromatographed on a silica gel column (22 g) with hexane–ethyl acetate (1 : 2, v/v) to yield **2b** (150 mg, 39%). δ_H(500 MHz; $CDCl_3$; TMS) 0.04 (6H, s × 2), 0.87 (9H, s), 2.26–2.31 (1H, m), 2.42–2.48 (1H, m), 3.38–3.48 (2H, m), 3.79 (3H, s), 3.80–3.83 (1H, m), 4.23–4.25 (1H, m), 5.85 (2H, s), 6.36 (1H, dd, J = 6.2), 6.81–6.84 (2H, m), 7.23–7.37 (12H, m), 7.97 (1H, s), 8.34 (1H, s); δ_C(126 MHz; $CDCl_3$; $CDCl_3$) −4.6, 18.1, 25.9, 40.9, 55.3, 55.4, 71.9, 72.5, 72.6, 84.5, 86.5, 113.5, 120.2, 127.50, 128.2, 130.1, 131.3, 134.2, 139.0, 139.1, 143.1, 143.1, 149.8, 153.1, 155.6, 158.9; m/z (ESI) 670.2808 $[M + H]^+$. $C_{36}H_{44}N_5O_4SSi^+$ requires 670.2878.

4-*N*-Benzoyl-3′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-[(4-methoxytrityl)sulfenyl]-2′-deoxycytidine (2c). 4-*N*-Benzoyl-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine (**1c**, 200 mg, 0.45 mmol) was rendered anhydrous by repeated co-evaporation with dry THF and finally dissolved in dry THF (0.9 cm^3). The solution was added to LHMDS (1.6 M in THF, 0.62 mL, 1.0 mmol) in a flask. After being stirred at r.t. for 20 min, MMTrSCl (0.72 mmol, 245 mg) was added and the resulting solution stirred for 1 h. The reaction was quenched by the addition of concentrated NH_3 (1 cm^3). The solution was diluted with ethyl acetate (30 cm^3), washed with sat. $NaHCO_3$ (100 cm^3) and water (20 cm^3 × 2). The organic layer was collected, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was chromatographed on a silica gel column (20 g) with hexane–ethyl acetate (4 : 1, v/v) to yield **2c** (149 mg, 44%).; δ_H(500 MHz; $CDCl_3$; TMS) 0.01 (6H, s), 0.84 (9H, s), 1.94–1.99 (1H, m), 2.43–2.49 (1H, m),3.39 (1H, dd, J = 11.5 and 2.7), 3.50 (1H, dd, J = 11.2 and 3.2), 3.76 (1H, dd, J = 6.6 and 3.2), 3.80 (3H, s), 4.10 (1H, dd, J = 4.5, and 10.1), 6.17 (1H, t, J = 5.9 Hz), 6.84–6.87 (2H, m), 7.25–7.38 (12H, m), 7.50–7.54 (3H, m), 7.60–7.63 (1H, m), 7.92 (2H, d, J = 7.6) 8.08 (1H, d, J = 7.6), 8.74 (1H, s); δ_C (126 MHz; $CDCl_3$; $CDCl_3$) −4.8, 17.9, 25.7, 35.1, 40.6, 41.2, 55.2, 71.8, 72.4, 84.18, 86.2, 113.3, 126.4, 127.3, 128.0, 130.0, 131.1, 134.1, 139.9, 143.0, 151.3, 152.6, 158.1, 158.7, 159.6. m/z (ESI) 750.3173 $[M + H]^+$. $C_{42}H_{48}N_3O_6SSi^+$ requires 750.3028.

3′-*O*-(*tert*-Butyldimethylsilyl)-4-*N*-[(dibutylamino)methylene]-2′-deoxycytidine (1d). 3′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxycytidine[25] (5.6 g, 16 mmol) was rendered anhydrous by repeated co-evaporation with dry pyridine, toluene and MeOH, and finally dissolved in dry MeOH (32 cm^3). Dibutylformamide dimethyl acetal (32 mmol, 7.2 cm^3) was added to the solution. After being stirred at room temperature for 2 h, the solvent was evaporated, diluted with ethyl acetate (200 cm^3) and washed twice with water (200 cm^3). The organic layer was collected, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was chromatographed on a silica gel column (100 g) with hexane–ethyl acetate (2 : 3, v/v) to yield **1d** (7.6 g, quant). δ_H(500 MHz; $CDCl_3$; TMS) 0.07 6H, s),

0.89 (9H, s), 0.92–0.96 (6H, m), 1.29–1.36 (4H, m), 1.55–1.62 (4H, m), 2.24–2.30 (1H, m), 2.57–2.63 (1H, m), 3.33 (2H, t, J = 7.3), 3.53 (2H, t, J = 7.6 Hz), 3.74 (1H, d, J = 12.0), 3.82 (1H, br), 3.92–3.97 (2H, m), 4.52–4.55 (1H, m), 6.01–6.04 (2H, m), 7.69 (1H, d, J = 7.3), 8.83 (1H, s); δ_C (126 MHz; $CDCl_3$; $CDCl_3$) −4.8, −4.6, 13.8, 13.8, 18.1, 19.9, 20.2, 25.9, 29.2, 31.1, 40.7, 45.6, 46.2, 52.3, 62.2, 71.8, 88.3, 90.4, 103.2, 143.3, 156.5, 158.5, 172.1; m/z (ESI) 481.3259 $[M + H]^+$. $C_{24}H_{45}N_4O_4Si^+$ requires 481.3205.

3′-*O*-(*tert*-Butyldimethylsilyl)-4-*N*-[(dibutylamino)methylene]-5′-*O*-[(4-methoxytrityl)sulfenyl]-2′-deoxycytidine (2d). Compound **1d** (200 mg, 0.42 mmol) was rendered anhydrous by repeated co-evaporation with dry THF and finally dissolved in dry THF (1.8 cm^3). The solution was cooled to −78 °C and then, *n*-BuLi (1.59 M in THF, 0.46 mmol, 0.3 cm^3) was added. After being stirred at −78 °C for 20 min, the reaction was warmed to ambient temperature. Subsequently, MMTrSCl (0.67 mmol, 230 mg) was added and the resulting solution stirred for 1 h. The reaction was quenched by the addition of concentrated NH_3 (1 cm^3). The solution was diluted with ethyl acetate (50 cm^3) and then washed with sat. $NaHCO_3$ (50 cm^3 × 3). The organic layer was collected, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was chromatographed on a silica gel column (30 g) with hexane–ethyl acetate (7 : 3, v/v) containing 0.5% triethylamine to yield **2d** (181 mg, 58%). δ_H(500 MHz; $CDCl_3$; TMS) 0.01 (6H, s × 2), 0.84 (9H, s), 0.94–0.99 (6H, m), 1.32–1.40 (4H, m), 1.58–1.64 (4H, m), 1.91–1.97 (1H, m), 2.36–2.42 (1H, m), 3.35 (2H, t, J = 7.3), 3.41 (1H, dd, J = 3.2 and 11.2), 3.48 (1H, dd, J = 3.4 and 11.2), 3.54–3.58 (2H, m), 3.71 (1H, dd, J = 3.3 and 7.4), 3.82 (3H, s), 4.08–4.11 (1H, m), 5.99 (1H, d, J = 7.3), 6.24 (1H, t, J = 6.1), 6.83–6.87 (2H, m), 7.26–7.39 (12H, m) 7.72 (1H, d, J = 7.3), 8.85 (1H, s); δ_C(126 MHz; $CDCl_3$; $CDCl_3$) −4.8, −4.6, 13.8, 13.9, 18.1, 19.9, 20.2, 25.8, 29.2, 31.1, 42.0, 45.4, 52.2, 55.3, 55.3, 71.0, 71.9, 86.2, 86.4, 102.9, 113.5, 127.5, 128.1, 130.0, 131.2, 134.1, 140.9, 143.0, 143.0,156.4, 158.2, 158.9, 172.0; m/z (ESI) 785.4144 $[M + H]^+$. $C_{44}H_{61}N_4O_5SSi^+$ requires 785.4126.

3′-*O*-(*tert*-Butyldimethylsilyl)-2-*N*-isobutyryl-5′-*O*-[(4-methoxytrityl)sulfenyl]-2′-deoxyguanosine (2e). 3′-*O*-(*tert*-Butyldimethylsilyl)-2-*N*-isobutyryl-2′-deoxyguanosine (**1e**, 11 g, 24 mmol) was rendered anhydrous by repeated co-evaporation with dry THF and finally dissolved in dry THF (120 cm^3). LHMDS (1.6 M in THF, 53 mmol, 33 cm^3) was added to the solution, which was then stirred at room temperature for 20 min. Subsequently, MMTrSCl (38 mmol, 13 g) was added and the resulting solution was stirred for 1 h. The reaction was quenched by the addition of concentrated NH_3 (20 cm^3). The solution was diluted with ethyl acetate (200 cm^3) and then washed with sat. $NaHCO_3$ (250 cm^3 × 3). The organic layer was collected, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was subjected to flash column chromatography on a silica gel column (145 g) with hexane–ethyl acetate (3 : 1, v/v) to yield crude **2e** (13 g). This material was used without further purification in the preparation of **3e**.

6-*N*-[(Dimethylamino)methylene]-5′-*O*-[(4-methoxytrityl)-sulfenyl]-2′-deoxyadenosine (3a). Compound **2a** (2.9 g, 4.0 mmol) was rendered anhydrous by repeated co-evaporation with dry THF and finally dissolved in dry THF (36 mL). TEA·3HF (2.0 cm^3, 12 mmol) and TEA (1.7 cm^3, 12 mmol) were added to the solution. After being stirred for 24 h, the reaction was quenched by the addition of water (10 cm^3). The solution was diluted with ethyl acetate (150 cm^3) and then washed with water (100 cm^3 × 3). The organic layer was collected, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was chromatographed on a silica gel column (100 g) with ethyl acetate–methanol (100 : 3, v/v) to yield **3a** (1.5 g, 62%). δ_H(500 MHz; $CDCl_3$; TMS) 2.37–2.43 (1H, m), 2.48–2.54 (1H, m), 3.09 (1H, s), 3.20 (3H, s), 3.26 (3H, s), 3.43–3.53 (2H, m), 3.78 (3H, s), 3.86 (1H, dd, J = 7.4 and 3.8), 4.29 (1H, m), 6.43 (1H, t, J = 6.6), 6.80–6.83 (2H, m), 7.22–7.36 (12H, m), 8.08 (1H, s), 8.51 (1H, s), 8.98 (1H, s); δ_C(126 MHz; $CDCl_3$; $CDCl_3$) 35.1, 40.5, 41.2, 55.2, 71.7, 71.8, 83.9, 85.6, 113.3, 126.2, 127.3, 128.0, 129.9, 131.0, 134.0, 139.8, 142.8, 142.9, 151.1, 152.5, 158.3, 158.7, 159.6; m/z (ESI) 611.2487 $[M + H]^+$. $C_{33}H_{35}N_6O_4S^+$ requires 611.2435.

4-*N*-[(Dibutylamino)methylene]-5′-*O*-[(4-methoxytrityl)sulfenyl]-2′-deoxycytidine (3d). Compound **2d** (3.5 g, 4.5 mmol) was treated with TEA·3HF (2.2 cm^3, 13.4 mmol) and TEA (1.9 cm^3, 13.4 mmol) according to the procedure described for **3a** to yield **3d** (1.7 g, 58%). δ_H(500 MHz; $CDCl_3$; TMS) 0.92–0.98 (6H, m), 1.28–1.34 (4H, m), 1.56–1.64 (4H, m), 1.92–1.98 (1H, m), 2.32 (1H, br), 2.44–2.50 (1H, m), 3.33 (2H, t, J = 7.4), 3.41 (1H, dd, J = 3.1 and 11.1), 3.50–3.57 (3H, m), 3.75–3.78 (1H, dd, J = 6.8 and 3.2), 3.80 (1H, s), 4.02–4.04 (1H, m), 6.02 (1H, d, J = 7.3), 6.24 (1H, t, J = 6.2), 6.81–6.85 (2H, m), 7.23–7.37 (12H, m), 7.75 (1H, d, J = 7.1) 8.83 (1H, s); δ_C(126 MHz; $CDCl_3$; $CDCl_3$) 13.8, 13.9, 19.9, 20.2, 29.2, 31.1, 41.8, 45.5, 52.2, 55.4, 71.2, 72.0, 85.7, 86.5, 103.1, 113.5, 127.5, 128.2, 130.0, 131.2, 134.1, 140.9, 142.9, 143.0, 156.5, 158.3, 158.9, 172.0; m/z (ESI) 671.3294 $[M + H]^+$. $C_{38}H_{47}N_4O_5S^+$ requires 671.3262.

2-*N*-Isobutyryl-5′-*O*-[(4-methoxytrityl)sulfenyl]-2′-deoxyguanosine (3e). Crude **2e** (13 g, 18 mmol) was treated with TEA·3HF (8.6 cm^3, 53 mmol) and TEA (7.4 cm^3, 53 mmol) according to the procedure described for **3a** to yield **3e** (7.5 g, 48% in 2 steps from **1e**). δ_H(500 MHz; $CDCl_3$; TMS) 1.20–1.26 (m, 6H), 2.23–2.34 (m, 2H), 2.77–2.83 (m, 1H), 3.39–3.46 (m, 2H), 3.73 (s, 3H), 3.85 (d, J = 3.2 Hz, 1H), 4.01 (s, 1H), 4.30 (s, 1H), 6.01 (t, J = 6.6 Hz, 1H), 6.77 (d, J = 8.8 Hz, 2H), 7.13–7.30 (m, 12H), 7.83 (s, 1H), 10.23 (s, 1H), 12.32 (s, 1H); δ_C(126 MHz; $CDCl_3$; $CDCl_3$) 19.1, 19.2, 36.4, 40.75, 46.0, 55.4, 71.7, 72.0, 78.3, 84.2, 86.3, 113.5, 121.2, 127.5, 128.1, 130.0, 131.2, 134.1, 137.5, 143.0, 143.1, 148.0, 148.5, 156.0, 159.0, 179.8. ESI-MS: calc. for $C_{34}H_{36}N_5O_6S$ $[M + H]^+$, 642.2381; found, 642.2381.

6-*N*-[(Dimethylamino)methylene]-5′-*O*-[(4-methoxytrityl)-sulfenyl]-2′-deoxyadenosine 3′-(2-cyanoethyl-*N*,*N*-diisopropyl-phosphoramidite) (4a). Compound **3a** (1.4 g, 2.3 mmol) was rendered anhydrous by repeated co-evaporation with dry CH_2Cl_2, and finally dissolved in dry CH_2Cl_2 (4.5 cm^3). Then, 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite

(0.87 cm^3, 2.8 mmol), diisopropylamine (0.13 cm^3, 0.92 mmol) and 1*H*-tetrazole (64 mg, 0.92 mmol) were added to the solution. The resulting solution was stirred for 8 h. The reaction was quenched by adding H_2O–CH_3CN (1 : 1, v/v, 10 cm^3). The mixture was diluted with Et_2O (150 cm^3) and washed with sat. Na_2CO_3 (5 × 100 cm^3). The organic layer was collected, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was chromatographed on a silica gel column (100 g) with ethyl acetate to yield **4a** (1.4 g, 70%): δ_H(500 MHz; $CDCl_3$; TMS) 1.14–1.20 (12H, m), 2.44–2.63 (4H, m), 3.27 (3H, s), 3.21 (3H, s), 3.41–3.51 (2H, m), 3.54–3.63 (2H, m), 3.73–3.85 (5H, m), 3.99–4.04 (1H, m), 4.40–4.45 (1H, m), 6.41 (1H, m), 6.82 (2H, dd, *J* 9.0 and 2.5), 7.23–7.36 (12H, m), 8.07 (1H, m), 8.53 (1H, m), 8.97 (1H, s); δ_C(126 MHz; $CDCl_3$; $CDCl_3$) 20.4, 20.4, 20.5, 24.6, 24.6, 24.6, 24.7, 24.7, 35.2, 39.7, 41.3, 43.3, 43.3, 43.4, 43.4, 55.3, 58.4, 58.5, 71.9, 71.5, 73.59, 73.7, 73.9, 74.0, 78.0, 84.2, 84.2, 85.2, 85.4, 113.5, 117.5, 126.4, 127.4, 128.1, 130.0, 130.0, 131.2, 134.1, 140.0, 143.0, 143.1, 151.5, 152.7, 158.3, 158.8, 159.7; δ_P($CDCl_3$, 202 MHz, 85% H_3PO_4) 149.25, 149.45; *m*/*z* (ESI) 811.3535 $[M + H]^+$. $C_{42}H_{52}N_8O_5PS^+$ requires 811.3514.

4-*N*-[(Dibutylamino)methylene]-5′-*O*-[(4-methoxytrityl)sulfenyl]-2′-deoxycytidine 3′-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite) (4d). Compound **3d** (1.7 g, 2.5 mmol) was treated with 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (0.95 cm^3, 3.0 mmol), diisopropylamine (0.14 cm^3, 1.0 mmol) and 1*H*-tetrazole (70 mg, 1.0 mmol) according to the procedure described for **4a** to yield **4d** (1.3 g, 60%): δ_H(500 MHz; $CDCl_3$; TMS) 0.92–0.98 (6H, m), 1.09–1.25 (14H, m), 1.30–1.38 (4H, m), 1.56–1.64 (4H, m), 1.90–1.96 (1H, m), 2.53–2.64 (3H, m), 3.31–3.35 (2H, m), 3.38–3.41 (1H, m), 3.46–3.59 (6H, m), 3.68–3.78 (1H, m), 3.80 (3H, m), 3.88–3.94 (1H, m), 4.21–4.23 (1H, m), 5.95 (1H, d, *J* 7.1), 6.25–6.30 (1H, m), 6.82–6.85 (2H, m), 7.23–7.37 (12H, m), 7.64–7.69 (1H, m), 8.83 (1H, m); δ_C(126 MHz; $CDCl_3$; $CDCl_3$) 13.8, 13.9, 19.8, 20.1, 20.3, 20.4, 24.5, 24.6, 24.7, 29.1, 31.1, 40.8, 43.2, 43.2, 43.3, 43.3, 45.4, 52.1, 55.3, 58.2, 58.4, 58.5, 71.9, 72.0, 72.6, 72.8, 73.3, 77.3, 77.6, 85.1, 85.2, 85.4, 86.5, 103.0, 103.1, 113.5, 117.5, 117.6, 127.5, 128.1, 130.0, 131.2, 134.0, 140.8, 140.9, 142.9, 143.0, 156.4, 158.2, 158.9, 171.9; δ_P($CDCl_3$, 202 MHz, 85% H_3PO_4) 149.39, 149.10; *m*/*z* (ESI) 871.4347 $[M + H]^+$. $C_{47}H_{64}N_6O_6PS^+$ 871.4340.

2-*N*-Isobutylyl-5′-*O*-[(4-methoxytrityl)sulfenyl]-2′-deoxyguanosine 3′-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite) (4e). Compound **3e** (7.0 g, 11 mmol) was treated with 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (4.2 cm^3, 13 mmol), diisopropylamine (0.6 cm^3, 4.4 mmol) and 1*H*-tetrazole (0.3 g, 4.4 mmol) according to the procedure described for **4a** to yield **4e** (7.5 g, 81%): δ_H(500 MHz; $CDCl_3$; TMS) 1.15–1.23 (18H, m), 2.24–2.30 (0.5H, m), 2.35–2.41 (0.5H, m), 2.47–2.53 (1H, m), 2.57–2.71 (3H, m), 3.38 (1H, d, *J* 4.2), 3.50–3.63 (3H, m), 3.73–3.87 (5H, m), 3.96 (0.5H, s), 4.16 (0.5H, d, *J* 2.9), 4.31 (0.5H, s), 4.50 (0.5H, d, *J* 3.2), 6.10–6.13 (1H, m), 6.82 (2H, d, *J* 7.3), 7.21–7.34 (12H, m), 7.74 (1H, m), 8.53 (0.5H, s), 8.87 (0.5H, s), 11.97 (1H, s); δ_C(126 MHz; $CDCl_3$; $CDCl_3$) 18.9, 19.0, 19.1, 20.6, 24.6, 24.7, 36.2, 36.3, 39.5, 40.3, 43.3, 43.4, 43.4, 55.4, 57.7, 57.9, 71.9, 73.4, 73.5, 74.5, 74.7, 77.8, 78.0, 84.1, 84.9, 85.4, 85.6, 113.5, 117.8, 121.7, 127.5, 128.1, 130.0, 131.2, 131.3, 134.0, 136.6, 137.4, 142.9, 143.0, 147.4, 147.6, 147.8, 155.6, 155.7, 158.9, 178.8; δ_P($CDCl_3$, 202 MHz, 85% H_3PO_4) 148.28, 149.31. *m*/*z* (ESI) 842.3415 $[M + H]^+$. Calc. for $C_{43}H_{53}N_7O_7PS^+$ requires 842.3459.

Solid-phase synthesis of TpT in manual operation

A thymidine-loaded CPG (0.5 μmol, 46 μmol g^{-1}, succinyl linker) was used. The CPG was treated with 3% trichloroacetic acid in CH_2Cl_2 (0.5 mL, 30 s × 3) then washed [CH_2Cl_2 (1 mL × 3), CH_3CN (1 mL × 3)]. Each cycle of chain extension consisted of the following steps: (1) coupling [phosphoramidite unit (20 μmol), 1*H*-tetrazole (2.8 mg, 40 μmol) and CH_3CN (250 μL), 2 min]; (2) washing [CH_3CN (1 mL × 3)]; (3) capping [Ac_2O-Py (1 : 9, v/v, 500 μL) in the presence of 0.1 M DMAP for 2 min]; (4) oxidation [0.1 M I_2, 2 min] and (5) washing [CH_3CN (1 mL × 3)]. After chain extension, the oligomer was released from the polymer support by treatment with concentrated aqueous NH_3 (1 mL) for 15 min. The polymer support was removed by filtration and washed with water (1 mL × 3). The filtrate was evaporated and analyzed by reverse-phase HPLC.

Solid-phase synthesis of T10-mer in automated synthesis

The T10-mer was synthesized on an automated DNA/RNA synthesizer using phosphoramidite **4f** and a thymidine-loaded CPG (1 μmol, 46 μmol g^{-1}, succinyl linker). I_2/pyridine–H_2O–CH_3CN (0.1 M) (45 : 5 : 50–20 : 30 : 50, v/v as shown in Table 1) was used in place of the commercially available I_2 solution. The detailed parameters of the synthetic protocol are shown in the ESI.‡ After chain extension using the various activators shown in Table 1, deprotection and cleavage from the solid supports were carried out by treatment with aqueous NH_3 for 30 min. The materials were analyzed and purified by anion-exchange HPLC. The structures were confirmed by MALDI-TOF mass analyses. **T10-mer**: *m*/*z* 2976.93 $[M + H]^+$ requires 2979.51.

Automated synthesis of ODN1 and ODN2

The ODNs were synthesized on an automated DNA/RNA synthesizer using phosphoramidite **4a** and **4d–f**, and a thymidine-loaded CPG (1 μmol, 46 μmol g^{-1}, succinyl linker). I_2/pyridine–H_2O–CH_3CN (0.1 M) (15 : 15 : 70, v/v) was used in place of the commercially available I_2 solution. The detailed parameters of the synthetic protocol are shown in the ESI.‡ After chain extension, the deprotection and cleavage from the solid supports were carried out by treatment with aqueous NH_3 at 55 °C for 15 h. The materials were eluted, lyophilized, analyzed and purified by anion-exchange HPLC. The structures were confirmed by MALDI-TOF mass analyses. **ODN1**: *m*/*z* 6093.15 $[M + H]^+$ requires 6089.05; **ODN2**: *m*/*z* 3000.44 $[M + H]^+$ requires 3001.55.

Acknowledgements

This study was supported by a grant in aid from the Industrial Technology Research Grant Program in '05 from New Energy

and Industrial Technology Development Organization (NEDO) of Japan, and by a grant in aid for Scientific Research (B) 20350074 from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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