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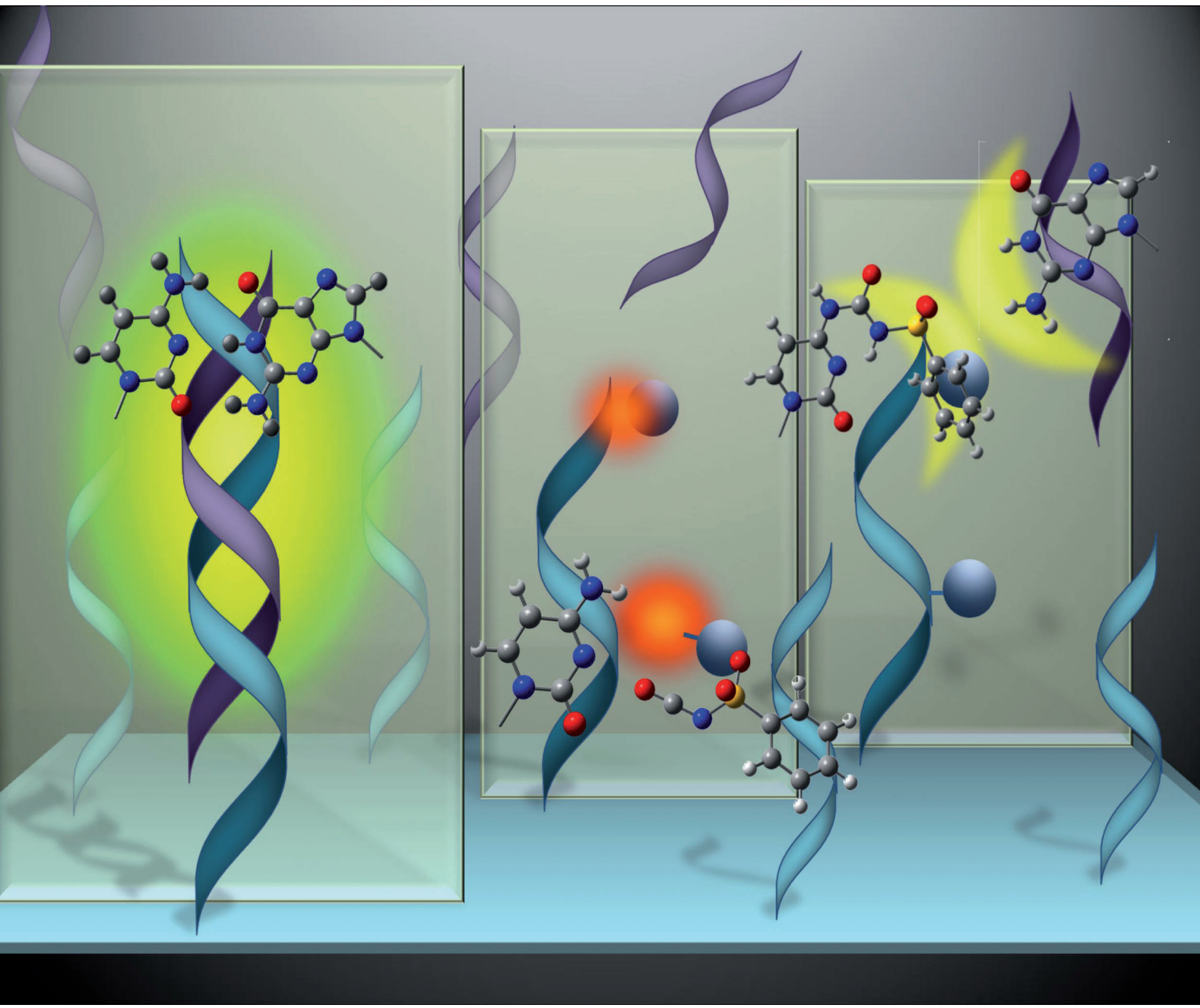

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FULL PAPER
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New thermolytic carbamoyl groups for the protection of nucleobases

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New thermolytic carbamoyl groups for the protection of nucleobases†

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It was found that *N*-arylcarbamoyl and *N*-(phenylsulfonyl)carbamoyl (psc) groups could be effectively introduced onto the amino groups of deoxycytidine and deoxyadenosine derivatives and could be removed thermolytically. We succeeded in synthesizing DNA probes incorporating these thermo-removable protecting groups and developed a new system for molecular switching by changing the protection- and deprotection-modes using simple heating and re-carbamoylation with isocyanates. This reversible process enabled us to control the hybridization ability of the DNA probes.

Introduction

In a number of previous studies, various protecting groups for amino and phosphate groups have been reported in the synthesis of oligonucleotides.[1] Among them, acyl and 2-cyanoethyl groups are commonly used for the base and phosphate groups, respectively, in the general method for DNA or RNA synthesis. They can be removed under basic conditions such as aqueous ammonia. However, oligonucleotides having base-labile functional groups, such as aminoacylated RNAs[2,3] or *N*-acylated DNAs,[4] are not tolerant to such basic conditions at the deprotection step. To overcome this serious problem, it is necessary to develop a new protecting group that can be rapidly removed under neutral conditions in the final stage of DNA synthesis. Previously, Beaucage and co-workers reported 4-methylthio-1-butyl[5] and 3-(2-pyridyl)-1-propyl[6] as thermolytic protecting groups for the phosphate group. These protecting groups can be cleaved by heat-induced deprotection involving an intramolecular cyclization. If new thermolytic protecting groups for the nucleobases could be developed, in the synthesis of oligonucleotides all base and phosphate protecting groups on solid supports could be removed by just a one-step treatment of "heating" without additional treatment with conc. NH_4OH.

In our recent study, it was found that the carbamoyl group of (4-*N*-naphth-2-yl)carbamoyl-dC (dC^{napcm}),[7] which has a unique property as a universal base, was removed at 80 °C in DMSO-H_2O (T_{comp} = 15 h). Although this elimination by heating was unexpected, there was a possibility that this reaction might be useful for the synthesis of oligonucleotides because, generally, carbamoyl groups are very stable under the conditions of detritylation, coupling, capping, and oxidation in phosphoramidite chemistry. In this paper, we report the thermal stability of several carbamoyl-type protecting groups for the amino groups of nucleobases (C, A, and G) and the development of a new method for the synthesis of oligonucleotides incorporating the thermolytic protecting groups that could be used as new molecular switches.

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† Electronic supplementary information (ESI) available: ^1H and ^{13}C NMR spectra of compounds **2a–2j**, **3a** and **3b**; ^1H NMR and HPLC analysis of the thermolytic deprotection. See DOI: 10.1039/b816831h

Results and discussion

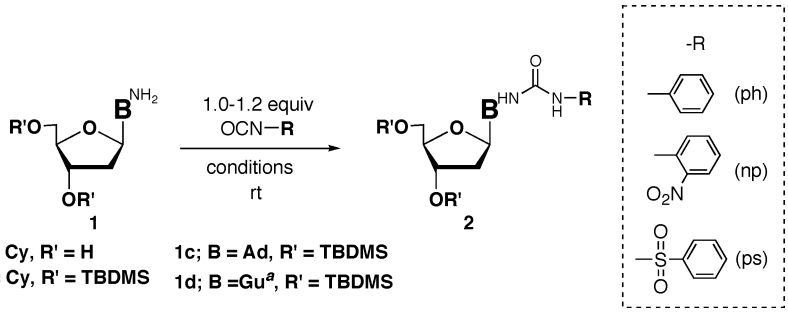
Introduction of *N*-arylcarbamoyl and *N*-(phenylsulfonyl)carbamoyl groups into 2′-deoxynucleosides

To estimate the efficiency of the introduction of carbamoyl groups onto the amino groups of the nucleobases, 4-*N*-arylcarbamoyl- and 4-*N*-[*N*-(phenylsulfonyl)carbamoyl]-2′-deoxynucleoside derivatives were synthesized, as shown in Table 1. *N*-Phenylcarbamoyl (pcm) and *N*-(2-nitrophenyl)carbamoyl (ncm) could be easily introduced selectively onto the amino group of 2′-deoxycytidine **1a** in good yields without protection of the hydroxyl groups by treatment with the corresponding isocyanates for 30 min (entries 1 and 2). When phenylsulfonyl isocyanate was used to obtain 4-*N*-[(*N*-phenylsulfonyl)carbamoyl]-dC, it was difficult to purify the product because of its extremely low solubility. Therefore, 3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-dC **1b** was used for the introduction of an *N*-(phenylsulfonyl)carbamoyl (psc) group onto the cytosine base. As a result, the reaction proceeded rapidly in CH_2Cl_2 in 5 min to give the product **2c** (entry 3). Entries 4–6 show the results of the introduction of carbamoyl groups onto dA derivatives. In these cases, protection using the TBDMS group was also necessary because the reactivity of the 5′-hydroxyl group toward the isocyanate compounds is higher than that of the amino group of dA, unlike dC. The reactions of 3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-dA **1c** with these isocyanate reagents were slower than those of **1b** owing to the low nucleophilicity of its amino group, but the products **2d-f** could be obtained in high yields of 94–99%. In the case of the synthesis of the 2-*N*-psc-dG derivative **2g**, a 2,4,6-triisopropylphenylsulfonyl group was introduced into the 6-*O*-position of the guanine moiety to increase the nucleophilicity of the 2-amino group. As shown in entry 7, compound **2g** was synthesized in 95% yield by treatment with phenylsulfonyl isocyanate for 1.5 h. These carbamoyl groups incorporated into the nucleobases were stable during purification at room temperature.

Thermolytic deprotection of *N*-arylcarbamoyl and *N*-(phenylsulfonyl)carbamoyl groups incorporated into deoxynucleosides

The thermolytic deprotection of the pcm, ncm, and psc groups from the *N*-carbamoylated deoxynucleoside derivatives (**2a–c**, **2f**,

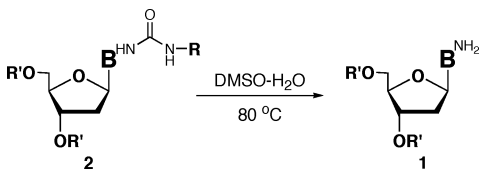
Table 1 Introduction of arycarbamoyl and phenylsulfonylcarbamoyl groups into nucleosides



Entry	*N*-Carbamoylated nucleoside	B	R′	-R	Equiv.	Conditions	Yield
1	**2a**	Cy	H	ph	1.0	DMF, 30 min	94%
2	**2b**	Cy	H	np	1.0	DMF, 30 min	90%
3	**2c**	Cy	TBDMS	ps	1.0	CH_2Cl_2, 5 min	87%
4	**2d**	Ad	TBDMS	ph	1.2	CH_2Cl_2, 4 h	94%
5	**2e**	Ad	TBDMS	np	1.2	CH_2Cl_2, 5 h	99%
6	**2f**	Ad	TBDMS	ps	1.2	CH_2Cl_2, 30 min	99%
7	**2g**	Gu[a]	TBDMS	ps	1.2	CH_2Cl_2, 1.5 h	95%

[a] 6-*O*-(2,4,6-triisopropylphenyl)sulfonylguanine.

Table 2 Thermolytic deprotection of *N*-arylcarbamoyl and *N*-(phenylsulfonyl)carbamoyl groups incorporated into deoxynucleosides



Entry	*N*-Carbamoylated nucleoside	B	R′	-R	$t_{1/2}$[a]
1	2a	Cy	H	ph	11 h
2	2b	Cy	H	np	30 min
3	2c	Cy	TBDMS	ps	<5 min (t_{comp})
4	2h	Ad	H	ph	2.5 h
5	2i	Ad	H	np	2 h
6	2f	Ad	TBDMS	ps	<5 min (t_{comp})
7	2j	Gu	TBDMS	ps	48 h

[a] Estimated by ^{1}H NMR.

and **2h–j**) was examined in detail by ^{1}H NMR analysis, as shown in Table 2. The deprotection of the pcm group of 4-*N*-pcm-dC **2a** was slow ($T_{1/2} = 11$ h) (entry 1). In sharp contrast to this result, the carbamoyl groups of 4-*N*-ncm-dC **2b** and 3′,5′-*O*-bis(TBDMS)-4-*N*-psc-dC **2c** were easily removed in DMSO-H_2O (entries 2 and 3). In particular, the latter was quickly deprotected within only 5 min. Similarly, the psc group of 3′,5′-*O*-bis(TBDMS)-4-*N*-psc-dA **2f** was also found to be removed completely within 5 min (entry 6). Much slower deprotection was observed in the case of the pcm and ncm groups (entries 4 and 5). In entry 7, the deprotection rate of the psc group on the dG derivative **2j** was also much slower ($T_{1/2} = 48$ h) than those of the dC and dA derivatives. These results indicated that the psc group was not suitable as a protecting group for dG in the synthesis of oligonucleotides having base-labile functional groups. Therefore, we decided to use the thermolytic carbamoyl-type protecting groups only for dC and dA since the base protecting groups of dT and dG have proved to be unnecessary in the usual phosphoramidite approach.[8–10]

The carbamoylated dC and dA derivatives (**2a–c**, **f**, **h**, **i**) have the corresponding conformers containing an intramolecular hydrogen bond *via* a 6-membered ring between the carbamoyl group and the ring nitrogen atom of the nucleobase,[11] as shown in Fig. 1-A and -B. The protic *N*-H proton of such 6-membered ring carbamoyl groups might accelerate the thermolytic deprotection. On the other hand, the carbamoylated deoxyguanosine derivative **2j** has two conformers, of open and closed forms,[12] as shown in Fig. 1-C. Previously, we reported that the closed form is more stable than the open one containing an intramolecular hydrogen bond between the carbamoyl group and the nitrogen atom at position 3 of the guanine residue. Therefore, the deprotection rate of the carbamoyl group on the guanine moiety was much slower than those on dC

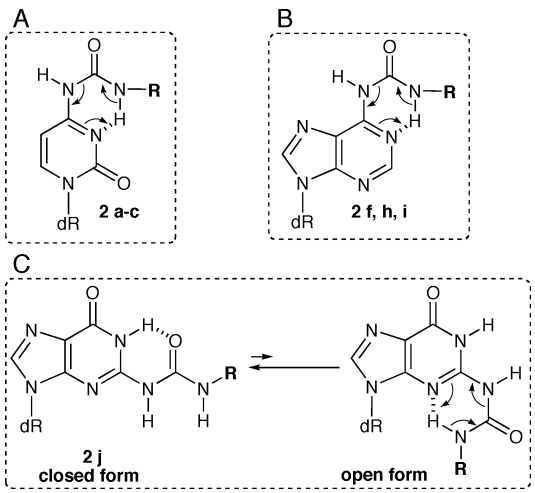


Fig. 1 Conformers of *N*-carbamoylated deoxynucleosides containing an intramolecular hydrogen bond and electron migration giving rise to NH_2-deoxynucleosides and isocyanates.

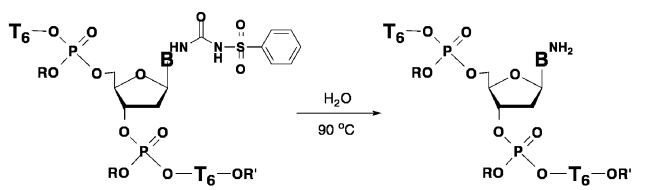
or dA because of the necessity for the conversion of the closed form to the open one, which allows elimination of an isocyanate molecule, as shown in Fig. 1C.

Moreover, it was found that we could regulate the rate of thermolytic deprotection by changing the contents of the solvent. For example, the rate of deprotection of psc^{4}C **2c** decreased remarkably in toluene (t_{comp} = 72 h). These results indicate that the deprotection of the psc groups introduced into oligonucleotides can be easily controlled by masking the surface of a device containing a number of DNA probes, such as DNA chips, using different solvents. Namely, we expected that the site-specifically deprotected species having an unprotected C could be obtained by heating for a short time at the position masked using a polar solvent that contains H_2O to realize rapid deprotection. In contrast, by using a non-polar solvent such as toluene, a position having a protected psc^{4}C that is covered with this solvent can be kept in a protected state because of slow deprotection.

Synthesis of oligonucleotides using the 3′-phosphoramidite building blocks of psc^{4}C and psc^{6}A

To examine the rate of thermolytic deprotection in oligonucleotides, we synthesized two 3′-phosphoramidite units **3a** and **3b** of psc^{4}C and psc^{6}A and six kinds of oligonucleotides **4a-f** in phosphoramidite chemistry. (The HPLC profiles of oligonucleotides **4a** and **4b** are shown in Fig. 2-A and -C.) Table 3 shows the deprotection rate of the psc group incorporated into an oligonucleotide. As a result, the rate of deprotection decreased, compared with that of nucleosides (entries 1 and 2), though the peaks of side reactions (depurination and depyridination) could not be observed in HPLC analysis, as shown in Fig. 2-B and -D. A similar tendency was also observed in the deprotection of oligodeoxynucleotides immobilized on resins (entries 3 and 4). In particular, the deprotection of the psc group on the cytosine moiety was slow in entries 1 and 3. The decrease might result from the presence of anion charges on the neighboring phosphate groups.

Table 3 Thermolytic deprotection of the psc groups incorporated into oligonucleotides



Entry	*N*-Carbamoylated oligonucleotide	B	R	R′	t_{comp}
1	**4a**	Cy	H	H	60 min[a]
2	**4b**	Ad	H	H	15 min[a]
3	**4c**	Cy	H	succinyl-CPG[b]	60 min[c]
4	**4d**	Ad	H	succinyl-CPG[b]	15 min[c]
5	**4e**	Cy	2-cyanoethyl	succinyl-CPG[b]	20 min[c]
6	**4f**	Ad	2-cyanoethyl	succinyl-CPG[b]	15 min[c]

[a] Estimated by anion-exchange HPLC, [b] succinyl linker immobilized on CPG resin, [c] estimated by anion-exchange HPLC after treatment of the resins with 28% NH_4OH for 1 h.

Therefore, we performed thermolytic deprotection of the psc groups introduced into oligonucleotides without removal of the 2-cyanoethyl groups (entries 5 and 6). Although the deprotection rate of the oligonucleotide **4f** with psc^{6}A was similar to those of entries 2 and 4, that of the oligonucleotide **4e** with psc^{4}C significantly increased (t_{comp} = 20 min).

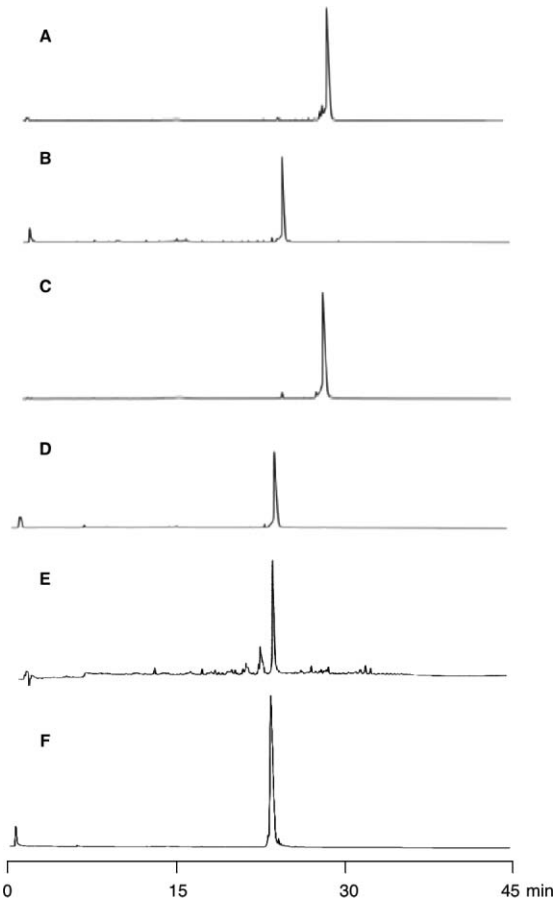


Fig. 2 Anion-exchange HPLC profile of the crude mixture obtained in the synthesis of (A) d[T_6-psc^{4}C-T_6], (B) d[T_6-C-T_6], (C) d[T_6-psc^{6}A-T_6], (D) d[T_6-A-T_6], (E) d[GATACATTGACCT], and (F) d[ATACATT-*i*bu^{2}G-ACCT].

Moreover, we could also synthesize an oligonucleotide with a mixed-sequence, d[GATACATTGACCT] **6** using the psc^{4}C and psc^{6}A phosphoramidite units **3a** and **3b** with commercially available T and *N*-unprotected dG phosphoramidite units.[8–10] Fig. 2-E shows the HPLC profile of the crude mixture. The target oligonucleotide was observed as the main product and was satisfactorily isolated in 25% yield. Furthermore we synthesized a modified oligonucleotide, d[ATACATT-*i*bu^{2}G-ACCT] **7** incorporating 2-*N*-isobutyrylguanine (ibu^{2}G) as a base-labile functional group, as shown in Fig. 2-F. Elongation of the oligomer was carried out on highly cross-linked polystyrene resins[13] having a silyl-type linker[14] by use of general procedures for DNA synthesis.

After elongation, the psc groups were removed by treatment of the resin with H_2O at 90 °C for 20 min. Subsequently, deprotection of all the 2-cyanoethyl groups on the phosphate groups was carried out by treatment with 10% DBU in CH_3CN for 1 min. The oligomer was released from the resin by use of 0.2 M Et_3N-3HF

in THF and isolated in 31% yield. The modified DNA 12mer was characterized by MALDI-TOF mass spectroscopy.

These results demonstrate that psc is a useful group for protecting the amino groups of cytosine and adenine bases, which can be cleaved under neutral conditions. Since protecting groups that can be cleaved by heating have been reported for the hydroxyl[15,16] and phosphate groups[5,6] of oligonucleotides, our present results could provide the final part of a complete set of thermo-removable protecting groups required for the synthesis of oligonucleotides without using basic conditions.

Molecular switching by modification of an oligonucleotide using the psc group

As described above, the psc group can be easily incorporated into the amino groups of the nucleobases using phenyl isocyanate and can be rapidly cleaved by thermolytic deprotection. These properties might be useful not only for the synthesis of oligonucleotides but also for molecular switching[17–20]; *i.e.*, a reversible change of base recognition can be regulated between the ON and OFF states by chemical reactions, as shown in Fig. 3. Therefore, we tried to synthesize oligonucleotides containing the psc group on solid supports to examine their hybridization with the complementary DNA oligomers (match targets) on a glass surface. The deprotection of the psc groups incorporated into oligonucleotides without anion charges on the internucleotidic phosphate groups was faster than that of those with anion charges on the internucleotidic phosphate groups, as described above. However, oligonucleotides having 2-cyanoethyl groups as the phosphate protecting groups cannot hybridize with the complementary DNA. Therefore, to omit the effect of anion charges, we synthesized an oligonucleotide (13-mer) having methylphosphonate linkages[21] as a DNA probe instead of the one having phosphodiester linkages on 10-μm controlled-pore glass (CPG) particles.[22] Treatment of the particles with methylamine for removal of the acetyl groups on the cytosine bases[23] after chain elongation, according to the general procedure of phosphoramidite chemistry, gave the particles having probe 1. Subsequently, the particles having probe 1 were treated with phenylsulfonyl isocyanate for 1 h in CH_2Cl_2 to synthesize probe 2, containing three consecutive psc groups, as shown in Fig. 4.

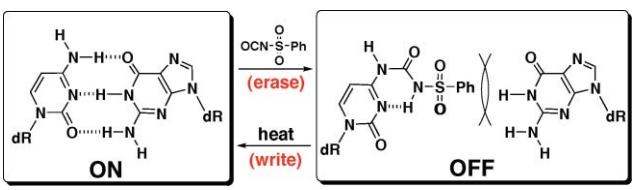


Fig. 3 ON-OFF switching of a G-C base pair by modification of an oligonucleotide using the psc group.

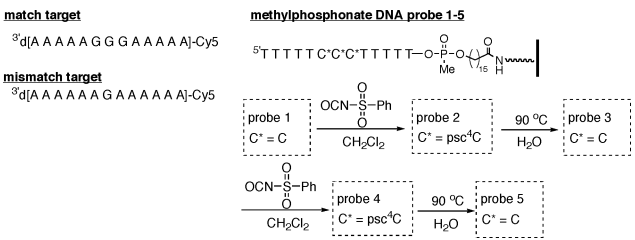


Fig. 4 Sequences of match and mismatch targets, and DNA 1–5.

The particles having probe 3 were prepared by treating probe 2 with H_2O at 90 °C for 1 h. Moreover, particles having probes 4 and 5 were obtained by repeating the same cycle of introduction and deprotection of the psc group. Finally, these CPG particles were immobilized on the surface of a glass slide coated with a polybutadiene adhesive.

We conducted hybridization experiments of probes 1–5 with a Cy3-labeled match target (13-mer). After hybridization of the target using 1.0 mM sodium phosphate buffer for 1 h at room temperature on the glass slide, the glass was treated with 2 × SSC buffer for 10 min at room temperature at the washing step.

It was found by analysis of the fluorescence images that the fluorescence strength of probes 1, 3, and 5 was much stronger than those of probes 2 and 4. A five-fold difference in discrimination between three successive C–G and psc[4]C–G base pairs was observed, as shown in Fig. 5. These results indicate that the hybridization affinity of the oligonucleotide can be regulated by a reversible change of base recognition between C and psc[4]C. Therefore, the ON and OFF states can be optionally obtained by repeating this cycle.

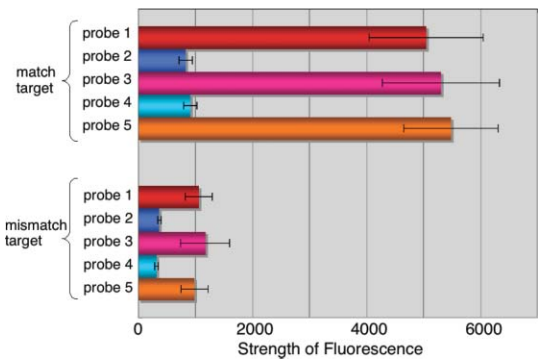


Fig. 5 Fluorescence analysis of the hybridization affinity of probes 1–5 for match target and mismatch target.

Similarly, we also conducted hybridization experiments on probes 1–5 with a Cy3-labeled mismatch target having two mismatches. The strength of fluorescence decreased in all the probes compared with that observed when a match target was used. As a result, if the threshold line is set to about 2000, the state between ON (probes 1, 3, and 5 in the case of the match target) and OFF (probes 2 and 4 in the case of the match target, and probes 1–5 in the case of the mismatch target) can be easily distinguished.

Conclusions

In summary, we determined that *N*-arylcarbamoyl and *N*-(phenylsulfonyl)carbamoyl groups can be effectively introduced onto amino groups of cytosine and adenine, and can be thermolytically removed from the nucleobases. Since the psc group could be rapidly introduced and removed, we succeeded in synthesizing DNA oligomers using psc[4]C and psc[6]A phosphoramidite units. Moreover, we developed a new system for molecular switching by changing base recognition using this high reactivity. If protected DNA probes[3] having ac[4]C,[24] ac[6]az[8]c[7]A,[4] and cm[2]G,[12] which can be hybridized with a complementary

oligonucleotide without removal of the base protection, were used in place of unmodified DNA probes, the sequences of oligonucleotides for reversible molecular switches might not be limited, because these protected bases are inert toward phenylsulfonyl isocyanate. In the future, oligonucleotides may be required to use more stable DNA mimics such as PNA[25] oligomers, because there is a possibility of the gradual decomposition of the methylphosphonate linkages with a number of heating steps. Further studies are now under way in this direction.

Experimental

4-*N*-(*N*-Phenylcarbamoyl)deoxycytidine 2a

Deoxycytidine hydrochloride (246 mg, 1 mmol) was allowed to react with triethylamine (141 μL, 1 mmol) and phenyl isocyanate (108 μL, 1 mmol) in DMF and the mixture was stirred at room temperature for 30 min. After the mixture was evaporated under reduced pressure, the residue was purified by 60 N silica gel chromatography with $CHCl_3$-MeOH to give **2a** (324 mg, 94%).

^{1}H NMR (DMSO) δ 1.99–2.08 (1H, m), 2.23–2.31 (1H, m), 3.56–3.61 (2H, m), 3.83–3.87 (1H, m), 4.18–4.23 (1H, m), 5.04–5.07 (1H, m), 5.27–5.29 (1H,m), 6.13 (1H, t, J = 6.3 Hz), 6.39 (1H, d, J = 7.2 Hz), 7.06 (1H, t, J = 7.4 Hz), 7.30–7.36 (2H, m), 7.47 (1H, d, J = 7.7 Hz), 8.24 (1H, d, J = 7.4 Hz), 10.17 (1H, brs), 11.35 (1H, brs); ^{13}C NMR (DMSO-d_6) δ 40.7, 61.0, 70.0, 86.1, 87.9, 94.7, 119.3, 123.4, 129.0, 138.1, 134.5, 144.0, 151.2, 153.3 162.2. HRMS (ESI) m/z (M + h) calcd for $C_{16}H_{18}N_4O_5^+$ 347.1350, found 347.1355.

4-*N*-[*N*-(2-Nitrophenyl)carbamoyl]deoxycytidine 2b

Deoxycytidine hydrochloride (246 mg, 1 mmol) was allowed to react with triethylamine (141 μL, 1 mmol) and 2-nitrophenyl isocyanate (164 mg, 1 mmol) in DMF and the mixture was stirred at room temperature for 30 min. After the mixture was evaporated under reduced pressure, the residue was purified by 60 N silica gel chromatography with $CHCl_3$-MeOH to give **2b** (352 mg, 90%).

^{1}H NMR (DMSO) δ 1.97–2.08 (1H, m), 2.25–2.34 (1H, m), 3.50–3.64 (2H, m), 3.84–3.89 (1H, m), 4.16–4.21 (1H, m), 5.05 (1H, brs), 5.21 (1H, brs), 6.07 (1H, t, J = 6.3 Hz), 6.32 (1H, d, J = 7.2 Hz)), 7.32 (1H, m), 7.67 (1H, m), 7.91 (1H, m), 8.02 (1H, m), 8.19 (1H, d, J = 7.2 Hz); ^{13}C NMR (DMSO-d_6) δ 40.7, 61.0, 70.0, 86.1, 87.9, 94.6, 124.4, 124.8, 125.2, 132.2, 134.5, 140.1, 144.3, 151.5, 153.1, 161.9. HRMS (ESI) m/z (M + h) calcd for $C_{16}H_{18}N_5O_7^+$ 392.1206, found 392.1210.

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-4-*N*-[*N*-(phenylsulfonyl)carbamoyl]deoxycytidine 2c

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)deoxycytidine (911 mg, 2 mmol) was dissolved in dry CH_2Cl_2 (20 mL). To the solution was added phenylsulfonyl isocyanate (271 mL, 2.0 mmol), and the mixture was stirred at room temperature for 5 min. The mixture was diluted with $CHCl_3$ (20 mL), and the mixture was washed with brine and saturated $NaHCO_3$. The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by C200 silica gel chromatography with hexane-$CHCl_3$ to give **2c** (1.1 g, 87%).

^{1}H NMR ($CDCl_3$) δ 0.08–0.11 (12H, m), 0.90–0.92 (18H, m), 2.07–2.17 (1H, m), 2.59–2.67 (1H, m), 3.76–3.95 (2H, m), 3.95–4.03 (1H, m), 4.34–4.42 (1H, m), 6.26 (1H, dd, J = 5.9 Hz, J = 5.6 Hz), 7.42–7.75 (4H, m), 8.09–8.13 (2H, m), 8.32 (1H, d, J = 7.6 Hz), 11.22 (1H, brs), 12.99 (1H, brs); ^{13}C NMR (DMSO-d_6) δ −5.59, −5.50, −5.0, −4.6, 17.8, 18.2, 25.1, 25.6, 25.8, 42.1, 61.7, 70.2, 77.2, 87.3, 88.1, 97.4, 128.0, 128.1, 128.6, 128.7, 133.7, 139.7, 144.3, 149.7, 156.2, 164.0. HRMS (ESI) m/z (M + h) calcd for $C_{28}H_{47}N_4O_7SSi_2^+$ 639.2704, found 639.2708.

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-6-*N*-(*N*-phenylcarbamoyl)deoxyadenosine 2d

3′,5′-*O*-Bis (*tert*-butyldimethylsilyl)deoxyadenosine (480 mg, 1 mmol) was rendered anhydrous by repeated co-evaporation with dry CH_3CN and finally dissolved in dry CH_2Cl_2(10 mL). To the solution was added phenyl isocyanate (130 μl, 1.2 mmol), and the mixture was stirred at room temperature for 4 h. The mixture was diluted with $CHCl_3$ (50 mL) and washed with brine. The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by C200 silica gel chromatography with hexane-$CHCl_3$ to give **2d** (563 mg, 94%).

^{1}H NMR (270 MHz, $CDCl_3$) δ −0.02–0.00 (12H, m), 0.80–0.81 (18H, m), 2.35–2.39 (1H, m), 2.52–2.57 (1H, m), 3.65–3.81 (2H, m), 3.93 (1H, s), 4.52 (1H, s), 6.39 (1H, dd, J = 6.2 Hz, J = 6.2 Hz), 7.01 (1H, dd, J = 6.5 Hz, J = 7.0 Hz), 7.25 (2H, dd, J = 7.6 Hz, J = 7.6 Hz), 7.53 (2H, d, J = 7.6 Hz), 8.06 (1H, s), 8.24 (1H, s), 8.51, (1H, s), 11.65 (1H, s); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.7, −5.6, −4.9, −4.8, 17.9, 18.2, 25.6, 25.8, 40.5, 62.6, 71.8, 84.3, 87.9, 118.7, 120.1, 120.7, 123.5, 128.5, 128.8, 138.1, 142.4, 150.0, 150.2, 150.5, 151.6; ESI-mass m/z calcd for $C_{29}H_{47}N_6O_4Si_2^+$ 599.3197; found [M + H] 599.3192.

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-6-*N*-[*N*-(2-nitrophenyl) carbamoyl]deoxyadenosine 2e

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)deoxyadenosine (480 mg, 1 mmol) was rendered anhydrous by repeated co-evaporation with dry CH_3CN and finally dissolved in dry CH_2Cl_2(10 mL). To the solution was added 2-nitrophenyl isocyanate (187 mg, 1.2 mmol), and the mixture was stirred at room temperature for 5 h. The mixture was diluted with $CHCl_3$ (50 mL) and washed with brine. The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by C200 silica gel chromatography with hexane-$CHCl_3$ to give **2e** (640 mg, 99%).

^{1}H NMR (270 MHz, $CDCl_3$) δ −0.03–0.00 (12H, m), 0.80–0.81 (18H, m), 2.36–2.42 (1H, m), 2.53–2.60 (1H, m), 3.64–3.82 (2H, m), 3.93–3.94 (1H, m), 4.54 (1H, s), 6.40 (1H, dd, J = 6.2 Hz, J = 6.2 Hz), 7.01 (1H, dd, J = 6.5 Hz, J = 7.0 Hz), 7.07–7.12 (1H, m), 7.54 (1H, dd, J = 7.3 Hz, J = 8.4 Hz), 8.07 (1H, d, J = 8.4 Hz), 8.27–8.31 (2H, m), 8.59, (1H, d, J = 8.4 Hz), 8.67 (1H, s), 13.35 (1H, s); ^{13}C NMR (67.8 MHz, CDCl3) δ −6.1, −5.8, −5.7, −5.4, −5.3, −5.0, −4.9, −4.6, 17.3, 17.6, 17.7, 18.1, 18.2, 25.6, 39.9, 62.5, 71.8, 84.4, 87.7, 120.2, 122.3, 123.5, 125.0, 134.2, 134.3, 138.1, 142.9,

148.9, 150.3, 151.8; ESI-mass m/z calcd for $C_{29}H_{45}N_7NaO_6Si_2$+ 666.2862; found [M + Na] 666.2858.

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-6-*N*-[*N*-(phenylsulfonyl) carbamoyl]deoxyadenosine 2f

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)deoxyadenosine (480 mg, 1 mmol) was rendered anhydrous by repeated co-evaporation with dry CH_3CN and finally dissolved in dry CH_2Cl_2(10 mL). To the solution was added phenylsulfonyl isocyanate (160 μL, 1.2 mmol), and the mixture was stirred at room temperature for 0.5 h. The mixture was diluted with $CHCl_3$ (50 mL) and washed with brine. The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by C200 silica gel chromatography with hexane-$CHCl_3$ to give **2f** (655 mg, 99%).

^{1}H NMR (270 MHz, $CDCl_3$) δ −0.04–0.00 (12H, m), 0.80–0.81 (18H, m), 2.34–2.41 (1H, m), 2.49–2.56 (1H, m), 3.63–3.80 (2H, m), 3.91–3.95 (1H, m), 4.48–4.53 (1H, m), 6.36 (1H, dd, $J = 6.2$ Hz, $J = 6.2$ Hz), 7.41–7.55 (3H, m), 8.04–8.08 (2H, m), 8.28 (1H, s), 8.43 (1H, s), 8.54 (1H, s), 12.99 (1H, s); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.5, −4.8, −4.7, 18.0, 18.4, 25.7, 25.9, 41.3, 62.6, 71.7, 84.9, 88.2, 120.8, 128.5, 128.9, 133.7, 139.0, 142.4, 148.5, 148.9, 150.5, 150.6; ESI-mass m/z calcd for $C_{29}H_{47}N_6O_6SSi_2^+$ 663.2811; found [M + H] 663.2812.

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-2-*N*-[*N*-(phenylsulfonyl) carbamoyl]-6-*O*-(2,4,6-triisopropylbenzenesulfonyl) deoxyguanosine 2g

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-6-*O*-(2,4,6-triisopropylbenzenesulfonyl)deoxyguanosine (3.2 g, 4.2 mmol) was rendered anhydrous by repeated co-evaporation with dry CH_3CN and finally dissolved in dry CH_2Cl_2(40 ml). To the solution was added phenylsulfonyl isocyanate (680 μl, 5 mmol), and the mixture was stirred at room temperature for 1.5 h. The mixture was diluted with $CHCl_3$ (100 mL) and washed with brine. The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by C200 silica gel chromatography with hexane-$CHCl_3$ to give **2g** (3.8 g, 95%).

^{1}H NMR (270 MHz, $CDCl_3$) δ 0.01–0.04 (12H, m), 0.78–0.88 (18H, m), 1.18–1.23 (18H, m), 2.38–2.48 (2H, m), 2.81–2.88 (1H, m), 3.68–3.83 (2H, m), 3.96–3.99 (1H, m), 4.10–4.20 (2H, m), 4.48–4.53 (1H, m), 6.28 (1H, dd, $J = 5.9$ Hz, $J = 6.2$ Hz), 7.16–7.21 (5H, m), 7.45–7.57 (3H, m), 8.06–8.09 (2H, m), 8.22 (1H, s), 11.69 (1H, s); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.5, −5.4, −4.9, −4.7, 17.9, 18.4, 23.5, 24.6, 25.7, 25.9, 29.9, 34.3, 41.8, 53.5, 62.5, 71.4, 84.9, 88.2, 118.9, 124.0, 128.3, 128.5, 128.8, 128.9, 130.2, 133.6, 139.1, 142.4, 148.4, 150.5, 151.2, 155.1; ESI-mass m/z calcd for $C_{44}H_{69}N_6O_9S_2Si_2^+$ 945.4101; found [M + H] 945.4100.

6-*N*-(*N*-Phenylcarbamoyl)deoxyadenosine 2h

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-6-*N*-[(*N*-phenylcarbamoyl)] deoxyadenosine (560 mg, 0.94 mmol) was rendered anhydrous by repeated co-evaporation with dry CH_3CN and finally dissolved in dry THF (9 mL). To the solution was added tetrabutylammonium fluoride (730 mg, 2.8 mmol), and the mixture was stirred at room temperature for 3 h. The mixture was diluted with $CHCl_3$ (50 mL) and washed with brine. The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by C200 silica gel chromatography with $CHCl_3$–MeOH to give **2h** (180 mg, 52%).

^{1}H NMR (270 MHz, DMSO-d_6) δ 2.29–2.38 (1H, m), 2.70–2.80 (1H, m), 3.50–3.65 (2H, m), 3.87–3.89 (1H, m), 4.44 (1H, s), 5.00–5.02 (1H, m), 5.35–5.37 (1H, m), 6.44 (1H, dd, $J = 7.0$ Hz, $J = 6.5$ Hz), 7.04–7.10 (1H, m), 7.32–7.40 (2H, m), 7.60–7.63 (2H, m), 8.67 (1H, s), 8.68 (1H, s), 9.26, (1H, s), 11.78 (1H, s); ^{13}C NMR (67.8 MHz, DMSO-d_6) δ 61.6, 70.6, 83.8, 88.0, 119.4, 120.6, 123.2, 129.0, 138.5, 142.3, 149.9, 150.3, 150.7, 151.0; ESI-mass m/z calcd for $C_{17}H_{19}N_6O_4^+$ 371.1462; found [M + H] 371.1465.

6-*N*-[*N*-(2-Nitrophenyl)carbamoyl]deoxyadenosine 2i

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-6-*N*-[*N*-(2-nitrophenyl)carbamoyl]deoxyadenosine (640 mg, 1 mmol) was rendered anhydrous by repeated co-evaporation with dry CH_3CN and finally dissolved in dry THF (10 mL). To the solution was added tetrabutylammonium fluoride (780 mg, 3 mmol), and the mixture was stirred at room temperature for 2 h. The mixture was diluted with $CHCl_3$ (50 mL) and washed with brine. The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by C200 silica gel chromatography with $CHCl_3$–MeOH to give **2i** (280 mg, 67%).

^{1}H NMR (270 MHz, $CDCl_3$) δ 2.34–2.39 (1H, m), 2.72–2.79 (1H, m), 3.56–3.61 (2H, m), 3.90 (1H, s), 4.45 (1H, s), 5.04 (1H, s), 5.39 (1H, s), 6.45 (1H, dd, $J = 6.5$ Hz, $J = 6.2$ Hz), 7.26 (1H, dd, $J = 7.8$ Hz, $J = 7.6$ Hz), 7.71 (1H,, dd, $J = 8.1$ Hz, $J = 7.6$ Hz), 8.08 (1H, d, $J = 8.1$ Hz), 8.45 (1H, d, $J = 8.4$ Hz), 8.62 (1H, s), 8.69 (1H, s), 10.59 (1H, s), 13.12 (1H, s); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 61.6, 70.6, 83.6, 88.1, 120.5, 123.5, 125.4, 133.5, 134.9, 138.9, 142.6, 149.3, 150.1, 150.6, 151.3; ESI-mass m/z calcd for $C_{17}H_{18}N_7O_6^+$ 416.1313; found [M + H] 416.1318.

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-2-*N*-[*N*-(phenylsulfonyl) carbamoyl]deoxyguanosine 2j

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-2-*N*-[*N*-(phenylsulfonyl)carbamoyl]-6-*O*-(2,4,6-triisopropylbenzenesulfonyl)deoxyguanosine (1.9 g, 2 mmol) was rendered anhydrous by repeated co-evaporation with dry CH_3CN and finally dissolved in dry CH_3CN (20 mL). To the solution was added *syn*-*o*-nitrobenzaldoxime (1.0 g, 6 mmol) and 1,1,3,3-tetramethylguanidine (750 μl, 6 mmol), and the mixture was stirred at room temperature for 1 h. The mixture was diluted with $CHCl_3$ (100 mL), and the mixture was washed with brine. The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by C200 silica gel chromatography with $CHCl_3$–MeOH to give **2j** (1.6 g, quant.).

^{1}H NMR (270 MHz, DMSO-d_6) δ −0.08–0.05 (12H, m), 0.64–0.88 (18H, m), 2.14–2.16 (1H, m), 2.57–2.78 (1H, m), 3.51–3.64 (2H, m), 3.71 (1H, s), 4.40 (1H, s), 6.03 (1H, dd, $J = 7.0$ Hz, $J = 6.5$ Hz), 7.33–7.36 (3H, m), 7.66–8.74 (3H, m), 7.91 (1H, s), 9.69 (1H, s), 12.81 (1H, s); ^{13}C NMR (67.8 MHz, DMSO-d_6) δ −5.5, −4.9, −4.8, 17.7, 18.0, 24.8, 25.7, 25.8, 62.8, 72.2, 79.2, 82.4, 87.2, 118.6, 126.8, 127.9, 130.3, 136.3, 145.1, 149.6, 150.4, 155.3, 160.9; ESI-mass m/z calcd for $C_{29}H_{47}N_6O_7SSi_2^+$ 679.2760; found [M + H] 679.2766.

General protocol for the thermolytical deprotection of *N*-carbamoylnucleoside

After *N*-carbamoylnucleoside (8.4 μmol) was dissolved in D_2O-DMSO-d_6 (1:5, v/v, 1.2 mL), the reaction mixture was heated at 80 °C. The rate of deprotection was analyzed by ^{1}H NMR.

5′-*O*-(4,4′-Dimethoxytrityl)-6-*N*-[*N*-(phenylsulfonyl) carbamoyl]deoxycytidine-3′-*O*-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite 3a

5′-*O*-(4,4′-Dimethoxytrityl)-deoxycytidine-3′-*O*-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (730 mg, 1 mmol) was rendered anhydrous by repeated co-evaporation with dry CH_3CN and finally dissolved in dry CH_2Cl_2(10 mL). To the solution was added phenylsulfonyl isocyanate (160 μL, 1.2 mmol), and the mixture was stirred at room temperature for 2 h. The mixture was diluted with $CHCl_3$ (50 mL) and washed with brine. The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by C200 silica gel chromatography with hexane-$CHCl_3$ to give **3a** (695 mg, 76%).

^{1}H NMR (270 MHz, $CDCl_3$) δ 1.06–1.17 (14H, m), 2.28–2.33 (1H, m), 2.43–2.48 (1H, m), 2.62–2.64 (1H, m), 2.81 (1H, br), 3.34–3.84 (14H, m), 4.21 (1H, s), 4.61 (1H, s), 6.24–6.26 (1H, m), 6.81–6.84 (4H, m), 7.24–7.27 (13H, m), 7.35–7.37 (2H, m), 7.46–7.57 (3H, m), 8.04–8.07 (2H, m), 8.17–8.27 (1H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 8.4, 20.1, 20.2, 20.4, 24.4, 24.5, 24.6, 40.7, 41.1, 43.1, 43.3, 45.8, 55.2, 58.0, 58.2, 58.3, 58.5, 61.9, 62.2, 71.4, 71.7, 72.2, 72.5, 85.9, 86.8, 87.4, 97.6, 113.2, 117.4, 117.9, 127.1, 127.9, 128.0, 128.1, 128.6, 129.9, 130.0, 133.3, 135.1, 135.2, 143.9, 156.2, 158.6, 158.6, 163.9; ^{31}P NMR (109.4 MHz, $CDCl_3$) δ 149.71, 149.78; ESI-mass m/z calcd for $C_{46}H_{54}N_6O_{10}PS^+$ 913.3354; found [M + H] 913.3352.

5′-*O*-(4,4′-Dimethoxytrityl)-6-*N*-[*N*-(phenylsulfonyl) carbamoyl]deoxyadenosine 3′-*O*-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite 3b

5′-*O*-(4,4′-Dimethoxytrityl)deoxyadenosine 3′-*O*-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (720 mg, 1 mmol) was rendered anhydrous by repeated co-evaporation with dry CH_3CN and finally dissolved in dry CH_2Cl_2(10 mL). To the solution was added phenylsulfonyl isocyanate (160 μL, 1.2 mmol), and the mixture was stirred at room temperature for 2 h. The mixture was diluted with $CHCl_3$ (50 mL) and washed with brine. The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by C200 silica gel chromatography with hexane-$CHCl_3$ to give **3b** (625 mg, 69%).

^{1}H NMR (270 MHz, $CDCl_3$) δ 1.10–1.24 (13H, m), 2.43–2.48 (1H, m), 2.58–2.71 (2H, m), 2.88–2.95 (1H, m), 3.35 (1H, s), 3.54–3.84 (11H, m), 4.29 (1H, s), 4.77 (1H, s), 6.42 (1H, s), 6.72–6.75 (4H, m), 7.17–7.24 (11H, m), 7.33–7.35 (2H, m), 7.49–7.60 (3H, m), 8.13–8.20 (3H, m), 8.53 (1H, s), 13.05 (1H, s); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 8.3, 20.0, 20.1, 20.2, 20.3, 24.3, 24.4, 24.5, 38.4, 43.0, 43.2, 55.0, 57.9, 58.0, 58.2, 58.3, 63.2, 63.3, 73.1, 73.3, 73.7, 73.9, 85.1, 85.7, 85.8, 86.0, 86.1, 112.8, 117.4, 117.6, 120.9, 126.6, 127.6, 127.9, 128.2, 128.7, 129.8, 129.9, 133.4, 135.4, 135.5, 139.0, 143.8, 144.4, 148.7, 150.1, 150.5, 150.6, 158.2, 158.3; ^{31}P NMR (109.4 MHz, $CDCl_3$) δ 149.47, 149.49; ESI-mass m/z calcd for $C_{47}H_{54}N_8O_9PS^+$ 937.3467; found [M + H] 937.3469.

Synthesis of oligonucleotides 4a and 4b

A thymidine-loaded CPG resin (1 μmol, 34 μmol/g, succinate linker) was used. The elongation cycle of oligodeoxyribonucleotides **4a** and **4b** by the use of an ABI 392 DNA synthesizer was carried out according to a standard procedure. (Activator: 1*H*-tetrazole, oxidizer: 0.02 M I_2, capping reagent; Ac_2O.) After chain elongation, the oligomers were released from the resins by treatment with 28% NH_4OH for 1 h. The polymer supports were removed by filtration and washed with H_2O (1 mL × 3). The filtrate was evaporated and purified by anion-exchange HPLC.

TTTTTT-psc^{4}C-TTTTTT **4a**; MALDI-TOF mass calcd for $C_{136}H_{175}N_{28}O_{91}P_{12}S^+$ 4061.7; found [M + H] 4058.7

TTTTTT-psc^{6}A-TTTTTT **4b**; MALDI-TOF mass calcd for $C_{137}H_{175}N_{30}O_{90}P_{12}S^+$ 4085.8; found [M + H] 4082.9

Synthesis of oligonucleotides 4c–f

A thymidine-loaded CPG resin (1 μmol, 34 μmol/g, succinate linker) was used. The elongation cycle of oligodeoxynucleotides **4c-f** by the use of an ABI 392 DNA synthesizer was carried out according to the standard procedure. (Activator: 1*H*-tetrazole, oxidizer: 0.02 M I_2, capping reagent; Ac_2O.) After chain elongation, the oligomers **4e** and **4f** immobilized on resins were obtained.

4e: TTTTTT-psc^{4}C-TTTTTT-CPG (with 2-cyanoethyl groups)

4f: TTTTTT-psc^{6}A-TTTTTT-CPG (with 2-cyanoethyl groups)

Subsequently, the resins were treated with 10% DBU in pyridine-BSA (*N,O*-bistrimethylsilylacetoamide) for 10 min to deprotect the 2-cyanoethyl groups. The polymer supports were washed with CH_3CN and dried under reduced pressure to obtain the oligomers **4c** and **4d** immobilized on CPG resin.

4c: TTTTTT-psc^{4}C-TTTTTT-CPG (without 2-cyanoethyl groups)

4d: TTTTTT-psc^{6}A-TTTTTT-CPG (without 2-cyanoethyl groups)

Synthesis of oligonucleotide 6

A thymidine-loaded CPG resin (1 μmol, 34 μmol/g, succinate linker) was used. The elongation cycle of oligodeoxynucleotide **6** was carried out by the use of an ABI 392 DNA synthesizer in DNA synthesis without capping steps. (Activator: 1*H*-tetrazole, oxidizer: 0.02 M I_2.) After chain elongation, the resin was heated at 90 °C in H_2O (1 mL) for 20 min to deprotect the psc groups. Subsequently, the oligomers were released from the resins by treatment with 28% NH_4OH for 1 h. The polymer supports were removed by filtration and washed with H_2O (1 mL × 3). The filtrate was evaporated and purified by anion-exchange HPLC to give the oligonucleotide in 25% yield.

d[GATACATTGACCT] **6**; MALDI-TOF mass calcd for $C_{127}H_{162}N_{47}O_{76}P_{12}^+$ 3932.7; found [M + H] 3927.7

Synthesis of oligonucleotide 7

A thymidine-loaded HCP (1 μmol, 22 μmol/g, succinate linker) having a silyl-type linker was used. The elongation cycle of oligodeoxyribonucleotide **6** by the use of an ABI 392 DNA

synthesizer was carried out according to the standard procedure. (Activator: 1*H*-tetrazole, oxidizer: 0.02 M I_2, capping reagent; Ac_2O.) After chain elongation, the resin was heated at 90 °C in H_2O (1 mL) for 20 min to remove the psc groups. Subsequently, the resin was treated with 10% DBU in CH_3CN for 1 min. The oligomer was released from the resin by treatment with 0.2 M Et_3N-3HF in THF (500 μL) for 4 h. The polymer supports were removed by filtration and washed with H_2O (1 mL × 3). The filtrate was evaporated and purified by anion-exchange HPLC to give the oligonucleotide in 31% yield.

d[ATACATT-*i*bu^{2}G-ACCT] **7**; MALDI-TOF mass calcd for $C_{121}H_{156}N_{42}O_{71}P_{11}^+$ 3673.7; gound [M + H] 3673.8

Synthesis of probes 1–5 on glass slide

The probes on 10 μm CPG particles (15.0 μmol/g, 61 pmol/cm^2, 16-hydroxyhexadodecanoyl linker) were synthesized in a Gene World H-8 synthesizer. After chain elongation, the acetyl groups of the oligonucleotides were deprotected by treatment of the CPG particles (60 mg) with excess 2 M methylamine/THF (1 mL) for 2 h to give the particles containing probe 1. Subsequently the particles having probe 1 (50 mg) were treated with phenylsulfonyl isocyanate for 1 h in CH_2Cl_2 (1 mL) to synthesize probe 2 containing three continuous psc groups. The particles having probe 3 were prepared by treatment of the particles containing probe 2 (40 mg) with H_2O (1 mL) at 90 °C for 1 h. Moreover, the particles having probe 4 were obtained by treatment of the particles (30 mg) with phenylsulfonyl isocyanate for 1 h in CH_2Cl_2 (1 mL). Finally, the particles having probe 5 were prepared by treatment of the particle containing probe 4 (20 mg) with H_2O (1 mL) at 90 °C for 1 h.

After each particle (10 mg) was suspended in triethylene glycol (20 mL), the mixture was spotted on a glass slide coated with polybutadiene adhesive by using Shotmaster (Musashi Engineering Incorporated). The glass slide was dried under reduced pressure at 50 °C for 1 h to immobilize the particles.

Fluorescence analysis of the hybridization affinity of probes 1–5 toward match target and mismatch target

A glass slide plate having probes 1–5 was added to a 1.0 μM solution of the target oligoDNA having a Cy3 residue at the 5′ position in 100 mM sodium phosphate buffer (20 μL, 1M NaCl, pH 7.0). The mixture was incubated at room temperature for 1 h. Next, the glass plate was washed with 5 × SSC buffer (pH 7.0) at room temperature for 10 min. After drying of the glass plate, the fluorescence strength of the plate was measured by a fluorescence scanner. The value of the fluorescence was given as an average of the data from 3 or 4 spots.

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