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Synthesis and Hybridization Properties of Oligonucleotides Incorporating Bi- and Tricyclic Cytosine Derivatives

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DNA oligomers with cytosine bases (C^{pyr}, C^{tpp}, and C^{pnx}) with bi- and tricyclic ring structures were synthesized. Among the modified bases, C^{pyr} was found to enhance the duplex stability retaining the base recognition ability. C^{tpp} was effective in neither hybridization nor base recognition. C^{pnx} could form more strongly a matched base pair with guanine but lost more significantly base recognition than C^{pyr}.

In nucleic acid chemistry, a large number of artificial oligonucleotides[1] with various functional groups have been developed as useful DNA or RNA probes for gene therapies,[2] the exhaustive analysis of gene expression,[3] the detection of single nucleotide polymorphisms (SNPs),[4] and nanotechnology.[5] These probes should have strong hybridization and high base recognition to bind to target molecules efficiently and accurately. In addition, these probes should be easily synthesized for application to various fields as new tools.

Recently, we have proposed a new strategy called "protected DNA probe (PDP) method"[6] in which appropriately protected bases can bind selectively to the complementary bases. PDPs containing 4-*N*-acetylcytosine (ac^4C)[7] and 6-*N*-acetyl-8-aza-7-deazaadenine exhibited higher hybridization affinity for ssDNA and ssRNA than the corresponding unmodified DNA probes and similar base recognition abilities. Moreover, we have demonstrated that PDPs attached to CPG flat discs could be easily synthesized according to the conventional phosphoramidite approach without time-consuming ammonia treatment and could be used as new tools allowing high hybridization affinity for target DNA without decrease in base discrimination. However, the application of PDP is limited because the acyl groups used for PDPs are unstable under basic conditions. In this paper, we focused on base-stable cytosine derivatives with bi- and tricyclic structures in the place of ac^4C to overcome this serious problem.

In our previous study,[8] a bicyclic 4-*N*-carbamoyldeoxycytidine derivative (C^{hpp}) geometrically locked was synthesized as a new fluorescent material, as shown in Figure 1. The carbamoyl group of C^{hpp} was very stable under basic conditions and did not interfere with the formation of a base pair with G like the acetyl group of ac^4C that is oriented to the 5-vinyl proton via a unique intramolecular hydrogen bond. However, C^{hpp} proved to form not only a matched base pair with G but also a stable mismatched base pair with A, as shown in Figure 2. To destabilize this mismatched base pair, our interest was focused on a bicyclic base C^{tpp} (Figure 1) that could weaken the base pair formation with adenine by an unfavorable hydrogen bond between the thiocarbonyl group and the amino group, as depicted in Figure 2. We previously reported that this modified base was nonfluorescent but the other properties are unknown. In this study, two bi- and tricyclic cytosine bases (C^{pyr} and C^{pnx})[9,10] were also tested as PDP bases. It was expected that these modified bases could not form the mismatched base pairs since the carbonyl group on the 4-amino group of cytosine was eliminated. In previous studies, oligonucleotides containing these modified cytosine bases have been reported as fluorescent probes,[9a–9e] to enhance the hyblidization ability,[9f] or as triplex forming oligonucleotides[9g] but their base discrimination abilities in oligonucleoties have not been disclosed.

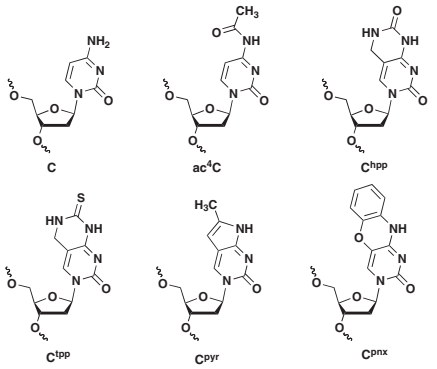


Figure 1. Structures of bi- and tricyclic protected C analogs.

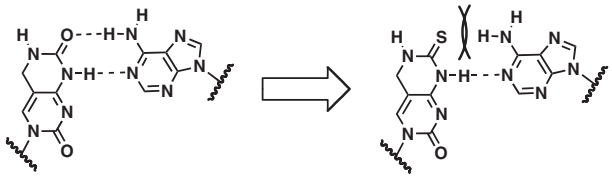
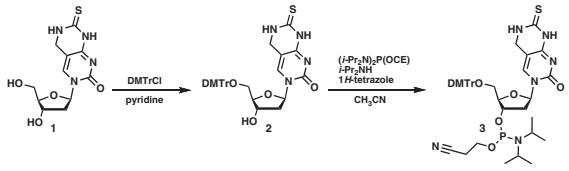


Figure 2. Base pairs of C^{htp} and C^{ttp} with adenine base.



Scheme 1. Synthesis of C^{ttp} phosphoramidite unit **3**.

The C^{tpp} phosphoramidite unit **3** was synthesized by tritylation of deoxynucleoside **1**[11] followed by 3′-phosphitylation, as shown in Scheme 1. The C^{pnx} phosphoramidite unit was prepared according to a method reported by Matteucci et al.[12] The phosphoramidite unit[13] of C^{pyr} was commercially available. Thus, we synthesized modified DNA oligomers **1**–**3** containing C^{tpp}, C^{pyr}, and C^{pnx} by using these phosphoramidite units in the standard procedure used for DNA synthesis. Additional protecting groups were not required for their nucleobases because they did not react with activated phosphoramidite intermediates and a

Table 1. T_m values of DNA 13mer duplexes containing C, C^{tpp}, pyrrolo-C, and phenoxazine

unmodified DNA **5'-d(T T C T T C C C T T C T T)**
modifield DNA **1-3** **5'-d(T T C T T C C* C T T C T T)**
complementary DNA **4-7** **d(A T G G A T X T A G G T A)-5'**

X	Unmodified DNA		Modified DNA **1** C* = C^{tpp}		Modified DNA **2** C* = C^{pyr}		Modified DNA **3** C* = C^{pnx}	
	T_m/°C[a]	ΔT_m/°C[b]	T_m/°C[a]	ΔT_m/°C[b]	T_m/°C[a]	ΔT_m/°C[b]	T_m/°C[a]	ΔT_m/°C[b]
G (**4**)	49.5	—	47.4	—	53.7	—	55.8	—
A (**5**)	29.7	−19.8	34.4	−13.0	32.9	−20.8	41.8	−14.0
T (**6**)	30.7	−18.8	30.6	−16.8	36.2	−17.5	40.9	−14.9
C (**7**)	25.7	−23.8	23.5	−23.9	23.9	−29.8	32.4	−23.4

[a]The T_m values are accurate within ±0.5 °C. The T_m measurements were carried out in a buffer containing 150 mM sodium phosphate (pH 7.0), 100 mM NaCl, 0.1 mM EDTA, and 2 μM duplex. [b]ΔT_m is the difference in the T_m value between the duplex having a guanine and those having other bases at the X position.

capping reagent of acetic anhydride. It was also found that the C^{tpp}, C^{pyr}, and C^{pnx} residues incorporated into oligonucleotides were stable in concd NH_4OH. The modified DNA oligomers **1**–**3** were isolated by anion-exchanged HPLC and characterized by MALDI-TOF mass spectrometry.[14]

The T_m experiments of the duplexes formed between these modified DNA oligomers **1**–**3** and the DNA oligomers **4**–**7** having the complementary or single mismatch sequences were carried out. These results are summarized as in Table 1. Unexpectedly, modified DNA oligomer **1** showed lower binding affinity for the complementary strand **4** than the unmodified oligomer [T_m: 47.4 °C (C^{ttp}) vs. 49.5 °C (C)]. On the other hand, the modified DNA oligomers **2** and **3** exhibited higher T_m values by 4.2 and 6.3 °C than the unmodified oligomer [T_m: 53.7 °C (C^{pyr}) or 55.8 °C (C^{pnx}) vs. 49.5 °C (C)] probably because of increasing stacking effects of the bi- or tricyclic nucleobases.

Moreover, it was found that the modified DNA oligomers **1** and **3** showed significantly lower base discrimination than the unmodified DNA [ΔT_m: −13.0 °C (C^{ttp}), −14.0 °C (C^{pyr}) vs. −18.8 °C (C)]. These nucleobases, particularly the latter, stabilized the mismatched base pairs with A compared with the unmodified cytosine base though their base recognition was improved compared with C^{hpp}. However, the marked stabilization of the mismatched base pair with A was not observed in the case of DNA oligomer **2** though base recognition of DNA oligomer **2** to thymine was slightly lower than that of the unmodified DNA oligomer [ΔT_m: −17.5 °C (C^{pyr}) vs. −18.8 °C (C)].

In conclusion, the results above mentioned suggest that C^{pyr} might be useful for our PDP strategy in the place of ac^4C because of its high chemical stability and DNA duplex-forming ability. The hybrdization ability of oligomer **2** with C^{pyr} was superior to that having C without significant decrease of base recognition. These results encouraged us to develop chemically stable PDPs containing C^{pyr}. Further studies are now under way in this direction.

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14 Oligomer **1**: $[M + H]^+$ calcd 3889.6, found 3890.0. Oligomer **2**: $[M + H]^+$ calcd 3856.5, found 3851.6. Oligomer **3**: $[M + H]^+$ calcd 3905.3, found 3908.6.

