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Synthesis and Properties of a New Fluorescent Bicyclic 4-*N*-Carbamoyldeoxycytidine Derivative

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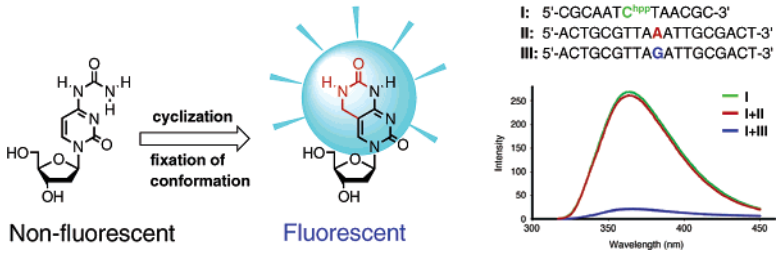
ABSTRACT



A bicyclic 4-*N*-carbamoyldeoxycytidine derivative (1, dC^{hpp}) geometrically locked was synthesized as a new fluorescent nucleobase. The hybridization properties of oligodeoxynucleotides containing dC^{hpp} were investigated by use of T_m analysis. It was found that dC^{hpp} forms stable base pairs not only with the complementary guanine base, but also with the adenine base. Interestingly, the fluorescence of dC^{hpp} was suppressed only when a dC^{hpp}-dG base pair was formed.

A variety of base-modified nucleosides have been synthesized to introduce new attractive functions into oligonucleotides. Recently, much attention has been paid to modified nucleosides having fluorescent nucleobases that are sensitive to the proximal environment as easily detectable probes for DNA hybridization and SNP detection. Most of these fluorescent nucleobases have been designed on the basis of the following requirements: (a) conjugation of fluorescent molecules such as pyrene or fluorene to pyrimidine and deazapurine bases[1] and (b) expansion of the conjugation

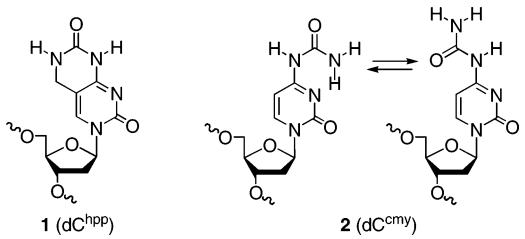


Figure 1. Chemical structures of dC^{hpp} (**1**) and dC^{cmy} (**2**) nucleosides.

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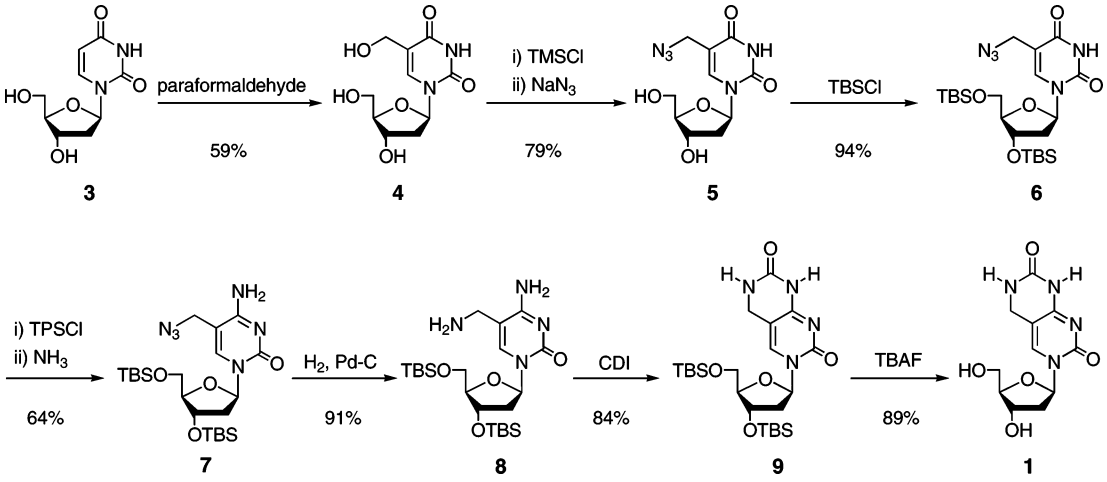
Scheme 1. Synthesis of Bicyclic 4-*N*-Carbamoyldeoxycytidine (**1**, dC^{hpp})



system of natural nucleobases.[2] Here, we describe a new fluorescent nucleoside dC^{hpp} (**1**) (Figure 1).

Recently, we have reported the synthesis and properties of oligodeoxynucleotides containing 4-*N*-carbamoyldeoxycytidine derivatives.[3] It was revealed that the geometry of the carbamoyl group of 4-*N*-carbamoyldeoxycytidine (C^{cmy}) derivatives changes depending on the polarity of the solvent. The NMR analysis of dC^{cmy} in D_2O suggested that the carbamoyl group formed an intramolecular hydrogen bond with the cytosine ring nitrogen atom so that formation of a Watson−Crick base pair with the complementary guanine base was inhibited. However, the T_m analysis showed that carbamoylation of the 4-amino group of deoxycytidine allowed the base pairing with a guanine base following the conformational change of the carbamoyl group. The stability of DNA duplexes containing a dC^{cmy}−dG base pair was somewhat low ($\Delta T_m = -0.6$ °C) compared with that of the unmodified DNA duplex. On the other hand, the acylation or alkoxycarbonylation[4] of the 4-amino group of deoxycytidine did not affect significantly the base pairing with the guanine. These results imply that destabilization of DNA duplexes containing dC^{cmy} is due to the energy loss resulting from the necessity of conformational change of the 4-*N*-carbamoyl group. In this paper, we report the synthesis and properties of a new bicyclic deoxycytidine derivative that has a conformationally locked 4-*N*-carbamoyl group. The carbamoyl group of dC^{hpp} and the 5-position of the cytosine ring are bridged via a methylene linker so that the modified group does not inhibit the formation of a Watson−Crick base pair.

The synthesis of dC^{hpp} (**1**) is outlined in Scheme 1. Treatment of deoxyuridine (**3**) with paraformaldehyde gave the 5-hydroxymethyldeoxyuridine (**4**). The reaction of **4** with chlorotrimethylsilane in dioxane followed by treatment with sodium azide in DMF gave 5-azidomethyldeoxyuridine (**5**).[5] The usual silylation of **5** with TBSCl gave the 3′,5′-*O*-protected compound **6**. The reaction of **6** with 2,4,6-triisopropylbenzenesulfonyl chloride followed by ammonolysis gave 5-azidomethyldeoxycytidine derivative (**7**).[6] This strategy proved to be much superior to the well-known alternative method by use of $POCl_3$/1,2,4-triazoles[7] that gave a complex mixture. The Pd/C-catalyzed hydrogenation of **7** gave the 5-aminomethyldeoxycytidine **8** in 91% yield. Intramolecular cyclization of **8** with 1,1′-carbonyldiimidazole afforded the bicyclic derivative **9** in 84% yield. The silyl groups of **9** were removed by treatment with tetrabutylammonium fluoride hydrate to give dC^{hpp} (**1**) in 89% yield.

The UV absorption spectra of dC, dC^{cmy}, and dC^{hpp} nucleosides in phosphate buffer are shown in Figure 2A. Different from dC and dC^{cmy}, the spectrum of dC^{hpp} exhibited an interesting absorption band at 300 nm. Furthermore, dC^{hpp} exhibited an emission spectrum (λ_{max} = 360 nm) when excited at 300 nm, as shown in Figure 2B.

The quantum yield of dC^{hpp} in 10 mM sodium phosphate (pH 7.0) was caluculated to be 0.12. The absorption spectra of 4-*N*-(*N*-methylcarbamoyl)deoxycytidine derivatives which have acyclic *N*-methylcarbamoyl groups at the cytosine amino group are similar to those of dC^{cmy}, but these acyclic derivatives did not exhibit any fluorescent properties (data not shown).

The synthesis of the phosphoramidite **14** is shown in Scheme 2. For the convenient synthesis of **14**, the DMTr group was first introduced to the 5′-OH group of compound **5**. To avoid the deprotection of the DMTr group during

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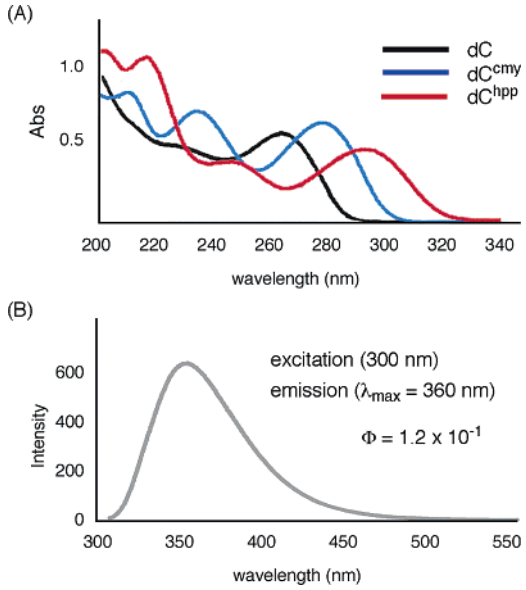


Figure 2. (A) UV absorption spectra of dC, dC^{cmy}, and dC^{hpp}: 80 μM nucleoside, 10 mM sodium phosphate, pH 7.0. (B) Fluorescent spectra of dC^{hpp}: 5 μM nucleoside, 10 mM sodium phosphate, pH 7.0.

reduction of the azide group with Pd−C, ammonium formate was used as a hydrogen source for the catalytic hydrogenation. Oligonucleotides containing dC^{hpp} were synthesized by use of a DNA/RNA synthesizer according to the standard protocol.

The thermal stability of DNA duplexes containing dC^{hpp} was investigated in sodium phosphate buffer (pH 7.0) containing 1.0 M NaCl. As shown in Table 1, the T_m value of the duplex containing dC^{hpp} (entry 2, 58.2 °C) was slightly higher by 1.1 °C than that of the control unmodified duplex (entry 1, 57.1 °C). We have recently reported that modification of the cytosine amino group with an acyclic *N*-methylcarbamoyl group destabilized the duplex stability (ΔT_m = −1.5 °C).[3] These results indicate that the conformational fixation of the 4-*N*-carbamoyl group resulted in direct binding to the guanine base without energy loss.

Table 1. T_m Values[a] for DNA 13mer Duplexes Containing dC^{hpp}

5′-CGCAATXTAACGC-3′
3′-GCGTTAYATTGCG-5′

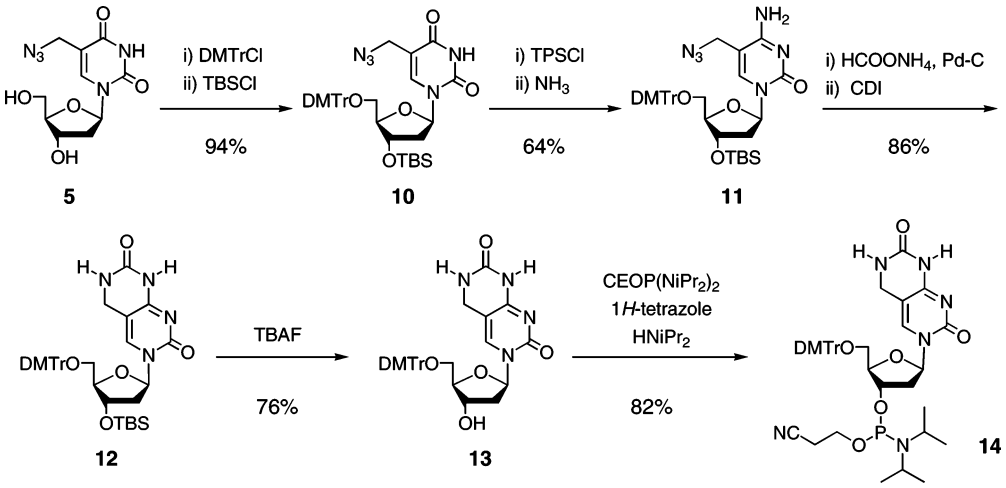
entry	X	Y	T_m[b] (°C)	ΔT_m[c] (°C)
1	C	G	57.1	
2	C^{hpp}	G	58.2	+1.1
3	C	A	40.6	
4	C^{hpp}	A	50.8	+10.2
5	C	C	30.6	
6	C^{hpp}	C	30.4	−0.2
7	C	T	41.4	
8	C^{hpp}	T	44.3	+2.9

[a] 2 μM duplex, 100 mM NaCl, 0.1 mM EDTA, 50 mM sodium phosphate buffer, pH 7.0. [b] The T_m values are accurate within ±0.5 °C. [c] ΔT_m is the difference in the T_m value between the duplex having a modified base and that having a natural base.

The T_m values of DNA duplexes having mismatched base pairs (Y = A, C, and T) are also summarized in Table 1. Interestingly, modification of the cytosine amino group with the bridged carbamoyl group stabilized markedly the C−A mismatched base pair. The T_m value of the duplex containing C^{hpp}−A was significantly higher (ΔT_m = +10.2 °C) than that of the duplex containing a C−A mismatch base pair (entry 3, 40.6 °C).

dC^{hpp} can form a base pair with an adenine base with the geometry of a reverse-Watson−Crick base pair. However, it is not clear whether dC^{hpp} changes the tautomerism of cytosine from the amino form to the imino form in order to form a Watson−Crick base pair with the adenine base. Further experiments are needed to determine the tautomerism of the C^{hpp}−A base pair in DNA duplexes. To investigate the local environmental effect of this fluorescence nucleo-

Scheme 2. Synthesis of Bicyclic 4-*N*-Carbamoyldeoxycytidine Phosphoramidite (**14**)



base, the fluorescent spectra of single- or double-strand oligodeoxynucleotides containing dC^{hpp} were measured (Figure 3). It was found that the fluorescent properties of

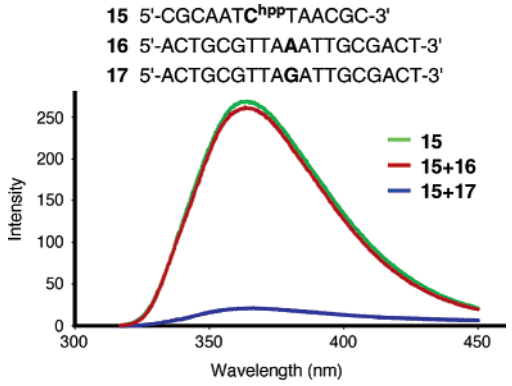


Figure 3. Fluorescent spectra of oligodeoxynucleotides containing dC^{hpp} (5 μM nucleoside, 10 mM sodium phosphate, pH 7.0, excitation 300 nm).

dC^{hpp} remained in an oligodeoxynucleotide 13mer **15** containing dC^{hpp} at the center position. The λ_{max} value of the excitation and the emission spectra of the oligonucleotide were identical to those of dC^{hpp} (Figure 2B and Figure 3, green line).

When the C^{hpp} base faced a mismatch base dA as exemplified by a DNA duplex formed between the modifed oligomer **15** and a mismatched oligomer **16**, the fluorescence intensity (Figure 3, red line) was very similar to that (Figure 3, green line) of the single-strand oligonucleotide containing C^{hpp}. In contrast, the fluorescent spectrum of a DNA duplex containing a C^{hpp}−G base pair was significantly suppressed (Figure 3, blue line). These phenomena are similar to those of benzopyridopyrimidine (BPP) nucleoside derivatives reported by Okamoto et al.[8] However, it is interesting that the conjugated system of the cytosine ring of dC^{hpp} is much smaller than that of BPP derivatives, suggesting the minimized structure for the base-discriminating fluorescent properties of BPP derivatives.

In conclusion, we synthesized a new fluorescence nucleobase that can form a stable base pair with both guanine and adenine bases. Although the conjugated system of the cytosine ring of dC^{hpp} is similar to dC^{cmy} reported previously, dC^{hpp} was found to serve as a strong fluorescent analogue of deoxycytidine, which proved to have a base-discriminating fluorescent property. A tautomeric study of dC^{hpp} is now underway.

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Supporting Information Available: Experimental procedure and characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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