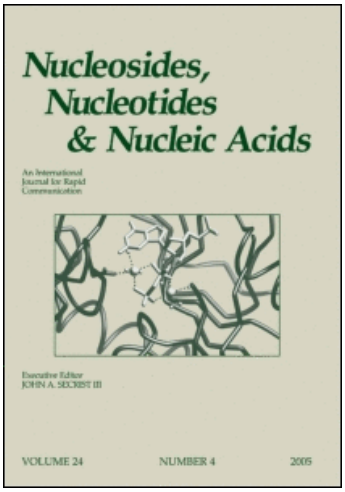

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A Pyrimidopyrimidoindole Nucleoside (dC^{PPI}): Photophysical Properties and Thermal Stability of the Modified Dna Duplexes

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A PYRIMIDOPYRIMIDOINDOLE NUCLEOSIDE (dC^{PPI}): PHOTOPHYSICAL PROPERTIES AND THERMAL STABILITY OF THE MODIFIED DNA DUPLEXES

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□ *A new fluorescent deoxycytidine analog, 10-(2-deoxy-β-D-ribofuranosyl)-pyrimido[4′,5′:4,5]-pyrimido[1,6-a]indole-6,9(7H)-dione (dC^{PPI}) was synthesized. Its fluorescent properties were studied in detail. It was found that this fluorescent nucleoside dC^{PPI} could be used as a fluorescent label for DNA probes with minimal disturbance of their overall structure.*

Keywords Cytosine; Fluorescent nucleoside; Thermal stability

INTRODUCTION

Many biochemical experiments require labeling of nucleic acids with fluorescent tags. Typically, a fluorophore is attached to an oligonucleotide *via* an appropriate linker.[1] However, the free motion of the linker-based fluorophores precludes gathering full data of molecular dynamics. In contrast to such simple fluorescence labels, fluorescent bases that imitate the natural DNA bases have served as sensitive real-time probes of base-stacking

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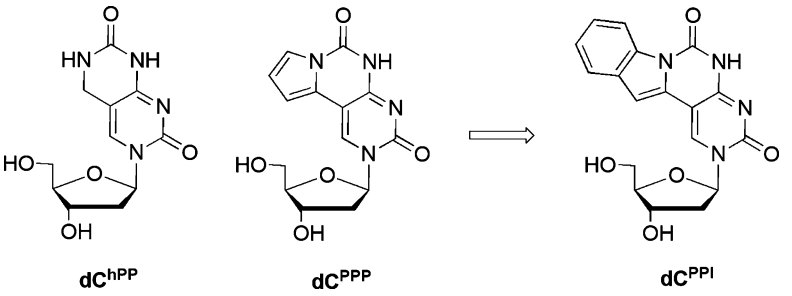


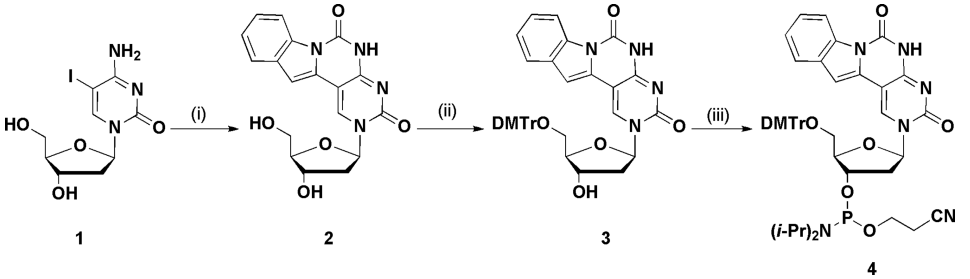
FIGURE 1 The strucuture of dC^{hPP}, dC^{PPP}, and dC^{PPI}.

and base-pairing in their vicinity.[2] However, there are few fluorescent nucleosides whose colors could be kept without reduction of their fluorescent intensities and distortion of the nucleic acid conformation. Recently, we have reported new fluorescent pyrimidine nucleosides, a bicyclic 4-*N*-carbamoyldeoxycytidine derivative (dC^{hpp})[3] and pyrrolopyrimidopyrimidine (dC^{PPP}),[4] having intensive quantum yields and large Stokes shifts of 60 nm and 119 nm, respectively. In this study, we report the synthesis and photophysical properties of dC^{PPI} as a ring-expanded analog of these new fluorescent pyrimidine nucleosides (Figure 1).

RESULTS AND DISCUSSION

Synthesis and Properties of Pyrimidopyrimidoindole Nucleoside Derivatives

dC^{PPI} (**2**) was synthesized in 75% yield via a Suzuki-Miyaura coupling reaction of 5-iodo-2′-deoxycytidine **(1)** with *N*-Boc-indole-2-borate[5] in the presence of $Pd(OAc)_2$ and TPPTS [tris(3-sulfonatophenyl)phosphine] followed by spontaneous cyclization (Scheme 1). The absorption and emission



SCHEME 1 Reagents and conditions: i) *N*-Boc-indole-2-borate (2.0 equiv.), $Pd(OAc)_2$ (0.03 eqiuv.), TPPTS (0.08 equiv.), Na_2CO_3 (2.2 equiv), H_2O-CH_3CN (2:1, v/v), 45°C, 8 hours, 75%; ii) DMTrCl (1.5 equiv.), triethylamine (1.5 equiv.), DMF, 12 hours, 73%; (iii) (2-cyanoethoxy)bis(diisopropylamino)phosphine (1.5 equiv.), diisopropylamine (0.8 equiv.), 1*H*-tetrazole (0.8 equiv.), CH_2Cl_2, 8 hours, 71%.

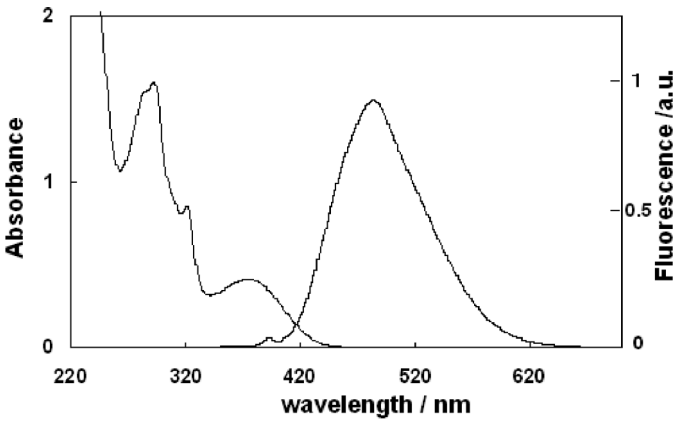


FIGURE 2 Absorption and emission spectra of 0.1 mM dC^{PPI} in 50 mM phosphate buffer (pH 7.0).

spectra were measured in an aqueous buffer. The absorption spectrum of dC^{PPI} showed its absorption maxima at around 310 nm and 374 nm. It was also proved that the excitation at 366 nm gave maximum emission at 513 nm (Figure 2).

The fluorescence emission spectrum was obtained at $\lambda_{ex} = 366$ nm.

Thermal Stabilities and Fluorescent Properties of Modified Oligodeoxynucleotides

Next, the phosphoramidite **(4)** was successfully prepared *via* the DMTr derivative **3**, as shown in Scheme 1. and dC^{PPI} was incorporated into an oligonucleotide to give d(CGCAAT[dC^{PPI}]TAACGC) (ODN 1). To characterize the oligonucleotide incorporating dC^{PPI}, the T_m values of the duplexes of ODN 1 and ODN 2: 3′-d(GCGTTAYATTGCG)-5′, where Y = dG

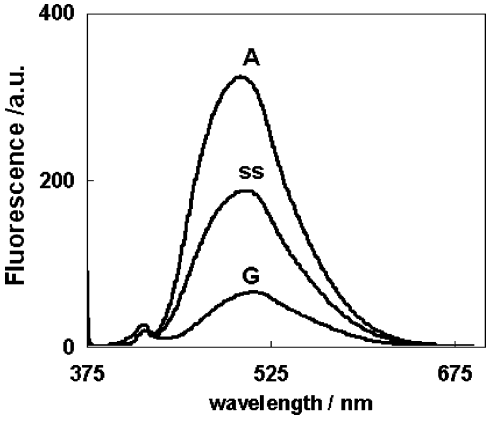


FIGURE 3 The change of fluorescence spectra of 2.5 μM ODN 1 hybridized with 2.5 μM ODN 2 (Y = dG or dA). The spectrum in the single strand ODN 1 was represented by ss.

TABLE 1 Melting temperature (T_m) of duplexes d(CGCAATXTAACGC)/d(GCGTTAYATTGCG)

X	Y	T_m
dC^{PPI}	dG	58.1
dC	dG	57.8
dC^{PPI}	dA	54.2
T	dA	56.2

or dA, were compared with those of the corresponding unlabeled duplexes having dC-dG and T-dA base-pairs in place of dC^{PPI}-dG and dC^{PPI}-dA base pairs, respectively (Table 1). The T_m values of these duplexes were measured at pH 7.0 in the presence of 0.1 M sodium chloride. When the base at the opposite site was guanine, the T_m value (58.1°C) of the duplex containing dC^{PPI} was almost the same as that of unmodified double-stranded DNA (57.8°C). Moreover, the T_m value (54.2°C) of the dA-dC^{PPI} base-pair was only slightly lower by 2.0°C than that (56.2°C) of the canonical A-T base-pair, and much more stable than the dA-dC mismatch (data not shown). These results indicated the ability of dC^{PPI} to recognize both dG and dA.

Finally, the fluorescent intensities of dC^{PPI} in the single strand ODN 1 and ODN 1/ODN 2 duplexes were measured in the same medium as described above (Figure 3). The fluorescence was stronger in the duplex state (Y = dA) than in the single strand state. However, when dC^{PPI} was paired with dG, the fluorescence reduced drastically. This observation suggests that dC^{PPI}forms a base-pair with G that induced significant quenching of the fluorescence of dC^{PPI}.

CONCLUSION

The dC^{PPI}-labeled DNA duplexes are a good model to study the accommodation of a bulky molecule in DNA and check if the hydrogen bonds with both adenine and guanine bases in the duplexes are maintained. We showed dC^{PPI} as a fluorophore of DNA probes with minimal disturbance of their overall structure.

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