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Synthesis of a fluorescent 2′3′-dideoxycytosine analog, tCdd

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ABSTRACT

The pathway leading to the preparation of a novel tricyclic 2′3′-dideoxycytosine analog, tCdd (**1**) is reported. A protected 2′3′-dideoxyribose prepared from L-glutamic acid was coupled to a silylated fluorescent base to yield a mixture of the α- and β-anomers of the 2′3′-dideoxyribonucleoside of 1,3-diaza-2-oxophenothiazine, tCdd (**1**). The fluorescent base analog retains a high fluorescence emission over a large pH range and should be useful in a variety of probe applications.

Oligonucleotide labeling methods often involve attaching a fluorophore to a natural nucleoside through a linker. While this method of attachment can be quite useful, one drawback is that the variable position of the fluorophore complicates structural and conformational analysis studies. Recently, many novel fluorescent nucleobases have been developed leading to an increase in their biochemical and biotechnological applications. Fluorescent nucleobases can more advantageously be placed closer to the site of chemical or structural activity as compared to linker-based fluorophores.

Several environment sensitive nucleobases have base-discriminating fluorescence properties.[1] These molecules have been shown to be useful as probes of DNA hybridization and reporters of single nucleotide polymorphisms. The sensitivity of a nucleobase to environment, while often valuable, can be detrimental to its utility. For example, at times the nucleobase fluorescence is quenched during duplex formation or by neighboring bases,[2] thereby limiting the sequence options for an oligonucleotide containing the nucleobase probe.

Environment insensitive fluorescent nucleobases can be used for various applications. For example, they can be used as probes for studies of the dynamics and conformations of nucleic acids as part of a fluorescence resonance energy transfer (FRET) pair.[3] Specifically, they may prove useful in studies involving enzymatic processes such as DNA elongation and repair.

The fluorescent DNA base analog, 3,5-diaza-4-oxophenothiazine, tC,[4] is a nucleobase, that is, relatively insensitive to its environment. It has a high fluorescent quantum yield when incorporated into single and double-stranded oligonucleotides, making it a useful reporter group.[5] It has been demonstrated that DNA duplexes containing tC adopt normal B-form structures without distortion of the backbone[6] and that tC forms Watson–Crick base pairs with guanine.[7] The fluorescence emission spectrum of tC is not perturbed either during DNA duplex formation[8] or by the formation of ternary complexes with large biological macromolecules.[9]

Recently, tC has been used as a FRET donor paired with Alexa-555 to study the conformational changes of a polymerase–DNA complex during the selection and binding of nucleotide substrates.[3] The introduction of the dideoxynucleoside tCdd (**1**) herein will expand the utility of similar studies and present new applications since this useful fluorescent probe can now be placed in any position of an elongating or terminated oligonucleotide.

To accomplish biophysical studies with tCdd (**1**), the fluorescent dideoxynucleobase can be paired with a FRET acceptor or a quencher molecule. By positioning the fluorophore in the 3′-terminal position, it can be used to study binding to non-extendable sequences with the fluorescence reporter group likely proximal to the site of activity. This is a considerable advantage over using tC or another extendable fluorophore in the penultimate position with a non-reporter terminating group in the 3′ position. Based on the fixed position of tCdd, information regarding the distance changes between the tCdd and its FRET pair would provide important information regarding structural and conformational changes that occur during any binding events monitored. Additional

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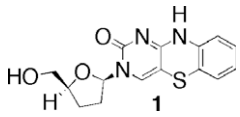


Figure 1. Compound 1, dideoxy-tC nucleoside, tCdd.

information can be gathered from kinetics studies and other photophysical measurements. It is expected that significant contributions in addition to those suggested for tCdd will be realized.

We report herein the synthesis and characterization of the fluorescent 2′3′-dideoxyribonucleoside of 1,3-diaza-2-oxophenothiazaine, tCdd (**1**) (see Fig. 1).

The successful synthetic approach involves the formation of a protected dideoxyribose, a protected fluorescent base, and the linking of the two to produce the desired product anomer (complete synthetic details and spectroscopic analysis available online in Supplementary data). The protected dideoxyribose (**4**) can be prepared in a straightforward fashion from L-glutamic acid.[10] The tC base is synthesized from 2-aminothiophenol and 5-bromouracil.[11] Coupling of the protected sugar (**4**) with the silylated tC base (**3**) using ethylaluminum dichloride as a Lewis acid (Scheme 1) afforded an equal mixture of the α- and β-anomers (**5**). The anomers were separated by chromatography on silica gel then deprotected to give tCdd (**1**) and its α-anomer. Characterization of the compounds was determined by analysis of the ROESY and dqCOSY spectra of the anomers.

Several different synthetic strategies were considered and tested. There are two dominant synthetic schemes prevalent in the literature. Some groups have used nucleosides or deoxynucleosides as starting materials and then deoxygenated them to yield dideoxynucleosides.[12] The conditions required for the removal of the OH group(s) proved to be too harsh for the tC base. The other generally reported method begins with L-glutamic acid, which is converted to a dideoxy sugar acetate for coupling to a base.[10] Our final method of coupling the protected dideoxyribose (**4**), synthesized from L-glutamic acid, with silylated tC (**3**) followed the method that Okabe had used to successfully synthesize dideoxycytidine.[13]

Okabe et al. had tested several different Lewis acids to obtain both the best yields and favorable α/β-anomer product ratio. With their system, they achieved a 2:3 ratio. With our system, we did not achieve any anomer preference, getting an almost equal amount of each anomer. Likely due to the low solubility of the tC base, we obtained low yields of the anomers, but the yields are similar to those reported for the synthesis of the tC nucleoside which suffered from the same complications.[11]

We found it easiest to separate the tCdd anomers before the final deprotection step. On silica gel, the desired β-anomer was retained on the column longer. We tried several reagents to remove the *tert*-butyldimethylsilyl protecting group. We found that most reagents likely destroyed the tC base and did not observe any of the desired product formed with the use of *p*-toluenesulfonic acid followed by basic resin or triethylamine trihydrofluoride. Tetrabutylammonium fluoride, however, proved to be mild enough to provide the product in good yield.

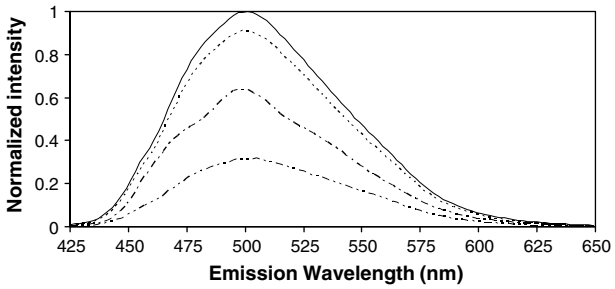
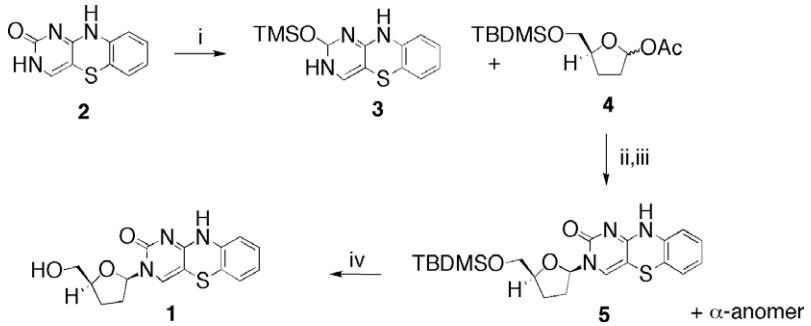


Figure 2. Normalized emission spectra of tCdd nucleoside (1) at different pH and 25 °C. The buffers used were 50 mm glycine–NaOH for pH 10 (—), 13 (—·—) and 50 mm citrate with the addition of HCl for pH 4 (----) and 1.8 (—··—).

In order to determine the identity of the α- and β-anomers, 1-D and 2-D NMR techniques were used. The strongest evidence was obtained from the analysis of the coupling pattern in the dqCOSY between the 1′ and 2′/2″ protons.[14] Both anomers were characterized completely by ^{1}H NMR, ^{13}C NMR and mass spectrometry.

By inference to the fluorescent tC nucleoside, tCdd is in its neutral, base-pairing form in a wide pH interval, 4–12.[15] The relative insensitivity of the fluorescence of tCdd to pH is important to its utility as a useful structural probe. Figure 2 shows an emission versus pH profile for tCdd that is similar to that reported for the tC nucleoside. The emission for tCdd decreases as pH decreases. At high pH, the emission of tCdd is also decreased. At pH 13, when tCdd is predicted to be largely deprotonated, the emission spectrum is more ordered. It has been surmised that the ordering of the spectrum is due to the planar, more rigid structure. Overall, there is little sensitivity over a pH range of 4–10, making tCdd a useful reporter in a large variety of systems and experimental conditions.

In conclusion, the synthesis of a fluorescent 2′3′-dideoxynucleoside has been described. The dideoxycytosine analog, tCdd, has spectral properties that will make it useful as a probe to study the conformational changes associated with the interaction of DNA with biological macromolecules and potentially other types of structural studies. Further studies involving the incorporation of tCdd into DNA are currently underway in our laboratory.



Scheme 1. Reagents and conditions: (i) HMDS, NH_4SO_4, reflux, 1 h; (ii) CH_2Cl_2, $EtAlCl_2$; (iii) sat. $NaHCO_3/CH_2Cl_2$, 0 °C; (iv) TBAF, THF.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.11.015.

References and notes

1. Okamoto, A.; Saito, Y.; Saito, I. *J. Photochem. Photobiol. C Photochem. Rev.* **2005**, *6*, 108.
2. Wilson, J. N.; Cho, Y.; Tan, S.; Cuppoletti, A.; Kool, E. T. *ChemBioChem* **2008**, *9*, 279.
3. Stengel, G.; Gill, J. P.; Sandin, P.; Wilhelmsson, L. M.; Albinsson, B.; Norden, B.; Millar, D. *Biochemistry* **2007**, *46*, 12289.
4. Lin, K.-Y.; Jones, R. J.; Matteucci, M. *J. Am. Chem. Soc.* **1995**, *117*, 3873.
5. Sandin, P.; Borjesson, K.; Li, H.; Martensson, J.; Brown, T.; Wilhelmsson, L. M.; Albinsson, B. *Nucleic Acids Res.* **2008**, *36*, 157.
6. Engman, K. C.; Sandin, P.; Osborne, S.; Brown, T.; Billeter, M.; Lincoln, P.; Norden, B.; Albinsson, B.; Wilhelmsson, L. M. *Nucleic Acids Res.* **2004**, *32*, 5087.
7. Wilhelmsson, L. M.; Holmen, A.; Lincoln, P.; Nielsen, P. E.; Norden, B. *J. Am. Chem. Soc.* **2001**, *123*, 2434.
8. Sandin, P.; Wilhelmsson, L. M.; Lincoln, P.; Powers, V. E. C.; Brown, T.; Albinsson, B. *Nucleic Acids Res.* **2005**, *33*, 5019.
9. Tahmassebi, unpublished data.
10. Farina, V.; Benigni, D. A. *Tetrahedron Lett.* **1988**, *29*, 1239.
11. Sandin, P.; Lincoln, P.; Brown, T.; Wilhelmsson, L. M. *Nat. Protoc.* **2007**, *2*, 615.
12. Manchand, P. S.; Belica, P. S.; Holman, M. J.; Huang, T. N.; Maehr, H.; Tam, S. Y. K.; Yang, R. T. *J. Org. Chem.* **1992**, *57*, 3473.
13. Okabe, M.; Sun, R. C.; Tam, S. Y. K.; Todaro, L. J.; Coffen, D. L. *J. Org. Chem.* **1988**, *53*, 4780.
14. Wuthrich, K. *NMR of Proteins and Nucleic Acids*; John Wiley & Sons, Inc, 1986. pp 224.
15. Wilhelmsson, L. M.; Sandin, P.; Holmen, A.; Albinsson, B.; Lincoln, P.; Norden, B. *J. Phys. Chem. B* **2003**, *107*, 9094.