Published in final edited form as:
Org Biomol Chem. 2010 November 21; 8(22): 5053–5055. doi:10.1039/c0ob00413h.

Fluorescent nucleoside analogue displays enhanced emission upon pairing with guanine†

Yun Xie, **Tucker Maxson**, and **Yitzhak Tor**
Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA, 92093-0358, USA. ytor@ucsd.edu; Fax: +1 858 534 0202; Tel: +1 858 534 6401

Abstract

A fluorescent nucleobase analogue, 7-aminoquinazoline-2,4-(1*H*,3*H*)-dione, is incorporated into a DNA oligonucleotide and senses mismatched pairing by displaying G-specific fluorescence enhancement.

Single nucleotide polymorphisms (SNPs),[1] mutated base pairs, have been linked to specific diseases or susceptibility to particular therapeutics.[2] While there are several developed and commercialized approaches for detecting SNPs,[3] many recent advancements have centered around the design of base-discriminating fluorescent nucleosides.[4–7] Following incorporation into DNA hybridization probes and duplex formation with target oligonucleotides, the emissive nucleosides display characteristic photophysical signature, depending on their pairing partner.[4,8]

To develop base discriminating probes, it is important to identify heterocycles that are structurally similar to native nucleobases and capable of Watson–Crick pairing. Red shifted absorption spectra relative to native nucleosides, permitting selective excitation, are highly desirable. The emission of the fluorescent analogs should be sensitive to its hybridization microenvironment, and perhaps more importantly, fluorescence enhancement rather than quenching should be associated with positive identification of a mismatch. Detecting mismatched G residues has, therefore, presented a challenge, as guanine, being the easiest nucleobase to oxidize,[9–10] frequently quenches the emission of most commonly used fluorophores.[11–14] Here we present a new fluorescent pyrimidine analog that, when hybridized against G, displays an enhanced emission when compared to a perfect duplex or all other mismatches.

In accordance with our design principles,[5–7] we have synthesized a polarizable nucleobase, 7-aminoquinazoline-2,4-(1*H*,3*H*)-dione **1** and the corresponding 2′-deoxynucleoside **2**, which contain an electron-rich ring fused into an electron-deficient pyrimidine (Scheme 1). We surmise that placing the electron donating amine group in a conjugated position to the pyrimidine's carbonyl would facilitate a charge transfer transition and greater sensitivity of the photophysical characteristics to environmental changes. To assess the nucleoside's sensitivity to its microenvironment, its absorption and emission spectra were recorded in solvents of distinct polarity (Fig. 1 and Table 1). Solvent polarity has little effect on the lowest energy absorption maximum of nucleoside **2** (316 ± 1 nm), but the absorption band

†Electronic supplementary information (ESI) available: Details of synthesis, characterization of compounds, thermal melting data and additional fluorescence data. CCDC reference number 784395. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c0ob00413h

Correspondence to: Yitzhak Tor.

around 288 nm is sensitive to polarity changes, resulting in a greater molar absorptivity in nonpolar solvents.

Importantly, both emission wavelength and intensity are affected by solvent polarity. In water, the most polar solvent examined, **2** exhibits the most quenched and bathochromically shifted emission band (Fig. 1), peaking around 361 nm ($\Phi_F = 0.039 \pm 0.006$, Stoke Shift = 3.9×10^3 cm^{-1}). In methanol, nucleoside **2** displays the most intense emission with an emission band at 352 nm ($\Phi_F = 0.14 \pm 0.01$, Stoke Shift = 3.2×10^3 cm^{-1}). In solvents of lower polarity, **2** shows more hyperchromically shifted emission with decreasing intensity (Table 1, Stoke Shifts = 1.9–2.1×10^3 cm^{-1}). These observations suggest an enlarged dipole and charge transfer character of the excited state when compared to the ground state.

To incorporate the non native nucleoside into a DNA oligonucleotide, phosphoramidite **3** was prepared (Scheme 1). 7-Aminoquinazoline-2,4(1*H*,3*H*)-dione **1** was glycosylated to provide the modified nucleoside **2** after saponification of all esters and isolation of the β-anomer (X-ray Structure: Figure S1 and Table S1†).[15] Protection of the 5′-hydroxyl as the 4,4′-dimethoxytrityl (DMTr) derivative, followed by phosphitylation of the 3′-hydroxyl, provided phosphoramidite **3** (Scheme 1). Standard solid-phase oligonucleotide synthesis was utilized to prepare the 13-mer DNA construct **4**, where probe **2** was placed in the middle of the sequence (Fig. 2). The oligonucleotide was purified by PAGE, and MALDI-TOF mass spectrometry confirmed its full length and the presence of the intact emissive nucleoside **2** (Figure S2†).[15]

The fluorescent single strand DNA oligonucleotide **4** exhibits a similar, albeit broader, emission profile to the nucleoside in water with an emission band around 361 nm. Upon hybridization to its complement **7**, a quenched emission at 363 nm is observed (Fig. 3 and Table 2). In contrast, when the fluorescently labeled DNA oligonucleotide **4** is hybridized with **5**, an oligonucleotide with a G mismatch opposite nucleoside **2**, its emission is greatly enhanced and hyperchromically shifted to 353 nm, displaying an emission more similar to nucleoside **2** in methanol (Fig. 3 and Table 2). Other oligonucleotides with mismatches (**6** and **8**) failed to produce a dramatic increase in fluorescence intensity and all displayed emission bands around 362 nm, where nucleoside **2** emits in water. Importantly, thermal denaturation measurements (Table 2 and Figure S4†),[15] determined by monitoring changes in absorbance at 260 nm as a function of temperature, show that stable duplexes were formed for all oligonucleotide pairs. The T_m value for the complemented duplex **4·7** (T_m = 57 ± 1 °C) was within error of the melting temperature of an unmodified control duplex (T_m = 58 ± 1 °C) (Figure S3–S4†). Hybridization with DNA strands containing mismatches do show, as expected, destabilization (Table 2).

Nucleoside **2** uniquely reports the presence of a G mismatch with over a two-fold enhanced emission, compared to its emission intensity in a perfect duplex when found opposite A, a feature rarely seen with isosteric/isomorphic fluorescent nucleoside analogs.[11–14] While the underlying molecular factors governing this behavior are unclear at present, a disparity between the redox potential of G and the new nucleobase, coupled to environmental factors influencing the solvation of the modified base are likely to be influencing factors. It is tempting to speculate that a formation of a wobble G·**2** pair anchors the emissive nucleoside in a partially exposed geometry, while still preserving a partially stacked and desolvated microenvironment.[16–18] Regardless of these putative structural features, the results reported

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here demonstrate that new emissive nucleobase analogs can display unique photophysical features and potentially find utility for mismatch detection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the National Institutes of Health for their generous support (GM 069773), the National Science Foundation (instrumentation grant CHE-0741968), and Nicholas Greco for his assistance with MALDI experiments.

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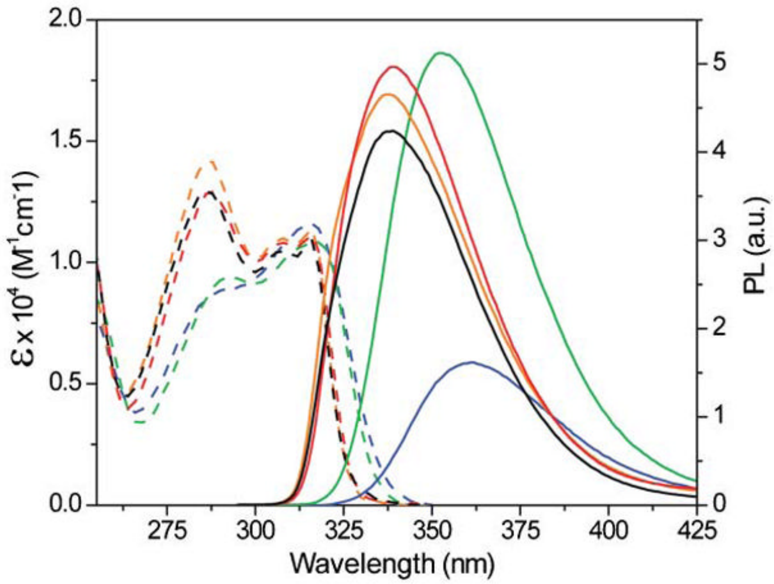

Fig. 1.

Absorption (—) and emission (—) spectra of nucleoside **2** in water (blue), methanol (green), acetonitrile (red), dioxane (orange), and dichloromethane (black).

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4	5'-GCG	ATG	**2**	GTA	GCG-3'
5	3'-CGC	TAC	**G**	CAT	CGC-5'
6	3'-CGC	TAC	**T**	CAT	CGC-5'
7	3'-CGC	TAC	**A**	CAT	CGC-5'
8	3'-CGC	TAC	**C**	CAT	CGC-5'

Fig. 2.

Synthesized oligonucleotide **4** and oligonucleotides used in hybridization and fluorescence experiments.

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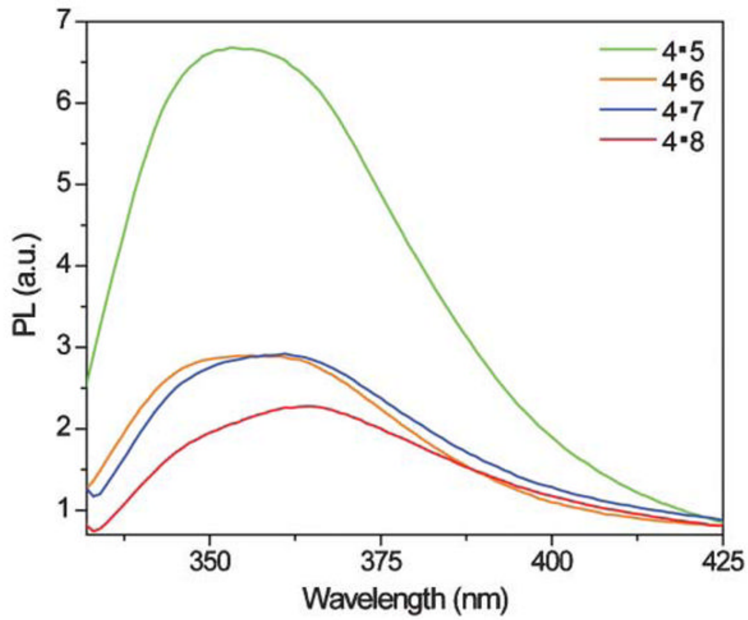
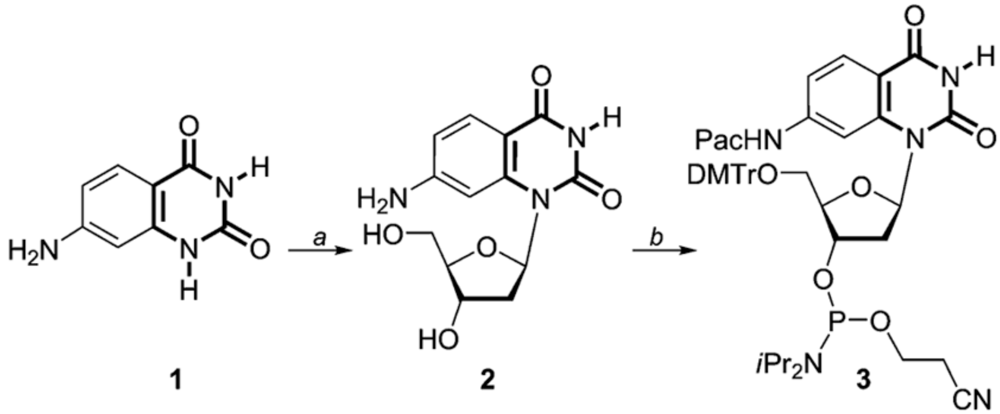


Fig. 3.
Emission spectra of **4·5** (green), **4·6** (orange), **4·7** (blue), and **4·8** (red). Conditions same as listed in Table 2.



Scheme 1.

Synthesis of the nucleoside and phosphoramidite based on 7-aminoquinazoline-2,4-(1*H*, 3*H*)-dione. *Reagents*: (*a*) (*i*) $(NH_4)_2SO_4$, *N*, *O*-bis(trimethylsilyl)acetamide, $CF_3SO_3Si(CH_3)_3$,2-D-3,5-di-*O*-*p*-toluoyl-α-L-*erythro*-pentofuranosyl chloride, CH_3CN; (*ii*) conc. NH_4OH, 40%. (*b*) (*i*) $(CH_3)_3SiCl$, phenoxyacetic anhydride, H_2O, conc. NH_4OH, pyridine, 75%; (*ii*) DMTrCl, Et_3N, pyridine, 85%; (*iii*) *i*Pr_2NEt, $(iPr_2N)P(Cl)O\text{-}CH_2CH_2CN$, $ClCH_2CH_2Cl$, 65%.[15]

Table 1

Photophysical data of nucleoside **2**[a]

Solvent	**E_T (30)**[b]	**λ_{bs}**[c]**/nm**	**λ_{em}/nm**	**I_{rel}**[d]
Water	65.3	316	361	1.0
Methanol	55.7	316	352	3.2
Acetonitrile	45.9	316	339	3.1
Dichloromethane	40.9	316	338	2.6
Dioxane	36.4	316	336	2.9

[a]Conditions for absorption and emission spectra: 5.0 and 0.5 × 10^{-5} M, respectively.

[b]Units are kcal mol^{-1}.

[c]The lowest energy maximum is given.

[d]Relative emission intensity with respect to intensity in water.

Table 2

Photophysical data of oligonucleotide **4** and its duplexes[a]

Duplexes	**4·5**	**4·6**	**4·7**	**4·8**
λ_{em}/nm	353	362	361	365
I_{rel}[b]	2.2	1.3	1.0	0.8
TM °C^{-1}	50 ± 1	51 ± 1	57 ± 1	50 ± 1

[a]Conditions: 5.0×10^{-6} M in 2.0×10^{-2}M Na_3PO_4, pH 7.0.

[b]Relative emission intensity with respect to intensity of **4·7**.