

Simple Fluorescent Pyrimidine Analogues Detect the Presence of DNA Abasic Sites

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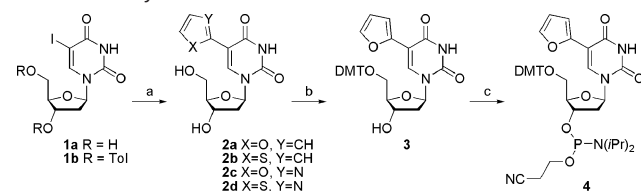
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Nucleic acids experience a variety of perturbations, including strand cleavage, depurination and depyrimidination, local conformational changes, base flipping, as well as more subtle and transient effects induced by ligand binding. Fluorescent nucleoside analogues that are sensitive to their local environment have become powerful tools for investigating nucleic acid structure, dynamics, and recognition.¹ These probes can be broadly classified into the following categories: (1) isomorphous base analogues that resemble the natural nucleobases with respect to their dimension, hydrogen bonding face, and ability to form isostructural Watson–Crick base pairs (e.g., 2-aminopurine);^{2,3} (2) pteridines, intensely fluorescent purine analogues;^{1b} (3) expanded nucleobases, where the natural bases are extended by conjugation to additional aromatic rings (e.g., 1,N⁶-ethenoadenine);^{4,5} (4) base analogues, where the natural heterocycle is replaced with a fluorescent aromatic residue;⁶ and (5) conjugated base analogues, where fluorescent aromatic moieties are linked to the natural nucleobases.^{7,8} Notably, favorable photophysical characteristics (e.g., long emission wavelengths and high emission quantum efficiencies) are typically associated with significant structural perturbation when compared to the native nucleobases.

Our research program is directed toward the design, synthesis, and implementation of new fluorescent isosteric nucleosides that satisfy the following criteria: (a) maintain the highest possible structural similarity to the natural nucleobases, (b) display emission at long wavelengths (preferably in the visible range), (c) retain adequate emission quantum efficiency, and, importantly, (d) exhibit emission that is sensitive to the microenvironment. Here, we describe the synthesis and photophysical characteristics of a series of simple and responsive thymidine analogues, where a 2'-deoxy-U core is conjugated to aromatic five-membered heterocycles, including furan, thiophene, oxazole, and thiazole (Scheme 1). When incorporated into oligonucleotides, the furan analogue **2a** is shown to positively signal the presence of DNA abasic sites.

The one-step synthesis of the modified pyrimidines is straightforward (Scheme 1).^{9,10} It entails a palladium-mediated cross coupling of the commercially available 5-iodo-2'-deoxyuridine¹¹ (or 3',5'-diTol-Iodo-dU) and the corresponding stannylated heterocycles. Standard protection of the 5'-hydroxyl with 4,4'-dimethoxytrityl chloride followed by phosphitylation of the unprotected 3'-

Scheme 1. Synthesis of Nucleosides **2a–d** and Amidite **4a**



^a Reagents: (a) **2a**: **1a**, 2-(Bu₃Sn)furan, PdCl₂(Ph₃P)₂, dioxane, 94%. **2b**: **1a**, 2-(Bu₃Sn)thiophene, PdCl₂(Ph₃P)₂, dioxane, 53%. **2c**: (i) **1b**, 2-(Bu₃Sn)oxazole, Pd(Ph₃P)₄, toluene; (ii) K₂CO₃, 5% THF/methanol, 10%. **2d**: (i) **1b**, 2-(Bu₃Sn)thiazole, PdCl₂(Ph₃P)₂, dioxane; (ii) K₂CO₃, 5% THF/methanol, 34%. (b) DMTCl, pyridine, Et₃N, 71%. (c) (iPr₂N)₂POCH₂CH₂CN, 1H-tetrazole, CH₃CN, 65%.

Table 1. Photophysical Data of Nucleosides **2a–d**^a

	λ_{max} Et ₂ O (nm)	λ_{max} H ₂ O (nm)	Φ H ₂ O	λ_{em} Et ₂ O (nm)	λ_{em} H ₂ O (nm)	I H ₂ O/Et ₂ O
2a	314	316	0.03	395	431	5.6
2b	320	314	0.01	421	434	1.6
2c	292	296	<0.01	390	400	1.0
2d	318	316	<0.01	397	404	2.1

^a 5.0×10^{-5} M (λ_{max}), 1.0×10^{-5} M (λ_{em}), H₂O (ref 13), Φ (ref 9).

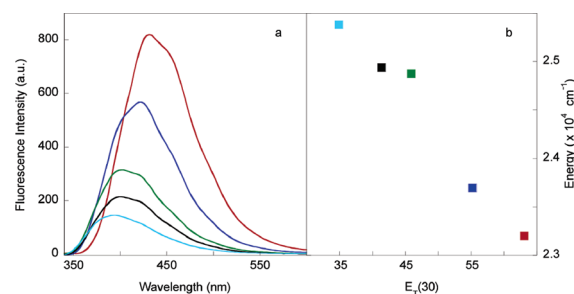


Figure 1. (a) Steady-state emission spectra of nucleoside **2a** in (red) buffer,¹³ (dark blue) methanol, (green) acetonitrile, (black) dichloromethane, (light blue) ether. (b) A linear relationship between emission energy and $E_T(30)$ values.⁹

5 5' - GCG - ATG - XGT - AGC - G - 3'
6 5' - CGC - TAC - ACA - TCG - C - 3'
7 5' - CGC - TAC - YCA - TCG - C - 3'
8 5' - GCG - ATG - TGT - AGC - G - 3'

Figure 2. Synthesized oligonucleotides. X = **2a** and Y = THF residue.⁹

hydroxyl affords the building blocks necessary for solid-phase DNA synthesis (Scheme 1).¹²

Photophysical properties of nucleosides **2a–d** were examined prior to incorporation into oligonucleotides (Table 1). To evaluate the nucleoside's potential to respond to polarity changes, their photophysical characteristics have been evaluated in different solvents. Increasing solvent polarity has little influence on the absorption maxima of the conjugated nucleosides. In contrast, both emission wavelength and intensity are markedly affected by solvent polarity. In ether, the least polar solvent tested, nucleoside **2a** displays a relatively weak emission with a maximum at 395 nm (Figure 1). In water, the most polar solvent examined, **2a** exhibits an intense emission band (Figure 1), which peaks around 430 nm and decays deeply into the visible (>550 nm). Solvents of intermediate polarity display an intermediate behavior with a clear emission bathochromic and hyperchromic effects with increasing solvent polarity (Figure 1).¹⁴ Nucleoside **2a**, containing a conjugated furan, exhibits the highest sensitivity to solvent polarity (Table 1) and is, therefore, selected for incorporation into oligonucleotides (Figure 2).

The absorption and emission spectra of the singly modified single-stranded oligonucleotide **5** (Figure 3) are similar to those exhibited by the free nucleoside **2a** in buffer.^{13,15} When hybridized

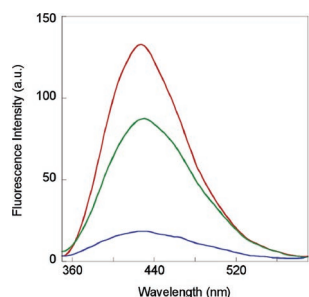


Figure 3. Steady-state emission of 5·6 (blue, 1.0×10^{-6} M), 5 (green, 1.0×10^{-6} M), and 5·7 (red, 1.0×10^{-6} M) in buffer.^{13,17}

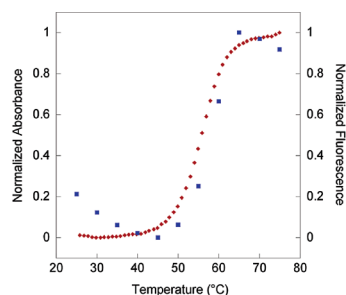


Figure 4. Denaturation of duplex 5·6 monitored by absorbance (red triangles, 1.0×10^{-6} M) and fluorescence (blue squares, 2.0×10^{-6} M) in buffer.^{13,16}

to its perfect complement **6**, a duplex (5·6) that is as stable as the control unmodified duplex 6·8 is obtained ($T_m = 56$ °C for both).⁹ Similar to other emissive nucleosides (e.g., 2-aminopurine), the emission of the furan containing dU is significantly quenched when found in a perfectly base-paired duplex (Figure 3). Importantly, thermal denaturation curves (Figure 4), determined by either absorbance at 260 nm or emission at 430 nm, yield the same melting temperature ($T_m = 56$ °C).¹⁶

Abasic sites are important DNA lesions that can be generated either spontaneously or via enzymatic base excision of damaged nucleosides. Several methods have been developed for detecting the presence of these cytotoxic abasic sites, most require irreversible modifications of isolated DNA.¹⁸ When oligo **5** is hybridized to the tetrahydrofuran-containing oligo **7**, a duplex containing an abasic site is formed. Remarkably, the emission of duplex 5·7 is enhanced 7-fold when compared to that of duplex 5·6, formed upon hybridization to the perfect complement (Figure 3). Nucleoside **2a**, when incorporated into a reporter oligonucleotide, positively signals the presence of a DNA abasic site.

An unpaired base opposite an abasic site can be intrahelical or extrahelical, depending on the sequence context. Our current working hypothesis is that **2a** is intrahelical, assuming a syn conformation. This stacked conformation protects the hydrophobic furan moiety, while projecting the hydrogen bonding face toward the major groove. Support is offered by the following observations. (a) Duplex 5·7 is more stable than the control duplex 7·8 that contains a dT residue opposite the abasic site ($T_m = 39$ and 35 °C, respectively).⁹ The increased stability of the modified abasic duplex ($\Delta T_m = +4$ °C) suggests a favorable accommodation of the modified nucleobase by the duplex. (b) The emission band observed for duplex 5·7 decays sharper (> 500 nm) than when compared to the emission exhibited by the free nucleoside in solution. This is consistent with flattening of the chromophore that can be associated with the restricted rotation of the conjugated furan ring upon inclusion within the DNA duplex.¹⁹

In summary, an accessible and simple fluorescent dT analogue is demonstrated to effectively probe the DNA microenvironment

and display different emission in a duplex versus single-stranded oligonucleotides. Of particular importance is its ability to positively report the presence of DNA abasic sites with significantly increased emission.

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Supporting Information Available: Synthetic details, thermal denaturation, absorption/fluorescence spectra, and crystal structures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- (12) Incorporation of **4** into oligonucleotides was accomplished using standard protocols for solid-phase oligonucleotide synthesis (88% unoptimized coupling efficiency). Deprotection using concentrated ammonium hydroxide was followed by PAGE purification. Oligonucleotides were sequenced using MALDI-TOF MS.⁹
- (13) Aqueous buffer = 0.01 M sodium phosphate, 0.1 M NaCl, pH = 7.0.
- (14) This behavior typically occurs in polar molecules that are likely to have enlarged dipoles and charge-transfer character in their excited state.
- (15) The λ_{max} values of 5·6 and 5·7 are identical (Figure S7.4) and similar to that of **2a**.⁹
- (16) The emission of **2a** is inherently temperature dependent and decreases as temperature increases. See Figures S8.1–S8.3 in the Supporting Information.⁹
- (17) Duplex 6·8 was subtracted for background correction.⁹
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