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Design, Synthesis, and Spectroscopic Properties of Extended and Fused Pyrrolo-dC and Pyrrolo-C Analogs

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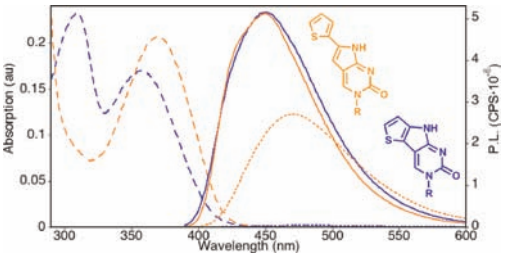
ABSTRACT



The syntheses of four fluorescent nucleoside analogs, related to pyrrolo-C (PyC) and pyrrolo-dC (PydC) through the conjugation or fusion of a thiophene moiety, are described. A thorough photophysical analysis of the nucleosides, in comparison to PyC, is reported.

Fluorescence spectroscopy is a powerful tool for the detailed investigation of nucleic acids and their diverse biomolecular interactions, due to its sensitivity and molecular specificity.[1] These features become accessible when suitable fluorescent probes exist. The insignificant emission of the native nucleobases[2] presents unique opportunities for the design of nonperturbing fluorophores. Creative modifications with minimal structural perturbations have resulted in the development of isomorphic fluorescent nucleoside analogs.[3] These fluorophores have been successfully employed in the monitoring of real-time biochemical events such as drug binding,[4] RNA folding and cleavage,[5] and RNA−protein interactions.[6] The specific utility of a fluorescent nucleoside depends on its photophysical behavior under the desired assay conditions.[3] Analogs that display sensitivity to their microenvironment, through wavelength shifts or changes in intensity, can provide information about local parameters such as polarity,[7] viscosity,[8] and pH.[9] Equally, analogs with minimal sensitivity to the microenvironment exhibiting desired absorption and emission bands may be utilized in a FRET-based system.[3b,4d,4e,6b,10] The photophysical properties of any given analog cannot be predicted through simple structural analysis of the fluorophore. Only upon the synthesis of a probe and analysis of its photophysical characteristics can its properties, such as quantum yield, Stokes shift, brightness, and sensitivity to polarity, be determined to indicate its most apt implementation.

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Pyrrolo-dC (PydC) and pyrrolo-C (PyC) are structurally modified fluorescent deoxycytidine and cytidine analogs, which maintain a proper Watson–Crick H-bonding face (Figure 1).[11] A nominal structural modification leads to a fluorophore with a significant quantum yield and an absorbance band, which is red-shifted from those of the native nucleosides and aromatic amino acid residues. The quantum yield of PyC decreases upon incorporation into oligonucleotides and is quenched even further upon duplex formation.[12] Still, PyC has been used in numerous biophysical assays including spectroscopic visualization of the elongation complex of an RNA polymerase[12,13] and the monitoring of RNA-folding dynamics.[5b,14] While modifications of PyC have gained popularity in recent years,[3b,15] new analogs may expand the structural repertoire and diversify the photophysical properties attainable to facilitate the development of novel assays.

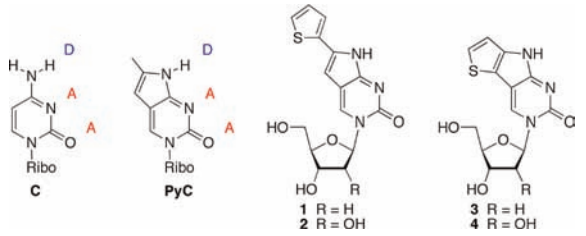

Figure 1. Watson–Crick hydrogen-bonding faces of C and PyC. Extended (**1** and **2**) and fused (**3** and **4**) PyC analogs.

Our program has focused on the development of diverse nonperturbing fluorescent nucleoside analogs via the conjugation or fusion of aromatic heterocycles to the native bases, especially the pyrimidines.[3a] For example, placing a furan or thiophene moiety at the 5-position of uridine leads to sensitive nucleoside analogs, which have been used to detect abasic sites[16] and oxidatively damaged nucleosides[17] and monitor RNA–drug interactions.[4c] Although these useful nucleosides possess measurable sensitivity, low fluorescence quantum yields leave room for improvement.[18] Studies have demonstrated that hampering the free rotation of the furan or thiophene moieties in viscous media dramatically increases the quantum yield.[8] This inspired the design and synthesis of nucleoside analogs **1**–**4** (Figure 1). In correlation to our 5-modified pyrimidines, analogs **1** and **2** represent a PyC core, which is extended via conjugation to a thiophene. Analogs **3** and **4**, in contrast, represent a fused system, similar to both PyC and our previously reported nucleoside alphabet,[19] in which the chromophore possesses no rotatable bonds. As such, deoxy- and ribonucleosides **1**–**4** can be viewed as new PyC analogs. We report the synthesis and evaluation of these analogs, as well as compare and contrast their photophysical features with one another and with PydC and PyC.

The syntheses of **1**–**4** were based upon the implementation of a Pd-mediated cross-coupling reaction followed by a crucial intramolecular cyclization step (Schemes 1 and 2). This approach employs native nucleosides as starting materials, eliminating ambiguities commonly associated with glycosylation reactions regarding the isolation of the correct anomer and regioisomer. Previously reported syntheses of PyC and its analogs were based upon one-pot Sonogashira coupling and ensuing cyclization through the use of Pd and Cu catalysts. This cross-coupling reaction was performed successfully between 5-iodoridine and a variety of substituted alkynes.[15b,20] The resulting furanopyrimidines were fluorescent but lacked a proper Watson–Crick H-bonding face. Following solid-phase incorporation into oligonucleotides, ammonolysis resulted in the efficient conversion of the furanopyrimidines into cytidine analogs.[21] This conversion may also be achieved by treating the furanopyrimidines with methanolic ammonia before incorporation into oligonucleotides.

The initial synthetic attempts to obtain extended thiophene analogs **1** and **2** were based upon this approach. Unfortunately, all efforts to convert the furanopyrimidines resulted in complex mixtures and low yields of the desired cytidine analogs. An alternate synthesis was attempted (Scheme 1) using 5-iodo-2′-deoxycytidine (**5**) and cytidine as starting materials. Cytidine (**6**) was acetylated and iodinated using established methods,[22] while compound **5** was purchased from a commercial source. Compounds **5** and **7** reacted readily with 2-ethynylthiophene under standard cross-coupling conditions, but the included copper catalyst failed to facilitate further cyclization. Compounds **8** and **9** were, therefore, isolated, purified, and screened for an alternate metal-based cyclization catalyst. Gold catalysts activate alkyne moieties to a greater degree than other metal ions,[23] so a sodium tetrachloroaurate(III) dihydrate was employed based upon successful cyclization of related heterocycles.[24] Although the cyclization yields were not optimal, this concise synthesis produced **1** in an overall yield of 41% in 2 steps and **2** in an overall yield of 12% in 4 total steps.

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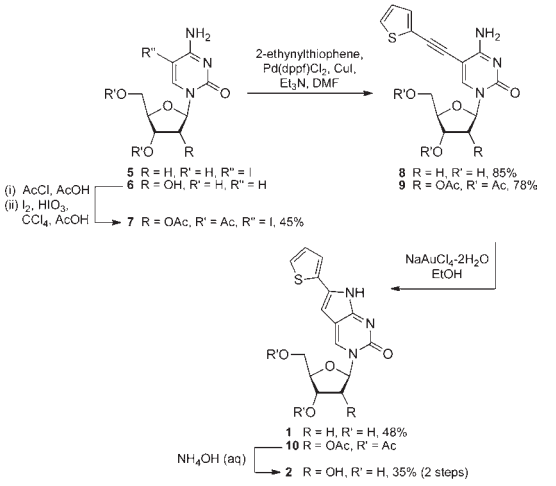
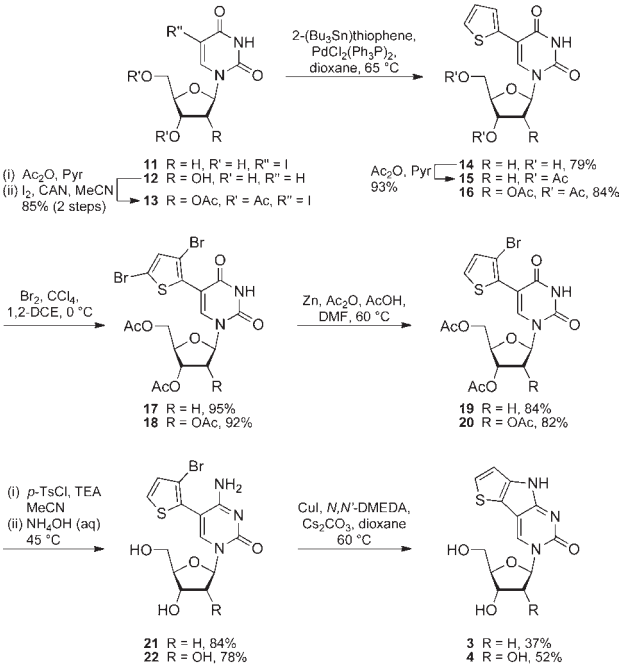
Scheme 1. Synthesis of Extended Analogs **1** and **2**



Scheme 2. Synthesis of Fused Analogs **3** and **4**



The syntheses of the fused thiophene PyC analogs **3** and **4** (Scheme 2) were initiated with the use of commercially available **11** and known modifications of uridine to afford **13**.[25] Cytidine starting materials were explored, but all reactions involving cytidine analogs were lower yielding and more problematic to purify. As numerous methods exist for the exchange of a C-4 carbonyl for an amine, the conversion of a uridine to a cytidine core was left until the penultimate step. The Pd-mediated Stille coupling reaction was high yielding for both the unprotected 2′-deoxynucleoside **11** and the acetylated ribonucleoside **13** and afforded mildly fluorescent nucleosides **14** and **16**.[26] Installing a halogen at the 3-position of the thiophene allowed for the screening of catalysts capable of inducing the desired intramolecular cyclization to the pyrrole moiety. Since the 5-position of the thiophene undergoes electrophilic aromatic substitution more readily than the 3-position, an excess of bromine was added to afford dibrominated products **17** and **18**.[27] Selective zinc-mediated debromination of the 5-position yielded **19** and **20**, respectively.[28] Following the activation of the C-4 carbonyl, a one-pot conversion of the U to C analog and *O*-deacetylation produced **21** and **22**.

The intramolecular cyclization of **21** and **22** to yield the desired fused PyC analogs proved to be the biggest synthetic hurdle. Buchwald–Hartwig amination reactions were first attempted through the use of various ligands and Pd catalysts. All reactions yielded, however, only starting material and/or the debrominated product 5-(thiophen-2-yl)-cytidine or 5-(thiophen-2-yl)-2′-deoxycytidine. Copper catalysts with various ligands were explored next. Of all attempted reactions, only a single set of conditions which include copper(I) iodide, cesium carbonate, and *N*,*N*′-dimethylethylenediamine (*N*,*N*′-DMEDA) provided the cyclized products **3** and **4**.[29] Although the cyclization reactions were not as high yielding as the preceding steps, they provided ample quantities of nucleosides **3** and **4** for full analytical and photophysical studies. Compound **3** was synthesized in an overall yield of 18% over 6 steps, while compound **4** was prepared in an overall yield of 22% over 6 steps.

The photophysical properties of nucleosides **1**–**4** along with PyC and PydC are summarized in Table 1. As expected, the presence of a ribose or deoxyribose moiety (**1** vs **2**, or **3** vs **4**) had little impact on the photophysics of the respective chromophores. The nucleosides displayed red-shifted absorption bands at 369 nm (**1**), 371 nm (**2**), and 357 nm (**3** and **4**), longer wavelengths than those of the parent PydC (343 nm) and PyC (342 nm) chromophores in water (Figure 2A). These bands were further red-shifted when the nucleosides were dissolved in dioxane, an aprotic, less polar solvent. Somewhat surprisingly, in aqueous solution, the thiophene extended analogs displayed only slight shifts in emission maxima, 469 nm (**1**) and 473 nm (**2**), from the parent nucleosides PydC (463 nm) and PyC (461 nm). However, the significantly higher quantum yields of 0.41 and 0.43 for **1** and **2**, respectively, are over nine times that of PyC (0.04) and PydC (0.05). The quantum yields of **1** and **2** showed moderate sensitivity to solvent polarity, exhibiting a hypsochromic shift and increase of fluorescence in dioxane. The exceptional property of **1** and **2** is the brightness value ($\Phi\varepsilon$), which is primarily due to large extinction coefficient (ε) values of

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Table 1. Photophysical Data for Nucleosides **1**–**4**

compd	solvent	λ_{abs} (nm)[a]	λ_{em} (nm)	ε[b]	Φ[c]	Stokes shift[d]	brightness ($\Phi\varepsilon$)	polarity sensitivity[e]
PydC	H_2O	342	461	4.03	0.05	7.6	0.20	71
	dioxane	351	439	5.00	0.10	5.7	0.50	
PyC	H_2O	343	463	5.04	0.04	7.6	0.20	67
	dioxane	349	439	5.00	0.10	6.0	0.50	
1	H_2O	369	469	11.6	0.41	5.7	4.8	70
	dioxane	383	449	13.6	0.50	3.9	6.8	
2	H_2O	371	473	12.9	0.43	5.9	5.5	73
	dioxane	379	449	15.0	0.47	4.0	7.1	
3	H_2O	357	474	2.38	0.01	7.0	0.024	75
	dioxane	367	447	3.16	0.70	4.9	2.2	
4	H_2O	357	478	2.42	0.01	7.1	0.024	74
	dioxane	365	447	3.21	0.74	5.0	2.4	

[a] Long λ maximum. [b] 10^3 $M^{-1}cm^{-1}$. [c] Relative quantum yields. [d] 10^3 cm^{-1}. [e] Polarity sensitivity values are expressed in $cm^{-1}/(kcal \cdot mol^{-1})$ and represent the slope of the line in the plot of Stokes shift versus solvent polarity (see SI for details).

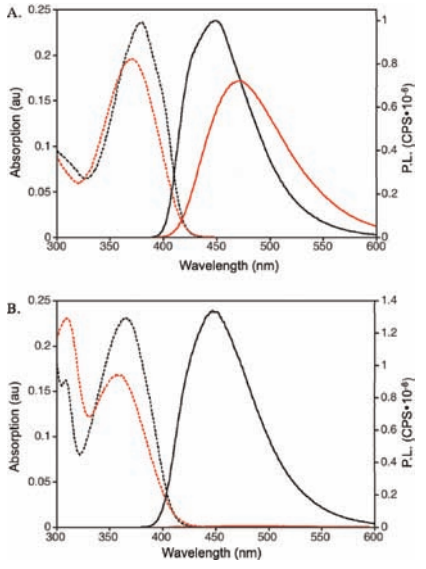

Figure 2. Absorption (dashed) and emission (solid) spectra of **2** (A) and **4** (B) in water (red) and dioxane (black).

11.6×10^3 and 12.9×10^3 M^{-1} cm^{-1} (**1** and **2**, respectively, in water). Both nucleosides show a linear correlation between Stokes shift and solvent polarity with reasonable polarity sensitivity values (see Supporting Information (SI)). Notably, both the absorption and emission spectra display sensitivity to solvent polarity for the entire nucleoside series.

The fused thiophene PyC analogs exhibited photophysical characteristics that were distinctive from the thiophene extended system (see Figure 2B). In water, nucleosides **3** and **4** exhibited the most red-shifted emission maxima (474 and 478 nm, respectively) along with the lowest emission intensity of this nucleoside series. In dioxane, the increased emission intensity is dwarfing in comparison to that in water, with maxima at 447 nm. While the Stokes shifts of nucleosides **3** (7.0×10^3 cm^{-1}) and **4** (7.1×10^3 cm^{-1}) were comparable to the parent PydC and PyC (7.6×10^3 cm^{-1}), they were higher than **1** (5.7×10^3 cm^{-1}) and **2** (5.9×10^3 cm^{-1}). Most striking is the extreme sensitivity that the quantum yields of **3** and **4** exhibited to solvent polarity. In the polar protic environment of water, **3** and **4** both displayed a very low quantum yield of 0.01. However, in an aprotic and less polar environment, the quantum yields of **3** and **4** increased dramatically. These quantum yield values of 0.70 and above are exceptional, considering the minimal structural modification from the virtually nonemissive cytidine nucleosides. This increase occurs concomitantly with a decrease in Stokes shift resulting in values of approximately 5×10^3 cm^{-1} for both nucleosides. Nucleosides **3** and **4** have similar ε in both water (2.38×10^3 and 2.42×10^3 M^{-1} cm^{-1}) and dioxane (3.16×10^3 and 3.21×10^3 M^{-1} cm^{-1}), which are significantly lower than those of nucleosides **1** and **2**. This results in comparatively lower brightness values for **3** (0.024 in water and 2.2 in dioxane) and **4** (0.024 in water and 2.4 in dioxane). Notably, the brightness values for **3** and **4** are nearly 100-fold higher in dioxane than in water.

In summary, the PyC family is joined by new members. The effective syntheses of nucleosides **1**–**4** via novel, metal-catalyzed cyclization reactions resulted in four nucleosides displaying complementary photophysical properties. These nucleosides make a significant and diverse addition to the growing toolbox of nonperturbing, isomorphic, fluorescent analogs.

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Supporting Information Available. Experimental procedures, NMR data, and detailed photophysical data and calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.