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SYNTHESIS OF *N*1-UNSUBSTITUED 5-ALKYNYLCYTOSINE AND DERIVATIVES THEREOF

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Abstract – The first examples of 5-alkynylcytosines (**3**) lacking other ring substitution have been synthesized directly from Sonogashira coupling of 5-iodocytsoine (**1**) with terminal alkynes. Alternatively, 7*H*-pyrrolo[2,3-*d*]-pyrimidin-2(*H*)-ones (**4**), via domino cross-coupling and cyclization reactions were prepared from *N*4-benzoyl-5-iodocytosine (**2**). Both the 5-phenylethynyl-cytosines and bicyclic derivatives show substituent-dependent fluorescence.

Common nucleosides consist of an aglycone or heterocycle linked to D-ribose or 2'-deoxy-D-ribose by a glycoside bond. Some synthetic nucleosides have found use as luminescent reporter groups when incorporated into oligonucleotides (Figure 1).[1,2,3] Alternatively, the aglycone has potential for use as a selective small molecule probe for a complementary nucleobase[4] or may be a substrate in a glycosylation reaction to prepare unusual nucleosides.[5] Herein we report the synthesis of 5-alkynylcytosines (**3**) from 5-iodocytosine and the related 7*H*-pyrrolo[2,3-*d*]pyrimidin-2(*H*)-ones (**4**), otherwise known as pyrrolocytosine, from *N*4-benzoyl-5-iodocytosine (**2**). Notably, these derivatives lack any other ring substitution.[6,7] The pyrrolocytosines are water-soluble and intrisincally luminescent as are the 5-phenylethynylcytosines.[8]

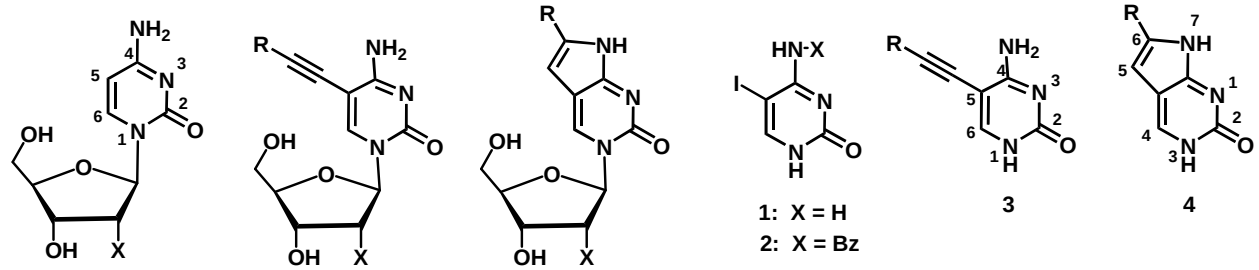
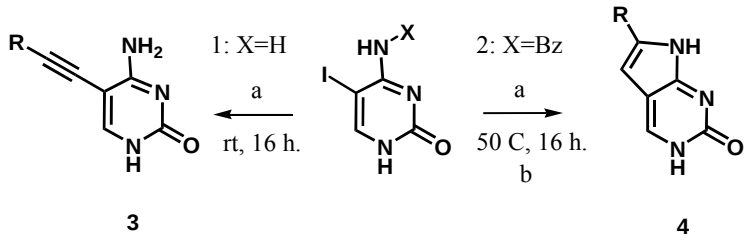


Figure 1

The key step in the synthesis of compounds (**3**) or (**4**) begins with the use of the appropriate aryl iodide. When 5-iodocytosine (**1**)[9] is used in the Sonogarshira reaction, the expected cross-coupling with terminal alkynes is clean, without any annulated byproducts observed. When the exocyclic amino group is converted to a benzamide (**2**), a domino cross-coupling, annulation sequence of reactions occurs without observation or isolation of the supposed 5-alkynylcytosine intermediate (Scheme 1).[10]



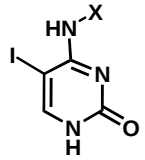
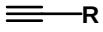
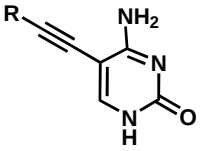
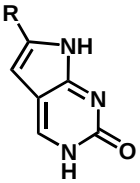
3 **4**

Scheme 1. Reaction conditions, 2.1 mmol scale for **1** and **2**: a. 10 mol % $Pd(PPh_3)_4$, 20 mol % CuI, 3 eq. NEt_3, 3 eq. alkyne, in 10 mL of dry DMF. b. reflux EtOH, 16 h to remove the benzoyl group.

The reactions proceed to complete consumption of the aryl iodide according to thin-layer chromatographic analysis of the reaction mixtures, but the purified yields are substantially less because of difficulty in isolation the desired product. The synthesis of the 5-alkynylcytosines is facile for the four alkynes reported, and the products were conveniently isolated by precipitation from DCM followed by elution through a silica plug using neat MeOH. The pyrrolocytosines were prepared in two steps: cross-coupling and annulation; followed by debenzoylation by refluxing in ethanol. The formation of the bicyclic nucleobase is conveniently followed in the ^{1}H NMR spectrum wherein there is the appearance of a characteristic alkenyl resonance associated with the pyrrole ring (δ (ppm, DMSO-d_6): **4a**: 6.68; **4b**: 6.52; **4d**: 6.14) and a downfield shift of the proton on C4 and the protons associated with the substituent on C6. The isolation of the pyrrolocytosines is more difficult than the 5-alkynyl cogeners because they possess significant solubility in water and a simple extractive workup must be avoided. As well, these highly polar compounds were troublesome to purify by silica gel column chromatography and these factors resulted in a diminished overall yield (Table 1). Despite the modest yield, this route is comparable to other modern methods for the synthesis of unsubstituted pyrrolocytosine (30% over 3 steps).[11] In addition, the *p*-nitrophenylethynylcytosine resisted cyclization and compound (**3c**) was isolated despite starting with compound (**2**), under conditions which succeed for the other alkynes. The use of silver nitrate[12] to catalyze the 5-*endo-dig* cyclization reaction of 5-phenylethynylcytosine was not successful. The lack of reactivity of this compound is likely related to poor Lewis Acid binding ability of the electron deficient alkyne because of the strong electronic effect of the *para*-nitrophenyl subsitutent. Evidence for the formation of compound (**4c**) was found only after extended reaction times (Sonogashira conditions, 8

d) by examination of the ^{1}H NMR spectra, but always as a mixture with **3c**. Attempts to separate **4c** from **3c** proved unsuccessful due to their poor solubility and near identical chromatographic behavior under a variety of conditions. Due to these difficulties: necessity of prolonged reaction time; exceedingly difficult purification; and poor solubility, compound (**4c**) was not pursued further.

Table 1. Spectral Characterization of 5-Alkynyl- and Pyrrolocytosines.

Aryl iodide (X)	Alkyne (R)	Product (% yield)		Spectral Properties[a]			
				ε ($l \cdot mol^{-1} \cdot cm^{-1}$)	λex (nm)	λem (nm)	Rel. Int.
H	Ph	**3a**, 82%	-	9600	303	390	22
H	*p*-(MeO)Ph	**3b**, 84%	-	1750	291	436	60
H	*p*-(NO_2)Ph	**3c**, 71%	-	5400[b]	397[b]	598[b]	-
H	CH_3OCH_2	**3d**, 77%	-	2260	310	401	1
Bz	Ph	-	**4a**, 44%	1260	356	448	350
Bz	*p*-(MeO)Ph	-	**4b**, 49%	2500	362	462	490
Bz	*p*-(NO_2)Ph	**3c**, 70%[c]	**4c**, NA	-	-	-	-
Bz	CH_3OCH_2	-	**4d**, 36%	2550	335	438	260

a. UV-vis and fluorescence spectra were measured as 12.5 μM solutions in reverse-osmosis purified waters (18 MΩ). Molar extinction coefficients (ε) were determined at the excitation wavelength (λex). Relative fluorescence intensity (Rel. Int.) was determined by comparing the steady-state fluorescence (counts per second) measured under identical instrumental conditions at the wavelength of maximum emission (λem). Degassing was not necessary for the organic or aqueous solutions. b. Measured in DMSO. c. Only compound (**3c**) was isolated under the standard conditions (16 h, 50 °C), under extended reaction time, (**4c**) was identified in the reaction mixture but could not be isolated.

Under the conditions of measurement, phenyl-substituted pyrrolocytosines (**4a**, **4b**) demonstrate better relative fluorescence than the alkyl substituted pyrrolocytosine (**4d**). Further, the bicyclic heterocycles show better fluorescence than the 5-phenylethynylcytosines (**3a-d**). It is interesting to note that the 5-phenylethynylcytosines are intrinsically luminescent – neither the phenylacetylene nor the cytosine possess any fluorescence of their own. For both the monocyclic and bicyclic heterocycles, the presence of a *para*-methoxy substituent increases the relative fluorescence. Alkylethynylcytosine (**3d**) is the least emissive compound in this series and is not visibly fluorescent at the concentrations used.

Although it is well recognized that pyrrolocytosines are luminescent and have found some use as a reporter molecule, this work has shown that simple 5-phenylethynylcytosines also exhibit substituent-

dependent luminescent properties. These types of molecules are potential fluorescent probes or components for the synthesis of unnatural nucleosides.

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