Blue fluorescent deoxycytidine analogues: convergent synthesis, solid-state and electronic structure, and solvatochromism†

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We report the synthesis and photospectroscopic characterisation of intrinsically fluorescent triazole-appended cytidines. Fluorescence was found to be highly dependent on solvent conditions. X-Ray crystallographic data show the proton of the exocyclic amine of the nucleobase and the triazole N^3 engaged in a H-bond.

Introduction

Modified nucleosides have been used in nucleic acid chemistry for over half a century. Luminescent derivatives have enjoyed much success, finding use as nucleosides,[1] nucleotides,[2] or within the context of oligomers.[3] Fluorescent probes may be divided into two general classes: (1) those bearing a remotely appended fluorophore, separated electronically and in-space from the base-pairing face, and (2) those in which the nucleobase is integral to the fluorophore. Members of the latter class are especially attractive because their fluorescent response can report on the microenvironment of the nucleobase.

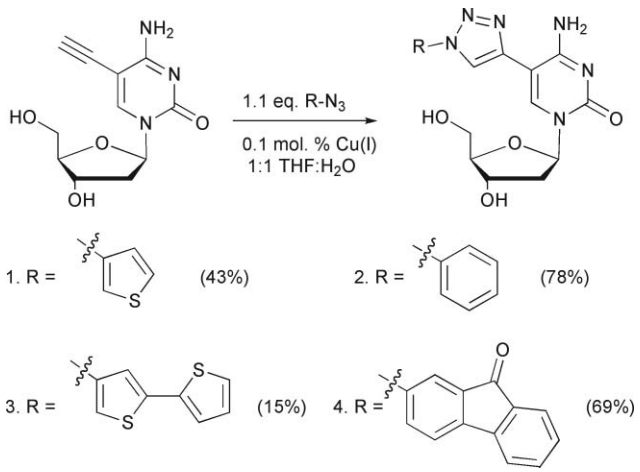
The Cu(I)-catalysed Huisgen cycloaddition of azides and alkynes, to give 1,4-disubstituted 1,2,3-triazoles,[4] has been exploited for rapid drug discovery,[5] for conjugation of modified biological macromolecules,[6] for derivatization of nanoparticles[7] and, more relevant to this work, for the modification of nucleosides and DNA.[8] Ethynyl- and octadiynyl-modified uracil are the most commonly used substrates for this "click" chemistry, with coumarin dyes, benzyl groups and biotin labels being attached at the 5-position. Generally, the 5-position of pyrimidines is a useful site for manipulation due to the ease of synthesis of such derivatives and the propensity for substituents appended here to be well accommodated in the major groove whilst leaving the Watson–Crick face unhindered.[3] The purine analogues, 7-deazaadenosine and 7-deazaguanosine, have been used to similar ends,[8a,b] and recently, 8-ethynyladenosine has been employed in "click" chemistry.[8c]

The corresponding cytidine analogues are notable by their absence. At the time of writing, there are no reports of the reactivity of 5-ethynylcytidine towards azides, nor have there been studies of the fluorescence of any conjugated triazolylnucleosides in the absence of extraneous fluorophores with respect to cytidine.[9] Therefore, we deemed fluorescent triazolyldeoxycytidines to be attractive targets for potential use in the various facets of nucleic acid chemistry.

Results and discussion

Synthesis

Our synthesis of these compounds is shown in Scheme 1. The ethynyl-containing starting material was readily prepared in two steps from 2′-*O*,5′-*O*-diacetyl-5-iodo-2′-deoxycytidine, which itself was made from the parent 2′-deoxycytidine using slightly modified literature procedures.[10] Sonogashira coupling of the iodonucleoside with trimethylsilylacetylene in deoxygenated THF at room temperature using CuI and $Pd(PPh_3)_4$ (0.1 mol%) as catalysts, and subsequent deprotection, yielded 5-ethynyldeoxycytidine in 75% overall yield from 2′-deoxycytidine. This alkyne was then coupled to four aryl azides in the presence of Cu(I) (generated *in situ* from $CuSO_4$ and sodium ascorbate) in a 1 : 1 THF–H_2O solution. The resulting triazole-containing compounds **1–4** were characterised by ^1H and $^{13}C\{^1H\}$ NMR spectroscopies, and HRMS. X-Ray diffraction data were collected for **2** (see the ESI†).



Scheme 1

Derivatives **1** and **3** were targeted for their likely visible fluorescence and also for onward electrochemical polymerization

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Table 1 Fluorescence quantum yields (Φ), and excitation (Ex) and emission (Em) maxima (λ, nm) for compounds **1–4**

	$\Phi_{(H_2O)}$	$\Phi_{(EtOH)}$	$\lambda_{Ex\ (EtOH)}$	$\lambda_{Em\ (EtOH)}$
1	0.0019	0.011	330	375
2	0.0025	0.013	330	380
3	0.0062	0.066	320	380
4	0.0055	0.089	320	385

and attachment to surfaces; this work is currently under study. A fluorenyl-substituted nucleoside (not shown) was similarly investigated for fluorene's inherent fluorescence; however, repeated *in situ* oxidation of this compound to **4** under the synthetic conditions led us instead to develop a rational synthesis of the fluorenonyl derivative from the corresponding azide.

Photoluminescence

All of the deoxycytidine analogues were fluorescent in the blue region (375–385 nm). Quantum yields ranged from low to moderate, the highest being observed for **3** and **4**, for which Φ = 0.066 and 0.089, respectively in EtOH (Table 1).[11] These values are comparable to the useful fluorophore methylpyrrolocytosine, which has been employed for DNA structural elucidation in recent years.[12]

Unusual, mutually consistent solvatochromatic effects were observed for all compounds. On changing the solvent composition from H_2O to EtOH in *ca.* 10 vol% increments, dramatic variations in emission intensity resulted (Fig. 1). Similar non-linear solvatochromism has been observed for fluorenone-containing compounds and has been explained by the presence of multiple excited states that are differentially stabilized by the solvation shell.[13] The triazole compounds had single broad emission bands at r.t., but at low temperature showed three distinct, closely-separated emission maxima (data not shown). These indicated multiple excitation states that could, in principle, be the source of the observed solvatochromism, although more investigation is required to establish this unequivocally. Only very small shifts in excitation and emission maxima (<5 nm) were observed on varying the solvent composition, although the quantum yields changed substantially, accounting for the observed trends in fluorescence emission intensity.

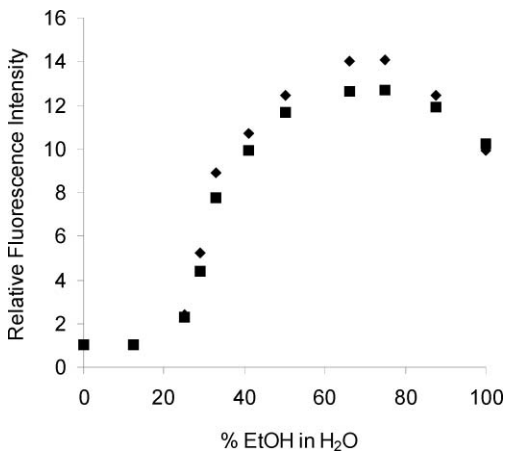

Fig. 1 Variation in steady state room temperature emission intensity of compounds **3** (♦) and **4** (■) at their respective maxima relative to emission intensity in neat H_2O as a function of solvent composition.

The response of a fluorescent nucleobase to changes in solvent is a good predictor of its performance as a base-pairing discriminator because match and mismatch combinations result in differential solvation of the excited state.[3] It is interesting that the triazolylnucleosides synthesised herein react similarly to solvent perturbations regardless of the aryl substituent, which implies that the triazole and the base are an integral part of the fluorophore. A variety of solvents were investigated (CH_2Cl_2, EtOH, 1,4-dioxane, acetone and cyclopentanone; see the ESI†) with no discernable trend when fluorescence emission intensity was plotted against solvent $E_T(30)$ values (see the ESI†).[14]

Structural studies

Single-crystal X-ray diffraction studies revealed that the three aromatic rings of **2** are nearly coplanar in the solid state: torsion angles around the ring junctions are ≤6° (Fig. 2). A proton of the exocyclic amine of the nucleobase and *N*3 of the triazole ring are separated by *ca.* 2 Å, which implies stabilization of coplanarity by an intramolecular H-bond.

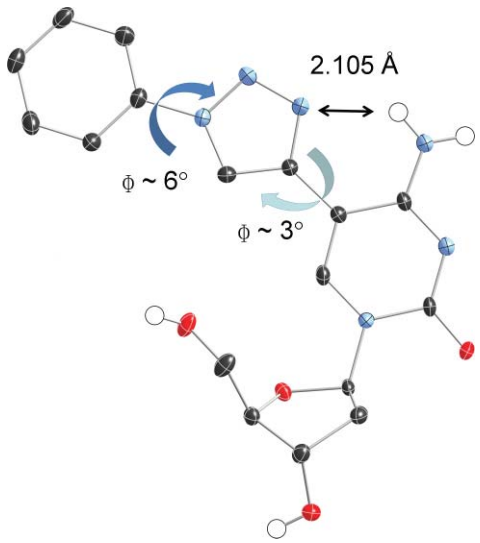

Fig. 2 ORTEP representation of the molecular structure of **2** with ellipsoids at 30% probability showing the dihedral angles about ring junctions and an interatomic distance suggesting a hydrogen-bonding arrangement.‡

Computational studies

Gas-phase DFT calculations were used to elucidate the effect of this H-bond on the HOMO–LUMO gap in **1**, which should be sensitive to the coplanarity of the rings, and also to determine the molecular orbitals (MOs) responsible for the observed fluorescence. Mapping these MOs revealed that the nucleobase

‡ Selected crystal data for **2**·0.5H_2O·0.5MeOH, $C_{17.5}H_{21}N_6O_5$, M = 395.40, monoclinic, $P2_1$ (No. 4), a = 11.3994(14), b = 7.5273(9), c = 21.096(3) Å, β = 97.767 (2)°, V = 1793.6(4) Å^3, Z = 4, T = –80 °C, D_c = 1.464 Mg m^{-3}, μ = 0.110 mm^{-1}, GoF = 1.034, R_1 [$F_o^2 \geq 2\sigma(F_o^2)$] = 0.0521 and w$R_2$ [$F_o^2 \geq -3\sigma(F_o^2)$] = 0.1169. Structural parameters have been deposited with the CCDC.

itself is indeed an integral part of the fluorophore (Fig. 3). In all compounds **1–4**, the HOMO is derived predominantly from the triazole and nucleobase heterocycles, and in all but one case (**4**), there is also a small but significant contribution to the LUMO from the base. This suggests that these analogues may exhibit greater sensitivity to hybridization events than conventional fluorophore-appended nucleosides.

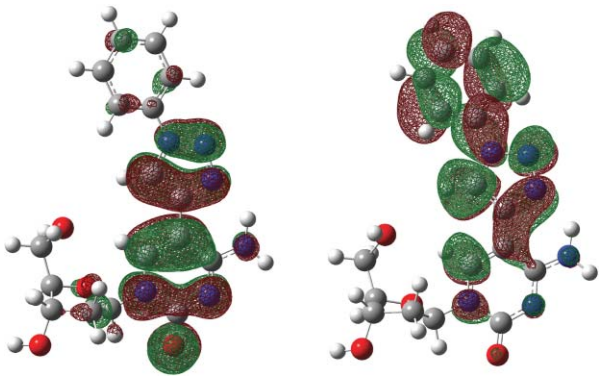

Fig. 3 Representation of the HOMO (left) and LUMO (right) for compound **2**. For **1**, **3** and **4**, see the ESI.†

Fig. 4 shows the variation in the calculated single point energy of **1** with rotation about the triazole–nucleobase bond. As expected, the lowest energy molecular configuration engages the H-bond and has approximately coplanar rings; this is coincident with the smallest HOMO–LUMO gap (4.15 eV). Increasing the interplanar angle to 90° breaks both conjugation and the H-bond and results in an increase in total energy content (by *ca.* 7 kcal mol^{-1}) and in the HOMO–LUMO gap (by *ca.* 0.5 eV). Continued rotation to 180° lowers the HOMO–LUMO gap by reintroducing coplanarity

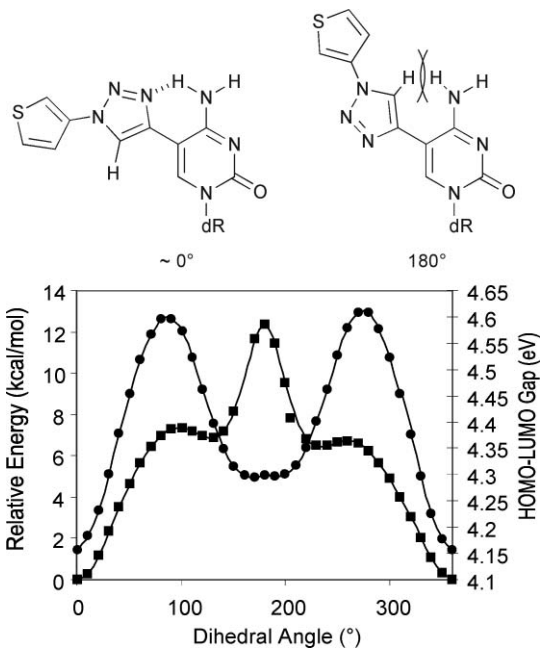

Fig. 4 Representations of **1** at the highest (180°, above right) and lowest energy conformations (0°, above left), and a plot of single-point energies (■) and HOMO–LUMO gap energy (●) *versus* the torsion angle about the nucleobase–triazole bond.

but at the expense of total energy, which climbs steeply a further 5 kcal mol^{-1} on account of steric clash between a nucleobase N–H and the triazolyl C–H groups (Fig. 4).

These data are consistent with our general observation of increasing fluorescence intensity with decreasing solvent polarity: H-bond stabilization in less polar solvents gives enhanced planarity and greater conjugation. This stabilization should be further increased by Watson–Crick base pairing, which magnifies the protic nature of the exocyclic amine. These studies are currently in progress.

Conclusions

A new family of blue fluorescent nucleoside analogues has been made and their potential for use as reporter groups on their environment has been evaluated through study of their photophysical properties. Compounds **3** and **4** possess comparable fluorescence to probes currently being applied in nucleic acid chemistry and are unusually sensitive to the nature of the solvent. The crystal structure of **2** reveals the presence of an intramolecular H-bond which enhances the coplanarity of the heterocycles. These X-ray diffraction data are the first for any triazolyl-nucleosides, despite the great attention paid to the class represented in the literature.

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