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PAPER

Click fleximers: a modular approach to purine base-expanded ribonucleoside analogues†

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The synthesis of nucleoside analogues incorporating 4-(5-pyrimidinyl)-1,2,3-triazole aglycons as expanded purine nucleobase mimics were accessed using the copper-catalyzed azide–alkyne Huisgen cycloaddition between a ribosyl azide and 5-alkynylpyrimidines. Depending on the nature of the alkyne employed, other nucleoside analogues that possess fluorescence or potential metal-binding properties were prepared. Computational studies were undertaken on the purine analogues and indicate that the heterocycles of the unfused nucleobase prefer a coplanar arrangement and the *anti*-glycosidic conformer is favoured in most instances.

Introduction

The synthesis of a new class of expanded nucleobases termed "fleximers" was first described by Seley in 2001.[1,2] The separation of a purine nucleobase into its imidazole and pyrimidine components attached *via* a single C–C bond effectively converted a rigid fused bicyclic system into one that permitted conformational freedom.[3,4] These compounds were designed to be flexible bioprobes for enzymes. In this vein, a fleximer inhibitor of *S*-adenosyl-L-homocysteine hydrolase has been discovered[5] and an azido derivative of a G-fleximer has been used in photoaffinity labelling of catalytically important amino acids in the active site of GTP fucose pyrophosphorylase.[6]

The first generation fleximers possessed a *C*5-imidazole to *C*6-pyrimidine attachment. The second generation fleximers were constructed with a *C*4-imidazole to *C*5-pyrimidine linkage which alters the trajectory of the base-pairing moiety relative to the former attachment (Fig. 1).[7,8]

The synthesis of both generation fleximers was synthetically challenging and given the potential utility of fleximers, we were inspired to examine an alternative route to related analogues. Some recent research in our group has focused on the derivatization of 5-ethynyl-2′-deoxycytidine *via* the copper catalyzed azide–alkyne Huisgen cycloaddition (CuAAC),[9] and recognizing the structural similarity between this class of molecule and fleximers motivated the current synthetic study (Fig. 2). By replacing the imidazole in Seley's second generation fleximers with a 1,2,3-triazole we envisioned a facile synthesis using the CuAAC.

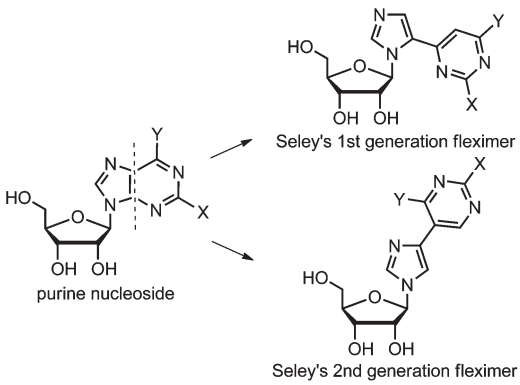

Fig. 1 Comparison of a generic purine nucleoside to fleximer analogues.

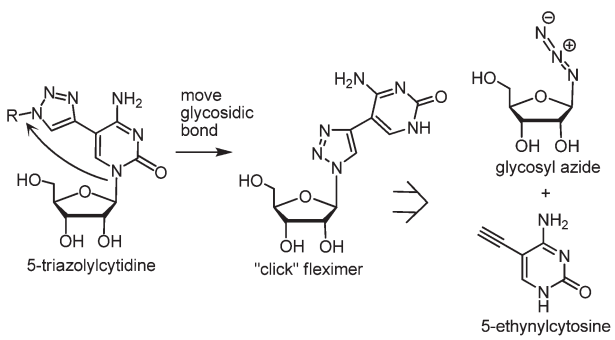

Fig. 2 Design of nucleoside analogues accessed by click chemistry.

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† Electronic supplementary information (ESI) available: Complete experimental details and characterization of compounds **2c**, **2d**, **3c**, **3d**, **4a–g**, **5a–g** and the fluorescence data for **4f**, **4g**, **5d**, **5f**, and **5g** and details of the computational studies. See DOI: 10.1039/c2ob25678a

Of course, the popularity of CuAAC has led to its wide exploitation in nucleoside, nucleotide and oligonucleotide chemistries.[10] Ribosyl azides have been employed in CuAAC to produce *N*-triazolylglycosides predominately with a preponderance of aromatic or alkyl alkynes that do not possess nucleobase-like functionality.[11–14] In related work, *N*-1 or *N*-9 propargyl nucleobases have been reacted with 1-azido-2-deoxyribofuranose under CuAAC conditions to produce tethered bases which may be viewed as methylene-spaced fleximer homologues.[10] To date, there have been no reported synthetic efforts toward nucleoside fleximers accessed *via* CuAAC, which we now report, along with two new nucleoside fluorophores conceptually related to "fluorosides".[15]

Results and discussion

We chose to target the ribonucleosides in order to exploit the β-directing ability of the 2′-hydroxyl protecting group during Lewis-acid catalyzed glycosyl azide formation. The peracylated riboses, acetyl or benzoyl, were synthesized from D-ribose following literature procedures.[12,16] Although the preparation of the peracyl-β-azidoribose is reported as being highly stereoselective,[16] under a variety of reaction conditions we were unable to reproduce these results. Consequently, the α-anomer was removed by column chromatography, which was easier to achieve for the benzoyl-protected ribose (**1**).

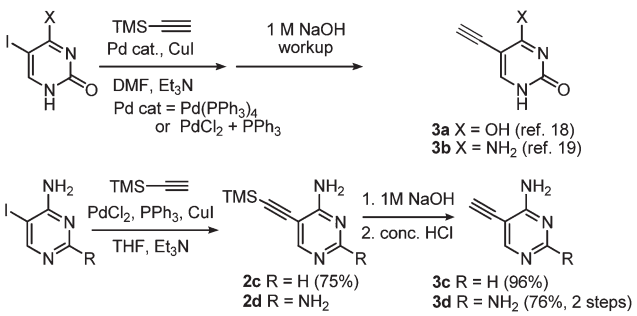
For the selection of alkynes the easily accessible uracil (**3a**), cytosine (**3b**) and 4-aminopyrimidine (**3c**) alkynes were chosen to produce analogues of xanthosine (X), isoguanosine (isoG) and adenosine (A), respectively. The 2,4-diaminopyrimidine alkyne (**3d**) was selected as a mimic of diaminopurine riboside (DAP). The 2-pyridinyl (**3e**) alkyne was chosen for its potential ability as a bidentate ligand for metal chelation.[17] The alkynes **3f** (fluorene) and **3g** (pyrene) were selected as fluorescent reporter groups with the potential to form intercalation complexes.

The 5-ethynylpyrimidines of uracil (**3a**) and cytosine (**3b**) were synthesized based on published procedures.[18,19] The 5-iodopyrimidine was used in a Sonogashira coupling with TMS-acetylene, followed by removal of the TMS group under alkaline conditions. The 4-aminopyrimidine and 2,4-diaminopyrimidine alkynes (**3c** and **3d**) were synthesized from the 5-iodopyrimidines in 72% and 76% yield, respectively (Scheme 1).

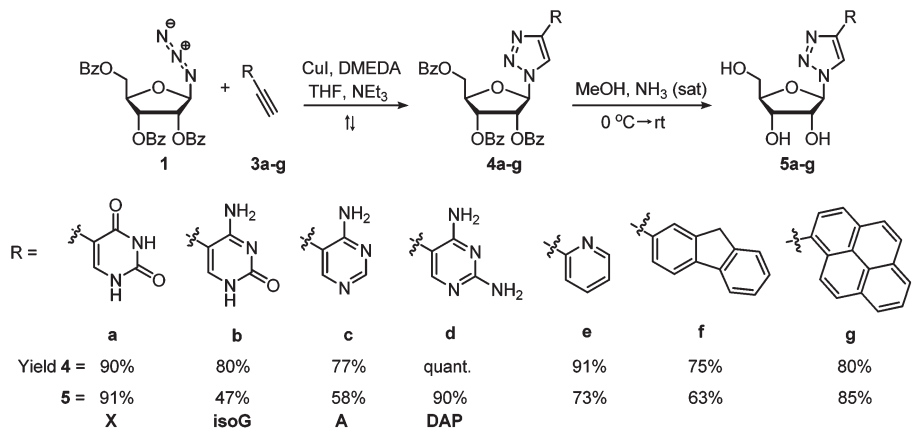
These nucleobases lack an acidic imide which then dictated an acidic work up to afford water solubility. The synthesis of the 2-ethynylpyridine (**3e**) followed literature precedent from 2-bromopyridine.[20] The fluorescent alkyne 2-ethynylfluorene (**3f**) was synthesized following literature procedure from fluorene[21] and 1-ethynylpyrene (**3g**) was obtained commercially.

The CuAAC to produce the benzoyl-protected click fleximers required more forcing conditions than typical click reactions.[22] The optimized conditions were refluxing THF and CuI paired with the metal chelator *N,N′*-dimethylethylenediamine thus producing **4a–g** in 75% to quantitative yield (Scheme 2). Compounds **4a–c** were discovered to be organogelators while attempting to characterize the compounds in $CDCl_3$. Previously, the ability of nucleosides to gelate solvents has been investigated for use as supramolecular templates or as drug delivery assemblies.[23] 1,2,3-Triazole containing nucleosides have been discovered to be hydro- and organogelators, a property attributed to the perpendicular nature of their interactions (base pairing and π–π stacking),[24,25] so it was not completely surprising that the benzoyl-protected nucleoside click fleximers **4a–c** were able to gelate organic solvents. The gelation properties were not investigated fully; however, it was observed that reversible gels were formed in $CHCl_3$, EtOAc, and DMSO. Despite its structural similarity to aminopyrimidine **4c**, the 2,4-diaminopyrimidine click fleximer **4d** was found to be freely soluble in $CHCl_3$.

The pyridine compounds **4e** and **5e** have been reported previously but were not examined for metal binding properties at that time.[14] Subsequently, it has been reported that the



Scheme 1 Synthesis of 5-ethynyl-pyrimidines.



Scheme 2 Synthesis of "click fleximers" **5a–g**.

Table 1 Steady state fluorescent properties of click fleximers

	Excitation λ_{max} (nm)	Emission λ_{max} (nm)	Φ_F EtOH	Φ_F H_2O
4f	292	317	0.16	—
4g	352	383	0.16	—
5d	311	356	0.48	0.05
5f	311	331	—	0.06
5g	346	382	—	0.46

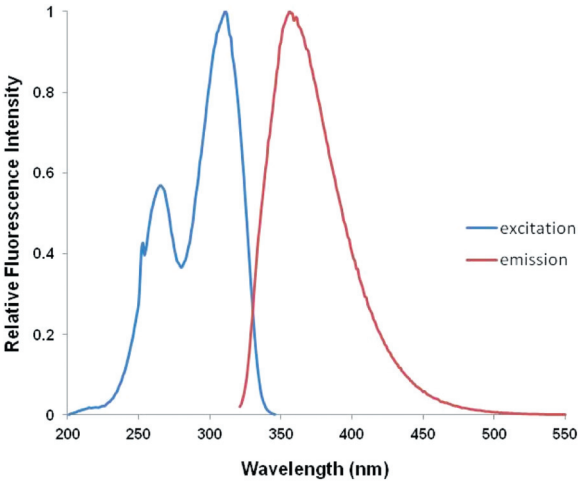


Fig. 3 Steady state fluorescence spectra for **5d** (10^{-5} M in EtOH): excitation (blue line) and emission (red line).

2-pyridinyl-4-[1,2,3-triazole] moiety acts as a metal chelator for several metals including Pt^{2+}, but not in the context as a nucleoside analog.[17] Preliminary studies in our lab with a 2-pyridinyl-4-[1,2,3-triazole] analogue have shown the ability to form a complex with Ni^{2+}, in the context of modified peptide nucleic acids.[26]

The fluorescence properties of the selected fleximers were studied (Table 1). The fluorescent quantum yields (Φ_F) were determined in EtOH, for solubility of the benzoyl-protected click fleximers, or in water for the free nucleosides. Removal of the benzoyl protecting groups in compounds **4a–g** was achieved by suspension in methanolic ammonia to yield **5a–g** in 47–91%. Somewhat to our surprise, the diaminopyrimidine fleximer **5d** displayed purple fluorescence when examined under long-wavelength UV irradiation (360 nm) on a TLC plate, whereas the other nucleobase triazoles were not obviously emissive. The fluorescence properties of **5d** (Fig. 3) were characterized along with the other fleximers incorporating known luminophores.

The fluorescence emission spectrum of the pyrene fleximer **4g** displayed an elongated tail from 450–550 nm which is not due to excimer formation as revealed by examination of the concentration dependence of the spectra (see ESI†). Both the fluorene and pyrene exhibited significant solvatochromism. The Φ_F of the fluorene fleximer **5f** increases approximately 3-fold from water (0.06) to the less polar EtOH, indicating its potential usefulness an environmentally sensitive fluorescence reporter group. The pyrene derivatives **5g** and **4g** displayed the reverse trend, where the Φ_F increases about 3-fold upon moving from EtOH to H_2O.

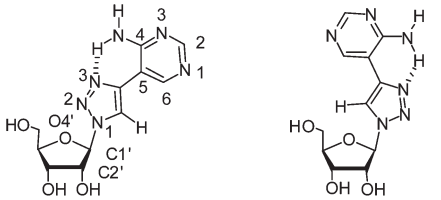


Fig. 4 Nucleoside numbering scheme.

Table 2 Summary of relative conformational energies

	Glycosidic conformation (kcal mol^{-1}) *syn-*	*anti-*	Minimum barrier to rotation of heterobiaryl bond[a] (kcal mol^{-1})
X: **5a**	0.509	0	11.9
IsoG: **5b**	−1.338	0	4.8
A: **5c**	1.965	0	7.1
DAP: **5d**	2.167	0	6.1

[a] Estimate of the minimal barrier from the energy *vs.* torsion profiles for the N^1-methyl base analogues. The transition state energy was found rigorously only for **5c**, and had a relative energy of 7.28 kcal mol^{-1}.

The click fleximer **5d** is strongly emissive in EtOH (Φ_F = 0.48) and much less so in H_2O (Φ_F = 0.05). This large change in emissivity may be useful for studying its interaction with enzymes or as a reporter group if incorporated into oligomers.

The conformations for the flexible nucleoside analogues were studied computationally using DFT methods (Bb3lyp/6-31+G**) implemented by Gaussian 09 (see ESI† for details).[27] Favoured glycosidic conformers and the favoured orientation of the heterobiaryl bond were identified as well as barriers to rotation about this bond. The nucleosides are described by the numbering scheme and nomenclature given in Fig. 4. The conventional numbering for the ribose and pyrimidine are preserved and the glycosidic linkage is assigned to N^1 of the triazole. The *anti* glycoside is defined when the triazole H^5 is *anti* to the ribose ($O^{4'}$–$C^{1'}$–N^1–C^5 torsion angle ~ −130°, or +230°) whereas the syn conformer places the triazole H^5 over the ribose ring ($O^{4'}$–$C^{1'}$–N^1–C^5 torsion angle ~63°).

The relaxed structures of the fleximers **5a–5d** maintained 3′-endo-like ring pucker that is usual for ribosides. The *anti*-glycosidic conformer was the lowest energy structure for fleximers A (**5c**, shown below, Fig. 5), X and DAP, whereas the *syn* conformation was slightly preferred by isoG (see ESI†), Table 2. Observed in both the *anti*- and *syn*- conformations, the triazole ring (either N^2 or H^5) is oriented to nearly bisect the furanose ring (Fig. 5a). The energy profile for rotation about the glycosidic bond indicates that the *anti*-conformer is favoured by nearly 2 kcal mol^{-1}. The higher energy structures correspond to eclipsing interactions of N^1–C^5(triazole) with $C^{1'}$–$C^{2'}$(points 21, 22) or $O^{4'}$–$C^{1'}$ (point 13, Fig. 5b).

The *anti*-conformers are potentially stabilized by an $O^{5'}$–H to N^2 hydrogen bond, which is realized in the calculations for fleximers A, X and DAP. The nonconventional hydrogen bond from C^5–H(triazole) to $O^{5'}$, although possible in all the

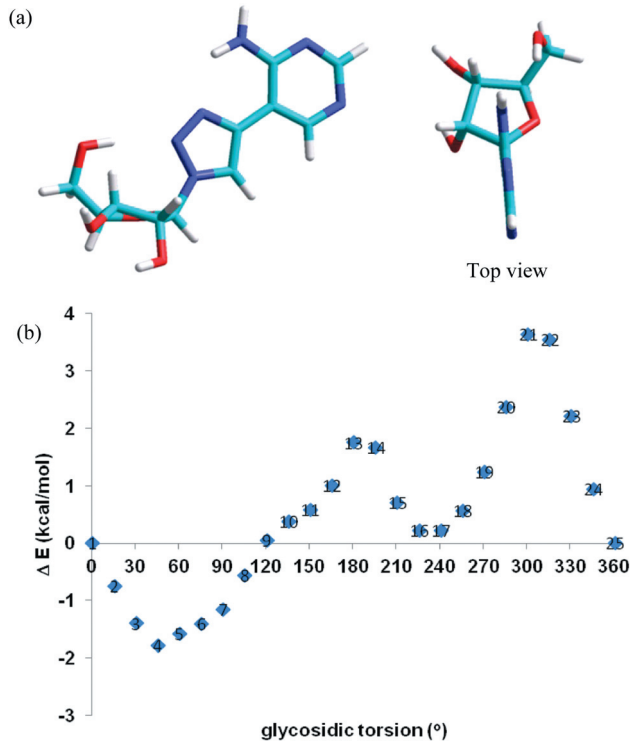


Fig. 5 (a) Molecular representations of fleximer A, **5c**, illustrating ring pucker and orientation of the aglycone of the lowest energy structure #4. (b) Energy profile for stepwise rotation about the glycosidic bond ($O^{4'}$–$C^{1'}$–N^1–C^5 torsion angle) in 15° increments.

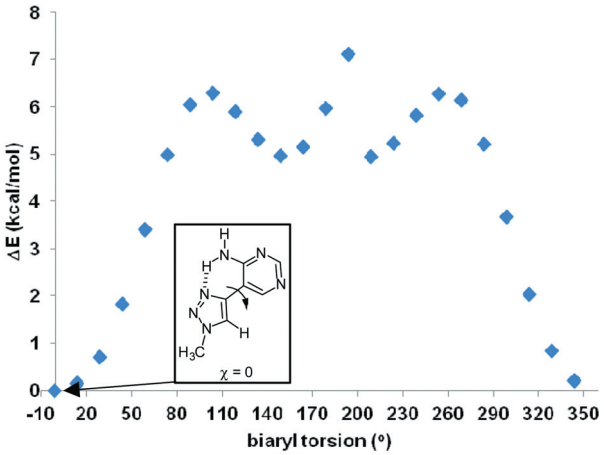


Fig. 6 Energy profile for rotation about the biaryl bond for fleximer A, wherein the ribose was replaced with a methyl group, see ESI.†

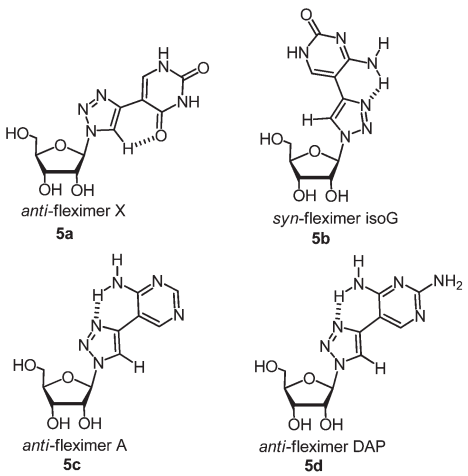


Fig. 7 Representations of the relaxed conformations of the fleximers.

fleximers, is only preferred for isoG. There are no obvious structural features noted that are responsible for this change in glycosidic conformational preference.

The preferred conformation of the biaryl bond was examined for derivatives wherein the ribose was replaced with a methyl group to reduce the computational burden. The conformations are dictated by the presence of an intramolecular H-bond between the triazole ring and the pyrimidine. Each fleximer possesses the ability to form a conventional H-bond between the exocyclic amino group and the triazole N^3 (A, DAP, isoG) or a non-conventional H-bond between the triazole C^5–H and carbonyl O^4 (X) which favours a coplanar arrangement of the rings (Fig. 6).

For example, the lowest energy structure corresponding to a torsion of ~0° (N^3–C^4–C^5–C^4), for fleximer A (**5c**), (Fig. 6), is given along with representations of the relaxed structures of the other fleximers (Fig. 7, details for the calculations are found in the ESI†).

Calculations showed the heterocycles were coplanar which is favoured due to the bonding interactions as shown in Fig. 7. Whereas calculations on Seley's fleximers showed that the aryl groups were not coplanar, the triazole fleximers appear to have a preference for coplanarity.

Conclusions

We have prepared ribonucleoside analogues possessing substituted triazole aglycons by CuAAC. This represents a convergent approach to new fleximer analogues, of which four have been synthesized. These analogues possess subtly different conformational properties than those already known and may usefully augment studies on enzymatic nucleoside substrate acceptance. Importantly, the expanded DAP analogue was found to be luminescent, a property that may be useful for studying its interaction with enzymes or upon incorporation into oligonucleotides. Additionally, it was demonstrated that new ribo-"fluorosides" could be accessed by the same chemical route.

Acknowledgements

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