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Sonogashira alkynylation of unprotected 8-brominated adenosines and guanosines: fluorescence properties of compact conjugated acetylenes containing a purine ring

Andrew G. Firth,[a,b] Ian J. S. Fairlamb,[a,*] Kate Darley[c] and Christoph G. Baumann[b,*]

[a]*Department of Chemistry, University of York, Heslington, York YO10 5DD, UK*
[b]*Department of Biology, Area 10, University of York, Heslington, York YO10 5YW, UK*
[c]*Replizyme Ltd, Genesis 2 Building, York Science Park, Heslington, York YO10 5DQ, UK*

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Abstract—A practical Sonogashira alkynylation protocol for the preparation of 8-alkynylated adenosines and guanosines has been developed. Protection of the sugar hydroxyl substituents is not required; protection hinders the purification of these products. A preliminary fluorescent study is reported, which shows that the presence of a substituent on the phenylene ring influences the fluorescent properties considerably, an outcome that could be utilized in biological applications.

C-Modified nucleosides are used in numerous applications, particularly in pharmaceutical agents and biological probes or mimetics.[1] Covalently modified fluorescent nucleosides are valuable probes of DNA and RNA helix-to-coil transitions, DNA and RNA chain elongation, protein–nucleic acid complexes, mechano-chemical coupling in motor proteins and cellular signal transduction pathways.[2] This drives the innovative discovery of fluorescent nucleoside analogues, especially those that are sterically less bulky, but which still possess desirable fluorescent properties for use in biological systems.

We recognized that donor–acceptor phenylene–ethynylene substituted π-conjugated organic derivatives,[3] possessing low-lying charge-transfer excited states, could facilitate the employment of relatively small fluorescent nucleoside analogues. Clearly, lengthening, shortening, or broadening (through ring expansion) the π-conjugated electron network would alter the fluorophore properties, in essence allowing one to tailor-make analogues for specific applications. It was speculated that the incorporation of a conjugated rigid-rod, attached directly by C-modification at the 8-position on the purine (guanosine and adenosine), would represent a useful way to introduce a mutually compact and effective fluorescent moiety (Fig. 1).

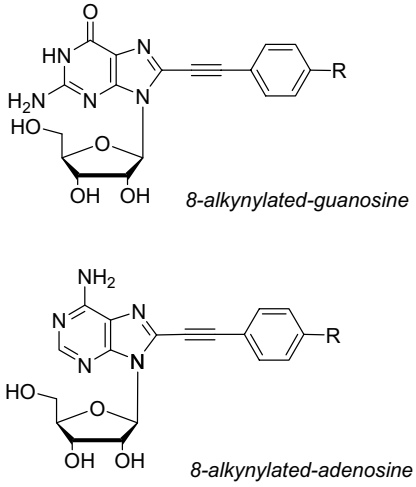

Figure 1. 8-Alkynylated purine nucleosides with potential as compact fluorescent probes.

* Corresponding authors. Tel.: +44 (0)1904434091; fax: +44 (0)1904432516 (I.J.S.F.); tel.: +44 (0)1904328828; fax: +44 (0)1904328825 (C.G.B.); e-mail addresses: ijsf1@york.ac.uk; cb17@york.ac.uk

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The low lying charge transfer energy states of the alkynyl/aryl groups in these derivatives could also be tuned through alteration of the *para*-substituent to modulate the fluorescence properties, thus minimizing the degree of steric changes to the parent phenylene–ethynylene–purine compound. We thus set about preparing a library of linear π-conjugated 8-alkynylated adenosines and guanosines.

Pd-catalyzed cross-coupling reactions[4] have proven effective for the efficient preparation of substituted nucleosides.[5] Alkynylated nucleosides may be accessed by Sonogashira cross-coupling, although surprisingly there are no literature examples detailing the alkynylation of the unprotected 8-bromoguanosine **1** with terminal acetylenes to give **5** (Fig. 2). There are two studies detailing the Sonogashira alkynylation of 8-bromoadenosine **3** to give 8-alkynylated-adenosines **7**. In the first report,[6] no yields or structural characterization for **7** were provided. In the second report, however, some very interesting lipophilic 8-alkynylated adenosines **7** were prepared from **3**,[7] although the successful conditions involved use of a large excess of Et_3N (30 equiv w.r.t. **3**) and terminal acetylene (6 equiv w.r.t. **3**). It has generally been assumed that protection of the hydroxyl or amino substituents in guanosines is required for efficient cross-coupling, which is also required for solubility in common organic solvents.[2e,7]

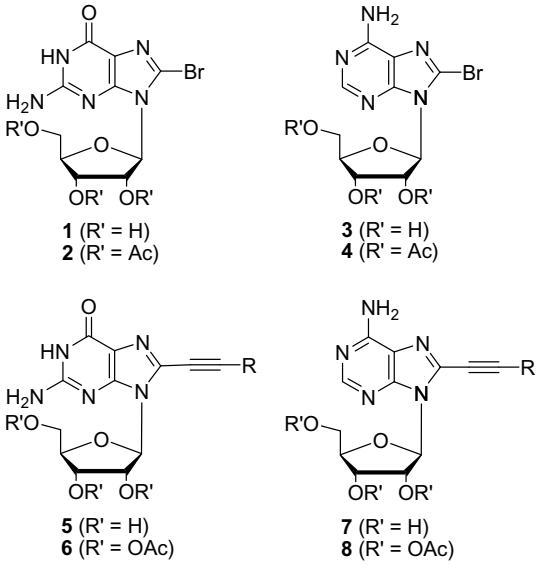


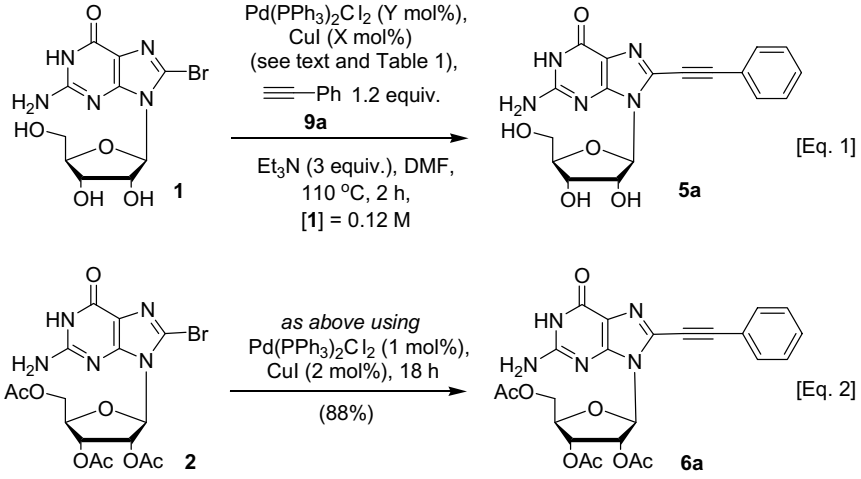
Figure 2.

On a general note, the Pd-mediated processes appear to be more facile with adenosine derivatives.[8] Various guanine coordination modes are possible, for example, *N*-1/*N*-7 and *O*-6 coordination, to Pd (and presumably Cu), which could be a hindrance in cross-coupling brominated guanosine derivatives such as **1**. This could in part explain the unusual lack of studies detailing the cross-coupling reactions of **1**.

Mindful of this, and the potential inhibitory effects of the guanine moiety in particular, we initially investigated the development of an efficient Sonogashira alkynylation protocol for the cross-coupling of **1** with phenylacetylene **9a** to give **5a** (Scheme 1, Eq. 1).

Standard conditions[2e] for Sonogashira alkynylation of **1** were chosen (10 mol % $(Ph_3P)_2PdCl_2$, 10 mol % CuI, 1.2 equiv **9a** and 3 equiv Et_3N in DMF at 110 °C for 18 h). This proved to be a generally ineffective protocol, affording **5a** in yields of ca. 20% with poor purity (believed to be due to the uptake of Pd and/or Cu), which led us to optimize the conditions. Rapid Pd agglomeration and precipitation were thought to contribute to the low yields. An established means to overcome this problem is to employ lower Pd loadings (lower Pd-concentration), an observation derived from our previous experiments involving Sonogashira cross-coupling of aryl halides with terminal acetylenes.[9] It was eventually established that 1 mol % $(Ph_3P)_2PdCl_2$ and 2 mol % CuI proved the most effective catalyst/co-catalyst combination, affording **5a** in 95% yield (after only 2 h).

Under similar conditions, the acetate protected guanosine derivative **2** reacted with **9a** to produce **6a** in 88%



Scheme 1. Sonogashira alkynylation of unprotected and protected 8-bromoguanosines (**1** and **2**, respectively) with phenylacetylene **9a**.

yield (Scheme 1, Eq. 2), which was more cumbersome to purify than **5a** vide infra.[10]

The recent reports of Cu(I)-free Sonogashira alkynylation (formally a Heck alkynylation) led us to assess the affect of Cu(I) on the yields of **5a**.[11] Maintaining the initial Pd(II) concentration constant at 1 mol % it was found that in the absence of Cu(I) **5a** was produced in 18% yield after 2 h (Table 1, entry 1). Moving to 0.5 and 1 mol % Cu(I) gave **5a** in 30% and 38% yields, respectively (Table 1, entries 2 and 3). On going to higher Cu(I) loadings (Table 1, entry 5), the yield of **5a** was reduced (77%). The purity of **5a** was also questionable at the higher Cu(I) loading; the solid is a light green colour, rather than an off-white, which we interpret as being due to the presence of trace quantities of guanosine derived copper complexes (bidentate coordination through *N*-1 and *O*-6).

The purification of **5a** is straightforward. On complete reaction, the mixture is cooled to 40 °C and the DMF removed in vacuo to leave a dark colored solid, which is transferred to a sintered glass funnel and washed with boiling water (this removes $Et_3N{\cdot}HCl$ and any unreacted **1**). The solid is then washed with small quantities of EtOAc and Et_2O to remove any traces of homo-coupled product (produced through the pre-reduction of $(Ph_3P)_2PdCl_2$ with alkynylcuprate). The limited solubility of compound **5a** in boiling water, and the majority of chlorinated and ethereal solvents, facilitates its purification (**5a** is soluble in DMF, DMSO and DMSO/H_2O mixtures, etc.).

Using these optimized conditions,[12] a series of Sonogashira alkynylation reactions of **1** were performed with several terminal acetylenes **9b-g** (Table 2). Good yields were recorded for electron-rich phenylacetylenes **9b** and **9c** (Table 2, entries 1 and 2). The electron-poor phenylacetylenes **9d** and **9e** (Table 2) also worked well. However, for 4-nitrophenylacetylene **9f**, no cross-coupling occurred (Table 2, entry 5). In the last example, 4-chlorophenylacetylene **9g** faired better, although the low yield could not be improved upon under the reaction conditions employed.

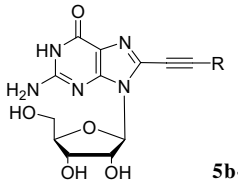
With a series of derivatives of **5** prepared, we turned our attention toward cross-coupling the unprotected 8-bromoadenosine **3** with the same terminal acetylenes (Table 3). High yields were recorded for compounds **6a** and **b** (Table 3, entries 1 and 2).

4-Diethylaminophenylacetylene **9c** reacted to give **7c** in a satisfactory 52% yield (Table 3, entry 3).

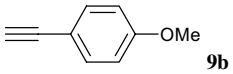
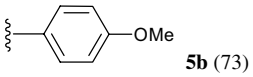
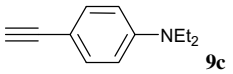
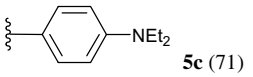
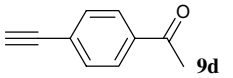
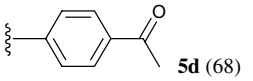
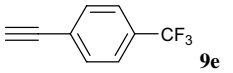
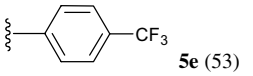
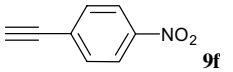
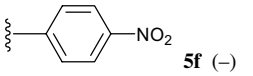
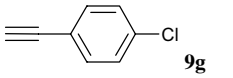
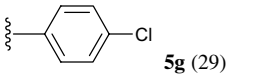
Table 1. Effect of Cu(I) loading on Sonogashira alkynylation of **1**

Entry	CuI loading/mol %	Cu(I):Pd(II) ratio	Yield/%
1	0	—	18
2	0.5	1:2	30
3	1.0	1:1	38
4	2.0	2:1	95
5	4.0	4:1	77

Table 2. Sonogashira alkynylation of unprotected 8-bromoguanosine **1**[a]



5b-g

Entry	Terminal acetylene	Product (R=)/yield (%)
1	 **9b**	 **5b** (73)
2	 **9c**	 **5c** (71)
3	 **9d**	 **5d** (68)
4	 **9e**	 **5e** (53)
5	 **9f**	 **5f** (–)
6	 **9g**	 **5g** (29)

[a] Reagents and conditions: **1** (1 equiv), $(Ph_3P)_2PdCl_2$ (1 mol %), CuI (2 mol %), terminal acetylene (1.2 equiv), Et_3N (3 equiv), DMF, 110 °C, 18 h.

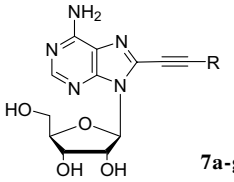
Both 4-acetophenylacetylene **9d** and 4-trifluoromethylphenylacetylene **9e** gave cross-coupled products **7d** and **7e** in 74% and 64% yields, respectively (Table 3, entries 4 and 5). As seen with **1**, the cross-coupling of **3** with 4-nitrophenylacetylene **9f** resulted in negligible cross-coupling.[13] The reaction of **3** with 4-chlorophenylacetylene **9g** again produced the cross-coupled product **7g** in poor yield (Table 3, entry 7).

The difference observed in the reactivity of the terminal acetylenes suggests that the rate of transmetallation and reductive elimination in the catalytic cycle are both affected.[14] In the latter step, a highly sensitive electronic relationship clearly exists between the guanine and adenine heterocycles and terminal acetylene on palladium(II). This is not unusual for heterocyclic compounds, albeit not fully understood.[15]

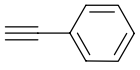
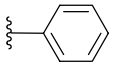
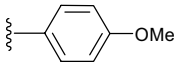
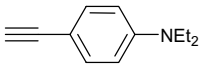
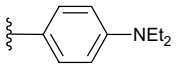
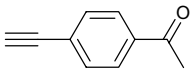
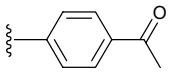
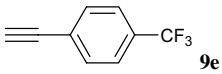
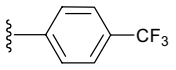
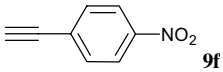
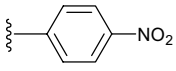
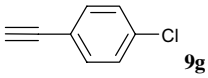
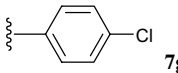
From the results illustrated in Tables 2 and 3, a valuable library of 8-alkynylated guanosines **5** and adenosines **7** are now available (easily prepared in one step from **1** and **3**, respectively), which can be performed on gram scale.

UV–visible spectra reveal an intense absorption in the UV region for all the compounds evaluated. The fluorescence spectral properties of six compounds are presented in Table 4, which are consistent with the S_0 absorption and S_1 emission wavelengths. As expected, the spectrum

Table 3. Sonogashira alkynylation of unprotected 8-bromoadenosine **3**[a]



7a-g

Entry	Terminal acetylene	Product (R=)/yield (%)
1	 **9a**	 **7a** (85)
2	 **9b**	 **7b** (80)
3	 **9c**	 **7c** (52)
4	 **9d**	 **7d** (74)
5	 **9e**	 **7e** (64)
6	 **9f**	 **7f** (–)
7	 **9g**	 **7g** (25)

[a] Reagents and conditions: **3** (1 equiv), $(Ph_3P)_2PdCl_2$ (1 mol %), CuI (2 mol %), terminal acetylene (1.2 equiv), Et_3N (3 equiv), DMF, 110 °C, 18 h.

Table 4. Fluorescence spectroscopic properties of select compounds in DMSO

Compound	λ_{ex}/nm	λ_{em}/nm	Stokes shift[a]/cm^{-1}
5a	370	438	4196
5c	398	502	5205
5e	386	468	4539
7a	350	397	3383
7c	408	506	4747
7e	362	440	4897

[a] Stokes shift $= (1/\lambda_{ex} - 1/\lambda_{em})$.

of **5c** is red-shifted from that of compound **5a** (the parent compound), revealing a smaller HOMO–LUMO gap. Compound **5e**, also red-shifted relative to **5a**, exhibits a slightly higher HOMO–LUMO gap than **5c**. Similar trends are seen with the adenosine compounds **7a**,**c** and **7e**, although the excitation maximum shift of **7c** relative to compound **7a** (the parent compound) is greater (see Fig. 3).

The linear π-conjugated 8-alkynylated purine nucleosides were designed to allow through-bond energy transfer. The spectroscopic properties detailed in Table 4 show that the S_0 absorption wavelength and Stokes shift can be tuned by altering the electron-donating characteristics of the *para*-substituent on the phenyl ring. The larger Stokes shifts observed for **5c** and **7e** are consistent with energy transfer to the guanine and adenine rings being enhanced by both electron-donating and electron-withdrawing substituents through the aromatic ring, respectively.

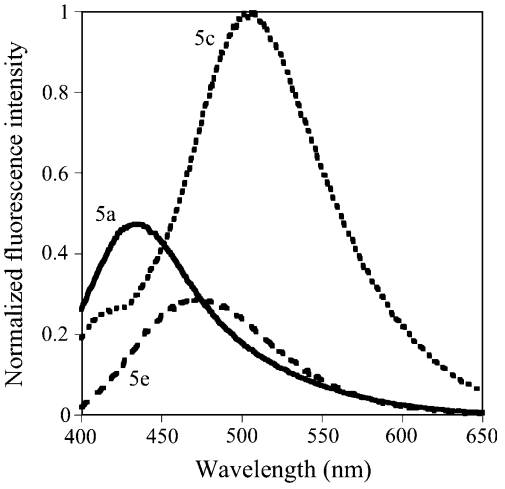


Figure 3. Normalized fluorescence emission spectra for compounds **5a**,**c** and **5e** in DMSO.

In summary, we have a prepared several novel alkynylated guanosines and adenosines. Many of these derivatives exhibit interesting fluorescent properties that could prove useful in biochemical applications. The key to the success of the Sonogashira cross-coupling was low palladium loading (1 mol %; c 1.18×10^{-3} mol dm^{-3}) and an optimum Pd(II)/Cu(I) ratio (1:2). The effect of a Cu(I) co-catalyst in these reactions is substantial, and one which indicates a potential secondary role for this metal in the catalytic cycle (particularly in reactions employing **1**).[16] Further mechanistic studies are underway to uncover a dual role for Cu(I) in these reactions. In due course, the biological applications of the fluorescent analogs detailed herein will be reported.

Acknowledgements

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12. *General procedure*: A solution of 8-bromoguanosine (520 mg, 1.5 mmol, 1 equiv) in dry DMF (12 mL) was added to the substituted phenylacetylene (1.8 mmol, 1.2 equiv) and dry triethylamine (0.63 mL, 4.5 mmol, 3 equiv) in a vacuum dried Schlenk tube. $PdCl_2(PPh_3)_2$ (11 mg, 0.015 mmol, 1 mol %) and CuI (6 mg, 0.030 mmol, 2 mol %) were added and the reaction mixture was left to stir at 110 °C for 18 h, after which time it was allowed to cool to 40 °C and the DMF removed in vacuo to leave a brown solid. The solid was transferred to a sintered glass filter and was washed with boiling water (5×200 mL), EtOAc (5×25 mL) and diethyl ether (2×25 mL) yielding the product as a light cream solid (0.53 g, 91%). *Representative data for 8-(2′-phenylethynyl)guanosine*: Mp 234–236 °C (decomp.); ν_{max} (DMSO solution)/cm^{-1} 1695 (CO), 3126 and 3328 (NH), 3421 (OH); δ_H (400 MHz; DMSO-d_6) 3.65 (1H, m), 3.88 (1H, m), 3.84 (1H, s), 4.19 (1H, s), 4.99 (2H, m), 5.15 (1H, br s), 5.50 (1H, d, *J* 6.4), 5.89 (1H, d, *J* 6.4), 6.62 (2H, s), 7.51 (3H, m), 7.64 (2H, m), 10.90 (1H, s); δ_C (128 MHz; DMSO-d_6) 61.9, 70.4, 70.8, 79.4, 85.6, 88.2, 92.6, 117.5, 120.4, 128.9, 129.3, 129.9, 131.5, 151.1, 153.9, 156.0; *m/z* (FAB) MH^+ 384 (2.5%), 369, 354, 277, 185 (100%); HRMS (MH^+): 384.1299 (calcd for $C_{18}H_{18}N_5O_5$ 384.1307).
13. The molecular ion for the cross-coupled product was detected by FAB-MS, but shown to be impure by ^1H and ^{13}C NMR spectroscopy.
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16. Preliminary ^{31}P NMR spectroscopic experiments indicate that the excess Cu(I) reduces the amount of Pd(0) species bound to **1** or **5**, thereby releasing active catalyst into the catalytic cycle. Complete details of these experiments will be reported in a full paper.