Design and synthesis of fluorescent 7-deazaadenosine nucleosides containing π-extended diarylacetylene motifs

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COMMUNICATION

Design and synthesis of fluorescent 7-deazaadenosine nucleosides containing π-extended diarylacetylene motifs

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***C*-Modified 7-deazaadenosines containing a diphenylacetylene moiety have been synthesised using cross-coupling approaches. The *C*-modified nucleosides exhibit remarkable fluorescence properties, including high quantum yields. Solvatochromic studies show a near linear correlation between the Stokes shift and solvent polarity which is indicative of intramolecular charge transfer. DFT calculations have allowed us to correlate the experimentally observed photophysical properties with the calculated HOMO-LUMO energy gaps within a series of real and model compounds.**

Introduction

Fluorescently-labelled nucleosides and nucleotides are invaluable tools in the study of biological systems.[1] The addition of compact substituents at C8 in adenosine, *e.g.* phenyl- (**1**) or phenylethynyl- (**2**), induces significant fluorescence.[2] This property cannot be exploited fully however, as C8-modified analogues are incompatible with base-pair formation (due to an unfavourable conformational preference).[3]

On the other hand, 7-deazaadenosine nucleosides adopt conformations commensurate with adenosine, allowing the C7-position to be utilised for functionalisation.[4] C7-groups orientate into the major groove of double helices, resulting in minimal duplex destabilisation.[5] There are a limited number of 7-deaza-2′-deoxyadenosine analogues incorporating compact fluorophores (*e.g.* **3**), but they typically exhibit weak fluorescence.[6] It is therefore necessary to develop highly fluorescent C7-modified analogues, which is the principal aim of the current study.

In this paper, we report the synthesis and photophysical properties of novel 7-deazaadenosine nucleosides containing compact π-extended fluorophores. An extrinsic fluorophore, *i.e.* a diarylacetylene group (tolan), has been introduced at the C7-position (as illustrated for generic compound **4** in Fig. 1). The design of the series of compounds **4a-e** was guided by computational studies, which predict that such molecules should function as classical 'push-pull' diarylacetylene systems, which are known to exhibit strong fluorescence.[7] The theoretical predictions go some way to explain the experimentally observed photophysical properties of compounds within the series **4a-e**. The fluorescence lifetimes and quantum yields make these artificial *C*-modified nucleosides potentially useful as probes in biological systems.

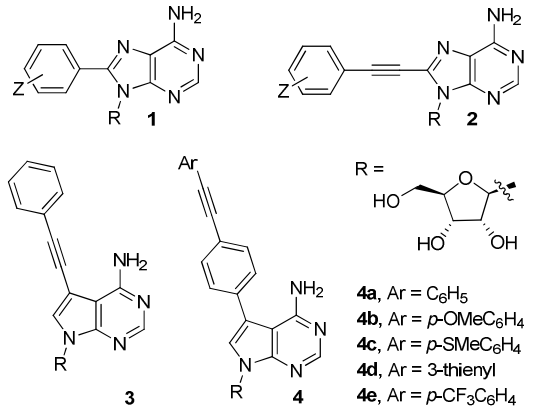


Fig. 1. Fluorescent nucleosides, including C7 and C8 modifications. The series of compounds **4a-e** are the target molecules in this paper.

Results and Discussion

The following section is divided into theoretical (DFT calculations) and experimental (synthesis and photophysical properties) parts. The theoretical work establishes the credibility of **4** as a potentially useful new class of fluorescent nucleosides.

Density Functional Theory (DFT) calculations. We studied structures containing both *N*-ribose and *N*-methyl groups, *i.e.* **3** and **4a**, and **5** and **6a**, respectively. Several conformations within the ribose sugar and the π-system within each molecule were considered in the calculations. In these derivatives, the ribose with a C3'-*endo* conformation was selected. The methyl substituent was viewed as an electronically similar group to the more conformationally-flexible ribose. Decoupling the potential complexity of the latter was necessary, especially when studying a larger series of compounds (**4a-e**), *vide infra*. All of the calculated structures were optimised at the (RI-)PBE0/def2-TZVPP level and their frontier molecular orbitals (MOs) and vertical excitation energies (from TDDFT studies at the same level of theory) were used to probe the effect of chemical structure on their photophysical properties.

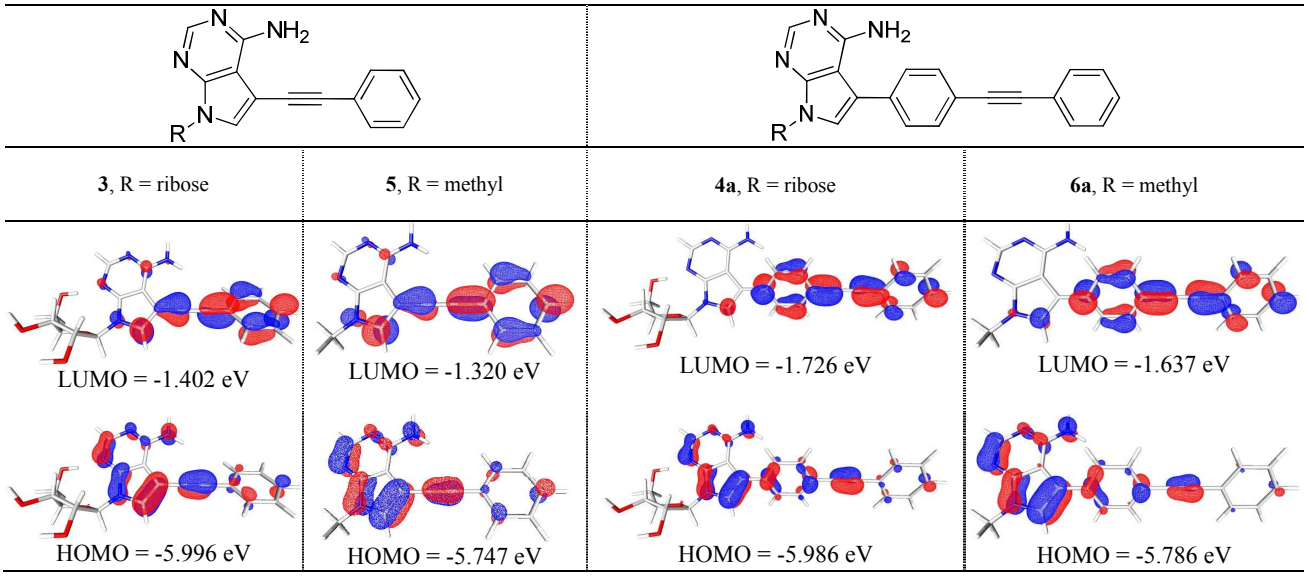


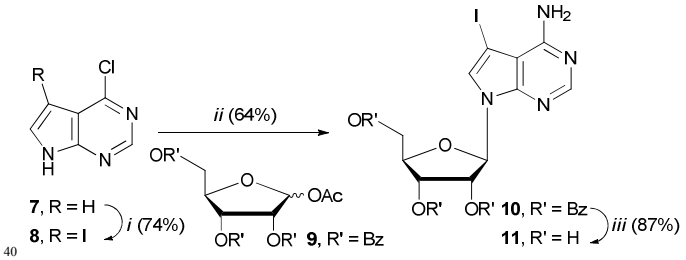
Fig. 2. (RI-)PBE0/def2-TZVPP optimised structures for *N*-substituted-7-phenylethynyl-7-deazaadenosines **3** and **5** (left) and *N*-substituted-7-[4-(phenylethynyl)-phenyl]-7-deazaadenosines **4a** and **6a** (right). In all cases, the frontier MOs (HOMO/LUMO) are illustrated (isosurface at 0.05 a.u.).

Interestingly, a qualitative analysis of the MOs of **3** and **5** (Fig. 2) goes some way to explaining a general preference for non-radiative decay and the weak fluorescence observed[6] experimentally. The HOMO/LUMO in both **3** and **5** are characteristic of a weaker push-pull system and suggests limited intramolecular charge transfer upon excitation.

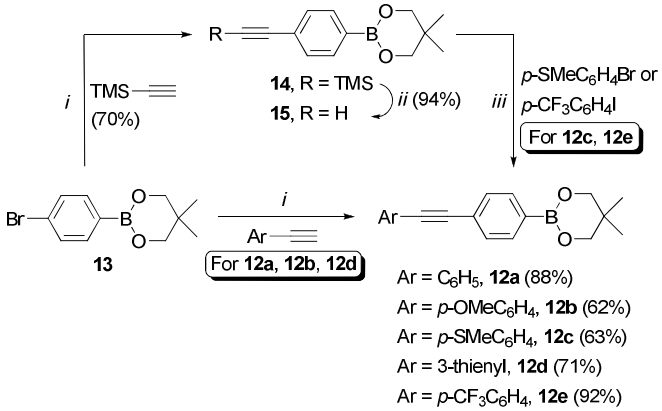
By contrast, the MOs for ribose derivative **4a** and *N*-methyl derivative **6a** (shown in Fig. 2) show localisation of the HOMO primarily on the 7-deazaadenine unit and the LUMO on the diarylacetylene. This observation suggests that there is potential for intramolecular charge transfer upon excitation in this type of structure, *i.e.* as expected in a 'push-pull'-type system, and that the ribose and methyl groups do not appreciably affect the distribution of electron density within the π-system.

Synthesis of target compounds (4a-4e). We set about synthesising the core structure of **4a** {and derivatives (**4b-e**)} using 7-iodo-7-deazaadenosine **11** as a convenient starting material (Scheme 1). The synthesis of **11** involves reaction of 6-chloro-7-deazapurine **7** with iodine in DMF to give **8**. An alternative iodination method using *N*-iodosuccinimide required recrystallisation of **8** from MeOH, which led to the undesired formation of 7-iodo-6-methoxy-7-deazapurine. Compound **8** was coupled to a protected-ribose sugar **9** using Vorbrüggen glycosylation conditions to give **10**.[8] Optimal yields of **10** were achieved using sub-stoichiometric quantities of trimethylsilyl triflate and *N,O*-bis(trimethylsilyl)acetamide (BSA). Sugar deprotection of **10** and installation of the exocyclic amine was carried out with aqueous NH_3 in dioxane (in a sealed tube at 60 °C), which avoids methanolic ammonia and an autoclave, affording **11** in good overall yield.

For the diarylacetylene arm, an organoboron species was required for coupling to **11**. Diarylacetylene boronate esters **12a-e** were synthesised by chemoselective Pd-catalysed cross-coupling of 4-bromophenyl boronic acid neopentyl glycol ester **13**.[9] Two approaches were used. Firstly, **13** was cross-coupled with three terminal arylacetylenes to give **12a**, **12b** and **12d**, respectively.



Scheme 1. Synthesis of key starting material **11**. Reaction conditions: *i*. I_2 (1.1 eq), KOH (2.5 eq), DMF, rt, 30 min. *ii*. **9** (1.1 eq), BSA (0.5 eq), TMSOTf (0.5 eq), MeCN, 80 °C, 1.5 h. *iii*. NH_3 (aq, 25%) / dioxane (1:1), 60 °C, 3 days.

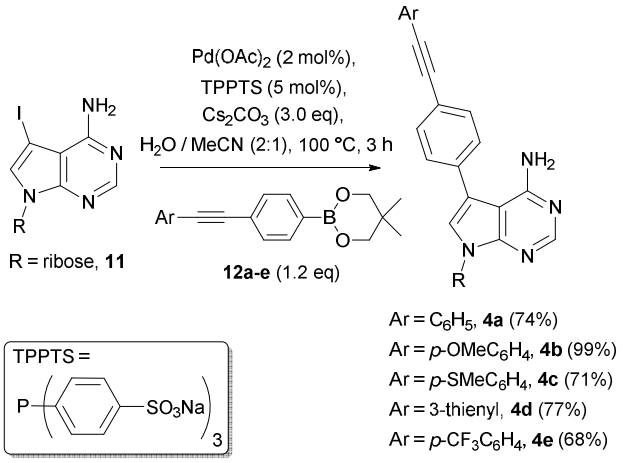


Scheme 2. Synthesis of compounds **12a-e**. Reaction conditions: *i*. $PdCl_2(PPh_3)_2$ (5 mol%), CuI (5 mol%), PPh_3 (20 mol%), Et_2NH, DMF, 120 °C (MW), 20 min. *ii*. TBAF, THF. *iii*. As for *i*, without DMF, 25 min.

For access to **12c** and **12e**, **13** was cross-coupled with trimethylsilylacetylene to give **14**, which was then deprotected quantitatively with TBAF to reveal terminal acetylene **15**. The latter compound was then cross-coupled with the respective aryl halide to give boronate esters **12c** and **12e**.

With the diarylacetylene boronate esters **12a-c** in hand, they were cross-coupled with 7-iodo-7-deazaadenosine **11** under reported aqueous conditions,[10,4a] with the water-soluble

phosphine ligand TPPTS. Pure C7-diarylacetylene functionalised nucleosides **4a-4e** were all isolated by column chromatography on silica gel or by preparative thin-layer chromatography in good yields (see E.S.I. for characterisation details).



Scheme 3. Synthesis of target compounds **4a-e**.

Table 2 UV-visible absorption and fluorescence data for compounds **4a-4e** in dilute DMSO solution.

Cpd	λ_{max} / nm (exp.)	λ_{max} / nm (calc.)[a]	ε / 10^4 M^{-1} cm^{-1}	λ_{em} / nm	Stokes Shift / cm^{-1}	Φ	τ /ns
4a	321	345	2.2	428	7790	0.74	2.2 (92%), 0.5 (8%)
4b	321	341	2.3	405	6460	0.78	1.6 (90%), 0.5 (10%)
4c	331	353	2.5	431	7010	0.76	2.0 (92%), 0.5 (8%)
4d	313	349	2.1	404	7200	0.32	1.5 (88%), 0.5 (12%)
4e	328	363	1.5	491	10000	0.41	2.1 (87%), 0.8 (12%)

[a] Calculated λ_{max} are for model complexes **6a-6e**.

Photophysical studies. We turned our attention to studying the photophysical properties of 7-modified-7-deazaadenosines **4a-e**. UV-visible absorption spectra of the modified nucleosides exhibit maxima at wavelengths >310 nm, with the lowest λ_{max} observed for the thiophene-containing compound **4d** (Table 2). The absorbance maxima of **4a-c** (at *ca.* 320 nm) are shifted away from the intrinsic absorbance of nucleic acids and proteins (*ca.* >280 nm), which is important when considering the potential applications of these novel nucleosides as fluorescent probes in biological systems. The 7-modified-7-deazaadenosines **4a-e** possess high molar absorbance coefficients (*ca.* 2 x 10^4 M^{-1} cm^{-1}), suggesting the diphenylacetylene motif connected to the 7-deaza-adenine unit is an effective chromophore.

The 7-modified-7-deazaadenosines **4a-e** exhibit promising fluorescence properties (Table 2). High emission quantum yields were recorded for all compounds in DMSO, but of particular note are compounds **4a**, **4b** and **4c**, which have quantum yields > 0.70. The thiophene-containing analogue **4d** has a somewhat lower quantum yield (Φ = 0.32), as does the trifluoromethyl-compound **4e** (Φ = 0.41). The variation in wavelength of the emission maxima for these compounds is much larger than the variation in their absorption maxima. This gives rise to large differences in their Stokes shifts, *e.g.* trifluoromethyl compound **4e**, has a remarkably large Stokes shift of 1 x 10^4 cm^{-1}.

In order to further explore the substituent effects seen in **4a-e**, the model structures (**6a-e**, R = Me rather than ribose) were optimised computationally at the (RI-)PBE0/def2-TZVPP level, allowing their UV-visible absorption spectra to be calculated in the gas phase (TDDFT at the same level of theory, 50 singlet excitations considered). Vertical excitation energies were used to probe the effects of various substituents on the photophysical properties of these molecules. The frontier MOs and their energies are shown in Fig. 3. The calculated λ_{max} values for **6a-e** are given in Table 2, where there are differences with the experimental data. Crucially, the trend within the series can be considered. For example, when all calculated (which includes **4a**, **5** and **6a-e**) and experimental λ_{max} data are plotted against each other, there is a good correlation between the experimentally and theoretically derived data (Fig. 4). Even in the case of the 3-thienyl-system (**4d** and **6d**), a correlation is seen within the compound series. Interestingly, the 3-thienyl group in **6d** contributes to the LUMO, but not appreciably to the HOMO (see Fig. 3), which is perhaps surprising given the electron-rich nature of the thienyl group.

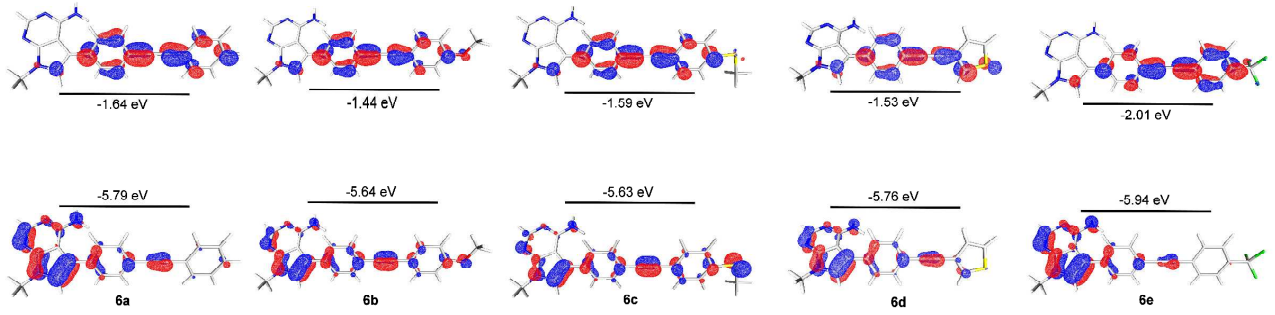


Figure 3. HOMO and LUMO surfaces (isosurface at 0.05 a.u.) and energies for compounds **6a-6e**, calculated by DFT at the (RI-)PBE0/def2-TZVPP) level.

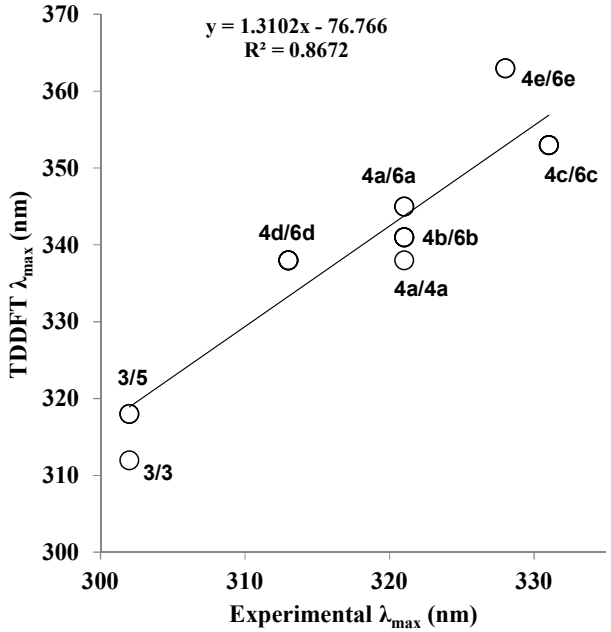


Figure 4. Correlation between calculated and experimental λ_{max} (nm) for all optimised structures and conformational isomers (see ESI for details). The structures that are plotted against each other are indicated as their compound numbers (*e.g.* **4d**/**6d**). TDDFT refers to Time Dependent Density Functional Theory; calculated λ_{max} values for structures **5**, **6a**-**e** are shown on the y-axis. The experimental λ_{max} values for compounds **3** and **4a**-**e** are shown on the x-axis.

Fluorescence lifetimes were measured using time-correlated single photon counting (TCSPC), see Table 2.[11] All of the 7-modified-7-deazaadenosines **4a**-**e** were found to exhibit bi-exponential decays, with a major component that varied according to the substituent at the 7-position, and a minor component (*ca.* 10%) that was consistently *ca.* 0.5 ns. The fluorescence lifetime values recorded are in-keeping with measurements on related tolan derivatives made by Marder and co-workers.[12]

Solvatochromism of fluorophores can be informative about the nature of the excited state and the electronic transitions giving rise to the absorption and emission spectra. For example, solvatochromic fluorescent probes can be used to gain insight into the polarity of the local environment.[11] The variation in the absorption and emission spectra of 7-[4-(phenylethynyl)-phenyl]-7-deazaadenosine **4a** was, therefore, investigated in a variety of solvents (Table 3).

Both the absorption and emission maxima vary slightly when the solvent is changed. Whilst no distinct correlation was observed between wavelength and solvent polarity, the data shows a reasonable correlation between ($\bar{\upsilon}_A - \bar{\upsilon}_F$) and the orientation polarizability (Δf) in a Lippert-Mataga plot (Fig. 5), indicating the sensitivity of the fluorophore to its local chemical environment. The linear correlation is improved if one omits water ($R^2 = 0.89$), which could suggest that **4a** is behaving quite differently in this solvent. It is of particular note that the fluorescence quantum yield of **4a** in water was found to be 0.15, which is significantly higher than 7-phenylethynyl-7-deaza-2′-deoxyadenosine ($\Phi = 0.02$)[6] and, indeed, the majority of other bespoke fluorescent *C*-modified nucleosides reported to date.

Table 3 Effect of solvent on the fluorescence properties of **4a**.

Solvent	λ_{max} /nm	ε / 10^4 M^{-1} cm^{-1}	λ_{em} / nm	Stokes shift / cm^{-1}	Φ	Solvent dielectric constant [b]
H_2O[a]	302	2.0	429	9800	0.15	80.1
DMSO	321	2.2	428	7790	0.74	47.2
MeCN	308	2.4	409	8020	0.62	36.6
MeOH	306	2.5	407	8110	0.46	33.0
EtOH	304	2.6	393	7450	0.31	25.3
^{i}PrOH	304	3.1	396	7640	0.29	20.2
CH_2Cl_2[a]	312	2.5	394	6670	0.61	9.0
EtOAc[a]	311	2.4	389	6450	0.33	6.1
DMF	318	2.7	420	7640	0.78	38.3

[a] Solutions made from stocks of **4a** in DMSO (2.5×10^{-3} M), due to poor compound solubility in non-polar solvents, which were diluted to 1-100 μM, so that $A = 0.1$ for each sample. The % (v/v) DMSO in the final solutions was typically <1%. [b] Measured at 20°C.

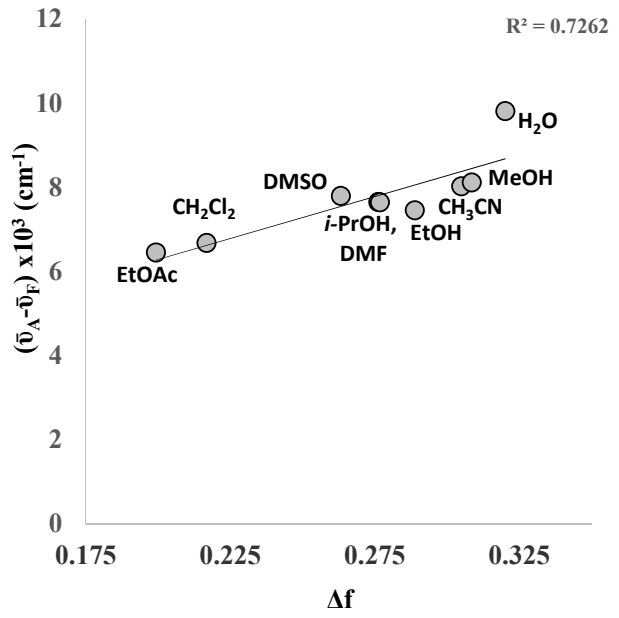


Fig. 5. Lippert-Mataga plot for compound **4a**.

In conclusion, a novel class of 7-modified-7-dezaadenosine nucleosides **4a**-**e** have been designed and synthesised from the corresponding 7-iodo nucleoside **11**, using multi-step Pd-catalysed cross-coupling approaches. The nucleosides exhibit promising UV-visible absorption and fluorescence properties, with large quantum yields and absorption maxima shifted away from the intrinsic absorption associated with amino acid and nucleic acid residues. The changes in the absorption and emission wavelengths in different solvents indicate that the fluorescence properties of **4a** are quite complex, whereas the Stokes shift exhibits a reasonable linear correlation with the orientation polarizability of the solvent. DFT calculations provided valuable insight into the fluorescent properties observed experimentally for reported compound **3**, and the herein described novel

compounds **4a-e**.

We were particularly pleased with the quantum yield for **4a** in water (Φ = 0.15). The incorporation of a compact fluorophore means that the chemical modification should not have a pronounced effect on either: (i) the biological compatibility of the nucleoside, or (ii) the stability of nucleic acids containing these C7-modified nucleotides. Applications for this class of fluorescent biomolecules will be reported in due course.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [Characterisation data of new compounds and detailed flurorescence measurements and spectra]. See DOI: 10.1039/b000000x/

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