A sequential direct arylation/Suzuki–Miyaura cross-coupling transformation of unprotected 2′-deoxyadenosine affords a novel class of fluorescent analogues†

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Novel rigid 8-biaryl-2′-deoxyadenosines with tuneable fluorescent properties can be accessed by an efficient sequential catalytic Pd^0-coupling approach.

Fluorescent nucleosides are used widely as probes of enzymatic turnover and biomolecular structure, and as labels in high-throughput biotechnologies.[1] Common organic, *e.g.* pyrenyl or fluorescein, and inorganic metal-containing extrinsic fluorophores can be attached to either sugar[2] or nucleobase.[3] Direct coupling of a conjugated aromatic group yields a more compact fluorescent analogue. Examples include aryl,[4] heteroaryl[5] or arylalkynyl[6] nucleosides, but also modified nucleobase mimetics exhibiting unique fluorescent properties (Fig. 1).[7]

This communication describes the synthesis of novel 8-biaryl-2′-deoxyadenosines (*e.g.* **II**, Scheme 1) which function as *Rigid Organo-fluorescent Nucleosides* (RONs). These π-conjugated systems are accessible using our recently developed direct arylation methodology for unprotected adenine nucleosides, which facilitates the chemoselective installation of an aryl group at the 8-position.[8] The use of a dihaloaromatic coupling partner would provide a secondary handle for further chemical manipulation, allowing addition of aryl groups *via* a classical cross-coupling (hereafter 'coupling') approach. Suzuki–Miyaura coupling has been used effectively with unprotected nucleosides[9] and nucleotides,[10] therefore it should be applicable

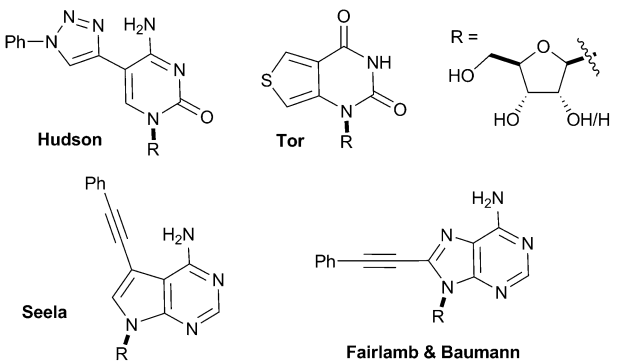
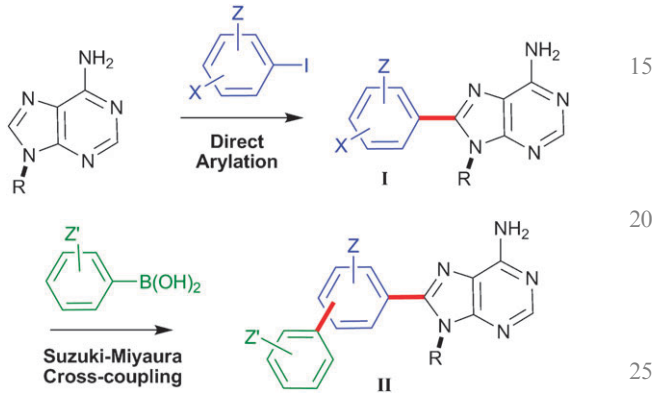


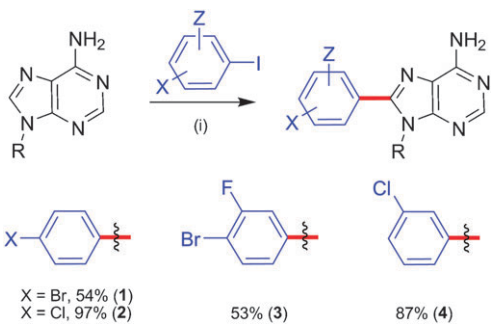
Fig. 1 Fluorescent nucleosides.



Scheme 1 Proposed targets and sequential direct arylation/Suzuki–Miyaura approach (R = 2′-deoxyribose).

to these substrates. This sequential coupling approach would provide a divergent synthetic route to differently substituted 8-biaryl-2′-deoxyadenosines (*via* **I**).

For the synthesis of target **I**, the use of diiodobenzene was dismissed as our prior findings indicated that double C–H arylation occurs.[8a] However, we rationalised that an aromatic coupling partner possessing two sites with different chemical reactivity towards Pd^0 might provide an elegant solution. Therefore, as a first step towards the selective synthesis of the intermediate compounds, we evaluated iodobromobenzenes and iodochlorobenzenes as direct arylation substrates (Scheme 2). Using our established direct arylation conditions,[8b] both 4-bromo and 4-chloro-iodobenzene reacted with 2′-deoxyadenosine to give compounds **1** and **2** in 54% and 97% yields, respectively.



Scheme 2 Direct arylation of 2′-deoxyadenosine. *Reagents and conditions*: (i) ArI (2.0 eq.), $Pd(OAc)_2$ (5.0 mol%), CuI (3.0 eq.), piperidine (0.4 eq.), Cs_2CO_3 (2.5 eq.), DMF, 80 °C, 15 h (R = 2′-deoxyribose; Z = H or F).

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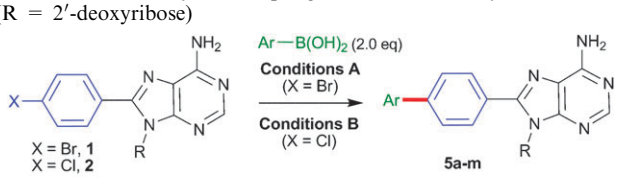
† Electronic supplementary information (ESI) available: Experimental details (including characterisation data for all compounds) and X-ray data for **5d** and **5i**, including cif files. CCDC [CCDC NUMBER(S)]. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c0cc02043e

3-Fluoro-4-bromoiodobenzene was a robust substrate giving **3** in 53% yield, whilst 3-chloro-iodobenzene provided **4** in 87% yield.

With the 8-haloaryl-2′-deoxyadenosines (**1–4**) in hand we focussed on the Suzuki–Miyaura coupling methodology. We employed a Pd : TPPTS (tris-(3-sulfophenyl)phosphine trisodium salt = TPPTS) water soluble catalyst system. A slightly modified version of Shaughnessy's protocol was employed, specifically $Pd(OAc)_2$ (1.25 mol%), TPPTS (2.5 mol%), Na_2CO_3 (2.0 eq.), MeCN : H_2O (2 : 1), 80 °C, 1.5 h (hereafter Conditions A).[9a,b] Interestingly, the employment of a catalyst preformation/preactivation step {$Pd(OAc)_2$, ligand, solvent and heating} led to a poor catalyst system resulting in incomplete reaction (Table 1; numbers in parentheses). This has implications for product isolation as it has a similar polarity to the starting material. The addition of the pre-catalyst and ligand with the solid reagents to a dry vessel (under an argon atmosphere) prior to addition of the solvent mixture worked most effectively (Table 1, all other yields).

The coupling of arylboronic acids with bromo analogue **1** using Conditions A proceeded well. Both electron-rich and electron-deficient arylboronic acids were efficient coupling partners (entries 1, 3, 5–7 and 9, Table 1). It is not surprising that chloro analogue **2** failed to serve as a substrate using Conditions A. Indeed, the difference in reactivity of C–Cl and C–Br bonds is apparent in the high yield observed for **5e** (81%, entry 5). We identified Buchwald's bulky electron-rich phosphine Xphos (2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl = Xphos) as an enhancing ligand.

Table 1 Suzuki–Miyaura couplings of **1** and **2** with arylboronic acids (R = 2′-deoxyribose)



Conditions A: $Pd(OAc)_2$ (1.25 mol%), TPPTS (2.5 mol%), Na_2CO_3 (2.0 eq.), MeCN:H_2O (2:1), 80 °C, 1.5 h
Conditions B: $Pd(OAc)_2$ (5 mol%), XPhos (10 mol%), aq. Na_2CO_3 (2M), THF, 80 °C, 3.5 h

Entry	Ar	Cpd	Yield/% Conditions A (from **1**)	Conditions B (from **2**)
1	4-$MeOC_6H_4$	**5a**	71 (38)[a]	88
2	4-MeC_6H_4	**5b**	—	72
3	C_6H_5	**5c**	89 (74)[a]	91 [81][b]
4	4-FC_6H_4	**5d**	—	92
5	4-ClC_6H_4	**5e**	81[c,d]	—
6	3,5-$F_2C_6H_3$	**5f**	82	94[e]
7	4-$CF_3C_6H_4$	**5g**	77	88
8	3-$CF_3C_6H_4$	**5h**	—	79
9	4-AcC_6H_4	**5i**	89[c]	76
10	4-$CHOC_6H_4$	**5j**	—	84
11	2-Furyl	**5k**	—	71
12	2-Thienyl	**5l**	—	52[f]
13	3-Thienyl	**5m**	—	63

[a] Using a preformed catalyst (see main text for details). [b] Using $PhBF_3K$ as a substitute for $PhB(OH)_2$. [c] 3 h reaction time. [d] Residual boronic acid starting material remaining. [e] 2 h reaction time. [f] 18 h reaction time.

Reactions were conducted using standard 'in-house' conditions, namely $Pd(OAc)_2$ (5 mol%), XPhos (10 mol%), aq. Na_2CO_3 (2 M), 80 °C, 3.5 h (hereafter Conditions B). Nine substituted biphen-4-yl-2′-deoxyadenosines were generated in good yields (entries 1–4, 6–10, Table 1) and three heteroaromatics were also coupled successfully (entries 11–13, Table 1). Using $PhBF_3K$ as a substitute for $PhB(OH)_2$, a slightly lower yield of **5c** (entry 3, Table 1) was obtained with Conditions B. The structures of compounds **5d** and **5i** were determined by single crystal X-ray diffraction; **5d** is shown as a representative example in Fig. 2.

The crystal structure of **5d** shows that this nucleoside adopts a *syn*-C2′-*endo* conformation and exhibits an intramolecular H-bond, consistent with related 8-aryl-2′-deoxyadenosines.[8b]

The Suzuki–Miyaura couplings using **3** (Conditions A) provided the 8-(2-fluoro-biphen-4-yl)-2′-deoxyadenosines **6a** and **6b** in good yields (Table 2, entries 1 and 2). 8-(Biphen-3-yl)-2′-deoxyadenosines **7a–c** were accessible from **4** and $ArB(OH)_2$ using Conditions B (Table 2, entries 3–5). Finally, the chloro-substituted analogue **5e** was subjected to Conditions B to give terphenyl analogue **8** in 76% yield (Scheme 3).

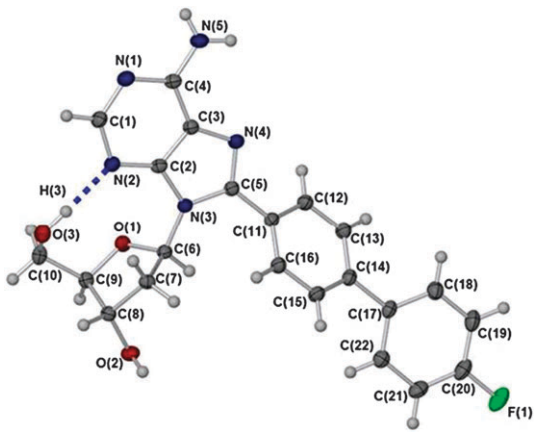
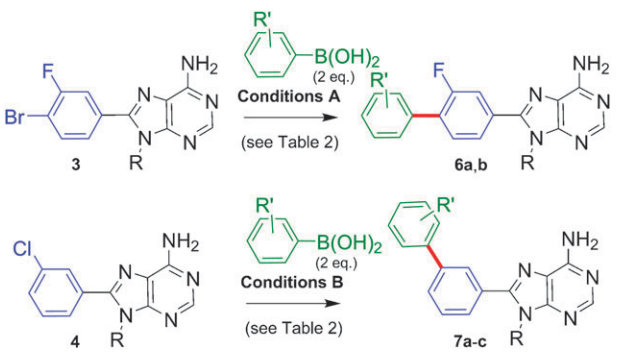
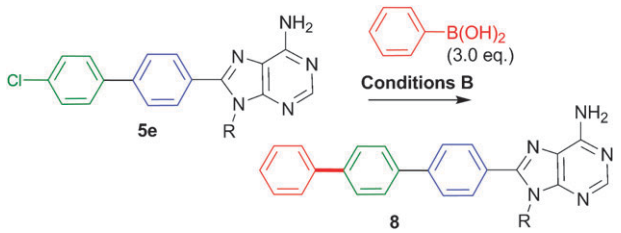


Fig. 2 Crystal structure of compound **5d** (using arbitrary numbering).

Table 2 Suzuki–Miyaura couplings of **3** and **4** with arylboronic acids



Entry	Substrate	Conditions	R′	Cpd	Yield/%
1	**3**	A	4′-MeO	**6a**	65
2	**3**	A	H	**6b**	93
3	**4**	B	4′-MeO	**7a**	88
4	**4**	B	H	**7b**	87
5	**4**	B	3′,5′-F	**7c**	87



Scheme 3 Synthesis of terphenyl derivative **8**.

Table 3 Photophysical properties of 8-substituted-2′-deoxyadenosines in DMSO at 25 °C

Cpd	λ_{max}/nm	$\varepsilon \times 10^4$/M^{-1} cm^{-1}	λ_{em}/nm	Stokes shift/cm^{-1}	Φ
5a	309	3.13	400	7362	0.65
5b	305	2.26	409	8337	0.68
5c	304	2.07	414	8740	0.69
5d	304	2.30	412	8623	0.69
5f	307	1.71	434	9532	0.72
5g	307	1.75	440	9846	0.75
5h	304	1.76	427	9475	0.77
5i	314	2.02	491	11 481	0.27
5j	318	2.53	399	6384	0.10
5k	320	2.71	407	6680	0.70
5l	322	2.25	419	7190	0.59
5m	307	2.14	403	7759	0.64
6a	310	2.21	408	7748	0.60
6b	304	1.89	420	9085	0.69
7a[a]	275	3.30	393	10 918	—
7b[a]	261	1.93	380	11 278	—
7c[a]	260	1.98	383	12 352	—
8	315	3.14	424	8161	0.77
9[b]	289	1.29	384	8560	0.81

[a] Measurements determined using methanol solutions. [b] Compound **9** is 8-phenyl-2′-deoxyadenosine.[8b]

The photophysical properties of all RONs (**5a–j**, **6a**, **6b**, **7a–c**, **8** and **9**) were determined (λ_{max}, ε, λ_{em} and Φ are listed in Table 3). A sequential increase in the UV λ_{max} and decrease in fluorescence lifetime is observed as the π-system is extended: phenyl (289 nm, 2.6 ns, **9**), biphen-4-yl (304 nm, 1.9 ns, **5c**) and terphenyl (315 nm, 1.7 ns, **8**). The biphen-3-yl derivatives (**7a–c**) possess unfavourable λ_{max} values which overlap with biomolecular absorption bands.[11] In the **5a–m** series the λ_{max} values are either similar to **5c** or shifted to longer wavelengths. The 8-aryl-2′-deoxyadenosines (**5a–h**, **5k–m**, **6a**, **b**, **8** and **9**) have excellent fluorescence quantum yields (0.59–0.81).

In summary, a library of novel RONs has been synthesised using an efficient sequential direct arylation/Suzuki–Miyaura coupling approach. The spectroscopic properties of RONs can be tuned by changing the terminal aryl group, in most cases without adversely affecting the quantum yield. Once incorporated into oligonucleotides using solid-phase synthesis, the RONs could be exploited as thymine specific base-discriminating fluorescent probes.[10a]

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11 Absorption bands at 260 and 280 nm for nucleic acids and proteins, respectively.