Synthesis and Photophysical Evaluation of New Fluorescent 7-Arylethynyl-7-Deazaadenosine Analogs

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Dedicated to the memory of Professor J. Peter Guthrie

ABSTRACT

Three new fluorescent 7-deaza-2′-deoxyadenosine analogs were synthesized via the Sonogashira cross-coupling reaction of 7-iodo-7-deaza-2′-deoxyadenosine with 1-ethynylpyrene, 2-ethynyl-6-methoxynaphthalene, and 9-ethynylphenanthrene. The spectral properties of these analogs were evaluated in dioxane, EtOH, and H_2O to determine their potential for use as environmentally sensitive fluorescent probes. All three analogs displayed large solvatofluorochromicity in H_2O, relative to their emission wavelengths in dioxane or EtOH. Moreover, all three analogs exhibited microenvironmental sensitivity of their fluorescence emission intensity, being moderate to high quantum yields in dioxane and EtOH and significantly lower in H_2O. Various attempts to perform domino cross coupling/annuation reactions on 7-deaza-7-alkynyladenine derivatives to form a new fused tricyclic adenine analog were unsuccessful.

KEYWORDS

fluorescence, environmentally sensitive fluorescence, solvatochromicity, 7-deazaadenine, Sonogashira cross-coupling

INTRODUCTION

Fluorescent nucleosides and analogs have long interested chemists and biochemists involved in the study of nucleic acids. Due to the relative scarcity of naturally occurring fluorescent nucleosides, there has been increasing focus on the synthesis of analogs.[1,2,3,4] 2-Aminopurine nucleoside, a highly fluorescent isomer of adenosine, has been one of the most utilized adenosine analogs for the past forty years.[5,2] However, this compound does have some limitations for studying nucleic acids. For instance, 2-aminopurine nucleoside (2-AP), suffers dramatically reduced emission once incorporated into single-stranded oligonucleotides and further yet upon duplex formation.[6,2] This leaves relatively weak emission for studying the structure and dynamics of duplex species. Therefore recent developments have sought to overcome the limitations of 2-AP.[7]

Although adenosine analogs may be substituted at various positions[1] (Figure 1), our interest is specifically focused on adenosines which are capable of Watson-Crick base pairing and are substituted at the 7-position with alkynes that engender fluorescence. The benefit of substitution at the 7-position of the 7-deazapurine is that large groups can be accommodated in the major groove of resultant duplexes.[8]

INSERT Figure 1

Seela and co-workers have synthesized a library of fluorescent 7-deazaadenosine and the related 7-deaza-8-aza-2'-deoxyadenosine analogs via attachment of various alkynes to the 7-position via the Sonogashira reaction.[9,10,11,12] Fluorescence quantum yields (Φ_F) in H_2O of the various 7-substituted-7-deazaadenosine analogs reported by Seela *et al*. range from relatively weak

to moderate (Φ_F = 0.002 – 0.27).[9-12] Although many of these modified nucleobases exhibit fairly large Stoke shifts, a drawback associated with these fluorescent analogs is that their absorption wavelength maxima range from 270 – 320 nm. The shorter wavelengths substantially overlaps with the excitation wavelength of the natural nucleobases which implies that selective excitation of the modified nucleobases may not be possible in all cases and there exists the hazard of photochemical damage to the nucleic acids.

In 2004, Saito and co-workers developed a tethered pyrene adenosine analog, 7-deaza-7-(1-pyrenecarboxamido)propyl-2'-deoxyadenosine (**A^{py}**).[13] The authors did not report the spectroscopic properties of the monomer, rather they showed that this fluorescent modification exhibited a fluorescence quantum yield of 0.064 in the single strand 5'-d(CGCAAT**A^{py}**TAACGC)-3'. The corresponding **A^{py}**-C, **A^{py}**-G and **A^{py}**-A mismatched duplexes showed Φ_F = 0.097, 0.098 and 0.054 respectively.[13] Interestingly, the fluorescence quantum yield of the fully complementary **A^{py}**-T duplex was significantly lower, Φ_F = 0.006, than the single strand or the mismatched duplexes. The authors hypothesized that the observed fluorescence quenching of the matched duplex was due to intercalation of pyrene into the duplex, which was supported by molecular modelling studies.[13]

Later, in 2008, Hocek and co-workers reported on the synthesis and photophysical properties of 7-deaza-2'-deoxyadenosine derivatives bearing bipyridine ligands and their Ru(II)-complexes.[14,15] These Ru(II)-complexes showed rather weak red luminescence (MeCN, Φ_F = 0.021 - 3 x 10^{-4}),[14] but nonetheless one compound was transformed into the triphosphate and incorporated into oligonucleotides that were used for SNP detection.[15]

Saito and co-workers, in 2011, synthesized a novel push-pull fluorescent 7-deazaadenosine analog based on an electron-withdrawing 4-cyanophenyl group (acceptor) and 7-deaza-2'-deoxyadenosine (donor) bridged by a pyrene via rigid alkyne linkers.[16] The nucleoside analog showed strong emission in chloroform (Φ_F = 0.448; $\lambda_{Ex.}$ = 416 nm, $\lambda_{Em.}$ = 470 nm) and markedly weaker emission 416 nm in DMF (Φ_F = 0.089; $\lambda_{Ex.}$ = 416 nm, $\lambda_{Em.}$ = 530 nm) but displayed considerable solvatofluorochromicity ($\Delta\lambda$ = 60 nm). More recently, Saito and co-workers reported a structurally similar, but smaller, environmentally sensitive fluorescent 7-deaza-2'-deoxyadenosine derivative possessing a 7-cyanonaphthylethyne substitution. The cyano-naphthylethynylated 7-deaza-2'-deoxyadenosine exhibited large solvatochromatic properties ($CHCl_3$: $\lambda_{Ex.}$ = 345 nm, $\lambda_{Em.}$ = 423 nm; MeCN: $\lambda_{Ex.}$ = 345 nm, $\lambda_{Em.}$ = 494 nm), and, in the context of oligonucleotide probes, signaled the difference between a perfectly matched complementary strand versus a mismatch by a change in emission wavelength and intensity.[17]

Although there exists a variety of 7-deaza-7-substituted adenine fluorophores, at the time of planning this work,[18] there was still a need to develop brighter and more responsive probes for use in nucleic acids research. Initially, we were interested in the synthesis of a novel fluorescent tricyclic adenine analog by possibly extending the substrate scope of the Sonogashira cross-coupling/heteroannulation chemistry that has been exploited in our group for cytosine analogs[19] to adenine analogs, Figure 2. Heteroannulation of nucleosides via the formation and reaction of 5-pyrimidine- or 7-deazapurine-biaryl nucleoside adducts was first described by Matteucci and coworkers. They demonstrated the formation of a carbazaole analog of 2'-deoxycytidine by an intramolecular nucleophilic substitution on a suitably derivatized C4-position of a pyrimidine

yielding a fused 5-membered ring. Matteucci's work mainly focused on the base-pairing and helix stabilizing properties of modified nucleosides once incorporated into oligonucleotides and not on their photophysical properties. Much later, Hudson and Suchy recapitulated the synthesis of a carbazole nucleobase analog and reported its bright blue, environmentally responsive fluorescence.[20] Following on their work with cytosine analogs, Matteucci showed that a similar approach was feasible to produce tetracyclic adenine analogs.[21] Since its first report, Wilhelmsson and collaborators have extended the variety of fused aromatic ring adenine analogs greatly and have shown that they are bright and tunable fluorophores and have employed them in FRET-based probes.[22,23,24,25] Given the structural similarity between the carbazole cytosine analog (a benzofused pyrrolocytosine) and pyrrolocytosine (Figure 2b), which is obtained via Larock-type cyclization[26] of 5-alkynylcytosine derivatives, we reasoned that a tricyclic adenine analog derived from the 7-deaza-7-alkynyladenine may possess useful and interesting fluorescence. This approach was attractive because such adenine analogs are derived from readily available and diverse alkynes.

INSERT FIGURE 2

However, our efforts toward the annulation of 7-deaza-7-ethynyladenine analogs were unsuccessful, yet nonetheless are described herein. The photophysical characterization of three new 7-deazaadenosine analogs substituted at the 7-position with 9-ethynylphenanthrene, 2-ethynyl-6-methoxynaphthalene and 1-ethynylpyrene are reported. The photophysical properties of these analogs were evaluated in three different solvents, H_2O, EtOH and 1,4-dioxane in order to judge their microenvironment responsiveness.

EXPERIMENTAL

General experimental procedures

Reagents were commercially available and all solvents were HPLC grade except for water (18.2 $M\Omega \bullet cm^{-1}$ millipore water) and DMF (dried over Al_2O_3 in a solvent purification system). Solvents were removed under reduced pressure in a rotary evaporator, aqueous solutions were lyophilised and organic extracts were dried over Na_2SO_4. Flash column chromatography (FCC) was carried out using silica gel (SiO_2), mesh size 230 - 400 Å and basic alumina (Al_2O_3), mesh size 80 - 200 Å. Thin-layer chromatography (TLC) was carried out on Al backed silica gel plate with compounds visualised by I_2 vapours, 5% ninhydrin stain, phosphomolybdic acid stain, and UV light, as appropriate for the compounds under study. NMR spectra were recorded on either 600 MHz or 400 MHz spectrometers for ^1H NMR spectra, as noted; δ values were referenced to residual proton signal in the solvents as follows: $CDCl_3$ (7.27 ppm); CD_3OD (4.80, 3.30 ppm); DMSO-D_6 (2.49 ppm); D_2O (4.75 ppm). Proton decoupled NMR for ^{13}C were measured at 125

MHz; chemical shift values (δ, ppm) were referenced to the solvent signals as follows: $CDCl_3$ (77.0 ppm); CD_3OD (49.0 ppm); DMSO-D_6 (39.5 ppm). High resolution (HR) mass spectra (MS) were obtained using electron impact (EI), chemical ionization (CI) or electrospray ionisation (ESI).

4-chloro-7H-pyrrolo[2,3-d]pyrimidine (1): 7H-pyrrolo[2,3-d]pyrimidin-4-ol (4.01 g, 29.7 mmol) was suspended in 45 mL of $POCl_3$ and refluxed for 1 hour. The solution was cooled to room temperature and excess $POCl_3$ was removed by rotary evaporation. A mixture of dichloromethane (600 mL) and ice water (200 mL) was then added to the dark-brown residue and stirred for 24 hours, or until the dark-brown residue was completely dissolved. The aqueous layer was then neutralized by adding increments of a saturated sodium bicarbonate solution (3 × 300 mL). The organic layer was collected, dried over $MgSO_4$, and gravity filtered. The product was dried by rotary evaporation, producing a pale yellow solid (2.35 g, 52%). ^{1}H-NMR (DMSO-d_6, 400 MHz): δ = 12.57 (br s, 1 H), 8.59 (s, 1 H), 7.69 (d, *J* = 2.3 Hz, 1 H), 6.61 (d, *J* = 2.3 Hz, 1 H); ^{13}C-NMR (DMSO-d_6, 125 MHz): δ = 151.75, 150.41, 150.26, 128.35, 116.52, 98.76; HRMS (EI): calcd. for $C_6H_3ClN_3[M]^+$ 153.0094, found 153.0090.

4-chloro-5-iodo-7H-pyrrolo[2,3-d]pyrimidine (2): Compound **1** (2.33 g, 15.2 mmol) was dissolved in 54 mL of DMF, followed by the addition of *N*-iodosuccinimide (NIS, 3.58 g, 15.9 mmol). The reaction was stirred at room temperature for 2 hours. The solvent was removed by rotary evaporation and 60 mL of ice water was added to the orange residue. The precipitate was then collected by vacuum filtration, washed with ice water (3 × 15 mL), and concentrated in vacuo. The product was obtained as a pale brown solid (3.75 g, 88%). ^{1}H-NMR (DMSO-d_6, 400 MHz): δ = 12.94 (br s, 1 H), 8.59 (s, 1 H), 7.93 (s, 1 H). ^{13}C-NMR (DMSO-d_6, 125 MHz): δ = 151.95,

151.18, 150.93, 134.31, 116.23, 52.12; HRMS (EI): calcd. for $C_6H_3ClIN_3[M]^+$ 278.9060, found 278.9052.

(2R,3S,5R)-5-(4-chloro-5-iodo-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-((4-chlorobenzoyloxy)methyl)tetrahydrofuran-3-yl 4-chlorobenzoate (3): Compound **2** (1.00 g, 3.60 mmol) was dissolved in 28 mL of anhydrous acetonitrile, followed by the addition of sodium hydride (0.15 g, 6.6 mmol). The reaction was stirred at room temperature under an atmosphere of nitrogen for 30 minutes. Next, 1-chloro-2-deoxy-3,5-di-*O*-*p*-chlorobenzoyl-α-D-ribose (1.57 g, 3.6 mmol) was added to the reaction and the mixture was stirred at 50 °C for 2 hours. The solvent was removed by rotary evaporation and the crude product was purified by flash chromatography eluting with 2 % acetone in toluene to afford compound **3** as a pale yellow solid (1.36 g, 57%). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 8.61 (s, 1H), 8.10 – 7.90 (m, 4H), 7.55 (s, 1H), 7.47 (m, 4H), 6.77 (t, *J* = 4.0 Hz, 1H)**,** 5.76 (m, 1H), 4.80 – 4.50 (m, 3H), 2.90 – 2.70 (m, 2H). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ = 165.0, 164.9, 152.8, 150.9, 150.4, 140.1, 139.9, 131.2, 131.0, 130.5, 128.9, 128.8, 127.5, 127.2, 117.4, 84.3, 82.3, 75.0, 63.9, 52.9, 37.9. HRMS (EI): calcd. for $C_{25}H_{17}Cl_3IN_3O_5[M]^+$ 670.9278, found 670.9546.

(2R,3S,5R)-5-(4-amino-5-iodo-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-(hydroxymethyl)tetrahydrofuran-3-ol (4): Compound **3** (0.55 g, 0.80 mmol) was added to a thick-walled pressure vessel and suspended in 50 mL of methanol. The suspension was cooled to 0 ^{o}C followed by the addition of ammonia, which was bubbled into the suspension for 30 minutes. The reaction vessel was tightly sealed and the reaction was stirred at 100 ^{o}C for 24 hours. The solvent was removed by rotary evaporation and the crude product was purified by flash chromatography on SiO_2, eluting with 10 % methanol in dichloromethane. The product was

obtained as a white solid in 80 % yield. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.09 (s, 1H), 7.65 (s, 1H), 6.48 (m, 1H), 5.25 (d, J = 6.0 Hz, 1H), 5.02 (t, J = 6.0 Hz, 1H), 4.32 (m, 1H), 3.80 (m, 1H), 3.56 (m, 2H), 2.47 (m, 1H), 2.17 (m, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 157.3, 152.1, 150.0, 126.9, 103.3, 87.5, 83.1, 71.1, 62.0, 52.0, C(2') overlapped with DMSO; HRMS (EI): *m/z* calcd for $C_{11}H_{13}IN_4O_3$: 376.0032; found: 376.0025.

7-(2-ethynyl-6-methoxynaphthalene)-7-deaza-2'-deoxyadenosine A^{mon} (5): Compound **4** (85 mg, 0.20 mmol) was dissolved in dry, deoxygenated DMF (1.8 mL) to which CuI (8 mg, 0.042 mmol), Et_3N (0.060 mL, 0.43 mmol), 2-ethynyl-6-methoxynaphthalene (163 mg, 0.80 mmol) and tetrakis(triphenylphosphine) palladium ($Pd(PPh_3)_4$, 24 mg, 0.021 mmol) were sequentially added. The reaction mixture was stirred in the dark at 60 ^{o}C for 24 hours. The solvent was removed in vacuo and the residue diluted with 15 mL of dichloromethane. The organic layer was washed with 3 % EDTA solution (3 x 5 mL) and brine (3 x 5 mL). The organic layer was dried and concentrated in vacuo. The crude product was purified by FCC eluting with 10 % methanol in dichloromethane to afford compound **5** in 33 % yield. ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 8.16 (s, 1H), 8.12 (s, 1H), 7.89 (s, 1H), 7.90 -7.82 (m, 1H), 7.60 (dd, J_1 = 12.0 Hz, J_2 = 6.0 Hz, 1H), 7.55-7.37 (m, 1H), 7.22 (dd, J_1 = 12.0 Hz, J_2 = 6.0 Hz, 1H), 6.53 (m, 1H), 5.29 (m, 1H), 5.07 (t, J = 6.0 Hz, 1H), 4.37 (m, 1H), 3.88 (s, 3H), 3.83 (m, 1H), 3.59 (m, 1H), 3.57 (m, 1H), 2.23 (m, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 158.2, 157.7, 149.5, 138.5, 133.9, 129.4, 128.2, 119.5, 117.4, 102.2, 95.0, 91.8, 87.9, 82.7, 71.08, 62.0, 51.5, 50.0, C(2') overlapped with DMSO; HRMS (EI): *m/z* calcd for $C_{24}H_{22}N_4O_4$: 430.1641; found: 430.1636.

7-(9-ethynylphenanthrene)-7-deaza-2'-deoxyadenosine A^{phen} (6): Compound **4** (100 mg, 0.27 mmol) was dissolved in dry, deoxygenated DMF (2.0 mL) to which CuI (10 mg, 0.054 mmol),

Et_3N (0.075 mL, 0.52 mmol), 9-ethynylphenanthrene (215 mg, 1.08 mmol) and $Pd(PPh_3)_4$ (31 mg, 0.026 mmol) were sequentially added. The reaction mixture was stirred in the dark at 60 ^oC for 24 hours. The solvent was removed in vacuo and the residue diluted with 15 mL of dichloromethane. The organic layer was washed with 3 % EDTA solution (3 x 5 mL) and brine (3 x 5 mL). The organic layer was dried and concentrated in vacuo. The crude product was purified by FCC eluting with 10 % methanol in dichloromethane to afford compound **6** in 55 % yield. ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 8.93-8.86 (m, 2H), 8.45 (m, 1H), 8.31 (s, 1H), 8.20 (s, 1H), 8.08 (s, 1H), 8.04 (d, J = 6.0 Hz, 1H), 7.81-7.70 (m, 4H), 6.56 (m, 1H), 5.30 (d, J = 6.0 Hz, 1H), 5.10 (t, J = 6.0 Hz, 1H), 4.39 (m, 1H), 3.87 (m, 1H), 3.63 (m, 1H), 3.57 (m, 1H), 2.57 (m, 1H), 2.26 (m, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 157.6, 152.8, 149.5, 132.3, 131.7, 131.4, 130.7, 130.5, 130.2, 129.6, 129.5, 128.5, 127.9, 127.5, 127.3, 122.9, 102.1, 94.6, 89.0, 87.6, 83.2, 70.9, 61.8, 30.7, C(2') overlapped with DMSO; HRMS (EI): *m/z* calcd for $C_{27}H_{22}N_4O_3$: 450.1692; found: 450.1685.

7-(1-ethynylpyrene)-7-deaza-2'-deoxyadenosine A^{pyr} (7): Compound **4** (100 mg, 0.26 mmol) was dissolved in dry, deoxygenated DMF (2.0 mL) to which CuI (10 mg, 0.052 mmol), Et_3N (0.075 mL, 0.52 mmol), 1-ethynylpyrene (240 mg, 1.04 mmol) and $Pd(PPh_3)_4$ (31 mg, 0.026 mmol) were sequentially added. The reaction mixture was stirred in the dark at 60 ^oC for 24 hours. The solvent was removed in vacuo and the residue diluted with 15 mL of dichloromethane. The organic layer was washed with 3 % ethylene diamine tetraacetic acid disodium salt solution (EDTA, 3 x 5 mL) and brine (3 x 5 mL). The organic layer was dried and concentrated in vacuo. The crude product was purified by FCC eluting with 10 % methanol in dichloromethane to afford compound **7** in 33 % yield. ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 8.59 (d, J = 12.0 Hz, 1H), 8.49 – 8.00 (m 10H), 6.58 (m, 1H), 5.31 (d, J = 6.0 Hz, 1H), 5.11 (t, J = 6.0 Hz, 1H), 4.41 (m, 1H), 3.88 (m, 1H), 3.75 - 3.50 (m, 2H), 2.58 (m, 1H), 2.27 (m, 1H). ^{13}C-NMR (DMSO-d_6, 125 MHz): δ

157.7, 152.8, 149.5, 131.0, 130.8, 130.7, 130.4, 129.5, 128.7, 128.2, 127.3, 127.2, 126.3, 126.0, 125.9, 125.8, 124.7, 123.6, 123.4, 117.1, 102.1, 94.9, 89.9, 88.9, 87.6, 83.2, 70.9, 61.9, 54.9, C(2') overlapped with DMSO; HRMS (EI): *m/z* calcd for $C_{29}H_{22}N_4O_3$: 474.1692; found: 474.1697.

Methods of photophysical analysis

All photophysical measurements were made in 1 cm × 1 cm quartz cuvettes (ℓ = 1 cm) using spectroscopic grade EtOH, dioxane, and H_2O (18.2 MΩ•cm^{-1} millipore water). All absorption experiments were carried out using a CARY 300 UV-Visible spectrophotometer and all fluorescence experiments were carried out using a PTI-QM-4-SE spectrofluorometer. Molar extinction coefficients (ε) of A^{mon}, A^{phen} and A^{pyr} were calculated from plots using Beer-Lambert's law ($A = \varepsilon c\ell$) from five different solutions of known concentration. Error in the molar extinction coefficients was calculated based on a 95% confidence interval. Due to insufficient solubility of A^{mon}, A^{phen} and A^{pyr} in water, they were first dissolved in 0.20 mL of spectroscopic grade DMSO, prior to dilution with water. However, after dilution [DMSO] < 1% and was considered to be negligible.

The relative fluorescence quantum yield (Φ_F) for A^{mon}, A^{phen} and A^{pyr} in EtOH, dioxane, and H_2O were calculated using five solutions of different concentrations for both the analogue and its corresponding fluorescence standard. The samples used for analysis were diluted such that their absorbance was below 0.1, in order to minimize re-absorption effects. Excitation was done at the wavelength of maximum absorption for each analog in the corresponding solvent (Table 1). Integrated fluorescence intensities were then plotted against the absorbance for each sample, for both the analog and its corresponding fluorescence standard. The slopes of these two plots were then used in conjunction with the known fluorescence quantum yield of the fluorescence standard

(Φ_{STD}), in order to determine the fluorescence quantum yield of the compound of interest. These calculations are based on the following equation:

$$\Phi_F = \Phi_{STD}\left(\frac{Slope_F}{Slope_{STD}}\right)\left(\frac{\eta_F^2}{\eta_{STD}^2}\right) \qquad \textbf{eq. 1}$$

In order to determine the fluorescence quantum yields of A^{mon}, A^{phen} and A^{pyr} the fluorescence standard's quantum yield in the solvent of interest must be known. However, there were no established fluorescence quantum yields in the literature for some of the fluorescent standards in the solvents of interest. Thus, the fluorescence quantum yields of these standards were measured relative to a known fluorescence quantum yield of the same standard in a different solvent. The difference in solvent was accounted for by including a ratio of the refractive indices in the calculation, as seen in **eq. 1**. The fluorescence quantum yields that were used for analysis are presented in Table S1. Quinine sulfate in 0.5 M H_2SO_4 was used as the fluorescent standard for the determination of the quantum yields of $\mathbf{A^{mon}}$, $\mathbf{A^{phen}}$ and $\mathbf{A^{pyr}}$ in H_2O.

RESULTS and DISCUSSION

The syntheses of the 7-deaza-7-alkynyladenine derivatives began with chlorination of commercially available 7-deaza-6-hydroxypurine using $POCl_3$,[27] to afford the desired compound **1** in reasonable yield (Scheme 1). Iodination of **1** at C7 was accomplished by treatment with NIS in DMF to obtain compound **2** in high yield.[28] Glycosylation was effected by deprotonation of **2** (NaH in acetonitrile) followed by treatment with 1-chloro-2-deoxy-3,5-di-*O*-*p*-chlorobenzoyl-α-D-ribose[29] to afford exclusively the β-anomer, compound **3**, in 57 % yield. Conversion of the 6-chloro to 6-amino group and removal of the ester protecting groups was accomplished in one step

via treatment of compound **3** with a saturated NH_3/MeOH solution[30] to afford compound **4** in good yield. Lastly compound **4** was reacted with various alkynes under Sonogashira conditions[19] to obtain the desired 7-substituted-7-deazaadenosine nucleosides **5 – 7**, Scheme 1. No annulated product was observed to accompany the formation of the cross-coupled product, which is a departure from the chemistry displayed by the analogous cytosine system.

INSERT Scheme 1

PHOTOPHYSICAL EVALUATION

The spectral properties of A^{mon}, A^{phen} and A^{pyr} were evaluated in dioxane, ethanol, and water in order to determine the influence of solvent polarity on the fluorescence properties. Based on the solvent's dielectric constant (ε), dioxane represents a non-polar, aprotic solvent (ε = 2), EtOH represents a polar, protic solvent (ε = 24), and water represents a highly polar, protic solvent (ε = 80).[31] The results obtained are presented in Table 1 and selected excitation-emission profiles are shown in Figure 3 and Figure S15-17.

INSERT Table 1

INSERT Figure 3

All three adenosine analogs were found to have absorption bands a longer wavelengths than the naturally occurring nucleobases that roughly correlated with the number of polyaromatic rings such that $\mathbf{A^{pyr}}$ had the most red-shifted absorption wavelengths (λ_{ab} ~ 365 nm), followed by $\mathbf{A^{phen}}$ (λ_{ab} ~ 334 nm), and $\mathbf{A^{mon}}$ (λ_{ab} ~ 318 nm). Similarly, in the three solvents examined (dioxane, ethanol, water), $\mathbf{A^{pyr}}$ exhibited the most red-shifted fluorescence emission (λ_{Em} = 429, 432, 474 nm), followed by $\mathbf{A^{phen}}$ (λ_{Em} = 390, 392, 444 nm), and $\mathbf{A^{mon}}$ (λ_{Em} = 370, 370, 435 nm). Furthermore, the emission wavelengths in water for all three analogs were significantly red-shifted compared to emission wavelengths in ethanol and dioxane. Thus, all three analogs show solvatofluorochromicity in water, relative to ethanol and dioxane. This result suggests that highly polar aqueous solvents stabilize the excited states to a greater extent than organic solvents of both high and low polarity. In addition, this result also indicates that polyaromatic substituents containing conjugated electron-donating groups do not enhance the solvatofluorochromicity of 7-deaza-2′-deoxyadenosines, as was found with conjugated electron-withdrawing groups.[17]

Due to the stabilizing effect of solvent polarity on the excited states of fluorophores, all three analogs had Stokes shifts that increased with solvent polarity. In addition, the Stokes shifts were found to increase with the number of aromatic rings present, such that $\mathbf{A^{pyr}}$ had the largest Stokes shifts (62, 66, 110 nm), followed by $\mathbf{A^{phen}}$ (54, 60, 109 nm), and $\mathbf{A^{mon}}$ (51, 52, 118 nm). However, one interesting exception to this trend was that $\mathbf{A^{mon}}$ displayed a larger Stokes shift in water than both $\mathbf{A^{pyr}}$ and $\mathbf{A^{phen}}$. This effect is believed to be a direct consequence of the presence

of the methoxy group, since fluorophores of higher polarity are more sensitive to the stabilizing effects of polar solvents.[32]

Due to the high fluorescence quantum yield of pyrene (Φ_F in ethanol = 0.65),[33] $\mathbf{A^{pyr}}$ was expected to display a high fluorescence quantum yield, which it did (dioxane, Φ_F = 0.52, and ethanol, Φ_F = 0.43). Conversely, $\mathbf{A^{pyr}}$ had a dramatically reduced fluorescence quantum yield in water, Φ_F = 0.01. These large differences in quantum yield are attractive because they enable $\mathbf{A^{pyr}}$ to be used as an environmentally sensitive fluorescent probe across all three solvents. In addition, when the molar extinction coefficients of $\mathbf{A^{pyr}}$ are taken into consideration, its high brightness values (16.9, 14.1, 0.2) indicate the potential of $\mathbf{A^{pyr}}$ as a sensitive fluorescence probe. Similarly, due to the moderate fluorescence quantum yield of phenanthrene (Φ_F in ethanol = 0.13),[32] $\mathbf{A^{phen}}$ was expected to display useful fluorescence properties. Overall, $\mathbf{A^{phen}}$ displayed modest fluorescence quantum yield differences across dioxane Φ_F = 0.14, ethanol Φ_F = 0.08, and water Φ_F = 0.02. Although it was expected that $\mathbf{A^{phen}}$ would display lower quantum yields than $\mathbf{A^{pyr}}$, it was interesting to find that $\mathbf{A^{phen}}$ also displayed lower fluorescence quantum yields than $\mathbf{A^{mon}}$, despite the inherent benefit of additional size of the aromatic system. These moderate fluorescence quantum yields significantly impacted the brightness values determined for $\mathbf{A^{phen}}$ (2.3, 1.2, 0.1 $\times 10^3$ M^{-1} cm^{-1}), despite having high molar absorptivity. Lastly, due to previous reports that conjugated electron-donating groups can be used to increase fluorescence quantum yields, $\mathbf{A^{mon}}$ was expected to show a marked increase in quantum yields, relative to the 2-ethynylnaphthalene derivative.[13] $\mathbf{A^{mon}}$ exhibited its largest fluorescence quantum yield in ethanol Φ_F = 0.24, followed by dioxane Φ_F = 0.15, and water, Φ_F = 0.01. These moderately high fluorescence quantum yields had a positive effect on fluorophore brightness (2.0, 1.9, 0.04 $\times 10^3$ M^{-1} cm^{-1}), compensating for the compound's relatively low molar absorptivity. Thus, $\mathbf{A^{mon}}$ displays fair potential for use as an

environmentally sensitive fluorescent probe across all three solvents. In addition, the 2-ethynylnaphthalene derivative was synthesized and photophysically characterized by Saito and co-workers.[13] The authors reported similar spectral characteristics to $\mathbf{A^{mon}}$ in ethanol, λ_{ab} = 318 nm, λ_{Em} = 371 nm, however the fluorescence quantum yield in ethanol was significantly lower, Φ_F = <0.01.[13] This result confirms the ability of conjugated electron-donating groups to enhance the fluorescence quantum yield of 7-deaza-2′-deoxyadenosines. Overall, the fluorescence properties of these derivatives is consonant with the emission from monomeric species in solution from similarly derivatized purine nucleosides.[34]

Attempted cyclization of 7-alkynyl derivatives

Initially, the approach to this work was envisioned as employing sequential cross-coupling/annulation sequences to access tricyclic adenine analogs from commercially available diaminopyrimidine, Scheme 2. The attractive feature to this approach was the possibility that diverse groups could be installed at both the C8 position of the purine (R_1) and on the new C7-N6 fused ring (R_2).

INSERT Scheme 2

Once it became clear that the nucleosides smoothly cross-coupled without further cyclization, we prepared a nucleobase derivative (*N*9-methylene carboxyl, Scheme 3), that is more robust to vigorous treatment due to the absence of an *N*-glycoside linkage, to serve as a substrate to test a variety of reaction conditions. Furthermore, compounds of this type could be used in the synthesis of peptide nucleic acids making the efforts for their preparation even more worthwhile.

Commercially available diaminopyrimidine hemisulfate hydrate was iodinated, cross-coupled and cyclized to yield 7-deazaadenine **10** according to a literature procedure.[35] Iodination was performed similarly as to 6-chloropurine, described earlier, and then alkylation at *N*9 by either ethyl bromoacetate to give **12**, or by *t*-butyl bromoacetate to give **15**. The exocyclic amino group of compound **12** was benzoylated giving **13**, in order to study its effect on the ability to cyclize after installation of the alkyne. Our previous studies on the annulation of 5-alkynyl cytosines indicated that acylation of *N*4 facilitated the annulation reaction,[19] although cyclization is possible under more forcing conditions in the absence of acylation, see for example **9** → **10**. Sonogashira cross-coupling was performed on both **13** and **15** to give the 7-phenylalkynyl derivatives **14** and **16**, respectively, which served as substrates subjected to various condition designed to promote annulation, Figure 3.

INSERT Scheme 3

Initial attempts to promote the tandem Sonogashira/cyclization reaction on the adenine scaffold included carrying out the reaction at higher temperatures (90 ºC or 120 ºC) while holding all other reaction conditions constant. However, the increased temperatures did not promote the cyclization reaction and only the straight cross-coupled product was formed. In all cases, the isolated product showed the presence of alkyne carbons at δ = 92.8 ppm (^{13}C-NMR analysis) and an alkyne stretch ($\tilde{\nu}$ = 2150 cm^{-1}) in the IR spectrum confirming the lack of cyclization.

Since the problematic step was determined to be the annulation reaction rather than the Sonogashira reaction, compound **14** was used as the substrate for the cyclization reaction using greater amounts of CuI catalyst. At 30 mol % CuI, reacting for 24 h at 80 ºC, only **14** was

recovered; however at 50 mol % CuI and allowing the reaction to proceed for 48 hours (80 °C) there was extensive decomposition of the material as determined by TLC analysis. Next, the possibility of promoting the annulation reaction of substrate **16**, that lacked the *N*6-benzoyl group, in the presence of other alkynophilic metals, namely silver[36,37] and gold[38] under prolonged or forcing conditions was briefly examined. Unfortunately, treating compound **16** with silver nitrate in refluxing acetone for up to eleven days resulted in unreacted starting material. Similarly, treatment of **16** with auric acid ($HAuCl_4$) with heating (70 °C) in ethanol for four days, with periodic monitoring, resulted in nothing more than the *t*-butyl to ethyl transesterification product.

To aid in understanding the difficulty encountered in the cyclization, we turned to a computational examination of the structures, specifically comparing the 5-alkynylcytosine scaffold that successfully undergoes a 5-*endo-dig* reaction and the 7-alkynyl-7-deazaadenine which resisted 6-*endo-dig* cyclization. In general terms, both of these reactions are predicted to be favourable by Baldwin's rules;[39,40] however, the outcome depends on the length and nature of the linking chain to permit the terminal atoms to achieve the required trajectory for ring formation. Transition metal catalysis of alkyne reactions are well-established,[41,42] especially by late transition metal species. The coordination of a metal Lewis acid to the alkyne can promote nucleophilic addition but distortion of alkyne geometry is not severe. For example, X-ray crystallographic studies on a homoleptic series of monomeric Cu(I), Ag(I), and Au(I)-alkyne complexes revealed modest deviation from linearity of the alkyne, thus implying in the present case that for a first approximation that closing the distance of the terminal ends for ring formation by metal coordination could be neglected.

Using DFT methods (B3LYP/6-31+G**) implemented by Spartan14 (Figure 4), revealed the distance between the exocyclic nitrogen and the alkyne carbon in the ground state structures. The distance for the 7-deazaadenine substrate was calculated to be 3.84 Å, whereas the distance between the exocyclic nitrogen and the alkyne carbon in the cytosine substrate was calculated to be 3.64 Å. Perhaps the 0.2 Å greater distance between the nucleophile and electrophile in the 7-deazaadenine substrate is the reason behind the difficulty in the annulation reaction, although differences in the electronics of both systems may also influence the outcome.

INSERT Figure 4

A slightly less rigid system, *N*9-propargyl adenine was shown to undergo Ag(I) catalyzed 6-*endo-dig* cyclization with the *N*3 ring nitrogen,[43] the main difference is the presence of a methylene in the linker which permits the alkyne closer approach to the nucleophilic nitrogen. As well, Furstner and Davis[44] demonstrated the $PtCl_2$ -catalyzed carboalkoxylation reaction of an *o*-alkynylbenzoate giving the product of a 6-*endo-dig* cyclization in which all the linking atoms are sp- or sp^2-hybridized as is the case for the 7-deaza-7-alkynyladenine substrate; thus, all avenues of investigation have not yet been exhausted.

CONCLUSIONS

Three new fluorescent 7-deaza-2′-deoxyadenosine derivatives connected to rigid, polyaromatic chromophores via an ethyne bridging group were successfully synthesized employing the Sonogashira cross-coupling reaction of 7-iodo-7-deaza-2′-deoxyadenosine (**4**) with 1-ethynylpyrene, 2-ethynyl-6-methoxynaphthalene, and 9-ethynylphenanthrene. These 7-deaza-

2′-deoxyadenosine analogs were spectrally characterized in dioxane, EtOH, and H_2O in order to evaluate their potential for use as environmentally sensitive fluorescent probes.

Among them, $\mathbf{A^{pyr}}$ showed the most promise, due to its red-shifted absorption wavelengths, red-shifted emission wavelengths, large molar extinction coefficients, high fluorescence quantum yields in dioxane and EtOH, and dramatic decrease in fluorescence quantum yield in H_2O. Such sizable differences in quantum yields and molar absorption coefficients allow for A^{pyr} result in relatively large brightness values. These characteristics, in conjunction with the benefit that large substituents at the 7-position of the 7-deazapurine can be accommodated in the major groove of resultant duplexes, suggests that $\mathbf{A^{pyr}}$ has considerable potential for use as an environmentally sensitive fluorescent probe. In addition, $\mathbf{A^{mon}}$ and $\mathbf{A^{phen}}$ were found to display modest potential for use as environmentally sensitive fluorescent probes. Both $\mathbf{A^{mon}}$ and $\mathbf{A^{phen}}$ had red-shifted absorption and emission wavelengths above those of the naturally occurring nucleobases. However, the combination of lower quantum yields and lower molar absorptivity values result in significantly lower brightness for these fluorophores. However, the fluorescence quantum yield of the 2-ethynyl-6-methoxynaphthalene derivative ($\mathbf{A^{mon}}$) was found to be dramatically larger (EtOH: $\Phi_F = 0.24$) than that of the 2-ethynylnaphthalene derivative (EtOH: $\Phi_F = <0.01$),[13] thus confirming the ability of polyaromatic substituents containing electron-donating groups to increase the fluorescence quantum yields of 7-deaza-2′-deoxyadenosines.

The 7-alkynyl-7-deazaadenine analogs that were examined resisted undergoing annulation reactions which are prevalent for 5-alkynylpyrimine derivatives. Reactions using archetypal alkynophilic metals such Cu(I), Pd(0), Ag(I) or Au(III) to induce nucleophilic addition by the exocyclic amino group failed to produce the desired products.

ACKNOWLEDGEMENTS

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Figure 1: 2´-deoxyadenosine with the heterocycle numbering scheme shown, its 7-deaza-7-alkynyl-substituted analog.

Figure 2: Synthetic approaches to the annulation of nucleobase derivatives for cytosine (left) and adenine (right): a) intramolecular nucleophilic aromatic substitution on biaryl derivatives derived from Suzuki-Miyaura type reactions at the 5-position of pyrimindine (left) or 7-position of 7-deazapurine (right); X = leaving group, b) 5-*endo-dig* intramolecular cyclization of 5-alkynylcytosine (left) and the proposed 6-*endo-dig* of 7-alkynyl-7-deazaadenine.

Figure 3: Excitation (blue) and emission (red) profiles of 5, A^{mon} (left), 6, A^{phen} (middle) and 7, A^{pyr} (right) in EtOH.

Figure 4: Calculated equilibrium geometries of the *N*9- or *N*1-methyl 7-deazaadenine (left) and cytosine (right) analog substrates for cyclization. The distance between the exocyclic amine and distal alkyne carbon indicated by the double-headed arrow.

Scheme 1: Synthesis of 7-deaza-2'-deoxyadenosine analogs.

Scheme 2: Proposed approach to 7-deazaadenine analogs via sequential cross-coupling and annulation reactions.

Scheme 3: Synthesis of model compounds for cyclization attempts. See the supplemental information for experimental details. Various conditions for the attempted annulation of 14 or 16 described in the text.

Table 1: Photophysical properties of fluorescent 7-deaza-2'-deoxyadenosines.

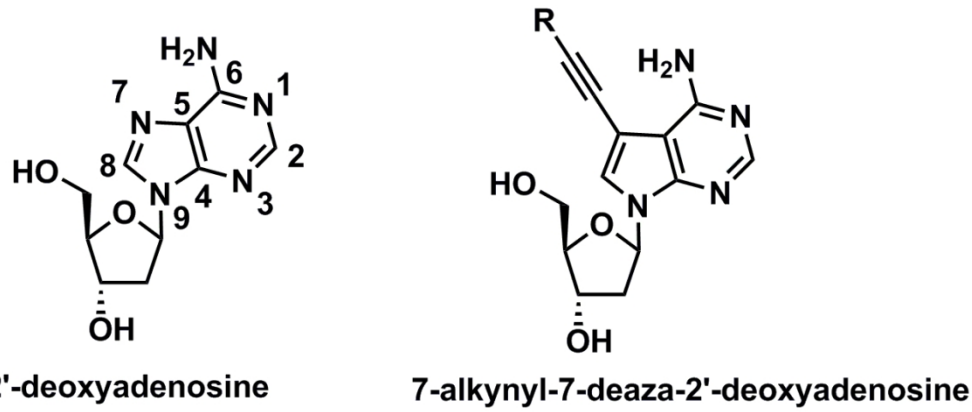

Figure 1: 2′-deoxyadenosine with the heterocycle numbering scheme shown, its 7-deaza-7-alkynyl-substituted analog.

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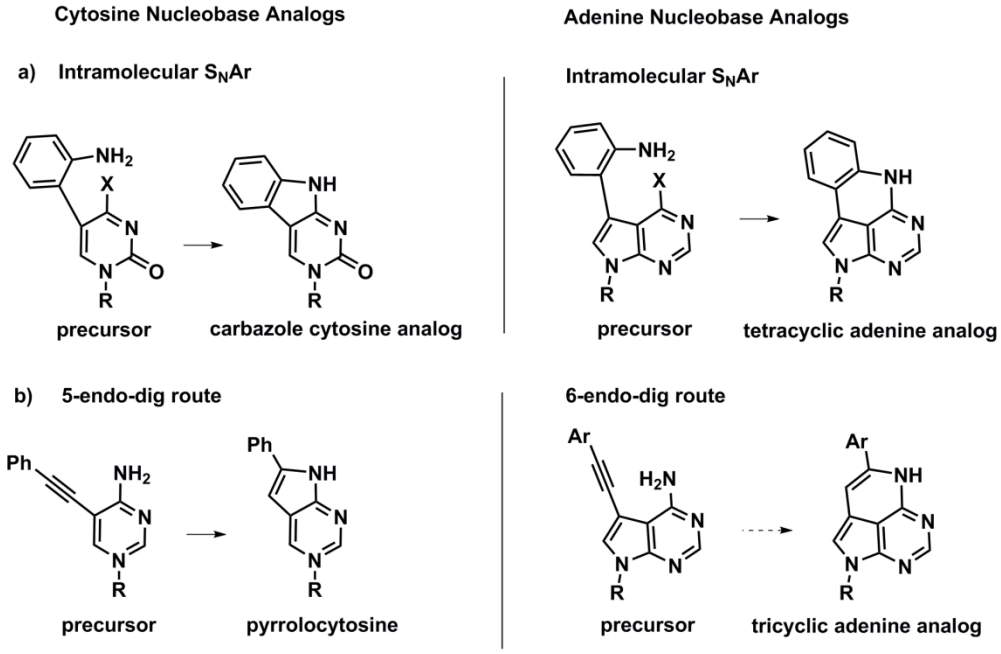

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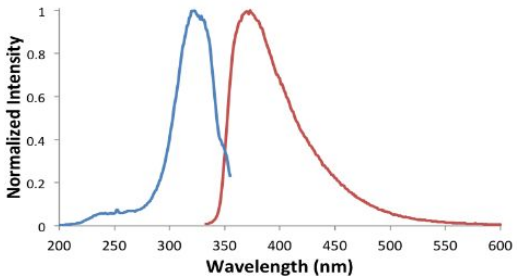

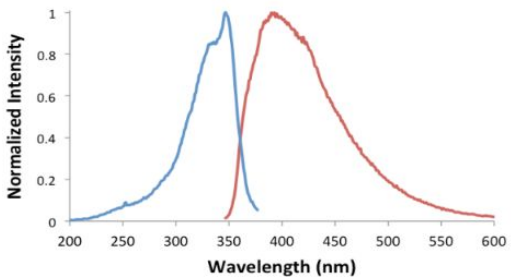

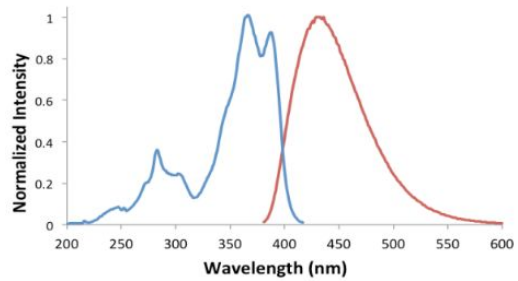

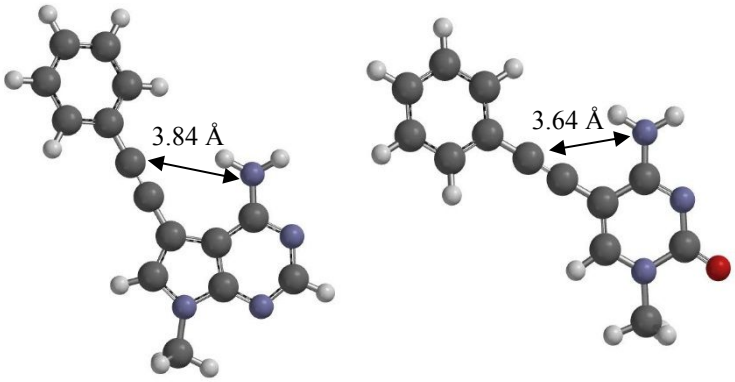

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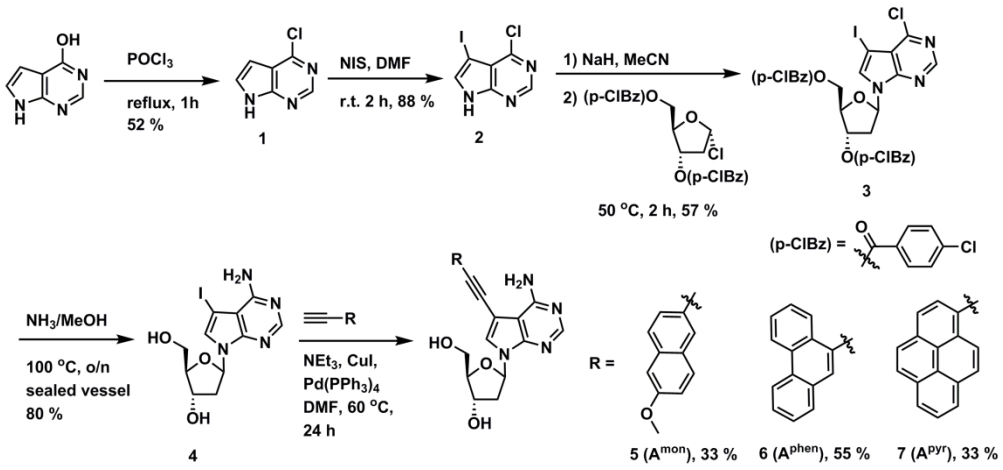
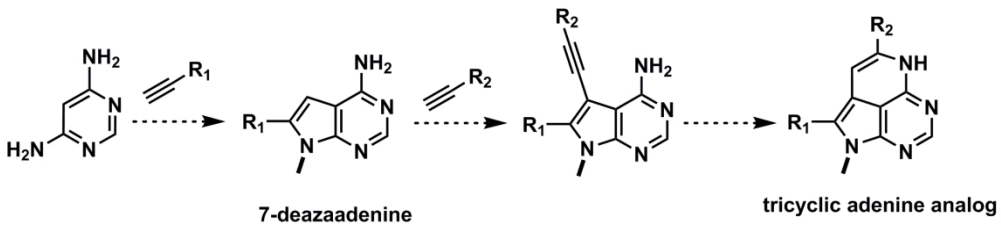
Table 1: Photophysical properties of fluorescent 7-deaza-2'-deoxyadenosines.

Analog	Solvent	λ_{ab} (nm)	λ_{fl} (nm)	Stokes Shift (nm)	ε ($\times 10^3$ $M^{-1}cm^{-1}$)	Φ_F	Brightness $\varepsilon \times \Phi_F$ ($\times 10^3$ $M^{-1}cm^{-1}$)
	dioxane	319	370	51	12.5 ± 0.2	0.15	1.9
5, A^{mon}	EtOH	318	370	52	8.3 ± 0.4	0.24	2.0
	H_2O	317	435	118	2.2 ± 0.2	0.02	0.04
	dioxane	336	390	54	16.1 ± 0.3	0.14	2.3
6, A^{phen}	EtOH	332	392	6 0	15.0 ± 0.6	0.08	1.2
	H_2O	335	444	109	6.2 ± 0.5	0.02	0.1
	dioxane	367	429	62	27.1 ± 1.6	0.52	14.1
7, A^{pyr}	EtOH	366	432	66	39.4 ± 1.0	0.43	16.9
	H_2O	364	474	110	18.7 ± 0.6	0.01	0.2



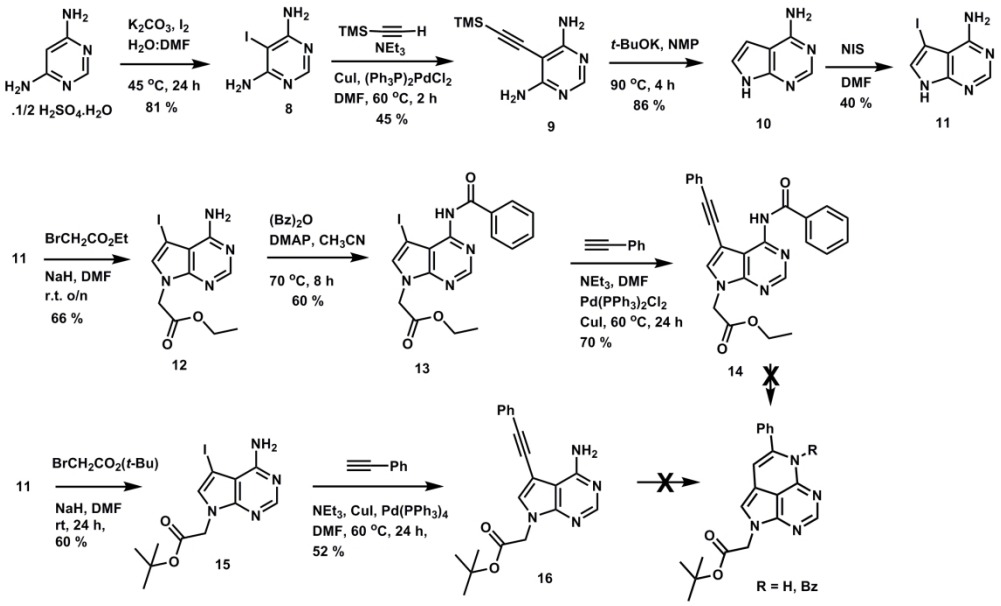
Scheme 1: Synthesis of 7-deaza-2′-deoxyadenosine analogs.

217x101mm (300 x 300 DPI)



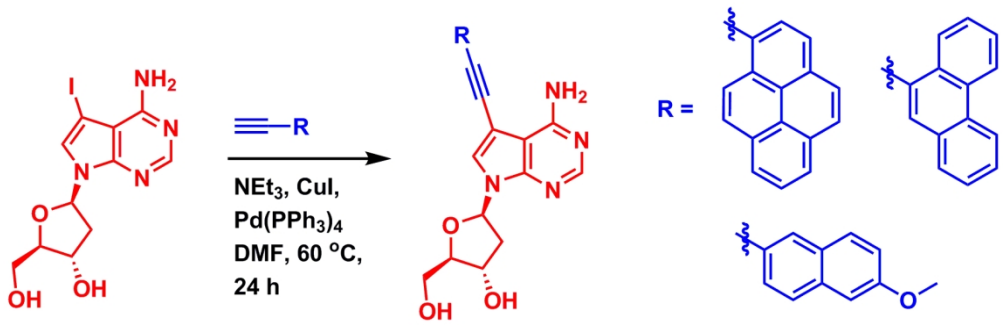
Scheme 2: Proposed approach to 7-deazaadenine analogs via sequential cross-coupling and annulation reactions.

170x39mm (300 x 300 DPI)



Scheme 3: Synthesis of model compounds for cyclization attempts. See the supplemental information for experimental details. Various conditions for the attempted annulation of 14 or 16 described in the text.

211x127mm (300 x 300 DPI)



152x49mm (300 x 300 DPI)