HHS Public Access

Author manuscript

J Org Chem. Author manuscript; available in PMC 2019 October 18.

Published in final edited form as:

J Org Chem. 2019 March 15; 84(6): 3624–3631. doi:10.1021/acs.joc.8b03135.

Fluorescent 5-Pyrimidine and 8-Purine Nucleosides Modified with an *N*-Unsubstituted 1,2,3-Triazol-4-yl Moiety

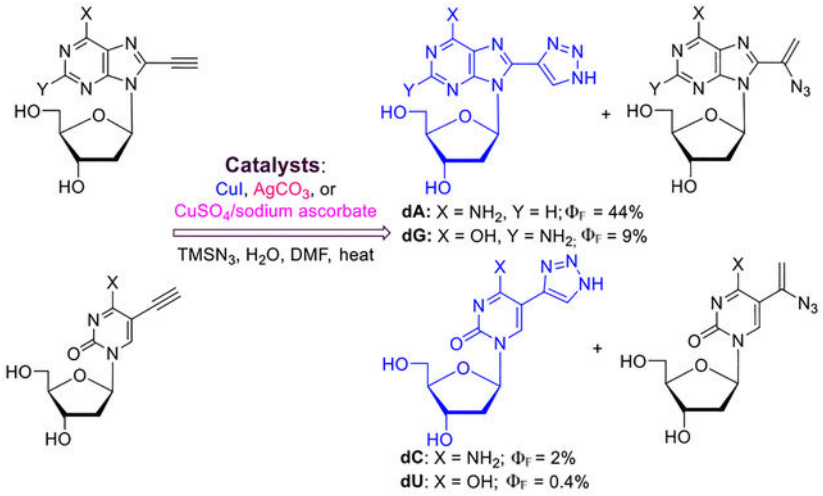
Zhiwei Wen, **Paloma R. Tuttle**, **A. Hasan Howlader**, **Anna Vasilyeva**, **Laura Gonzalez**, **Antonija Tangar**, **Ruipeng Lei**, **Eduardo E. Laverde**, **Yuan Liu**, **Jaroslava Miksovska**, **Stanislaw F. Wnuk***

Department of Chemistry and Biochemistry, Florida International University, Miami, Florida 33199, United States

Abstract

The Cu(I)- or Ag(I)-catalyzed cycloaddition between 8-ethynyladenine or guanine nucleosides and $TMSN_3$ gave 8-(1-*H*-1,2,3-triazol-4-yl) nucleosides in good yields. On the other hand, reactions of 5-ethynyluracil or cytosine nucleosides with $TMSN_3$ led to the chemoselective formation of triazoles via Cu(I)-catalyzed cycloaddition or vinyl azides via Ag(I)-catalyzed hydroazidation. These nucleosides with a minimalistic triazolyl modification showed excellent fluorescent properties with 8-(1-*H*-1,2,3-triazol-4-yl)-2′-deoxyadenosine (8-TrzdA), exhibiting a quantum yield of 44%. The 8-TrzdA 5′-triphosphate was incorporated into duplex DNA containing a one-nucleotide gap by DNA polymerase *β*.

Graphical Abstract



***Corresponding Author**: wnuk@fiu.edu.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.8b03135.

NMR spectra, mechanistic study, and fluorescent characterization (PDF)

The authors declare no competing financial interest.

The natural nucleic acid has weak fluorescent properties.[1] However, modified nucleoside analogues with fluorescent properties serve as powerful tools to study nucleic acid interactions, activities, and structures.[2–9] The nucleoside derivatives with fluorescent nucleobases have been recently reviewed.[10] The criteria[4] for designing fluorescent nucleosides are (i) sensitiveness to the microenvironment, (ii) emission at long wavelengths, (iii) high quantum efficiency, and (iv) minimalistic modifications, which cause the least amount of distortion of natural hydrogen bonding.

Fluorescent properties of nucleosides bearing triazolyl modifications varied mostly by the (i) position of nucleobases, at which a triazolyl unit is attached, (ii) site of triazolyl attachment to the nucleobase (N1 vs C4), and (iii) additional substitutions either at nucleobase or triazolyl units. The syntheses of the *N*-substituted triazolyl analogues are mainly based on CuAAC and strain promoted click chemistry.[6,11–18] For example, the 8-(1*H*-1,2,3-triazol-4-yl)adenosine derivatives **A** and **B** (Figure 1), with triazolyl carbon attached to the C8 position of the purine ring, have quantum yields up to 64%,[14,19] while (1,2,3-triazol-1-yl)adenosines **C** and **D**, with triazolyl nitrogen attached to the C8 position of purine, display significantly lower quantum yields (0.21–1.7%).[6,15] The 2-(1,2,3-triazol-1-yl)adenosine analogues **E** and **F** with a triazole moiety at the C2 position have a moderate to relatively high quantum yield (2–20%).[6,11] The 5-azidouracil nucleosides click with cyclooctyne substrates, generating 5-(1,2,3-triazol-1-yl) nucleosides **G** for the living cell fluorescent imaging.[6]

So far, only a few examples of nucleosides modified with the minimalistic (*N*-unsubstituted) (1*H*-1,2,3-triazol-4-yl) unit are reported. Thus, 5-(1*H*-1,2,3-triazol-4-yl)-2′-deoxyuridine (5-TrzdU) was synthesized by microwave-assisted CuAAC between the corresponding 5-ethynyluracil and in situ generated pivaloyloxymethyl (POM) azide followed by *N*-deprotection[20] or by the nucleobase-exchange reaction.[21] *N*-POM-protected 5-TrzdU was incorporated into DNA via solid-phase synthesis.[20] The 5-TrzdU[20] and its analogues[17,22] stack in the major groove and increase the stability of the duplex DNA.

Strategies developed for the synthesis of *N*-unsubstituted triazoles include: (i) CuI-catalyzed [3+2] cycloaddition of terminal alkynes and trimethylsilyl azide ($TMSN_3$),[23] (ii) Pd-[24] or *p*-toluenesulfonic acid-catalyzed[25] cycloaddition between activated alkene and NaN_3, and (iii) deprotection of *N*-substituted triazoles.[12,26,27] Herein, we report the catalyst-dependent cycloaddition of acetylenic nucleosides with $TMSN_3$ for the synthesis of 5-pyrimidine and 8-purine nucleosides modified with a minimalistic *N*-unsubstituted 1,2,3-triazol-4-yl moiety and their fluorescent properties.

Synthesis.

Treatment of 8-ethynyl-2′-deoxyadenosine **1a**[28] with $TMSN_3$ in the presence of CuI as a catalyst[23] gave 8-(1*H*-1,2,3-triazol-4-yl)-2′-deoxyadenosine (8-TrzdA, **2a**; method A; Table 1, entry 1). Analogous treatment of TBDMS-protected 2′-deoxyadenosine **1b**[28] with $TMSN_3$ yielded protected 8-TrzdA (**2b**, 47%; entry 2). 8-Ethynyl-2′-deoxyguanosine **1c**[29] under analogous conditions provided 8-(1*H*-1,2,3-triazol-4-yl)-2′-deoxyguanosine (8-

TrzdG, **2c**; entry 3). Thus, cycloaddition of the 8-ethynylpurine nucleosides with $TMSN_3$ in the presence of CuI produced triazoles as sole products.

To avoid both loss of catalytic ability of Cu(I) and colorization of the reaction mixture, we found that treatment of **1a** with $TMSN_3$ in the presence of in situ generated Cu(I) from $CuSO_4$/sodium ascorbate gave **2a** with a 62% isolated yield (method B, entry 4). Similar treatment of **1b** and **1c** with $TMSN_3$ afforded triazoles **2b** (entry 5) and **2c** (entry 6) in higher yields than that by method A.

We also found that Ag_2CO_3-catalyzed hydroazidation of **1a** with $TMSN_3$ (in DMF with 2 equiv of H_2O) produced **2a** (58%) in addition to the vinyl azide **3a** (15%; method C, entry 7). Analogous treatment of **1b** provided **2b** without a detected trace of vinyl azide **3b** (entry 8). Subjection **1c** to method C for 4 h also yielded **2c** as the sole product (entry 9). It is noteworthy that Ag_2CO_3-catalyzed reactions between arylacetylenes and $TMSN_3$ provide vinyl azides as main products.[30,31]

Transition-metal-catalyzed cycloaddition of $TMSN_3$ with alkyne has been extended into the 5-ethynyl pyrimidine nucleosides **4a–d** (methods A–C, Table 2). Thus, CuI-catalyzed cycloaddition of 3′,5′-di-*O*-acetyl-5-ethynyl-2′-deoxycytidine **4a**[32] yielded 5-(1*H*-1,2,3-triazol-4-yl) product **5a** (method A; 45%, entry 1), while unprotected 2′-deoxycytidine substrate **4b**[32] provided 5-(1*H*-1,2,3-triazol-4-yl)-2′-deoxycyti-dine **5b** (5-TrzdC, entry 2). Protected **4c**[33] and unprotected 5-ethynyl-2′-deoxyuridine **4d**[33] gave 5-(1*H*-1,2,3-triazol-4-yl) products **5c** (65%, entry 3) and **5d** (5-TrzdU; 62%, entry 4). It appears that H_2O is required for the reaction to proceed[23] since the mixture of DMF/H_2O (9:1) as a solvent gave the best yields of products **5a–d**. Hydroazidation of **4d** in the presence of 2 equiv of H_2O in DMF gave **5d** (50%, entry 5) as a sole product, showing that a stoichiometric amount of H_2O was sufficient for cycloaddition to occur.

The $CuSO_4$/sodium ascorbate-catalyzed cycloaddition of $TMSN_3$ to alkynes **4a–d** produced **5a–d** in higher yields (60–82%; method B, entries 6–9) than by method A. However, treatment of alkyne **4a** with $TMSN_3$ in the presence of Ag_2CO_3 provided triazole **5a** in a low yield (7%), affording instead the 5-(1-azidovinyl)cytosine nucleoside **6a**[34] as a major product (51%, entry 10). The analogous hydroazidation of **4c** produced 5-(1-azidovinyl) **6c** (52%) as the sole product (entry 11). It appears that paths for these reactions between nucleoside alkynes and $TMSN_3$ depends not only on the catalyst used but also on the nature of the nucleobases, to which the alkyne group is attached, leading selectively to triazoles or vinyl azides.

The $CuSO_4$/sodium ascorbate-catalyzed synthesis of triazoles from alkynes and $TMSN_3$ (method B) has a general character as illustrated with *p*-substituted phenylacetylenes **7a–c** (Scheme 1). Thus, aryl alkyne **7a** with EDG (CH_3O) gave triazole product **8a** (63%), while aryl alkyne **7c** with EWG (CF_3) yielded triazole **8c** in a higher yield of 83%. Electron-withdrawing groups (EWGs) are known to promote the formation of triazoles in higher yields.[23,35]

In summary, *N*-unsubstituted triazolyl analogues of the four natural bases of DNA were prepared by treatment of 5-ethynylpyrimidine or 8-ethynylpurine 2′-deoxynucleosides with $TMSN_3$ in the presence of CuI or $CuSO_4$/sodium ascorbate as a catalyst. Interestingly, Ag-catalyzed reactions of $TMSN_3$ with 8-alkyne purines gave mainly triazole products **2a–c** (Table 1), while with 5-alkyne pyrimidines provided mainly Markovnikov addition products **6a** or **6c** (Table 2), probably due to electronic differences between pyrimidine and purine imidazole rings. NMR studies showed that triazolyl nucleosides **2** and **5** are stable in D_2O/DMSO-d_6 solution (500 μL/50 μL; 2 mg/mL) at 37 °C for 72 h.

A reaction mechanism for the formation of triazoles might involve [3+2] cycloaddition or an initial formation of vinyl azide followed by 1,5-electrocyclization and tautomerization. However, attempts to validate the latter mechanism by treatment with 8-(1-azidovinyl)-2′-deoxyadenosine **3a** using method C failed to produce the desired triazole **2a** (Scheme 2). Also treatment of 5-(1-azidovinyl)-2′-deoxyuridine **6d** using method B did not produce triazole **5d**. Thus, the formation of the triazolyl unit involving [3+2] cycloaddition[23] of alkyne with in situ generated HN_3 seems to be plausible (Figure S1).

Enzymatic Incorporation of 8-TrzdATP into DNA.

Treatment of 8-TrzdA **2a** with $POCl_3$ in the presence of a proton sponge[36] followed by the addition of tributylammonium pyrophosphate (TBAPP) and tributylamine (TBA) yielded 8-TrzdATP **9** (Scheme 3).

We also examined the incorporation of the triazolyl nucleotides into an oligonucleotide DNA substrate containing a nucleotide gap by pol β. The pol β efficiently incorporated 8-TrzdATP **9** into the one-nucleotide gap substrate containing a phosphate group on the downstream strand (Figure 2, Table S3). Incorporation of 8-TrzdATP increased with increasing concentrations of pol β (10–200 nM; lanes 2–6). Ten nM pol β was sufficient to insert 8-TrzdATP into the DNA to generate ~10% of the DNA synthesis product (lane 2). The 200 nM concentration of pol β resulted in 40% of incorporation of 8-TrzdATP (lane 6). Incorporation of **9** was extended by pol β in duplex DNA in the presence of dGTP (Figure S4). Polymerase-catalyzed incorporation of the 8-substituted purine nucleotides is known to depend on the size of the substituent and preference for *syn*/*anti* conformation of the base.[37,38]

Fluorescent Properties.

The normalized fluorescence emission, absorption, and excitation spectra for 1*H*-1,2,3-triazol-4-yl nucleosides in methanol are shown in Figure 3. Their photophysical data are summarized in Table 3. The 8-TrzdA **2a** with the C4 of triazolyl attaching to the C8 of adenine exhibits the highest quantum yield (Φ_F) of 44% and emits at 300–480 nm with the maximum emission at 355 nm. The silyl-protected 8-TrzdA analogue 2b has a Φ_F of 48% (Table S1). The emission of 8-TrzdG **2c** starts at 300 nm but extends to 540 nm, and the maximum emission is at 364 nm with a Φ_F of 9%. Emission λ_{max} of **2a** is pH- and solvent-independent (Figure S2, Table S2).

The 5-pyrimidine analogues 5-TrzdU **5d** and 5-TrzdC **5b** showed a larger Stokes shift of ~110 nm with a maximum emission approximately at 408 nm and lower quantum yields. The 5-TrzdC emits at 320–550 nm (Φ_F = 2%), while 5-TrzdU emits at 320–500 nm (Φ_F = 0.4%). Acetyl-protected **5a** and **5c** have similar fluorescent properties (Table S1). The emission spectra of **5b** show a strong pH dependence in aqueous phosphate buffer with a bathochromic shift of the emission band maximum from 378 nm (pH 4.0) to 435 nm (pH 7.0) and to 433 nm (pH 12.0). Emission maxima in DMSO and ACN are similar to those observed in phosphate buffer at a neutral and basic pH (Figure S3, Table S2).

All triazoles showed biphasic fluorescence decay. The triazoles present a fast lifetime of 0.1–4.4 ns (Table 3). Compound **5b** shows the longest lifetime of 4.35 ns and longest average lifetime of 3.7 ns. Compounds **2a** (63%), **2c** (71%), **5b** (85%), and **5d** (73%) showed a larger contribution of the long lifetime (τ_2).

Fluorescent *N*-unsubstituted 1,2,3-triazol-4-yl deoxynucleo-sides have been synthesized by catalyst-dependent reactions between 5-ethynylpyrimidine or 8-ethynylpurine nucleosides with $TMSN_3$. CuI or $CuSO_4$/sodium ascorbate-catalyzed cycloaddition gave triazoles products for both purine and pyrimidine nucleosides. In contrast, Ag_2CO_3-catalyzed reactions with 8-ethynylpurine nucleosides produced 8-triazolyls, whereas 5-ethynylpyrimidine nucleosides followed the hydroazidation pathway to give 5-(1-azidovinyl) as a major product. The triazoles showed good fluorescent properties with 8-TrzdA, exhibiting the highest quantum yield of 44%. 8-TrzdATP was incorporated into duplex DNA containing a one-nucleotide gap by human DNA polymerase β. Metabolic incorporation of 8-purine and 5-pyrimidine 1,2,3-triazol-4-yl nucleosides into DNA for fluorescent imaging and their antiviral and anticancer evaluation will be published elsewhere.[39]

EXPERIMENTAL SECTION

General Information.

^{1}H NMR spectra at 400 MHz and ^{13}C NMR at 100.6 MHz were recorded in DMSO-d_6 unless otherwise noted. All chemical shift values are reported in parts per million (ppm) and referenced to the residual solvent peaks of DMSO-d_6 (2.50 ppm) for ^{1}H NMR and DMSO-d_6 (39.52 ppm) peaks for ^{13}C NMR spectra, with coupling constant (J) values reported in hertz (Hz). HRMS were obtained in TOF (ESI) mode. TLC was performed on Merck silica gel 60-F_{254}, and products were detected with 254 nm light. Merck silica gel 60 (230–400 mesh) was used for column chromatography. All reagents and solvents were purchased from commercial suppliers and used without further purification. Experimental procedures for the fluorescent characterization of the triazolyl nucleosides and protocols for the incorporation of 8-TrzdATP 9 by human DNA polymerase are detailed in the Supporting Information.

8-(1H-1,2,3-Triazol-4-yl)-2′-deoxyadenosine (2a).

Procedure A: CuI as a Catalyst.: The stirred solution of **1a**[28] (27.5 mg, 0.1 mmol) in DMF/H_2O (1 mL, 9:1, v/v) was degassed with Ar for 15 min. $TMSN_3$ (26.3 μL, 23 mg, 0.2 mmol) and CuI (1 mg, 0.005 mmol) were then added, and the resulting mixture was further degassed for another 5 min and was stirred at 90 °C for 5 h. After the mixture cooled to

ambient temperature, the volatiles were evaporated and the residue was column chromatographed ($CHCl_3$/MeOH, 100:0 → 85:15) to give **2a** (15.0 mg, 48%): UV (MeOH) λ_{max} 203, 228, 287 nm (ε 15 900, 16 050, 17 950), λ_{min} 213, 250 nm (ε 13 800, 5100); ^{1}H NMR δ 2.20 (ddd, J = 12.9, 5.9, 1.7 Hz, 1H), 3.12–3.19 (m, 1H), 3.49–3.55 (m, 1H), 3.67–3.72 (m, 1H), 3.90 (q, J = 3.7 Hz, 1H), 4.46–4.51 (m, 1H), 5.27 (d, J = 3.7 Hz, 1H), 5.77–5.80 (m, 1H), 7.07 (t, J = 7.2 Hz, 1H), 7.51 (s, 2H), 8.13 (s, 1H), 8.44 (s, 1H), 15.72 (s, 1H); ^{13}C{^{1}H} NMR δ 38.1, 62.4, 71.6, 85.9, 88.4, 119.4, 130.6, 137.8, 141.6, 149.8, 152.1, 156.1; HRMS (ESI) m/z calcd for $C_{12}H_{14}N_8O_3Na$ $[M + Na]^+$ 341.1081, found 341.1062.

Note that purification of the crude reaction mixture on RP-HPLC (solvent A, 100% ACN; solvent B, 5% ACN/H_2O; gradient 0% A → 15% A in 30 min, flow rate = 2 mL/min) gave **2a** (52%).

Procedure B: $CuSO_4$/Sodium Ascorbate as a Catalyst.: The stirred solution of **1a** (27.5 mg, 0.1 mmol) and $CuSO_4 \cdot 5H_2O$ (2.5 mg, 0.01 mmol) in DMF/H_2O (1 mL, 9:1, v/v) was degassed with Ar for 15 min. $TMSN_3$ (26.3 μL, 23 mg, 0.2 mmol) and sodium ascorbate (4 mg, 0.02 mmol) were then added, and the resulting mixture was further degassed for another 5 min and was stirred at 90 °C for 5 h. After the mixture was cooled to ambient temperature, the volatiles were evaporated and the residue was column chromatographed ($CHCl_3$/MeOH, 100:0 → 85:15) to give **2a** (19.7 mg, 62%) with the spectroscopic data as described above.

Procedure C: Ag_2CO_3 as a Catalyst.: Ag_2CO_3 (2.8 mg, 0.01 mmol) was added to a solution of **1a** (27.5 mg, 0.1 mmol), $TMSN_3$ (26.3 μL, 23 mg, 0.2 mmol), and H_2O (3.6 μL, 3.6 mg, 0.2 mmol) in DMF (1 mL). The resulting mixture was stirred at 80 °C for 1 h. After the mixture cooled to ambient temperature, the volatiles were evaporated under the reduced pressure and the residue was column chromatographed ($CHCl_3$/MeOH, 100:0 → 85:15) to give **3a** (4.8 mg, 15%, see below for spectroscopic characterization) followed by **2a** (18.4 mg, 58%).

3′,5′-Di-O-tert-butyldimethylsilyl-8-(1H-1,2,3-triazol-4-yl)-2′-deoxyadenosine (2b).—Treatment of **1b**[28] (201.6 mg, 0.4 mmol) with CuI by Procedure A (column chromatography; hexane/EtOAc 50:50 → 0:100) gave **2b** (103.2 mg, 47%): UV (MeOH) λ_{max} 225, 285 nm (ε 17 500, 14 100), λ_{min} 247 nm (ε 3900); ^{1}H NMR δ −0.13 (s, 3H), −0.07 (s, 3H), 0.12 (s, 6H), 0.77 (s, 9H), 0.90 (s, 9H), 2.20–2.27 (m, 1H), 3.57–3.66 (m, 2H), 3.77 (dd, J = 9.0, 4.7 Hz, 1H), 3.88 (dd, J = 10.8, 6.0 Hz, 1H), 4.88 (“q”, J = 4.2 Hz, 1H), 7.01 (t, J = 6.5 Hz, 1H), 7.36 (s, 2H), 8.13 (s, 1H), 8.41 (s, 1H); ^{13}C{^{1}H} NMR δ −5.6, −5.5, −4.9, −4.7, 17.8, 17.9, 25.6, 25.7, 36.4, 62.3, 72.2, 84.5, 86.6, 119.3, 130.6, 138.0, 142.0, 150.2, 152.3, 156.0; HRMS (ESI) m/z calcd for $C_{24}H_{43}N_8O_3Si_2$ $[M + H]^+$ 547.2991, found 547.3004.

Treatment of **1b** (201.6 mg, 0.4 mmol) with $CuSO_4$/sodium ascorbate by Procedure B (column chromatography; hexane/EtOAc 50:50 → 0:100) gave **2b** (155.6 mg, 71%).

Treatment of **1b** (201.6 mg, 0.4 mmol) with Ag_2CO_3 by Procedure C (column chromatography; hexane/EtOAc 50:50 → 0:100) gave **2b** (120.4 mg, 55%).

8-(1H-1,2,3-Triazol-4-yl)-2′-deoxyguanosine (2c).—Treatment of **1c**[29] (29.1 mg, 0.1 mmol) with CuI by Procedure A (column chromatography; $CHCl_3$/MeOH, 95:5 → 80:20) gave **2c** (10.2 mg, 31%): UV (MeOH) λ_{max} 205, 283 nm (ε 14 450, 14 800); λ_{min} 238 (ε 2600); ^{1}H NMR δ 2.07–2.13 (m, 1H), 3.12–3.19 (m, 1H), 3.50 (dd, J = 11.7, 5.3 Hz, 1H), 3.65 (dd, J = 11.8, 4.8 Hz, 1H), 3.78–3.81 (m, 1H), 4.38–4.44 (m, 1H), 5.05 (“s”, 1H), 5.18 (d, J = 3.7 Hz, 1H), 6.43 (s, 2H), 8.34 (t, J = 7.8 Hz, 1H), 10.83 (s, 1H), 15.42 (s, 1H); ^{13}C{^{1}H} NMR δ 37.4, 62.3, 71.4, 84.8, 88.0, 117.6, 128.8, 138.8, 151.9, 153.1, 154.0, 156.5; HRMS (ESI) m/z calcd for $C_{12}H_{15}N_8O_4$ $[M + H]^+$ 335.1211, found 335.1214.

Note that purification of the crude reaction mixture on RP-HPLC (C18, A, 100% ACN; B, 5% ACN/H_2O; 0% A → 15% A in 30 min, flow rate = 2 mL/min) gave **2c** (36%).

Treatment of **1c** (29.1 mg, 0.1 mmol) with $CuSO_4$/sodium ascorbate by Procedure B (column chromatography; $CHCl_3$/MeOH, 95:5 → 80:20) gave **2c** (26.0 mg, 52%).

Treatment of **1c** (29.1 mg, 0.1 mmol) with Ag_2CO_3 by Procedure C (column chromatography; $CHCl_3$/MeOH, 95:5 → 80:20) gave **2c** (30.1 mg, 60%).

8-(1-Azidovinyl)-2′-deoxyadenosine (3a): 4.8 mg, 15%; Procedure C, see above; ^{1}H NMR δ 2.19 (ddd, J = 13.1, 6.3, 2.3 Hz, 1H), 3.20–3.27 (m, 1H), 3.47–3.54 (m, 1H), 3.67 (dt, J = 11.8, 4.0 Hz, 1H), 3.89 (q, J = 4.1 Hz, 1H), 4.46–4.51 (m, 1H), 5.31 (d, J = 4.1 Hz, 1H), 5.40 (d, J = 2.0 Hz, 1H), 5.46 (dd, J = 8.1, 4.0 Hz, 1H), 5.56 (d, J = 2.0 Hz, 1H), 6.36 (dd, J = 8.1, 6.4 Hz, 1H), 7.56 (s, 2H), 8.15 (s, 1H); ^{13}C{^{1}H} NMR δ 37.4, 62.1, 71.3, 85.6, 88.4, 108.7, 118.6, 134.2, 143.9, 149.5, 152.8, 156.4; HRMS (ESI) m/z calcd for $C_{12}H_{14}N_8O_3Na^+$ $[M + Na]^+$ 341.1081, found 341.1088.

3′,5′-Di-O-Acetyl-5-(1H-1,2,3-triazol-4-yl)-2′-deoxycyti-dine (5a).—Treatment of **4a**[40] (134.0 mg, 0.4 mmol) with CuI by Procedure A (column chromatography; $CHCl_3$/MeOH, 100:0 → 90:10) to give **5a** (67.6 mg, 45%): UV (MeOH) λ_{max} 208, 238, 293 nm (ε 13 050, 9400, 4150), λ_{min} 225, 271 nm (ε 8350, 3150); ^{1}H NMR δ 1.99 (s, 3H), 2.08 (s, 3H), 2.36 (ddd, J = 14.1, 5.8, 2.0 Hz, 1H), 2.44–2.47 (m, 1H), 4.20–4.23 (m, 1H), 4.26–4.35 (m, 2H), 5.20–5.22 (m, 1H), 6.21 (dd, J =7.6, 6.2 Hz, 1H), 7.67 (s, 1H), 7.90 (s, 1H), 8.07 (s, 1H), 8.24 (s, 1H), 15.28 (s, 1H); ^{13}C{^{1}H} NMR δ 20.5, 20.7, 36.6, 63.7, 74.3, 81.6, 86.0, 97.2, 126.9 (HMQC showed a cross peak to proton at 8.24 ppm), 139.8, 153.6, 162.4, 170.0, 170.2; HRMS (ESI) m/z calcd for $C_{15}H_{19}N_6O_6^+$ $[M + H]^+$ 379.1361, found 379.1372.

Treatment of **4a** (134.0 mg, 0.4 mmol) with $CuSO_4$/sodium ascorbate by using Procedure B (column chromatography; $CHCl_3$/MeOH, 100:0 → 90:10) gave **5a** (97.6 mg, 65%).

5-(1H-1,2,3-Triazol-4-yl)-2′-deoxycytidine (5b).—Treatment of **4b**[40] (25.1 mg, 0.1 mmol) with CuI by using Procedure A (column chromatography; $CHCl_3$/MeOH, 100:0 → 80:20) gave **5b** (13.9 mg, 48%): UV (MeOH) λ_{max} 207, 238, 296 nm (ε 18 750, 13 900, 5500), λ_{min} 224, 273 nm (ε 11 900, 3500); ^{1}H NMR δ 2.08–2.14 (m, 1H), 2.21 (ddd, J = 13.2, 6.1, 4.7 Hz, 1H), 3.59–3.66 (m, 1H), 3.68–3.75 (m, 1H), 3.82 (q, J = 3.3 Hz, 1H), 4.24–4.30 (m, 1H), 5.24 (s, 1H), 5.29 (s, 1H), 6.18 (t, J = 6.1 Hz, 1H), 7.68 (s, 1H), 7.80 (s, 1H), 8.07 (s, 1H), 8.60 (s, 1H), 15.18 (s, 1H); ^{13}C{^{1}H} NMR δ 41.4, 61.2, 70.0, 85.9, 88.0,

96.9, 126.8, 140.6, 142.0, 154.2, 162.7; HRMS (ESI) *m*/*z* calcd for $C_{11}H_{15}N_6O_4^+$ $[M + H]^+$ 295.1149, found 295.1160.

Note that the purification of the crude reaction mixture on RP-HPLC (C18, A, 100% ACN; B, 5% ACN/H_2O; 0% A → 15% A in 30 min, flow rate = 2 mL/min) gave **5b** (53%).

Treatment of **4b** (25.1 mg, 0.1 mmol) with $CuSO_4$/sodium ascorbate by using Procedure B (column chromatography; $CHCl_3$/MeOH, 100:0 → 80:20) gave **5b** (17.4 mg, 60%).

3′,5′-Di-O-Acetyl-5-(1H-1,2,3-triazol-4-yl)-2′-deoxyuridine (5c).—Treatment of **4c**[33] (33.6 mg, 0.1 mmol) with CuI by using Procedure A (column chromatography; $CHCl_3$/MeOH, 100:0 → 92:8) gave **5c** (24.6 mg, 65%): UV (MeOH) λ_{max} 231, 293 nm (ε 10 700, 9600), λ min 258 nm (ε 2900); ^{1}H NMR δ 2.08 (s, 3H), 2.13 (s, 3H), 2.36–2.46 (m, 2H), 4.22–4.26 (m, 2H), 4.27–4.31 (m, 1H), 5.21–5.26 (m, 1H), 6.25 (t, J = 6.3 Hz, 1H), 8.18 (s, 1H), 8.31 (s, 1H), 11.82 (s, 1H), 15.19 (s, 1H); $^{13}C\{^1H\}$ NMR δ 20.7, 20.8, 36.7, 63.8, 74.2, 81.7, 84.9, 105.5, 128.2 (HMQC showed a cross peak to proton at 8.18 ppm), 135.8, 149.6, 161.2, 170.1, 170.4; HRMS (ESI) *m*/*z* calcd for $C_{15}H_{18}N_5O_7^+$ $[M + H]^+$ 380.1201, found 380.1208.

Treatment of **4c** (33.6 mg, 0.1 mmol) with $CuSO_4$/sodium ascorbate by using Procedure B (column chromatography; $CHCl_3$/MeOH, 100:0 → 92:8) gave **5c** (31.0 mg, 82%).

5-(1H-1,2,3-Triazol-4-yl)-2′-deoxyuridine (5d).—Treatment of **4d**[33] (25.1 mg, 0.1 mmol) with CuI by using Procedure A (column chromatography; $CHCl_3$/MeOH, 100:0 → 85:15) gave **5d**[20,21] (18.4 mg, 62%): UV (MeOH) λ_{max} 231, 292 nm (ε 12 400, 11 450), λ_{min} 259 nm (ε 3700); ^{1}H NMR δ 2.18 (dd, J = 6.3, 4.7 Hz, 2H), 3.55–3.63 (m, 2H), 3.84 (q, J = 3.4 Hz, 1H), 4.25–4.31 (m, 1H), 5.04 (“s”, 1H), 5.29 (d, J = 4.1 Hz, 1H), 6.22 (t, J = 6.6 Hz, 1H), 8.14 (s, 1H), 8.49 (s, 1H), 11.68 (s, 1H), 15.10 (s, 1H); $^{13}C\{^1H\}$ NMR δ 39.9, 61.3, 70.6, 84.7, 87.6, 105.1, 131.8, 135.8, 137.0, 149.7, 161.2; HRMS (ESI) *m*/*z* calcd for $C_{11}H_{14}N_5O_5^+$, $[M + H]^+$ 296.0989, found 296.0983.

Treatment of **4d** (25.1 mg, 0.1 mmol) with CuI by using modified Procedure A with 2 equiv of H_2O and DMF as a solvent (column chromatography; $CHCl_3$/MeOH, 100:0 → 85:15) gave **5d** (14.9 mg, 50%).

Treatment of **4d** (25.1 mg, 0.1 mmol) with $CuSO_4$/sodium ascorbate by using Procedure B (column chromatography; $CHCl_3$/MeOH, 100:0 → 85:15) gave **5d** (21.2 mg, 72%).

3′,5′-Di-O-Acetyl-5-(1-azidovinyl)-2′-deoxycytidine (6a).—Treatment of **4a** (134.0 mg, 0.4 mmol) with Ag_2CO_3 by using Procedure C (column chromatography; $CHCl_3$/MeOH, 100:0 → 90:10) gave **6a** (75.6 mg, 51%) followed by **5a** (10.4 mg, 7%). Compound **6a**: ^{1}H NMR ($CDCl_3$) δ 2.02–2.14 (m, 1H), 2.08 (s, 3H), 2.10 (s, 3H), 2.75 (ddd, J = 14.3, 5.5, 2.0 Hz, 1H), 4.31–4.41 (m, 3H), 5.03 (d, J = 1.5 Hz, 1H), 5.11 (d, J = 1.4 Hz, 1H), 5.21 (dt, J = 6.3, 1.8 Hz, 1H), 5.83 (s, 2H) 6.28 (dd, J = 8.0, 5.6 Hz, 1H), 7.80 (s, 1H); $^{13}C[^1H]$ NMR ($CDCl_3$) δ 20.8, 21.0, 39.0, 64.0, 74.4, 82.9, 86.8, 101.8, 103.0, 139.9, 140.2, 154.3, 162.7, 170.4, 170.6; HRMS (ESI) *m*/*z* calcd for $C_{15}H_{19}N_6O_6^+$ $[M + H]^+$ 379.1361, found 379.1355.

3′,5′-Di-O-Acetyl-5-(1-azidovinyl)-2′-deoxyuridine (6c).—Treatment of **4c** (67.2 mg, 0.2 mmol) with Ag_2CO_3 by using Procedure C (hexane/EtOAc 50:50) gave **6c**[41] (41.2 mg, 52%): ^{1}H NMR δ 2.07 (s, 6H), 2.33–2.46 (m, 2H), 4.24–4.29 (m, 3H), 5.06 (“s”, 1H), 5.19–5.21 (m, 1H), 6.00 (“s”, 1H), 6.14 (t, J = 6.4 Hz, 1H), 7.83 (s, 1H), 11.73 (s, 1H); ^{13}C[^{1}H] NMR δ 20.4, 20.8, 36.7, 63.8, 74.2, 81.8, 85.4, 101.6, 107.3, 136.9, 138.3, 149.3, 160.9, 170.1, 170.2; HRMS (ESI) m/z calcd for $C_{15}H_{18}N_5O_7^+$ $[M + H]^+$ 380.1201, found 380.1195.

4-(4-Methoxyphenyl)-1H-1,2,3-triazole (8a).—Treatment of **7a** (13.2 mg, 0.1 mmol) with $CuSO_4$/sodium ascorbate by using Procedure B (column chromatography; hexane/EtOAc, 100:0 → 70:30) gave **8a**[23,42] (11 mg, 63%): ^{1}H NMR δ 3.80 (s, 3H), 7.02 (d, J = 8.8 Hz, 2H), 7.78 (d, J = 8.8 Hz, 2H), 8.22 (s, 1H); ^{13}C{^{1}H} NMR δ 55.6, 114.5, 114.8, 123.3, 127.4, 145.8, 159.7; HRMS (ESI) m/z calcd for $C_9H_{10}N_3O^+$ $[M +H]^+$ 176.0818, found 176.0822.

4-Phenyl-1H-1,2,3-triazole (8b).—Treatment of **7b** (20 mg, 0.2 mmol) with $CuSO_4$/sodium ascorbate by using Procedure B (column chromatography; hexane/EtOAc, 100:0 → 70:30) gave **8b**[23,42] (21 mg, 73%): ^{1}H NMR δ 7.39 (t, J = 7.2 Hz, 1H), 7.50 (t, J = 7.5 Hz, 2H), 7.86 (d, J = 7.3 Hz, 2H), 8.34 (s, 1H); ^{13}C{^{1}H} NMR δ 126.0, 127.7, 128.5, 129.4, 130.8, 145.6; HRMS (ESI) m/z calcd for $C_8H_8N_3^+$ $[M + H]^+$ 146.0713, found 146.0708.

4-(4-Trifluoromethylphenyl)-1H-1,2,3-triazole (8c).—Treatment of **7c** (17 mg, 0.1 mmol) with $CuSO_4$/sodium ascorbate by using Procedure B (column chromatography; hexane/EtOAc, 100:0 → 70:30) gave **8c**[42] (17.6 mg, 83%): ^{1}H NMR δ 7.82 (d, J = 8.4 Hz, 2H), 8.10 (d, J = 8.1 Hz, 2H), 8.54 (s, 1H); ^{13}C{^{1}H} NMR δ 124.7 (q, J = 271.8 Hz), 126.4 (q, J = 3.8 Hz), 126.5, 127.9, 128.8 (q, J = 31.9 Hz), 135.0, 144.7; ^{19}F NMR δ −1.0; HRMS (ESI) m/z calcd for $C_9H_7F_3N_3^+$ $[M +H]^+$ 214.0587, found 214.0593.

8-(1H-1,2,3-Triazol-4-yl)-2′-deoxyadenosine 5′-triphosphate (8-TrzdATP, 9).— $POCl_3$ (28 μL, 46 mg, 0.3 mmol) was added to a stirred solution of 8-TrzdA **2a** (48 mg, 0.15 mmol) and proton sponge (80 mg, 0.375 mmol) in $(MeO)_3PO$ (2 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 30 min; then tributylammomium pyrophosphate solution in DMF (0.5 M; 1.5 mL, 0.75 mmol) followed by Bu_3N (106.8 μL, 83.4 mg, 0.45 mmol) were added, and stirring was continued at 0 °C for 2 min. The reaction was quenched by adjusting the pH to 7.5 with 2 M TEAB buffer. The residue was dissolved in H_2O (5 mL) and was extracted with EtOAc (3 × 5 mL). The water layer was evaporated and coevaporated with a mixture of EtOH/H_2O (1:1, 5 mL). The residue was column chromatographed (DEAE–Sephadex, TEAB 0.1 M → 0.6 M) and the appropriate fractions were evaporated in a vacuum and coevaporated 5 times with a mixture of EtOH/H_2O (1:1, 10 mL) to give 8-TrzdATP triethylammonium salt **9** (33.4 mg, 30%): ^{1}H NMR (D_2O) δ 2.33 (ddd, J = 13.4, 6.5, 3.9, 1H), 3.22–3.26 (m, 1H), 4.04–4.10 (m, 1H), 4.14 (“q”, J = 5.2 Hz, 1H), 4.18–4.24 (m, 1H), 4.66 (“quint”, J = 3.9 Hz, 1H), 6.74 (t, J = 7.8 Hz, 1H), 8.20 (s, 1H), 8.41 (s, 1H); ^{31}P NMR (D_2O) δ –23.22 (t, J = 21.0 Hz, 1P$_\beta$), −11.34 (d, J = 21.0 Hz, 1P$_\alpha$), −10.22 (d, J = 21.0 Hz, 1P$_\gamma$); ^{13}C{^{1}H} NMR (D_2O) δ 36.2, 65.2, 70.6, 84.3, 84.8, 118.6, 128.8, 136.1,

143.0, 149.9, 152.6, 155.0; HRMS *m*/*z* calcd for $C_{12}H_{16}N_8O_{12}P_3$ $[M - H]^-$ 557.0106, found 557.0091.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

Z.W. and A.H.H. are recipients of the FIU Dissertation Year and Presidential Fellowships, respectively. This work was partially supported by NIH (S.F.W., SC1CA138176; Y.L., ES023569).

REFERENCES

(1). Daniels M; Hauswirth W Fluorescence of the Purine and Pyrimidine Bases of the Nucleic Acids in Neutral Aqueous Solution at 300 K. Science 1971, 171, 675–677. [PubMed: 5540307]

(2). Holz B; Klimasauskas S; Serva S; Weinhold E 2-Aminopurine as a fluorescent probe for DNA base flipping by methyltransferases. Nucleic Acids Res 1998, 26, 1076–1083. [PubMed: 9461471]

(3). Seo YJ; Ryu JH; Kim BH Quencher-Free, End-Stacking Oligonucleotides for Probing Single-Base Mismatches in DNA. Org. Lett 2005, 7, 4931–4933. [PubMed: 16235925]

(4). Greco NJ; Tor Y Simple Fluorescent Pyrimidine Analogues Detect the Presence of DNA Abasic Sites. J. Am. Chem. Soc 2005, 127, 10784–10785. [PubMed: 16076156]

(5). Srivatsan SG; Tor Y Fluorescent Pyrimidine Ribonucleotide: Synthesis, Enzymatic Incorporation, and Utilization. J. Am. Chem. Soc 2007, 129, 2044–2053. [PubMed: 17256858]

(6). Zayas J; Annoual M; Das JK; Felty Q; Gonzalez WG; Miksovska J; Sharifai N; Chiba A; Wnuk SF Strain Promoted Click Chemistry of 2- or 8-Azidopurine and 5-Azidopyrimidine Nucleosides and 8-Azidoadenosine Triphosphate with Cyclooctynes. Application to Living Cell Fluorescent Imaging. Bioconjugate Chem 2015, 26, 1519–1532.

(7). Srivatsan SG; Tor Y Using an emissive uridine analogue for assembling fluorescent HIV-1 TAR constructs. Tetrahedron 2007, 63, 3601–3607. [PubMed: 18431440]

(8). Tanpure AA; Pawar MG; Srivatsan SG Fluorescent Nucleoside Analogs: Probes for Investigating Nucleic Acid Structure and Function. Isr. J. Chem 2013, 53, 366–378.

(9). Wilhelmsson LM Fluorescent nucleic acid base analogues. Q. Rev. Biophys 2010, 43, 159–183. [PubMed: 20478079]

(10). Xu W; Chan KM; Kool ET Fluorescent nucleobases as tools for studying DNA and RNA. Nat. Chem 2017, 9, 1043–1055. [PubMed: 29064490]

(11). Ozols K; Cirule D; Novosjolova I; Stepanovs D; Liepinsh E; Bizdena E; Turks M Development of N6-methyl-2-(1,2,3-triazol-1-yl)-2′-deoxyadenosine as a novel fluorophore and its application in nucleotide synthesis. Tetrahedron Lett 2016, 57, 1174–1178.

(12). Kavoosi S; Rayala R; Walsh B; Barrios M; Gonzalez WG; Miksovska J; Mathivathanan L; Raptis RG; Wnuk SF Synthesis of 8-(1,2,3-triazol-1-yl)-7-deazapurine nucleosides by azide–alkyne click reactions and direct CH bond functionalization. Tetrahedron Lett 2016, 57, 4364–4367. [PubMed: 28239199]

(13). Lawson CP; Dierckx A; Miannay F-A; Wellner E; Wilhelmsson LM; Grotli M Synthesis and photophysical characterisation of new fluorescent triazole adenine analogues. Org. Biomol. Chem 2014, 12, 5158–5167. [PubMed: 24912077]

(14). Dyrager C; Börjesson K; Dinér P; Elf A; Albinsson B; Wilhelmsson LM; Grøtli M Synthesis and Photophysical Characterisation of Fluorescent 8-(1H-1,2,3-Triazol-4-yl)adenosine Derivatives. Eur. J. Org. Chem 2009, 1515–1521.

(15). Redwan IN; Bliman D; Tokugawa M; Lawson C; Grøtli M Synthesis and photophysical characterization of 1- and 4-(purinyl)triazoles. Tetrahedron 2013, 69, 8857–8864.

(16). Bag SS; Gogoi H Design of “Click” Fluorescent Labeled 2′-deoxyuridines via C5-[4-(2-Propynyl(methyl)amino)]phenyl Acetylene as a Universal Linker: Synthesis, Photophysical Properties, and Interaction with BSA. J. Org. Chem 2018, 83, 7606–7621. [PubMed: 29877080]

(17). Kumar P; Hornum M; Nielsen LJ; Enderlin G; Andersen NK; Len C; Hervé G; Sartori G; Nielsen P High-Affinity RNA Targeting by Oligonucleotides Displaying Aromatic Stacking and Amino Groups in the Major Groove. Comparison of Triazoles and Phenyl Substituents. J. Org. Chem 2014, 79, 2854–2863. [PubMed: 24611639]

(18). Hong SW; Hwang GT Photophysical Study of 2-Fluorenyl-1,2,3-Triazole-labeled 2′-Deoxyuridine and Its Oligonucleotide. Bull. Korean Chem. Soc 2018, 39, 78–83.

(19). Dierckx A; Dinér P; El-Sagheer AH; Kumar JD; Brown T; Grøtli M; Wilhelmsson LM Characterization of photophysical and base-mimicking properties of a novel fluorescent adenine analogue in DNA. Nucleic Acids Res 2011, 39, 4513–4524. [PubMed: 21278417]

(20). Kočalka P; Andersen NK; Jensen F; Nielsen P Synthesis of 5-(1,2,3-Triazol-4-yl)-2′-deoxyuridines by a Click Chemistry Approach: Stacking of Triazoles in the Major Groove Gives Increased Nucleic Acid Duplex Stability. ChemBioChem 2007, 8, 2106–2116. [PubMed: 17969214]

(21). Hatano A; Kurosu M; Yonaha S; Okada M; Uehara S One-pot approach to functional nucleosides possessing a fluorescent group using nucleobase-exchange reaction by thymidine phosphorylase. Org. Biomol. Chem 2013, 11, 6900–6905. [PubMed: 24057401]

(22). Hornum M; Djukina A; Sassnau A-K; Nielsen P Synthesis of new C-5-triazolyl-functionalized thymidine analogs and their ability to engage in aromatic stacking in DNA : DNA and DNA : RNA duplexes. Org. Biomol. Chem 2016, 14, 4436–4447. [PubMed: 27089052]

(23). Jin T; Kamijo S; Yamamoto Y Copper-Catalyzed Synthesis of N-Unsubstituted 1,2,3-Triazoles from Nonactivated Terminal Alkynes. Eur. J. Org. Chem 2004, 3789–3791.

(24). Barluenga J; Valdés C; Beltrán G; Escribano M; Aznar F Developments in Pd Catalysis: Synthesis of 1H-1,2,3-Triazoles from Sodium Azide and Alkenyl Bromides. Angew. Chem., Int. Ed 2006, 45, 6893–6896.

(25). Quan X-J; Ren Z-H; Wang Y-Y; Guan Z-H p-Toluenesulfonic Acid Mediated 1,3-Dipolar Cycloaddition of Nitroolefins with NaN3 for Synthesis of 4-Aryl-NH-1,2,3-triazoles. Org. Lett 2014, 16, 5728–5731. [PubMed: 25343314]

(26). Kalisiak J; Sharpless KB; Fokin VV Efficient Synthesis of 2-Substituted-1,2,3-triazoles. Org. Lett 2008, 10, 3171–3174. [PubMed: 18597477]

(27). Cha H; Lee K; Chi DY Synthesis of N-unsubstituted 1,2,3-triazoles via aerobic oxidative N-dealkylation using copper (II) acetate. Tetrahedron 2017, 73, 2878–2885.

(28). Reddy MR; Shibata N; Kondo Y; Nakamura S; Toru T Design, Synthesis, and Spectroscopic Investigation of Zinc Dodecakis-(trifluoroethoxy)phthalocyanines Conjugated with Deoxyribonucleosides. Angew. Chem 2006, 118, 8343–8346.

(29). Shinohara Y; Matsumoto K; Kugenuma K; Morii T; Saito Y; Saito I Design of environmentally sensitive fluorescent 2′-deoxyguanosine containing arylethynyl moieties: Distinction of thymine base by base-discriminating fluorescent (BDF) probe. Bioorg. Med. Chem. Lett 2010, 20, 2817–2820. [PubMed: 20363628]

(30). Liu Z; Liao P; Bi X General Silver-Catalyzed Hydroazidation of Terminal Alkynes by Combining TMS-N_3 and H_2O: Synthesis of Vinyl Azides. Org. Lett 2014, 16, 3668–3671. [PubMed: 24971828]

(31). For a review on the chemistry of vinyl azides see:Hu B; DiMagno SG Reactivities of vinyl azides and their recent applications in nitrogen heterocycle synthesis. Org. Biomol. Chem 2015, 13, 3844–3855. [PubMed: 25731154]

(32). Suzol SH; Howlader AH; Wen Z; Ren Y; Laverde EE; Garcia C; Liu Y; Wnuk SF Pyrimidine Nucleosides with a Reactive (β-Chlorovinyl)sulfone or (β-Keto)sulfone Group at the C5 Position, Their Reactions with Nucleophiles and Electrophiles, and Their Polymerase-Catalyzed Incorporation into DNA. ACS Omega 2018, 3, 4276–4288. [PubMed: 29732453]

(33). Liang Y; Suzol SH; Wen Z; Artiles AG; Mathivathanan L; Raptis RG; Wnuk SF Uracil Nucleosides with Reactive Group at C5 Position: 5-(1-Halo-2-sulfonylvinyl)uridine Analogues. Org. Lett 2016, 18, 1418–1421. [PubMed: 26933954]

(34). For studies on the generation of aminyl and iminyl radicals from 5-(1-azidovinyl)pyrimidinyl nucleosides and their potential application as radiosensitizers, see:Wen Z; Peng J; Tuttle PR; Ren Y; Garcia C; Debnath D; Rishi S; Hanson C; Ward S; Kumar A; Liu Y; Zhao W; Glazer PM; Liu Y; Sevilla MD; Adhikary A; Wnuk SF Electron-Mediated Aminyl and Iminyl Radicals from C5 Azido-Modified Pyrimidine Nucleosides Augment Radiation Damage to Cancer Cells. Org. Lett 2018, 20, 7400–7404. [PubMed: 30457873]

(35). Journet M; Cai D; Kowal JJ; Larsen RD Highly efficient and mild synthesis of variously 5-substituted-4-carbaldehyde-1,2,3-triazole derivatives. Tetrahedron Lett 2001, 42, 9117–9118.

(36). Kovács T; Ötvös L Simple synthesis of 5-vinyl- and 5-ethynyl-2′-deoxyuridine-5′-triphosphates. Tetrahedron Lett 1988, 29, 4525–4528.

(37). For selected papers on the polymerase-catalyzed incorporation of 8-substituted purine nucleotides into DNA, see:(a)Cahova H; Pohl R; Bednarova L; Novakova K; Cvacka J; Hocek M Synthesis of 8-bromo-, 8-methyl- and 8-phenyl-dATP and their polymerase incorporation into DNA. Org. Biomol. Chem 2008, 6, 3657–3660 [PubMed: 18843392] (e.g., 8-bromo dATP = good substrate, 8-methyl dATP = moderate substrate, and 8-phenyl dATP = poor substrate).(b)Kamath-Loeb AS; Hizi A; Kasai H; Loeb LA Incorporation of the Guanosine Triphosphate Analogs 8-Oxo-dGTP and 8-NH_2- dGTP by Reverse Transcriptases and Mammalian DNA Polymerases.J. Biol. Chem 1997, 272, 5892–5898. [PubMed: 9038207] (8-NH_2-dGTP is a better substrate than 8-Oxo-dGTP.)

(38). For selected papers on the effects of the 8-substituted purine nucleotides on polymerases-catalyzed DNA synthesis, see:(a)Vivet-Boudou V; Isel C; Sleiman M; Smyth R; Ben Gaied N; Barhoum P; Laumond G; Bec G; Götte M; Mak J; Aubertin A-M; Burger A; Marquet R 8-Modified-2'-Deoxyadenosine Analogues Induce Delayed Polymerization Arrest during HIV-1 Reverse Transcription. PLoS One 2011, 6, e27456. [PubMed: 22087320] (b)Chen LS; Sheppard TL Chain Termination and Inhibition of Saccharomyces cerevisiae Poly(A) Polymerase by C-8-modified ATP Analogs. J. Biol. Chem 2004, 279, 40405–40411. [PubMed: 15265873] (c)Jäger S; Rasched G; Kornreich-Leshem H; Engeser M; Thum O; Famulok M A Versatile Toolbox for Variable DNA Functionalization at High Density. J. Am. Chem. Soc 2005, 127, 15071–15082. [PubMed: 16248646]

(39). N-Unsubstituted triazolyl nucleosides might enhance the anticancer activity of cisplatin by augmenting binding between cisplatin and the triazole moiety. For studies on the complexation of triazoles with cisplatin, see:(a)Komeda S; Lutz M; Spek AL; Chikuma M; Reedijk J Inorg. Chem 2000, 39, 4230–4236. [PubMed: 11196916] (b)Komeda S; Lutz M; Spek AL; Yamanaka Y; Sato T; Chikuma M; Reedijk JJ Am. Chem. Soc 2002, 124, 4738–4746.

(40). Dodd DW; Swanick KN; Price JT; Brazeau AL; Ferguson MJ; Jones ND; Hudson RHE Blue fluorescent deoxycytidine analogues: convergent synthesis, solid-state and electronic structure, and solvatochromism. Org. Biomol. Chem 2010, 8, 663–666. [PubMed: 20090985]

(41). Kumar R; Wiebe LI; Knaus EE Synthesis of 5-(1-azidovinyl) and 5-[2-(1-azirinyl)] analogs of 2′-deoxyuridine. Can. J. Chem 1996, 74, 1609–1615.

(42). Lee H; Lee JK; Min S-J; Seo H; Lee Y; Rhee H Copper(I)-Catalyzed Synthesis of 1,4-Disubstituted 1,2,3-Triazoles from Azidoformates and Aryl Terminal Alkynes. J. Org. Chem 2018, 83, 4805–4811. [PubMed: 29600859]

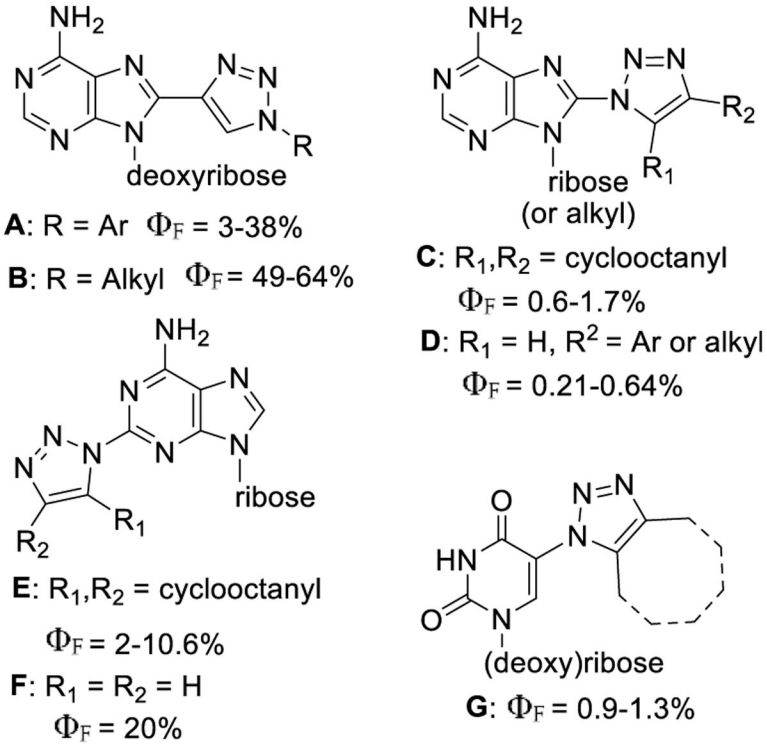


Figure 1.
Structures and quantum yields of the selected triazoles

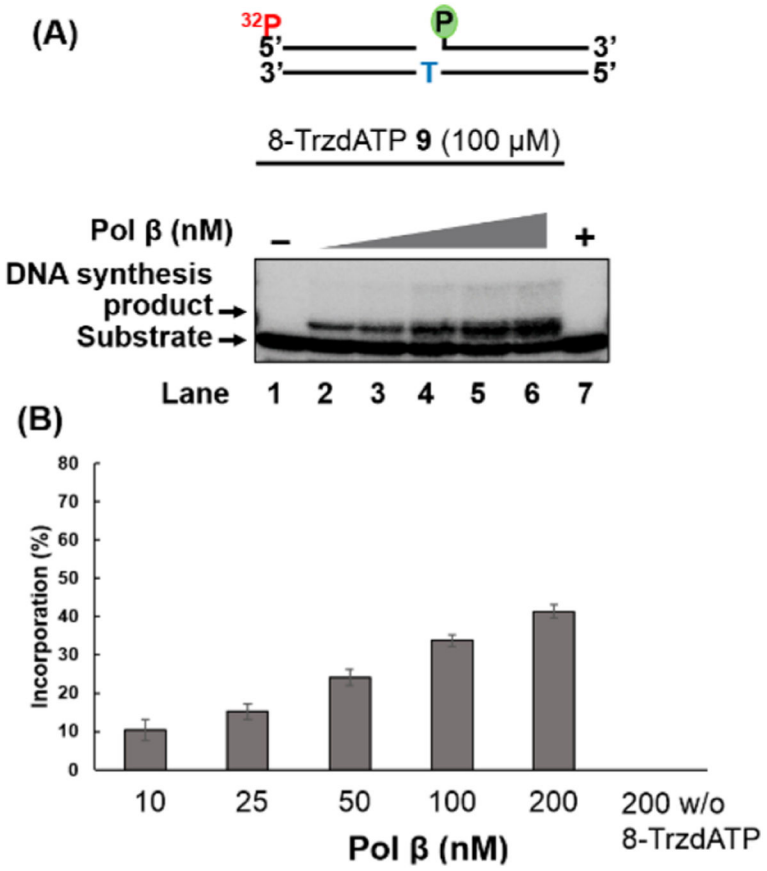


Figure 2.

(A) Incorporation of 8-TrzdATP 9 into duplex DNA containing a one-nucleotide gap by pol *β*. Substrates were ^{32}P-labeled at the 5′-end of the upstream strand of the substrate. Lane 1 indicates substrate only. Lanes 2–6 indicate the DNA synthesis product at increasing concentrations of pol *β* (10–200 nM). Lane 7 indicates the reaction with pol *β* without 8-TrzdATP. (B) Bar chart illustrating the quantification of the pol *β* DNA synthesis product.

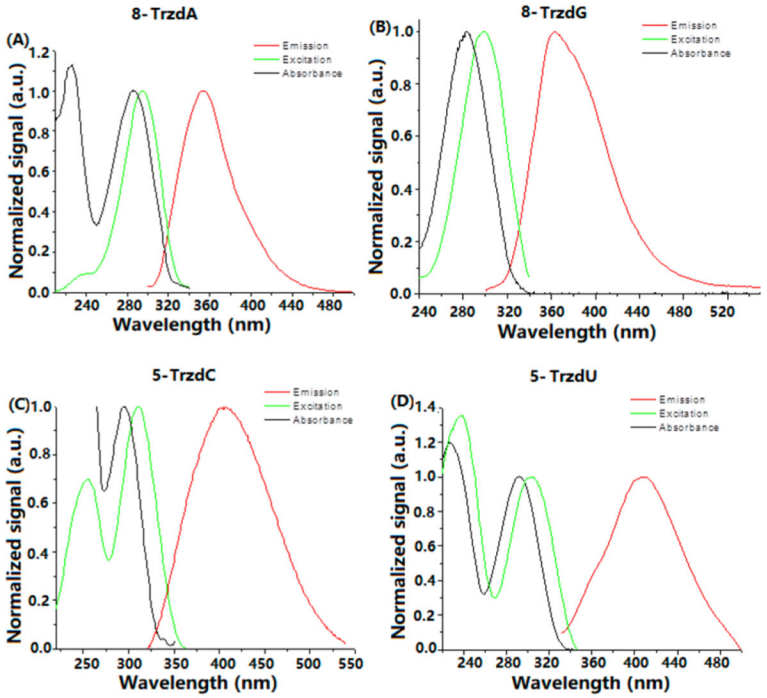
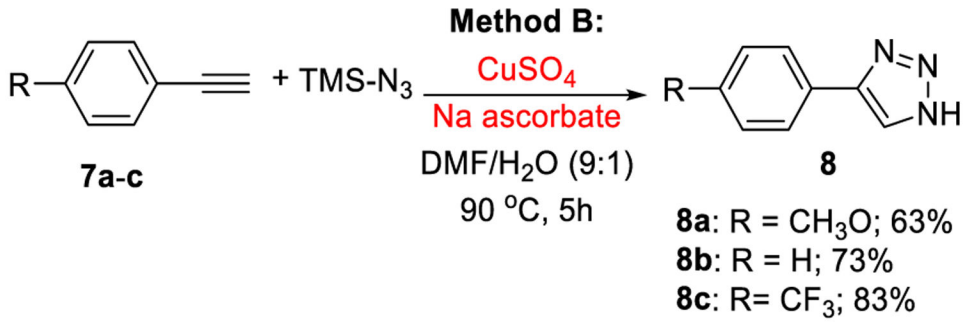
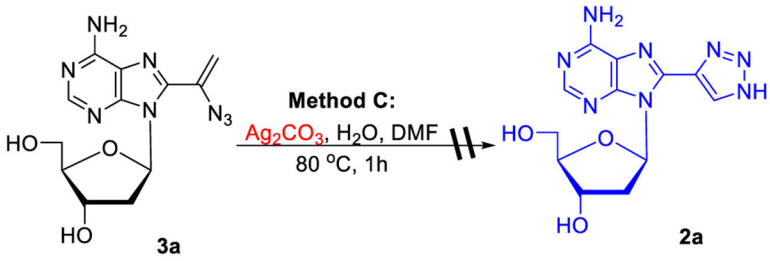


Figure 3.

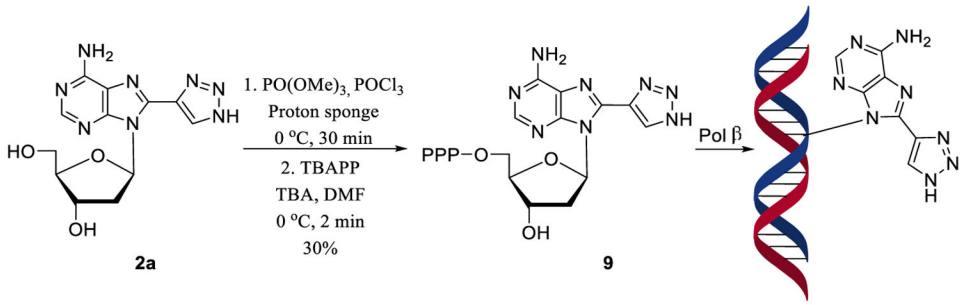
Normalized fluorescence emission, absorption, and excitation spectra for (A) 8-TrzdA, (B) 8-TrzdG, (C) 5-TrzdC, and (D) 5-TrzdU in MeOH. Absorption and excitation spectra were normalized to one at the absorption band of the lowest energy.



Scheme 1.
Synthesis of *p*-Substituted Phenyl Triazoles by Method B



Scheme 2.
Attempted Conversion of Vinyl Azide to Triazole



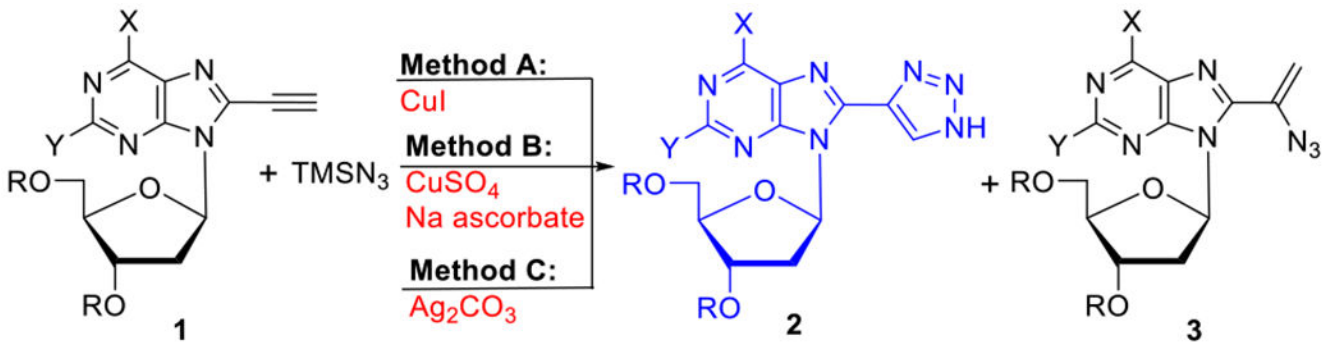
Scheme 3.

Synthesis of 8-TrzdATP and Its Incorporation into DNA by Human DNA Polymerase β (Pol β)

J Org Chem. Author manuscript; available in PMC 2019 October 18.

Table 1.

Synthesis of 8-(1*H*-1,2,3-Triazol-4-yl)purine Nucleosides



Method A and B: DMF/H_2O (9:1), 90 °C, 5h; Method C: DMF, 2 equiv H_2O, 80 °C, 1h

Series a: X = NH_2, Y = R = H; **b**: X = NH_2, Y = H, R = TBDMS; **c**: X = OH, Y = NH_2, R = H

entry	compound 1	method	compound 2	yield[a] [%]	compound 3	yield[a] [%]
1	**1a**	A	**2a**	48 (52[b])	**3a**	0
2	**1b**	A	**2b**	47	**3b**	0
3	**1c**	A	**2c**	31 (36[b])	**3c**	0
4	**1a**	B	**2a**	62	**3a**	0
5	**1b**	B	**2b**	71	**3b**	0
6	**1c**	B	**2c**	52	**3c**	0
7	**1a**	C	**2a**	58	**3a**	15
8	**1b**	C	**2b**	55	**3b**	0
9	**1c**	C[c]	**2c**	60	**3c**	0

[a] Isolated yields. Products were purified on a silica gel column.

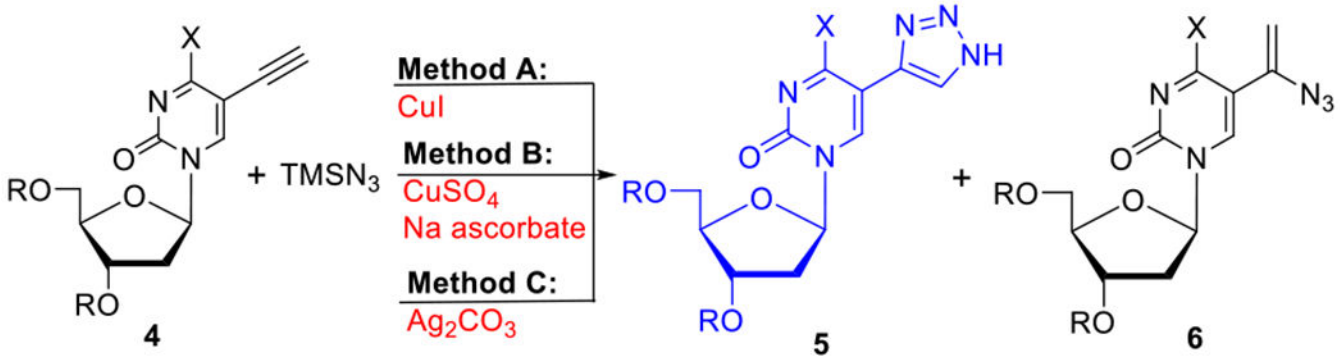
[b] Products were purified on RP-HPLC.

[c] 4 h.

J Org Chem. Author manuscript; available in PMC 2019 October 18.

Table 2.

Synthesis of 5-(1*H*-1,2,3-Triazol-4-yl) and 5-(1-Azidovinyl)pyrimidine Nucleosides



Method A and B: DMF/H_2O (9:1), 90 °C, 5h; Method C: DMF, 2 equiv H_2O, 80 °C, 1h

Series a: X = NH_2, R = Ac; **b**: X = NH_2, R = H; **c**: X = OH, R = Ac; **d**: X = OH, R = H

entry	compound 4	method	compound 5	yield[a] [%]	compound 6	yield[a] [%]
1	**4a**	A	**5a**	45	**6a**	0
2	**4b**	A	**5b**	48[b]	**6b**	0
3	**4c**	A	**5c**	65	**6c**	0
4	**4d**	A	**5d**	62	**6d**	0
5	**4d**	A[c]	**5d**	50	**6d**	0
6	**4a**	B	**5a**	65	**6a**	0
7	**4b**	B	**5b**	60	**6b**	0
8	**4c**	B	**5c**	82	**6c**	0
9	**4d**	B	**5d**	72	**6d**	0
10	**4a**	C	**5a**	7	**6a**	51
11	**4c**	C	**5c**	0	**6c**	52

[a] Isolated yields. Products were purified on a silica gel column.

[b] Yield was 53% when **5b** was purified on RP-HPLC.

Author Manuscript

Author Manuscript

Author Manuscript

$^{c}_{H_2O}$ (2 equiv).

Table 3.

Photophysical Data for Compounds 2a, 2c, 5b, and 5d[a]

	2a	2c	5b	5d
ε_{max} (M^{-1} cm^{-1})	17950	14800	18750	12400
λ_{max} (abs) (nm)	287	283	295	291
λ_{max} (exc) (nm)	295	299	311	238
λ_{max} (exc) (nm)	235		254	302
λ_{max} (emi) (nm)	355	364	407	408
Stokes shift (nm)	68	65	112	117
Φ_F	0.44	0.09	0.02	0.004
τ_1 (ns)	0.69	0.61	0.10	0.14
τ_2 (ns)	2.07	3.22	4.35	1.06
$\tau_{average}$ (ns)	1.55	2.47	3.72	0.805
f_1 (%)	37	28	15	27
f_2 (%)	63	71	85	73

[a]In MeOH