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Note

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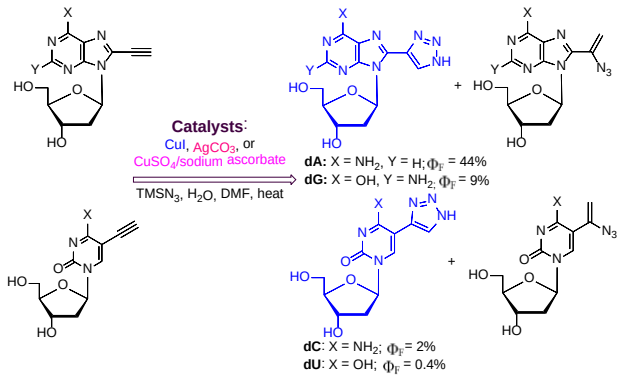
TOC



ABSTRACT: The Cu(I)- or Ag(I)-catalyzed cycloaddition between 8-ethynyladenine or guanine nucleosides and $TMSN_3$ gave 8-(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 minimalistic triazolyl modification showed excellent fluorescent properties with 8-(1,2,3-triazol-4-yl)-2'-deoxyadenosine (8-TrzdA) exhibiting quantum yield of 44%. The 8-TrzdA 5'-phosphate was incorporated into DNA duplex containing one-nucleotide gap by DNA polymerase β.

The natural nucleic acid have weak fluorescent properties.[1] However, modified nucleoside analogs 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 least distortion of natural hydrogen bonding.

Fluorescent properties of nucleosides bearing triazolyl modifications varied mostly by (*i*) position of nucleobases at which triazolyl unit is attached, (*ii*) site of triazolyl attachment to nucleobase (N1 vs. C4), and (*iii*) additional substitutions either at nucleobase or triazolyl units. Synthesis of the *N*-substituted triazolyl analogs 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 C8 position of purine ring, have quantum yield up to 64%,[14,19] while (1,2,3-triazol-1-yl)adenosines **C** and **D**, with triazolyl nitrogen attached to C8 position of purine, display significantly lower quantum yields (0.21-1.7%).[6,15] The 2-(1,2,3-triazol-1-yl)adenosine analogs **E** and **F** with triazole moiety at C2 position have 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 cells fluorescent imaging.[6]

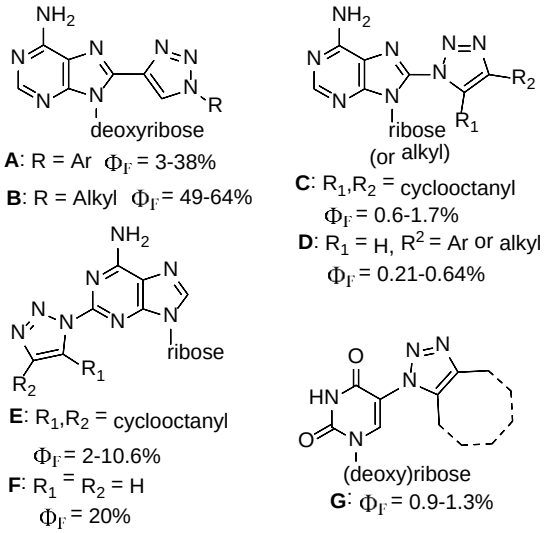


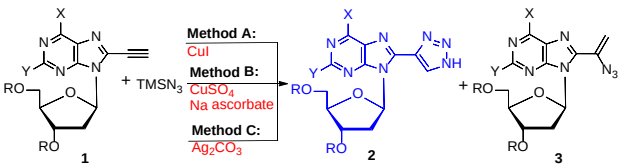
Figure 1. Structures and quantum yields of the selected triazoles

So far only 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 corresponding uracil-5-alkyne and in-situ generated pivaloyloxymethyl (POM) azide followed by *N*-deprotection[20] or by 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 DNA duplex.

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

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	1	Meth	2	Yield[a] [%]	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

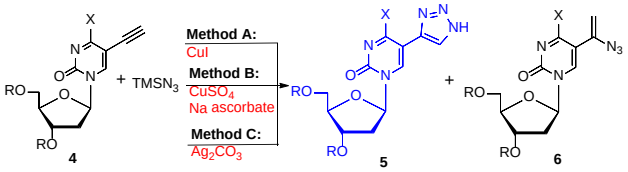
[a]Isolated yields. Products were purified on silica gel column; [b]Products were purified on RP-HPLC. [c]4 h.

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 2**b** 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'-deoxycytidine **5b** (5-TrzdC, entry 2). The 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 mixture of DMF/H_2O (9:1) as solvent gave 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 stoichiometric amount of H_2O was sufficient for cycloaddition to occur.

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

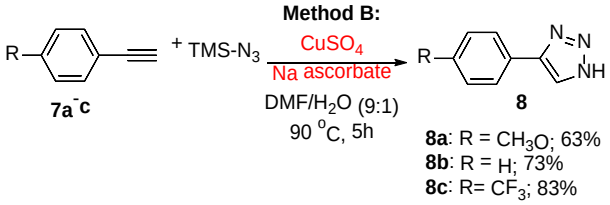
Entry	4	Meth	5	Yield[a] [%]	6	Yield[a] [%]
1	**4a**	A	**5a**	45	**6a**	0
2	**4b**	A	**5b**	48[b]	**6b**	0
3	**4c**	A	**5c**	65	**6c**	0
4	**4d**	A	**5d**	62	**6d**	0
5	**4d**	A[c]	**5d**	50	**6d**	0
6	**4a**	B	**5a**	65	**6a**	0
7	**4b**	B	**5b**	60	**6b**	0
8	**4c**	B	**5c**	82	**6c**	0
9	**4d**	B	**5d**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

[a]Isolated yields. Products were purified on silica gel column; [b]Yield was 53% when **5b** was purified on RP-HPLC; [c]H_2O (2 equiv.).

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 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 illustrates 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 higher yield of 83%. EWGs are known to promote the formation of triazoles in higher yields.[23,35]

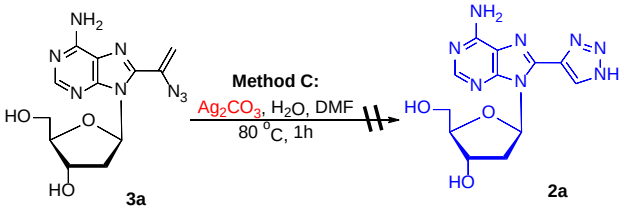
Scheme 1. Synthesis of *p*-substituted phenyl triazoles by Method B



In summary, *N*-unsubstituted triazolyl analogs of the four natural bases of DNA were prepared by treatment of the 5-ethynylpyrimidine or 8-ethynylpurine 2'-deoxynucleosides with $TMSN_3$ in the presence of CuI or $CuSO_4$/sodium ascorbate as 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.

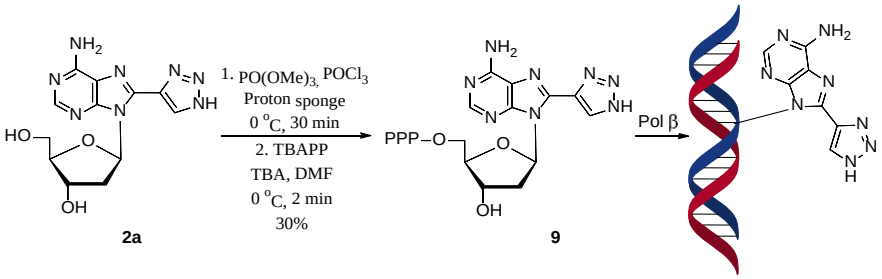
A reaction mechanism for the formation of triazoles might involve [3+2] cycloaddition or initial formation of vinyl azide followed by 1,5-electrocyclization and tautomerization. However, attempts to validate the latter mechanism by treatment 8-(1-azidovinyl)-2'-deoxyadenosine **3a** by Method C failed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

Scheme 2. Attempted conversion of vinyl azide to triazole



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

Scheme 3. Synthesis of 8-TrzdATP and its incorporation into DNA by human DNA polymerase β (Pol β)



We also examined incorporation of the triazolyl nucleotides into 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 nM-200 nM; lanes 2-6). 10 nM pol β was sufficient to insert 8-TrzdATP into the DNA to generate ~10% of 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 DNA duplex in the presence of a dGTP (Figure S4). Polymerase-catalyzed incorporation of the 8-substituted purine nucleotides are known to depend on the size of the substituent and preference for *syn/anti* conformation of the base.[37,38]

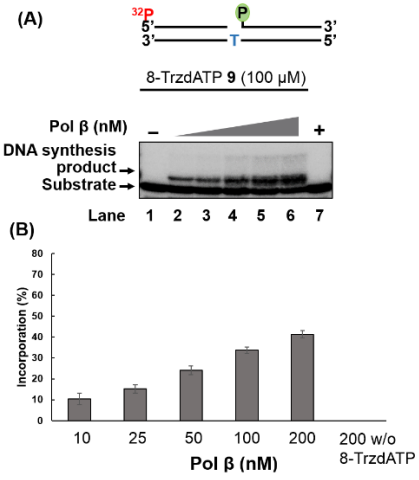


Figure 2. (**A**) Incorporation of 8-TrzdATP **9** into DNA duplex containing one-nucleotide gap by pol β. Substrates was ^{32}P-labeled at the 5'-end of the upstream strand of the substrate. Lane 1 indicates substrate only. Lanes 2-6 indicates DNA synthesis product at increasing concentrations of pol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

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 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 Φ_F of 9%. Emission λ_{max} of **2a** is pH and solvent independent (Figure S2, Table S2).

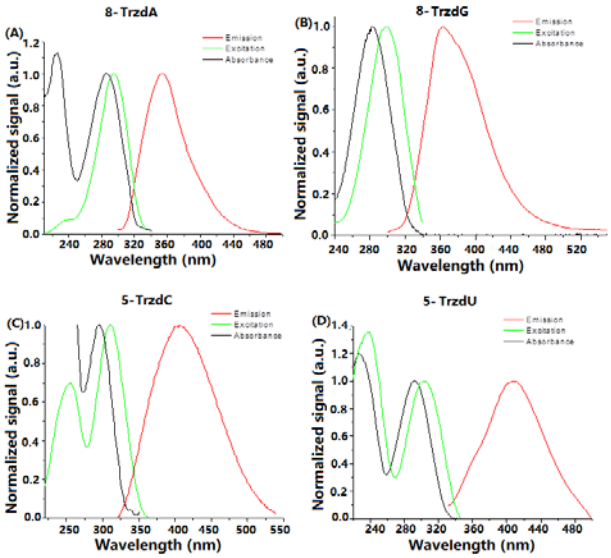


Figure 3. Normalized fluorescence emission, absorption, and excitation spectra for (A) 8-TrzdA, (B) 8-TrzdG, (C) 5-TrzdC, and (D) 5-TrzdU in MeOH. Absorption and excitation spectra were normalized to one at the absorption band of lowest energy.

The 5-pyrimidine analogs 5-TrzdU **5d** and 5-TrzdC **5b** showed a larger Stokes shift of ~110 nm with 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%). The acetyl protected **5a** and **5c** have similar fluorescent properties (Table S1). The emission spectra of **5b** shows 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 maximum in DMSO and ACN are similar to those observed at phosphate buffer at neutral and basic pH (Figure S3, Table S2).

Table 3. Photophysical data for 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[b]	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.

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

Fluorescent *N*-unsubstituted 1,2,3-triazol-4-yl deoxynucleosides 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 hydroazidation pathway to give 5-(1-azidovinyl) as major products. The triazoles showed good fluorescent properties with 8-TrzdA exhibiting the highest quantum yield of 44%. The 8-TrzdATP was incorporated into DNA duplex containing one-nucleotide gap by human DNA polymerase β. Metabolic incorporation of the 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]

EXPERIMETNAL 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 Hz. HRMS were obtained in TOF (ESI) mode. TLC was performed on Merck kieselgel 60-F_{254}, and products were detected with 254 nm light. Merck kieselgel 60 (230-400 mesh) was used for column chromatography. All reagents and solvents were purchased from commercial suppliers and used without further purification. Experimental procedure for the fluorescent characterization of the triazolyl nucleosides and protocols for the incorporation of 8-Trz-dATP **9** by human DNA polymerase are detailed in SI section.

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

Procedure A. CuI as 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 cooling 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: 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 catalyst:

The stirred solution of **1a** (27.5 mg, 0.1 mmol) and $CuSO_4$•$5H_2O$ (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 cooling 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 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 cooling 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); $^{13}C[^1H]$ 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: 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**).* Yield (4.8 mg, 15%; Procedure C, see above): ^1H 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); $^{13}C[^1H]$ 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'-deoxycytidine (**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); ^1H 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); $^{13}C[^1H]$ 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 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 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: 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 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 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), λmin 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[^{1}H] 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 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 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[^{1}H] 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 modified Procedure A with 2 equivalent of H_2O and DMF as 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 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 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 had: ^{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[^{1}H] 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 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 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 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 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. and 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 x 5 mL). The water layer was evaporated and coevaporated with 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 vacuum and coevaporated 5 times with 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.

ASSOCIATED CONTENT

Supporting Information

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

NMR spectra, mechanistic study and fluorescent characterization.

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Notes

The authors declare no competing financial interest.

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