S1 **Supporting Information**

Ratiometric Analysis of Zidovudine(ZDV) Incorporation by Reverse Transcriptases or Polymerases via Bio-orthogonal Click Chemistry

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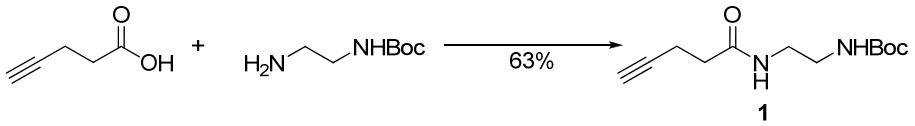
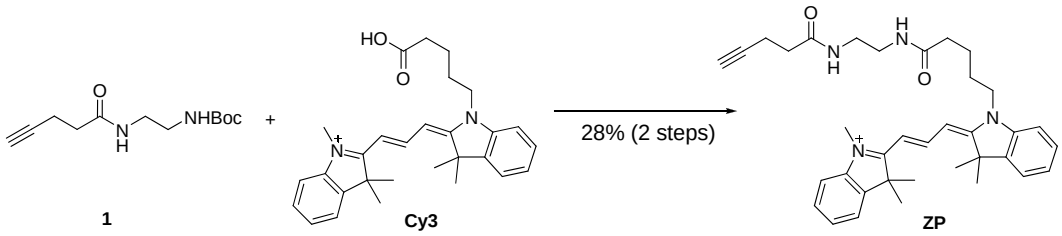
1. Synthesis and characterization of ZP

General Information: All reactions were performed using oven-dried glassware under dry argon atmosphere. Dichloromethane (DCM) was dried by distillation from CaH_2. Other solvents and organic reagents were purchased from commercial venders and used without further purification unless otherwise mentioned. *N*-Boc-ethylenediamine, *N,N'*-dicyclohexylcarbodiimide (DCC), *N,N*-dimethylformamide (DMF), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC hydrochloride), *N,N*-diisopropylethylamine (DIPEA), and 4-pentanoic acid were purchased from Sigma-Aldrich, USA. Trifluoroacetic acid (TFA) was purchased from TCI [Tokyo chemical industry Co., Ltd., Japan]. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Inova-500 [Varian Assoc., USA], and chemical shifts were measured in ppm downfield from internal tetramethylsilane (TMS) standard. Multiplicity was indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), td (triplet of doublet), etc. Coupling constants were reported in Hz. Low resolution mass spectrometry (LRMS) analysis was performed with Finnigan MSQ Plus Surveyer HPLC/MS system [Thermo Electron Corp., USA] using electron spray ionization (ESI). Reverse phase HPLC analysis was performed on a VPODS C-18 column (150 x 4.6 mm) at a flow rate of 1.0 mL/min for analytical analysis, and PRC-ODS C-18 column (250 x 20 mm) at a flow rate of 10.0 mL/min for preparation by LC-6AD pump with SPD-10A detector equipped with photodiode array (PDI) [Shimadzu, Japan]. HPLC solvents consist of water [HPLC grade, B&J, USA] containing 0.1% TFA as eluent A and acetonitrile [HPLC grade, B&J, USA] containing 0.1% TFA as eluent B.



Scheme S1. Synthesis of compound **1**

To a solution of DCC (505 mg, 2.447 mmol) in anhydrous DCM (12 mL), was added 4-pentynoic acid (200 mg, 2.039 mmol) at 0°C and stirred for 30 min. *N*-Boc-ethylenediamine (359 mg, 2.243 mmol) was added in dropwise to the reaction mixture. The resulting mixture was allowed to warm up to room temperature and stirred for 1 h. The precipitated white solid was removed by filtration and washed with DCM. The filtrate was concentrated *in vacuo* and purified with silica-gel flash column chromatography (1:20 = MeOH:DCM, v/v) to provide **1** (309 mg, 63% w/w). ^{1}H NMR (500 MHz, CD_3OD) δ 3.25 (t, J = 6.0, 2H), 3.15 (t, J = 6.0, 2H), 2.46 (td, J = 6.8, 2.3 Hz, 2H), 2.38 (t, J = 7.0 Hz, 2H), 2.26 (t, J = 2.2 Hz, 1H), 1.43 (s, 9H); LRMS (ESI+) *m/z* calcd for $C_{12}H_{20}N_2O_3$ $[M+H]^+$ 241.15, found *m/z* 241.08



Scheme S2. Synthesis of the **ZP**

To a solution of compound **1** (20 mg, 0.0832 mmol) in DCM (500 µL), was added TFA (500 µL) at room temperature and the resulting mixture was stirred for 1.5 h. DCM and TFA were removed under the reduced pressure by azeotrope with acetonitrile for several times. The crude mixture was diluted with DMF (600 µL) and added with DIPEA (21.7 µL, 0.125 mmol) followed by stirring for 10 min. Cy3 (47.5 mg, 0.0832 mmol) and EDC hydrochloride (31.9 mg, 0.167 mmol) were added to the reaction mixture and stirred at room temperature for overnight. The crude reaction mixture was purified with reverse phase HPLC charged with C18 column to provide **ZP** (13 mg, 28% w/w). The elution protocol for analytical HPLC is following: (1) 95%

eluent A for 5 min, (2) a linear gradient to 5% eluent over 75 min, (3) a linear gradient to 0% eluent A over 5 min, (4) a constant flow with 0% eluent for 5 min. ^{1}H NMR (500 MHz, CD_3OD) δ 8.54 (t, J = 13.5 Hz, 1H), 7.54 (d, J = 7.5 Hz, 2H), 7.44 (td, J = 7.7, 3.8 Hz, 2H), 7.36 (dd, J = 7.7, 2.2 Hz, 2H), 7.31 (td, J = 7.5, 4.3 Hz, 2H), 6.44 (dd, J = 13.5, 5.5 Hz, 2H), 4.16 (t, J = 7.2 Hz, 2H), 3.68 (s, 3H), 3.26 (s, 4H), 2.41 (td, J = 6.6, 2.2 Hz, 2H), 2.33 (t, J = 7.0 Hz, 2H), 2.28 (t, J = 7.0 Hz, 2H), 2.25 (q, J = 2.3 Hz, 1H), 1.87–1.79 (m, 4H), 1.77 (s, 6H), 1.76 (s, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 176.7, 176.0, 175.6, 174.3, 152.1, 144.1, 143.4, 142.2, 142.1, 130.0, 129.9, 126.8, 126.7, 123.5, 123.4, 112.4, 112.3, 103.8, 103.7, 83.5, 70.4, 50.6, 50.6, 44.9, 40.1, 39.9, 36.3, 36.0, 28.3, 28.1, 27.9, 23.9, 15.6; MS (ESI+) *m/z* calcd for $C_{36}H_{45}N_4O_2$ $[M]^+$ 565.35, found *m/z* 565.48.

2. Click Reaction Profile of ZP with ZDV

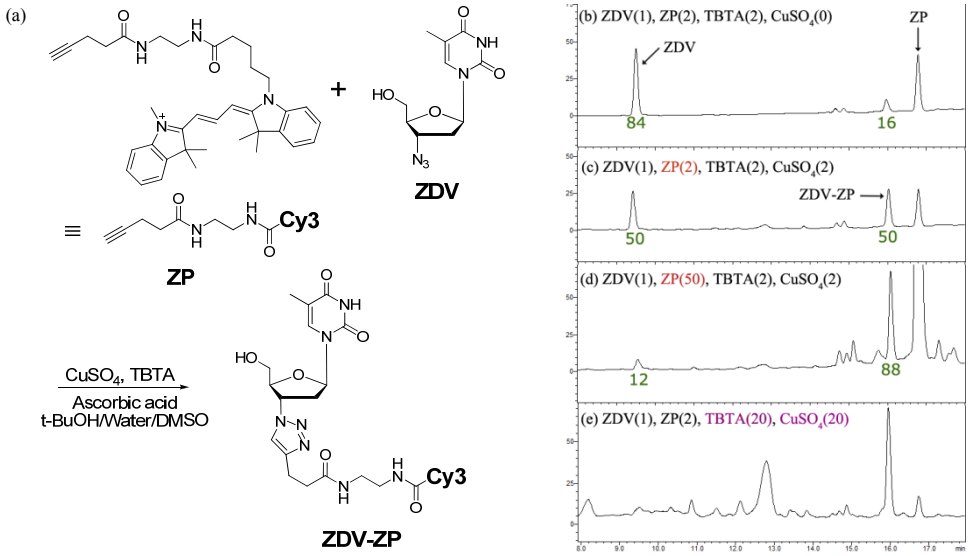


Fig. S1 Reaction profile of **ZP** with ZDV via various conditions of click reaction. (a) Chemical equation of the click reaction between **ZP** and ZDV. The reaction is monitored by HPLC at 254 nm under each condition; (b) TBTA (87 μM), ascorbic acid (43 mM), ZDV (43 μM) and **ZP** (87 μM); (c) $CuSO_4$ (87 μM), TBTA (87 μM), ascorbic acid (43 mM), ZDV (43 μM) and **ZP** (87 μM); (d) $CuSO_4$ (87 μM), TBTA (87 μM), ascorbic acid (43 mM), ZDV (43 μM) and **ZP** (2.2 mM); (e) $CuSO_4$ (870 μM), TBTA (870 μM), ascorbic acid (43 mM), ZDV (43 μM) and **ZP** (87 μM). The numbers inside parentheses stand for the equivalent quantities of reagents to the ZDV. All reactions were performed for 2 h at 37°C in aqueous solution containing *t*-BuOH (2%) and DMSO (4%). Tris-(benzyltriazolylmethyl)amine (TBTA), *tert*-butanol (*t*-BuOH) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich, USA.

The reaction condition was optimized by screening various concentrations of reagents such as $CuSO_4$/TBTA and **ZP**. The formation of the desired product, **ZDV-ZP**, was confirmed by LC/MS of the indicated peak [MS (ESI+) *m/z* calcd for $C_{48}H_{58}N_9O_6$ $[M]^+$ 832.45, found *m/z* 832.40]. Although the high concentration of $CuSO_4$ and TBTA (870 μM each) provides the complete conversion of ZDV, the resulting mixture contains many unidentifiable by-products caused by high concentration of reactive Cu(I) species [Fig. S1(e)], which is not desirable for bio-orthogonal detection of ZDV. In contrast, most of ZDV (~90%) was converted to the desired adduct, ZDV-ZP, using 50 equivalent of **ZP** in the presence of relatively low concentrations of $CuSO_4$/TBTA within 2 h [Fig. S1(d)]. Therefore, we concluded that the bio-orthogonal detection of ZDV-incorporated DNA can be robustly achieved without damages on DNA and biopolymers in the presence of high excess of **ZP** (at least 400 equiv. to ZDV) with $CuSO_4$ (87 μM), TBTA (87 μM), and ascorbic acid (43 mM).

3. ZDV Detection on the Polyacrylamide Gel

Primer extension assay

Template

3'-CAGATAGTCTTCACGAGGCAGGTCGTCTTGTCCTGGTACTCGTTTGCGTTGCG-5'

Primer

Cy5-5'-GTCTATCAGAAGTGCTCCGTCC-3'

SFP

Cy5-5'-GTCTATCAGAAGTGCTCCGTCCAGCAGAACAGGACCATGAGCAAACGCAACGC-3'

STP

Cy5-5'-GTCTATCAGAAGTGCTCCGTCCAGCAGAACAGGACCAT-3'

For the primer extension reaction of HIV reverse transcriptase (RT) [Ambion, Inc.,USA] or M-MLV RT [Ambion, Inc.,USA], the reaction was performed in Tris buffer [Tris HCl (pH 8.3, 50 mM), KCl (75 mM), $MgCl_2$ (3 mM), DTT (5 mM)] which contains the following in a volume of 20 μL:

Template (50 nM) [Bioneer Corp., S. Korea]
Primer (100 nM) [Bioneer Corp., S. Korea]
HIV or M-MLV RT (20 U, unit defined by the manufacturer)
dATP/dGTP/dCTP (1 μM each)
ZDV-TP (described concentration) [eEnzyme®,USA].

In the case of Taq polymerase [Genenmed Inc., S. Korea, 20 U], slightly modified Tris buffer [Tris HCl (pH 9.1, 50 mM), $(NH_4)_2SO_4$ (16 mM), $MgCl_2$ (2.5 mM) and BSA (15 μg/ml)] were used instead of abovementioned Tris buffer. The template-primer annealing was proceeded in advance of the primer extension reaction by mixing the abovementioned components without reverse transcriptases or polymerase, followed by denaturing at 85 °C for 5 min and cooling to 55 °C for 8 min and 37 °C for 10 min. After the annealing step, the corresponding DNA synthesizing enzymes were added and the reaction vessel was incubated for 60 min at 42 °C (for HIV or M-MLV RT) or 72 °C (for Taq polymerase).

Click chemistry

To the product mixture of the primer extension reaction, $CuSO_4$ (87 μM), TBTA (87 μM), ascorbic acid (43 mM) and **ZP** (17 μM) were added in final volume of 23 μL solution and incubated for 2 h at 37 °C for the proof-of-concept experiment (Fig. 2). The direct comparison experiments of ZDV incorporation were performed with up to 2174 μM of **ZP** in the presence of three different DNA synthesizing enzymes.

Denaturing urea polyacrylamide gel electrophoresis (d-PAGE)

The resulting reaction mixture (2 μL) was mixed with gel-loading buffer (8 μL, containing 1× TBE buffer, 7 M urea, 12% v/v glycerol) and heated at 95 °C for 5 min. The samples were directly incubated at ice for 5 min and three microliters of samples were run by d-PAGE (10% v/v) under 120 V and 10 A for 120 min.

Fluorescence detection and quantification

The *in-gel* fluorescence signal was visualized at the Cy3 (532 nm excitation) or Cy5 (633 nm excitation) channel by Typhoon Trio [Amersham Bioscience, USA] and quantified by ImageQuant TL program [Amersham Bioscience, USA].

4. ZDV Incorporation Efficiency of M-MLV-RT in the presence of dTTP

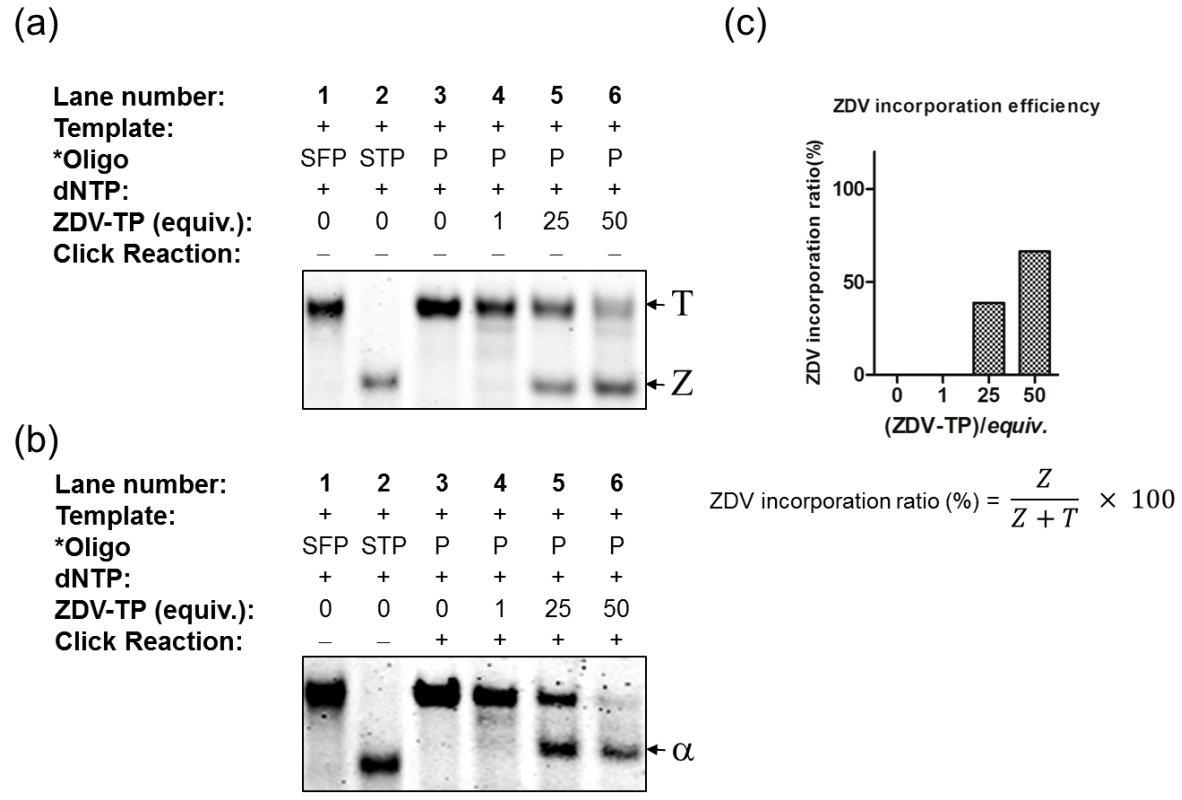


Fig. S2 ZDV incorporation efficiency of M-MLV-RT in the presence of dTTP. (a) d-PAGE analysis of primer extension products by increasing concentrations of ZDV-TP without Click reaction or (b) with Click reaction. (c) The quantification data of *in-gel* fluorescence click reaction with **ZP**. * denotes Cy5-labeled oligonucleotides. P: primer, 22-mer; SFP: synthetic full-length product, 53-mer; STP: synthetic terminated product, 38-mer.

5. ZDV Detection on the Chromosome

Cell incubation and chromosome spread preparation

CHO-K1 cells were cultured in HAM's F12 medium [WelGENE Inc., S. Korea] supplemented with 10% v/v fetal bovine serum (FBS) [Gibco, USA]. CHO-K1 cells were incubated in thymidine deficient HAM's F12 medium containing 10% v/v dialyzed FBS [WelGENE Inc., S. Korea] for 24 h prior to the treatment of ZDV. The cells were incubated for 4 h at 37 ^{o}C in the presence of 1 mM 3′-azido-3′-deoxythymidine (ZDV) [Sigma-Aldrich, USA] or DMSO and washed with Ca^{2+}- and Mg^{2+}-free phosphate buffered saline (PBS) [WelGENE Inc., S. Korea]. After washing, the cells were incubated under fresh medium containing 3.0 μM thymidine and 10% v/v FBS for 24 h. The chromosome spread was prepared by the 1 h treatment of demecolcine (1 μg/mL) [Aldrich, USA] followed by the trypsinization and hypotonic treatment with 75 mM KCl solution, and subsequent fixation with methanol and acetic acid.[1]

Chromosome staining

ZP (10 μM), $CuSO_4$/TBTA (1 mM), and ascorbic acid (100 mM) were added to the chromosome mixture after fixation for 1 h at room temperature in the dark. To remove the residual **ZP**, 1 mM of 2-azido-2-deoxy-D-glucose [Aldrich, USA] was added to the chromosome mixture for additional 1 h at room temperature. This treatment provides the click reaction of remaining **ZP** with 2-azido-2-deoxy-D-glucose and enhances the hydrophilicity of **ZP**, which allows the effective removal of **ZP** in resulting samples via washing twice with PBS. Then, 20 μM of Hoechst solution [Molecular Probe, USA] was treated to the slide glass spreaded with chromosome samples for 5 min.

Fluorescence Imaging

We carried out fluorescence microscopy studies with Olympus Inverted Microscope Model IX71 [Model: IX71-F22FL/PH], equipped for epi-illumination using a halogen bulb [Philips No. 7724]. Emission signal of each experiments were observed at two spectral settings: blue channel using a 330-385 band pass exciter filter, a 400 nm center wavelength chromatic beam splitter, and a 420 nm-long pass barrier filter [Olympus filter set U-MWU2]; red channel using a 510-550 band pass exciter filter, 570 nm center wavelength chromatic beam splitter, and a 590 nm long pass barrier filter [Olympus filter set, U-MWG2]. Emission signals of each experiment were detected with 12.5M pixel recording digital color camera [Olympus, DP71].

Chromosome imaging: blue channel, exposal time (1/2000 s)

ZP imaging: red channel, exposal time (1/5 s)

6. The Complete List of the Authors in the Reference

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