Electronic Supplementary Information

Cu^{II}-mediated DNA base pairing of triazole-4-carboxylate nucleoside prepared by click chemistry

Lingyun Hu, Yusuke Takezawa* and Mitsuhiko Shionoya*

Department of Chemistry, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. E-mail: takezawa@chem.s.u-tokyo.ac.jp; shionoya@chem.s.u-tokyo.ac.jp

Contents

1. Experimental procedures

General. All reagents used for the organic synthesis were purchased from FUJIFILM Wako Pure Chemical Industries, Tokyo Chemical Industry (TCI), and Aldrich, and were used without further purification. Unless otherwise noted, all reactions were carried out under argon atmosphere with commercial dehydrated solvents (FUJIFILM Wako Pure Chemical Industries). Silica gel column chromatography was carried out using Merck Silica Gel 60 (230–400 mesh), and reverse-phase column chromatography using Wakogel 50C18 (38–63 μm). All NMR spectra were measured on a Bruker AVANCE 500 spectrometer (500 MHz for ^{1}H, 202 MHz for ^{31}P, and 126 MHz for ^{13}C). The spectra were referenced to tetramethylsilane (TMS) in $CDCl_3$ (δ 0 ppm) or to the residual solvent signals in CD_3OD (δ 3.31 ppm). Electrospray ionization-time-of-flight (ESI-TOF) mass spectra were recorded on a Waters LCT Premier XE. Metal sources were purchased from FUJIFILM Wako Pure Chemical Industries ($MnCl_2·4H_2O$ (99%), $FeCl_2·4H_2O$ (99.0–102.0%), $CoCl_2$ (99.0–102.0%), $NiCl_2·6H_2O$ (99.9%), $CuSO_4·5H_2O$ (99.5% purity), $AgNO_3$ (99.8%)) and Soekawa Chemical Co. ($ZnSO_4·7H_2O$ (99.9%)).

Compound 3. 2-Deoxy-3,5-di-*O*-(*p*-toluoyl)-*β*-D-*erythro*-pentafuranosyl azide (**1**) (1.04 g, 2.6 mmol) was dissolved in CH_2Cl_2 (26 mL), and acetic acid (181 μL, 1.2 eq.), diisopropylethylamine (2.3 mL, 5.0 eq.) and ethyl propiolate (321 μL, 1.2 eq.) were sequentially added. After CuI powder (1.0 g, 2.0 eq.) was added, the suspension was vigorously stirred at room temperature for 20 min. Then, the reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with saturated EDTA aqueous solution (25 mL) for several times until the aqueous layer became colorless. The organic phase was further washed with water (50 mL) and was dried over Na_2SO_4 before evaporation. The crude product was purified by silica gel column chromatography (*n*-hexane → *n*-hexane:AcOEt = 2:1) to afford compound **2** as a yellow-brown solid (1.16 g, 2.4 mmol). Although the color indicated the existence of impurities, the obtained compound was used for the next reaction without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.28 (s, 1H), 7.94 (d, J = 8.2 Hz, 2H), 7.82 (d, J = 8.2 Hz, 2H), 7.28 (d, J = 8.1 Hz, 2H), 7.22 (d, J = 8.1 Hz, 2H), 6.51 (dd, J = 6.3, 6.3 Hz, 1H), 5.76–5.74 (m, 1H), 4.69 (ddd, J = 3.5, 3.5, 3.5 Hz, 1H), 4.65–4.56 (m, 2H), 4.38 (q, J = 7.1 Hz, 2H), 3.15 (ddd, J = 14.3, 6.6, 6.3 Hz, 1H), 2.44 (ddd, J = 14.3, 6.3, 3.3 Hz, 1H), 2.44 (s, 3H), 2.40 (s, 3H), 1.36 (t, J = 7.1 Hz, 3H). The obtained compound **2** (1.16 g, 2.4 mmol) was dissolved in a mixture of EtOH (50 mL) and CH_2Cl_2 (20 mL). After the addition of K_2CO_3 (0.81 g, 5.9 mmol), the mixture was stirred at 45 °C for 4 h. The reaction mixture was slowly decanted to remove excess K_2CO_3, and the remaining solution was evaporated to dryness. The crude product was purified by silica gel

column chromatography ($CHCl_3$ → $CHCl_3$:MeOH = 10:1) to afford compound **3** as a colorless solid (0.58 g, 2.3 mmol, 86% in 2 steps). ^{1}H NMR (500 MHz, CD_3OD): δ 8.78 (s, 1H), 6.48 (dd, J = 5.9, 5.9 Hz, 1H), 4.57 (ddd, J = 5.1, 5.1, 5.1 Hz, 1H), 4.40 (q, J = 7.1 Hz, 2H), 4.08–4.05 (m, 1H), 3.76 (dd, J = 12.0, 3.8 Hz, 1H), 3.66 (dd, J = 12.0, 4.8 Hz, 1H), 2.82–2.78 (m, 1H), 2.60–2.56 (m, 1H), 1.40 (t, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD): δ 160.5, 139.5, 126.9, 89.3, 88.5, 70.6, 61.6, 60.9, 40.5, 13.2. HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{10}H_{15}N_3O_5$: 258.1090, found: 258.1070.

Compound 4. Compound **3** (0.26 g, 1.0 mmol) and DMAP (1.2 mg, 0.01 eq.) were dissolved in dry pyridine (20 mL). After the addition of 4,4′-dimethoxytrityl chloride (0.41 g, 1.2 mmol), the mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL), and then washed with saturated $NaHCO_3$ aqueous solution (50 mL) and H_2O (50 mL). The organic layer was dried over Na_2SO_4 before being evaporated to dryness. The crude product was purified by silica gel column chromatography (*n*-hexane:AcOEt:MeOH = 5:15:2 with 1% of triethylamine) to afford compound **4** as colorless foam (0.50 g, 0.90 mmol, 89%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.27 (s, 1H), 7.37–7.20 (m, 9H), 6.82 (dd, J = 8.9, 1.2 Hz, 4H), 6.35 (dd, J = 6.5, 4.4 Hz, 1H), 4.66–4.60 (m, 1H), 4.35 (q, J = 7.1 Hz, 2H), 4.15–4.12 (m, 1H), 3.80 (s, 6H), 3.37 (dd, J = 10.4, 4.6 Hz, 1H), 3.29 (dd, J = 10.4, 4.8 Hz, 1H), 2.96 (ddd, J = 13.6, 6.3, 4.4 Hz, 1H), 2.62 (ddd, J = 13.3, 6.5, 6.5 Hz, 1H), 1.34 (t, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 160.5, 158.5, 144.4, 140.1, 135.6, 135.5, 129.9, 128.0, 127.9, 126.9, 126.3, 113.2, 89.0, 86.6, 71.5, 63.2, 61.2, 55.2, 40.8, 14.2. HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{31}H_{33}N_3O_7$: 560.2397, found: 560.2387.

Compound 5. To a solution of compound **4** (0.17 g, 0.30 mmol) in CH_2Cl_2 (3.0 mL), diisopropylethylamine (198 μL, 4.0 eq) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (100 μL, 1.5 eq) were added slowly. After stirred at room temperature for 30 min, the reaction mixture was diluted with AcOEt (25 mL) and washed with saturated Na_2CO_3 aqueous solution (25 mL × 2) and brine (25 mL). The organic layer was dried over anhydrous Na_2SO_4 and then evaporated to dryness. The residue was purified by silica gel column chromatography (*n*-hexane:AcOEt = 1:1 with 1% triethylamine) to afford compound **5** as colorless foam (184 mg, 0.16 mmol, 82%, a diastereomeric mixture). ^{1}H NMR and ^{31}P NMR spectra indicated the existence of two diastereomers. The compound **5** was immediately used for the DNA synthesis. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.31 (s, 0.5H), 8.28 (s, 0.5H), 7.37–7.11 (m, 9H), 6.82–6.78 (m, 4H), 6.39–6.34 (m, 1H), 4.74–4.65 (m, 1H), 4.39–4.28 (m, 3H), 3.88–3.52 (m, 10H), 3.34–3.24 (m, 2H), 2.98–2.88 (m, 1H), 2.77–2.65 (m, 1H), 2.62 (t, J = 6.3, 1H),

2.46 (t, J = 6.5, 1H), 1.34–1.29 (m, 3H) 1.20–1.08 (m, 12H). ^{31}P NMR (202 MHz, $CDCl_3$, 300 K): δ 149.34, 149.04. HRMS (ESI-TOF) m/z: $[M + Na]^+$ calcd for $C_{40}H_{49}N_5O_8PNa$: 782.3295, found: 782.3260.

DNA synthesis. DNA strands containing **Taz^C** were synthesized on an automated DNA synthesizer (NTS M-4-MX DNA/RNA synthesizer) according to the standard procedure. The **Taz^C** phosphoramidite (**5**) was dissolved in dry MeCN to prepare a 0.08 M solution. DNA synthesis was carried out on a 1-μmol scale in a DMTr-on mode with ultramild deprotection phosphoramidites and reagents (Glen Research). The coupling time for the **Taz^C** phosphoramidite was extended to 15 min. The oligonucleotides were detached from the solid supports by 2-hour incubation with a 0.3 M NaOH aqueous solution at room temperature. The supernatants were further incubated at 37 °C for 22 h. The obtained products were detritylated and purified using Glen-pak cartridges (Glen research) according to the manufacturer's protocol. Further purification by reverse-phase HPLC (Waters XBridge Oligonucleotide BEH C18 column, buffers: A = MeCN, B = 0.1 M TEAA (pH 7.0) + 2% MeCN, 60 °C) afforded the desired DNA strands, which were identified by ESI-TOF mass spectrometry.

TTaz^{C}T. 5′-T**Taz^C**T-3′. HPLC retention time: 14.4 min (gradient: 2%A (0 min), 4%A (40 min)). ESI MS: m/z calcd for $[C_{28}H_{37}N_7O_{19}P_2 - H]^-$: 836.15; found: 836.06.

ODN1. 5′-CAC ATT A**Taz^C**T GTT GTA-3′. HPLC retention time: 15.6 min (gradient: 5%A (0 min), 6.5%A (30 min)). ESI MS: m/z calcd for $[C_{146}H_{185}N_{51}O_{91}P_{14} - 3H]^{3-}$: 1513.58; found: 1513.59.

ODN2. 5′-TAC AAC A**Taz^C**T AAT GTG-3′. HPLC retention time: 15.0 min (gradient: 5%A (0 min), 6.5%A (30 min)). ESI MS: m/z calcd for $[C_{146}H_{183}N_{57}O_{87}P_{14} - 3H]^{3-}$: 1519.26; found: 1519.31.

Concentration determination of DNA strands. The concentration of DNA strands was determined based on the UV absorbance at 260 nm. The molar extinction coefficients (ε_{260}) of the DNA strands were calculated by the nearest neighbor method. For the calculation of the ε_{260} values of **Taz^C**-containing DNA strands, the fully deprotected **Taz^C** nucleoside was prepared by incubating compound **2** (50 mg, 0.2 mmol) with equimolar amount of NaOH (0.4 M NaOH aqueous solution, 0.5 mL) at room temperature for 2 h. Subsequent reverse-phase column chromatography (eluent: H_2O) afforded fully deprotected **Taz^C** nucleoside (50 mg as a sodium salt, over 100%), which was identified by ^{1}H NMR. ^{1}H NMR (500 MHz, CD_3OD): δ 8.33 (s, 1H), 6.42 (dd, J = 6.0, 6.0 Hz, 1H), 4.55 (m, 1H), 4.04 (m, 1H), 3.73 (dd, J = 12.0, 4.1 Hz, 1H),

3.64 (dd, J = 12.0, 5.2 Hz, 1H), 2.79 (ddd, J = 13.6, 6.0, 5.8 Hz, 1H), 2.53 (ddd, J = 13.6, 6.6, 6.0 Hz, 1H). The UV spectrum of the **Taz**^{C} nucleoside was measured on Thermo Scientific NanoDrop 2000 spectrophotometer with a path length of 0.1 cm (Fig. S3). The spectrum showed that **Taz**^{C} has no absorption at 260 nm. Therefore, the ε_{260} values of the **Taz**^{C} nucleosides were deemed to be zero to calculate the ε_{260} values of **Taz**^{C}-containing DNA strands.

Duplex melting analysis. All samples were prepared by combining the DNA strands (2.0 μM each) in a 10 mM HEPES buffer (pH 7.0) solution containing 100 mM NaCl or 100 mM $NaNO_3$ (for Ag^{I}). After the addition of metal ions, the samples were annealed from 60 °C to 4 °C at the rate of 1.0 °C/min. Absorbance at 260 nm (A_{260}) was recorded on UV-1700, UV-1800 and UV-1900 spectrophotometers (Shimadzu) equipped with a TMSPC-8 temperature controller. The temperature was raised from 4 °C to 60 °C at the rate of 0.2 °C/min. The melting curves were depicted after normalization as follows:

$$\text{Normalized } A_{260} = \{A_{260}\ (t\ ^\circ\text{C}) - A_{260}\ (4\ ^\circ\text{C})\} / \{A_{260}\ (60\ ^\circ\text{C}) - A_{260}\ (4\ ^\circ\text{C})\} \times 100.$$

The melting temperature (T_m) was determined as an inflection point of a melting curve using a T_m analysis software LabSolutions (Shimadzu) with a 17-point adaptive smoothing program. Average T_m values of at least three independent runs are shown.

Mass spectrometry. For characterization of single DNA strands, 20 μM samples were used for the measurements. For the analysis of the duplex with Cu^{II} ions (**ODN1**·**ODN2**·Cu^{II}), each DNA strand (100 μM) and Cu^{II} (100 μM) were mixed in a 20 mM NH_4OAc buffer (pH 7.0) and annealed prior to the measurements (60 °C → 4 °C, 1.0 °C/min). ESI-TOF mass spectra were recorded in the negative mode.

2. Supplementary figures

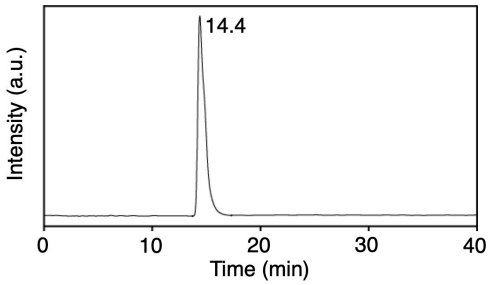


Fig. S1. HPLC analysis of 5′-T**Taz**^{C}T-3′ after deprotection using NaOH aqueous solution. Waters XBridge Oligonucleotide BEH C18 column, flow rate: 1.0 mL/min, temperature: 60 °C, monitored at 260 nm. Gradient: 0 to 40 min, 2% A to 4% A (solvent A = MeCN, solvent B = 0.1 M TEAA buffer (pH 7.0) + 2% MeCN).

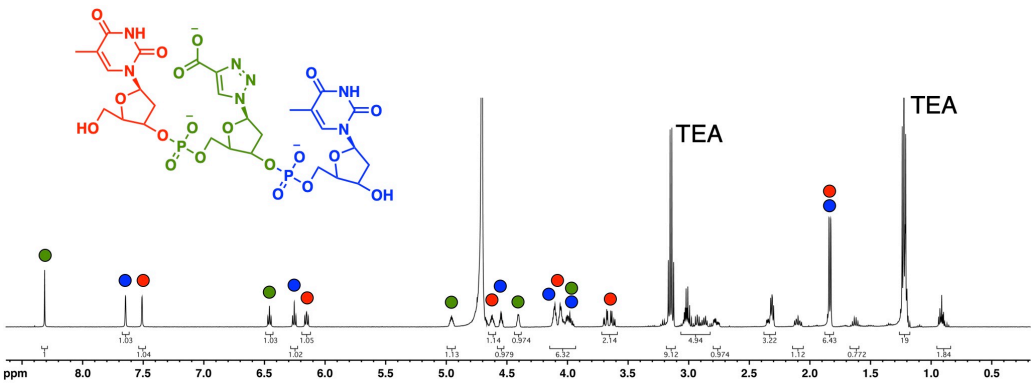


Fig. S2. ^{1}H NMR spectrum of 5′-T**Taz**^{C}T-3′ after HPLC purification (500 MHz, 300 K, D_2O). Protons belonging to different nucleotides are labeled with the corresponding colors.

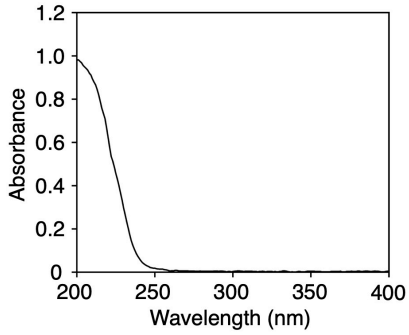


Fig. S3. UV spectrum of **Taz**^{C} nucleoside. [nucleoside] = ca. 1.0 mM in 10 mM HEPES buffer (pH 7.0). l = 0.1 cm, rt. Note that there is no absorption at 260 nm.

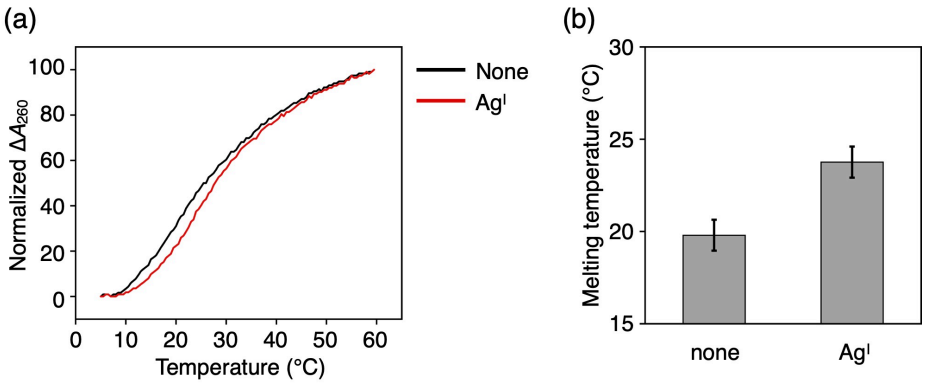


Fig. S4. (a) Melting curves of a DNA duplex containing a **Taz**^{C}–**Taz**^{C} base pair (**ODN1**·**ODN2**) in the presence of Ag^{I} ions. [duplex] = 2.0 μM, [Ag^{I}]/[duplex] = 0 or 1.0 in 10 mM HEPES buffer (pH 7.0), 100 mM $NaNO_3$, 0.2 °C/min. (b) Melting temperatures. N = 3. The error bars represent the standard errors.

3. Supplementary tables

Table S1. Melting temperatures of duplexes **ODN1**·**ODN2** in the presence of 1 equiv. of transition metal ions.[a]

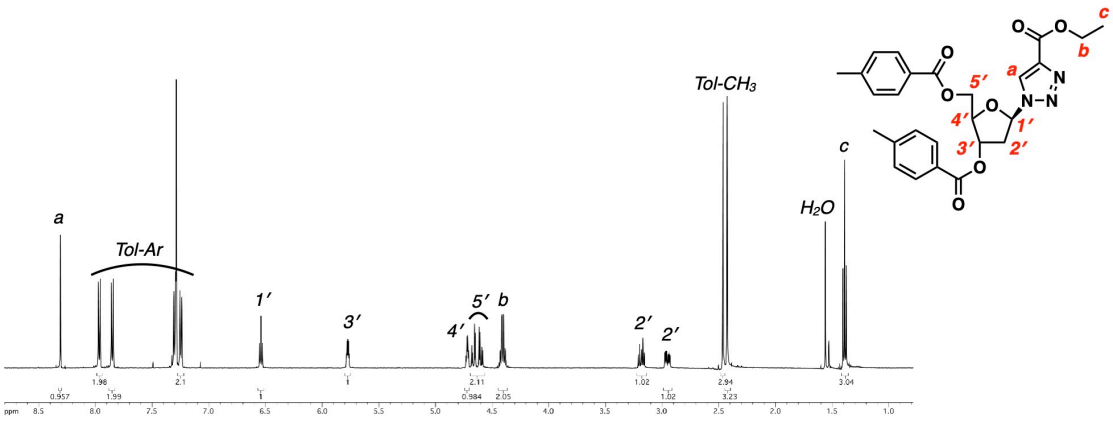
Metals	**1**·**2**	
	T_m/ °C	ΔT_m/ °C[b]
None	20.4 ± 0.9	–
Mn^{II}	20.4 ± 0.5	± 0
Fe^{II}	21.8 ± 1.0	+1.4
Co^{II}	21.8 ± 0.3	+1.4
Ni^{II}	22.0 ± 0.9	+1.6
Cu^{II}	28.1 ± 0.8	+7.7
Zn^{II}	21.4 ± 1.0	+1.0
Ag^{I} [c]	23.8 ± 0.8	+4.0 [d]

[a] [duplex] = 2.0 μM, [metal ion] = 2.0 μM in a 10 mM HEPES buffer (pH 7.0), 100 mM NaCl. N = 4. The standard errors are shown. [b] ΔT_m represents the temperature difference from the T_m value of the metal-free duplex. [c] [duplex] = 2.0 μM, [Ag^I ion] = 2.0 μM in a 10 mM HEPES buffer (pH 7.0), 100 mM $NaNO_3$. N = 3. [d] The T_m of the metal-free duplex was 19.8 ± 0.8 °C under the same conditions.

4. NMR spectra

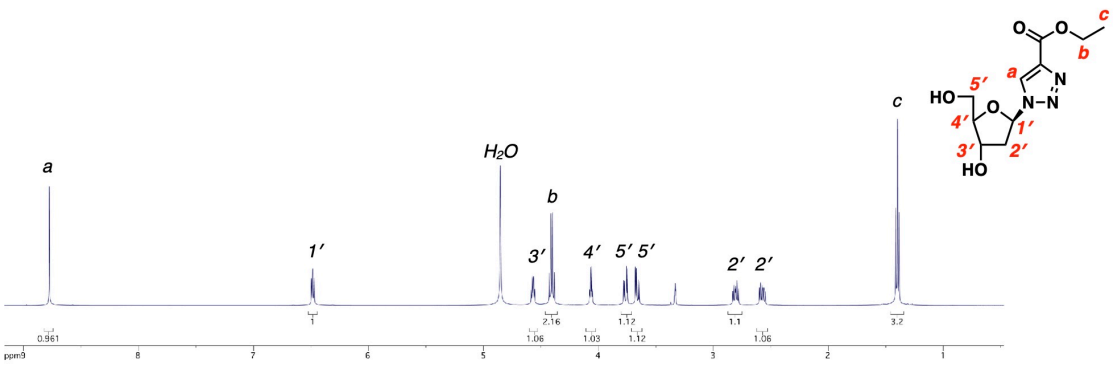
Compound 2

^{1}H NMR (500 MHz, $CDCl_3$, 300 K).

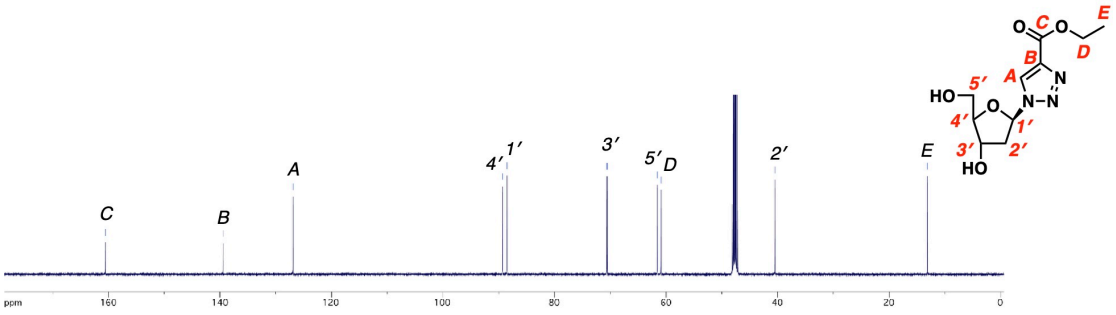


Compound 3

^{1}H NMR (500 MHz, CD_3OD, 300 K).

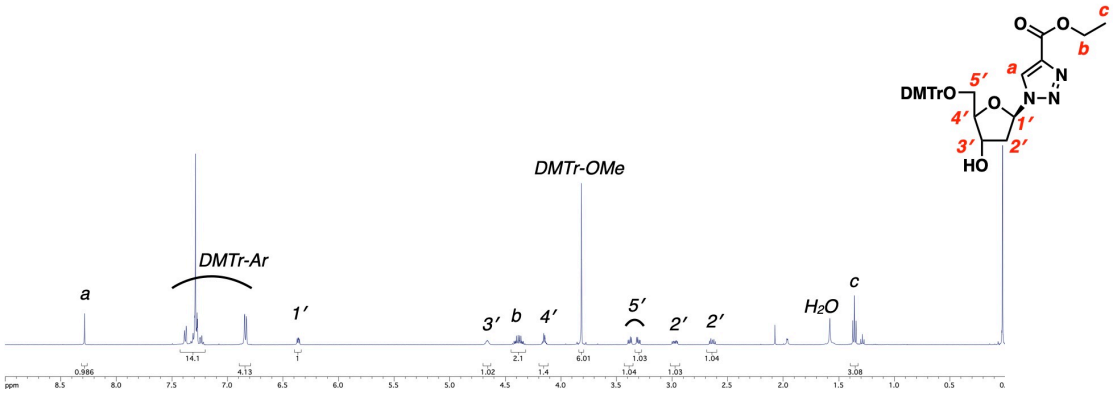


^{13}C NMR (126 MHz, CD_3OD, 300 K).

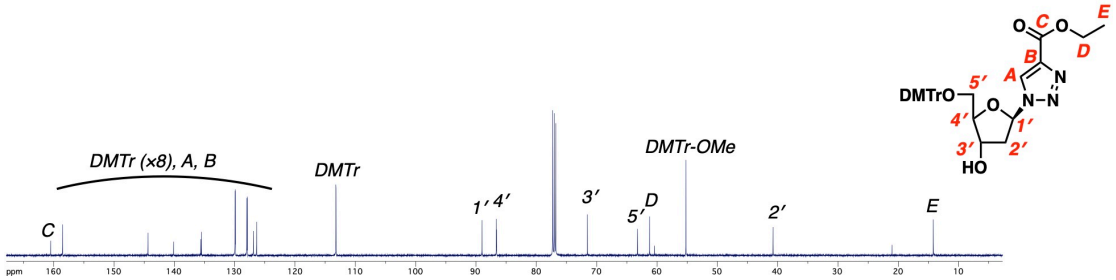


Compound 4

^{1}H NMR (500 MHz, $CDCl_3$, 300 K).

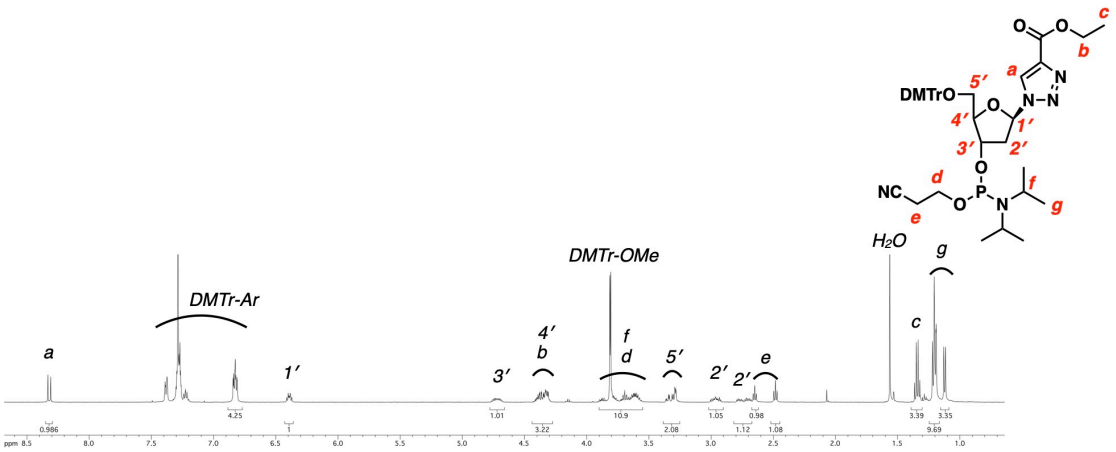


^{13}C NMR (126 MHz, $CDCl_3$, 300 K).



Compound 5

^{1}H NMR (500 MHz, $CDCl_3$, 300 K).



^{31}P NMR (202 MHz, $CDCl_3$, 300 K).