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# One-step to get 5-azidomethyl-2′-deoxyuridine from 5-hydroxymethyl-2′-deoxyuridine and detection of it through click reaction

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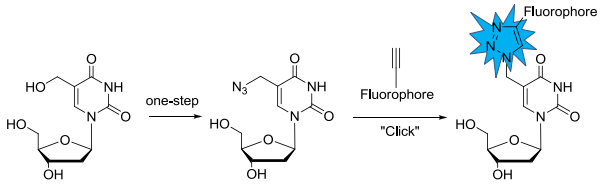
ABSTRACT

Nowadays a few ways to synthesize 5-azidomethyl-2′-deoxyuridine from 5-hydroxymethyl-2′-deoxyuridine have been reported. But none of them was one-step. And many of them need to protect the hydroxyl group on the pentose ring. The detection of 5-hydroxymethyl-2′-deoxyuridine is also very important in many biological processes. However few fluorescence detection strategies have been tried to do this. Herein, we reported a one-step protocol to synthesize 5-azidomethyl-2′-deoxyuridine, which was then used for detecting 5-hydroxymethyl-2′-deoxyuridine through a click reaction.



## 1. Introduction

DNA methylation is related to a variety of diseases, making the development of good detection methods important. Recently, much work has been done to study DNA methylation; particularly, the detection of 5-hmC and 5-mC has attracted enormous attention.[1–10] In addition, 5-hydroxymethyl-2′-deoxyuridine (5-hmdU) is a widely researched form of oxidative damage to DNA,[11,12] and the level of 5-hmdU in DNA from blood is a marker of breast cancer, as found by Zora and co-workers.[13] However, the detection of 5-hydroxymethyl-2′-deoxyuridine, which is involved in the demethylation of DNA, has attracted little attention.[14] Herein, we provide a simple method to detect 5-hmU in vitro. First, the hydroxyl group at the C-5 position group of 5-hydroxymethyl-2′-deoxyuridine selectively reacted with sodium azide in trifluoroacetic acid to produce 5-azidomethyl-2′-deoxyuridine (5-amdU) in onestep, which was followed by reaction with an alkynyl fluorescent probe (Scheme 1). Previously, 5-azidomethyl-2′-deoxyuridine has already been synthesized through several routes[15–18] but the yields of these routes are not satisfactory and more reagents are required in these routes.




**Scheme 1.** Schematic illustration of fluorescence turn-on detection of 5-hmdU based on click reaction.

Our original intention was to detect 5-hydroxymethyl-cytosine, but interestingly, 5-hydroxymethylcytosine did not react with sodium azide in trifluoroacetic acid (TFA). This phenomenon also demonstrated that the sodium azide did not reacted with the hydroxy group on the furan ring. We next tried 5-hydroxymethyl-2′-deoxyuridine and found that the yield was surprisingly high (85%). This phenomenon may be attributed to the intramolecular hydrogen bond formed between the 5-hydroxyl group and the 4-amino group; this feature can help us distinguish 5-hmdU from 5-hmC. In order to differentiate 5-hmdU from the four natural nucleosides

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in DNA, the four natural bases were treated using the procedure described and the mixture of the four nucleosides also exhibited weak fluorescence (Fig. 1). Thus, we can conclude that the selectivity of this strategy is relatively good.

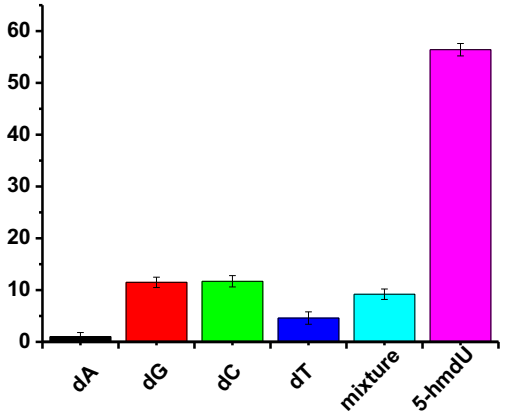


**Fig. 1.** Fluorescence intensity of selectivity experiments.

## 2. Experimental section

### 2.1. Materials

The following solvents, compounds, and reagents were commercially available: 2′-deoxyuridine, 7-hydroxyl coumarine, HPLC/spectroscopy grade acetonitrile were bought from Sigma–Aldrich. Dimethyl Formamide, triethylamine, copper sulfate pentahydrate, sodium ascorbate were bought from SCRC (Shanghai, China). The DNA sequence was bought from Suzhou Ribo Life Science Co. Ltd. (China).

### 2.2. Instruments

$^1$H and $^{13}$C NMR spectra were recorded on Varian Mercury 300 and 400 spectrometers, respectively. HRMS were recorded on a Bruker Daltonics, Inc. APEXIII 7.0 TESLA FTMS, and Varian Pro-MALDI. API-ES were recorded on a Agilent LC/MSD. Fluorescent emission spectra were collected on PerkinElmer LS 55. UV absorption spectra were collected on SHIMADZU UV-2550. HPLC spectra were recorded on a Laballiance Series III. Quartz cuvettes with 600 μL volume were used for emission measurements. Unless otherwise specified, all spectra were taken at an ambient temperature.

### 2.3. Synthesis

*2.3.1. 5-Azidomethyl-2′-deoxyurdine (5-amdU).*[19] 2′-Deoxyuridine (5.25 g, 23.0 mmol) and paraformaldehyde (3.11 g, 103.5 mmol) were added into 250 mL bottle with two necks and dissolved in 80 mL 0.5 mol/L triethylamine aqueous solution. The mixture was stirred at 60 °C for 4 days. During the reaction, more paraformaldehyde (4.49 g, 149.5 mmol), triethylamine (1 mL), and water (10 mL) was added into reaction mixture each day. After reaction finished, the mixture was concentrated in vacuo to remove the solvent. The residue was recrystallized in methanol to obtain 4.11 g 5-hmdU as white solid, yield=62%. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ (ppm): 11.34 (s, 1H), 7.71 (s, 1H), 6.17 (t, $J$=6.8 Hz, 1H), 5.26 (s, 1H), 4.97 (s, 2H), 4.20 (q, $J$=3.2 Hz, 1H), 4.10 (s, 2H), 3.75 (q, $J$=3.2 Hz, 1H), 3.57–3.47 (m, 2H), 2.09–1.99 (m, 2H); $^{13}$C NMR (DMSO-$d_6$, 75 MHz) $\delta$ (ppm): 162.5, 150.2, 136.7, 114.1, 87.1, 83.7, 70.4, 61.3, 55.9, 35.2. 5-hmdU (258 mg, 1 mmol) was dissolved in 15 mL trifluoroacetic acid in a 50 mL bottle, and then sodium azide (130 mg, 2 mmol) was added slowly into it. The mixture was stirred at room temperature overnight. Then the mixture was neutralized by saturated sodium bicarbonate to pH=7. The mixture was concentrated in vacuo to remove the solvent. The residue was purified by column chromatography ($SiO_2$, $CH_2Cl_2$/methanol=10:1) to get 5-amdU (241 mg, 0.85 mmol) as white solid, yield=85%. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ (ppm): 11.56 (s, 1H), 8.03 (s, 1H), 6.14 (t, $J$=6.3 Hz, 1H), 5.28 (d, $J$=3.9 Hz, 1H), 5.07 (s, 1H), 4.23 (s, 1H), 4.05 (s, 2H), 3.78 (q, $J$=2.7 Hz, 1H), 3.52–3.62 (m, 2H), 2.01–2.11 (m, 2H); $^{13}$C NMR (DMSO-$d_6$, 75 MHz) $\delta$ (ppm): 162.82, 150.11, 139.83, 108.19, 87.35, 84.11, 70.14, 61.09, 46.77, 40.13; HRMS (ESI) calcd for $C_{10}H_{13}N_5O_5$ $[M-H]^-$: 282.0838; found: 282.0841.

*2.3.2. 7-Ethynyl-2H-chromen-2-one (CA).* CA was synthesized according to Mélanie Chtchigrovsky and co-workers.[20] $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ (ppm): 8.06 (d, 1H, $J$=9.9 Hz), 7.71 (d, 1H, $J$=7.8 Hz), 7.51 (s, 1H), 7.43 (d, 1H, $J$=7.5 Hz), 6.52 (d, 1H, $J$=9.9 Hz), 4.50 (s, 1H); $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ (ppm): 160.46, 153.74, 142.97, 128.27, 128.01, 125.81, 120.38, 119.31, 117.40, 82.28, 80.97; HRMS (ESI) $m/z$: calcd for $C_{11}H_6O_2$ $[M+Na]^+$ 193.0265, found: 193.0259.

*2.3.3. 1-((2R,4S,5R)-4-Hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-((4-(2-oxo-2H-chromen-7-yl)-1H-1,2,3-triazol-1-yl)methyl)pyrimidine-2,4(1H,3H)-dione (CZ).* 5-amdU (28.3 mg, 0.1 mmol) was dissolved in 5 mL $H_2O$ and 5 mL DMF, then catalytic amount of copper sulfate pentahydrate and sodium ascorbate were added into the flask. At last, CA (17 mg, 0.1 mmol) was poured into mixture. The mixture was stirred overnight at room temperature. The solvents were removed in vacuo and the residue was washed with water and methanol and then recrystallized from methanol to get CZ (43.5 mg, 0.096 mmol) as white solid. Yield=96%. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ (ppm): 11.62 (s, 1H), 8.68 (s, 1H), 8.23 (s, 1H), 8.07 (d, $J$=9.6 Hz, 1H), 7.70–7.90 (m, 3H), 6.48 (dd, $J$=9.6 Hz, 1H), 6.17 (t, $J$=3 Hz, 1H), 5.20–5.32 (m, 3H), 5.07 (s, 1H), 4.26 (s, 1H), 3.80 (s, 1H), 3.58 (m, 2H), 2.10–2.20 (m, 2H); $^{13}$C NMR (DMSO-$d_6$, 75 MHz) $\delta$ (ppm): 162.57, 159.96, 154.08, 150.27, 144.68, 143.97, 141.43, 141.40, 134.25, 129.12, 122.71, 121.18, 118.17, 115.81, 112.15, 107.04, 87.56, 84.54, 70.15, 61.09, 46.58; HRMS (ESI) calcd for $C_{21}H_{19}N_5O_7$ $[M+H]^+$: 454.1363, found: 454.1359, $[M+Na]^+$: 476.1182, found: 476.1178.

*2.3.4. 2-Ethyl-6-ethynyl-1H-benzo[de]isoquinoline-1,3(2H)-dione (NP).* NP was synthesized according to Sawa and co-workers.[21] $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ (ppm): 8.64 (m, 2H), 8.52 (d, 1H, $J$=5.7 Hz), 7.92 (d, 1H, $J$=5.7 Hz), 7.82 (m, 1H), 4.23 (m, 2H), 3.73 (s, 1H), 1.33 (m, 3H); $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ (ppm): 163.97, 132.38, 131.86, 130.32, 127.91, 126.40, 123.24, 86.75, 80.57, 35.86, 13.60.

*2.3.5. 2-Ethyl-6-(1-((1-(5-hydroxy-4-(hydroxymethyl)tetrahydrofuran-2-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl)-1H-1,2,3-triazol-4-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (NZ).* The synthesis route of NZ was the same as CZ. NZ is dark red solid and the yield is 90%. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ (ppm): 11.63 (s, 1H), 9.13 (s, 1H), 8.77 (s, 1H), 8.49 (s, 1H), 8.26 (s, 1H), 8.07 (s, 1H), 7.88 (s, 1H), 6.18 (s, 1H), 5.36 (s, 3H), 5.08 (s, 1H), 4.27 (s, 1H), 4.06 (s, 1H), 3.59 (s, 1H), 2.18 (s, 2H); $^{13}$C NMR (DMSO-$d_6$, 75 MHz) $\delta$ (ppm): 163.12, 162.80, 162.66, 150.24, 144.40, 141.31, 133.92, 130.74, 130.30, 128.17, 128.08, 127.50, 127.01, 125.35, 122.35, 121.49, 107.15, 87.53, 84.55, 70.14, 61.22, 46.68, 34.79, 13.08; HRMS (ESI) calcd for $C_{26}H_{24}N_6O_7$ $[M+Na]^+$: 555.1598, found: 555.1599.

*2.3.6. 10-(4-Ethynylphenyl)-5,5-difluoro-1,3,7,9-tetramethyl-5H-dipyrrolo[1,2-c:2′,1′-f][1,3,2]diazaborinin-4-ium-5-uide (AB).* AB was synthesized according to Li and co-workers.[22] $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ (ppm): 7.62 (d, 2H, $J$=7.2 Hz), 7.27 (d, 2H, $J$=6.9 Hz), 5.98 (s,