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# Highly Efficient and Facile Synthesis of 5-Azido-2'-Deoxyuridine

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#### HIGHLY EFFICIENT AND FACILE SYNTHESIS OF 5-AZIDO-2'-DEOXYURIDINE

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□ Previously reported syntheses of the photoaffinity label 5-azido-2'-deoxyuridine are rather inefficient and involve the tedious preparation of a 5-nitro intermediate. To overcome these inconveniences, we have developed a new approach from the commercially available 5-bromo-2'-deoxyuridine nucleoside. Our synthetic route makes use of a benzylamination reduction sequence. Using this strategy, the 5-azido-2'-deoxyuridine photolabel is prepared in three steps and quantitative yields.

Keywords 5-Azidouracil nucleosides; benzylamination; azidation

Photoactivable molecules incorporating the 5-azidouracil moiety are widely used to study a large variety of proteins including nucleoside drug metabolizing enzymes,<sup>[1a]</sup> polymerases,<sup>[1b-d]</sup> transcription factors,<sup>[1e]</sup> ribosomal proteins,<sup>[1f]</sup> triphosphatases,<sup>[1g]</sup> and sugar transferases.<sup>[2]</sup> Recently, we studied the photochemical properties of 5-azido-2'-deoxyuridine<sup>[3]</sup> (1) and faced the problem of its convenient and efficient synthesis. Herein, we disclose a simple and highly efficient method to prepare 1 from the commercially available 5-bromo-2'-deoxyuridine **2**.<sup>[4]</sup>

Compound 1 has already been synthesized<sup>[5,6]</sup> and the synthetic approach follows a main protocol developed in the nucleoside monophosphate series.<sup>[7,8]</sup> The first step always consists in the nitration of 2'-deoxyuridine **3** into the 5-nitro derivative **4** using nitrosonium tetrafluoroborate (Scheme 1). Compound **4** is then reduced by the action of  $Zn/HCl^{[5,7]}$  or 5% Pd- $C/H_2^{[6,8]}$  to afford the corresponding amino derivative **5**. Diazotization of

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SCHEME 1<sup>5,6</sup> Known syntheses of 1 using a 5-nitro intermediate.

5 in the presence of sodium nitrite and 1N HCl followed by treatment with  $NaN_3$  gives rise to 1. However, detailed experimental descriptions are lacking and the overall yield, from 3 to 1, when reported, is rather low since it oscillate between 10% and 20%.<sup>[5]</sup> In addition, such synthesis requires a large excess (10 to 25 equiv.) of moisture sensitive and expensive NOBF<sub>4</sub> reagent as well as tedious purification protocols. More embarrassingly, in our hands, this first step that is described to be quantitative was not always reproducible depending greatly on the quality of NOBF<sub>4</sub> and the DMF moisture content. Therefore, alternative and improved routes for the synthesis of 1 are highly desirable.

5-Aminouracil nucleosides necessary to access 5-azido derivatives according to this synthetic route can be directly prepared by nucleophilic substitution of 5-bromouracil precursors.<sup>[9]</sup> Applied to **2**, this procedure that requires treatment with liquid ammonia in a steel bomb and heating for several days yielded **5** in 63% to 75% yield.<sup>[9b,c,f]</sup> However, this amination reaction presents moderate and variable yields and also hazards associated with handling anhydrous liquid NH<sub>3</sub>. It also requires appropriate material and training. Owing to these drawbacks, we searched for a safer, more convenient and efficient approach to introduce the amine function at position C5 of 2'-deoxyuridine starting from the bromo precursor **2**. Benzylamination of uracil aglycones from their 5-bromo counterpart is an efficient reaction.<sup>[10]</sup> Therefore, we selected the benzylamination/reduction sequence to prepare **5** (Scheme 2) assuming that the presence of the 2-deoxyribose would not interfere with this reactions sequence.



SCHEME 2 Synthesis of 1 using a benzylamination/reduction sequence.

Treatment of 2 in the presence of neat benzylamine at 90°C yielded quantitatively the 5-benzylamino derivative 6 after an aqueous extraction procedure. Then, catalytic hydrogenolysis of 6 using ammonium formate and 10% palladium-carbon catalyst in refluxing methanol afforded quantitatively **5** after filtration.

In our hands, the azidation conditions described in the literature  $(NaNO_2/1N \text{ HCl at } 0^{\circ}\text{C} \text{ and } NaN_3)^{[7,8]}$  led to the hydrolysis of the *N*-glycosidic bond of **5**. Therefore, we used a milder protocol for the generation of nitrous acid which makes used of 80% acetic acid and NaNO<sub>2</sub>.<sup>11</sup> Under these milder conditions, the azido compound **1** was formed quantitatively after 1 hour at 0°C in the presence of 1.1 equiv. NaN<sub>3</sub>.

In conclusion, we have herein described a quantitative, safe, convenient and easily reproducible synthesis of 1 starting from commercially available nucleoside 2. This synthetic route provides as well a new and reliable access to 5-amino-2'-deoxyuridine 5 that is also an important biological tool.<sup>[9c,f]</sup> Finally, our synthetic route to 1, in addition to its usefulness with regard to photoaffinity labeling, should be also profitable for applications in the copper-catalyzed Huisgen cycloaddition chemistry. Indeed, a recent example illustrates the first use of 1 to attach an alkyne fluorescent dye for the detection of DNA synthesis in cells.<sup>[6]</sup>

#### EXPERIMENTAL

#### 5-Benzylamino-2'-deoxyuridine 6

5-Bromo-2'-deoxyuridine **2** (1g, 3.26 mmol.) was dissolved in benzylamine (7.2 mL) and the solution was heated at 90°C for 3 hours. After cooling to room temperature, the reaction mixture was co-evaporated with toluene. The residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) and extracted with water (3 × 100 mL). The aqueous extract was concentrated to give quantitatively **6** as a white powder. <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD) δ 7.40–7.21 (m, 5H), 6.76 (s, 1H), 6.34 (dd, J = 6.3; 7.3 Hz, 1H), 4.24 (d, J = 13.7 Hz, 1H, ddd, J = 3.3; 3.6; 6.4 Hz, 1H), 4.14 (d, J = 13.7 Hz, 1H), 3.82 (ddd, J =3.3; 3.6; 4.0 Hz, 1H), 3.63 (dd, J = 3.6; 6.13; 13.5 Hz, 1H), 1.98 (ddd, J = 6.4; 7.3; 13.5 Hz, 1H); <sup>13</sup>C NMR (75 MHz, CD<sub>3</sub>OD) δ 162.5, 150.4, 139.0, 129.2, 128.1, 128.0, 125.8, 113.5, 87.8, 85.6, 71.7, 62.6, 48.6, 40.2; HRMS (ESI): m/z: [M+Na]<sup>+</sup> calcd for C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>5</sub>Na: 356.1222, found 356.1221; Anal. Calcd for C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>5</sub>N.1/4 H<sub>2</sub>O: C, 56.88; H, 5.82; N, 12.44. Found: C, 56.65; H, 5.66; N, 12.47.

#### 5-Amino-2'-deoxyuridine 5

To a solution of **6** (288 mg, 0.86 mmol.) in methanol (12 mL) under nitrogen was added 10% Pd-C (83 mg, 0.78 mmol., 0.9 equiv.) then ammonium formate (97 mg, 1.54 mmol., 1.78 equiv.). The reaction mixture was

vigorously stirred and refluxed for 1 hour. After cooling to room temperature, the reaction mixture was sonicated for 1h then filtered through Celite. The Celite was washed with methanol and the filtrate and methanolic wash were combined and evaporated in vacuo to furnish **5** as a white powder in quantitative yield. <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD)  $\delta$  7.29 (s, 1H), 6.29 (t, *J* = 6.8 Hz), 4.42 (dt, *J* = 3.6; 5.4 Hz, 1H), 3.99 (ddd, *J* = 3.5; 3.6; 5.0 Hz, 1H), 3.80 (dd, *J* = 3.5; 12.5 Hz, 1H), 3.72 (dd, *J* = 5.0; 12.5 Hz, 1H), 2.32 (dd, *J* = 5.4; 6.8 Hz, 2H); <sup>13</sup>C NMR (75 MHz, CD<sub>3</sub>OD)  $\delta$  162.7, 150.8, 123.8, 119.5, 88.2, 85.7, 72.2, 63.1, 40.1; HRMS (ESI) *m/z*: [M+Na]<sup>+</sup> calcd for C<sub>9</sub>H<sub>13</sub>N<sub>3</sub>O<sub>5</sub>Na: 266.0755, found 266.0753.

#### 5-Azido-2'-deoxyuridine 1

To a stirred solution of 5 (263 mg; 1.08 mmol) in 80% acetic acid (2.8 mL) at 0°C was added NaNO<sub>2</sub> (82 mg; 1.19 mmol., 1.1 equiv.). After 5 minutes,  $NaN_3$  (77 mg, 1.19 mmol., 1.1 equiv.) was added and the reaction mixture was allowed to stir at 0°C for 1 hour in the dark. Solvents were removed by evaporation under reduced pressure without heating then coevaporated with toluene. Compound 1 was quantitatively obtained with 16 equiv. of acetic acid. When necessary, full removal of acetic acid was achieved by a silicagel purification of the residue using a gradient of methanol in CH<sub>2</sub>Cl<sub>2</sub> (0 to 10%). Fractions eluting at 10% were collected and concentrated to dryness to yield 1 as a white powder (116 mg, 40% yield): IR (thin film): 2119, 1690, 1468, 1403, 1359, 1310, 1266 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O) δ 7.71 (s, 1H), 6.25 (t, I = 6.5 Hz, 1H), 4.43 (ddd, I = 3.9; 4.8; 6.3 Hz, 1H), 4.00 (ddd, I = 3.4; 3.9; 4.6 Hz, 1H), 3.82 (dd, I = 3.4; 12.6 Hz, 1H), 3.73 (dd, J = 4.6; 12.6 Hz, 1H), 2.34 (m, 2H); <sup>13</sup>C NMR (75 MHz, D<sub>2</sub>O)  $\delta$  160.9, 149.9, 128.7, 115.7, 86.4, 85.2, 69.8, 60.9, 37.2; HRMS (ESI) m/z: [M+Na]<sup>+</sup> calcd for C<sub>9</sub>H<sub>11</sub>N<sub>5</sub>O<sub>5</sub>Na: 292.0658, found 292.0664.

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