

## Efficient Preparation of Diuridine $P^2$ , $P^3$ - (Dihalo) Methylenetetraphosphates

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**Abstract.** Diuridine  $P^2$ ,  $P^3$ - (dihalo) methylenetetraphosphates ( $Up_2CF_2p_2U$  and  $Up_2CBr_2p_2U$ ) were synthesized via the activation of P (V) – N bond. The key uridine 5'-phosphoropiperidate intermediate was obtained via a redox condensation method.

### Introduction

Dinucleoside polyphosphates are ubiquitous compounds involved in the biological processes of different tissues and organs, such as the cardio-vascular system, eye, and neuron system<sup>[1–5]</sup>. They have been shown to activate a variety of purinergic receptors and act as substrates for different DNA polymerases. In recent years, their biological functions and pharmaceutical applications have been extensively studied. Artificial dinucleoside polyphosphates, such as  $Up_4U$  and  $Up_4dC$ , have also been developed as therapeutic agents for purinergic receptor-related diseases<sup>[6–8]</sup>. Though their chemical and metabolic lability in vivo is significantly improved compared to their mononucleoside counterparts<sup>[9]</sup>, hydrolysis-resistant dinucleoside polyphosphates are still highly desired. Accordingly, medicinal chemists have attempted to address this problem by synthesizing of  $Np_nN$  analogs with oxo-bridge modified polyphosphate chains<sup>[10–11]</sup>.

Currently there are only a few chemical synthetic methods available for the preparation of  $Np_nN$  analogs. In the present work, we report the application of the P (V) – N activation strategy for the preparation  $Up_4U$  analogs ( $Up_2CF_2p_2U$  and  $Up_2CBr_2p_2U$ ) from uridine 5'-phosphoropiperidate. After the uridine 5'-phosphoropiperidate was converted into  $\beta$ ,  $\gamma$ -bridging oxygen-modified uridine 5'-triphosphate, the triphosphate analog was coupled with the phosphoropiperidate precursor in the presence of 4, 5-dicyanoimidazole (DCI) activator.

### Experimental

Chemical reagents and solvents were obtained from commercial suppliers and used without further purification. Uridine 5'-phosphoropiperidate (1) was synthesized according to the reported method<sup>[12]</sup>. All reactions were performed under an atmosphere of dry argon. Ion exchange chromatography employed DEAE Sephadex A-25 anion exchanger. All NMR spectra were obtained with a 400 MHz instrument with chemical shifts reported in parts per million (ppm,  $\delta$ ) and referenced to  $D_2O$ . IR spectra were recorded on a FT-IR spectrometer. Low-resolution mass spectra were obtained with an ion trap mass spectrometer and reported as  $m/z$ .

Uridine 5'- $\beta,\gamma$ -difluoromethylenetriphosphate, tetrasodium salt (2). To a solution of uridine 5'-phosphoropiperidate (28mg, 0.06mmol) in DMF (1mL) were added tris (tetra-*n*-butylammonium) difluoridized methylenediphosphonate (112 mg, 0.12mmol) and 4, 5-dicyanoimidazole (DCI, 43mg, 0.36mmol). The reaction was stirred at 40°C for 1h and concentrated in vacuo. The residue was dissolved in NaOAc aqueous solution (10M, 0.5mL). Then, EtOH (50mL) was added. The resulting white precipitate was collected by centrifuge. The crude product was dissolved in deionized  $H_2O$

(0.5mL) and loaded on a DEAE Sephadex A-25 ion exchange column (1.6 × 25cm). Elution with  $\text{NH}_4\text{HCO}_3$  buffer (linear gradient 0.2 to 0.6 M), combination of appropriate fractions, and lyophilization afforded the product in ammonium salt form. Passage of the solution of the ammonium salt in deionized  $\text{H}_2\text{O}$  through a bed of Dowex 50W-X8 ion exchange resin ( $\text{Na}^+$  form) and lyophilization afforded the product as tetrasodium salt (26mg, 69%), a white solid;  $^1\text{H}$  NMR (400MHz,  $\text{D}_2\text{O}$ ):  $\delta$  7.95 (d,  $J = 8.1\text{Hz}$ , 1H), 5.97–5.94 (m, 2H), 4.41–4.37 (m, 2H), 4.26–4.23 (m, 3H) ppm;  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  165.4, 151.0, 140.8, 101.8, 87.4, 82.5, 72.8, 68.7, 64.0 ppm;  $^{31}\text{P}$  NMR (162 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  3.02 (dt,  $J_{\text{P-}\beta,\text{P-}\gamma} = 57.8\text{ Hz}$ ,  $J_{\text{P-}\gamma,\text{F}} = 76.2\text{ Hz}$ , 1P), –4.23 (ddt,  $J_{\text{P-}\beta,\text{P-}\gamma} = 57.8\text{ Hz}$ ,  $J_{\text{P-}\beta,\text{P-}\alpha} = 31.2\text{ Hz}$ ,  $J_{\text{P-}\beta,\text{F}} = 89.6\text{Hz}$ , 1P), –11.23 (d,  $J_{\text{P-}\beta,\text{P-}\alpha} = 31.2\text{Hz}$ , 1P) ppm; IR (KBr):  $\nu_{\text{max}}$  3384, 2972, 2894, 1681, 1424, 1275, 916, 826  $\text{cm}^{-1}$ ; LRMS (ESI–):  $m/z$  Calcd. for  $\text{C}_{10}\text{H}_{15}\text{F}_2\text{N}_2\text{O}_{16}\text{P}_3$  [ $\text{M-H}$ ] $^-$  548.8; found 548.9.

Uridine 5'- $\beta,\gamma$ -dibromomethylenetriphosphate, tetrasodium salt (3). To a solution of uridine 5'-phosphoropiperidate (28mg, 0.06mmol) in DMF (1mL) were added tris (tetra-*n*-butylammonium) dibromodized methylenediphosphonate (126mg, 0.12mmol) and 4, 5-dicyanoimidazole (DCI, 43mg, 0.36mmol). The reaction was stirred at 40°C for 1h and concentrated in vacuo. The residue was dissolved in NaOAc aqueous solution (10 M, 0.5mL). Then, EtOH (50mL) was added. The resulting white precipitate was collected by centrifuge. The crude product was dissolved in deionized  $\text{H}_2\text{O}$  (0.5mL) and loaded on a DEAE Sephadex A-25 ion exchange column (1.6 × 25 cm). Elution with  $\text{NH}_4\text{HCO}_3$  buffer (linear gradient 0.2 to 0.6 M), combination of appropriate fractions, and lyophilization afforded the product in ammonium salt form. Passage of the solution of the ammonium salt in deionized  $\text{H}_2\text{O}$  through a bed of Dowex 50W-X8 ion exchange resin ( $\text{Na}^+$  form) and lyophilization afforded the product as tetrasodium salt (29mg, 65%), a white solid;  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  7.94 (d,  $J = 8.1\text{ Hz}$ , 1H), 5.97–5.94 (m, 2H), 4.42–4.37 (m, 2H), 4.27–4.23 (m, 3H) ppm;  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  165.3, 151.1, 140.8, 101.8, 87.5, 82.5, 72.8, 68.6, 64.0 ppm;  $^{31}\text{P}$  NMR (162 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  7.27 (d,  $J_{\text{P-}\alpha,\text{P-}\beta} = 17.6\text{ Hz}$ , 1P), –0.21 (dd,  $J_{\text{P-}\beta,\text{P-}\gamma} = 17.7\text{ Hz}$ ,  $J_{\text{P-}\beta,\text{P-}\alpha} = 29.3\text{ Hz}$ , 1P), –11.13 (d,  $J_{\text{P-}\alpha,\text{P-}\beta} = 29.4\text{ Hz}$ , 1P) ppm; IR (KBr):  $\nu_{\text{max}}$  3386, 2977, 2899, 1677, 1261, 1068, 914, 814  $\text{cm}^{-1}$ ; LRMS (ESI–):  $m/z$  Calcd. for  $\text{C}_{10}\text{H}_{15}\text{Br}_2\text{N}_2\text{O}_{16}\text{P}_3$  [ $\text{M-H}$ ] $^-$  664.7; found 664.8.

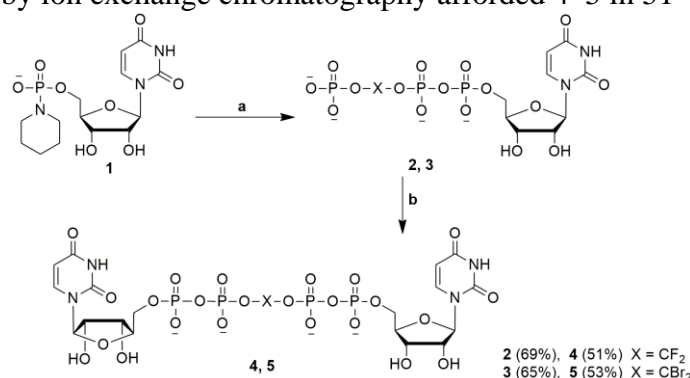
$\text{P}^1,\text{P}^4$ -Diuridine-5',5'- $\text{P}^2,\text{P}^3$ -difluoromethylene-tetraphosphate, tetrasodium salt (4). To a solution of uridine 5'-phosphoropiperidate (37mg, 0.08mmol) in *N*-methylpyrrolidone (2mL) were added tris (tetra-*n*-butylammonium) uridine 5'- $\beta,\gamma$ -difluoromethylenetriphosphate (51 mg, 0.04 mmol) and 4, 5-dicyanoimidazole (DCI, 24mg, 0.2mmol). The reaction was stirred at 20°C for 20h. Then, diethyl ether (20mL) was added into the solution, and the resulting white precipitate was collected by centrifuge. The crude products were dissolved in deionized  $\text{H}_2\text{O}$  (1mL) and loaded onto an ion exchange gel column. Elution with  $\text{NH}_4\text{HCO}_3$  buffer (linear gradient 0.3 to 0.6M), combination of appropriate fractions, and lyophilization gave  $\text{Up}_2\text{CF}_2\text{p}_2\text{U}$  as the ammonium salts. Passage of the solution of the ammonium salts in deionized  $\text{H}_2\text{O}$  through a bed of Dowex 50W-X8 ion exchange resin ( $\text{Na}^+$  form) and lyophilization afforded the product as tetrasodium salt (19mg, 51%), a white solid;  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  7.95 (d,  $J = 8.1\text{Hz}$ , 2H), 5.96 (s, 2H), 5.95 (d,  $J = 4.0\text{ Hz}$ , 2H), 4.39–4.34 (m, 4H), 4.23–4.20 (m, 6H) ppm;  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  166.0, 151.6, 141.3, 102.4, 87.8, 83.2, 73.4, 69.4, 64.7ppm;  $^{31}\text{P}$  NMR (162MHz,  $\text{D}_2\text{O}$ ):  $\delta$  –1.8 (d,  $J = 21.0\text{ Hz}$ , 2P), –11.4 (d,  $J = 21.0\text{Hz}$ , 2P) ppm; IR (KBr):  $\nu_{\text{max}}$  3388, 2976, 2897, 1684, 1424, 1265, 1065, 916, 816  $\text{cm}^{-1}$ ; LRMS (ESI–):  $m/z$  calcd for  $\text{C}_{19}\text{H}_{26}\text{F}_2\text{N}_4\text{O}_{24}\text{P}_4$  [ $\text{M-H}$ ] $^-$  855.0; found 854.9.

$\text{P}^1,\text{P}^4$ -Diuridine-5',5'- $\text{P}^2,\text{P}^3$ -dibromomethylene-tetraphosphate, tetrasodium salt (5). To a solution of uridine 5'-phosphoropiperidate (28mg, 0.06mmol) in *N*-methylpyrrolidone (2mL) were added tris (tetra-*n*-butylammonium) uridine 5'- $\beta,\gamma$ -dibromomethylenetriphosphate (42mg, 0.03mmol) and 4, 5-dicyanoimidazole (DCI, 18mg, 0.15mmol). The reaction was stirred at 20°C for 20h. Then, diethyl ether (20mL) was added into the solution, and the resulting white precipitate was collected by centrifuge. The crude products were dissolved in deionized  $\text{H}_2\text{O}$  (1mL) and loaded onto an ion exchange gel column. Elution with  $\text{NH}_4\text{HCO}_3$  buffer (linear gradient 0.3 to 0.6M), combination of appropriate fractions, and lyophilization gave  $\text{Up}_2\text{CBr}_2\text{p}_2\text{U}$  as the ammonium salts. Passage of the solution of the ammonium salts in deionized  $\text{H}_2\text{O}$  through a bed of Dowex 50W-X8 ion exchange

resin (Na<sup>+</sup> form) and lyophilization afforded the product as tetrasodium salt (17mg, 53%), a white solid; <sup>1</sup>H NMR (400MHz, D<sub>2</sub>O): δ 7.94 (d, *J* = 8.1 Hz, 2H), 5.97 (s, 2H), 5.95 (d, *J* = 4.0 Hz, 2H), 4.38–4.33 (m, 4H), 4.23–4.21 (m, 6H) ppm; <sup>13</sup>C NMR (100MHz, D<sub>2</sub>O): δ 166.0, 151.6, 141.3, 102.4, 87.8, 83.2, 73.4, 69.4, 64.7 ppm; <sup>31</sup>P NMR (162MHz, D<sub>2</sub>O): δ –1.9 (d, *J* = 21.0 Hz, 2P), –11.5 (d, *J* = 21.0 Hz, 2P) ppm; IR (KBr): ν<sub>max</sub> 3386, 2978, 2899, 1687, 1426, 1261, 1065, 914, 818cm<sup>–1</sup>; LRMS (ESI<sup>–</sup>): *m/z* calcd for C<sub>19</sub>H<sub>26</sub>N<sub>4</sub>O<sub>24</sub>P<sub>4</sub>Br<sub>2</sub> [M–H]<sup>–</sup> 970.8; found 970.7.

## Results and Discussion

As shown in Scheme 1, uridine phosphoropiperidate (1) was treated with 2.0 equiv of tris(tetra-*n*-butylammonium) dihalogenated methylenediphosphonate and 6 equiv of DCI at 40°C in DMF for 1 h. Ethanol precipitation followed by ion exchange chromatography afforded 2–3 in 65–69% isolated yields. In the following step, treatment of 2–3 with 2 equiv of uridine 5'-phosphoropiperidate and 5 equiv of DCI at 20°C in *N*-methylpyrrolidone for 12h. Diethylether precipitation followed by ion exchange chromatography afforded 4–5 in 51–53% isolated yields.



Reagents and conditions: (a) diphosphonates, DCI, DMF, 40 °C, 1h; (b) uridine 5'-phosphoropiperidate, DCI, *N*-methylpyrrolidone, 20 °C, 12h

Fig.1 Synthesis of Diuridine P<sup>2</sup>, P<sup>3</sup>- (Dihalo) Methylenetetraphosphates

## Summary

In conclusion, the work described in this paper presents an efficient procedure for the preparation of diphosphonate-modified dinucleoside tetraphosphates. Compared to the previously reported synthetic methods, the P (V)–N activation approach provided a facile and efficient approach for the synthesis of dinucleoside P<sup>2</sup>, P<sup>3</sup>-(dihalo) methylenetetraphosphate analogs.

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