Synthesis of Dinucleoside Triphosphates Containing Antiviral Nucleoside

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Abstract—A general and efficient method for the synthesis of asymmetrical dinucleoside triphosphates with an antiviral nucleoside moiety has been developed. The coupling of adenosine diphosphate with antiviral nucleoside phosphoropiperidate via the DCI activation of P-N bond afforded the desired products in good yield.

Keywords—Zidovudine; Stavudine; Dinucleoside triphosphate; Antiviral nucleoside; Phosphoropiperidate

I. INTRODUCTION

Dinucleoside polyphosphates (DNPPs or NpnN') constitute a family of nucleotides containing two nucleoside moieties joined at their 5'-positions by a linear polyphosphate chain. The polyphosphate chain ranges from 2 to 7 phosphorus atoms. In the past several decades, naturally occurring dinucleoside polyphosphates have been elucidated to involve in a variety of intra and extracellular processes including DNA replication and repair [1–4], regulation of purine nucleotide metabolism, alarmones action in heat shock and oxidative stresses, catalysis of certain kinases, and control of cell division [5]. In addition, modified artificial dinucleoside triphosphates are also of important therapeutic antivirals and diagnostic value [6–8]. For instance, Ap₃AZT and Ap₃d4T have been synthesized and tested for the therapy of HIV-infected patients [9–11]. The increasing interest of the biological roles and pharmaceutical applications of modified dinucleoside triphosphates have promoted the development of general and efficient methods for this type of compounds.

Previously, dinucleoside triphosphates have been mostly prepared by the conventional methods in low yields. A practical synthesis of dinucleoside triphosphates remains a challenge for phosphorus chemists. Up to now, enzymatic methods have also been used for the synthesis of NpnN derivatives [12, 13]. However, the enzymatic approaches have limitations in reaction scale and specificity to limited natural nucleoside substrates. In the present work, we report an method for the preparing of asymmetric dinucleoside triphosphates from the corresponding antiviral nucleoside phosphoropiperidates promoted by the activation of the P(V)–N bond.

II. EXPERIMENTAL

Chemical reagents and solvents were obtained from commercial suppliers. The triethylammonium salt of zidovudine and stavudine 5'-phosphoropiperidate was synthesized according to the procedures described in a

previous report. All reactions were performed under an atmosphere of dry argon. All NMR spectra were obtained with a 400 MHz instrument with chemical shifts reported in parts per million (ppm, δ) and referenced to D₂O. IR spectra were recorded on a FT-IR spectrometer. Low-resolution and high-resolution mass spectra were reported as m/z and obtained with an ion trap and a TOFQ mass spectrometer, respectively.

A. *P*¹-Adenosine-5'-P³-zidovudine-5'-triphosphate, trisodium salt (2).

To a solution of zidovudine 5'-phosphoropiperidate (46 mg, 0.1 mmol) in DMF (1 mL) were added adenosine 5'-diphosphate bis (tetra-n-butylammonium salt, 45 mg, 0.05 mmol) and DCI (35 mg, 0.3 mmol). The reaction was stirred at 40 °C for 10 h and concentrated in vacuo. The residue was dissolved in NaOAc aqueous solution (3.0 M, 0.5 mL) and then EtOH (30 mL) was added. The white precipitation was collected by centrifuge. The crude product was dissolved in deionized H₂O (0.5 mL) and loaded on a DEAE Sephadex A-25 ion exchange column (1.6 × 25 cm). Elution with NH₄HCO₃ buffer (linear gradient 0.3 to 0.5 M), combination of appropriate fractions, and lyophilization afforded dinucleoside triphosphate in ammonium salt form. Passage of the solution of the triethylammonium salt in deionized H₂O through a bed of Dowex 50W-X8 ion exchange resin (Na⁺ form) and lyophilization afforded Ap₃AZT (2, 40 mg, 53%) as trisodium salt, a white solid. ¹H NMR (400 MHz, D₂O): δ 8.42 (s, 1H), 8.12 (d, *J* = 6.8 Hz, 1H), 7.73 (s, 1H), 6.26 (t, *J* = 6.8 Hz, 1H), 6.03 (d, *J* = 6.4 Hz, 1H), 4.68–4.51 (m, 4H), 4.31–4.11 (m, 5H), 2.57–2.37 (m, 3H), 1.91 (s, 3H); ¹³C NMR (100 MHz, D₂O): δ 166.5, 155.6, 152.8, 151.8, 149.3, 139.9, 137.1, 118.6, 111.8, 86.5, 84.9, 84.3, 84.2, 83.0, 74.1, 70.4, 65.8, 60.9, 36.2, 11.6; ³¹P NMR (162 MHz, D₂O): δ 10.8 (d, *J*_{P,P} = 20.2 Hz, 2P), 23.2 (t, *J*_{P,P} = 20.2 Hz, 1P); IR (KBr): $\nu_{\max}$ 3730, 3595, 2935, 2813, 2379, 2348, 2314, 2111, 1706, 1484, 1368, 1278, 1127, 1062, 975, 518, 418 cm^{−1}; LRMS (ESI[−]): m/z calcd for C₂₀H₂₆N₁₀O₁₆P₃ [M–H][−] 755.1; found: 755.2.

B. *P*¹-Adenosine-5'-P³-stavudine-5'-triphosphate, trisodium salt (3).

To a solution of stavudine 5'-phosphoropiperidate (43 mg, 0.1 mmol) in DMF (1 mL) were added adenosine 5'-diphosphate bis (tetra-n-butylammonium salt, 45 mg, 0.05

mmol) and DCI (35 mg, 0.3 mmol). The reaction was stirred dissolved in NaOAc aqueous solution (3.0 M, 0.5 mL) and then EtOH (30 mL) was added. The white precipitation was collected by centrifuge. The crude product was dissolved in deionized H₂O (0.5 mL) and loaded on a DEAE Sephadex A-25 ion exchange column (1.6 × 25 cm). Elution with NH₄HCO₃ buffer (linear gradient 0.3 to 0.5 M), combination of appropriate fractions, and lyophilization afforded dinucleoside triphosphate in ammonium salt form. Passage of the solution of the triethylammonium salt in deionized H₂O through a bed of Dowex 50W-X8 ion exchange resin (Na⁺ form) and lyophilization afforded Ap₃d4T (**3**, 37 mg, 52%) as trisodium salt, a white solid. ¹H NMR (400 MHz, D₂O): δ 8.43 (s, 1H), 8.11 (d, *J* = 6.9 Hz, 1H), 7.59 (s, 1H), 6.94 (d, *J* = 1.2 Hz, 1H), 6.52 (d, *J* = 6.4 Hz, 1H), 6.03 (d, *J* = 6.3 Hz, 1H), 5.92 (d, *J* = 6.0 Hz, 1H), 5.20–5.02 (m, 5H), 4.67–4.11 (m, 6H), 1.85 (s, 3H); ¹³C NMR (100 MHz, D₂O): δ 166.8, 155.7, 152.8, 152.3, 149.5, 139.7, 138.1, 134.3, 125.1, 118.4, 111.5, 90.0, 86.5, 85.9, 84.3, 84.2, 74.1, 70.6, 66.5, 11.4; ³¹P NMR (162 MHz, D₂O): δ 11.2 (d, *J*_{P,P} = 19.4 Hz, 1P), 22.1 (t, *J*_{P,P} = 19.4 Hz, 1P); IR (KBr): ν_{max} 3732,

at 40 °C for 10 h and concentrated in vacuo. The residue was 3491, 3125, 2943, 2817, 2376, 2345, 2319, 1700, 1477, 1403, 1264, 1112, 1045, 987, 933, 835, 782, 595 cm⁻¹; LRMS (ESI⁻): *m/z* calcd for C₂₀H₂₅N₇O₁₆P₃ [M-H]⁻ 712.1; found: 712.

III. RESULTS AND DISCUSSION

As shown in Scheme 1, adenosine diphosphate (**1**) was treated with 2.0 equiv of zidovudine 5'-phosphoropiperidate and stavudine 5'-phosphoropiperidate in the presence of 6.0 equiv of DCI at 40 °C in DMF for 10 h. Ethanol precipitation followed by ion exchange chromatography afforded the desired Ap₃AZT and Ap₃d4T in over 50% yields. Due to the stability of adenosine diphosphate, the coupling reactions were performed at gradually elevated temperatures. As illustrated in Table 1, the reaction time for the synthesis of **2** and **3** was remarkably shortened to less than 10 h at higher temperatures (40 and 50 °C). However, when the reaction was performed at 50 °C, the amount of polyphosphate byproducts began to increase, thereby reducing the efficiency of the coupling reaction.

Figure 1. Synthesis of dinucleoside triphosphates (**2** and **3**). Reagents and conditions: (a) zidovudine 5'-phosphoropiperidate, DCI, DMF, 40 °C, 10 h; (b) stavudine 5'-phosphoropiperidate, DCI, DMF, 40 °C, 10 h

TABLE I. EFFECT OF TEMPERATURE ON THE SYNTHESIS OF **2** AND **3**

Entry	Temperature (°C)	Reaction Time (h)	Yield of Ap ₃ AZT (%)	Yield of Ap ₃ d4T (%)
1	20	24	46	45
2	30	18	50	49
3	40	10	52	53
4	50	6	47	45

IV. SUMMARY

In conclusion, an efficient method for the synthesis of asymmetrical dinucleoside triphosphates has been

developed in complementary to the reported approaches. The coupling of adenosine diphosphate with excess zidovudine and stavudine phosphoropiperidates in the

presence of DCI afforded Ap₃AZT and Ap₃d4T in good yields, respectively.

V. REFERENCES

- [1] A.G. McLennan, Boca Raton Vol. 43 (1992), p. 90.
- [2] A.G. McLennan, Pharmacol. Ther. Vol. 87 (2000), p. 68.
- [3] D. Clercq, Molecular Targets for Antiviral Agents. Vol. 297 (2000) p. 1
- [4] L.L. Kisselev, J. Justesen, A.D. Wolfson, L.Y. Frolova, FEBS Lett. Vol. 427 (1998), p. 157.
- [5] M.D. Baxi, J.K. Vishwanatha, J. Pharmacol. Vol. 58 (1995), p. 125.
- [6] O.I. Andreeva, E.V. Efimtseva, N.S. Padyukova, S.N. Kochetkov, S.N. Mikhailov, G.B. Dikson, M.Ya. Karpeiskii, Mol. Biol. Vol. 35 (2001), p. 846.
- [7] R. Yarchoan, H. Mitsuya, R.V. Thomas, J.M. Pluda, N.R. Hartman, C.F. Pemo, K.S. Marczyk, J.P. Allah, D.G. Job, S. Broder, Science Vol. 245 (1989), p. 414.
- [8] H. Mitsuya, K.J. Weinhold, P.A. Furman, M.H. St.Clair, Lehrman, S.N. Gallo, R.C. Bolognesi, D. Barry, D.W. Broder, P. Natl. Acad. Sci. Vol. 82 (1985), p. 7096.
- [9] Y.S. Skoblov, I.L. Karpenko, E.A. Shirokova, K.E. Popov, V.A. Andronova, G.V. Galegov, M.K. Kukhanova, Antiviral Res. Vol. 63 (2004), p. 107.
- [10] R. Yarchoan, H. Mitsuya, C.E. Myers, S. Broder, N. Engl. J. Med. Vol. 321 (1989), p. 728.
- [11] T.P. Cooley, L.M. Kunches, C.A. Saunders, J.K. Ritter, C.J. Perkins, C. McLaren, M.R.C. Path, R.P. McCaffrey, H.A. Liebman, N. Engl. J. Med. Vol. 322 (1990), p. 1340.
- [12] M.E. Theoclitou, T.S.H. El-Thaher, A.D.J. Miller, Chem. Common. Vol. 5 (1994), p. 659.
- [13] M.E. Theoclitou, E. Wittung, A. Hindley, T. Thaher, A.D.J. Miller, J. Chem. Soc. Perkin Trans. Vol. 16 (1996), p. 2009.