One-pot Synthesis of Diinosine Triphosphate and Tetraphosphate

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Abstract. An efficient one-pot protocol for the synthesis of symmetrical diinosine triphosphate (Ip_3I) and diinosine tetraphosphate (Ip_4I) from inosine 5′-phosphoropiperidate has been developed. The experimental results indicated that the employment of DCI activation could notably promote the reaction rate and yield.

Introduction

Inosine is the key nucleoside component and participates in the cellular functions and energy metabolism [1]. It has been reported that inosine exhibits radioprotective effect in animal models [2,3]. Moreover, inosine also has been proved to have immunomodulatory and neuroprotective effects [4,5]. Furthermore, the polyphosphates of inosine analogs with pharmaceutical activities have been of importance to the investigation of anticancer and antiviral drugs [6,7].

Compared to nucleoside triphosphates and tetraphosphates, symmetrical dinucleoside triphosphates and tetraphosphates are of better chemical and metabolism stability [8–10]. As a kind of potential pharmaceutical agents, symmetrical diinosine polyphosphates have been widely investigated in many other frontier fields including molecular biology, biochemistry, medicinal chemistry [11,12]. For instance, diinosine polyphosphates, such as Ip_3I and Ip_4I, have effects in the modulation of the intraocular pressure in normotensive New Zealand white rabbits.

So far, there are a few synthetic methods available for the preparation of dinucleoside triphosphates and tetraphosphates. The most widely used method is the condensation of nucleoside monophosphates and nucleoside diphosphates/ triphosphates with different activated reagents [13–15]. However, these methods are typically low yielding, and the products are difficult to isolate in high purity. More recently, we developed an efficient method for the synthesis of dinucleoside polyphosphates from the nucleoside phosphoropiperidate precursors [16]. In this paper, we report a one-pot and practical method for the synthesis of diinosine polyphosphates (Ip3I and Ip4I) without using inosine polyphosphates.

Experimental

Chemical reagents and solvents were obtained from commercial suppliers. Inosine 5′-phosphoropiperidate was prepared according to known procedures [16]. All reactions were performed under an atmosphere of dry argon. Ion exchange chromatography employed DEAE A-25 exchanger. IR spectra were recorded on a FT-IR spectrometer. All NMR spectra were obtained with a 400 MHz instrument with chemical shifts reported in parts per million (ppm, δ) and referenced to D_2O. Low-resolution mass spectra were obtained with an ion trap mass spectrometer and

reported as *m/z*.

P^1, P^3-Diinosine-5′,5′-triphosphate, Trisodium Salt (2)

To a solution of inosine 5′-phosphoropiperidate (41 mg, 0.1 mmol) in *N*-methylpyrrolidone (1.5 mL) were added bis (tetra-*n*-butylammonium) monophosphate (4 mg, 0.04 mmol) and DCI (35 mg, 0.3 mmol). The reaction was stirred at 20 °C for 24 h and concentrated in vacuo. The residue was dissolved in NaOAc aqueous solution (3 M, 0.5 mL) and EtOH (30 mL) was added. The resulting white precipitate was collected by centrifuge. The crude product was dissolved in deionized H_2O (1 mL) and loaded on a DEAE Sephadex A-25 ion exchange column (1.6 × 25 cm). Elution with NH_4HCO_3 buffer (linear gradient 0.3 to 0.6 M), combination of appropriate fractions, and lyophilization afforded Ip_3I in ammonium salt form. Passage of the solution of the ammonium salt in deionized H_2O through a bed of Dowex 50W-X8 ion exchange resin (Na^+ form) and lyophilization afforded **2** as trisodium salt, white solid (**2**, 47 mg, 57%); ^{1}H NMR (D_2O, 400 MHz): δ 8.12 (s, 2H), 7.97 (s, 2H), 5.84 (d, J = 5.2 Hz, 2H), 4.63 (dd, $J_1 = J_2$ = 5.2 Hz, 2H), 4.50 (dd, $J_1 = J_2$ = 4.4 Hz, 2H), 4.30–4.26 (m, 6H) ppm; ^{13}C NMR (D_2O, 100 MHz): δ 159.3, 154.3, 151.1, 136.9, 115.9, 87.1, 83.1, 73.9, 69.7, 64.7 ppm; ^{31}P NMR (D_2O, 162 MHz): δ 11.5 (d, J = 19 Hz, 2P), –23.2 (t, J = 19 Hz, 1P) ppm; IR (KBr): ν_{max} 3673, 3450, 2952, 2748, 2654, 2490, 2349, 1699, 1478, 1400, 1243, 1117, 1052, 850, 795, 723, 643, 511 cm^{-1}; LRMS (ESI–): *m/z* calcd for $C_{20}H_{24}N_8O_{18}P_3$ $[M–H]^-$ 757.0; found 757.1.

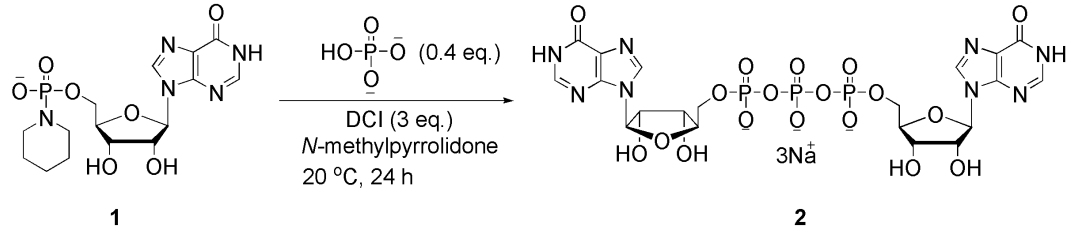
P^1, P^4-Diinosine-5′,5′-tetraphosphate, Tetrasodium Salt (3)

To a solution of inosine 5′-phosphoropiperidate (41 mg, 0.1 mmol) in *N*-methylpyrrolidone (1.5 mL) were added tris(tetra-*n*-butylammonium) hydrogen pyrophosphate (7 mg, 0.04 mmol) and DCI (35 mg, 0.3 mmol). The residue was dissolved in NaOAc aqueous solution (3 M, 0.5 mL) and EtOH (30 mL) was added. The resulting white precipitate was collected by centrifuge. The crude product was dissolved in deionized H_2O (1 mL) and loaded on a DEAE Sephadex A-25 ion exchange column (1.6 × 25 cm). Elution with NH_4HCO_3 buffer (linear gradient 0.3 to 0.6 M), combination of appropriate fractions, and lyophilization afforded Ip_4I in ammonium salt form. For characterization, passage of the solution of the ammonium salt in deionized H_2O through a bed of Dowex 50W-X8 ion exchange resin (Na^+ form) and lyophilization afforded **3** as tetrasodium salt, white solid (**3**, 32 mg, 34%); ^{1}H NMR (D_2O, 400 MHz): δ 8.13 (s, 2H), 7.98 (s, 2H), 5.83 (d, J = 5.2 Hz, 2H), 4.62 (dd, $J_1 = J_2$ = 5.2 Hz, 2H), 4.51 (dd, $J_1 = J_2$ = 4.4 Hz, 2H), 4.32–4.28 (m, 6H) ppm; ^{13}C NMR (D_2O, 100 MHz): δ 159.2, 154.6, 151.4, 136.7, 115.9, 87.2, 83.3, 73.7, 69.6, 64.7 ppm; ^{31}P NMR (D_2O, 162 MHz): δ –11.6 (d, J = 19 Hz, 2P), –23.4 (t, J = 19 Hz, 1P) ppm; IR (KBr): ν_{max} 3672, 3451, 2953, 2747, 2653, 2490, 2349, 1699, 1478, 1401, 1243, 1117, 1052, 857, 795, 721, 643, 510 cm^{-1}; LRMS (ESI–): *m/z* calcd for $C_{20}H_{25}N_8O_{21}P_4$ $[M–H]^-$ 837.0; found 837.1.

Results and Discussion

As shown in Scheme 1, symmetric diinosine triphosphate (2) was synthesized by treating inosine 5′-phosphoropiperidate (1) with 0.4 equiv of bis (tetra-*n*-butylammonium) monophosphate and 3.0 equiv of DCI at 20 °C in *N*-methylpyrrolidone for 24 h. Ethanol precipitation followed by ion exchange chromatography afforded Ip_3I in 57% yield. The effect of reaction temperature was

also tested for the formation of Ip_3I (Table 1). The reaction time could be significantly shortened with increasing temperature. The ^{31}P NMR spectra of the crude reaction mixture showed that the amount of nucleoside monophosphate and polyphosphate byproducts began to increase when the reaction temperature was elevated above 20 °C.



1 2

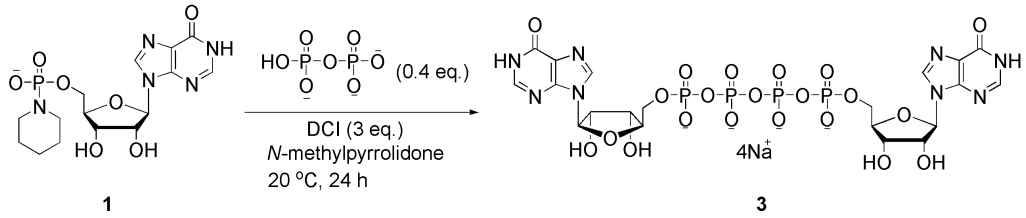
Scheme 1. One-pot synthesis of symmetrical diinosine triphosphate (Ip3I).

Table 1. The effect of temperature on the formation of Ip3I (2)

Entry	Temperature (°C)	Time (h)	Yield[a] (%)
1	10	48	55
2	20	24	57
3	30	18	45
4	40	12	31

[a] ^{31}P NMR yield

Similar to the synthesis of Ip_3I (**2**), treatment of inosine 5′-phosphoropiperidate (**1**) with 0.4 equiv of bis(tetra-*n*-butylammonium) hydrogen pyrophosphate and 3.0 equiv of DCI at 20 °C in *N*-methylpyrrolidone for 24 h followed by ion exchange chromatography afforded Ip_4I in 34% yield.



1 3

Scheme 2. Synthesis of symmetrical diinosine tetraphosphate (Ip4I).

Summary

In summary, a facile one-pot method has been developed for the synthesis of Ip_3I and Ip_4I. The synthesis of symmetrical dinucleoside polyphosphates based on nucleoside phosphoropiperidate /DCI system avoided the use of poorly soluble and expensive inosine polyphosphates reagents, and significantly simplified the synthesis of Ip_3I and Ip_4I.

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