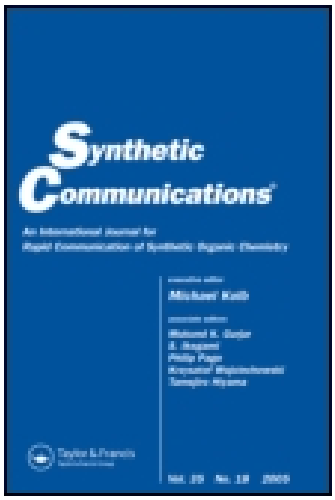

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Synthesis of Isonucleoside 5′-Triphosphates

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ABSTRACT

A series of isonucleoside 5′-triphosphates were synthesized via a rapid and efficient method. The structures of the triphosphates (**8a–8d**) were characterized by ^{31}P NMR and TOF-MS.

Key Words: Isonucleoside; Isonucleoside 5′-triphosphate; One-pot reaction.

Isonucleosides represent a class of nucleoside analogues in which the nucleobase is linked at various positions of ribose other than C-1′. It is believed that nucleoside inhibitors of HIV reverse transcriptase (AZT, ddI,

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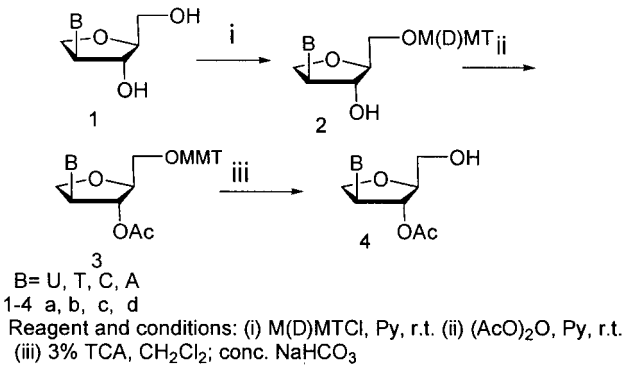
ddC, d4T, and 3TC) act as the nucleotides in vivo. We have synthesized isonucleoside triphosphates and found that some of these triphosphates show the inhibition to the DNA polymerase (unpublished data). We report here a convenient synthetic route to prepare the isonucleoside triphosphates.

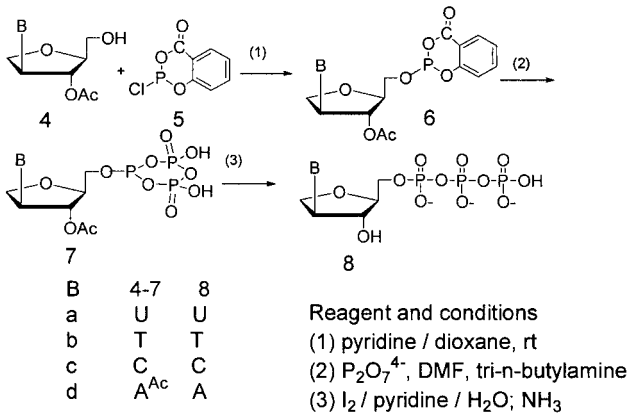
The isonucleosides were synthesized according to a published procedure[1] in our laboratory and the 3′-OH group was protected by a normal protecting method[2–4] in high yield (Sch. 1).

The protected isonucleosides were phosphorylated via a one-pot reaction,[5] as shown in Sch. 2.

3′-Protected isonucleoside **4** was reacted with reagent **5** in pyridine and dioxane at room temperature to give selectively an activated phosphite **6**



Scheme 1.



Scheme 2.

which was reacted with pyrophosphate to form the cyclic intermediate **7**. Compound **7** gives the corresponding isonucleoside triphosphate **8**. The advantage of this reaction is that protection of nucleobase for A, G, and C is not required and the reaction was carried out in "one-pot" with high yield.

EXPERIMENTAL

Preparation of the Protected Isonucleosides 3

General Procedure

Isonucleoside **1** (0.2 mmol) was dissolved in anhydrous pyridine (2 mL). In ice-water bath, MMTCl (for **1a** and **1b**) or DMTCl (for **1c** and **1d**) (0.24 mmol) was added. The mixture was kept stirring at room temperature. After 2 days, TLC showed that the reactant had converted completely. Anhydrous acetic anhydride (0.5 mL) was added. Then the mixture was kept stirring at room temperature. After 24 h, the solvent was evaporated and coevaporated with toluene. The residue was applied to a silica gel column which was pretreated with TEA (triethylamine) to avoid the hydrolysis of the DMT protecting group. The column was eluted by petroleum ether/acetone (10:1) for **3a**, **3b** and CH_2Cl_2/CH_3OH (50:1) for **3c**, **3d**, yielding 85–92%.

3a: ^{1}H NMR (300 MHz, DMSO-d_6) $\delta = 11.33$ (s 1H N-H); 7.60 (d $J_{5,6} = 7.8$ 1H H-6); 7.29 (m 12H Ph-H); 6.89 (d 2H Ph-H); 5.49 (d $J = 7.8$ 1H H-5); 5.18 (m 1H H-2′); 4.87 (m 1H H-3′); 4.09 (m 2H H-1′); 3.92 (d 1H H-4′); 3.74 (s 3H H-OCH_3); 3.30 (m 2H H-5′); 2.01 (s 3H $COCH_3$).

3b: ^{1}H NMR (300 MHz, DMSO-d_6) $\delta = 11.31$ (s 1H N-H); 7.59 (s 1H H-6); 7.29 (m 12H Ph-H); 6.89 (d 2H Ph-H); 5.20 (m 1H H-2′); 4.85 (m 1H H-3′); 4.02 (m 2H H-1′); 3.81 (m 1H H-4′); 3.72 (s 3H H-OCH_3); 3.68 (m 2H H-5′); 2.03 (s 3H $COCH_3$); 1.77 (s 3H 5-CH_3).

3c: ^{1}H NMR (300 MHz, DMSO-d_6) $\delta = 7.84$ (d $J_{5,6} = 5.4$ 1H 6-H); 7.44 (d $J = 7.8$ 2H Ph-H); 7.20–7.30 (m 7H Ph-H); 6.86 (d $J = 8.7$ 4H Ph-H); 6.87 (b 2H 4-NH_2); 6.09 (d $J_{6,5} = 5.7$ 1H 5-H); 5.19 (m 1H 2′-H); 4.04 (m 1H 3′-H); 3.87 (m 1H 1′-H); 3.72 (s 6H $2 \times OCH_3$); 3.15 (m 1H 5′-H); 2.07 (s 3H -$COCH_3$); 2.03 (s 3H $COCH_3$).

3d: ^{1}H NMR (300 MHz, DMSO-d_6) $\delta = 10.72$ (s 1H N-H); 8.58 (s 1H H-2); 8.46 (s 1H H-8); 7.18–7.39 (m 9H Ph-H); 6.85 (d $J = 6.9$ 4H Ph-H); 5.53 (t $J = 4.2$ 1H H-2′); 5.26 (m 1H H-3′); 4.48 (m 1H H-1′); 4.27 (m 1H H-1′); 4.08 (m 1H H-4′); 3.73 (s 6H $2 \times OCH_3$); 3.26 (m 2H 5′-H); 2.26 (s 3H -$COCH_3$); 2.02 (s 3H $COCH_3$).

Preparation of the Protected Isonucleosides 4

General Procedure

The protected isonucleoside **3** (1.5 mmol) was dissolved in 3% trifluoroacetic acid/CH_2Cl_2. The solution turn red immediately. The mixture was stirring at room temperature. After 10 min, TLC showed the reactant was disappeared. Concentrated $NaHCO_3$ was added to neutralize the reaction mixture. Then the solvent was evaporated and the residue was applied to a silica gel column which was eluted by CH_2Cl_2/CH_3OH (50:1–20:1) to get compound **4** (80–95%).

4a: ^{1}H NMR (300 MHz, DMSO-d_6) $\delta = 11.30$ (s 1H N-H); 7.68 (d 1H H-6); 5.60 (d 1H H-5); 5.13 (m 1H H-2′); 5.08 (t 1H 5′-OH); 4.93 (m 1H H-3′); 4.03 (m 2H H-1′); 3.81 (m 1H H-4′); 3.64 (m 2H H-5′); 2.04 (s 3H–$COCH_3$).

4b: ^{1}H NMR (300 MHz, DMSO-d_6) $\delta = 11.31$ (s 1H N-H); 7.59 (s 1H H-6); 5.16 (m 2H H-2′ 5′-OH); 4.97 (m 1H H-3′); 4.02 (m 2H H-1′); 3.81 (m 1H H-4′); 3.68 (m 2H H-5′); 2.04 (s 3H $COCH_3$); 1.776 (s 3H 5-CH_3).

4c: ^{1}H NMR (300 MHz, DMSO-d_6) $\delta = 7.82$ (d 1H H-6); 6.89 (s 2H -NH_2); 6.81 (d 1H H-5); 5.20 (m 1H H-2′); 5.08 (m 1H H-3′); 4.89 (t 1H 5′-OH); 4.02 (d 1H H-4′); 3.80 (m 2H H-1′); 3.51 (m 2H H-5′); 2.06 (s 3H $COCH_3$).

4d: ^{1}H NMR (300 MHz, DMSO-d_6) $\delta = 8.65$ (s 1H H-2); 8.53 (s 1H H-8); 8.19 (s 1H N-H); 5.33 (m 1H H-2′); 5.26 (m 1H-H-3′); 4.24 (m 3H H-1′, 5′-OH); 3.67 (m 2H 5′-H); 2.27 (s 3H -$COCH_3$); 2.07 (s 3H -OCH_3)

Preparation of Isonucleoside Triphosphates 8

General Procedure

The protected nucleoside **4** (100 μmol) was dissolved in anhydrous pyridine (2 mL) and evaporated to dryness in vacuum. The residue was dried further over P_2O_5 under vacuum for more than 1 h under room temperature. The reaction flask was filled with argon and the reaction was taken place under the atmosphere of argon. Compound **4** was dissolved into anhydrous pyridine (100 μL) and anhydrous dioxane (300 μL). A freshly prepared 1 M solution of **5** in anhydrous dioxane (300 μL) was then added into the well-stirred solution of the nucleoside. A white precipitate was formed. After 10 min, a mixture of a 0.5 M solution of *bis*(tri-*n*-butylamine)pyrophosphate in anhydrous DMF (300 μL) and tri-*n*-butylamine (100 μL) was quickly added. The white precipitate imme-

diately dissolved and the reaction mixture was stirred for 10 min at room temperature. Oxidation was completed by adding a solution of 1% iodine in pyridine/water (98/2, v/v) (2 mL, 157 μmol). After 15 min, the excess of iodine was removed by a 5% aqueous solution of $NaHSO_3$ and the reaction solution was evaporated to dryness. The residue was dissolved in water (10 mL) and allowed to stand at room temperature for 30 min then concentrated ammonia (20 mL) was added. After 1 h, the solution was evaporated to dryness. The residue was dissolved in water, and the solution applied to a DEAE Sephadex-A25 column (1.5 × 15 cm) which was eluted with a liner gradient of 500 mL of 0.005 M and 500 mL of 0.3 M TEAB (triethylammonium bicarbonate). Fractions containing product were combined and evaporated to dryness on a rotary evaporator and the residue was coevaporated with methanol to remove trace of buffer to yield 60–75% of triethylammonium isonucleoside 5′-triphosphates **8**.

The product was purified by HPLC which showed a purity of 97.06–99.50% (area%). Reverse-phase HPLC was formed with a Delta PAK C18 (7.8 × 300 nm, 100 Å) column which was eluted with 100 nM TEAB, pH 7.5, containing a linear gradient of acetonitrile from 0 to 15% in 20 min. The pure product was formed as a triethylammonium salt and then converted into sodium salt by using a cation-exchange chromatography D-72 resin (Na^+ form).

The structures of the triphosphates were confirmed by ^{1}H NMR, ^{31}P NMR, and ESI-TOF-MS (or MALDI-TOF-MS) spectra. ^{31}P NMR spectra of the isonucleoside triphosphates indicates three resonance at around −6, −9, and −20 ppm (in D_2O relative to H_3PO_4 external standard), corresponding to the γ, α and β phosphorus atoms, respectively.[6] Chemical shift values for these products are highly pH and counterion dependent. ^{1}H NMR data we report here are consisted with the salt of isonucleoside 5′-triphosphate **8**.

8a: $[\alpha]_D^{25} = 8.15$ (4.56 mg/mL, H_2O) ^{1}H NMR (300 MHz, D_2O) $\delta = 7.63$ (d $J_{5,6} = 7.86$ 1H H-6); 5.77 (d $J_{6,5} = 7.8$ 1H H-5); 4.83 (m 1H H-2′); 4.30 (m 1H H-3′); 3.99 (m 2H H-1′); 3.93 (m 1H H-4′); 3.37 (m 2H H-5′). ^{31}P NMR (121.41 MHz, D_2O) $\delta = -4.5$ (d 1P γP); −9.5 (αP); −20.2 (βP), MALDI-TOF-MS Calcd.: 468.14, Found: 467.32 (M − 1).

8b: $[\alpha]_D^{25} = 10.14$ (4.25 mg/mL, H_2O) ^{1}H NMR (300 Hz, D_2O) $\delta = 7.43$ (s 1H H-6); 4.87 (m 1H H-3′); 4.33 (t 1H H-4′); 4.07 (m 2H H-2′), 3.86 (m 2H H-6′), 1.75 (s 3H 5-CH_3). ^{31}P NMR (121.41 MHz, D_2O) $\delta = -7.4$ (d $J_{\gamma,\beta} = 30$ 1P γP), −9.3 (d $J_{\alpha,\beta} = 30$ 1P αP), −20.7 (t 1P βP), ESI-TOF-MS *m/z* Calcd.: 482.17, Found: 481.07 (M − 1), 503.06 (M − 1H + Na − 1), 525.02 (M − 2H + 2Na − 1), 547.00 (M − 3H + 3Na − 1).

8c: $[\alpha]_D^{25} = 6.43$ (5.36 mg/mL, H_2O) ^{1}H NMR (300 MHz, D_2O) δ 7.21 (d $J_{5,6} = 6.4$ 1H H-6); 6.18 (d $J_{6,5} = 6.4$ 1H H-5); 5.14 (s 1H H-2′); 4.27

(m 1H H-3′); 3.95–4.09 (m 5H H-1′, H-4′, H-5′). ^{31}P NMR (121.41 MHz, D_2O) $\delta = -7.8$ (d $J_{\gamma,\beta} = 42$ 1P γP); −9.2 (d $J_{\alpha,\beta} = 40$ 1P αP); −20.8 (t 1P βP), ESI-TOF-MS Calcd.: 467.16, Found: 466.05 (M 1).

8d: $[\alpha]_D^{25} = 10.10$ (6.21 mg/mL, H_2O) ^{1}H NMR (300 MHz, D_2O) $\delta = 8.18$ (s 1H H-2); 8.04 (s 1H H-8); 4.90 (m 1H H-2′); 4.60 (m 1H H-3′); 4.24 (m 2H H-1′); 4.08 (m 2H H-5′); 3.95 (m 1H H-4′). ^{31}P NMR (121.41 MHz, D_2O) $\delta = -4.4$ (d $J_{\gamma,\beta} = 48.0$ 1P γP); −8.9 (d $J_{\alpha,\beta} = 52.8$ 1P αP); −19.8 (m 1P βP), ESI-TOF-MS Calcd.: 491.18, Found: 490.11 (M − 1).

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