

Synthesis and Novel Properties of Alkyl Thiophosphoramidate Derivatives of Nucleosides

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Novel alkyl thiophosphoramidate derivatives of nucleoside analogues (**5**) have been prepared by phosphochloridothioate chemistry. *O*-Isopropyl 2', 3'-*O*-isopropylidene uridine-5'-yl *N*-thiophosphoryl threonine and serine methyl esters (**5a** and **5b**) underwent the intramolecular catalyzed hydrolysis reaction.

Keywords synthesis, alkyl thiophosphoramidate, nucleoside analogue, hydrolysis reaction, mechanism

Introduction

The development of nucleoside prodrugs capable of undergoing intracellular activation to the corresponding nucleotide has become an area of intense interest.¹ Several purine and pyrimidine bases and nucleoside analogs are important weapons in the anticancer and antiviral chemotherapeutic arsenal. The biological activity of most of these analogs requires intracellular metabolism to 5'-mononucleotides by kinase-mediated phosphorylation.² In order to overcome the problem of drug resistance and improve membrane penetration, a series of amino acid phosphoramidate di- and tri-esters of nucleotides have been developed to deliver phosphorylated nucleoside analogs as neutral derivatives into the cell.³ They have shown promise as potent antiviral agents, since in some cases they have exhibited enhancing antiviral activity and reducing cytotoxicity when compared to the parent nucleo-

side.^{4,5}

The relative metabolic stability of nucleoside-5'-phosphorothioates is well-documented. For instance, AMP-S is relatively resistant to enzymatic transformations by adenylate deaminase, adenylate kinase and 5'-nucleotidase,^{6,7} and ATP- α -S diastereoisomers exhibit selective metabolic stability.⁸ In addition to their relative metabolic stability, phosphorothioates are also characterized by their higher acidity relative to phosphates.

In this paper, the syntheses of alkyl thiophosphoramidates derivatives of nucleosides (**5**) and the novel properties of the synthesized compounds were reported.

Results and discussion

The target compounds were synthesized as shown in Scheme 1. *O*-Isopropyl phosphorodichloridothioate (**1**) was used as a starting material. The key step was the coupling of nucleosides or their analogues with alkyl methoxyalaninyl thiophosphorochloridate (**3**) to form a new conjugated compounds **5**.⁹

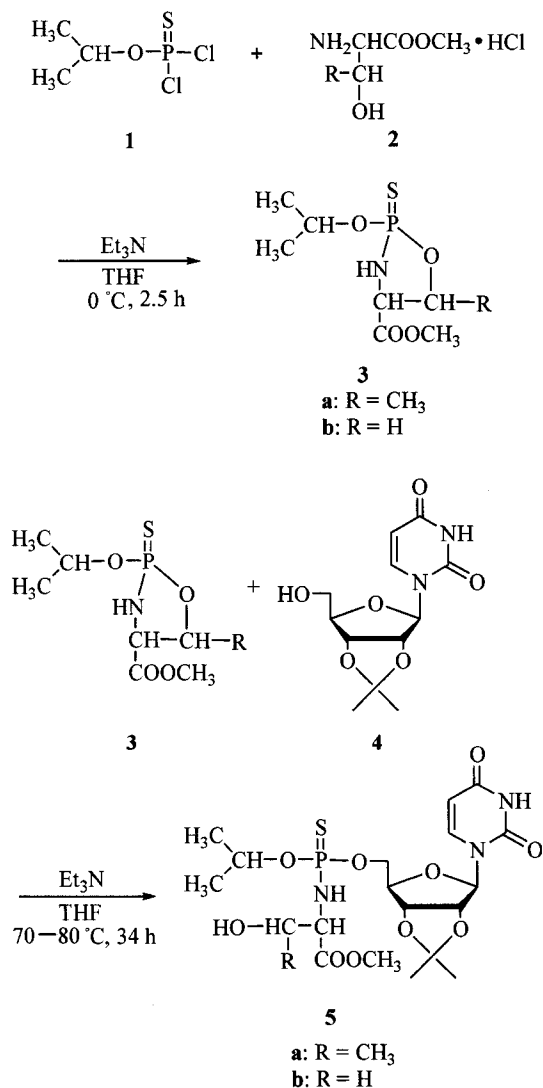
Reaction of amino acid methyl ester hydrochloride (**2**) with compound **1** was performed at 0 °C under nitrogen atmosphere (Scheme 1). Triethylamine was added via syringe to the stirring solution. The reaction was monitored by ³¹P NMR spectroscopy. It was found that compound **1** with a ³¹P NMR shift at δ 56.47 was transferred

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Received October 25, 2001; revised and accepted January 22, 2002.

Project supported by the National Natural Science Foundation of China (Nos. 29902003 and 39870415), the Ministry of Science and Technology, the Chinese Ministry of Education and Doctor Candidate Innovation Foundation in Tsinghua University (No. 092430305).

Scheme 1



into **3** which has a ^{31}P NMR signal at about δ 85 within approximately 2 h. Then a solution of 2',3'-*O*-isopropylidene uridine (**4**) and triethylamine in THF was added to the reaction solution (Scheme 1). After reacting for 34 h at 70–80 °C, the reaction mixture was filtered and concentrated *in vacuo*. The residue was dissolved in chloroform and washed with subsequently hydrochloric acid solution (1 mol/L), saturated sodium bicarbonate solution and water. The organic phase was dried (MgSO_4) and evaporated under vacuum, and the residue was purified by chromatography on a silica column by elution with 5% methanol in chloroform. Pooling and evaporation of appropriate fractions gave the product **5** in 81%–91% yields. Alkyl thiophosphoramidate derivatives of nucleoside (**5**) were obtained as a mixture of diastereoisomers due to the

chirality at the phosphorus center. Hence the ^{31}P NMR chemical shifts appeared as a pair of peaks at about δ 61.

Formation of **5a** was traced by ^{31}P NMR spectra shown in Fig. 1. ^{31}P NMR of the starting material **1** in THF shows at δ 56.47. After the solution of compound **2** and triethylamine was added to the solution of **1**, the peak at ^{31}P NMR δ 56.47 disappeared in 2.5 h with a pair of new peaks at ^{31}P NMR δ 85.42 and δ 85.10 corresponding to compound **3a** emerged. The threonine hydroxyl group was activated by Et_3N to increase its nucleophilicity to elevate the phosphorus into the penta-coordinated transition state. There was no attempt to isolate the reaction intermediate **3a**. However, according to the ESI/MS/ESI-MS spectra, **3a** existed m/z ($[\mathbf{3a} + \text{Na}^+]$, 276) (Fig. 2). Compound **3a** was considered as 2-isopropoxyl-3-methyl-5-methoxycarbonyl-1,3,2-oxazathiophosphorine-2-oxide. In comparison, **3b** was 2-isopropoxyl-5-methoxycarbonyl-1,3,2-oxazathiophosphorine-2-oxide.

When nucleoside **4** was added, the double peaks appeared at δ 61.40 and δ 60.56 corresponding to compound **5a**. After studying for 34 h only a pair of peaks at about ^{31}P NMR δ 61 were observed. Triethylamine acted as a catalytic reagent besides capturing hydrochloride produced in the reactions.

Compared with the other *O*-isopropyl 2',3'-*O*-isopropylidene uridine-5'-yl *N*-thiophosphoryl amino acid methyl ester which are stable compounds under basic conditions, it was interesting to find that 2',3'-*O*-isopropylidene uridine-5'-(*O*-isopropyl methoxythronenyl thiophosphoate) (**5a**) and 2',3'-*O*-isopropylidene uridine-5'-(*O*-isopropyl methoxyserinyl thiophosphoate) (**5b**) underwent thiophosphoryl transfer reaction once they were formed.

Compound **5a** (0.1 mmol) was incubated with triethylamine (1 mmol) and water (1 mmol) in acetonitrile (0.5 mL) at 60 °C. The progress of reaction was monitored by ^{31}P NMR spectra (Fig. 3). The starting material **5a** (δ 61.40 and 60.56) was slowly transferred into **7a** (δ 20.05) and **8** (δ 5.07). As the reaction continued, **8** reached its maximum within 36 h. After reaction, **8** and 2',3'-*O*-isopropylidene uridine were obtained by TLC (coated with silica gel) in almost quantitative yields. Similar result was also observed when the reaction was applied to **5b**.

Based on these results, the mechanism for the uracil intramolecularly catalytic dethiophosphorylation of compound **5a** was proposed in Scheme 2. There are two pairs

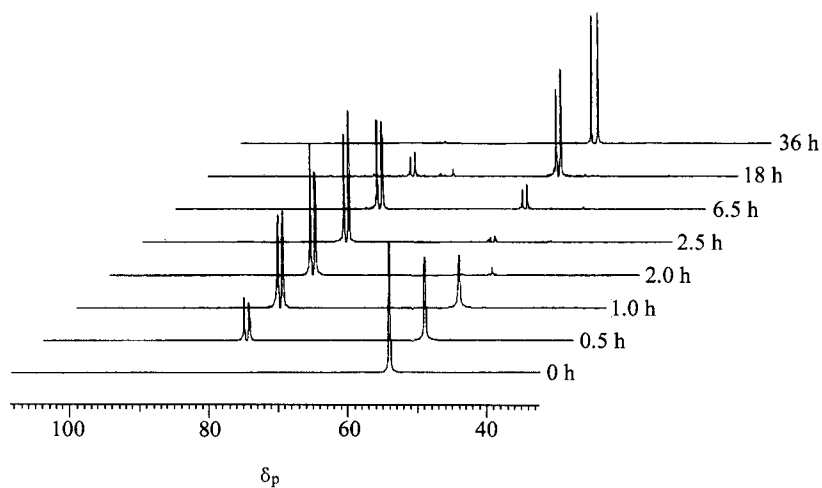


Fig. 1 Stack ^{31}P NMR spectra of formation of compound **5a**.

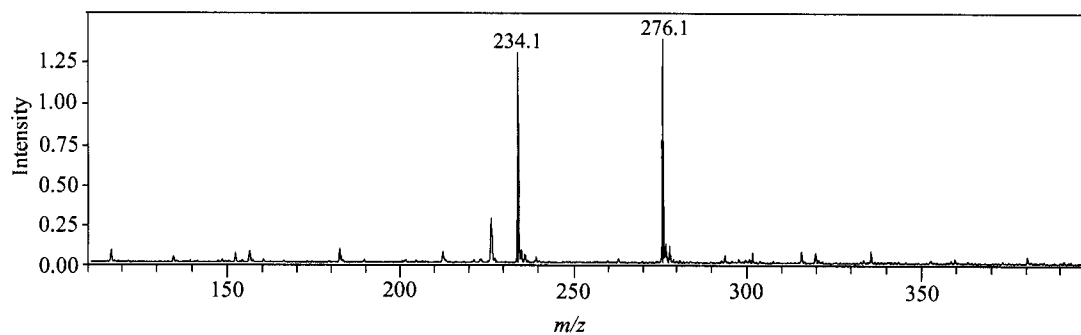


Fig. 2 Positive ion ESI mass spectrum of intermediate **3a**.

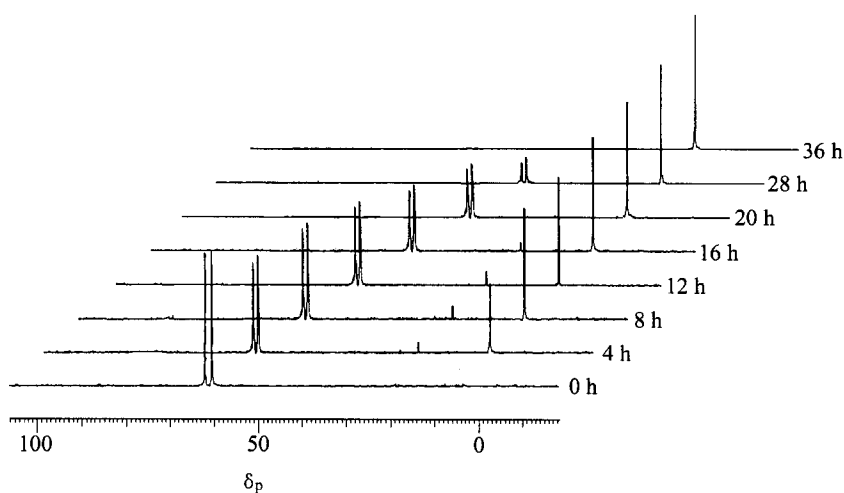
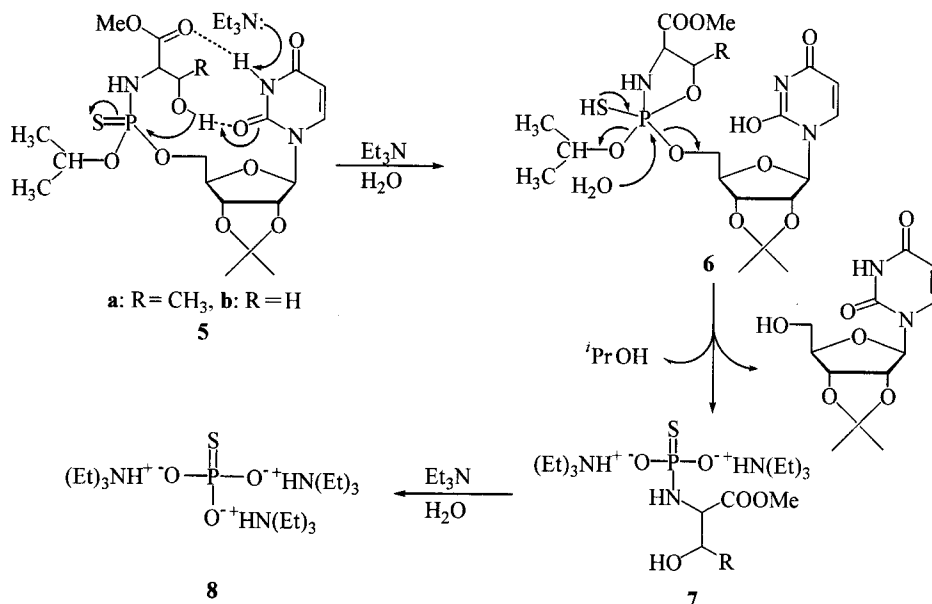


Fig. 3 Stack ^{31}P NMR spectra of hydrolysis reaction.

Scheme 2 Proposed mechanism of thiophosphoryl transfer reaction of compounds **5**

of hydrogen bonds between carbonyl and hydroxyl groups of threonine with uridine base. The threonine carbonyl group as the hydrogen acceptor and uracil base as hydrogen donors form the first hydrogen bond. While the second hydrogen bond, with the uracil base as the hydrogen acceptor and β -OH of threonine as the hydrogen donor, is the catalytic site. The threonine hydroxyl group was activated by 2-carbonyl group of uracil to increase its nucleophilicity to elevate the phosphorus into the penta-coordinated transition state which in turn would be hydrolyzed by water to release the nucleoside **4** and isopropanol. For compound **5b**, the similar reaction mechanism was proposed.

Experimental

General information

All glassware was dried in an oven for at least 3 h at 120 °C prior to use. Air sensitive materials were transferred under a nitrogen atmosphere. THF and triethylamine were dried over Na and CaH₂ respectively. ¹H NMR and ¹³C NMR spectra were recorded on a Bruker AM 500 spectrometer. TMS (δ 0.0) and CDCl₃ (δ 77.0) were references for ¹H NMR and ¹³C NMR spectra respectively. ¹³C NMR spectra were all taken under ¹H decoupled and ³¹P coupled conditions. ³¹P NMR spectra were taken on a Bruker AC 200 spectrometer at 81 MHz under

¹H decoupled conditions. ³¹P NMR chemical shifts were reported in δ downfield (+) or upfield (-) from external 85% H₃PO₄ as reference. Mass spectra were conducted on a Bruker Esquire-LC mass spectrometer operated in positive and negative ion mode.

Synthesis of amino acid methyl ester hydrochloride (**2**) and protected nucleoside (**4**)

The preparation of **2** and protected nucleoside **4** were carried out according to the literature.^{10,11} All physical constants and spectroscopies data of the products synthesized were in agreement with the literature.

General procedure for the syntheses of alkyl thiophosphoramidate derivatives of nucleosides (**5**)

A solution of triethylamine (1.4 mL, 1.0 g, 10.0 mmol) in THF (10 mL) was added dropwise with vigorous stirring to a solution of amino acid methyl ester hydrochloride (**2**) (5.02 mmol) and *O*-isopropyl phosphorodichloridothioate (**1**) (0.97 g, 5.02 mmol) in THF (10 mL) at -5 °C over a period of 15 min. The reaction mixture was slowly warmed to ambient temperature with stirring over 2 h, and the solvent was then removed in vacuum. The residue was treated with THF (15 mL), the mixture was filtered, and the filtrate was evaporated in vacuum to yield the product **3** as a colorless oil (5.02

mmol, 100%). A solution of nucleoside **4** (5.02 mmol) was dissolved in THF (10 mL), and alkyl methoxyalaninyl thiophosphorochloridate (**3**) (5.02 mmol) and triethylamine (0.7 mL, 0.5 g, 5.02 mmol) was added with vigorous stirring. After the reaction mixture reacted for 34 h at 70–80 °C the solvent was removed under vacuum. The residue was dissolved in chloroform (10 mL) and washed subsequently with 1 mol/L hydrochloric acid solution (2 × 15 mL), saturated sodium bicarbonate solution (2 × 10 mL), and water (3 × 15 mL). The organic phase was dried (MgSO₄) and evaporated under vacuum, and the residue was purified by chromatography on silica by elution with 5% methanol in chloroform. Pooling and evaporation of appropriate fractions gave the product **5** as foam.

5a (diastereoisomers) MeOH:CHCl₃ (1:20) as eluent ($R_f = 0.82$ for TLC), 2.20 g (yield 81.7%); ³¹P NMR (CDCl₃, 200 MHz) δ: 58.46, 57.90; ¹H NMR (CDCl₃, 500 MHz) δ: 9.56 (br, 1H, H-3), 7.88, 7.87 (dd, ³J = 5 Hz, ³J = 4.5 Hz, 1H, H-6), 5.81–5.97 (m, 2H, H-1', 5), 4.91–5.05 (m, 2H, H-2', 3'), 4.58–4.68 (m, 1H, OCHMe₂), 4.14–4.30 (m, 3H, H-4', OH, H-β), 4.00–4.08 (m, 2H, H-5'), 3.82–3.96 (m, 1H, P-NH), 3.65–3.73 (m, 4H, OCH₃, H-α), 1.61 (s, 3H, CH₃), 1.42 (s, 3H, CH₃), 1.10–1.30 [m, 9H, OCH(CH₃)₂, γ-CH₃]; ¹³C NMR (CDCl₃, 500 MHz) δ: 174.27 (COOMe), 163.13 (C-4), 151.28 (C-2), 144.36, 144.32 (C-6), 118.40, 117.59 (>CMe₂), 107.62, 107.43 (C-5), 98.76, 98.41 (C-1'), 86.23 (C-4'), 85.71, 85.63 (C-2'), 82.70, 82.58 (C-3'), 71.93 (OCH(CH₃)₂), 68.19 (C-β), 65.44 (C-5'), 56.87 (OCH₃), 50.73 (C-α), 26.53 (CH₃), 25.85 (CH₃), 23.68 (CH₃), 20.11 (C-γ); ESI-MS (positive) m/z : 538 (M + H)⁺; ESI-MS (negative) m/z : 536 (M - H)⁻.

5b (diastereoisomers) MeOH:CHCl₃ (1:20) as eluent ($R_f = 0.78$ for TLC), 2.24 g (yield 85.2%); ³¹P NMR (CDCl₃, 200 MHz) δ: 61.33, 60.56; ¹H NMR (CDCl₃, 500 MHz) δ: 11.42 (br, 1H, H-3), 7.59, 7.58 (d, ³J = 4.5 Hz, H-6), 5.74–5.90 (m, 2H, H-1', 5), 4.83–5.03 (m, 3H, H-2', 3', OH), 4.60–4.72 (m, 1H, OCHMe₂), 4.38–4.46 (m, 1H, H-4'), 3.98–4.04 (m, 2H, H-5'), 3.85–3.92 (m, 1H, P-NH), 3.63 (s, 3H, OCH₃), 3.53–3.58 (m, 1H, H-α), 3.44–3.50 (m, 2H, H-β), 1.53 (s, 3H, CH₃), 1.33 (s,

3H, CH₃), 1.30 (m, 6H, OCH(CH₃)₂); ¹³C NMR (CDCl₃, 500 MHz) δ: 171.25 (COOMe), 169.02 (C-4), 149.99 (C-2), 145.39, 145.36 (C-6), 117.02, 116.96 (>CMe₂), 104.33, 104.22 (C-5), 95.83, 95.16 (C-1'), 87.97 (C-4'), 87.20, 87.13 (C-2'), 83.64, 83.51 (C-3'), 72.44, 72.41 (OCH(CH₃)₂), 67.24 (C-5'), 63.10 (C-β), 55.09 (OCH₃), 45.80, 45.73 (C-α), 28.63 (CH₃), 26.86 (CH₃), 23.36 (CH₃); ESI-MS (positive) m/z : 524 (M + H)⁺; ESI-MS (negative) m/z : 522 (M - H)⁻.

Conclusion

Alkylthiophosphoramidates derivatives of nucleosides have been synthesized, and the direct interaction between the intramolecular uracil and 5'-serine and threonine residues was observed. We proposed the mechanism of the thiophosphoryl transfer reaction. This reaction was derived by the simultaneously existence of two pairs of hydrogen bonds. The loss of any pair of the hydrogen bonds will result into the diminishing of the self-cleavage reaction of the threonine-5'-nucleotide conjugates.

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(E0110254 SONG, J. P.; ZHAO, X. J.)