Studies on Synthesis and Intramolecular Catalyzed Hydrolysis of Thiophosphoramidate Derivatives of Nucleoside†

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Novel thiophosphoramidate derivatives of nucleoside analogue as membrane-soluble prodrugs of the bioactive free nucleotides have been prepared by thiophosphorylation reaction. 2′,3′-*O*, *O*′-Isopropylidene uridine-5′-yl *N*-thiophosphoryl serine and threonine methyl esters underwent the intramolecular catalyzed hydrolysis reaction.

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

Introduction

Nucleosides and their analogues as potential anticancer and antivirus agents have been studied extensively. Most of the currently licensed antiviral drugs and many of the drugs used in anticancer chemotherapy are nucleoside analogues.[1] For the majority of these analogues shown biological activities, the action of a kinase to form the 5′-monophosphate is required.[2-6] The latter is often metabolized further before the active compound is formed.[7]

Nucleotides themselves do not usually penetrate cells at a sufficient rate to show any significant chemotherapeutic effect.[8] 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.[9-11] They have shown promise as potent antiviral agents, since in some cases they have exhibited enhanced antiviral activity and reduced cytotoxicity compared to the parent nucleoside.

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,[12,13] and ATP-α-*S* diastereoisomers exhibit selective metabolic stability.[14] In addition to their relative metabolic stability, phosphorothioates are also characterized by their higher acidity relative to phosphates.[15] In this paper, we wish to report the synthesis of thiophosphoramidate derivatives of nucleosides, and their novel properties.

Results and discussion

The target compounds were synthesized as shown in Scheme 1. Thiophosphoryl chloride (**1**) was used as a starting material. The key step was the coupling of nucleosides with methoxyamino acid thiophosphorodichloridate (**3**) to form new compound **5**.

Reaction of amino acid methyl ester hydrochloride (**2**) with thiophosphoryl chloride (**1**) was performed at 0 ℃ under nitrogen atmosphere (Scheme 1). Triethylamine

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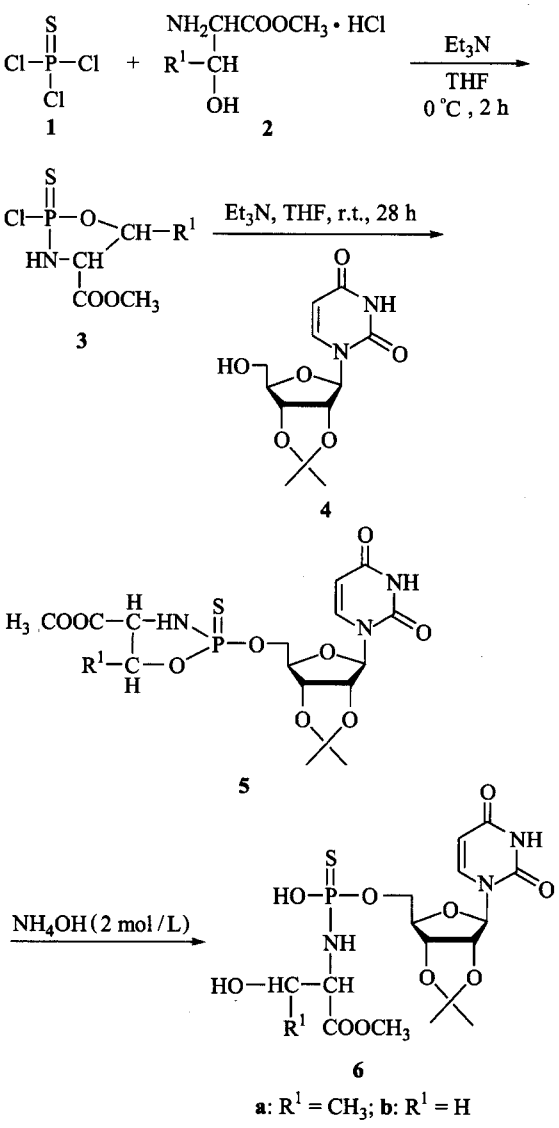
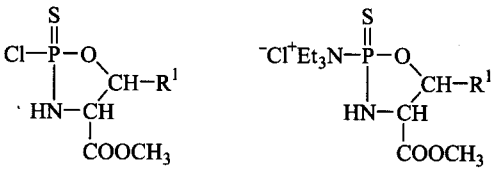
Scheme 1



1 2 3 4 5 6

a: $R^1 = CH_3$; **b**: $R^1 = H$

was added via syringe to the stirred solution. The reaction was monitored by ^{31}P NMR spectroscopy. It was found that thiophosphoryl chloride (**1**) with a ^{31}P NMR shift at δ 31.34 was converted into **3** and **3'** in two hours. Then a solution of 2',3'-*O*,*O'*-isopropylidene uridine (**4**) and triethylamine in THF was added to the reaction solution. The solution was stirred overnight, filtered and concentrated *in vacuo*. After hydrolysis of the crude product in NH_4OH, nucleoside 5'-thiophosphoramidates (**6**) were obtained in 73%—82% yields by chromatography on silica-gel. Being the existence of β-OH of threonine, the threonine hydroxyl group was activated by Et_3N to increase its nucleophility to elevate the phosphorus into the five membered circle transition state.



3 3'

Formation of **5a** was traced by ^{31}P NMR spectroscopy shown in Fig. 1. After the addition of the solution of amino acid methyl ester hydrochloride (**2**) and triethylamine to the solution of (**1**), the peak at ^{31}P NMR δ 31.34 disappeared in two hours and two new peaks at ^{31}P NMR δ 41.30, δ 75.71 emerged, corresponding to compounds **3** and **3'**.[15,16]

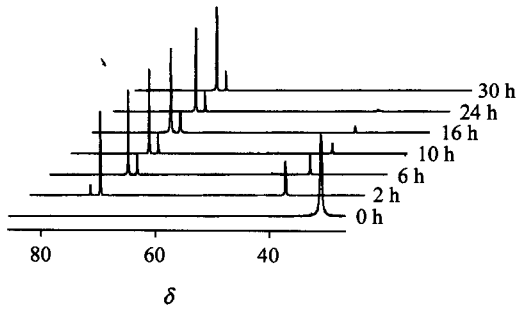


Fig. 1 The stack ^{31}P NMR spectra of formation of compound **5a**.

When nucleoside **4** was added, the double peaks at δ 72.73 and δ 71.05 appeared corresponding to compound **5a**. After 28 h only a pair of peaks at about ^{31}P NMR δ 72 were observed. 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 72. Triethylamine acted as a catalytic reagent besides capturing hydrochloride produced in the reaction.

Compared with the other 2',3'-*O*,*O'*-isopropylidene uridine-5'-yl *N*-thiophosphoryl amino acid methyl esters which are stable compounds under basic conditions, it was interesting to find that 2',3'-*O*,*O'*-isopropylidene uridine-5'-yl *N*-thiophosphoryl threonine and serine methyl esters **5a** and **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 ℃. The progress of reaction was monitored by ^{31}P NMR spectra (Fig. 2). The starting material **5a** (δ_p 72.73, 71.05) was slowly converted into **6a** (δ_p

56.82, 56.13). After 4 h, there were two new peaks at ^{31}P NMR δ 44.31 and δ 3.14 emerged, corresponding to **8a** and **9**.[17] As the reaction continued, **9** reached its maximum within 24 h. After reaction, threonine methyl ester and 2′,3′-*O*,*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**.

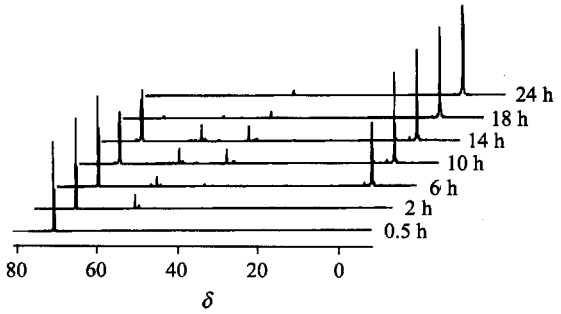


Fig. 2 The stack ^{31}P NMR spectra of thiophosphoryl transfer reaction.

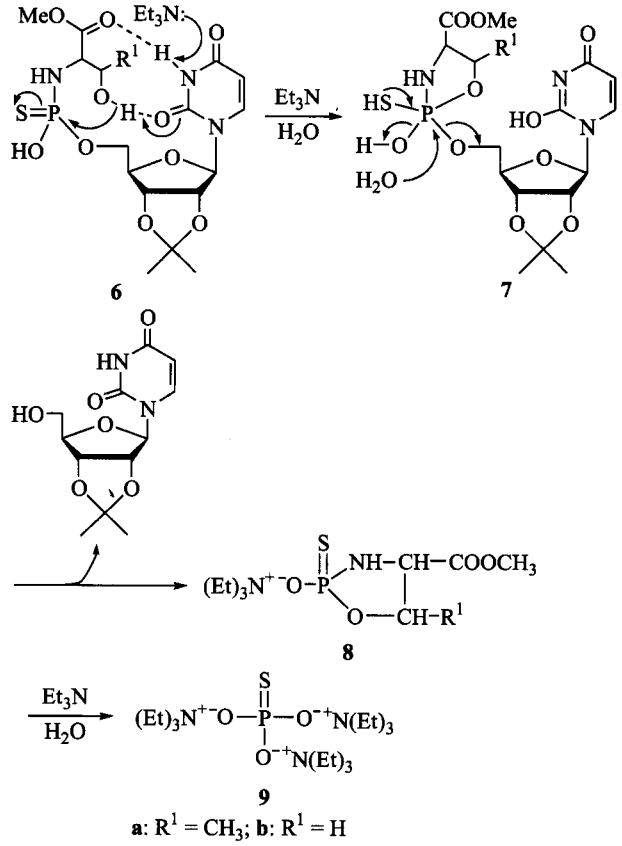
Based on these results, the mechanism for the intramolecularly catalytic dethiophosphorylation of compound **6a** was proposed in Scheme 2. There are two pairs of hydrogen bonds between carbonyl and hydroxyl groups of threonine with uridine base. The first hydrogen bond with the threonine carbonyl group as the hydrogen acceptor and uracil base as hydrogen donor is the binding site. While the second hydrogen bond, with the uracil base as the hydrogen acceptor, 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 pentacoordinated transition state which in turn would be hydrolyzed by water to release the nucleoside **4** isopropanol. For compound **6b**, the similar reaction mechanism was proposed.

Experimental

General information

All glassware was dried in an oven for at least 3 h at 120 ℃ prior to use. Air sensitive materials were transferred under a nitrogen atmosphere. THF and triethylamine were dried over Na and CaH_2 respectively. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker

Scheme 2 The proposed mechanism of thiophosphoryl transfer reaction of compound **6**



a: $R^1 = CH_3$; **b**: $R^1 = H$

AM 500 spectrometer. TMS ($\delta = 0.0$) and D_2O ($\delta = 4.80$) were references for ^1H and ^{13}C NMR spectra respectively. ^{13}C NMR spectra were all taken under ^1H decoupled and ^{31}P coupled conditions. ^{31}P NMR spectra were taken on a Bruker AC 200 spectrometer at 81 MHz under ^1H decoupled conditions. ^{31}P NMR chemical shifts are reported downfield (+) or upfield (−) from external 85% H_3PO_4 as reference. Mass spectra were conducted on a Bruker Esquire-LC mass spectrometer operated in positive and negative ion mode. All evaporations were carried out on a rotary evaporator under reduced pressure. Reaction temperatures were measured externally. Yields refer to chromatographically and spectroscopically pure compounds.

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

The preparation of amino acid methyl ester hydrochloride (**2**) and protected nucleoside (**4**) were carried out according to the reported methods.[18,19] All physi-

cal constants and spectroscopic data of the products synthesized were in agreement with those in the literature.

General procedure for synthesis of thiophosphoramidate derivatives of nucleosides (**6**)

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 thiophosphoryl chloride (**1**, 0.85 g, 5.02 mmol) in THF (10 mL) at 0 ℃ 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 vacuo*. The residue was treated with THF (15 mL), the mixture was filtered, and the filtrate was evaporated *in vacuo* to yield the products **3** and **3'** as colorless oil (5.02 mmol, 100%). A solution of 2',3'-*O*,*O'*-isopropylidene nucleoside (**4**, 5.02 mmol) and triethylamine (0.7 mL, 5.02 mmol) in THF (10 mL) was added and the reaction mixture was stirred overnight, filtered and concentrated *in vacuo*. After hydrolysis of the crude product in NH_4OH, the residue was purified by column chromatography on silica (200—300 mesh, 60 g) with elution by 2-propanol-NH_4OH-H_2O. Pooling and evaporation of appropriate fractions gave the product **6** as foam.

2',3'-*O*,*O'*-*Isopropylidene uridine* 5'-[*methoxythreonenyl thiophosphate*] (**6a**)

6a (diastereoisomers) 2-Propanol-NH_4OH-H_2O (33:1:1) as eluent (R_f = 0.51 for TLC). 1.83 g (yield 73.6%). ^{31}P NMR (81 MHz, D_2O) δ: 56.82, 56.13; ^1H NMR (500 MHz, D_2O) δ: 7.88, 7.87 (d, ^3J = 5 Hz, 1H, H-6), 5.86—5.92 (m, 2H, H-1', 5), 4.96—5.00 (m, 2H, H-2',3'), 4.14—4.30 (m, 2H, H-4', H-β), 4.00—4.08 (m, 2H, H-5'), 3.79—3.83 (m, 1H, H-α), 3.61—3.68 (m, 3H, OCH_3), 1.61 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 1.10—1.20 (m, 3H, γ-CH_3); ^{13}C NMR (125 MHz, D_2O) δ: 174.27 (**C**OOMe), 163.13 (C-4), 151.28 (C-2), 144.36, 144.32 (C-6), 118.40, 117.59 (>**C**Me_2), 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'), 68.19 (C-β), 65.44 (C-5'), 56.87 (OCH_3), 50.73 (C-α), 26.53 (CH_3), 25.85 (CH_3), 20.11 (C-γ); IR (KBr) ν: 3588, 3320, 2950, 1750, 1685, 1579 cm^{-1}; ESI-MS (pos.) *m/z*: 496 $(M+H)^+$; ESI-MS (neg.) *m/z*: 494 $(M-H)^-$; HRMS (FAB) *m/z* calcd for $C_{17}H_{26}N_3O_{10}PS$ $(M+H)^+$ 496.4463, found 496.4468.

2',3'-*O*,*O'*-*Isopropylidene uridine* 5'-[*methoxyserinyl thiophosphate*] (**6b**)

6b (diastereoisomers) 2-Propanol-NH_4OH-H_2O (33:1:1) as eluent (R_f = 0.47 for TLC). 1.97 g (yield 81.7%). ^{31}P NMR (81 MHz, D_2O) δ: 58.74, 58.06; ^1H NMR (500 MHz, D_2O) δ: 7.59, 7.58 (d, ^3J = 4.5 Hz, 1H, H-6), 5.79—5.85 (m, 2H, H-1', 5), 4.90—4.95 (m, 2H, H-2',3'), 4.38—4.46 (m, 1H, H-4'), 3.98—4.01 (m, 2H, H-5'), 3.63 (s, 3H, OCH_3), 3.55—3.59 (m, 1H, H-α), 3.48—3.54 (m, 2H, H-β), 1.53 (s, 3H, CH_3), 1.33 (s, 3H, CH_3); ^{13}C NMR (125 MHz, D_2O) δ: 171.25 (**C**OOMe), 169.02 (C-4), 149.99 (C-2), 145.39, 145.36 (C-6), 117.02, 116.96 (>**C**Me_2), 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'), 67.24 (C-5'), 63.10 (C-β), 55.09 (OCH_3), 45.80, 45.73 (C-α), 28.63 (CH_3), 26.86 (CH_3); IR (KBr) ν: 3600, 3295, 2880, 1775, 1665, 1544 cm^{-1}; ESI-MS (pos.) *m/z*: 482 $(M+H)^+$; ESI-MS (neg.) *m/z*: 480 $(M-H)^-$; HRMS (FAB) *m/z* calcd for $C_{16}H_{24}N_3O_{10}PS$ $(M+H)^+$ 482.4195, found 482.4197.

Conclusion

Thiophosphoramidate derivatives of nucleosides (**6**) have been synthesized, and the direct interaction between the intramolecular uracil and 5'-serine and threonine residues was observed. The possible mechanism of the thiophosphoryl transfer reaction was proposed. This reaction was derived by the simultaneous existence of two pairs of hydrogen bonds. The loss of any pair of the hydrogen bonds will result in the diminishing of the self-cleavage reaction of the threonine-5'-nucleotide conjugates.

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