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## Synthesis of Serine/Alanine Conjugated 3',5'-TpT

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### ABSTRACT

Serine and alanine phosphoramidates conjugates of 3',5'-TpT **4**, **5** were synthesized. The corresponding serine phosphoamidate possesses some unique properties due to the presence of the side chain hydroxyl group.

*Key Words:* Dithymidine; Amino acid; Conjugate; Phosphoramidate.

### INTRODUCTION

Amino acids phosphoramidate derivatives of some biologically active nucleosides have been studied as nucleoside prodrugs, which can be used as effective chemotherapeutic agents against cancer and viral diseases.<sup>[1–3]</sup> Among this kind of compounds, dinucleoside phosphoamidates are of potential interest since the dinucleotide analogs could exhibit modified biological activity vs. their parent nucleoside phosphates.<sup>[4]</sup> In this paper, Serine and alanine phosphoramidates of 3',5'-dithymidine (TpT) were synthesized. This kind of compound could be used as a model to study the nucleoside-releasing properties of nucleosides/nucleotides-amino acids phosphoamidates conjugates.

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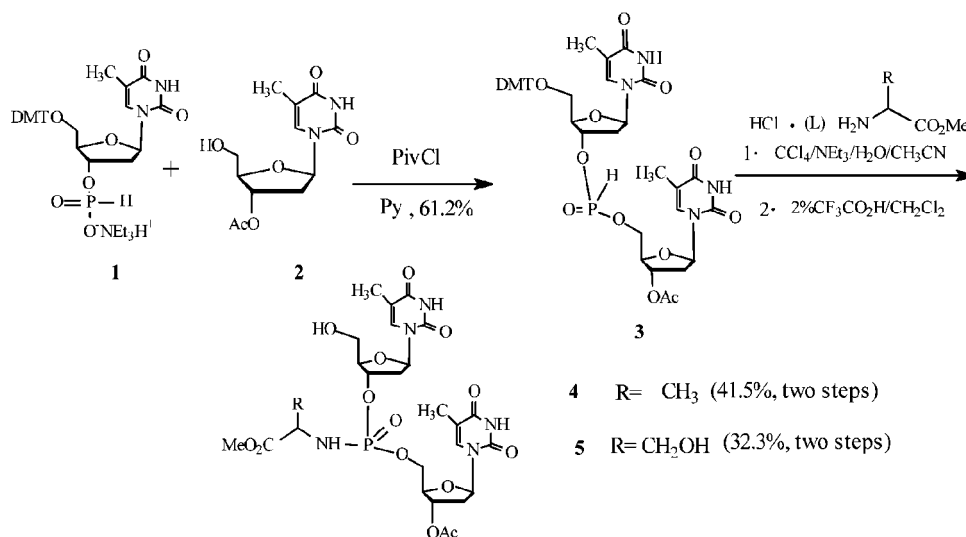


## RESULTS AND DISCUSSION

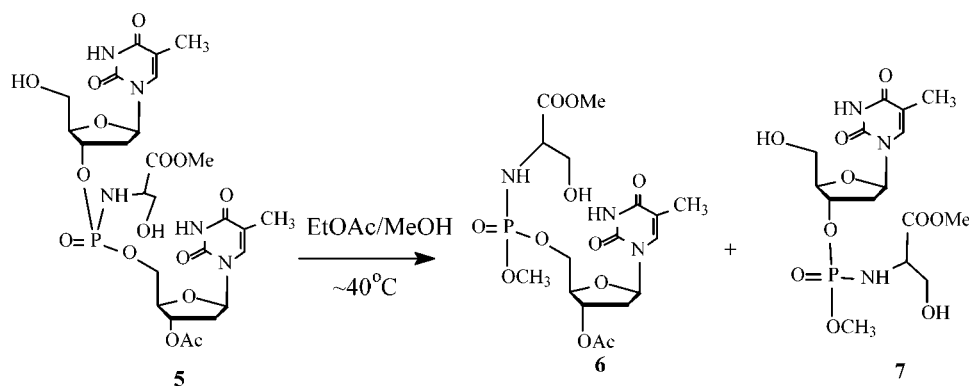
The synthetic strategy for the preparation of 3',5'-dithymidine phosphoramidates **4**, **5** consisted of two steps: (i) the coupling of **1**<sup>[5]</sup> and **2** with pivaloyl chloride (PivCl) in anhydrous pyridine to produce the H-phosphonate diester **3** and (ii) its conversion into the desired phosphoramidates via oxidative coupling with the corresponding L-amino acid methyl esters (Sch. 1). Since the serine conjugated 3',5'-TpT (compound **5**) was sensitive to basic conditions, the amount of NEt<sub>3</sub> used in step (ii) must be strictly controlled, usually two equivalent of NEt<sub>3</sub> was enough. The DMT group was removed with acid at the final stage.

Besides being sensitive to basic conditions, the serine-derivative **5** also exhibit unique properties compared with its alanine counterpart **4**. For instance, after purification with column chromatography eluted with EtOAc/MeOH, two byproducts **6** and **7** were easily produced during the solution of **5** was concentrated, especially when the temperature of water bath is about 40°C or higher (Sch. 2). However, the alanine derivative **4** was entirely stable under the same purification conditions. This ester exchange side reaction could be effectively suppressed by carefully controlling the water bath temperature below 20°C.

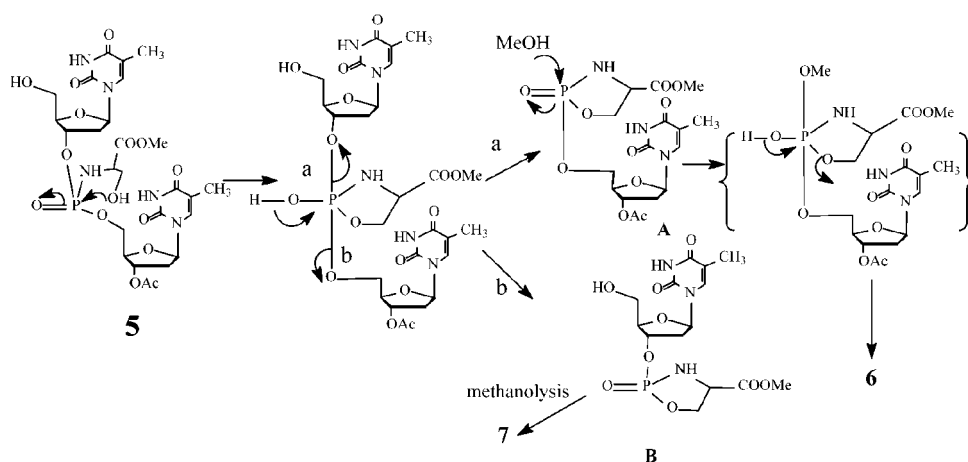
For compound **5**, its sensitivity to basic conditions and lability to methanol could be attributed to the presence of the serine side chain hydroxyl group. A mechanism for the ester exchange of this compound was proposed as follows (Sch. 3). The serine side chain hydroxyl group acted as the intramolecular catalyst to catalyze the methanolysis of **5** through intermediates **A**, **B** to yield compounds **6** and **7** respectively. To our knowledge, Shabarova et al. used a similar cyclic phosphoramidate intermediate to explain the instability of mononucleotidyl 5'-N serine esters to acid treatment<sup>[6]</sup>, here, we further demonstrated the serine hydroxyl group



Scheme 1. Synthesis of 3',5'-dithymidine phosphoramidates.



**Scheme 2.** Ester exchange side reaction of compound **5**.



**Scheme 3.** Mechanism of ester exchange of compound **5**.

also instabilizing the corresponding dinucleotide conjugate even at neutral conditions.

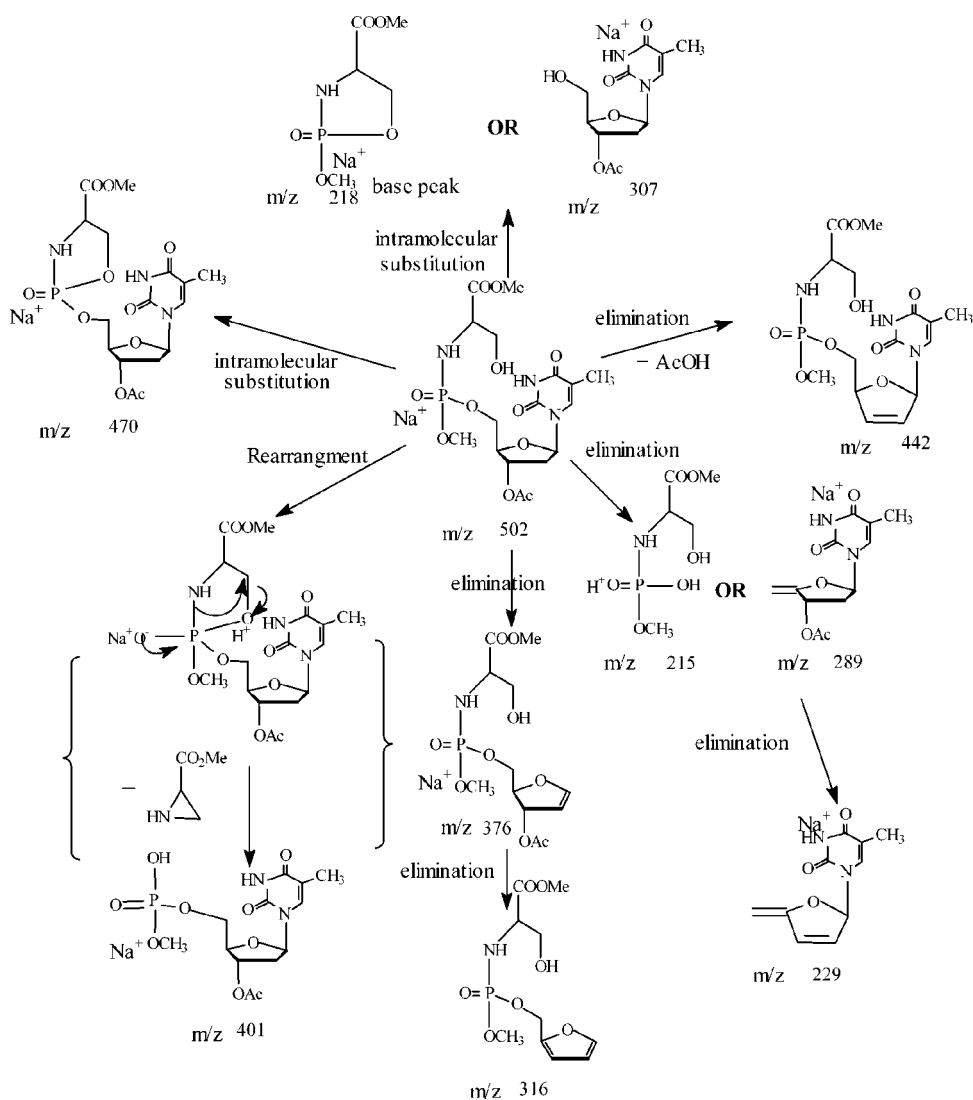
The structures of compounds **6** and **7** were easily deduced from their multiple stage electrospray ionization mass spectrometry (ESI-MS/MS, Table 1). Nearly every daughter ion originated from the  $[M + Na]^+$  could be elucidated by the rules we had found about serine conjugated nucleotide phosphoramidates.<sup>[7]</sup> The fragmentation pathway of compounds **6** and **7** were showed in Schs. 4 and 5. Their structures were further confirmed by HRMS.<sup>[8]</sup>

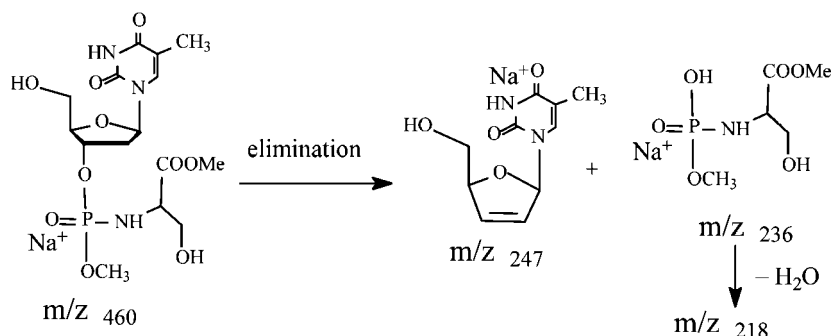
In Sch. 4, through intramolecular substitution, a pair of complementary ions were observed at  $m/z$  307 and  $m/z$  218, and the last one was the base peak. The ion at  $m/z$  401 corresponding to  $[M + Na - 101]^+$  is the consequence of rearrangement. It was generated via the loss of a neutral fragment with a formular weight of 101 which might be a cyclic three membered aza compound. The formation of some



**Table 1.** ESI MS/MS spectra of compounds **6**, **7**.

Compounds	Precursor ions	Fragment ions and relative intensity percentage (in parentheses)
<b>6</b> (FW = 479)	502	502 (12), 470 (43), 442 (16), 401(62), 376 (4), 341 (5), 316 (58), 307 (63), 289 (54), 284 (16), 275 (6), 247(8), 229 (11), 218 (100), 215 (29), 204 (43)
<b>7</b> (FW = 437)	460	460 (13), 247 (100), 236 (3), 218 (2)

**Scheme 4.** Fragmentation pathway of sodium adduct of **6**.



**Scheme 5.** Fragmentation pathway of sodium adduct of **7**.

other ions, such as  $m/z$  442,  $m/z$  376,  $m/z$  316,  $m/z$  289,  $m/z$  229,  $m/z$  215, could be explained through elimination processes. Similarly, ions originated from sodium adduct **7** could also be explained through elimination pathway (Sch. 5).

In conclusion, the serine and alanine conjugated 3',5'-TpT **4**, **5** were synthesized. The methanolysis of **5** gave products **6** and **7**, which could be characterized by ESI-MS/MS and HRMS. The alanine conjugated 3',5'-TpT **4**, however, was stable under the same conditions. This unique property of compound **5** was due to the presence of the side chain hydroxyl group which can attack the phosphorus atom to form a cyclic pentacoordinated phosphorane intermediate. These discoveries might also provide some clue to the biological function of serine in protein-nucleic acid interactions.

## EXPERIMENTAL

All reactions were monitored by TLC on silica gel GF<sub>254</sub>. Column chromatography was performed using silica gel H<sub>60</sub>. NMR spectra were recorded (internal standard tetramethylsilane) with a Bruker ARX-400 type spectrometer using CDCl<sub>3</sub> or CD<sub>3</sub>COCD<sub>3</sub> as the solvent. The mass spectra were obtained using a Bruker Esquire-LC ion-trap mass spectrometer. HRMS spectra were taken with Bruker APEX II FT-ICRMS.

**O-(5'-dimethoxytrityl-2'-deoxythmidin-3'-yl)-O'-(3'-acetyl-2'-deoxythymidin-5'-yl)hydrogenphosphonate (3).** 560 mg (0.79 mmol) 5'-O-dimethoxytritylthymidine 3'-phosphonate **1** and 210 mg (0.76 mmol) 3'-O-acetylthymidine **2** were dissolved in anhydrous pyridine and coevaporated twice. The residue was then dissolved in 15 mL anhydrous pyridine, 700  $\mu\text{L}$  PivCl (5.6 mmol) was added dropwise, the reaction mixture was stirred at room temperature for 20 min; after addition of a few drops of water, the solvent was removed under reduced pressure. The residue



was dissolved in 25 mL EtOAc and washed with saturated NaCl solution; the organic phase was separated and dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>. The crude product was purified with column chromatography (EtOAc/MeOH 50:1–20:1). After concentration, the product was obtained as white foam (61.2%). <sup>31</sup>P-NMR (acetone): δ 10.42, 9.11 ppm J<sub>P-H</sub> 723 Hz (mixture of diastereoisomers) <sup>1</sup>H-NMR(CD<sub>3</sub>COCD<sub>3</sub>): δ 10.26–10.27 (m, 4H, <sup>3</sup>N-H), 7.94 (1H, P-H, J<sub>P-H</sub> = 720 Hz), 6.88–7.62 (m, 30H, H-6 and Ar-H), 6.30–6.42 (m, 4H, H-1'), 6.140–6.149 (1H, P-H, J<sub>P-H</sub> = 720 Hz), 5.26–5.45 (m, 4H, H-3'), 4.32–4.42 (m, 8H, H-4' and H-5'), 3.45–3.50 (m, 4H, H-5'), 2.39–2.66 (m, 8H, H-2'), 2.08 (s, 6H, CH<sub>3</sub>CO), 1.84–1.87 (m, 6H, C<sub>5</sub>-CH<sub>3</sub>), 1.45–1.48 (m, 6H, C<sub>5</sub>-CH<sub>3</sub>). ESI MS: 897[M + Na]<sup>+</sup>.

**O-(5'-dimethoxytrityl-2'-deoxythmidin-3'-yl)-O'-(3'-acetyl-2'-deoxythymidin-5'-yl) N-alanine methyl ester phosphoramidate (4).** 20 mg (0.12 mmol) L-Alanine methyl ester hydrochloride was mixed with 50 μL H<sub>2</sub>O, 30 μL NEt<sub>3</sub> (0.21 mmol) and 0.5 mL CH<sub>3</sub>CN, 90 mg **3** in 1 mL CH<sub>3</sub>CN was added dropwise. The reaction mixture was stirred at room temperature for 20 min and concentrated to dryness. The residue was firstly purified with a short column, and then treated with 2% TFA/EtOAc, after neutralized with a few drops of saturated NaHCO<sub>3</sub>, the solution was concentrated under reduced pressure. The crude product was purified with column chromatography (EtOAc/MeOH 15:1). After concentration, the product was obtained as syrup (41.5%, two steps). <sup>31</sup>P-NMR (acetone): δ 8.86, 8.41 ppm (mixture of diastereoisomers) <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 8.39–8.46 (m, 2H, <sup>3</sup>N-H), 7.33–7.42 (m, 2H, H-6), 6.11–6.22 (m, 2H, H-1'), 5.31–5.33 (m, 1H, Ha-3'), 5.12–5.15 (m, 1H, H<sub>b</sub>-3'), 4.23–4.28 (m, 2H, Ha-5'), 4.16–4.18 (m, 2H, H<sub>b</sub>-5'), 3.92–3.98 (m, 1H, α-CH), 3.85–3.88 (m, 2H, H-4'), 3.74 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 3.60–3.62 (m, 1H, NH), 2.28–2.55 (m, 4H, H-2'), 2.093–2.097 (2s, 3H, CH<sub>3</sub>CO), 1.90–1.94 (m, 7H, 2 × C<sub>5</sub>-CH<sub>3</sub>, -OH), 1.40–1.42 (m, 3H, CH<sub>3</sub>) ESI-MS: 674[M + H]<sup>+</sup>, 696[M + Na]<sup>+</sup> HRMS: found [M + H]<sup>+</sup>, 674.2059 C<sub>26</sub>H<sub>37</sub>N<sub>5</sub>O<sub>14</sub>P requires M + H, 674.2068.

**O-(5'-dimethoxytrityl-2'-deoxythmidin-3'-yl)-O'-(3'-acetyl-2'-deoxythymidin-5'-yl) N-serine methyl ester phosphoramidate (5).** Compound (**5**) was obtained as described for compound **4** (32.3%, two steps). <sup>31</sup>P-NMR (acetone): δ 9.71, 9.33 ppm (mixture of two diastereoisomers) <sup>1</sup>H-NMR(CD<sub>3</sub>COCD<sub>3</sub>): 10.01 (s, 2H, <sup>3</sup>N<sub>a</sub>-H), 8.01 (s, 2H, <sup>3</sup>N<sub>b</sub>-H), 7.67–7.79 (m, 4H, H-6), 6.28–6.33 (m, 4H, H-1'), 4.82–5.39 (m, 4H, H-3'), 4.13–4.57 (m, 10H, H-5', α-CH), 3.67–4.09 (m, 20H, H-4', CO<sub>2</sub>CH<sub>3</sub>, CH<sub>2</sub>OH(Ser), NH(Ser), -OH), 2.20–2.58 (m, 8H, H-2'), 2.072–2.083 (2s, 6H, CH<sub>3</sub>CO), 1.80–1.87 (m, 12H, 4 × C<sub>5</sub>-CH<sub>3</sub>) ESI-MS: 712[M + Na]<sup>+</sup> HRMS: found [M + H]<sup>+</sup>, 690.2024 C<sub>26</sub>H<sub>37</sub>N<sub>5</sub>O<sub>15</sub>P requires M + H, 690.2018.

## ACKNOWLEDGMENTS

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8. HR ESI-MS of compounds **6** and **7**: For compound **6**, HRMS: found  $[M + H]^+$ , 480.1375  $C_{17}H_{27}N_3O_{11}P$  requires  $M + H$ , 480.1377 For compound **7**, HRMS: found  $[M + H]^+$ , 438.1267  $C_{15}H_{25}N_3O_{10}P$  requires  $M + H$ , 438.1272.

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