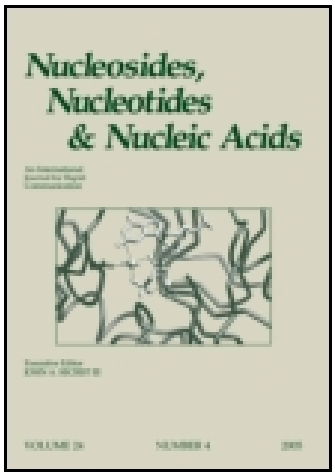

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn20

Synthesis of Serine/Alanine Conjugated 3′,5′-TpT

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Published online: 07 Feb 2007.

To cite this article: Zhi-Hui Qin , Chang-Xue Lin , Yong Ju & Yu-Fen Zhao (2003) Synthesis of Serine/Alanine Conjugated 3′,5′-TpT, Nucleosides, Nucleotides and Nucleic Acids, 22:1, 63-69, DOI: 10.1081/NCN-120018623

To link to this article: http://dx.doi.org/10.1081/NCN-120018623

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, No. 1, pp. 63–69, 2003

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 chemotheraputic agents against cancer and viral diseases.[1–3] 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.[4] 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 nuclesides/nucleotides-amino acids phosphoamidates conjugates.

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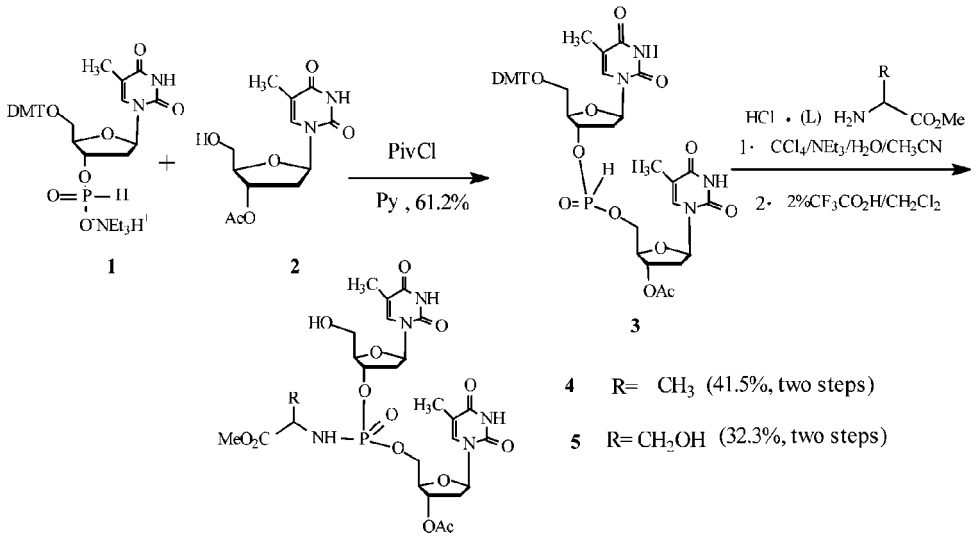
DOI: 10.1081/NCN-120018623
1525-7770 (Print); 1532-2335 (Online)

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**[5] 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_3 used in step (ii) must be strictly controlled, usually two equivalent of NEt_3 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[6], here, we further demonstrated the serine hydroxyl group



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

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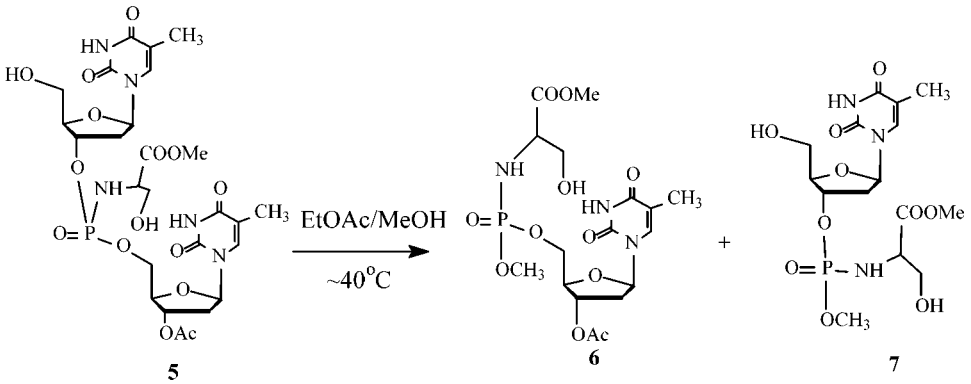
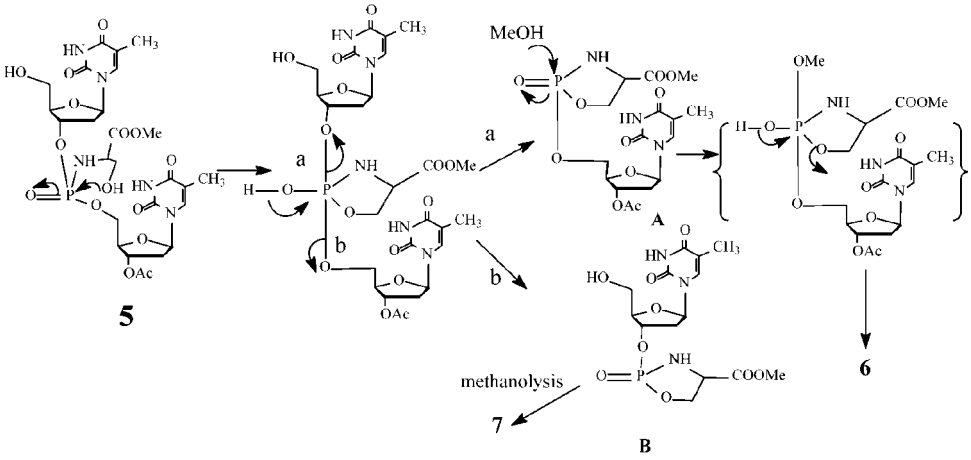


Scheme 2. Ester exchange side reaction of compound **5**.



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

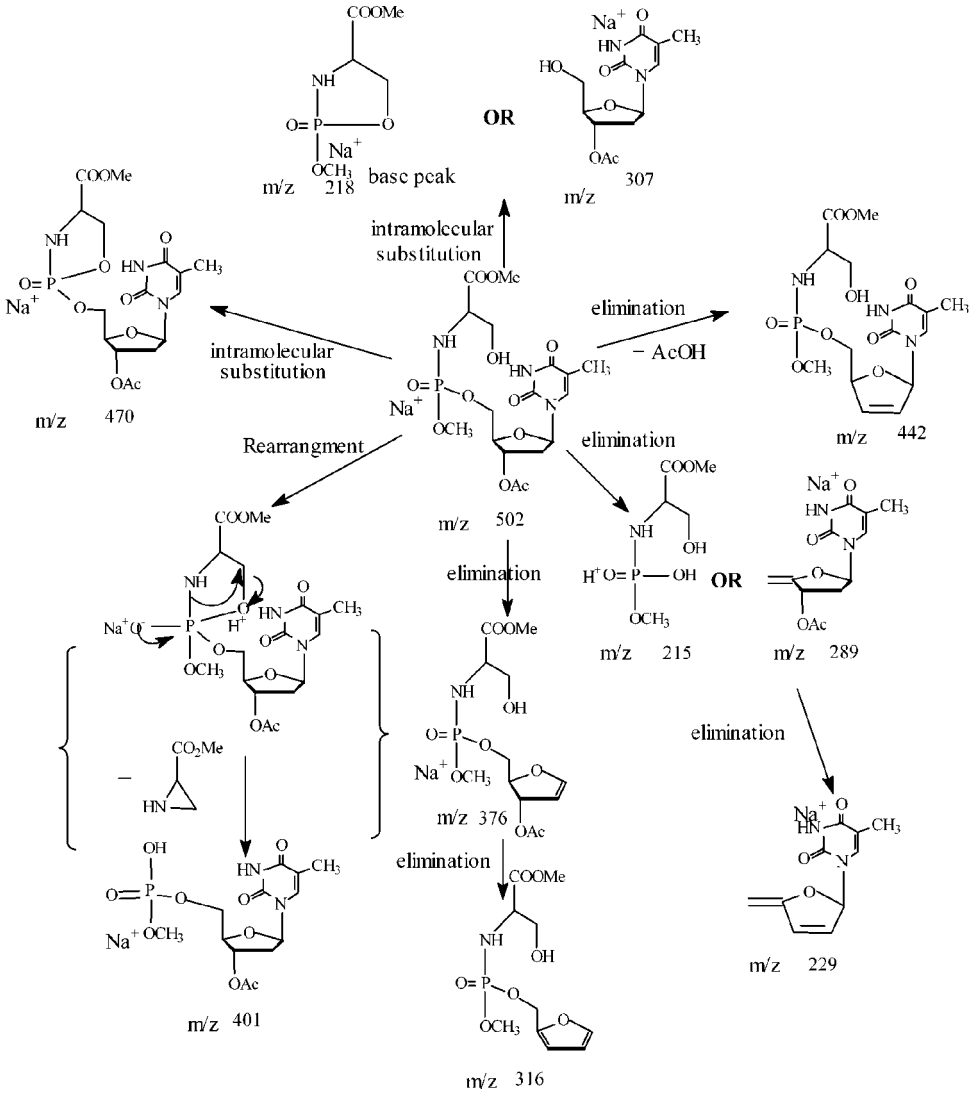
also instabilizing the corresponding dinucleotide conjugate even at neutral conditions.

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.[7] The fragmentation pathway of compounds **6** and **7** were showed in Schs. 4 and 5. Their structures were further confirmed by HRMS.[8]

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)
6 (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)
7 (FW = 437)	460	460 (13), 247 (100), 236 (3), 218 (2)



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

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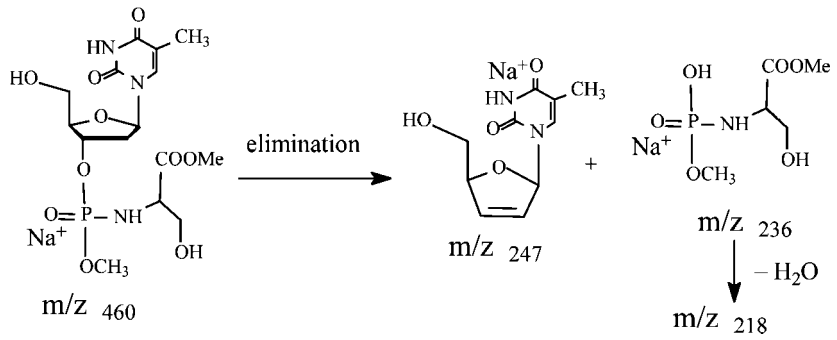

Scheme 5. Fragmentation pathway of sodium adduct of **7**.

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_{254}. Column chromatography was performed using silica gel H_{60}. NMR spectra were recorded (internal standard tetramethylsilane) with a Bruker ARX-400 type spectrometer using $CDCl_3$ or CD_3COCD_3 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 coevaperated twice. The residue was then dissolved in 15 mL anhydrous pyridine, 700 μL 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_2SO_4. 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%). ^{31}P-NMR (acetone): δ 10.42, 9.11 ppm $J_{P\text{-}H}$ 723 Hz (mixture of diastereoisomers) ^{1}H-NMR(CD_3COCD_3): δ 10.26–10.27 (m, 4H, ^{3}N-H), 7.94 (1H, P-*H*, $J_{P\text{-}H} = 720$ 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*, $J_{P\text{-}H} = 720$ 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, C*H*$_3$CO), 1.84–1.87 (m, 6H, C_5-C*H*$_3$), 1.45–1.48 (m, 6H, C_5-C*H*$_3$). ESI MS: 897$[M + Na]^+$.

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_2O, 30 μL NEt_3 (0.21 mmol) and 0.5 mL CH_3CN, 90 mg **3** in 1 mL CH_3CN was added dropwise. The reaction mixture was stired 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 $NaHCO_3$, 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). ^{31}P-NMR (acetone): δ 8.86, 8.41 ppm (mixture of diasteroisomers) ^{1}H-NMR ($CDCl_3$): 8.39–8.46 (m, 2H,^{3}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_b-3′), 4.23–4.28 (m, 2H, Ha-5′), 4.16–4.18 (m, 2H, H_b-5′), 3.92–3.98 (m, 1H, α-C*H*), 3.85–3.88 (m, 2H, H-4′), 3.74 (s, 3H, CO_2CH_3), 3.60–3.62 (m, 1H, N*H*), 2.28–2.55 (m, 4H, H-2′), 2.093–2.097 (2s, 3H, C*H*$_3$CO), 1.90–1.94 (m, 7H, 2 × C5-C*H*$_3$, -O*H*), 1.40–1.42 (m, 3H, C*H*$_3$) ESI-MS: 674$[M + H]^+$, 696$[M + Na]^+$ HRMS: found $[M + H]^+$, 674.2059 $C_{26}H_{37}N_5O_{14}P$ 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). ^{31}P-NMR (acetone): δ 9.71, 9.33 ppm (mixture of two diasteroisomers) ^{1}H-NMR(CD_3COCD_3): 10.01 (s, 2H, ^3N_a-H), 8.01 (s, 2H, ^3N_b-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-C*H*), 3.67–4.09 (m, 20H, H-4′, CO_2CH_3, C*H*$_2$O*H*(*Ser*), N*H*(*Ser*), -O*H*), 2.20–2.58 (m, 8H, H-2′), 2.072–2.083 (2s, 6H, C*H*$_3$CO), 1.80–1.87 (m, 12H, 4 × C5-CH_3) ESI-MS: 712$[M + Na]^+$ HRMS: found $[M + H]^+$, 690.2024 $C_{26}H_{37}N_5O_{15}P$ requires M + H, 690.2018.

ACKNOWLEDGMENTS

The authors would like to thank the financial supports from the Chinese National Science Foundation (No. 39870415, 20132020), the Ministry of Science and Technology, the Chinese Ministry of Education, and Tsinghua University.

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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.

Received April 15, 2002
Accepted October 22, 2002