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Synthesis and Chiral Separation of Dinucleotide(TpAZT) Phosphoramidates

Chang Xue LIN[1], Hua FU[1]*, Guang Zhong TU[2], Yu Fen ZHAO[1]

[1]Key Laboratory of Bioorganic Phosphorus Chemistry, Ministry of Education, Department of Chemistry, School of Life Sciences and Engineering, Tsinghua University, Beijing 100084
[2]Beijing Institute of Microchemistry, Beijing 100091

Abstract: Dinucleotide (TpAZT) phosphoramidates were synthesized by Todd reaction of dinucleoside H-phosphonates and amino acid methyl esters, and their diastereomers (R_p and S_p) were separated by crystallization, and the results showed that natural and cheap methyl esters of alanine and phenylalanine can be used for large-scale synthesis of dinucleotide analogs.

Keywords: Dinucleotide phosphoramidate, chiral auxiliary, chiral separation.

Intensive efforts have been made to develop effectively chemotherapeutic agents against the human immunodeficiency virus (HIV) [1-3]. Nucleoside analogues are widely used as antiviral agents in the treatments of AIDS and the AIDS related complex. 3′-Azido-5′-deoxythymidine (AZT), initially tested as anticancer agent, is an inhibitor of HIV-1 reverse transcriptase(RT) and the first drug used in clinic for the treatment of AIDS[4, 5].

However, it has been proved that AZT must be phosphorylated intracellularly to their active triphosphate form before acting as competitive inhibitor or alternate substrate (chain terminators) of HIV RT[6]. Because the cellular kinases involved in activating the nucleoside prodrugs are usually specific[7], it is thought that the replacement of ddNs with natural nucleoside, such as thymidine, could improve the rate of phosphorylation and inhibit the HIV-RT. Hakimelahi *et al.* also found that dinucleotide phosphoramidates conjugated with methyl ester of alanine were completely resistant to snake venom and spleen enzyme, and these phosphoramidates showed superior bioavailability and profound antiviral activity[8].

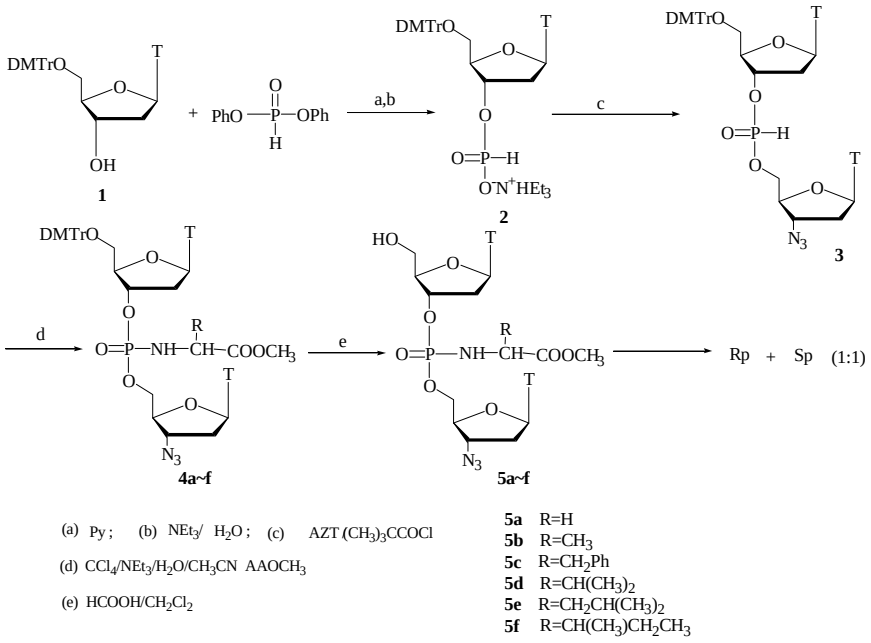
On the other hand, a pair of diastereomers are usually formed in the synthesis of nucleoside analogs due to phosphorus chiral center and show great difference in biological activity and cellular toxity for specificity of enzymes. For example, Meier *et al*. observed 3-80 folds difference of anitiviral activity between two diastereomers of cyclosal pronuclesides[9]. Dinucleotide phosphonamidates bearing with a D-amino ester moiety exhibited lower activity and less cellular toxity than the corresponding

*E-mail: fuhua@tsinghua.edu.cn, yfzhao@tsinghua.edu.cn

phosphoramidates bearing with a L-amino ester moiety[8].

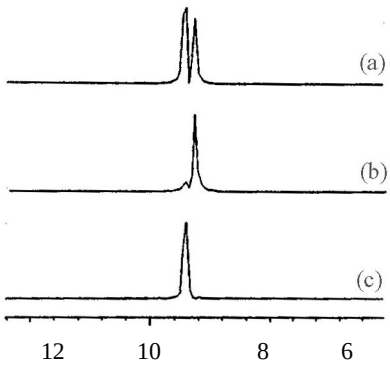
The single diastereomer of dinucleotide phosphoramidates usually was obtained *via* stereoselective synthesis[10] and chromatography isolation[11]. In this paper, we would like to report that a series of dinucleotide phosphoramidates were synthesized by Todd reaction and the single diastereomers, containing alanine and phenylalanine methyl esters were separated by crystallization.

Scheme 1 Synthetic route of dinucleotide phosphoramidates



Dinucleotide phosphoramidates were prepared as shown in **Scheme 1**[12]. Compounds **5** were obtained as a mixture of diastereoisomers and they were difficult to isolate. Their structures were confirmed by ^{31}P, ^{1}H and ^{13}C NMR. When the diastereomers of these compounds were dissolved in methanol and kept in refrigerator (-5°C) for about one week, they could be completely separated by crystallization. **Figure 1** (a), (b), (c) show ^{31}P NMR spectra of diastereoisomers **5b**, the mother solution and the precipitate re-dissolved in methanol respectively. The ^{31}P NMR spectrum of diastereoisomers **5b** in methanol showed two peaks at 9.23 and 9.04 ppm. The ^{31}P NMR of the white solid recrystalized from methanol and re-dissolved in methanol showed a single peak at 9.23 ppm and ^{31}P NMR of the mother solution exhibited another single peak at 9.04 ppm respectively. ^{1}H and ^{13}C NMR spectra also confirmed the structures of separated diastereomers. The ^{31}P NMR of dinucleotide phosphoramidates containing phenylalanine methyl ester showed the same results.

Figure 1 ^{31}P NMR spectra of compound **5b**



(a) The mixture of diastereomer in methanol
(b) (b) the mother solution
(c) (c) the precipitate re-dissolved in methanol

In conclusion, we have synthesized some dinucleotide (TpAZT) phosphoramidates. When the amino acid are alanine or phenylalanine, the diastereomers are easier to separated by crystallization. This method is cheap and could be used for chiral separation of large-scale PS-dinucleotides. The activity of the separated diastereomers (R_p and S_p) of dinucleotide phosphoramidates containing methyl esters of alanine and phenylalanine is being tested and the chiral separation of PS-dinucleotide phosphoramidates is in progress.

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12. The general procedure for synthesis of **5**: reaction of 5′- dimethoxytritylthymidine **1** and diphenyl phosphite in dry pyridine at room temperature under nitrogen atmosphere and following hydrolysis in the triethylamine and water led to **2**, dinucleoside H-phosphate **3** was obtained after coupling of **2** and AZT by pivaloyl chloride. Atherton-Todd reaction of **3** and

amino acid methyl ester in $CCl_4/NEt_3/H_2O/CH_3CN$ solution at room temperature gave product **4**. Product **5** was obtained as white foam after 5′-deprotection of **4** in formic acid and purification on silica gel column chromatography.

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