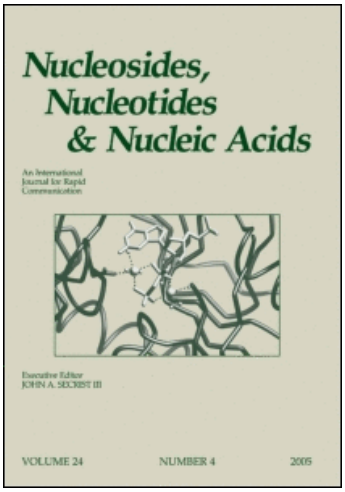

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Novel Diastereomeric Thymidine Cyclic 3',5'-*threo*- Phosphoramidates

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NOVEL DIASTEREOMERIC THYMIDINE CYCLIC 3',5'- *threo*- PHOSPHORAMIDATES

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Abstract: Novel diastereomeric thymidine cyclic 3',5'- *threo*- phosphoramidates were prepared by the treatment of 5'-azido derivative of *threo*-thymidine with triphenyl phosphite as well as by the treatment of the corresponding amino derivative with phenyl phosphodichloridate. Phosphoramidation of the regioisomeric 3'- and 5'-azido derivatives of *erythro*-thymidine by means of triphenyl phosphite afforded the open-chain 3'- and 5'-phosphoramidates. The reaction which afforded the cyclic products was assumed to proceed *via* the cyclic tetraoxazaphosphorane intermediates.

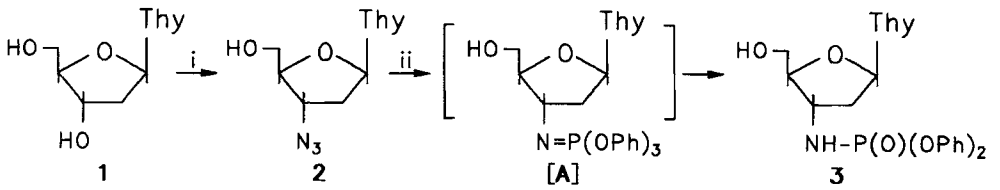
In living organisms, adenosine 3',5'-cyclic monophosphate (cAMP), isolated and purified in 1957[1] plays an important role in the regulation of metabolic process. For example, cAMP acts as a mediator of hormone action and as a modulator of enzymatic activity[2]. During the last decade a large number of cAMP analogues have been synthesized in order to study their interactions with specific enzymes, especially protein kinases and phosphodiesterases[3,4]. The most thorough investigations have been performed on the hydrolysis of cAMP and analogues. Much of what is known today concerning bimolecular displacement reactions at phosphorus can be traced back to the classic studies on phosphate ester hydrolysis by Westheimer in the in early 1960s[5]. Most likely a pentacoordinated phosphorus species is involved, either as an intermediate or as a transition state, in both the hydrolysis of cAMP to 5'-AMP and the activation of protein kinases by cAMP[6]. As a result, pentacoordinated phosphoranes became the focus of many experimental and theoretical studies[7,8]. To obtain more insight into the mechanism of action of cAMP, we planned to perform syntheses of six-membered cyclic tetraoxazaphosphorane derivatives of nucleosides as model systems of P(V) cyclic nucleotide intermediates. In a previous paper[9] we have described the preparation of tricyclic derivative of uridine with pentacoordinated phosphorus in the reaction of 1-(5-azido-5-deoxy-β-D-lyxofuranosyl)uracil with triphenyl phosphite. The cyclisation step includes the interaction of sterically favorably oriented hydroxyl groups with initially

formed phosphite imine. In an attempt to expand this methodology to the preparation of novel derivatives of nucleosides with six-membered tetraoxazaphosphorane rings, as starting compounds we selected a series of nucleosidic azidoalcohols **2, 4,** and **10.** The application of this method to the phosphorylation of **10** produces novel cyclic nucleotides **11a,b** which are diastereomeric at phosphorus.

3'-Azido-3'-deoxythymidine (AZT) (**2**) prepared in five steps (45 % overall yield) from thymidine (**1**) by an established sequence[10] was allowed to react with triphenyl phosphite in anhydrous dioxane at 100 °C. (Scheme 1). However, this reaction did not result in the formation of the expected cyclic tetraoxazaphosphorane. The 3'-deoxy-3'-phosphoramidothymidine (**3**) was formed instead in 66 % yield. The presence of two phenoxy groups (δ_H 7.22-7.44 ppm) and 3'-NH group signals (δ_H 6.29-6.37 ppm) in ^{1}H NMR spectrum[11] and resonance at δ_P = 0.36 ppm in the broadband ^{1}H decoupled ^{31}P NMR spectrum undoubtedly support this structure.

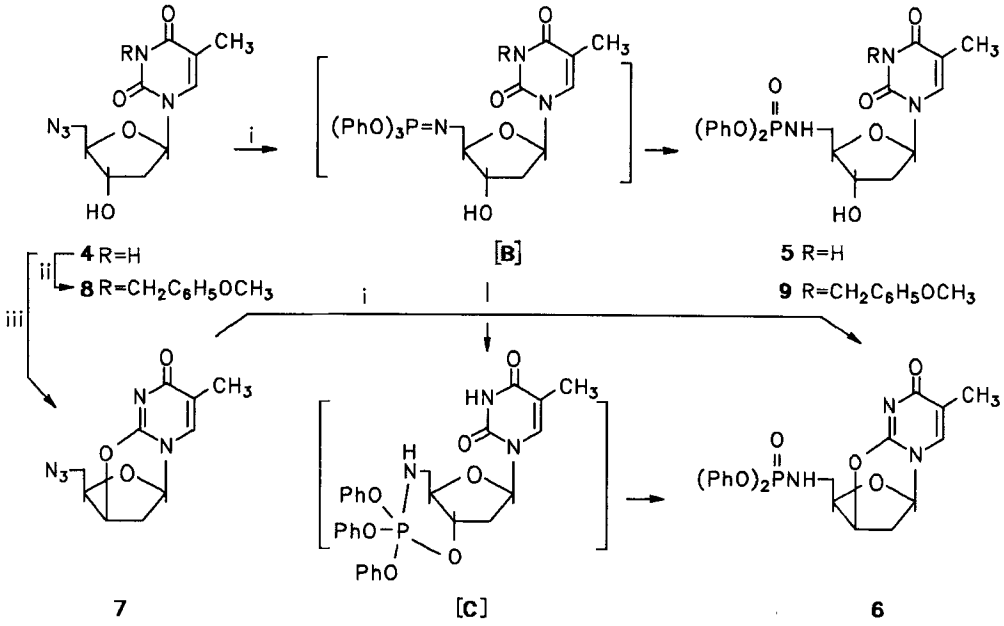
To obtain the regioisomeric azidoalcohol 5'-azido-5'-deoxythymidine[12] (**4**), thymidine (**1**) was selectively converted to the corresponding 5'-tosyloxy derivative, from which the desired compound **4** was prepared by the procedure developed by Horwitz and co-workers[13]. While the reaction of AZT with triphenyl phosphite gave only the product of phosphoramidation of azido group[14], the reaction of **4** with triphenyl phosphite in the same reaction conditions gave the 5'-phosphoramido nucleoside **5** (22 %) and the 2,3'-anhydro-5'-phosphoramido nucleoside **6** as the major product (49 %). The formation of the anhydro nucleoside **6** was evidenced by the appearance of the characteristic ultraviolet absorption peak in the 248 nm region and the absence of 3-NH and 3'-OH signals in ^{1}H NMR spectrum. Moreover, the signal of H-3' appeared at lower field than the corresponding signal of **4** ($\Delta\delta_H$ ~1 ppm) in accordance to anhydro bond formation. The signals of two phenoxy and 5'-NH groups in ^{1}H NMR spectrum, as well as the resonance at δ_P= 1.38 ppm in ^{31}P NMR spectrum indicate the open-chain phosphoramido group. The formation of both **5** and **6** in these reactions indicates that at least two competitive reaction pathways must be considered. The phosphoramidate **5** is apparently formed through direct phosphoramidation of 5'-azido group with triphenyl phosphite. The formation of anhydro product **6** could be explained *via* the formation of an intermediate cyclotetraoxazaphosphorane **C** which is subsequently opened by intramolecular nucleophilic attack by the 2-C=O group at C-3' (Scheme 2).

The cyclic 5'-phosphoramido compound **6** was also obtained in 72 % yield by direct phosphoramidation of 5'-azido-5'-deoxy-2,3'-anhydrothymidine (**7**)[15] with triphenyl phosphite. Samples of **6** prepared by both routes were identical in all respects. In the attempt to prevent the formation of anhydronucleoside and obtain the desired phosphorane, the 3-NH group of **4** was protected with *p*-methoxybenzyl group[16] giving the azidoalcohol **8** . However, in this reaction the 5'-phosphoramido compound **9** was



Reagents and conditions: i, Ref. 10.; ii, $(PhO)_3P$/dioxane, 100°C, 1.5 h

Scheme 1



Reagents and conditions: i, $(PhO)_3P$/dioxane, 100°C, 1.5 h; ii; p-$CH_3C_{H5}CH_2Cl$, DBU/CH_3CN, 60°C, 30 min; iii, Ref. 15.

Scheme 2

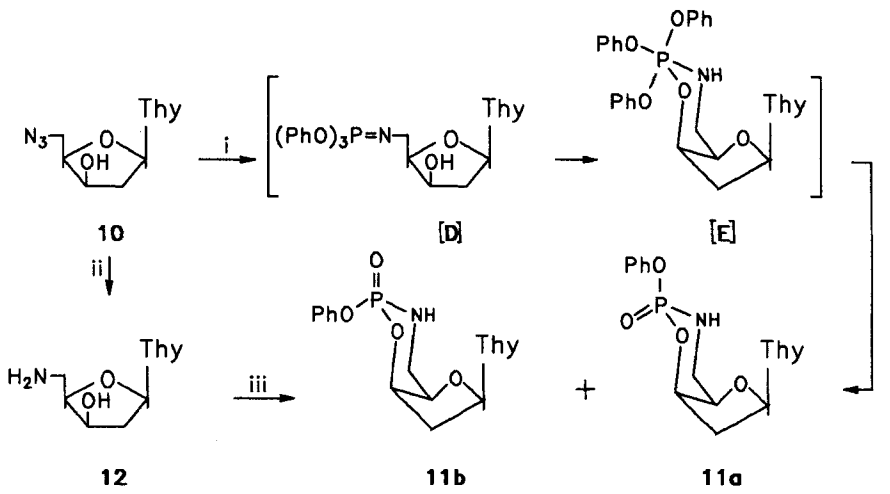
obtained in the 24 % yield together with the intractable material from which no definable product could be isolated.

The difference between 3'-azidothymidine **2** and its 5'-regioisomer **4** towards formation of tetraoxazaphosphorane must lie in the relative position of the phosphorimino group with respect to the 5'- and 3'-hydroxyl group in the two phosphorimino intermediates **A** and **B**, respectively. We have examined the conformational preferences of intermediates **A** and **B** by means of molecular mechanics

calculation using TRYPOS force field[17]. For **B,** smaller distance between P and 3'-OH (3.54 Å) was calculated compared to **A** (P to 5'-OH distance is 4.21 Å). Also, the calculated energy for cyclic intermediate **C** was lower for 6.3 kcal/mol than that calculated for similar cyclic intermediate generated from **A.**

In order to obtain the azidoalcohol with favorable structural characteristics and conformational flexibility to ensure the proximity of the phosphorimino group and hydroxyl groups, the synthesis of 5'-azido nucleoside containing "up" hydroxyl group was undertaken. The conversion of *erythro* azidoalcohol **4** to an azidoalcohol of the *threo* configuration **10**[18] was carried out by the reaction of the anhydro derivative **7** with NaOH. The *threo* compound was then allowed to react with triphenyl phosphite. However, no trace of the expected cyclic tetraoxazaphosphorane could be detected by TLC. Two other products, **11a** and **11b** (R_F=0.38 and 0.32, respectively; CH_2Cl_2:CH_3OH=10:1) were formed in 38 and 29 % yield, respectively. The analysis of ^1H, ^{13}C and ^{31}P spectra of those products allows the structure assignment as six-membered 3',5'-cyclophosphoramidates isomeric at phosphorus (Scheme 3).

Very similar ^1H NMR spectra of **11a** and **11b** show the absence of 3'-OH, and positions of H-3' signals at lower field ($\delta_{H\text{-}3'}$ 5.08 and 5.01 ppm, respectively) compared to that of **10** ($\delta_{H\text{-}3'}$ 4.26 ppm) indicating phosphorylation of 3'-hydroxyl group. The presence of only one phenoxy group resonance together with that of 5'-NH group signal is consistent with cyclophosphoramide structure in both, **11a** and **11b**. The ^{13}C NMR spectra of **11a** and **11b** showed downfield shifts ($\Delta\delta_C$ = 5.51 and 8.81 ppm) of the C-3' signals and upfield shifts (-1.8 and -3.03) of the C-4' signals, being in accord to chemical shifts changes of sugar carbons previously observed for various nucleoside 3',5'-cyclic phosphates[19]. In the ^{13}C NMR spectra resonances assigned to C-4', C-3', and C-2' appeared to be split by the phosphorus (**11a** : $J_{C\text{-}4',P}$ = 8.13, $J_{C\text{-}3',P}$ = 8.13; **11b** : $J_{C\text{-}4',P}$ = 4.6, $J_{C\text{-}3',P}$ = 4,6, $J_{C\text{-}2',P}$ = 9.28 Hz) which provides valuable information on the sugar-phosphorus connection. In the broadband ^1H decoupled ^{31}P NMR spectrum of the TLC fast-migrating compound **11a**, the ^{31}P chemical shift is smaller (δ_P= -3.12 ppm) compared to that for the slow migrating **11b** (δ_P= 3.51 ppm). The observed upfield positions of ^{31}P resonances suggest that **11a** and **11b** contain six-membered ring[20]. A possible explanation for the formation of the cyclic phosphoramidates **11a** and **11b** could be based on formation of tetraoxazaphosphorane nucleoside derivative **E** as an intermediate and its subsequent hydrolysis[21] leading to the diastereomeric **11a** and **11b**. The structures of the cyclophosphoramidates **11a** and **11b** were additionally confirmed chemically by synthesis from 1-(5-amino-2,5-dideoxy-β-D-*threo*-pentosyl)thymine[22] **(12)** . Phosphorylation of **12** with phenyl phosphodichloridate in DMF and Et_3N afforded cyclic compounds **11a** and **11b** in 47 and 36% yield, respectively. These materials were identical by UV, NMR, IR,



Reagents and conditions: i, $(PhO)_3P$/dioxane, 100°C, 1.5 h; ii, EtOH, Pd black, r.t., 18 h; iii, $PhOP(O)Cl_2$, Et_3N/DMF, r.t., 4 h

Scheme 3

and thin layer R_f values with those obtained from the 5'-azidoalcohol **9**. Assuming that P-containing six-membered ring are *cis* fused with 2'-deoxyribose moiety in a chair-like conformation the absolute configurations of diastereoisomeric **11a** and **11b** could be predicted. It is known that the ^{31}P NMR resonance of the axial stereoisomers of some nucleoside 3',5'-cyclophosphates is shifted upfield with respect to the equatorial one[23]. Hence, the axial (R_P) configuration can be assigned to the diastereoisomer **11a** (R_F=0.38, δ_P= -3.02ppm), and the equatorial (S_P) configuration to the **11b** diasteroisomer (R_F= 0.32, δ_P= 3.51ppm).

In conclusion, we report on the preparation of novel diastereomeric *threo* 3',5'-cyclophosphoramidates **11a,b** , and present the indirect evidence for the formation of the six-membered tetraoxazaphosphorane **E** as their intermediate. Further studies on absolute configurations of **11a** and **11b** by X-ray structure analysis and by means of enzymatic assay are currently in progress.

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