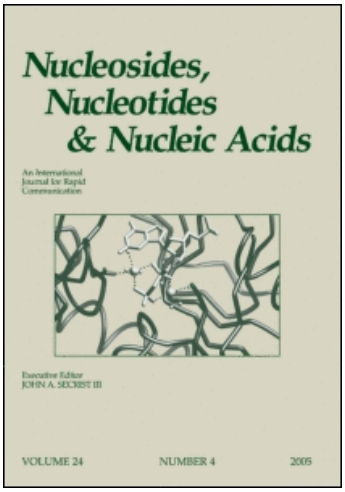

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Pyridoxal-Catalyzed Release of Nucleotides

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PYRIDOXAL-CATALYZED RELEASE OF NUCLEOTIDES

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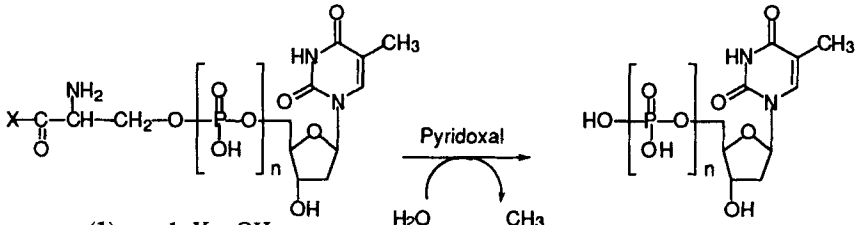
ABSTRACT : The synthesis of phosphoric esters linking the 5' alcohol of thymidine to the alcohol moiety of L-serine derivatives is reported, together with the pyridoxal-catalyzed ß-elimination reaction releasing thymidylate from such compounds. The process was extended to the release of thymidine di- and tri-phosphate.

We are currently developing a peptide-based carrier system for delivering nucleoside triphosphates or analogues thereof within cells. It is known that serine and serine-3-phosphate are deaminated by pyridoxal according to a ß-elimination reaction[1-3]. We thus investigated the ß-elimination of thymidylate grafted to the lateral chains of amino acids by pyridoxal as a model catalytic process for releasing nucleotides under physiological conditions.

For this purpose, we synthesized three different phosphodiesters linking the 5' alcohol of thymidine to the alcohol group of L-serine derivatives bearing a free carboxylic group (**1**), a carboxamide group (**2**) or an alanyl group (**3**).



(**1**) n = 1, X = OH
(**2**) n = 1, X = NH_2
(**3**) n = 1, X = Ala
(**4**) n = 2, X = NH_2
(**5**) n = 3, X = NH_2

The compounds **1-3** were prepared by P^{III} chemistry. The use of alkoxybis(dialkylamino)phosphine allowed us to introduce successively two substrates bearing a suitable OH function, firstly 3'-acetyl-thymidine and then N-protected serine amide or serine ethyl ester. Oxidation of the resulting phosphite triester followed by the selective cleavage of the initial alkoxy group and all the temporary protecting groups yielded the target phosphodiester. Compound **4** was obtained by condensation of Z-L-serinyl amide 3-phosphate and the morpholidate of thymidine 5'-monophosphate. Compound **5** was obtained by condensation of the morpholidate of Z-L-serinyl amide 3-phosphate and thymidine 5'-diphosphate. The purity of compounds **1-5** was checked by analytical reverse phase HPLC, NMR spectrometry (^{1}H, ^{13}C, ^{31}P) and electrospray mass spectrometry.

The release of thymidylate from substrates **1-3** was investigated under physiological conditions of pH and temperature. Cleavage tests were conducted by mixing at 37 °C equal volumes of pyridoxal and substrate solutions in MOPS buffer pH = 7.5. Aliquots were analyzed by reverse phase HPLC at intervals of time. When equimolar concentrations (10 mM) of pyridoxal and phosphodiesters (**1-3**) were used, dTMP was released in each case, with relative velocities of cleavage **2>3>1**. When a catalytic concentration of pyridoxal was used (1 mM), the carboxamide compound **2** was again found to be more prone to ß-elimination ($k = 0.1\ h^{-1}$) than the carboxylic compound **1** ($k = 0.005\ h^{-1}$) and the peptide compound **3** ($k = 0.02\ h^{-1}$).

Conjugates of thymidine 5'-di- and tri-phosphate to the alcohol moiety of L-serine amide (**4, 5**) were also synthesized. When compounds **4** and **5** (9 mM) were incubated with pyridoxal (45 mM) in MOPS buffer pH = 7.5 at 37 °C, dTDP and dTTP were cleanly formed without degradation of the phosphoanhydride bonds. Altogether, these results warrant further exploration of amino acid/nucleotide conjugates for enabling the therapeutic delivery of anti-metabolites and the diversification of nucleic acid biosynthesis *in vivo*.

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