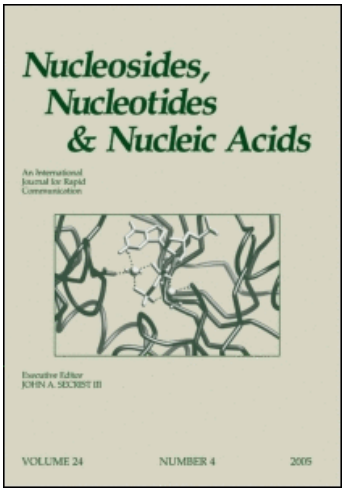

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A Possible Mechanism for the Base-Catalyzed Hydrolysis of Nucleoside Cyclic 3',5'-Monophosphates

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A POSSIBLE MECHANISM FOR THE BASE-CATALYZED HYDROLYSIS OF NUCLEOSIDE CYCLIC 3',5'-MONOPHOSPHATES

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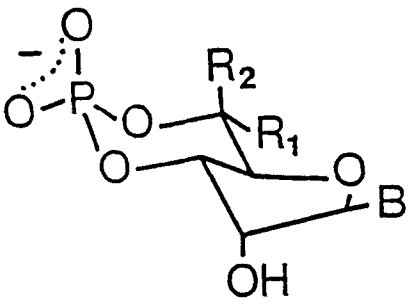
Abstract. The predominant formation of nucleoside 3'-monophosphates in the base-catalyzed hydrolysis of nucleoside 3',5'-monophosphates is interpreted in terms of the lone pair orientation effect that may decrease the transition state energy for P-O-C5' bond breaking.

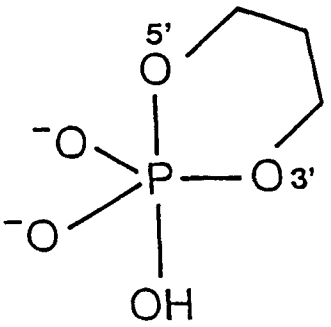
Nucleoside cyclic 3',5'-monophosphates (1) are six-membered cyclic phosphodiesters having one primary, the P-O-C5' and one secondary, the P-O-C3' phosphate ester bond. The two ester linkages show different stability towards base. For example, adenosine cyclic 3',5'-monophosphate (2) hydrolyzes under basic conditions [0.2 N $Ba(OH)_2$, 100°C] with complete inversion of configuration at phosphorus[1] to a 86:14 mixture of adenosine 3'- and adenosine 5'-monophosphate,[2] i. e. with dominant fission of the P-O-C5' linkage. The base-catalyzed hydrolysis of 1 was a key reaction in the first synthesis of diribonucleoside-monophosphates according to the phosphodiester method.[3] The stability differences between the primary and the secondary phosphate ester bonds were interpreted either on the analogy of the well-known stability differences between primary and secondary alkyl esters of carboxylic acids,[4] or on the basis of the stereochemical nonequivalence of the P-O-C5' and the P-O-C3' bonds.[5] However, no effort has been made to explain the base catalyzed hydrolysis of 1 in terms of the modern theory of displacement reactions at phosphorus. The present communication is an attempt to do this on the example of 2, the most important representative of 1.



	R_1	R_2	B
1:	H	H	purin-9-yl or pyrimidin-1-yl
2:	H	H	adenin-9-yl
3:	Me	H	adenin-9-yl
4:	H	Me	adenin-9-yl

1 - 4

A nucleophilic displacement reaction at phosphorus according to an $S_N2(P)$ mechanism proceeds via a trigonal bipyramidal transition state with apical attack of the entering group and apical departure of the leaving group.[6] Therefore, in the base catalyzed hydrolysis of 2, the transition states for P-O-C5' and P-O-C3' bond breaking may be formulated as structures 5 and 6, respectively, if we disregard the conformation of the dioxaphosphorinane ring.



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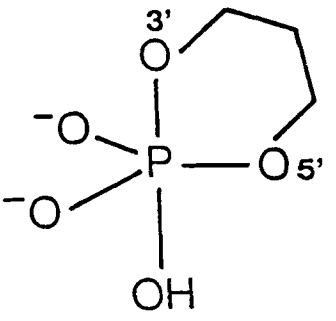

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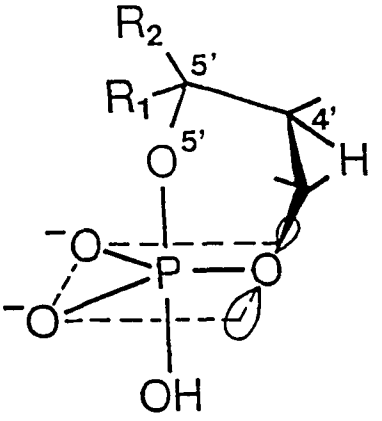
The dominant P-O-C5' bond breaking may be explained on the basis of the greater apicophilicity of the primary OC5' alkoxy group compared to that of the secondary OC3' alkoxy group.[7] If this interpretation is correct, substitution of a methyl group for any of the two H5' protons of 2 should

result in an increased P-O-C3' bond breaking at the expense of the P-O-C5' bond breaking. However, we found that only the substitution of the equatorial proton influences the hydrolytic process, the bond breakings being 37% (P-O-C5') and 63% (P-O-C3') for 9(6-deoxy- -D-allofuranosyl)adenine cyclic 3',5'-monophosphate (3, equatorial 5'-methyl group) and 80% (P-O-C5') and 20% (P-O-C3') for 9(6-deoxy- -L-talofuranosyl)adenine cyclic 3',5'-monophosphate (4, axial 5'-methyl group). Consequently, the greater apicophilicity of the primary alkoxy group may not be responsible for the stability differences between the P-O-C5' and the P-O-C3' bonds.

The 1,3,2-dioxaphosphorinane ring of 2 occupies apical-equatorial positions in the trigonal bipyramidal transition state of hydrolysis. For an apical-equatorial dioxaphosphorinane ring the boat form is the most stable conformer[8] as a result of the lone pair orientation effect.[9] Only an apical-equatorial boat-shaped dioxaphosphorinane ring provides for the lone pair on the equatorial sp^2 hybridized ring oxygen atom[10] to be in the favored equatorial plane.[8] Examinations of Dreiding models containing sp^2 hybridized O3' and O5' atoms and a boat-shaped dioxaphosphorinane ring, shows that in the transition state for P-O-C5' bond breaking (7), the lone pair on the equatorial O3' atom is located on the equatorial plane. On the contrary, in the transition state for P-O-C3' bond breaking (8), the lone pair on the equatorial O5' atom is perpendicular to the equatorial plane. Consequently, the transition state energy for P-O-C5' bond breaking may be smaller than that for the fission of the P-O-C3' linkage. Steric repulsion between the cis-oriented H4' and H5' protons in transition state 7, acts against the lone pair orientation effect and thus increases the transition state energy. Substitution of a methyl group of larger size for the equatorial H5' proton of 2 to give 3 should enlarge the steric repulsion and, in such a way, may further increase the transition state energy. A similar substitution of the axial H5' proton (the case of 4, trans-oriented H4' and 5'CH_3 in 7) is not expected to influence the steric repulsion.



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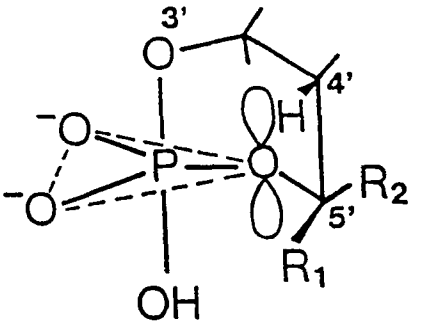

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Accordingly, the predominant formation of 3'-monophosphates in the base-catalyzed hydrolysis of 2 and 4 may be interpreted on the basis of the lone pair orientation effect that diminishes the transition state energy for P-O-C5' bond breaking. The lone pair orientation effect is sterically hindered, and thus the transition state energy for P-O-C5' bond breaking is increased for 3 resulting in a decrease of 3'-monophosphate formation.

The alkaline hydrolysis of the phosphoramidate derivatives of 2, 3 and 4 has been interpreted on similar grounds, recently.[11]

EXPERIMENTAL

Cyclic monophosphates 3 and 4 were prepared according to known procedures[12] and characterized by ^{1}H, ^{13}C and ^{31}P NMR.[11,13] Hydrolysis of 3 and 4 was performed under exactly the same conditions (0.05 mmol 3 or 4, 2.5 mL of 0.2 N $Ba(OH)_2$, 100°C, 30 min) as used by Khorana for that of 2.[2] After neutralization with Dowex-50x8 [H+] cation-exchange resin, the hydrolysates were separated on a DEAE-cellulose [HCO_3^-] (DE-32, Whatman) column (1.4x30.0 cm) by using a linear gradient of aqueous triethylammonium hydrogen carbonate, pH 7.5 (0-->0.3 M, 1 L). The quantity of the

cleanly separated monophosphates (elution order: 5'>3') was determined by UV at 260 nm. The isomeric monophosphates were distinguished by TLC comparison with authentic adenosine 5'- and 3'-monophosphates on silica gel (Kieselgel 60 F_{254}, Merck) chromatoplates in n-propanol/conc. NH_4OH/H_2O = 11/7/2 (v/v) developing solvent.

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