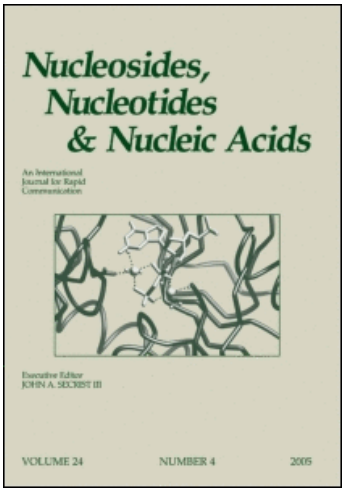

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Phosphonylmethyl Analogs of Nucleotides and Their Derivatives: Chemistry and Biology

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PHOSPHONYLMETHYL ANALOGS OF NUCLEOTIDES AND THEIR DERIVATIVES: CHEMISTRY AND BIOLOGY

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ABSTRACT. Ribonucleoside O-phosphonylmethyl derivatives are novel isopolar analogs of nucleotides. This review summarizes data on their synthesis and properties, as well as data on novel type of open-chain nucleotide analogs.

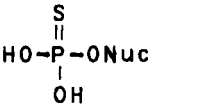
INTRODUCTION. - Our systematic investigation of analogs of nucleic acid components resisting the action of enzymes involved in catabolic pathways of nucleic acids and their precursors also included a development of nucleotide analogs modified at the phosphoric acid residue[1]. Biologically active nucleotide analogs are often converted *in vivo* into their 5'-phosphates which act either directly or after subsequent anabolic transformation as effectors of target enzymes. Except for certain cases[2], the direct application of these active antimetabolites *in vivo* did not bring the expected results. This failure is possibly due to the dephosphorylation during and/or after the transport of the nucleotide into the cell.

The *in vitro* studies revealed that phosphorus-modified nucleotides should preserve an isopolar character with natural nucleotides in order to interact efficiently with the enzymes of nucleic acid metabolism. Substitution at the phosphorus atom (Type I) nearly always diminishes the dissociability of the residue[3]. Only the phosphorothioates (II) (ref.[4]) do not obey this rule. The deoxynucleoside phosphonates (III) are both isopolar and enzymatically

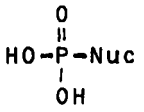
stable; various compounds of this type are effective inhibitors of enzymes[3].

The oxygen atom in the immediate vicinity of phosphorus plays an important role both in the recognition process and the catalytic step. Therefore, we have developed phosphonylmethyl ethers of nucleosides (IV) as a novel type of nucleotide analogs which contain both the resistant C-P linkage and the oxygen atom located near to the phosphorus.

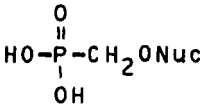
I R = H, alkyl, CH_2OH, CH_2NH_2



II



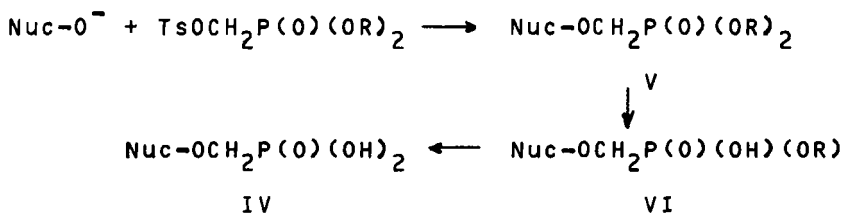
III



IV

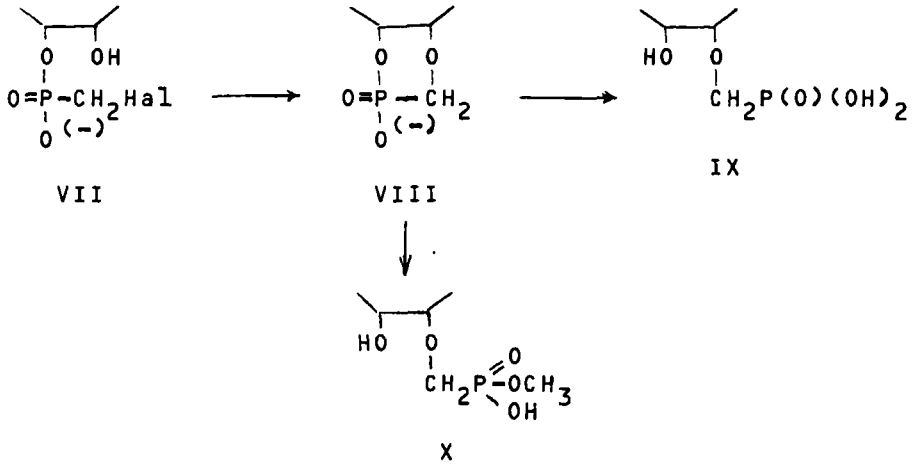
Nuc ... 5'-Deoxynucleosid-5'-yl residue

O-PHOSPHONYLMETHYL NUCLEOSIDES. 1. Chemical syntheses. The two synthetic approaches which have been used for the preparation of these compounds make use of methanephosphonic acid derivatives. The alkoxide anion formed from the hydroxy group of suitably protected nucleoside sugar moiety by sodium hydride reacts with p-toluenesulfonyloxymethanephosphonic acid diesters to afford a neutral intermediate V which loses one of the ester groups on hydrolysis. The remaining alkyl ester group in compounds VI is subsequently removed by treatment with iodo- or bromotrimethylsilane in DMF (ref.[5,6]):



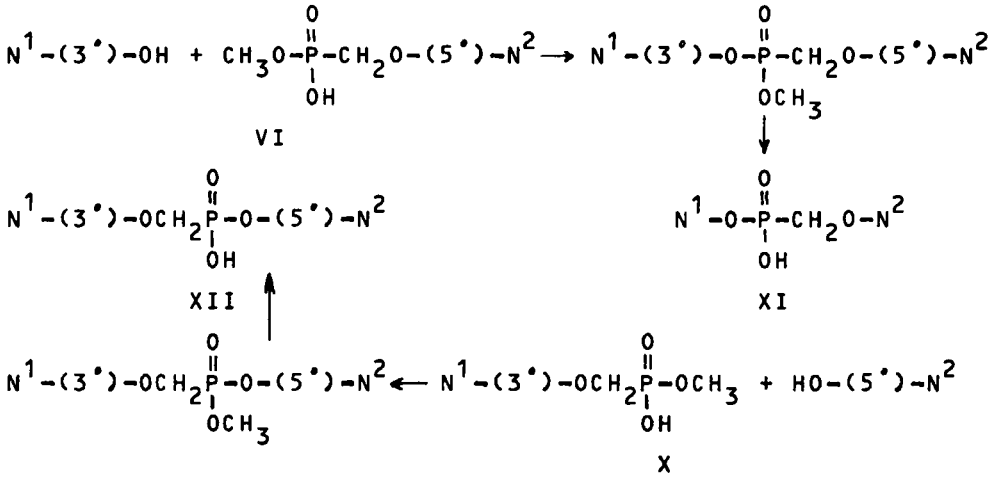
This method can be used for protected nucleosides which contain one isolated hydroxy group; the reaction conditions limit the choice of protecting groups and enforce protection of functional groups at the heterocyclic base.

The second alternative consists in a substitution of halogenomethanephosphonic acid derivatives by a nucleoside alkoxide anion. Sodium salts of halogenomethanephosphonic acids afford the phosphonylmethyl ethers in unsatisfactory yields[1,5] and the diesters or monoester salts completely fail to form the desired products. However, the halogenomethanephosphonic acid esters of nucleosides derived from the ribonucleoside cis-diol grouping (VII) yield the O--phosphonylmethyl ethers quantitatively by alkali treatment in anhydrous or aqueous medium[6,7]. The cyclic phosphonate VIII has been identified as a reaction intermediate. Such six-membered cyclic phosphonates are comparatively stable in acid and very unstable in alkaline solutions, thus differing from the six-membered cyclic phosphates. The phosphonylmethyl ethers IX can be obtained simply by treatment of ribonucleoside 2- or 3-O-chloromethanephosphonates with aqueous alkali. In alcoholic solutions of sodium alkoxides the alkyl esters X of the corresponding analogs result from the ring opening of the cyclic intermediate VIII (ref.[6]).



In the simplified procedure, 5'-O-acylnucleosides are treated first with chloromethylphosphonyl dichloride in the presence of a tertiary base and the mixture is then hydrolysed by aqueous alkali to form the ribonucleotide analogs in high yield[6,7].

The C-P linkage in the above nucleotide analogs is stable both under alkaline and acidic conditions. The 5'-ribonucleotide analogs can be transformed into the 5'-diphosphate or 5'-triphosphate analogs via morpholidates[5], the 2'- or 3'-O-phosphonylmethylribonucleosides afford the 2',3'-cyclic derivatives VIII by carbodiimide activations[6]. Also the analogs of diribonucleoside phosphates containing the $O-CH_2-P$ grouping were prepared by the usual diester[1] or triester procedure[8]. There are two main types of these compounds which differ in the position of the ether-linked phosphonic acid moiety. Compounds of the type XI can be prepared by reaction of protected 5'-O-phosphonylmethylnucleoside alkyl esters (VI) with a nucleoside derivative bearing a free 3'-hydroxyl group whereas their isomers XII arise from condensations of protected 3'-O-phosphonylmethylribonucleoside alkyl esters X with protected nucleoside derivatives containing a free 5'-hydroxyl function. The choice of protecting groups and condensation agents is the same as in oligonucleotide chemistry.



The ^{1}H NMR investigation proved that both in aqueous and non-aqueous solutions the conformation of 5'-ribonucleotide analogs is very similar to that of natural 5'-nucleotides[9]. This finding confirms the expectations arising from theoretical conformational adaptability of the CH_2-O-P grouping.

2. Biochemical properties. - The O-phosphonylmethyl analogs of ribonucleotides resist both non-specific and nucleotide-specific phosphomonoester hydrolases[1,5]. (The biodegradation of C-P linkage is known to proceed with phosphonate analog of amino acids as well as with alkanephosphonates and hydroxyalkanephosphonates[10].) Nonetheless, 5'-nucleotidases interact with 5'-ribonucleotide analogs, since their activity is strongly inhibited in the presence of the analogs[1]. The analogs of ribonucleoside 2',3'-cyclic phosphates (VIII) are equally indifferent to the hydrolytic reaction catalysed by ribonucleases (A, T2, U2) and spleen decyclizing phosphodiesterase[6]. This failure may be due to a different stereochemistry of the substrate and to a change induced by the methylene grouping at the phosphorus atom.

The specificity of ribonucleases in the transfer reaction is somewhat lower. Under extreme conditions, the phosphonate-containing diribonucleoside phosphate analogs XII can be cleaved by ribonucleases A and T2 to a minor extent; the analogs of the type XI are quite resistant. On the contrary, the snake venom exonuclease can catalyse a minor cleavage of type XI compounds, whereas the analogs XII resist its action. Consequently, the high stability of mononucleotide, cyclic nucleotide and oligonucleotide analogs derived from modified nucleotides of the above type can be taken for granted.

The ribonucleoside 5'-diphosphate analogs derived from 5'-O-phosphonylmethylribonucleosides are not substrates for polynucleotide phosphorylases from E. coli or M. luteus, either in homopolymerization or in copolymerization with natural 5'-diphosphates. This failure cannot be overcome by

the use of Mn^{2+} instead of Mg^{2+} ions. Nonetheless, these compounds act as strong competitive inhibitors of the enzyme, with K_m/K_i ratios in the range of $2x10^{-3}$ to 10^{-1}. This inhibition is not base-specific for the substrate, the most potent inhibitor is the GDP analog. It is of interest that the presence of minor quantities of an analog which still do not hinder substantially the polymerization of a natural 5'-diphosphate, significantly increase the length of the polymer chain formed. Such an enhancement of the chain length may amount up to 100% in the polyC synthesis performed in the presence of 1 mol.% of the CDP analog. This effect is not due to inhibition of the phosphorolytic reaction. The analog is incorporated neither in the chain, nor at the terminus[11,12].

The phosphonylmethyl analogs of ribonucleoside 5'-triphosphates are interesting as donors of phosphoric acid residue and as substrates for a transfer of 5'-nucleotide unit in the polymerization reactions catalysed by polymerases. Our studies have demonstrated that these compounds interact with nucleoside kinases: the analogs of UTP and CTP modified at the α-phosphorus inhibit the phosphorylation of uridine in the presence of L-1210 uridine kinase. Their effect consists probably in substituting for the natural 5'-triphosphates in the formation of a ternary complex[13]. However, the corresponding ATP and GTP analogs can function as phosphate donors in the same reaction with the same K_m value as the natural donor. The optimum Mg^{2+} concentration for this reaction is higher than that for ATP or GTP, possibly in order to reinforce the formation of transition state complex which is weakened by lower chelation capability of the analog[14].

Also, the polymerization of NTP in the presence of DNA dependent RNA polymerase from E. coli is efficiently inhibited by NTP analogs modified at the α-phosphorus. The base specificity of this inhibition confirms an interaction of the analog at the substrate specific binding sites.

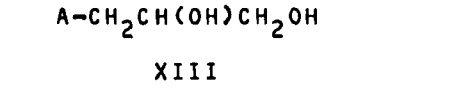
The O-phosphonylmethyl analogs of NTP can also be utilized as substrates for this enzyme. With poly(dA,dT) as a template, the reaction of ^{31}P-UTP with the ATP analog as the only adenine nucleotide affords a mixture of short-chain oligonucleotides in a considerable yield. The gel-electrophoresis proved the presence of dinucleotide and trinucleotide analogs as the prevailing components of this mixture. The compounds have been identified with the aid of enzymatic cleavage reactions as phosphonate-containing species. Thus, the initiation process and the first steps of elongation evidently take place quite efficiently, but the continuation of the chain growth to a polymer level is limited. A certain amount of polymer formation under the above conditions was also observed; a direct proof that this fraction contains modified phosphoric acid residues still remains to be given. The utilization of ribonucleoside 5'-triphosphate analogs by the DNA dependent RNA polymerase indicates a certain probability of incorporation of such analogs into nucleic acids in vivo; such linkages would resist the usual cellular degradation pathways.

None of the ribonucleotide analogs derived from natural or various modified bases exhibited any antibacterial, antiviral or cytostatic (L-1210) activities. There are no data available whether this lack of biological activity is due to a hindered transport of the analogs into the cells.

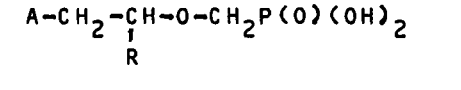
ACYCLIC NUCLEOTIDE ANALOGS. - Systematic search for nucleoside analogs which would not undergo catabolic degradation of the nucleosidic linkage resulted in the development of acyclic nucleosides. These analogs contain a hydroxy-substituted alkyl chain linked to the heterocyclic base instead of the pentafuranose ring. We have developed a group of chiral acyclic nucleosides some of which exhibit biological activities. 9-(S)-(2,3-Dihydroxypropyl)adenine ((S)-DHPA, XIII) has a broad-spectrum antiviral effect which is directed mainly against (-)-stranded RNA viruses. The mechanism of action of this compound and its congeners consists in an inhibiton of S-adenosylhomocysteinase (an

enzyme involved in the regulation of biological methylations)[11]. These compounds are not phosphorylated *in vivo* and their phosphates are inactive both *in vivo* and *in vitro* in various systems. In the context of the above studies we have also investigated a novel group of nucleotide analogs based on modification of both the carbohydrate and the phosphorus moieties - the O-phosphonylmethyl derivatives of acyclic nucleosides. The chemical approaches to their preparation make use of the above principles: the condensation of dialkyl p-toluenesulfonyloxymethanephosphonates for treatment of derivatives with an isolated hydroxy function or subsequent chloromethanephosphonyl chloride and alkali treatment for reactions starting from cis-diol containing nucleoside analogs.

An outstanding position in this series of nucleotide analogs occupy two adenine derivatives: 9-(2-phosphonylmethoxyethyl)adenine (PMEA, XIV) and 9-(S)-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA, XV). These compounds are very efficient antivirals which act specifically against DNA viruses. They are effective also against virus strains and mutants resistant to the antivirals currently used for treatment of DNA viruses (acyclovir, araA, PFA, PAA, BVDU, etc.)[17]. Compound XV which is the more active of the two is a derivative of (S)-DHPA; the conditions for its biological activity comprise the absolute configuration 2S and substitution by phosphonylmethyl residue at the position 2 of the side chain. The qualitative change of the biological target (RNA viruses for (S)-DHPA, DNA viruses for HPMPA as well as the lack of activity of compounds XIV, XV against the S-adenosylhomocysteinase suggest a different mechanism of action which probably implicates an interference with the biosynthesis of viral DNA.



XIII



XIV R = H

XV R = CH_2OH (S)

A = adenin-9-yl residue

This discovery opens a new field for research and design of novel analogs of nucleotides with potential biological applications.

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