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PHOSPHORYLATION OF 5'-AMINO-5'-DEOXYNUCLEOSIDES[+]

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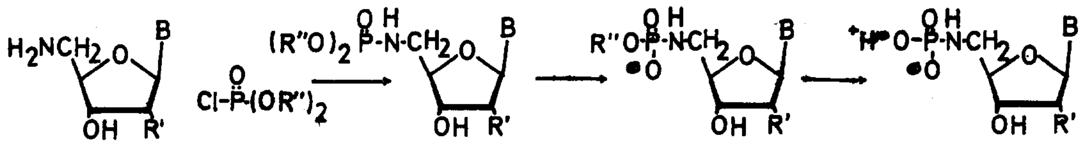
5'-Deoxy-5'-aminonucleosides have been amply described1)2)3)4). Though considerable effort has been spent on the synthesis of nucleotide analogues over some years N-5'-Nucleoside phosphoramidates apparently have not been previously described.

We report the synthesis of some N-phosphorylated 5'-amino-5'-deoxynucleosides (III, IV, V) using conventional methods.



(I) (II) (III) (IV) (V)

In the deoxy-series the unprotected 5'-amino-5'-deoxy-thymidine (Ia B=thymine, R'=H)4) served as a model compound and in the ribo-series 5'-amino-5'-deoxy-adenosine (Ib B=adenine, R'=OH) was used. The free amine(Ib) was prepared from the tosylate1) by means of an ion exchanger column (MERCK III) and subsequent freeze drying. Since the P-N bond is known to undergo acid catalyzed cleavage very readily6) the substituents -R'' on the phosphorylating agent should be removed in an alcaline or preferentially neutral medium. Hence bis-[ß,ß,ß-trichloroethyl]-phosphochloridate (IIa $R''=CH_2CCl_3$)7) and bis-p-nitrobenzylphosphochloridate (IIb $R''=CH_2$-Ph-(p)-NO_2)8) were chosen for phosphorylation. Thus the diester-phosphoamidates (IIIa1 B=thymine R'=H , $R''=CH_2CCl_3$; IIIa2 B=thymine R'=H, $R''=CH_2$-Ph-(p)-NO_2) and (IIIb1 B=adenine R'=OH, $R''=CH_2CCl_3$; IIIb2 B=adenine R'=OH, $R''=CH_2$-Ph-(p)-NO_2) were prepared in 5o-85% yield. For reacting nucleosides with the di-p-nitrobenzyl phosphochloridate (IIb) dioxane p.a. as a solvent and triethylamine as base gave the best results. Cleavage of one ß,ß,ß-trichloroethyl-ester group can be achieved quantitatively with pyridine/ ammonia (1:1)7) at room temperature. However the remaining ß,ß,ß-trichloroethyl group in (IVa1) and (IVb1) is stable to alcaline hydrolysis. Snake venom diesterase9) is capable of cleaving the monoester phosphoamidates (IVa1; IVb1). The expected phosphoamidate (V) is very unstable and is hydrolyzed, even in buffer solutions (p_H=6.5- p_H=9)

[+] Nucleoside-Phosphoamidates Part I

to yield (I) and phosphate. Consequently reductive fission7) of (IIIa1) and (IIIb1) with zinc under various conditions also leads to the 5'-amino-5'-deoxynucleosides (Ia) and (Ib) exclusively. To prepare (V) the dinitrobenzyl groups in (IIIa2) and (IIIb2) were removed by catalytic hydrogenation10) in ethanol/aqueous buffer solution (pH=10) during a minimum of time. Dinucleosidephospho-amidates have been prepared and their behaviour on enzymatic cleavage was investigated11).

Satisfactory analytical data were obtained on all new compounds. The structure of (IIIb1) could be confirmed by mass spectrometry ($\frac{m}{e}$=609)12) whereas in (IIIa1) the highest peak clearly corresponds to the molecule stripped of its base ($\frac{m}{e}$=459). NMR-data12) of the compounds are consistent with the assigned structures. Proton resonance spectra of the trichloroethylphosphate group (=R") are very likely determined by i) restricted rotation around the C-C bond causing non-equivalence of the two CH_2-protons and hence an AB-spectrum and ii) coupling with P. Only the center set of doublets is immediately recognized.

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