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A NEW TYPE OF NUCLEOSIDE 5'-TRIPHOSPHATE ANALOGUE: P_1-(NUCLEOSIDE 5'-) P_1-AMINO-TRIPHOSPHATES

A. Simoncsits and J. Tomasz*

Institute of Biophysics, Biological Research Centre,
Hungarian Academy of Sciences, H-6701 Szeged, Hungary

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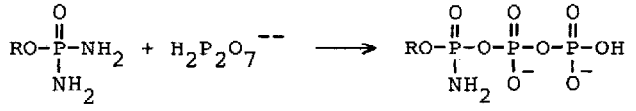
We report the synthesis of a new type of nucleoside 5'-triphosphate derivative, P_1-(nucleoside 5'-) P_1-amino-triphosphates (2), and some of their chemical properties, mainly their behaviour under different hydrolytic conditions. The synthesis is based on the selective replacement of one of the two amide groups of nucleoside 5'-phosphorodiamidates (1)[1] and may be formulated as follows:



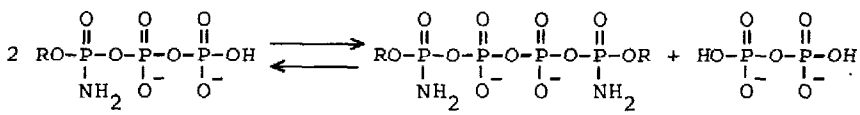
1 2

R= a; thymidine 5'
b; adenosine 5'

Treatment of a solution of thymidine 5'-phosphorodiamidate (1a)(0.1 mmole)[1] in anhydrous N.N-dimethylformamide (1 ml) with a fivefold molar excess of bis-tri-n-butylammonium pyrophosphate[2] at room temperature for 30 hr yielded P_1-(thymidine 5'-) P_1-amino-triphosphate (2a) as the main product (72%). In addition, the reaction mixture contained 1a (9%), thymidine 5'-phosphoramidate (3a)(4%), P_1-(thymidine 5'-) P_1-amino-diphosphate (4a)(6%), P_1P_4-di(thymidine 5'-) P_1P_4-diamino-tetraphosphate (5a)(6%) and thymidine 5'-triphosphate (6a)(3%). After separation on a DEAE-Sephadex column at 4°,

2a was isolated as chromatographically pure sodium salt; R_f (PEI-cellulose, 1.0 M sodium chloride) 0.28; UV spectra at pH values of 7.0 and 11.0 were identical with those of thymidine[3]; thymine : P_{total} = 1.00 : 2.91[4]. The structure of the compound was confirmed by acid and enzymatic hydrolyses. Due to the sensitivity of phosphoramidates to acid[5], 2a was quantitatively converted into 6a in 0.5 N hydrochloric acid at room temperature in 30 min. Upon hydrolysis with Escherichia coli alkaline phosphatase, 3a was detected as the only UV absorbing end product; $P_{enzyme\ labile}$: P_{total} = 0.67. The structure of by-products, 4a and 5a, was proved by synthesizing them from 1a with orthophosphate or 2a, respectively. These reactions could be responsible for the production of 4a and 5a during the synthesis of 2a.

As expected, enhanced reactivity of 2a was observed in comparison with 6a. As a result of the uncharged α-phosphorus atom and its decreased electron density, as well as the good leaving group property of the pyrophosphate group, 2a can easily be attacked by nucleophilic agents. Under slightly alkaline conditions (e.g. 1.0 N ammonium hydroxide, 25°), the substance was hydrolyzed to 3a and inorganic pyrophosphate (PP_i)[7]. In anhydrous N.N-dimethylformamide, at room temperature, a reversible exchange reaction was observed:



2a 5a

R=thymidine 5'

In this case, nucleophilic attack by the secondary hydroxyl group of the γ-phosphate of one molecule took place on the α-phosphorus atom of another molecule of 2a[8]. Under the conditions described 6a was quite stable.

2a behaved as a typical phosphoramidate upon acidic hydrolysis. It should be

noted, that the compound was split to 6a even at pH 6.5, the main product being, however, 3a at pH 7.5[9]. At the same time, acidic hydrolysis in the presence of molybdate resulted in the formation of 3a and PP_i. This indicates a marked difference in the complexing abilities of 3a and 6a.

P_1-(adenosine 5'-) P_1-amino-triphosphate (2b) was synthesized from adenosine 5'-phosphorodiamidate (1b) under identical conditions in a yield of 57%; adenine : P_{total} : $P_{enzyme\ labile}$ = 1.00 : 2.96 : 1.89; R_f (PEI-cellulose, 1.0 M sodium chloride) 0.29. Except for hydrolysis under slightly alkaline conditions, 2b behaved analogously with 2a. The slightly alkaline hydrolysis of 2b is a complex process giving at least eight different products[10]. The detailed study of this reaction is in progress and the results will be published shortly.

Compounds of type 2 as nucleoside 5'-triphosphate analogues containing chemically modified, chiral α-phosphorus atoms may be important from the point of view of different chemical and enzymatic studies. Experiments along these lines are in progress.

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References and Notes

(1) A. Simoncsits and J. Tomasz, Nucleic Acids Res., 2, 1223 (1975).

(2) Prepared from $Na_4P_2O_7.10H_2O$ according to J. G. Moffatt, Can. J. Chem., 42, 599 (1964), and used as 0.5 M stock solution in N.N-dimethylformamide.

(3) J. J. Fox and J. D. Shugar, Biochim. Biophys. Acta, 9, 369 (1952).

(4) Phosphorus was determined according to H. Eibl and W. E. M. Lands, Anal. Biochem., 30, 51 (1969).

(5) (a) R. W. Chambers and J. G. Moffatt, J. Am. Chem. Soc., 80, 3752 (1958); (b) J. G. Moffatt and H. G. Khorana, ibid., 83, 649 (1961).

(6) Escherichia coli alkaline phosphatase hydrolyses $(HO)_2P(O)$-O-P(O) pyrophosphate linkages, too. See L. A. Heppel, D. R. Harkness and R. G. Hilmoe, J. Biol. Chem., 237, 841 (1962).

(7) Quantitative hydrolysis was observed within 2 min at room temperature in 0.5 N sodium hydroxide.

(8) 5a could thus, be produced also in this way during the synthesis of 2a.

(9) At room temperature after 24 hr, quantitative decomposition was observed at pH 6.5, but more than 60% of 2a remained unaltered at pH 7.5.

(10) Similarly to 2a, only adenosine 5'-phosphoramidate was produced in 0.5 N sodium hydroxide.