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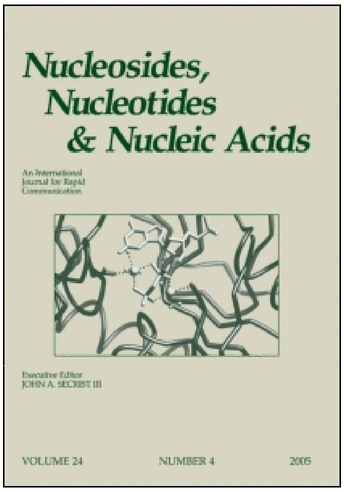

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Nonenzymatic Hydrolysis of an RNA-DIMER Containing a Thiophosphate Linkage

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NUCLEOSIDES & NUCLEOTIDES, 10(1-3), 653-655 (1991)

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NONENZYMATIC HYDROLYSIS OF AN RNA-DIMER CONTAINING A THIOPHOSPHATE LINKAGE.

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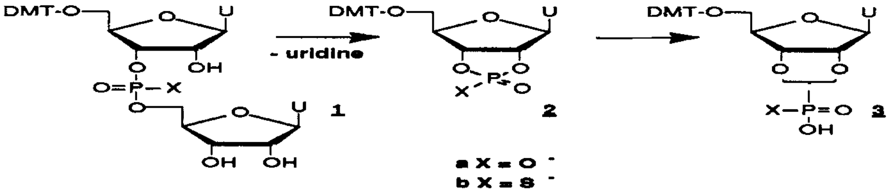
Abstract: A Diuridine (3´-5´)-thiophosphate (**1b**) was hydrolyzed under basic conditions. The rate of hydrolysis of the uridylyl (3´-5´)- uridine (**1a**) was three times higher compared to that of **1b**.

Phosphorothioates has been extensively used in investigations of enzymatic mechanisms[1] but not much has been reported in terms of chemical data for hydrolysis. When evaluating data from enzymatic processes it is valuable to have a reference point in terms of knowledge of the difference in rate for the corresponding chemical reactions. In this study the rate of cleavage of 5´-O-(4,4´-dimethoxytrityl)uridin-3´-yl uridin-5´-yl thiophosphate (**1b**) and 5´-O-(4,4´-dimethoxytrityl)uridylyl (3´-5´)-uridine (**1a**) under basic conditions was measured.



The hydrolyses were carried out in 0.067M NaOH (water:dioxane/2:1, ionic strength was 0.2M (NaCl)) at 50 C. Analysis was done by separating the products from the starting dimer using t.l.c. (SiO_2 , isopropanol:conc. ammonia:water / 7:1:2). The different spots were scraped off the plate and subjected to repeated extractions with 2.5% dichloroacetic acid in dichloroethane. These extracts were then analyzed spectrophotometrically at 498 nm (dimethoxytrityl cation) and the rate-curves obtained from the percentage of remaining dimer. The reactions were also followed using ^{31}P n.m.r. to make sure that no unexpected reactions occured.

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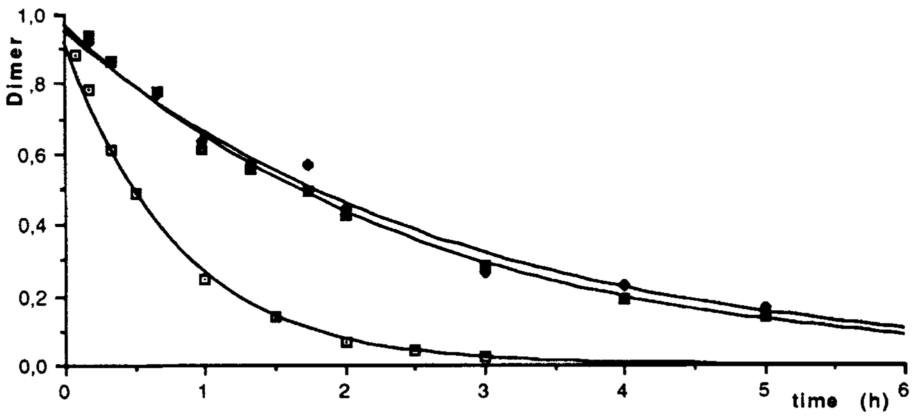

Cleavage of **1a** (bottom line) and **1b** (the two upper lines) in 0.067M NaOH (water:dioxane/2:1, ionic strength:0.2M (NaCl). All reactions follow first order kinetics.

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The rates of hydrolysis of the two diastereoisomers of **1b** (synthesized *via* an H-phosphonate approach[2]) were measured separately but little difference was observed. More interesting is that the rate of cleavage of **1a** and **1b** only differs by a factor of ~3. This is remarkable since a factor of 30-100[3,4] is often taken as the difference between the rate of hydrolysis of diesters of thiophosphoric and phosphoric acid. However, this is mainly based on values from triesters[5] and such an extrapolation does not seem to hold in light of the present work.

Whether the small difference in rate between the thiophosphates and the phosphate observed here is an inherent property of this particular system or general for diesters will be investigated further. In any case the present system, involving dialkyl esters and an internal nucleophile with a high degree of proximity, should be more relevant to enzymatic processes than the bimolecular substitutions on aryldialkyl and diarylalkyl triesters[5]. However, the important point from this work is that the difference between rates of cleavage of diesters of phosphoric acid and thiophosphoric acid can be substantially smaller than previously assumed.

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