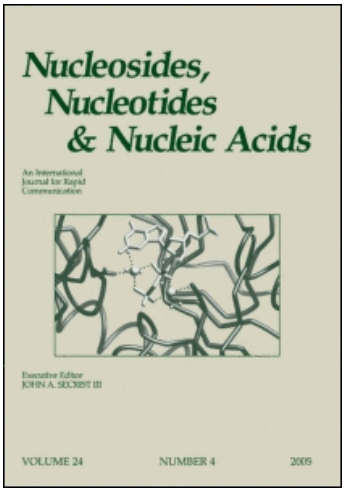

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Investigation of a Proposed Mechanism for Genotoxic Effects Induced by 2-Hydroxyalkylating Agents. Kinetics of Intramolecular Transesterification in Dithymidine 2-Hydroxyethyl-and 2-Hydroxypropyl Phosphate

E. Pettersson[a]; E. Bergmark[b]; U. Rannug[c]; L. Ehrenberg[d]; R. Ströumberg[a]
[a] Departments of Organic Chemistry, Stockholm University, Stockholm, Sweden [b] Departments of Organic Chemistry Organic Chemistry, Stockholm University, Stockholm, Sweden [c] Departments of Organic Chemistry Radiobiology, Arrhenius Laboratory, Stockholm University, Stockholm, Sweden [d] Genetic and Cellular Toxicology, Wallenberg Laboratory, Stockholm University, Stockholm, Sweden

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INVESTIGATION OF A PROPOSED MECHANISM FOR GENOTOXIC EFFECTS INDUCED BY 2-HYDROXYALKYLATING AGENTS. KINETICS OF INTRAMOLECULAR TRANSESTERIFICATION IN DITHYMIDINE 2-HYDROXYETHYL- AND 2-HYDROXYPROPYL PHOSPHATE

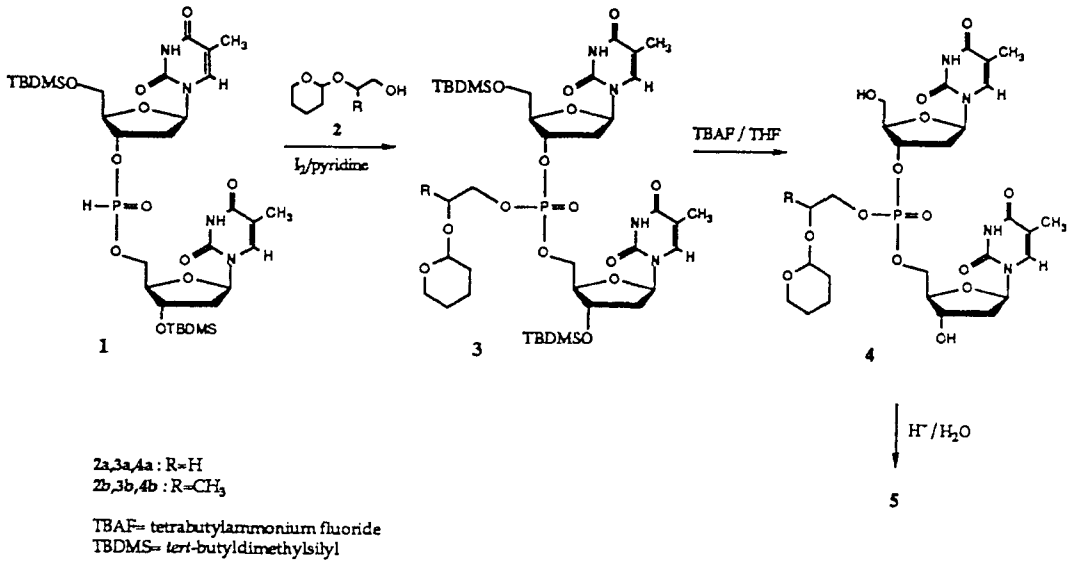
E. Pettersson[#], E. Bergmark[¤], U. Rannug[§], L. Ehrenberg[¤] and R. Strömberg[#*]

Departments of [#]Organic Chemistry and [¤]Radiobiology, Arrhenius Laboratory and [§]Genetic and Cellular Toxicology, Wallenberg Laboratory, Stockholm University, S-106 91 Stockholm, Sweden.

Alkylation of DNA gives rise to adducts, not only at the bases, but also at the phosphate groups giving phosphotriesters[1-3]. 2-Hydroxyalkylation of phosphodiester functions in DNA causes considerable strand breakage already in neutral solution[4]. This effect has been suggested to be involved in the higher genotoxicity of 2-hydroxyalkylating agents as compared to, for instance, the corresponding methoxy compounds[5].

Ethylene oxide (EO) is considerably more effective than propylene oxide (PO) in inducing various genotoxic effects[6,7]. It has been proposed that the difference in these effects for EO and PO could be due to the difference in rate of intramolecular cyclisation of the formed 2-hydroxyalkyl phosphotriester functions, and hence DNA strand cleavage[7].

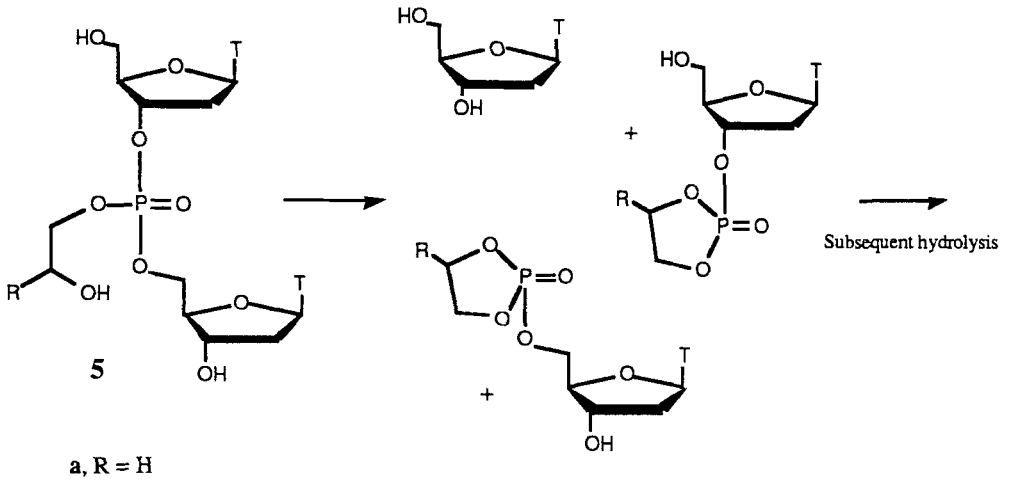
The Thorpe-Ingold effect[8,9] rather predicts that the propylene oxide adduct would cyclise faster as is also reported for hydroxyalkyl diesters[10]. However, since a mechanism violating this rule had been proposed, there was a need to establish the effect of an extra methyl group on intramolecular transesterfication of phosphotriesters and more specifically for a dinucleotide adduct.



Scheme 1. Synthesis of the model compounds dithymidine 2-hydroxyethyl phosphate (5a) and dithymidine 2-hydroxypropyl phosphate (5b).

To evaluate this potentially DNA-strand breaking reaction we decided to employ two model compounds, dithymidine 2-hydroxyethyl- and 2-hydroxypropyl-phosphate (**5a** and **5b**). These compounds were synthesized from 5'-O-*t*-butyldimethylsilylthymidine 3'-(3'-O-*t*-butyldimethylsilylthymidine 5'-H-phosphonate) (**1**) according to Scheme 1. In the oxidative coupling between **1** and the alcohol **2** we used the procedure with iodine as the oxidant[11].

The rates of intramolecular transesterification for these model compounds (Scheme 2) in aqueous buffers at 37° C was then determined. The kinetic parameters were obtained by reversed phase HPLC analysis of aliquots withdrawn from the reaction mixtures. The rates of cyclisation were determined and the results showed that the dithymidine 2-hydroxypropyl triester **5b** cyclises about four times faster than the 2-hydroxyethyl compound **5a** (Table).



a, R = H
b, R = CH_3

Scheme 2. Intramolecular transesterification in the dithymidine 2-hydroxyalkyl triesters **5**.

Table. Kinetic parameters for cyclisation of the model compounds **5**.

pH	$Tp_{(HOEt)}T$ (**5a**)		$Tp_{(HOPr)}T$ (**5b**)	
	k (10^{-5} s^{-1})	$\tau_{1/2}$ (h)	k (10^{-5} s^{-1})	$\tau_{1/2}$ (h)
7.0	1.16	16.5	4.76	4.0
7.4	2.23	8.6	9.36	2.0

The relative rate of **5a** and **5b** does indeed show that the Thorpe-Ingold effect also applies to this system but, as in the study on 2-hydroxyalkyl phosphodiesters[10], is not particularly high due to the rotational freedom of the hydroxyalkyl groups. From the observed rate differences we can then conclude that the higher ability of EO compared to PO in induction of genotoxic effects can not be caused by this type of reaction.

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