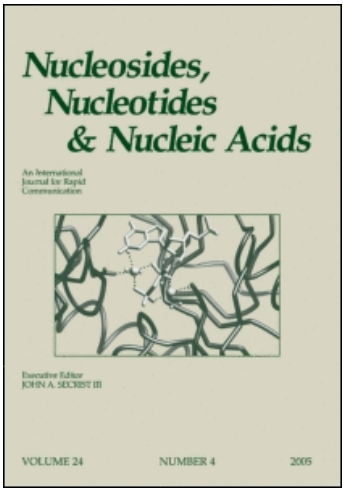

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Studies on the Stability of Dinucleoside *H*-Phosphonates

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ABSTRACT

The stability of two dinucleoside *H*-phosphonates under various conditions is reported.

Key Words: *H*-Phosphonates; Stability; Hydrolysis; Oligonucleotides.

The stability of two *H*-phosphonate dimers (**1** and **2**) under various conditions has been studied. The conditions are typically either used to remove some common protective groups in oligonucleotide chemistry, or useful for giving information about possible instability during storage and normal laboratory procedures. We hope that this practically oriented study will be a useful addition to the relatively sparse literature on the stability of *H*-phosponate diesters in solution.[1,2,3] A sample of **1** or **2** was dissolved in the test media to make a 30 mM solution. ^{31}P NMR was used to monitor the stability of various test solutions at 20°C. The results are summarized in Table 1. The *H*-phosphonate linkage is relatively stable in wet organic solvents, acidic, moderately basic and anhydrous conditions. The observed rate of hydrolysis is in close accordance with previously reported values in aqueous

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Table 1.

Conditions	1	2
0.5 M H_2O in MeCN	No cleavage $\leq$ 44 h	No cleavage $\leq$ 44 h
0.5 M H_2O in Pyridine	T $\frac{1}{2}$ = 43 h	T $\frac{1}{2}$ = 77 h
0.5 M N_2H_4 in Py/AcOH (4:1)	T $\frac{1}{2}$ = 6.8 h	T $\frac{1}{2}$ = 14.4 h
80% Acetic acid	T $\frac{1}{2}$ = 21 h	T $\frac{1}{2}$ = 30 h
2% DCA in DCM + 0.5 M pyrrole	No cleavage product observed during 19 h	No cleavage product observed during 20 h
1:3 TEA/MeCN MeCN with 35 ppm water TEA distilled over CaH_2	Material intact: $\geq$ 99% 30 min 95% 90 min 90% 210 min	Material intact: $\geq$ 99% 30 min 95% 5 h 90% 15 h
10% MeOH, 0.1% TEA in DCM	T $\frac{1}{2}$ = 12 h[a]	
10% MeOH in DCM	No reaction during 40 h	
10% MeOH, 0.1% Pyridine in DCM	No reaction during 20 h	
40% 2-propanol, 0.1% TEA in DCM	10% transesterification during 58 h	
3 eq TEA 3HF in DCM	T $\frac{1}{2}$ = 8.5 min[b]	
1.1 eq DBU in MeCN	Decomposing rapidly[c]	

[a]Transesterification by methanol.
[b]As evidenced from TLC and ^{31}P-NMR the dimer was cleaved to give both nucleosides and monoflourophosphonic acid (presumably by moisture related hydrolysis of the difluoro *H*-phosphonate), with no monoester or fluoro *H*-phosphonate monoester detected.
[c]Both hydrolysis products and what is likely to be 3′,3′ and 5′,5′ symmetrical diesters were detected; approximately 65% intact dimer after 3 h. Such side reaction has also been reported by others.[3]

solutions.[1] Contact of **1** or **2** with strong organic base (DBU) or fluoride ions cleave dinucleoside *H*-phosphonates rapidly. Also, caution is recommended in use of chromatography systems involving a protic solvent, e.g., methanol, in combination with a base. If such conditions are necessary, use of a more hindered alcohol such as 2-propanol is recommended. The stability difference of **1** vs. **2** is approximately 1 : 2 under the conditions described in Table 1. The greater stability of **2** may be attributed to the steric hindrance that the MOE[4] group is introducing, since the altered electronic properties of the sugar moiety carrying the *O*-alkyl group in close proximity would be expected to render the dimer more sensitive to hydrolysis.

EXPERIMENTAL

The dimers were synthesized by standard *H*-phosphonate couplings: the respective 3′-*H*-phosphonates ($TEAH^+$ salts) with 3′-MMT-T using either pivaloyl chloride or bis(2-oxo-3-oxazolidinyl) phosphinic chloride and was further purified by silica gel chromatography essentially as for protected diribonucleoside *H*-phosphonates.[5] *Stability tests.* Reactions were followed by ^{31}P NMR using a Bruker

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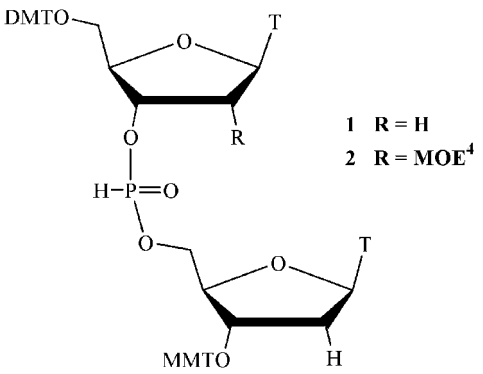

DRX400 instrument, 2 mL sample volume, 30 mM *H*-phosphonate dimer concentration, T = 20°C, proton decoupling, manual integration using external H_3PO_4 in D_2O as reference and standard. The first data point was typically taken after 5 to 10 min from starting the reaction.

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