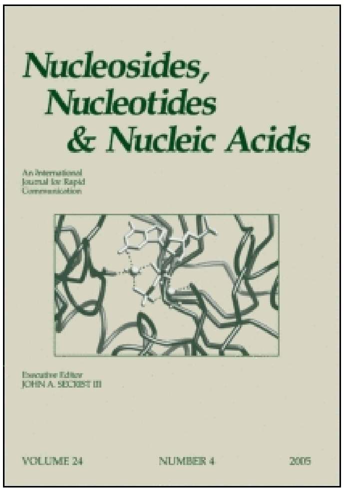

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A Cautionary Note on the Use of the ^{31}P NMR Spectroscopy in Stereochemical Correlation Analysis

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A CAUTIONARY NOTE ON THE USE OF THE ^{31}P NMR SPECTROSCOPY IN STEREOCHEMICAL CORRELATION ANALYSIS

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▫ *Stereoselectivity in condensation of protected ribonucleoside 3′-H-phosphonates with hydroxylic components was investigated using ^{31}P NMR spectroscopy. The correlation between absolute configuration at the phosphorus center and the chemical shifts of the produced H-phosphonate diesters and the corresponding phosphorothioates, was studied.*

Keywords *H*-Phosphonates, Stereospecific Coupling, ^{31}P NMR Spectroscopy

INTRODUCTION

In 1983 F. Eckstein formulated a postulate which correlated absolute configuration at the phosphorus centre of di(deoxyribonucleoside) phosphorothioates with their chemical shifts in ^{31}P NMR.[1] Thus, the diastereomers with R_P configurations were found to resonate at lower field while the S_P ones resonate at higher fields. This rule was later extended to the ribo series.[2]

A correlation between configuration at the phosphorus centre and the ^{31}P NMR chemical shifts was also observed for other classes of nucleotide analogues, e.g., aryl phosphorothioate triesteres,[3] tervalent phosphoramidites,[4] and oxathiaphospholanes,[5] although not all of these compounds followed Eckstein's rule.[1] Particularly, for protected pentavalent nucleotide derivatives, a tendency opposite

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to that suggested by Eckstein was frequently observed. For example, for ribonucleoside *H*-phosphonates[2,6] and *H*-phosphonothioates,[7] the S_P diastereomers resonate usually downfield relatively to the R_P ones. However, to the best of our knowledge, within the class of particular compounds, no changes in order of chemical shifts between R_P and S_P were observed; thus, the rule seemed reliable for assigning absolute configuration at the phosphorus centre for closely related compounds.

RESULTS AND DISCUSSION

To get better understanding of factors influencing stereoselectivity of ribonucleoside *H*-phosphonate diesters formation, we performed many reactions of 5′-*O*-dimethoxytrityl-2′-*O*-*t*-butyldimethylsilyl ribonucleoside *H*-phosphonates with suitably protected nucleosides or with simpler alcohols, to produce *H*-phosphonate diesters. The reactions were performed in various solvents and were monitored by ^{31}P NMR spectroscopy. The obtained diesters were then stereospecifically sulfurized with elemental sulfur[2,8] to the corresponding phosphorothioates and analysed again by ^{31}P NMR.

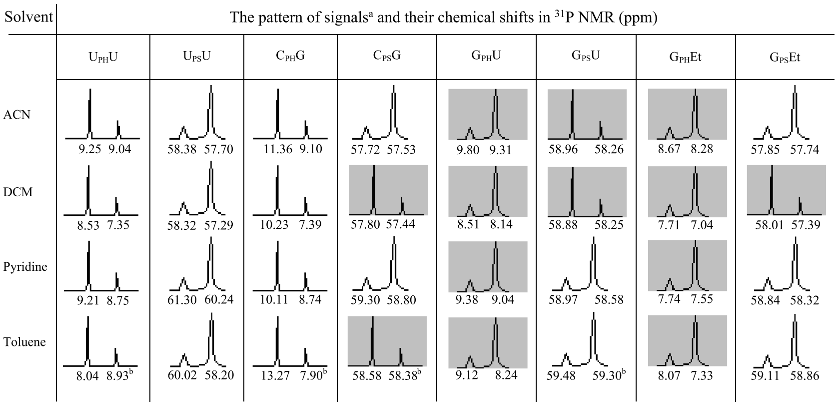
Almost always the main signal of *H*-phosphonate diester was the low field one, and of phosphorothioate diester, the high field one. This was expected and congruent with literature data.[6] However, in some cases an apparent swap of signals of P-diastereomers (i.e., a high field signal of *H*-phosphonate diester or a low field signal of the phosphorothioate diester became the major one) was observed.

A reversal of stereoselectivity in these instances seemed to be rather unlikely, taking into account that in most cases the anomalies for the *H*-phosphonate diesters and the corresponding phosphorothioates did not coincide. Thus, the most probable explanation was that the observed changes in relative positions of the phosphorus resonances of these compounds were not due to changes in stereochemistry but due to solvent effects, although this would violate the aforementioned Eckstein rule for this class of compounds.[2]

To have a closer look on this problem, we have chosen four representative cases in which different patterns of ^{31}P NMR signals were observed, namely the reactions of U_{PH} + U, C_{PH} + G, G_{PH} + U, and G_{PH} + EtOH. These condensations were carried out in acetonitrile (ACN) containing 3 equiv. of pyridine, and after completion, ACN was evaporated and replaced sequentially with dichloromethane (DCM), pyridine, and toluene and the ^{31}P NMR spectra were recorded. The same solvent exchange procedure was used after sulfurization of the above *H*-phosphonate diesters (Table 1).

From the data in Table 1 it seems apparent that for some diesters the order of the ^{31}P NMR signals was independent of the solvent used and was either a typical (as for $U_{PH}U$, $U_{PS}U$, $C_{PH}G$) or an anomalous ($G_{PH}U$, $G_{PH}Et$) one. However, for three phosphorothioates ($C_{PS}G$, $G_{PS}U$, $G_{PS}Et$) the order of the ^{31}P NMR signals was solvent-dependent and indicated that relative position of resonances of

TABLE 1 Positions of ^{31}P NMR Resonances of Protected *H*-Phosphonate Diesters and the Corresponding Phosphorothioates in Various Solvents.



Solvent	The pattern of signals[a] and their chemical shifts in ^{31}P NMR (ppm)							
	$U_{PH}U$	$U_{PS}U$	$C_{PH}G$	$C_{PS}G$	$G_{PH}U$	$G_{PS}U$	$G_{PH}Et$	$G_{PS}Et$
ACN	9.25 9.04	58.38 57.70	11.36 9.10	57.72 57.53	▨ 9.80 9.31	▨ 58.96 58.26	▨ 8.67 8.28	57.85 57.74
DCM	8.53 7.35	58.32 57.29	10.23 7.39	▨ 57.80 57.44	▨ 8.51 8.14	▨ 58.88 58.25	▨ 7.71 7.04	▨ 58.01 57.39
Pyridine	9.21 8.75	61.30 60.24	10.11 8.74	59.30 58.80	▨ 9.38 9.04	58.97 58.58	▨ 7.74 7.55	58.84 58.32
Toluene	8.04 8.93[b]	60.02 58.20	13.27 7.90[b]	▨ 58.58 58.38[b]	▨ 9.12 8.24	59.48 59.30[b]	▨ 8.07 7.33	59.11 58.86

The anomalies are marked with grey background

[a] The inserts are symbolic representations of the signals pattern, not the actual spectra.

[b] Due to poor solubility of the diester in neat toluene, a mixture containing 20% of DCM and 80% (v/v) of toluene was used.

diastereomeric compounds in ^{31}P NMR spectra may depend both on their stereochemistry at the phosphorus centre as well as on the solvent used. To substantiate these findings, P-diastereomers of the phosphorothioate diester $G_{PS}U$ were isolated, deprotected,* and subjected to a digestion with SVPD. As expected, only the major diastereomer was a substrate for the enzyme. This confirmed its R_P configuration and excluded the possibility of a reversal of stereochemistry in condensation for some nucleotides as might ^{31}P NMR data suggest. Thus, we could assign the R_P configuration to the main diastereomers of the phosphorothioate diesters and the S_P configuration to the main diastereomers of the *H*-phosphonate diesters.

In conclusion, these studies indicate that the published rules correlating absolute configuration at the phosphorus centre with their ^{31}P NMR chemical shifts have to be used with caution, particularly for derivatives containing guanosine.

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*Interestingly, even for deprotected dinucleoside phosphorothioates the order of the ^{31}P signals followed the Eckstein rule only in water, while in ACN their positions were reversed.

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