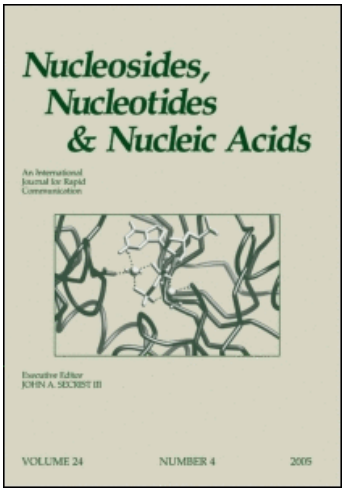


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Conversion of Nucleoside H-Phosphonate Monoesters to the Corresponding H-Phosphonothioates. ^{31}P NMR Studies

Jacek Stawiński[a]; Rula Zain[a]
[a] Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, Stockholm, Sweden

To cite this Article Stawiński, Jacek and Zain, Rula(1995) 'Conversion of Nucleoside H-Phosphonate Monoesters to the Corresponding H-Phosphonothioates. ^{31}P NMR Studies', Nucleosides, Nucleotides and Nucleic Acids, 14: 3, 711 — 714
To link to this Article: DOI: 10.1080/15257779508012455
URL: http://dx.doi.org/10.1080/15257779508012455

NUCLEOSIDES & NUCLEOTIDES, 14(3-5), 711-714 (1995)

CONVERSION OF NUCLEOSIDE H-PHOSPHONATE MONOESTERS TO THE CORRESPONDING H-PHOSPHONOTHIOATES. ^{31}P NMR STUDIES

Jacek Stawiński* and Rula Zain

Department of Organic Chemistry, Arrhenius Laboratory,
Stockholm University, 106 91 Stockholm, Sweden.

Abstract

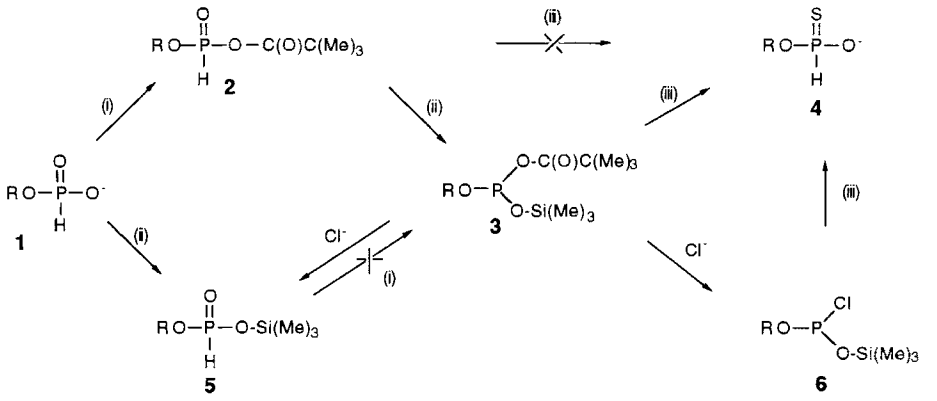
^{31}P NMR Studies on the conversion of nucleoside H-phosphonate monoesters into the corresponding H-phosphonothioates revealed that the key intermediate, the nucleoside trimethylsilyl pivaloyl phosphite ***3****, may undergo under the reaction conditions at least two parallel transformations to, most likely, the nucleoside trimethylsilyl chlorophosphite* ***6*** *and the monosilylated nucleoside H-phosphonate* ***5****.*

Introduction of various modifications at the phosphorus center of natural product derivatives may serve as a valuable means for investigation of mechanisms of enzymatic reactions[1]. Such knowledge is of prime importance for the development of new enzyme inhibitors or natural product analogues which are resistant to enzymatic degradation, while retaining some other biologically important properties.

As a part of our studies in H-phosphonate chemistry, we recently investigated the possibility of replacing one oxygen atom by sulfur at the H-phosphonate center to produce P-chiral H-phosphonothioates. For this purpose we proposed a conversion of nucleoside H-phosphonate monoesters into the P(III) derivatives of type **3a**, which upon treatment with hydrogen sulfide, underwent thiation to the desired H-phosphonothioate **4a**[2].

RESULTS AND DISCUSSION

Previously we have found that silylation of the mixed anhydride of type **2** *prior* thiation with hydrogen sulfide was necessary to avoid formation of



1a-6a, R=5'-O-dimethoxytritylthymidin-3'-yl; **1b-6b**, R=ethyl
(i) Pivaloyl chloride in quinoline/acetonitrile (1:4, v/v); (ii) Trimethylsilyl chloride in in quinoline/acetonitrile (1:4, v/v); (iii) Hydrogen sulfide in dioxane.

Scheme 1

nucleoside H-phosphono*di*thioate monoesters[2]. Since we postulated that the key synthetic intermediate in the reaction scheme is the P(III) derivative **3**[2], we carried out some ^{31}P NMR studies to investigate in more detail the formation of this intermediate and some of its chemical properties.

To this end, the mixed anhydride **2a**, formed *in situ* from the H-phosphonate **1a** and 1.5 equiv. of pivaloyl chloride (PV-Cl) in quinoline-acetonitrile (1:4, v/v), was treated with 5 equiv. of trimethylsilyl chloride (TMS-Cl). Two singlets characteristic for one spin system of P(III) phosphorus diastereomers appeared at ~121 ppm, and these were assigned to the silyl acyl phosphite **3a**. Together with this major product we always observed three additional resonances of variable intensities (up to ca 60%), at ~154 (singlet) and ~-2.5 (two singlets) ppm. The ratio of these signals was rather insensitive to larger excess of TMS-Cl. During time the signals from **3a** were gradually decreasing and finally disappeared completely after a few hours. Addition of hydrogen sulfide at various stages of the reaction resulted in an immediate disappearance of the signal at ~154 ppm and those from **3a**, and formation of nucleoside H-phosphonothioate **4a**. The resonances at ~-2.5 ppm remained unchanged. Upon addition of ethanol, the signals at ~154 and ~121 ppm were replaced by new ones at ~8 ppm, assigned to the nucleoside ethyl H-phosphonate.

From the above experiments it became apparent that irrespective of the chemical nature of the intermediate which resonated at ~154 ppm, it reacted similarly with hydrogen sulfide as the silyl acyl phosphite **3a**, to produce the desired monothio derivative **4a**. The signals at ~-2.5 ppm, however, should be considered as coming from a side product, since they could not be converted into **4a**.

To be able to eliminate or to diminish the extent of the side reaction during silylation of **2** (formation of a compound resonating at ~-2.5 ppm), we made an attempt to tentatively identify intermediates resonating at ~154 and ~-2.5 ppm. Changes in the pattern of signals in the ^{31}P NMR spectra suggested that both compounds are most likely formed from the key intermediate **3a**. For the first intermediate, the chemical shift value of ~154 ppm indicated on a tricoordinated species with one P-halogen bond. Since this intermediate in the reaction with hydrogen sulfide behaved similarly to **3a** (i.e., it acted as a monofunctional phosphitylating agent), we tentatively assigned the resonance at ~154 ppm to the silylphosphorochloridite **6a**. Such a compound could be formed form **3a** by replacement of pivaloyloxy group by chloride anion, or in a synchronous reaction of TMS-Cl with **3a**.

The signals at ~-2.5 ppm were assigned to the diastereomers of monosilylated H-phosphonate **5a** by virtue of their chemical shift and coupling pattern in the $\{^1H\}$-decoupled ^{31}P NMR spectra. Spurious water may account for the formation of **5a** from the acyl silyl phosphite **3a** but only in part since it was generated in the reaction mixture even when large excess of TMS-Cl was present. We assumed thus that a plausible mechanism for the formation of silyl H-phosphonate **5a** could be deacylation of **3a** by chloride anions. Since PV-Cl would be regenerated by this mechanism, one has to assume also that **5a** does not react significantly under the reaction conditions neither with PV-Cl nor with an excess of the silylating agent.

Since the reaction sequence with ethyl H-phosphonate **1b** followed the same pathway with similar intermediates formed, we used **1b** as a model compound for further investigations. The following experiments were designed to verify our hypothesis concerning formation of **5** and **6** under the reaction conditions [solvent, quinoline-acetonitrile or pyridine-acetonitrile (1:4, v/v)]: (i) silylation of the H-phosphonate **1a** and **1b** with an excess of TMS-Cl or N,O-bis(trimethylsilyl)acetamide (BSA); (ii) the reaction of **5b** with an excess of PV-Cl; (iii) silylation of the mixed anhydride **2b** with BSA; (iv) activation of **1b** with PV-Cl in the presence of an excess of pyridinium hydrochloride (Py·HCl), followed by silylation; (v) reaction of **3b** with an excess of Py·HCl.

The results of these experiments supported pattern of transformations presented in Scheme 1 and can be summarized as follows. H-Phosphonate monoesters of type **1** underwent, in the solvent systems investigated, silylation to the monosilyl derivatives **5** which have been found identical with the side products resonating at ~-2.5 ppm. A conversion to the disilyl derivatives did not occur to any noticeable extent even after prolonged treatment with 10 equiv. of the silylating reagent (TMS-Cl), or was rather slow (for BSA, ~10% of disilyl derivative after 15 min). In contrast to the mixed anhydride **2b**, the monosilyl H-phosphonate **5b** could not be converted efficiently into the P(III) intermediate **3b**, and once formed, stayed in the reaction mixture. The important difference between BSA and TMS-Cl when used for the silylation of the mixed anhydride **2b** was that the former reagent produced exclusively the key intermediate **3b** without concomitant formation of the side product **5b**. Activation of the H-phosphonate **1b** with PV-Cl in the presence of 10 equiv. of Py·HCl produced the expected mixed anhydride **2b**; however, the subsequent silylation with BSA afforded as a predominant product the monosilyl derivative **5b**. Addition of 10 equiv. of Py·HCl to the *in situ* produced **3b** triggered an immediate formation of **5b** and **6b**. It is worth noting that addition of excess of TMS-Cl to the intermediate **3b** (produced from **2b** and BSA) did not result in any noticeable changes in the reaction mixture.

EXPERIMENTAL PART

5'-O-Dimethoxytritylthymidine 3'-H-phosphonate **1** was prepared and purified by the published procedure[3]. The ^{31}P NMR experiments (JEOL GSX-270 FT, 2% H_3PO_4 in D_2O as an external reference) were carried out at 25 °C in 10 mm tubes using 0.05 mmol/2 mL concentrations of phosphorus containing compounds in quinoline-acetonitrile (1:4, v/v) or as indicated in the text.

Acknowledgements

We are indebted to Prof. Per J. Garegg for his interest, to the National Swedish Board for Technical Development and the Swedish Natural Science Research Council for financial support.

REFERENCES

1. Eckstein, F.; Gish, G. *Trends Biochem. Sci.* **1989**, 97.
2. Stawiński, J.; Strömberg, R.; Zain, R. *Nucleosides & Nucleotides* **1991**, *10*, 515.
3. Garegg, P. J.; Regberg, T.; Stawiński, J.; Strömberg, R. *Chemica Scripta* **1985**, *25*, 280.