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NUCLEOSIDE H-PHOSPHONATES. X. STUDIES ON NUCLEOSIDE HYDROGENPHOSPHONOTHIOATE DIESTER SYNTHESIS

Jacek Stawiński*, Mats Thelin, Rula Zain

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

Abstract
Synthesis and chemical properties of nucleoside H-phosphonothioates are discussed in the context of possible application of these compounds as intermediates in the synthesis of oligonucleotide analogues.

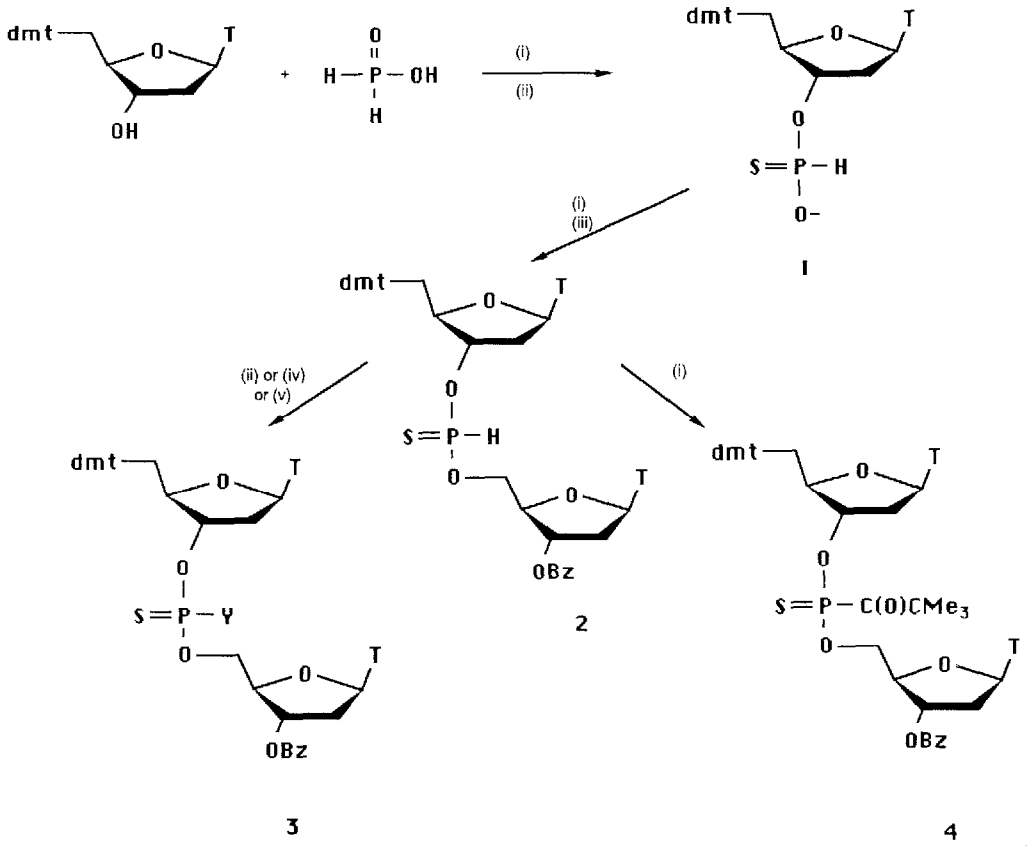
During our studies on nucleoside hydrogenphosphonates as starting materials for oligonucleotide synthesis[1] and as useful intermediates for the preparation of various phosphate esters and their analogues[2], we became interested in hydrogenphosphonothioates. The chemistry of this type of nucleotide analogues, potentially useful as precursors for introduction of a modified phosphate group into oligonucleotides, has not yet been explored. Recently, Caruthers *et al.*[3] reported on a synthesis of a new type of nucleotide analogue, phosphorodithioates, from dinucleoside phosphoramidites *via* H-phosphonothioates as intermediates. Since H-phosphonothioate diesters seems to be promising also for the preparation of other phosphate analogues (phosphorothioamidates, alkyl phosphorothioates and phosphorothioates)[3], easy access to such intermediates would be highly desired. However, attempted synthesis of H-phosphonothioate diesters from the appropriate monoesters, analogously to the H-phosphonate synthesis, failed to produced the desired compound of type **2**[3].

In this communication we would like to present some our studies on the chemistry of H-phosphonothioate esters, which are relevant to possible applications of these compounds as starting materials and intermediates for oligonucleotide analogues synthesis.

To investigate the feasibility of producing H-phosphonothioate diesters starting from nucleoside H-phosphonothioates, compound **1**[4] and 2´-O-benzoylthymidine (1.2 equiv.) were rendered anhydrous by repeated evaporation of added pyridine, and after dissolving in the same solvent, pivaloyl chloride (PV-Cl, 1.5 equiv.) was added. The TLC and ^{31}P NMR analyses have shown that condensation of the H-phosphonothioate monoester **1** with a hydroxylic component was as fast as an analogous reaction using H-phosphonates[1], and produced the desired H-phosphonothioate diester **2**[5]. After recording the second ^{31}P NMR spectrum, however, an additional signal at 139.45 ppm and two signals at 65.72 and 65.55 ppm (absence of $^{1}J_{PH}$ couplings, $^{3}J_{PH}$=9.8 Hz, quartets) appeared. The intensity of the signals at ca. 65 ppm increased at the expense of signals from the compound **2** when more PV-Cl was added. On the

other hand, when an additional portion of HO-T-OBz (1 equiv.) was added, the intensity of the signal at 139.45 ppm increased. On the basis of chemical shift values and the pattern of resonances in the ^{31}P NMR spectra, the signal at 139.45 ppm was assigned to a trinucleoside phosphite triester (comparison with the reference compound[6]) and the signals at ca. 65 ppm, to two diastereoisomers of the acylphosphonothioate **4**[7].

Scheme 1



Synthesis and reactions of nucleoside H-phosphonothioates. (i) pivaloyl chloride, (ii) sulfur; (iii) 2′-O-benzoylthymidine; (iv) selenium; (v) iodine in pyridine/water.

3a, $Y=S^-$; **3b**, $Y=Se^-$; **3c**, $Y=O^-$.

These experiments indicate that the P-H bond in H-phosphonothioates is much more reactive then the analogous bond in H-phosphonates, and that the desired product **2** may undergo fast P-acylation (ca. 5 min.) even when only a small excess of PV-Cl (1.5 equiv.) is used. The degree of acylphosphonothioate formation depends on the excess of PV-Cl, being almost quantitative with 5 equiv. of this condensing reagent during ca. 15 min. In contradistinction to H-phosphonate diesters, the thio analogues seem to undergo also an activation with PV-Cl. If an excess of a hydroxylic component is present in the reaction mixture, this results in elimination of sulfur and formation of phosphite triesters. Since these

two side reactions (P-acylation and activation of H-phosphonothioate diesters) are rather fast, a synthesis of H-phosphonothioate diesters is doomed to failure, if reaction conditions are not properly chosen (e.g. excess of PV-Cl and/or an hydroxylic component, long reaction time).

A similar course of the reaction was observed when **1** was allowed to react with dmt-T-OH in the presence of PV-Cl, and some selected ^{31}P NMR spectra are shown on Fig. 1.

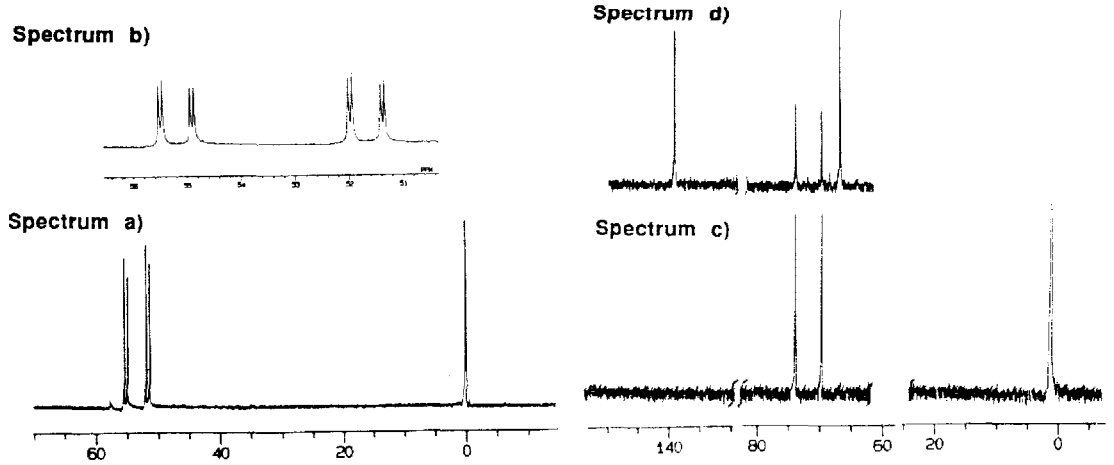


Fig. 1. ^{31}P NMR spectra (H_3PO_4 as a reference) of: a) 5′-O-dimethoxytritylthymidine 3′-H-phosphonothioate **1**; b) expanded fragment of the spectrum a; c) coupling reaction of **1** with 5′-O-dimethoxytritylthymidine (1.5 equiv.) in the presence of PV-Cl (1.2 equiv.); d) the reaction as in spectrum c, but in the presence of 3 equiv. of PV-Cl.

Our previous studies on the H-phosphonate chemistry have shown that H-phosphonate monoesters can be activated with a variety of condensing reagents of different chemical nature[8]. Since sulfur is a poor nucleophile for a phosphorus centre, using chlorophosphate as condensing reagents for the H-phosphonothioate diesters synthesis should reduce or even eliminate a phosphite triester formation, and obviously, will make the problem of P-acylation irrelevant. To check if chlorophosphates are more suitable condensing agents, compound **1** was allowed to react with 2′-O-benzoylthymidine (1.1 equiv.) in pyridine in the presence of diphenylphosphorochloridate (DPPC, 2 equiv.). Reaction proved to be fast and produced the desired H-phosphonothioate **2** without noticeable formation of a phosphite triester. When a hydroxylic component and/or the condensing agent were used in excess (2 and 4 equiv. respectively), no phosphite triesters formation was observed even when the reaction mixtures were kept for 1 h. Simple alcohols (e.g. methanol, ethanol) and nucleosides containing a secondary hydroxyl function (e.g. 5′-O-dimethoxytritylthymidine) reacted with H-phosphonothioate **1** in the presence of DPPC in a similar way. Less reactive chlorophosphates, e.g. *5,5-dimethyl-2-oxo-2-chloro-1,3,2-dioxaphosphorinane*[8], *can also be used to accomplish* a condensation to the H-phosphonothioate diesters.

Since abstraction of the proton from H-phosphonate esters is considered to be the rate limiting step during oxidation[9] of these compounds, it was expected that higher reactivity of the P-H bond in H-phosphonothioate diesters should result in their fast oxidation. Indeed, when sulfur (3 equiv.) was added to the H-phosphonothioate **2** in pyridine, a complete conversion into dinucleoside phosphorodithioate **3a**[10] was observed after recording the first

^{31}P NMR spectrum (ca. 3 min). Oxidation of **2** with iodine (2 equiv.) in aqueous pyridine proceeded smoothly to the phosphorothioate **3c**[10]. The H-phosphonothioate **2** was also converted into the phosphoroselenothioate **3b**[10] by treatment with elemental selenium (5 equiv.) in pyridine over night.

In conclusion, dinucleoside H-phosphonothioates can be produced from the appropriate nucleoside H-phosphonothioate monoesters and a suitably protected nucleoside in the presence of condensing agents. Pivaloyl chloride was found to cause side reactions (P-acylation, phosphite triester formation) and thus it is advisable to use condensing reagents of different chemical nature, e.g. chlorophosphates. Since sulfur is retained during the oxidation step with iodine, the H-phosphonothioate diesters can be considered as useful and easy accessible key intermediates in the synthesis of various phosphorothioate analogues.

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3. J. Nielsen, W. K.-D. Brill, Caruthers, M.H., *Tetrahedron Lett.*, **29**, 2911 (1988).
4. A convenient method for the synthesis of H-phosphonothioate monoesters has been developed, which consists of reaction of a nucleoside with triethylammonium phosphinate in pyridine in the presence of a condensing agent (pivaloyl chloride or diphenylphosphorochloridate), followed by oxidation with elemental sulfur (manuscript in preparation). Compounds of type **1** are stable and can be purified on silica gel analogously to the nucleoside 3′-H-phosphonates.
   Compound **1** (triethylammonium salt), ^{31}P NMR (pyridine) ∂=53.64 ($^{1}J_{PH}$=568.8 Hz, $^{3}J_{PH}$=12.2 Hz, dd) and 53.02 ($^{1}J_{PH}$=571.3 Hz, $^{3}J_{PH}$=12.2 Hz, dd). ^{1}H NMR (CDCl3): ∂=8.02 and 7.95 (2d, $^{1}J_{PH}$=579.4 Hz and $^{1}J_{PH}$=582.7 Hz, P-H, 1H), 7.57 (m, 1H, H-6), 7.3-6.6 (aromatic protons, 13 H), 6.41 (2 overlap. dd, 1H, 1′-H), 5.28 (m, 1H, 3′-H), 4.32 and 4.21 (2m, 1H, 4′-H), 3.75 (s, 6H, -OC$\underline{H}_3$), 3.45 (m, 2H, 5′-H), 2.60 and 2.35 (2m, 2H, 2′-H), 1.35 and 1.33 (2d, ^{4}J=1.2 Hz, 3H, Th-C$\underline{H}_3$), 1.20 and 2.80 (triethylammonium cation).
5. Compound **2**, ^{31}P NMR (pyridine), ∂=72.88 ($^{1}J_{PH}$=672.6 Hz, $^{3}J_{PH}$=11.0 Hz, dq) and 71.44 ($^{1}J_{PH}$=675.1 Hz, $^{3}J_{PH}$=11.0 Hz, dqq).
6. P.J. Garegg, I. Lindh, J. Stawinski, in *Biophosphates and Their Analogues. Synthesis, Structure, Metabolism and Activity*, eds. K.S. Brudzik and J.W. Stec, p. 89, Elsevier, Amsterdam 1987.
7. When HO-T-OBz in the above reaction was substituted by dmt-T-OH, only one signal at 65.36 ppm ($^{3}J_{PH}$=10.1 Hz, triplet) was observed (see also Fig.1).
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10. ^{31}P NMR data (in pyridine): **3a**, ∂=116.76 ppm; **3b**, ∂=104.99 and 104.56 ppm; **3c**, ∂=59.11 and 58.70 ppm.

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