Nucleoside H-Phosphonates, XXII: Synthesis and Properties of New Nucleotide Analogues – H-Phosphonothiolate Diesters

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Received 10 August 2007

Abstract: Condensation of nucleoside 3′-H-phosphonate monoesters with various thiols, promoted by condensing agents, provides a convenient access to a new class of H-phosphonate analogues, H-phosphonothiolate diesters. Chemical properties, relevant to possible applications of these compounds as a new type of synthetic intermediates in the preparation of nucleotide analogues bearing a sulfur atom at the bridging position of a phosphate group, were investigated.

Key words: H-phosphonates, H-phosphonothiolates, phosphate analogues, nucleotide analogues

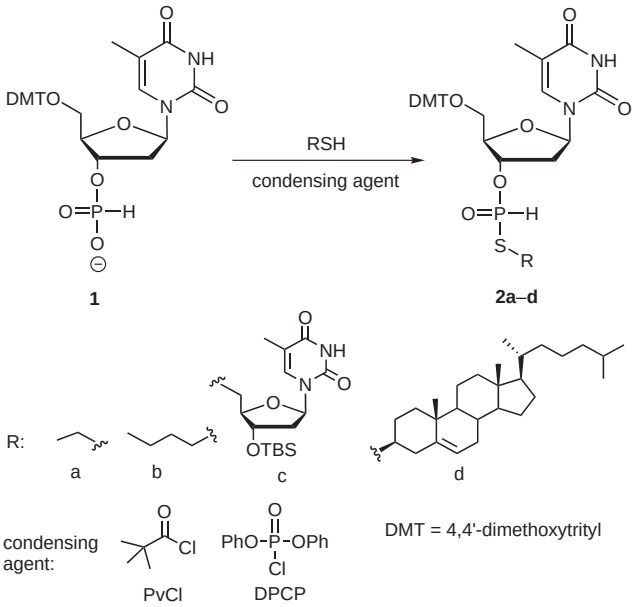
Oligonucleotide analogues containing modified internucleotide linkages are of growing interest as potential therapeutic agents[1] and tools in molecular biology for manipulation of RNA and DNA.[2] Among these, oligonucleoside phosphorothioates[3] and phosphorodithioates[4] are particularly attractive since they are isopolar and isosteric to their natural congeners.

In contrast to the P=S functionality, which can be relatively easy introduced into biologically important phosphates via sulfurization of the corresponding H-phosphonate[5] or phosphite triesters[6] precursors, the synthesis of analogues bearing sulfur atom at the bridging position of a phosphate group as in a P–S–C functionality, is usually a more complicated task. Due to inherently low reactivity of *S*-nucleophiles toward hard P(V) centers,[7] such compounds are usually prepared via alkylation of sulfur in phosphorothioate derivatives using suitable C-electrophiles,[8–10] by nucleophilic substitution at soft, tervalent P(III) center,[11] oxidation of the P(III) precursors with reactive disulfides,[12] or using dedicated thiophosphorylating reagent bearing already the P–S–C bond system.[13]

It is worth noting that due to different conformational preferences and the increased lability conferred by the P–S–C functionality, phosphorothiolates have distinct, from those of phosphorothioates (P=S), chemical and biological properties.[13–15] In the middle of the 1950s, numerous phosphorothiolate diesters were extensively investigated as potential antiradiation drugs,[9,16] and more recently, as potential antisense/antigene agents,[14] pronucleotides,[17] and tools in mechanistic investigations of ribozymes.[10,18]

SYNLETT 2007, No. 17, pp 2748–2752
Advanced online publication: 25.09.2007
DOI: 10.1055/s-2007-991072; Art ID: G24907ST

As part of our program in developing synthetic methods for biologically important phosphorus compounds based on H-phosphonate methodology,[5,19] we have embarked on exploration a new class of synthetic intermediates, H-phosphonothiolates, that could provide a convenient access to phosphorothiolates (the P–S–C functionality) and their analogues. Although thiols are poor nucleophiles in the $S_N2(P)$ reactions at P(V) centers,[7] we previously observed that activated H-phosphonates reacted with hydrogen sulfide[20,21] to form H-phosphonothioate and H-phosphonodithioates monoesters. Thus, as a viable approach to H-phosphonothiolate diesters of type **2** (Scheme 1) we considered a condensation of H-phosphonate monoesters with thiols, promoted by a condensing agent.



Scheme 1

To assess reactivity of thiols towards activated H-phosphonate monoesters, we subjected nucleoside H-phosphonate **1** to the reaction with ethanethiol (2 equiv) in pyridine, in the presence of pivaloyl chloride (PvCl, 3 equiv). ^{31}P NMR spectroscopy revealed that the starting material **1** (δ = 2.80 ppm, $^1J_{PH}$ = 640 Hz) was consumed within five minutes, but no product with a large $^1J_{PH}$ coupling constant, indicative of the presence of the desired H-phosphonothiolate **2a**, could be detected. Instead, two

phosphorus species, resonating at δ = 32.91 ppm ($^3J_{PH}$ = 9.2 Hz, m; ca 53%) and 159.0 ppm ($^3J_{PH}$ = 10.7 Hz, sext; ca 47%) were formed. On the basis of the chemical shifts and the observed splitting pattern in the ^{1}H-coupled ^{31}P NMR spectra, these signals were tentatively assigned to the *P*-acylphosphonate **3** (δ = 32.91 ppm)[22] and dithiophosphite **4** (δ = 159.0 ppm,[23] Figure 1). Since both compounds contained the P–S–C bonds, we assumed that apparently the desired H-phosphonothiolate **2a** was initially formed, but under the reaction conditions underwent subsequent reactions that ultimately led to the P-acylated product **3** and the corresponding dithiophosphite **4**.

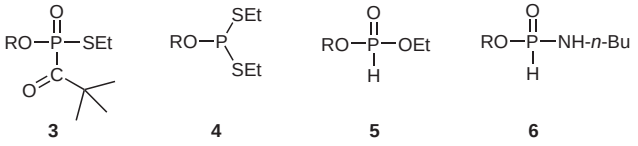


Figure 1

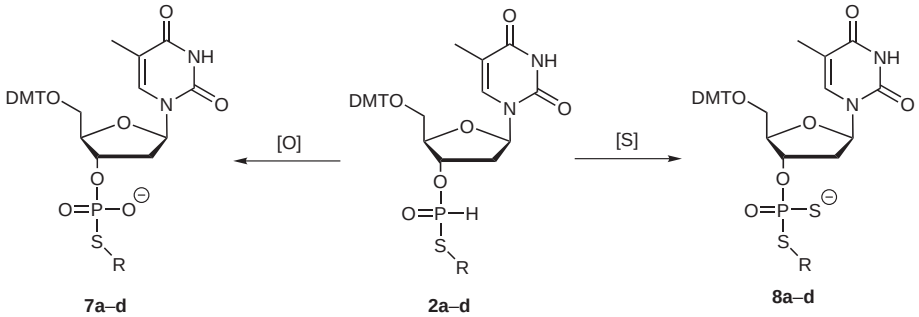
To suppress such undesired reactions, the condensation was carried out in less basic medium, namely in acetonitrile–pyridine mixture (4:1), and the amount of PvCl was reduced to 1.5 equivalents. Under these new reaction conditions, the condensation of H-phosphonate **1** with ethanethiol was still rapid (<5 min) but produced as a major product (>90%) a compound resonating at δ = 33.8 and 34.1 ppm ($^1J_{PH}$ = 658 Hz). Since the two signals of equal intensity were indicative of a one-spin system of phosphorus diastereomers, and their chemical shift values and a large ^{1}H–^{31}P coupling constant were consistent with the presence of a P–S–C and P–H functionalities, we assigned these signals to the expected H-phosphonothiolate **2a**. Although acylphosphonate **3** (10%; δ = 33.2 ppm) was initially the only byproduct present in the reaction, upon standing (ca 10 min), additional signals at 159.4 ppm (compound **4**) and at 2.8 ppm (H-phosphonate **1**) started to emerge, and after few hours, H-phosphonothioate **2a** was completely replaced by these two species (ca 1:1 ratio; ^{31}P NMR).

Since reactivity of H-phosphonothiolate **2a** seemed to differ significantly from that of the corresponding H-phosphonate diesters, in a separate experiment **2a** was generated as described above, and subjected to reactions with various nucleophiles and electrophiles. It was found (^{31}P NMR spectroscopy experiments) that addition of water to the reaction mixture (200 equiv) cause immediate hydrolysis of **2a** producing the starting nucleoside H-phosphonate **1**. As expected, primary amines, e.g. of *n*-butylamine (2 equiv) also rapidly reacted with H-phosphonothiolate **2a** affording within few minutes the corresponding *n*-butyl nucleoside H-phosphonamidate **6** (δ = 13.6 and 13.8 ppm, $^1J_{PH}$ = 640 Hz, $^3J_{PH}$ = 10.3 Hz; dq; >90%).[24] High susceptibility of this class of compounds to nucleophilic substitution at the phosphorus center was confirmed in the reaction of H-phosphonothioate **2a** with two equivalents of ethanol that produced quantitatively the corresponding nucleoside ethyl H-phosphonate **5** (δ = 7.9 ppm, $^1J_{PH}$ = 704 Hz, $^3J_{PH}$ = 8.9 Hz; dq).[25]

Since most relevant to possible applications of H-phosphonothiolates of type **2** as synthetic intermediates for the preparation of various nucleotide analogues were oxidative transformations, we subjected compound **2a** to oxidation under different experimental conditions.

As expected, standard oxidation system for the transformation of H-phosphonate diesters into the corresponding phosphates, consisting of iodine in 2% aqueous pyridine, gave poor results affording with one equivalent of iodine mainly the hydrolysis product (compound **1**, ca. 30%), dithiophosphite **4** (ca 50%), and only ca. 20% of the desired phosphorothiolate **7a** (δ = 18.8 ppm, $^3J_{PH}$ = 11.1 Hz, q). However, under the Atherton–Todd oxidation conditions,[26] using carbon tetrachloride as an oxidant in the presence of triethylamine and water (Scheme 2), the oxidation proceeded smoothly producing within few minutes exclusively the desired product, phosphorothiolate **7a** (isolated in 82% yield). Also sulfurization with elemental sulfur in the presence of triethylamine was uneventful and afforded cleanly the corresponding phosphorodithioate **8a** (δ = 74.2 and 75.3 ppm, $^3J_{PH}$ = 12.6 Hz, q; isolated in 67% yield).

Using the reaction conditions developed for **2a**, other representative H-phosphonothiolate diesters bearing a longer alkyl chain (**2b**), a cholesteryl moiety (**2d**), or another nucleosidic unit (**2c**), were efficiently generated



Scheme 2 *Oxidation conditions*: CCl_4 (10 equiv), H_2O (50 equiv), Et_3N (2 equiv). *Sulfurization conditions*: S_8 (3 equiv), Et_3N (2 equiv).

from nucleoside H-phosphonate **1** and the corresponding thiols (Scheme 1). All these compounds showed similar to **2a** chemical properties, and were converted cleanly into nucleotide analogues **7a**–**d** and **8a**–**d**.

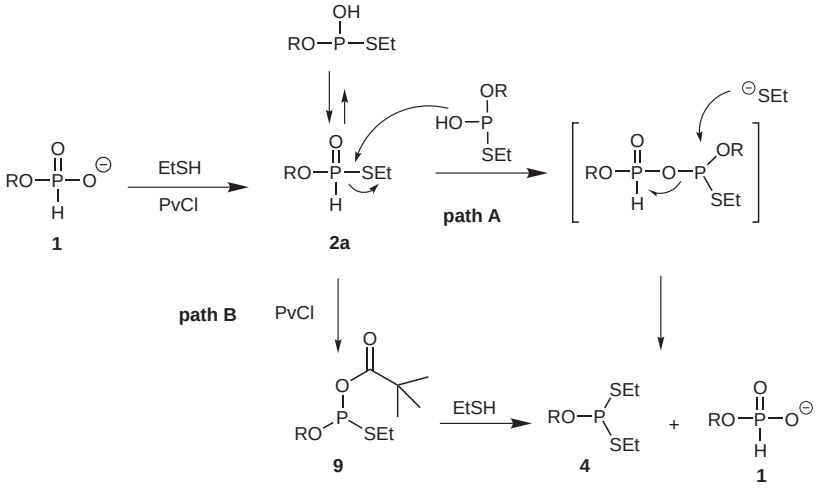
In light of the above, it was apparent that due to high reactivity towards nucleophiles and electrophiles, H-phosphonothiolate diesters, in most cases, probably could not be isolated by chromatographic methods, but instead, should be treated as reactive intermediates that have to be in situ converted into various phosphate analogues bearing single or multiple modifications at the phosphorus center. In this context, purity of the generated H-phosphonothiolates of type **2** might be a synthetically important issue, and for this reason we decided to carry out some additional studies on factors that can promote byproduct formation during synthesis of H-phosphonothiolate diesters.

Since formation of P-acylated products is known to be favored under basic conditions,[5,27] to suppress or to eliminate formation of this type of byproducts (compounds of type **3**), we attempted to carry out synthesis of H-phosphonothiolates **2** in acetonitrile containing 1% pyridine. Indeed, under such reaction conditions we did not observe (by ^{31}P NMR) formation of a P-acylated product of type **3** (due to a subsequent reaction of pivaloyl chloride with the produced H-phosphonothiolates **2**), however, the condensation became less efficient. In the reaction with ethanethiol (2 equiv), after five minutes the reaction mixture consisted of almost equimolar amounts of the desired H-phosphonothiolate **2a** and the corresponding H-phosphonic-pivalic mixed anhydride (δ = 2.18 and 2.28 ppm, $^{1}J_{PH}$ = 747 Hz, $^{3}J_{PH}$ = 9.2 Hz), and the reaction seemed to stop at this stage. With more ethanethiol (4 equiv), the reaction proceeded to ca. 70% completion, but still, the unreacted mixed anhydride was present in the reaction mixture. Extending the reaction time (40 min) did not provide any improvement in terms of yield of the produced H-phosphonothiolate **2a**, and only decomposition products of the mixed anhydride started to form.

Probably, by changing a pyridine content in the reaction mixture, a compromise between coupling efficiency and extent of P-acylation, could be achieved, however, since formation of byproducts of type **3** was obviously related to the condensing agent used, we investigated diphenyl phosphorochloridate (DPCP) as possible condensing agents. Indeed, this reagent turned out to be superior to PvCl in terms of purity of the generated H-phosphonothiolates **2**, and since the condensations were also rapid (<5 min), this reagent was used in further preparative runs.[28]

As to possible mechanisms of dithiophosphites of type **4** formation, some additional observations were pertinent. Irrespective of the condensing agent used, the reactions in neat pyridine afforded dithiophosphites **4** as major reaction products. When excess of DPCP and a thiol were added to a reaction mixture containing H-phosphonothiolates **2** in pyridine, within a few minutes a complete conversion of **2** into the corresponding dithiophosphite **4** was observed. In less basic reaction media, e.g. in acetonitrile–pyridine (4:1), this byproduct was usually formed after 10–15 minutes after completion of the condensation reaction, and in the presence of a limiting amount of a condensing agent, the formation of dithiophosphite **4** was accompanied by the appearance of the starting material, nucleoside H-phosphonate **1**. Finally, upon addition of a triethylamine (10 equiv) to a reaction mixture containing **2**, an immediate disappearance of the H-phosphonothiolate diester occurred, and formation of equimolar amounts of the corresponding dithiophosphite **4** and nucleoside H-phosphonate **1** was observed.

As a possible explanation for the above observations, we propose two mechanisms that can be involved in the formation of byproducts of type **4** during the investigated condensations. These are depicted in Scheme 3 as path A and path B. The first mechanism (path A, Scheme 3) is related to that of disproportionation of some aryl H-phosphonates[21,29] or phosphonic–carboxylic mixed anhydrides,[30] which under similar conditions afforded



Scheme 3

equimolar amounts of the corresponding tervalent and tetracoordinate P(III) species. This mechanism is consistent with the observed rapid transformation of H-phosphonothiolates **2** into equimolar mixture of dithiophosphites **4** and the staring H-phosphonate monoester **1**, in the presence of added triethylamine.

To find out if an alternative reaction pathway (path B, Scheme 3), i.e. activation of the produced H-phosphonothiolates **2** by a condensing agent, followed by the reaction with a thiol, could contribute also to the formation of dithiophosphites of type **4**, we carried out the condensation of nucleoside H-phosphonate **1** with excess of both ethanethiol (10 equiv) and PvCl (5 equiv) in acetonitrile–pyridine (4:1). Since no detectable increase in the rate of formation of dithiophosphite of type **4** compared to the reaction with less thiol and the condensing agent (2 equiv of both) was observed, we can tentatively conclude that this type of byproducts was mainly formed via disproportionation of H-phosphonothiolates **2**.

In conclusion, we developed an efficient protocol for generation of H-phosphonothiolate diesters of type **2**, consisting of condensation of H-phosphonate monoesters with various thiols, in the presence of a condensing agent. The coupling reactions were clean, rapid, and the produced H-phosphonothiolate diesters could be converted via oxidative transformations into various nucleotide analogues bearing sulfur atom at the bridging position in the phosphate group. The method is experimentally simple, makes use of easily accessible H-phosphonate monoesters,[31] and expands range of biologically important phosphate analogues that can be prepared via H-phosphonate methodology.

Acknowledgment

Financial support from the Swedish Research Council is gratefully acknowledged.

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(22) Compound **3** was obtained on independent way by reacting H-phosphonothiolate **2a** with pivaloyl chloride in MeCN–pyridine (4:1). The signals of two P-diastereomers were not resolved in the ^{31}P NMR spectrum.
(23) Ethyl H-phosphonate reacted with ethanethiol analogously to that of **1**, producing compound of type **4** (R = Et) that resonated at δ_P = 157.8 ppm ($^3J_{PH}$ = 9.4 Hz, hept). This compound was prepared independently by reacting ethyl phosphorodichloridite with 2 equiv of ethanethiol in MeCN–pyridine (4:1).
(24) Comparison with original sample obtained by reaction of **1** with phenol in the presence of pivaloyl chloride, followed by the addition of *n*-butylamine. See: Kers, A.; Stawinski, J.; Kraszewski, A. *Tetrahedron* **1999**, *55*, 11579.
(25) Comparison with original sample obtained by condensation of **1** with ethanol in the presence of pivaloyl chloride. The P-diastereomers were not resolved.
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(28) **General Procedure for Synthesis and Oxidative Transformations of H-Phosphonothiolates 2a–d**
Nucleoside H-phosphonate monoester **1** (0.15 mmol) was rendered anhydrous by evaporation of added pyridine, and the residue was dissolved in MeCN–pyridine (4:1; 2 mL) or in CH_2Cl_2–pyridine (4:1; 2 mL, for thiols **c** and **d**). To this solution, the appropriate thiol **a**–**d** (2 equiv) and a condensing agent (diphenyl phosphorochloridate; 1 equiv) were added. The reactions were complete within 5 min (^{31}P NMR analysis) producing the expected nucleoside H-phosphonothiolates **2**.
^{31}P NMR Data for Compounds 2
Compound **2a**: δ = 33.75 and 34.08 ppm ($^1J_{PH}$ = 658.2 Hz, $^3J_{PH}$ = 11.1 Hz, dq); **2b**: δ = 33.96 and 34.23 ppm ($^1J_{PH}$ = 659.9 Hz, $^3J_{PH}$ = 10.7 Hz, dq); **2c**: δ = 32.66 and 32.79 ppm ($^1J_{PH}$ = 672.2 Hz, $^3J_{PH}$ = 10.7 Hz, dq); **2d**: δ = 33.01 and 33.37 ppm ($^1J_{PH}$ = 652.0 Hz, $^3J_{PH}$ = 11.4 Hz, dt).
To the solution containing **2a**–**d**, a mixture of CCl_4 (10 equiv), H_2O (50 equiv), and Et_3N (2 equiv) was added. The

reactions were complete within 5 min (^{31}P NMR analyses), producing quantitatively the corresponding phosphorothiolates **7a**–**d**, that were isolated by silica gel column chromatography (purity >98%, ^{1}H NMR).

^{31}P NMR Data for Compounds 7

Compound **7a** (82%): δ = 18.76 ppm ($^{3}J_{PH}$ = 11.1 Hz, q); **7b** (72%): δ = 18.99 ppm ($^{3}J_{PH}$ = 11.1 Hz, q); **7c** (64%): δ = 16.64 ppm ($^{3}J_{PH}$ = 10.2 Hz, q); **7d** (56%): δ = 19.86 ppm ($^{3}J_{PH}$ = 10.1 Hz, t).

Sulfurization of the in situ generated H-phosphonothiolates **2a**–**d** was performed by the addition of elemental sulfur (3 equiv) and Et_3N (2 equiv) to the corresponding reaction mixtures. The reactions were complete within 5 min (^{31}P NMR analyses), affording quantitatively the corresponding phosphorodithioates **8a**–**d**, that were isolated by silica gel column chromatography (purity >98%, ^{1}H NMR).

^{31}P NMR Data for Compounds 8

Compound **8a** (67%): δ = 74.22 and 75.26 ppm ($^{3}J_{PH}$ = 12.6 Hz, q); **8b** (61%): δ = 74.39 and 75.26 ppm ($^{3}J_{PH}$ = 12.7 Hz, q); **8c** (65%): δ = 73.85 and 74.17 ppm ($^{3}J_{PH}$ = 12.6 Hz, q); **8d** (59%): δ = 74.54 and 76.35 ppm ($^{3}J_{PH}$ = 12.9 Hz, t).

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