Aryl *H*-Phosphonates. 12. Synthetic and ^{31}P NMR Studies on the Preparation of Nucleoside *H*-Phosphonothioate and Nucleoside *H*-Phosphonodithioate Monoesters

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Transformation of nucleoside *H*-phosphonate monoesters into the corresponding *H*-phosphonothioate and *H*-phosphonodithioate derivatives and possible side-reactions that may accompany this process were studied using ^{31}P NMR spectroscopy. These provided new insight into a possible mechanism involved in this transformation and constituted the basis for development of efficient methods for the preparation of nucleoside *H*-phosphonothioate and nucleoside *H*-phosphonodithioate monoesters using readily available *H*-phosphonate monoesters as starting materials.

Introduction

The pharmacological value of oligonucleotides with natural phosphodiester internucleotide linkages is seriously hampered by their rapid degradation in the presence of cell and serum nucleases.[1] As a result of this, most investigations have been focused on finding chemical modifications that would confer stability to nucleases while preserving the specificity and efficiency of complexation of the modified oligonucleotide with target DNA or RNA sequences.[2] Recent studies have shown that among nucleic acids analogues with modifications at the phosphorus center, oligonucleoside phosphorodithioates[3−6] possess most favorable biological and pharmacological properties as potential antisense or antigene agents[7] and also as nucleic acids based clinical diagnostics.[8,9]

During the past decade we have introduced and investigated various aspects of *H*-phosphonothioates[4,10−12] as a new type of synthetic intermediate that can supplement and extend applications based on *H*-phosphonate derivatives. In the context of nucleoside phosphorodithioates synthesis via P(III) intermediates, *H*-phosphonothioate building blocks[4,13] seem to be superior to phosphorothioamidite derivatives[3] because of their higher stability, ease of handling of the starting materials, and efficiency in solid-phase synthesis.[14] This stimulated interest in *H*-phosphonothioates as convenient starting materials for the preparation of biologically important nucleotide analogues that can be difficult to prepare in other ways, e.g., nucleoside phosphorodithioates,[8,14,15] nucleoside phosphoramidothioates,[16] nucleoside phosphorofluoridothioates,[17] etc.

Nucleoside *H*-phosphonothioate monoesters can be prepared either from suitably protected nucleosides using various thiophosphonylation protocols[10,18,19] or by nonoxidative thiation of nucleoside *H*-phosphonates.[11] This latter approach seems to be particularly attractive in

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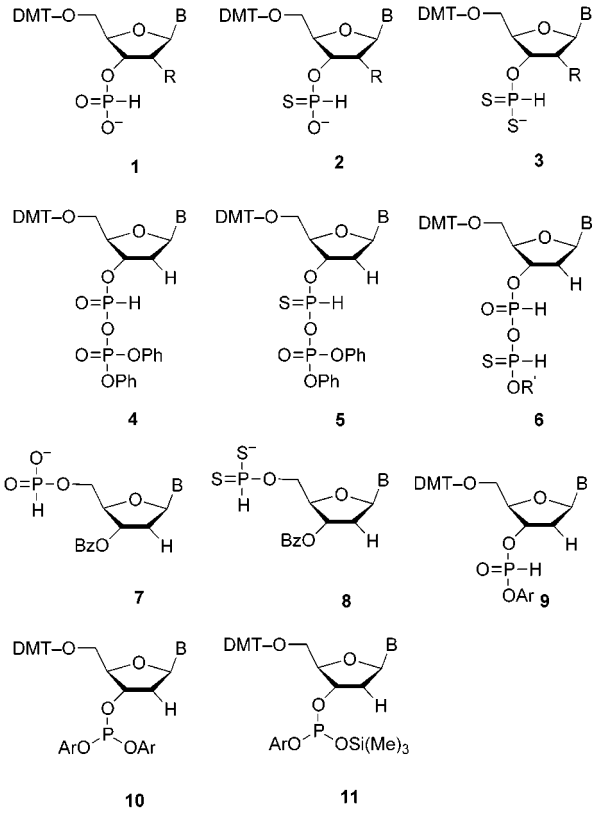
light of the growing availability of *H*-phosphonate monoesters[20,21] and has the added advantage that other synthetically useful intermediates, *H*-phosphonodithioate monoesters,[5,22,23] can also be prepared from the same substrate.[24] Unfortunately, at present there is only one protocol available for the transformation of *H*-phosphonate monoesters into the corresponding *H*-phosphonothioate derivatives.[11] This is based on a P(III) intermediate (a nucleoside pivaloyl silyl phosphite[12]) whose reactivity is difficult to modulate. To circumvent these shortcomings, we searched for a more versatile method for the *H*-phosphonates−*H*-phosphonothioates transformation that would permit control of reactivity of the intermediate involved depending on structural variation of the substrates and the reaction conditions required.

In this paper we describe ^{31}P NMR studies that enabled us to delineate the most important mechanistic aspects of the reactions of activated *H*-phosphonate derivatives with hydrogen sulfide (or its equivalent) under various experimental conditions. On the basis of these observations, it became possible to direct these reactions, which usually afforded various proportions of *H*-phosphonomono- and *H*-phosphonodithioates, to produce almost exclusively one of these products. According to the protocol developed, various nucleoside *H*-phosphonothioates and nucleoside *H*-phosphonodithioates were obtained in 85−95% yields in one-pot reactions from the corresponding *H*-phosphonate monoesters.

Results and Discussion

Reactions of nucleoside *H*-phosphonate monoesters of type **1** with hydrogen sulfide in the presence of pivaloyl chloride (or sulfhydrolysis of the in situ formed phosphonic-carboxylic anhydrides) usually afford as a major product the corresponding *H*-phosphonodithioate monoesters **3** with various proportions of an unreacted *H*-phosphonate monoester, depending on the amount of pivaloyl chloride used.[11,25] This was assumed to be due to relatively slow formation of the initial product, *H*-phosphonothioate monoester of type **2**, and its rapid reaction toward *H*-phosphonodithioate derivative **3**. To change the unfavorable kinetics of this transformation, we searched for an intermediate that would react fast with hydrogen sulfide to produce *H*-phosphonothioate monoesters **2** and in this way suppress the formation of dithio derivatives **3**. For this purpose we investigated the reaction of nucleoside *H*-phosphonate monoesters **1** with hydrogen sulfide in he presence of diphenyl phosphorochloridate (DPCP) and sulfhydrolysis of various nucleoside aryl *H*-phosphonates of type **9**.

Reactions of Nucleoside *H*-Phosphonates 1 with DPCP in the Presence of Hydrogen Sulfide. Nucleoside *H*-phosphonate **1a** (1 molar equiv) and hydrogen sulfide (3 molar equiv) in dichloromethane/pyridine (9:1, v/v) were allowed to react in the presence of DPCP (3 molar equiv), and progress of the reaction was monitored by ^{31}P NMR spectroscopy. The reaction was rapid, and the first spectrum recorded (<3 min) revealed a complete disappearance of the starting material **1a** (δ_P = 1.5 ppm) and the formation of nucleoside *H*-phosphonodithioate **3a** (δ_P = 85.4 ppm, $^1J_{PH}$ = 528.3 Hz, $^3J_{PH}$ = 13.9 Hz; dd) as the sole nucleotidic product.[26] The exclusive formation of dithio derivative **3a** indicated that, similarly to the pivaloyl chloride promoted reaction,[11] the second step (i.e., the transformation of *H*-phosphonothioate **2a** into **3a**) remained faster than the formation of the initial monothio product **2a**. However, somewhat surprisingly we found that although sulfhydrolysis of *H*-phosphonothioate **2a** under analogous conditions produced the corresponding *H*-phosphonodithioate **3a**, the reaction was significantly slower than that of *H*-phosphonate **1a** (ca 25 min vs <3 min for the completion). This suggested that besides mixed anhydride **5**, a putative common intermediate in transformations **1a** → **3a** and **2a** → **3a**, there was probably yet another reactive



DMT = 4,4′-dimethoxytrityl; Bz = benzoyl; Ph = phenyl

1a - 3a, B = thymin-1-yl, R = H
1b - 3b, B = adenin-9-yl, R = H
1c - 3c, B = cytosin-1-yl, R = H
1d - 3c, B = guanin-9-yl, R = H
1e - 3e, B = uracil-1-yl, R = *t*-butyldimethylsiloxyl
4, 5, 7, 8, B = thymin-1-yl
6, B = thymin-1-yl, R′ = 5′-dimethoxytritylthymidin-3′-yl
9a, 11a, B = thymin-1-yl, Ar = phenyl
9b, 11b, B = thymin-1-yl, Ar = 4-chlorophenyl
9c, 11c, B = thymin-1-yl, Ar = 2,4-dichlorophenyl
9d, 11d, B = thymin-1-yl, Ar = 4-nitrophenyl
9e, 10, 11e, B = thymin-1-yl, Ar = 2,4,6-trichlorophenyl

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(26) For assignments of signals in the ^{31}P NMR spectra to particular products or intermediates, see Experimental Section.

species involved in the former pathway[27] that made the formation of *H*-phosphono*di*thioate **3a** from *H*-phosphonate **1a** almost 1 order of magnitude faster. A possible reactive species that could participate in above reaction was assumed to be mixed phosphonic-thiophosphonic anhydride **6**. One can envisage its formation by the reaction of the initial product of sulfhydrolysis, *H*-phosphonothioate **2a**, with activated *H*-phosphonate derivative **4**, generated from *H*-phosphonate **1a** and the condensing agent. This activation pathway for **2a** is available only when *H*-phosphonate **1a** is subjected to sulfhydrolysis in the presence of DPCP.

The intermediacy of **6** in transformation **1a** → **3a** and its fast reaction to dithio derivative **3a** implied that in the presence of a limited amount of a condensing agent, the reaction should afford equimolar amounts of **3a** and **1a**. Indeed, the ^{31}P NMR spectra of the reaction mixture from sulfhydrolysis of *H*-phosphonate **1a** carried out in the presence of 1.5 equiv of DPCP revealed the formation of **1a** (47%) and **3a** (53%) in the expected ratio.

In separate experiments we found that when an equimolar mixture of nucleoside 5′-*H*-phosphonate **7**[28] and nucleoside 3′-*H*-phosphonothioate **2a** was subjected to sulfhydrolysis under analogous reaction conditions, it was practically only **2a** that underwent thiation to **3a**. The reaction was rapid (<3 min) and resulted in a complete consumption of 3′-*H*-phosphonothioate **2a**, while 5′-*H*-phosphonate **7** was converted to dithio derivative **8** only in ca. 10%. These results were in line with our assumption concerning the involvement of phosphonic-thiophosphonic anhydride **6** (vide supra) in the conversion of *H*-phosphonates **1** into *H*-phosphonodithioates **3**. The postulated higher reactivity of this intermediate vs thiophosphonic-phosphoric anhydride **5** can be rationalized on the grounds of a generally higher electrophilicity of phosphorus centers in uncharged tetracoordinated P(III) derivatives compared to those of the corresponding P(V) compounds.[29]

The other point that deserves some comments is chemoselectivity observed in the investigated reactions. The initial product of activation of *H*-phosphonate **1a**,[29] phosphonic-phosphoric anhydride **4**, has two electrophilic centers, P(III) and P(V), and since the former one is softer,[30] it should be attacked preferentially by soft nucleophiles, e.g., HS^-. In agreement with this, we observed (^{31}P NMR) exclusive formation of thiophosphonate derivatives and no products due to attack of sulfide anion on the P(V) center in **4**. The origin of chemoselectivity in sulfhydrolysis of the postulated intermediate **6** is less obvious, but this can also be explained on the ground of the HSAB principle.[30] In this instance, the presence of sulfur probably decreases electrophilicity of the thiophosphonyl center in **6**, but the higher acidity of the phosphorus-bound hydrogen[23] makes this center softer by virtue of its higher tendency to form tervalent species and thus more susceptible to attack by soft nucleophiles.

The above results lend support to our earlier observation[11] that, as a result of higher susceptibility of *H*-phosphonothioate vs *H*-phosphonate derivatives to sulfhydrolysis, it can be difficult to stop sulfhydrolysis of *H*-phosphonate monoesters promoted by condensing agents at the stage of monothio derivative of type **2**. However, the observed tendency of the reactions investigated to afford dithio derivatives **3a** could be exploited for preparative purposes. Indeed, using a synthetic protocol for sulfhydrolysis of *H*-phosphonate monoesters based on this reaction, various nucleoside *H*-phosphonodithioate monoesters of type **3** were obtained in over 90% yields (see Experimental Section).

Reactions of Aryl Nucleoside *H*-Phosphonates with H_2S and Hexamethyldisilathiane. Recently, we have showed that nucleoside phenyl *H*-phosphonates (generated in situ from suitably protected nucleosides with diphenyl *H*-phosphonates) afforded the expected nucleoside *H*-phosphonothioates of type **2** in high yields[18] when treated with hydrogen sulfide in the presence of triethylamine (TEA). However, since the indispensable component of this reagent system is a strong base (triethylamine),[31] these reaction conditions are not compatible with base-sensitive substrates. Therefore, we investigated the possibility of achieving similarly favorable kinetics under less basic reaction conditions by modulation of the reactivity of *H*-phosphonates of type **9** via varying electron density in aromatic rings of the aryl moieties.

To this end, nucleoside phenyl **9a** and nucleoside *p*-chlorophenyl **9b** *H*-phosphonates (prepared in situ as described previously[32]) were subjected to sulfhydrolysis (3 molar equiv of H_2S) in pyridine. As revealed by ^{31}P NMR spectroscopy, these compounds reacted with hydrogen sulfide at different rates (completion after ca. 60 min for phenyl derivative **9a** and 30 min for *p*-chlorophenyl derivative **9b)** but afforded similar mixtures of *H*-phosphonate **1a**, *H*-phosphonothioate **2a**, and *H*-phosphonodithioate **3a** (27%, 38%, and 35%, respectively, for **9a** and 23%, 37%, and 40% for **9b**).

These results can best be rationalized on the ground of a mechanism similar to that of sulfhydrolysis of **1a** in the presence of DPCP proposed above, involving phosphonic-thiophosphonic anhydride **6** as an intermediate, but with slightly different rates for the formation of monothio **2a** and dithio **3a** products.

Analogous reactions carried out on aryl *H*-phosphonates **9c**−**e**[33] bearing more acidic phenol moieties showed that the increasing electrophilicity at the phosphorus center in these compounds was counterproductive and favored instead the formation of dithio derivative **3a**. Irrespective of the substrate **9c**−**e** used, these reactions always afforded mixtures containing almost equimolar amounts of *H*-phosphonates **1** and dithio derivatives **3**,

(27) Since in condensation reactions, the activation of *H*-phosphonothioate monoesters is usually a rate determining step,[11] one can argue that the observed differences in overall rates of **1a** → **3a** and **2a** → **3a** are due to faster formation of **5** in the former pathway. However, there in no obvious reactive species that could more effectively than DPCP under the reaction conditions transform **2a** into phosphoric-thiophosphonic mixed anhydride **5**, and thus this option remains less likely.

(28) Reactivity of *H*-phosphonates **1a** and **7** are similar. For this particular experiment we chose nucleoside 5′-*H*-phosphonate **7** as its thiation product **8** has a ^{31}P NMR chemical shift and the splitting pattern distinctive from that of **3a**, and its use permitted the evaluation of two reaction pathways, **7** → **8** and **2a** → **3a**.

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(31) The presence of triethylamine made the sulfhydrolysis of **9a** proceed fast enough to suppress the formation of the secondary product, *H*-phosphonodithioate **3a**.

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(33) These reactions were carried out in dichloromethane in the presence of 10 equiv of pyridine as aryl *H*-phosphonates **9a**−**e** cannot be efficiently generated in neat pyridine.

with complete absence of monothio products **2**. Attempted sulfhydrolysis of **9c**−**e** with mixtures of hydrogen sulfide/TEA [also in the presence of trimethylsilyl chloride (TMSCl) to capture the initial product of sulfhydrolysis, **2a**] invariably led to *H*-phosphonate **1a** and *H*-phosphonodithioate **3a** in a 1:1 ratio.

Most striking was the absence of trimethylsilylated derivatives of *H*-phosphonothioate **2a** in the reaction mixtures when sulfhydrolysis of **1a** was carried out in the presence of TEA and TMSCl. This suggested that in the presence of TEA these reactions might proceed via another pathway that did not involve formation of monothio derivative **2a**. This assumption was corroborated by the results of two experiments. First, when aryl *H*-phosphonate **9e** was treated in dichloromethane in the presence of pyridine (10 equiv) with hydrogen sulfide and TMSCl (5 equiv), trimethylsilylated derivative of **2a** was formed exclusively (δ_P = 57.10 and 56.55 ppm, $^1J_{PH}$ = 658 and 660 Hz; $^3J_{PH}$ = 11.1 and 12.8 Hz, dd). Second, *H*-phosphonate **9e** in the presence of triethylamine underwent rapid disproportionation[34] to nucleoside bisaryl phosphite **10** and *H*-phosphonate monoester **1a**, and the former one underwent rapid conversion to dithio derivative **3a** upon the addition of hydrogen sulfide.

Since both transformations were clean and fast, we attempted to develop them as synthetic procedures for the preparation of nucleoside *H*-phosphonothioate and nucleoside *H*-phosphonodithioate monoesters, respectively. Considering that efficient formation of *H*-phosphonothioate monoesters of type **2** from the corresponding *H*-phosphonate derivatives **9** required silylation of the substrate prior to sulfhydrolysis, we investigated the possibility of using 1,1,1,3,3,3-hexamethyldisilathiane[35] (HMDST), a reagent that simultaneously can act as a silylating agent and as a source of sulfide ion.[36]

When aryl *H*-phosphonates **9a**−**e** (1 molar equiv) in dichloromethane containing pyridine (10 molar equiv) were treated with HMDTS (3 molar equiv), ^{31}P NMR spectroscopy showed rapid disappearance of the starting materials (<3 min) and clean formation of products resonating in the range of chemical shifts of tervalent phosphorus compounds (usually two signals at ca. 125 ppm, see Table 1). On the basis of spectral data and the observed chemical reactivity, we assigned to these species structures of aryl nucleoside silyl phosphites **11**. Although all **11a**−**e** were rapidly formed, they differed significantly in their reactivity toward trimethylsilylsulfide ion generated from HMDST. Thus, phenyl and *p*-chlorophenyl derivatives (**11a** and **11b)** required more than 24 h for a complete conversion to the corresponding silylated derivatives of **2**, 2,4-dichlorophenyl and 4-nitrophenyl derivatives (**11c** and **11d**) ca. 60 min, and for 2,4,6-trichlorophenyl derivative **11e**, the reaction was complete in less than 5 min. The latter reaction also proved to be most efficient when carried out on a preparative scale and afforded nucleoside *H*-phosphonothioates **2a**−**d** in high yield (> 90%) after silica gel chromatography.

Since nucleoside bisaryl phosphites of type **10** derived from highly acidic phenols (e.g., 2,4,6-trichlorophenol or 2,3,4,5,6-pentachlorophenol) can be efficiently generated in pyridine from the corresponding nucleoside *H*-phosphonates **1** and the appropriate phenols in the presence of DPCP,[32] we tried to use them as intermediates in the synthesis in of nucleoside *H*-phosphonodithioates **3**. Indeed, we found that these compounds when treated in pyridine with HMDST (5 equiv) rapidly (<5 min) and cleanly afforded the corresponding nucleoside *H*-phosphonodithioates of type **3**. A synthetic usefulness of this reaction was assessed on a preparative scale and nucleoside *H*-phosphonodithioates derived from thymidine, 2′-deoxyadenosine, 2′-deoxycytidine and 2′-deoxyguanosine (**3a**-**d**) were obtained in excellent yields (>90%) after silica gel chromatography (see Experimental Section).

It is worth noting that the methods developed seem to be applicable also to ribonucleoside 3′-*H*-phosphonates, as it was apparent from efficient conversion of uridine 3′-*H*-phosphonate **1e** (bearing a bulky 2′−O-*t*−*b*utyldimethylsilyl protective group) into the corresponding *H*-phosphonothioate **2e** or *H*-phosphonodithioate **3e** (yields > 90%).

In conclusion, on the basis of ^{31}P NMR studies, we developed new protocols for transformations of nucleoside *H*-phosphonate monoesters of type **1** into the corresponding *H*-phosphonothioate **2** and *H*-phosphonodithioate **3** derivatives under mild reaction conditions. Nucleoside *H*-phosphonothioates **2a**−**e** were obtained in high yields by reacting the in situ generated nucleoside aryl *H*-phosphonates of type **9** with HMDST. For the preparation of nucleoside *H*-phosphonodithioates **3a**−**e**, two alternative procedures were developed. These consisted of the reaction of *H*-phosphonate monoesters **1** with diphenyl chlorophosphate (DPCP) in the presence of hydrogen sulfide or sulfhydrolysis of the in situ produced nucleoside diaryl phosphites of type **10**. Since all these transformations were fast and efficient and could be carried out as one-pot reactions, they can be recommended as versatile and convenient methods for the preparation of nucleoside *H*-phosphonothioates and nucleoside *H*-phosphonodithioates from readily accessible nucleoside *H*-phosphonates.

Table 1. ^{31}P NMR Data of the Products of Type 2 and 3 and Some Reactions Intermediates[a]

compds	δ_P	$^1J_{HP}$ (Hz)	$^3J_{HP}$ (Hz)
2a	55.43, 55.85[b]	583.9, 579.3	12.6[c]
2b	54.42, 54.50[b]	583.0, 581.2	11.6[c]
2c	53.99, 55.29[b]	587.7, 578.4	11.6[c]
2d	54.10, 54.88[b]	582.8, 585.8	11.6, 13.4[c]
2e	54.96, 56.55[b]	579.3, 587.6	13.9[c]
3a	84.15	546.9	14.8[d]
3b	83.69	545.0	14.8[d]
3c	84.44	546.9	13.9[d]
3d	84.35	547.8	14.8[d]
3e	86.00	551.5	15.8[d]
7	4.41	637.7	7.4[e]
8	86.39	530.2	9.3[e]
9a−**e**	2.0−5.0[f]		
10	130.12		9.27[g]
11a	123.04, 124.07[b]		9.3, 10.2[h]
11b	127.31, 127.91[b]		10.3[h]
11c	122.95, 123.72[b]		10.3[h]
11d	121.61, 123.88[b]		9.3[h]
11e	125.42, 126.10[b]		9.3[h]

[a] Spectra recorded in dichloromethane containing pyridine (ca 10% v/v). [b] Two diastereoisomers. [c] Two doublets of doublets. [d] Doublet of doublets. [e] Doublet of triplets. [f] Compounds **9a**−**e** resonate a few ppm downfield from H_3PO_4, and their ^{31}P NMR data have been published earlier.[32] [g] Doublet. [h] Two doublets.

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(36) The transformation of aryl *H*-phosphonates **9** to the corresponding monothio derivatives **2** can also be effected using mixture of TMSCl with hydrogen sulfide.

Experimental Section

Material and Methods. ^{1}H and ^{31}P NMR spectra were recorded at 300 and 121 MHz respectively. The ^{31}P NMR experiments were carried out at 25 °C in 5 mm tubes using 0.1 M concentrations of phosphorus-containing compounds in appropriate solvents (0.6 mL), and the spectra were referenced to 2% H_3PO_4 in D_2O (external standard). TLC analyses were carried out on Merck silica gel 60 F_{254} precoated plates using the following solvent systems: (A) CH_3Cl/CH_3OH 9:1 (v/v); (B) ^{i}PrOH/NH_3 aqueous concentrated/H_2O 85:5:10 (v/v/v); (C) ^{i}PrOH/NH_3 aqueous concentrated/H_2O 7:2:1 (v/v/v). TLC mobilities (R_{TPH}) are reported relative to 5′-*O*-dimethoxytritylthymidine 3′-*H*-phosphonate. Pyridine (LabScan Ltd.) was stored over molecular sieves 4Å until the amount of water was below 20 ppm (Karl Fischer coulometric titration). Dichloromethane (POCH, Poland) was dried with P_2O_5, distilled, and stored over molecular sieves 4Å until the amount of water was below 10 ppm. 1,1,1,3,3,3-Hexamethyldisilathiane, diphenyl chlorophosphate, and trimethylsilyl chloride were commercial grade from Aldrich. Stock solution of hydrogen sulfide (1 M) in dioxane was prepared by passing H_2S through the solvent until saturation.

5′-*O*-Dimethoxytritylated nucleoside 3′-*H*-phosphonates **1a**−**e** were obtained according to published method.[21] Nucleoside aryl *H*-phosphonate **9a**−**e** were generated in situ as described previously.[32] Nucleoside 5′-*H*-phosphonate **7**[21] and nucleoside 5′-*H*-phosphonodithioate **8**[24] were obtained analogously to published procedures. The reference compounds used for the assignment of certain ^{31}P NMR resonances, were obtained as follows: 5′-*O*-dimethoxytritylthymidin-3′-yl trimethylsilyl *H*-phosphonothioate, by the reaction of **2a** with trimethylsilyl chloride in dichloromethane/pyridine;[37] bis(2,4,6-trichlorophenyl) 5′-*O*-dimethoxytritylthymidin-3′-yl phosphite **10**, by treatment of *H*-phosphonate **9e** with diisopropylamine;[32] 2,4,6-trichlorophenyl trimethylsilyl 5′-*O*-dimethoxytritylthymidin-3′-yl phosphites **11a**−**e**, by treatment of the corresponding *H*-phosphonates **9a**−**e** with trimethylsilyl chloride in dichloromethane/pyridine.

The assignments of signals in the ^{31}P NMR spectra to particular products or intermediates were done on the basis of their chemical shifts, multiplicity of the signals in ^{1}*H*-coupled and ^{1}*H*-decoupled spectra, by spiking the reaction mixtures with appropriate species, and if possible, by isolation of a compound in question from reaction mixtures. Multiplicity of some signals in ^{1}H NMR spectra of products **2** are due to P-diastereomers.

General Procedure for Synthesis of Nucleoside 3′-*H*-Phosphonothioates 2a−e. Nucleoside *H*-phosphonate **1** (triethylammonium salt, 0.1 mmol) and 2,4,6-trichlorophenol (1.1 molar equiv) were rendered anhydrous by repeated evaporation of added excess pyridine. The residue was dissolved in dichloromethane (1 mL) containing pyridine (12 molar equiv) and treated with diphenyl chlorophosphate (1.1 molar equiv). When the formation of aryl nucleoside *H*-phosphonate was complete (ca 5 min; ^{31}P NMR), 1,1,1,3,3,3-hexamethyldisilathiane (3 molar equiv) was added, and after 5 min the reaction mixture was diluted with dichloromethane (15 mL) and washed with saturated aqueous $NaHCO_3$ (3 × 10 mL). The organic phase was separated, dried over anhydrous Na_2SO_4, and concentrated to an oil. Nucleoside *H*-phosphonothioates **2** were purified by a silica gel column chromatography using dichloromethane/methanol/triethylamine (95:3:2 v/v/v) as an eluent. Products **2** (ca. 1:1 mixture of diastereomers) were obtained as white solids (triethylammonium salt) after freeze-drying from benzene.

5′-*O*-Dimethoxytritylthymidine 3′-*H*-Phosphonothioate, Triethylammonium Salt (2a). Yield, 0.21 g (96%); R_{TPH} 1.82 (A), 1.24 (B), 1.07 (C); δ_H ($CDCl_3$) 1.33 (9H, t, $^{3}J_{HH}$ = 7.2 Hz), 1.36 and 1.38 (3H, br s), 2.38 (1H, m), 2.61 (1H, m), 3.08 (6H, q, $^{3}J_{HH}$ = 7.2 Hz), 3.45 (2H, m), 3.79 (6H, s), 4.26 and 4.36 (1H, m), 5.32 (1H, m), 6.46 (1H, m), 6.81−7.60 (13H, m), 7.41 (1H, br s), 8.00 and 8.06 (1H, 2d, $^{1}J_{HP}$ = 583.7 and 579.8 Hz). For ^{31}P NMR data, see Table 1. Calcd for $C_{37}H_{48}N_3O_8PS$: C, 61.23; H, 6.67; N, 5.79. Found: C, 61.19; H, 6.72; N, 5.74.

5′-*O*-Dimethoxytrityl-*N*^{6}-benzoyl-2′-deoxyadenosine 3′-*H*-Phosphonothioate, Triethylammonium Salt (2b). Yield, 0.15 g (90%); R_{TPH} 1.91 (A), 1.99 (B), 1.39 (C); δ_H ($CDCl_3$) 1.15 (9H, t, $^{3}J_{HH}$ = 7.2 Hz), 2.75 (6H, q, $^{3}J_{HH}$ = 7.2 Hz), 2.81 (1H, m), 2.91 (1H, m), 3.42 (2H, m), 3.77 (6H, s), 4.44 and 4.46 (1H, m, m), 5.35 (1H, m), 6.60 (1H, m), 6.77−7.61 (18H, m), 8.05 and 8.10 (1H, 2d, $^{3}J_{HP}$ = 582.8 and 581 Hz), 8.19 (1H, s), 8.73 (1H, s). For ^{31}P NMR data see Table 1. Calcd for $C_{44}H_{51}N_6O_7PS$: C, 62.99; H, 6.13; N, 10.02. Found: C, 63.06; H, 6.18; N, 9.98.

5′-*O*-Dimethoxytrityl-*N*^{4}-benzoyl-2′-deoxycytidine 3′-*H*-Phosphonothioate, Triethylammonium Salt (2c). Yield, 0.22 g (90%); R_{TPH} 1.77 (A), 1.88 (B), 1.39 (C); δ_H ($CDCl_3$) 1.32 (9H, q, $^{3}J_{HH}$ = 7.2 Hz), 2.37 (1H, m), 2.90 (1H, m), 3.08 (6H, q, $^{3}J_{HH}$ = 7.2 Hz), 3.47 (2H, m), 3.80 (6H, s), 4.35 and 4.46 (1H, m, m), 5.16 and 5.30 (1H, m, m), 6.31 (1H, t, $^{3}J_{HH}$ = 6.0 Hz), 6.80−7.54 (18H, m), 7.88 (1H, d, $^{3}J_{HH}$ = 7.2 Hz), 8.00 and 8.06 (1H, 2d, $^{1}J_{HP}$ = 587.0 and 574.7 Hz), 8.24 (1H, d, $^{3}J_{HH}$ = 7.2 Hz). For ^{31}P NMR data see Table 1. Calcd for $C_{43}H_{51}N_4O_8PS$: C, 63.38; H, 6.31; N, 6.88. Found: C, 63.44; H, 6.39; N, 6.79.

5′-*O*-Dimethoxytrityl-*N*^{2}-isobutyryl-2′-deoxyguanosine 3′-*H*-Phosphonothioate, Triethylammonium Salt (2d). Yield, 0.22 g (87%); R_{TPH} 1.05 (A), 1.88 (B), 1.39 (C); δ_H ($CDCl_3$) 1.04 (3H, d, $^{3}J_{HH}$ = 6.9 Hz), 1.13 (3H, d, $^{3}J_{HH}$ = 6.9 Hz), 1.28 (9H, t, $^{3}J_{HH}$ = 7.2 Hz), 2.31 (1H, heptet, $^{3}J_{HH}$ = 6.9 Hz), 2.61 (1H, m), 3.04 (6H, q, $^{3}J_{HH}$ = 7.2 Hz), 3.12 (1H, m), 3.22 (1H, m), 3.37 (1H, m), 3.73 (1H, m), 3.76 (6H, s), 4.21 and 4.22 (1H, 2m), 5.74 (1H, m), 6.17 (1H, m), 6.73−7.43 (13H, m), 7.76 (1H, s), 7.93 and 8.06 (1H, 2d, $^{1}J_{HP}$ = 586.1 and 581.3 Hz), 11.91 (1H, br s, exch. D_2O). For ^{31}P NMR data see Table 1. Calcd for $C_{41}H_{53}N_6O_8PS$: C, 59.99; H, 6.51; N, 10.24. Found: C, 60.01; H, 6.59; N, 10.19.

5′-*O*-Dimethoxytrityl-2′-*O*-dimethyltertbutylsilyluridine 3′-*H*-Phosphonothioate, Triethylammonium Salt (2e). Yield, 0.16 g (91%); R_{TPH} 2.18 (A), 2.03 (B), 1.37 (C); δ_H ($CDCl_3$) 0.09−0.20 (6H, m), 0.89 and 0.91 (9H, 2s), 1.31 (9H, t, $^{3}J_{HH}$ = 7.2 Hz), 3.06 (6H, q, $^{3}J_{HH}$ = 7.2 Hz), 3.54 (2H, m), 3.79 (6H, s), 4.35 and 4.49 (1H, 2 m), 4.43 (1H, m), 5.06 and 5.18 (1H, 2m), 5.16 and 5.22 (1H, 2d, $^{3}J_{HH}$ = 8.4 Hz), 5.90 and 6.06 (1H, 2d, $^{3}J_{HH}$ = 3.0 Hz and 6.0 Hz), 6.82−7.43 (13H, m),7.85 and 8.08 (1H, 2d, $^{3}J_{HH}$ = 8.4 Hz), 7.96 and 8.17 (1H, 2d, $^{3}J_{HP}$ = 578.9 Hz and 586.9 Hz). For ^{31}P NMR data see Table 1. Calcd for $C_{42}H_{60}N_3O_9PSSi$: C, 59.91; H, 7.18; N, 4.99. Found: C, 59.94; H, 7.21; N, 5.02.

General Procedure for Synthesis of Nucleoside *H*-Phosphonodithioates 3a−e. Method A. Nucleoside *H*-phosphonate **1** (triethylammonium salt, 0.1 mmol) was rendered anhydrous by repeated evaporation of added excess pyridine and then dissolved in the same solvent (1 mL). To this, hydrogen sulfide (3 molar equiv; 0.3 mL of 1 M stock solution) was added, followed by diphenyl chlorophosphate (3 molar equiv). After 5 min the reaction mixture was quenched with water and evaporated to viscous oil. The residue was dissolved in a minimum volume of dichloromethane and purified by a silica gel column chromatography using dichloromethane/methanol/triethylamine (95:3:2 v/v/v) as an eluent. Fractions containing triethylammonium salts of pure product **3** were collected and freeze-dried from benzene to afford white solids.

Method B. Nucleoside *H*-phosphonate **1** (triethylammonium salt, 0.1 mmol) and 2,4,6-trichlorophenol or 1,2,3,4,5-pentachlorophenyl (3 molar equiv) were rendered anhydrous by repeated evaporation of added excess pyridine. The residue was dissolved in pyridine (1 mL) and treated with diphenyl chlorophosphate (3 molar equiv). When the formation of nucleoside bisaryl phosphite was complete (ca 30 min; ^{31}P NMR) 1,1,1,3,3,3-hexamethyldisilathiane (6 molar equiv) was added. After 10 min the reaction mixture was concentrated, and the residue was dissolved in dichloromethane and washed with saturated aqueous $NaHCO_3$. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated. Nucleoside *H*-phosphonodithioates **3** (triethylammonium salts) were isolated as described in Method A.

(37) Zain, R.; Stawiński, J. *J. Org. Chem.* **1996**, *61*, 6617−6622.

Compounds **3a**−**e** produced by methods A and B were indistinguishable by spectral methods (^{1}H, ^{31}P NMR), had identical TLC mobilities (systems A−C), and gave similar (within the experimental error) elemental analysis data.

5′-*O*-Dimethoxytritylthymidine 3′-*H*-Phosphonodithioate, Triethylammonium Salt (3a). Yields: Method A 0.21 g (92%), Method B 0.22 g (96%). R_{TPH} 1.73 (A), 2.00 (B), 1.39 (C); δ_H ($CDCl_3$) 1.37 (3H, br s), 1.37 (9H, t, $^3J_{HH}$ = 7.2 Hz), 2.38 (1H, m), 2.72 (1H, m), 3.25 (6H, q, $^3J_{HH}$ = 7.2 Hz), 3.45 (2H, m), 3.79 (6H, s), 4.37 (1H, m), 5.38 (1H, dm, $^3J_{HP}$ = 14.9 Hz), 6,45 (1H, m), 6.38−7.41 (13H, m), 7.61 (1H, br s), 8.37 (1H, br m, exch. D_2O), 8.70 (1H, d, $^1J_{HP}$ = 547.1 Hz). For ^{31}P NMR data, see Table 1. Calcd for $C_{37}H_{48}N_3O_7PS_2$: C, 59.90; H, 6.52; N, 5.66. Found: C, 59.81; H, 6.56; N, 5.64.

5′-*O*-Dimethoxytrityl-*N*^{6}-benzoyl-2′-deoxyadenosine 3′-*H*-Phosphonodithioate, Triethylammonium Salt (3b). Yields: Method A 0.15 g (95%), Method B 0.15 g (90%). R_{TPH} 1.95 (A), 2.41 (B), 1.57 (C); δ_H ($CDCl_3$) 1.36 (9H, t, $^3J_{HH}$ = 7.2 Hz), 2.39 (2H, m), 3.19 (6H, q, $^3J_{HH}$ = 7.2 Hz), 3.44 (2H, m), 3.77 (6H, s), 4.53 (1H, m), 5.45 (1H, dm, $^3J_{HP}$ = 12.3 Hz), 6.59 (1H, m), 6.75−8.0 (18H, m), 8.19 (1H, s), 8.72 (1H, s), 8.77 (1H, d, $^1J_{HP}$ = 547.1 Hz), 9.14 (1H, m, exch. D_2O). For ^{31}P NMR data, see Table 1. Calcd for $C_{44}H_{51}N_6O_6PS_2$: C, 61.81; H, 6.01; N, 9.83. Found: C, 61.91; H, 6.09; N, 9.76.

5′-*O*-Dimethoxytrityl-*N*^{4}-benzoyl-2′-deoxycytidine 3′-*H*-Phosphonodithioate, Triethylammonium Salt (3c). Yields: Method A 0.22 g (90%), Method B 0.22 g (90%). R_{TPH} 1.91 (A), 2.35 (B), 1.59 (C); δ_H ($CDCl_3$) 1.32 (9H, t, $^3J_{HH}$ = 7.2 Hz), 2.41 (1H, m), 2.94 (1H, m), 3.09 (6H, q, $^3J_{HH}$ = 7.2 Hz), 3.49 (2H, m), 3.80 (6H, s), 4.43 (1H, m), 5.29 (1H, dm, $^3J_{HP}$ = 14.1 Hz), 6.32 (1H, t, $^3J_{HH}$ = 6.0 Hz), 6.84−7.60 (18H, m), 7.88 (1H, d, $^3J_{HH}$ = 7.2 Hz), 8.28 (1H, d, $^3J_{HH}$ = 7.2 Hz), 8.76 (1H, d, $^1J_{HP}$ = 547.1 Hz). For ^{31}P NMR data, see Table 1. Calcd for $C_{43}H_{51}N_4O_7PS_2$: C, 62.15; H, 6.19; N, 6.74. Found: C, 62.07; H, 6.24; N, 6.69.

5′-*O*-Dimethoxytrityl-*N*^{2}-isobutyryl-2′-deoxyguanosine 3′-*H*-Phosphonodithioate, Triethylammonium Salt (3d). Yields: Method A 0.22 g (87%), Method B 0.23 g (91%). R_{TPH} 1.73 (A), 1.94 (B), 1.40 (C); δ_H ($CDCl_3$) 1.07 (3H, d, $^3J_{HH}$ = 6.8 Hz), 1.14 (3H, d, $^3J_{HH}$ = 6.7 Hz), 1.15 (9H, t, $^3J_{HH}$ = 7.2 Hz), 2.39 (1H, heptet, $^3J_{HH}$ = 6.8 Hz), 2.69 (1H, m), 3.05 (1H, m), 3.15 (6H, q, $^3J_{HH}$ = 7.2 Hz), 3.34 (2H, m), 3.76 (6H, s), 4.33 (1H, m), 5.73 (1H, dq, $^3J_{HP}$ = 14.9 Hz J_{HH} = 4.8 Hz), 6.20 (1H, t, $^3J_{HH}$ = 6.3 Hz), 6.74−7.42 (13H, m), 7.81 (1H, s), 8.70 (1H, d, $^1J_{HP}$ = 547.7 Hz), 12.01 (1H, br m, exch. D_2O). For ^{31}P NMR data, see Table 1. Calcd for $C_{41}H_{53}N_6O_7PS_2$: C, 58.83; H, 6.38; N, 10.04. Found: C, 58.91; H, 6.43; N, 9.97.

5′-*O*-Dimethoxytrityl-2′-*O*-dimethyltertbutylsilyluridine 3′-*H*-Phosphonothioate (3e). Yields: Method A 0.17 g (96%), Method B 0.16 g (90%). R_{TPH} 2.41 (A), 2.18 (B), 1.52 (C); δ_H ($CDCl_3$) 0.16 (3H, s), 0.21 (3H, s), 0.91 (9H, s), 1.35 (9H, t, $^3J_{HH}$ = 7.5 Hz), 3.21 (6H, q, $^3J_{HH}$ = 7.5 Hz), 3.58 (2H, m), 3.79 (6H, s), 4.45 (2H, m), 5.13 (1H, d, $^3J_{HH}$ = 8.2 Hz), 5.21 (1H, dm, $^3J_{HP}$ = 15.7 Hz), 5.93 (1H, d, $^3J_{HH}$ = 3.3 Hz), 6.82−7.43 (13H, m), 8.05 (1H, d, $^3J_{HH}$ = 8.2 Hz), 8.20 (1H, br m, exch. D_2O), 8.76 (1H, d, $^1J_{HP}$ = 551.3 Hz). For ^{31}P NMR data, see Table 1. Calcd for $C_{42}H_{60}N_3O_8PS_2Si$: C, 58.78; H, 7.05; N, 4.90. Found: C, 58.80; H, 7.11; N, 4.84.

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