Nucleoside H-Phosphonates. 15. Preparation of Nucleoside H-Phosphonothioate Monoesters from the Corresponding Nucleoside H-Phosphonates

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A convenient method for the preparation of nucleoside 3'-H-phosphonothioate monoesters from suitably protected nucleoside 3'-H-phosphonates has been developed. It consists of activation of nucleoside H-phosphonates with pivaloyl chloride in the presence of a limited amount of a base followed by treatment with 1,1,1,3,3,3-hexamethyldisilathiane (HMDST).

Introduction

Nucleoside H-phosphonothioates[1,2] represent an interesting class of compounds in the growing field of nucleotide analogues.[3] In recent years, a plethora of DNA and RNA surrogates with modified internucleotidic linkages,[4,5] modified sugar residues,[6,7] or the entire phosphate–sugar backbone replaced by another chemical entity[8] have been designed to act as modulators of gene expression *via* an antisense or an antigene approach.[9] Among these analogues, phosphorothioates[5] occupy a prominent place. They resemble the natural phosphodiester internucleotidic functions and can replace them often without significant loss of biological activity.[10,11] The potential usefulness of nucleoside H-phosphonothioates stems from the fact that they can be considered as convenient synthons for the introduction of an H-phosphonothioate diester group into oligonucleotides. This group can in turn be converted, *inter alia*, into a phosphoromonothioate or a phosphorodithioate function. Phosphorodithioates are known to confer favorable biological properties onto oligonucleotide analogues (*e.g.*, resistance to nucleases) without significantly impairing the ability of such analogues to form stable complexes with the target DNA or RNA sequences.[5,12]

In contradistinction to nucleoside H-phosphonate monoesters, the corresponding H-phosphonothioates are chiral at the phosphorus center. This feature can potentially be exploited in synthetic studies (*e.g.*, in stereospecific synthesis of phosphorothioates or other chiral phosphate analogues) or in mechanistic investigations of certain enzymatic reactions. Preliminary results from this laboratory showed[1] that nucleoside H-phosphonothioate monoesters can be converted into the corresponding diesters with an efficiency similar to that of H-phosphonate derivatives. Subsequent oxidation of H-phosphonothioate diesters under mild conditions opens new synthetic routes to phosphorodithioates,[1,13] phosphorothioselenoates,[1,13] and to other phosphate analogues, which are rather difficult to obtain *via* H-phosphonate or phosphite intermediates. Since the stereochemistry at the phosphorus center can be controlled[14,15] during oxidation of nucleoside H-phosphonothioates, one may obtain phosphate analogues with the same or with inverted configuration.

In order to investigate the chemical and physicochemical properties of this class of compounds in more detail, easy access to H-phosphonothioate monoesters is essential. Recently, we have developed an efficient method for the preparation of nucleoside H-phosphonothioates.[2] This involves phosphinylation of suitably protected nucleosides with triethylammonium phosphinate in the presence of a condensing agent, followed by *in situ* sulfurization of the intermediate nucleoside phosphinate.

Taking into account the increasing availability of H-phosphonate monoesters, which can be efficiently prepared under mild conditions *via* several routes,[16] we have recently embarked on a study directed toward transformation of the H-phosphonate monoester moiety into an H-phosphonothioate function. Since this transformation is a nonoxidative process it should, in principle, be compatible with the presence of other groups that are sensitive to oxidation. This novel procedure is complementary to the previously developed method for an oxidative formation of H-phosphonothioate monoesters.[2] In this paper we describe an efficient method for such a transformation.[17] It consists of the activation of nucleoside 3'-H-phosphonate monoesters with pivaloyl chloride in the presence of a limited amount of a base, followed by treatment with 1,1,1,3,3,3-hexamethyldisilathiane.

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Results and Discussion

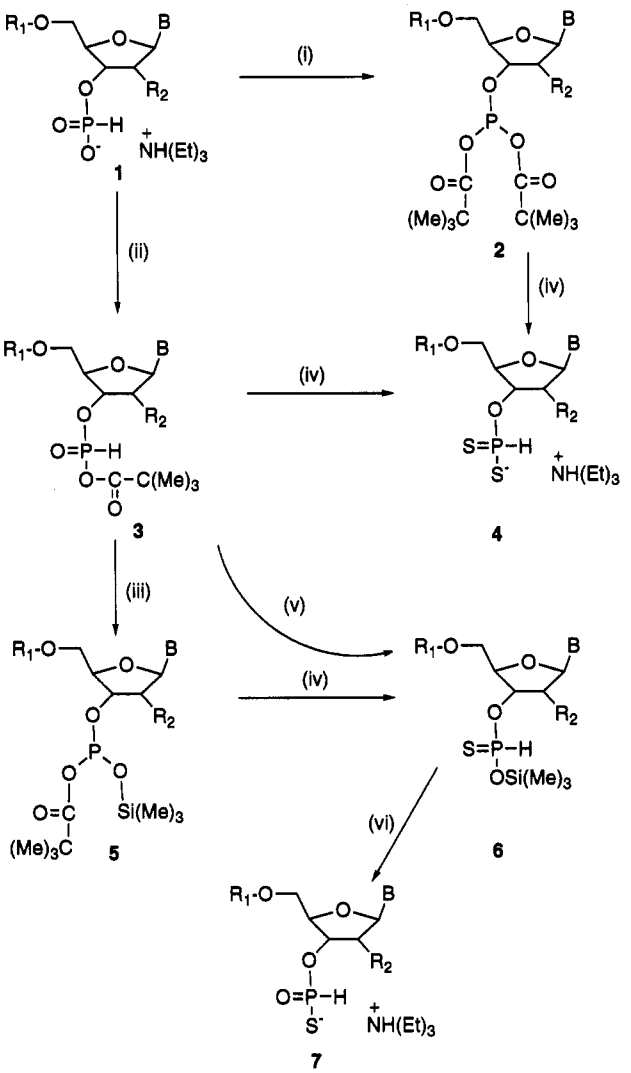
Since mono- and bis-activated species derived from H-phosphonate monoesters and acyl chlorides readily undergo nucleophilic substitution at the phosphorus center affording phosphorous acid esters,[18] we wanted to know if the same chemoselectivity holds when hydrogen sulfide is used as a nucleophile. We have recently reported that activation of nucleoside H-phosphonate monoesters **1** in pyridine with 3 equiv of pivaloyl chloride (PV-Cl) followed by treatment with hydrogen sulfide affords a high yield of the thio analogue in which two oxygen atoms have been replaced by sulfur (the H-phosphonodithioates **4**[19]). Although the most likely intermediate in the reaction is the bisacyl phosphite[18] (**2**), this *per se* does not preclude formation of nucleoside H-phosphonomonothioate (**7**) under the reaction conditions. Unfortunately, irrespective of any changes made in the synthetic protocol,[19] only minor amounts of the monothio derivatives **7** were formed.

We therefore attempted to take advantage of an alternative activation pathway of H-phosphonate monoesters which affords the mixed anhydrides of type **3** when the activation process is carried out in the presence of a limited amount of pyridine[18] or in a less basic solvent, *e.g.*, quinoline.[20] Although the intermediate **3** is a monofunctional phosphonylating agent, our preliminary observations[17] indicated that this species also tended to afford the dithio derivatives **4** predominantly, instead of the desired compounds **7**, when treated with hydrogen sulfide. Here again, all attempted modifications of the reaction conditions failed to produce any significant increase in the amount of the monothioate **7**. Considering the product distribution under various reaction conditions,[17,19] it seems most likely that in this instance the monothio derivative **7** is initially formed but apparently undergoes subsequent reactions with either the mixed anhydride **3** and/or with PV-Cl to form new intermediates which, in the presence of hydrogen sulfide, afford the H-phosphonodithioates **4**.

Since neither the mixed anhydrides **3** nor the bisacyl phosphites **2** could be efficiently converted to the H-phosphonomonothioates **7** by treatment with hydrogen sulfide we have been searching for a new type of P(III) intermediate which would possess chemical properties suitable for our purpose and which could be generated from the H-phosphonate monoesters **1**. Because hydrogen sulfide selectively attacks the silicon center in nucleoside bis(trimethylsilyl) phosphites,[21] we have chosen the acyl silyl phosphite **5** as a potentially useful intermediate on the way to the monothio derivatives **7**. We hypothesized that the intermediate **5** upon treatment with hydrogen sulfide should produce the silylated H-phosphonothioate **6**. This, in contradistinction to the H-phosphonothioate **7**, should not undergo a subsequent activation with **5**, and thus, even if the thiation would be slow, only the monothioated species of type **6** should be formed (Scheme 1).

To check the efficacy of such an approach, we attempted to form the acyl silyl phosphite **5a** and to investigate its reactivity toward hydrogen sulfide. To this end, the mixed anhydride **3a** [δ_P = 2.27 and 2.13 ppm, $^1J_{PH}$ = 748.3 Hz (d), $^3J_{PH}$ = 8.5 Hz (d)], produced *in situ* from the H-phosphonate **1a** and 1.5 equiv of PV-Cl in acetonitrile containing 20% of quinoline, was treated with trimethylsilyl chloride (TMS-Cl, 5 equiv), and to this a solution of hydrogen sulfide in dioxane was added. The ^{31}P NMR spectra recorded at various stages of the reaction showed that **3a** reacted immediately with TMS-Cl producing an intermediate which resonated in the range of chemical shifts expected for nucleoside acyl silyl phosphites. This, presumably the **5a** intermediate [δ_P = 121.60 and 121.50 ppm, $^3J_{PH}$ = 9.8 and 8.6 Hz, (d)], upon treatment with hydrogen sulfide rapidly afforded a compound which gave rise to two singlets in the ^{31}P NMR spectrum with the chemical shifts and the $^1J_{PH}$ coupling constants consistent with the silylated nucleoside H-phosphonothioate **6a** [δ_P = 57.30 and 56.70 ppm, and $^1J_{PH}$ = 659.6 and 660.0 Hz (d), $^3J_{PH}$ = 11.6 and 11.0 Hz (d), respectively]. During aqueous workup this com-

Scheme 1



(i) Pivaloyl chloride in pyridine; (ii) Pivaloyl chloride in CH_3CN/quinoline (4:1, v/v); (iii) Trimethylsilyl chloride; (iv) Hydrogen sulfide in dioxane; (v) $(Me)_3Si\text{-}S\text{-}Si(Me)_3$ (HMDST); (vi) Aqueous work-up

1a-7a, B=thymin-1-yl, R_1=dmt, R_2=H
1b, 3b, 5b, 6b, 7b, B=N^4-propionylcytosin-1-yl, R_1=dmt, R_2=H
1c, 3c, 5c, 6c, 7c, B=N^6-butyryladenin-9-yl, R_1=dmt, R_2=H
1d, 3d, 5d, 6d, 7d, B=N^2-phenoxyacetylguanin-9-yl, R_1=dmt, R_2=H
1e, 3e, 5e, 6e, 7e, B=uracil-1-yl, R_1=mmt, R_2= O-TBDMS
dmt - 4,4'-dimethoxytrityl; mmt - 4- monomethoxytrityl;
TBDMS - t-butyldimethylsilyl

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pound underwent spontaneous desilylation to afford the desired nucleoside H-phosphonothioate **7a**.

During silylation of the mixed anhydride **3a** with TMS-Cl, we observed, however, that apart from **5a**, two additional intermediates were always formed (δ_P ~154 and ~−2.9 ppm). The amount of the former one, as judged from the intensity of the ^{31}P NMR signal, tended to increase when more equivalents of TMS-Cl were used for the silylation. Since addition of hydrogen sulfide at various stages of the reaction invariably resulted in immediate disappearance of the signal at ~154 ppm and the formation of the monothioated product **6a**, we assumed that the intermediate in question probably contained, at the phosphorus center, only one group susceptible to substitution. These, together with the chemical shift value, suggested that the most likely structure of the intermediate resonating at ~154 ppm is that of 5′-*O*-(dimethoxytrityl)thymidin-3′-yl trimethylsilyl chlorophosphite.

The other intermediate, resonating at high field in ^{31}P NMR (δ_P ~−2.9 ppm), was unreactive toward hydrogen sulfide and afforded the parent H-phosphonate **1** upon aqueous workup of the reaction mixtures. On the basis of the ^{31}P NMR data (chemical shifts, multiplicity of signals, and the coupling patterns in the {^{1}H}-coupled spectra) and chemical reactivity and by comparison with a sample prepared by a different route,[22] it was identified as 5′-*O*-(dimethoxytrityl)thymidin-3′-yl (trimethylsilyl)-H-phosphonate (δ_P ~−2.9 ppm, two singlets).

Since nucleoside silyl H-phosphonate derivatives (the intermediate at δ_P −2.9 ppm) cannot be converted into the desired products **7**, it was important to pinpoint sources of their formation. Spurious water can in part be responsible for the generation of these side products, but more detailed studies seem to indicate that chloride anion can be involved in their formation.[23] Trying to overcome this problem, we turned our attention to 1,1,1,3,3,3-hexamethyldisilathiane (HMDTS),[24] a reagent which is known to be able to transfer trimethylsilyl groups *inter alia* to alcohols and carboxylic acids.[25] Inasmuch as hydrogen sulfide and/or silylated thiols are generated during this process,[25,26] the reagent seemed most attractive for our synthetic scheme owing to its ability of acting simultaneously as a silylating agent and a source of hydrogen sulfide.

The efficacy of HMDTS was checked by monitoring changes in the ^{31}P NMR spectrum of the reaction mixture resulting from addition of 5 equiv of the reagent to the mixed anhydride **3a**, formed *in situ* from the H-phosphonate **1a**. The intermediate **3a** disappeared quickly before the first ^{31}P NMR spectrum could be recorded (ca 3 min) and the only signals observed were those from the acyl silyl intermediate **5a** and the silylated H-phosphonothioate **6a**. Although the reaction proceeded rapidly to ca. 75% completion, it took usually 6−8 h until the intermediate **5a** was completely converted into the desired product (**6a**). We have found, however, that the time for conversion of the acyl silyl phosphites **5** to the H-phosphonothioate derivatives **6** can be substantially reduced (to 15−20 min) by adding a few equivalents of quinolinium hydrochloride (Q·HCl) to the reaction mixture. Since the intermediate **5a** reacted with hydrogen sulfide to completion within few minutes,[17] it is possible that the observed accelerating effect of Q·HCl in the reaction with HMDST can be due to generation of hydrogen sulfide or the silylated thiol from the reagent. The mechanism for generation of hydrogen sulfide or the silylated thiol from HMDST by Q·HCl is unknown. However, from our preliminary studies on the influence of different salts (pyridinium hydrochloride, triethylammonium hydrochloride, or pyridinium *p*-toluenesulfonate) on the rate of the thiation, it seems that the presence of chloride ions is essential for the catalytic activity.

One should bear in mind that the activation process, *i.e.*, the formation of mixed anhydrides **3**, is critical for the overall yield of the conversion of H-phosphonate monoesters **1** into H-phosphonothioates **7**. Since the intermediate **3**, even in acetonitrile−quinoline (4:1, v/v), slowly undergoes further activation to the bisacyl phosphite **2** (from which H-phosphonodithioates are formed upon treatment with hydrogen sulfide[19]), prolonged activation of H-phosphonate monoesters with PV-Cl should be avoided. The formation of **3** in quinoline is much faster than its subsequent reaction to the bisactivated species **2**. However, to suppress the formation of **2** further, we found it beneficial to carry out the reaction in acetonitrile in the presence of only 10−12 equiv of quinoline. Under such conditions the rate of the double activation of **1** was drastically reduced, while the basicity of the medium was still high enough to provide a proper stability for even rather acid sensitive protecting groups present in the nucleoside moieties (*e.g.*, 5′-*O*-(dimethoxytrityl) function).

The procedure, which we believe is close to optimal, for the conversion of H-phosphonate monoesters to the corresponding H-phosphonothioates thus consists of activation of **1** (0.05 mmol/mL) with pivaloyl chloride (1.5 equiv) for 5 min in acetonitrile−dichloromethane (4:1, v/v) in the presence of 10−12 equiv of quinoline, followed by treatment with HMDST. The mixture of dichloromethane in acetonitrile was found to be superior over neat acetonitrile for solubility reasons.

To evaluate the generality of the procedure we synthesized all four deoxyribonucleoside 3′-H-phosphonothioates **7a−d** starting from the corresponding H-phosphonate monoesters **1a−d**. The applicability of the method to the ribo series was assessed by carrying out a similar transformation on the uridine derivative **1e**. After aqueous work-up the nucleoside 3′-H-phosphonothioates **7a−e** were conveniently isolated by silica gel chromatography using chloroform−methanol as eluent. The compounds were chromatographically homogenous (purity ~98% by ^{1}H NMR) and did not require additional purification on a reversed-phase silica gel column as in the previously described procedure.[2]

The transformation of an H-phosphonate into an H-phosphonothioate function in the ribo series (conversion of **1e** to **7e**) proved to be a stereoselective process. The ratio of the diastereomers formed in this reaction (~4:1) was close to that observed in condensations of 2′-*O*-(*tert*-butyldimethylsilyl) ribonucleoside 3′-H-phosphonates with suitably protected nucleosides.[27,28] In contradistinction to this transformation, synthesis of the ribonucleoside 3′-H-phosphonothioate **7e** *via* phosphinate intermediates[2] hardly showed any stereoselectivity.

(22) 5′-*O*-(Dimethoxytrityl)thymidin-3′-yl (trimethylsilyl)-H-phosphonate was prepared in situ from **1** and trimethylsilyl chloride in acetonitrile in the presence of a limited amount of quinoline.

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In conclusion, we have developed a new, general method for the preparation of nucleoside H-phosphonothioates *via* transformation of an H-phosphonate function into an H-phosphonothioate. With the growing number of synthetic routes to H-phosphonate monoesters, the method may provide a convenient entry to nucleoside H-phosphonothioates and other natural product analogues bearing this functionality. The main advantages of this method are as follows: (i) efficiency and experimental simplicity; (ii) commercial availability of the required reagents; (iii) mild reaction conditions; (iv) significant reduction of side products compared to the procedure involving TMS-Cl and H_2S; and (v) stereoselectivity, which may be exploited in stereochemical studies involving ribonucleoside 3'-H-phosphonothioates.

Experimental Section

Materials and Methods. ^{31}P NMR experiments were carried out in 10-mm tubes, and 2% H_3PO_4 in D_2O was used as external standard (coaxial inner tube). The values of the chemical shifts for the intermediates produced *in situ* in some experiments varied (±1 ppm) depending on the reaction conditions. The nucleoside H-phosphonate monoesters **1** were prepared from the corresponding nucleosides using H-pyrophosphonate[29] (**1a**–**d**) or diphenyl H-phosphonate[16] (**1d** and **1e**) as a phosphonylating agent. Introduction of mono- and dimethoxytrityl[30] and *tert*-butyldimethylsilyl[31] groups was done by standard methods. Protecting groups for the heterocyclic bases are the same as those proposed previously[32] and used for RNA synthesis *via* the H-phosphonate approach.[33] Compounds **2**,[18] **3**,[18,20] **4**,[19] and **7**[2] used for the identification of some products or intermediates in the reaction mixtures were prepared according to known procedures. Compound **6a** was prepared *in situ via* silylation of H-phosphonothioates **7** with trimethylsilyl chloride (5 equiv) in pyridine. TLC analyses were carried out on Merck silica gel 60 F_{254} precoated plates using the following eluents: chloroform/methanol (8:2 v/v; system A); chloroform/methanol (9:1 v/v; system B); 2-propanol/33% aqueous ammonia/water (85:5:10, v/v/v; system C).

Pyridine, quinoline, acetonitrile, and triethylamine (TEA) were refluxed with CaH_2 and then distilled and stored over molecular sieves or CaH_2 (TEA). Pivaloyl chloride and 1,1,1,3,3,3-hexamethyldisilathiane (HMDST) were commercial grade (Fluka). The stock solution of 1 M H_2S was prepared by passing hydrogen sulfide through dioxane till saturation.

General Procedure for Synthesis of Nucleoside 3-H-Phosphonothioate Monoesters 7. A suitably protected nucleoside 3'-H-phosphonate (**1a**–**e**, triethylammonium salt, 1 mmol) was rendered anhydrous by evaporation of added acetonitrile. The residue was dissolved in acetonitrile–dichloromethane (4:1 v/v, 20 mL) containing quinoline (10 equiv) and treated with pivaloyl chloride (1.5 equiv) during 5 min. To this, 1,1,1,3,3,3-hexamethyldisilathiane (HMDST, 3 equiv) was added in one portion, and the reaction mixture was left overnight (procedure A). Alternatively (procedure B), the reaction was carried out in the presence of 3 equiv of quinolinium hydrochloride (completion within 15–20 min, ^{31}P NMR analysis).

The reaction was quenched with 1 M triethylammonium hydrogencarbonate (TEAB), and partitioned between dichloromethane (2 × 50 mL) and 0.5 M TEAB (30 mL). The organic phase was evaporated, and the residue was purified on a silica gel column using a stepwise gradient of methanol in chloroform (0–8%, v/v) containing TEA (0.02%). Fractions containing the desired product as a mixture of both diastereomers were pooled, concentrated under reduced pressure and dried overnight on a vacuum line.

5'-*O*-(4, 4'-Dimethoxytrityl)thymidin-3'-yl H-phosphonothioate, triethylammonium salt (7a): yield 82% (procedure A); R_f 0.27 (system A) and 0.53 (system C); ^{31}P NMR (CH_2Cl_2, δ in ppm) 53.95 and 53,20 ($^1J_{PH}$ = 580.5 and 585.9 Hz, d; $^3J_{PH}$ = 11.6 and 11.0 Hz, d); ^1H NMR ($CDCl_3$, δ in ppm) (multiplicity of some signals due to the presence of P-diastereomers) 8.10 and 8.00 (2 d, $^1J_{PH}$ = 580 and 585 Hz, 1H), 7.62 (s, 1H), 6.46 (m, 1H), 5.34 (m, 1H), 4.37 and 4.27 (2 m, 1H), 3.52 and 3.42 (2 m, 2H), 2.69 and 2.39 (2m, 2H), 1.38 and 1.36 (2s, 3H); HRMS(FAB) calcd for $C_{31}H_{32}O_8N_2PS$ (M − TEAH$^+$) 623.1617, found (M − TEAH$^+$) 623.1624.

5'-*O*-(4, 4'-Dimethoxytrityl))-N^4-propionyldeoxycytidin-3'-yl H-phosphonothioate, triethylammonium salt (7b): yield 82% (procedure B; R_f 0.26 (system A) and 0.59 (system C); ^{31}P NMR (CH_2Cl_2, δ in ppm) 53.96 and 53.19 ($^1J_{PH}$ = 574.3 and 580.5 Hz, d; $^3J_{PH}$ = 11.6 and 11.6 Hz, d); ^1H NMR ($CDCl_3$, δ in ppm) (multiplicity of some signals due to the presence of P-diastereomers) 8.05 and 8.0 (2d, $^1J_{PH}$ = 577 and 587 Hz, 1H), 8.18 (d, ^3J = 7.3 Hz, 1H), 7.1 (d, ^3J = 7.3 Hz), 6.27 (t, ^3J = 6.0 Hz, 1H), 5.15 and 5.29 (2m, 1H), 4.45 and 4.35 (2m, 1H), 3.48 (m, 2H), 2.64 and 2.34 (2m, 2H); HRMS(FAB) calcd for $C_{33}H_{35}O_8N_3PS$ (M − TEAH$^+$) 664.1883, found (M − TEAH$^+$) 664.1871.

5'-*O*-(4, 4'-Dimethoxytrityl))-N^6-butyryldeoxyadenosin-3'-yl H-phosphonothioate, triethylammonium salt (7c): yield 85% (procedure B); R_f 0.33 (system A) and 0.69 (system C); ^{31}P NMR (CH_2Cl_2, δ in ppm) 53.51 and 53.41 ($^1J_{PH}$ = 578.0 and 577.4 Hz, d; $^3J_{PH}$ = 10.3 and 12.3 Hz, d); ^1H NMR ($CDCl_3$, δ in ppm) (multiplicity of some signals due to the presence of P-diastereomers) 8.07 and 8.03 (2d, $^1J_{PH}$ = 581 and 582 Hz, 1H), 8.62 (s, 1H), 8.14 (s, 1H), 6.54 (m, 1H), 5.33 (m, 1H), 4.50 and 4.41 (2m, 1H), 3.45 (m, 2H), 3.07 and 2.66 (2m, 2H); HRMS(FAB) calcd for $C_{35}H_{37}O_7N_5PS$ (M − TEAH$^+$) 702.2151, found (M − TEAH$^+$) 702.2145.

5'-*O*-(4, 4'-Dimethoxytrityl)-N^2-(phenoxyacetyl)deoxyguanosin-3'-yl H-phosphonothioate, triethylammonium salt (7d): yield 71% (procedure A); R_f 0.29 (system A) and 0.51 (system C); ^{31}P NMR (CH_2Cl_2, δ in ppm) 53.83 and 53.33 ($^1J_{PH}$ = 578.6 and 577.4 Hz, d; $^3J_{PH}$ = 12.2 and 11.0 Hz, d); ^1H NMR ($CDCl_3$, δ in ppm) (multiplicity of some signals due to the presence of P-diastereomers) 8.09 and 8.03 (2d, $^1J_{PH}$ = 581 and 582 Hz, 1H), 7.82 (s, 1H), 6.36 (m, 1H), 5.40 (m, 1H), 4.50 and 4.35 (2m, 1H), 3.45 and 3.35 (2m, 2H), 3.10 and 2.61 (2m, 2H); HRMS(FAB) calcd for $C_{39}H_{37}O_9N_5PS$ (M − TEAH$^+$) 782.2050, found (M − TEAH$^+$) 782.2043.

5'-*O*-(4-Monomethoxytrityl)-2'-*O*-(*tert*-butyldimethylsilyl)uridin-3'-yl H-phosphonothioate, triethylammonium salt (7e): yield 90% (procedure A); R_f 0.34 (system A) and 0.73 (system C); ^{31}P NMR (CH_2Cl_2, δ in ppm) 55.65 and 53.82 ($^1J_{PH}$ = 582.3 and 576.2 Hz, d; $^3J_{PH}$ = 12.3 and 13.4 Hz, d); ^1H NMR ($CDCl_3$, δ in ppm) (multiplicity of some signals due to the presence of P-diastereomers) 8.17 and 7.98 (2d, $^1J_{PH}$ = 586 and 578 Hz, 1H), 8.07 and 7.86 (2d, ^3J = 8.1, 1H), 6.06 and 5.91 (2d, ^3J = 5.9 and 2.2 Hz, 1H), 5.15 (m, 2H), 4.50 and 4.45 (2m, 2H), 4.36 (m, 1H), 3.56 (m, 2H); HRMS(FAB) calcd for $C_{35}H_{42}O_8N_2PSSi$ (M − TEAH$^+$) 709.2169, found (M − TEAH$^+$) 709.2197.

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Supporting Information Available: Copies of NMR spectra (11 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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