Nucleoside *H*-Phosphonates. 18. Synthesis of Unprotected Nucleoside 5′-*H*-Phosphonates and Nucleoside 5′-*H*-Phosphonothioates and Their Conversion into the 5′-Phosphorothioate and 5′-Phosphorodithioate Monoesters

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A simple and efficient protocol for the preparation of unprotected nucleoside 5′-*H*-phosphonates and nucleoside 5′-*H*-phosphonothioates via a one-step deprotection of suitable precursors with methylamine has been developed. The synthetic utility of the unprotected nucleotide derivatives was demonstrated by converting them under mild conditions to the corresponding nucleoside 5′-phosphorothioate and nucleoside 5′-phosphorodithioate monoesters. Factors affecting oxidation of *H*-phosphonate, *H*-phosphonothioate, and phosphite derivatives with elemental sulfur are also discussed.

Introduction

In the last two decades phosphorothioate analogues of naturally occurring substances have become a firmly established tool in biochemical and biological studies directed toward unraveling of enzyme functions and mechanisms.[1] Due to their chirality at the phosphorus center and higher stability toward enzymatic hydrolysis, phosphorothioate derivatives have proven to be valuable as model constructs for designing enzyme inhibitors and transition state analogues, as stereochemical probes in elucidation of mechanisms of enzyme-catalyzed phosphoryl transfer reactions, or for probing structures of ribozymes, etc.[1−3]

Although nucleoside 5′-phosphate monoesters are central compounds in numerous biochemical and pharmacological studies,[2,4,5] their phosphorodithio analogues, in contradistinction to those of phosphate diesters, have received relatively little attention.[6−8] In fact, nucleoside phosphorodithioates, bearing two sulfur atoms at the nonbridging positions of the phosphomonoester moiety, have been prepared only recently[6,7,9,10] and their syntheses highlighted some fundamental problems connected with their preparation.

Due to the inherent instability of phosphorodithioate monoesters, most synthetic organic methods for thiophosphorylation of nucleosides[1] have been found inapplicable to the synthesis of these phosphate analogues. For example, an attempted synthesis of nucleoside phosphorodithioates via *S*-alkyl and *O*-alkyl phosphorodithioate diesters or the corresponding phosphotriesters failed, due to problems encountered during the removal of phosphate protecting groups.[6] An approach involving nucleoside *H*-phosphonodithioates as intermediates has been more successful, but also this synthesis was hampered by a low yield in some crucial steps and formation of significant amounts of side products during the final deprotection.[6] The route via 2-thio-1,3,2-dithiaphospholane derivatives, as proposed by Okruszek et al.,[7] produced nucleoside phosphorodithioates in acceptable yields (ca. 50%), but it required the inclusion of two extra steps (the introduction and removal of a 2-cyanoethyl group) into the synthetic protocol to prevent a severe decomposition of the products during the hydrolytic opening of the dithiaphospholane ring. The development of 9-fluorenemethyl *H*-phosphonothioate as a −(H)P(S)-

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O^- group transferring reagent[9,10] significantly improved the synthesis of nucleoside phosphorodithioates, although some degradation of the product during the removal of the 9-fluorenemethyl group was still observed.[10]

Nucleoside phosphorodithioates are interesting as potential inhibitors of several classes of enzymes, e.g. kinases, phosphatases, polymerases, etc. Preliminary biological studies indicate that phosphorodithioate monoesters derived from naturally occurring deoxyribonucleosides are resistant toward alkaline phosphatase treatment,[9,11] are neutral against DNA polymerase I Klenow fragment and human immunodeficiency virus reverse transcriptase (HIV RT),[7,9,11] and are competitive inhibitors of avian myeloblastosis virus reverse transcriptase (AMV RT)[11] and thymidylate synthase.[8] Since unprotected nucleotide analogues are required for most biochemical applications, an additional factor has to be taken into account when designing the synthesis of nucleoside phosphorodithioates, namely, the stability of the phosphorodithioate function during the final removal of sugar and heterocyclic base protecting groups.

To find a possibly general solution to the problem of instability of some functionalities during a final deprotection, we have recently embarked on studies directed toward preparation and evaluation of synthetic potential of unprotected nucleoside *H*-phosphonate and *H*-phosphonothioates. Since the *H*-phosphonate or *H*-phosphonothioate function can be selectively activated under oxidative conditions, access to such precursors would alleviate most problems often encountered during the synthesis of some labile nucleotide analogues, e.g. nucleoside phosphorodithioate monoesters. This kind of approach would also considerably expand applications of *H*-phosphonate and *H*-phosphonothioates as synthetic intermediates.

In this paper we describe synthesis of various unprotected nucleoside *H*-phosphonates and nucleoside *H*-phosphonothioates and demonstrate that they can serve as convenient precursors for the preparation of nucleoside phosphoro*mono*thioate and phosphoro*di*thioate monoesters.[12]

Results and Discussion

To avoid a possible degradation of a fragile modification in some nucleotide analogues during a multistep synthesis, we searched for an approach enabling the introduction of the most labile functionality in the last synthetic step. In the instance of nucleoside phosphorothioate monoesters, this would call for unprotected nucleotidic precursors,[13] which could be selectively converted in the desired product. As a viable approach for this purpose we considered sulfurization of unprotected nucleoside *H*-phosphonates and nucleoside *H*-phosphonothioates, which should provide a facile access to nucleoside phosphoro*mono*thioate and nucleoside phosphoro*di*thioate monoesters under exceedingly mild reaction conditions.

Unprotected nucleoside *H*-phosphonate and nucleoside *H*-phosphonothioate monoesters seem to be well suited as synthetic precursors, for at least two reasons. First, we expected, that these compounds should be equally stable and easy to handle as their protected counterparts, and with a variety of phosphonylation[14−17] and thiophosphonylation[10,18,19] methods available, they can also be relatively easily accessible. Second, being tetracoordinated P(III) compounds, they should undergo a variety of selective oxidative transformations at the phosphorus center[20] providing, under mild conditions, unprotected P(V) analogues.

Synthesis of Unprotected Nucleoside 5′-*H*-Phosphonates and Nucleoside 5′-*H*-Phosphonothioates. First, we have been searching for a simple method for the preparation of nucleoside 5′-*H*-phosphonates **3a**−**f** and nucleoside 5′-*H*-phosphonothioates **4a**−**f**. Due to a plethora of synthetic methods available for the phosphonylation[14−16] and thiophosphonylation[10,18,19] of protected nucleosides, we decided to use as starting materials for **3** and **4** standard nucleosidic precursors that are either easy to prepare or are commercially available. To this end, suitably protected *N*,*O*-acylated deoxyribonucleosides were converted into the corresponding 5′-*H*-phosphonates **1a**−**f** using the diphenyl *H*-phosphonate method.[15] These products without intermediate purification were subjected to various deprotection procedures. Among the protocols investigated, the most convenient was found to be that of utilizing aqueous methylamine (50%)[21] as a deprotecting reagent. In accordance with the reported data,[21] methylamine proved to be superior to aqueous concentrated ammonia, both in efficiency and in time required for a complete deprotection. The most significant differences between aqueous ammonia and methylamine as deprotecting reagents were observed for the deoxyguanosine derivative **1c** [1 h, RT (room temperature), for methylamine vs 24 h, 50 °C, for the concentrated ammonium hydroxide]. Thus, we adopted treatment with methylamine (1 h, RT) as a standard deprotection procedure[22] for the preparation of unprotected nucleoside 5′-*H*-phosphonates **3a**−**f** from the corresponding precursors **1a**−**f**. Since the deprotection reactions were virtually quantitative, **3a**−**f** could be isolated by simple flash silica gel chromatography, which provided the products of purity >98%, in satisfactory yields. Structures of the isolated compounds were confirmed by spectral and chromatographic analysis [^{1}H and ^{31}P NMR (Table 1) and HRMS and TLC].

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(12) Studies on synthesis of phosphate analogues utilizing unprotected *H*-phosphonates, *H*-phosphonothioates, and *H*-phosphonodithioates derived from various natural products are currently under investigation in these laboratories.

(13) Attempted dithiophosphorylation of unprotected (or partially protected) nucleosides with unsubstituted dithiophosphate dianion in dimethylformamide afforded exclusively mixtures of the corresponding 3′(5′) nucleoside phosphoro*mono*thioates. See: Dunaway-Mariano, D. *Tetrahedron* **1976**, *32*, 2991−2996.

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(22) Unprotected nucleoside *H*-phosphonates **3a**−**f** were stable under the reaction conditions even during a prolonged (12 h) treatment with aqueous methylamine (^{31}P NMR and TLC analysis).

Table 1. ^{31}P NMR Data for Nucleoside *H*-Phosphonates, *H*-Phosphonothioates, Nucleoside Phosphorothioates, and Phosphorodithioates

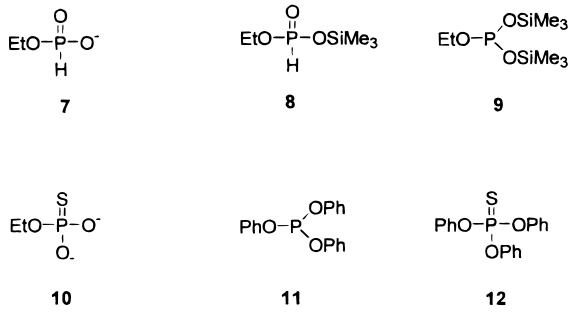
compd	δ_P (ppm)[a]	$^1J_{HP}$ (Hz)	$^3J_{HP}$ (Hz)
3a	6.54	639.6	6.5[b]
3b	5.13	621.0	
3c	6.63	639.6	7.0[b]
3d	5.00	619.2	
3e	6.42	638.7	6.0[b]
3f	6.48	639.0	6.3[b]
4a	55.40, 55.56[c]	598.1, 598.1	8.3, 8.3[b]
4b	55.35, 55.54[c]	593.2, 592.3	*d*
4c	55.40, 55.52[c]	594.2, 595.1	8.3, 8.3[b]
4d	55.23, 55.38[c]	592.3, 592.3	8.3, 7.4[b]
4e	55.44, 55.64[c]	594.2, 593.2	8.5, 7.5[b]
4f	55.67, 55.68[c]	575.6, 575.6	7.4, 7.4[b]
5a	45.09		*e*
5b	48.72		*e*
5c	44.82		*e*
5d	47.72		*e*
5e	44.38		*e*
5f	43.47		*e*
6a	87.94		6.5[f]
6b	87.74		4.6[f]
6c	87.95		7.4[f]
6d	87.68		7.5[f]
6e	88.10		6.5[f]
6f	87.92		6.5[f]

[a] Spectra recorded in H_2O containing NaOD (pH = 8.0) with heteronuclear decoupling (H_3PO_4 as external reference). [b] Two triplets. [c] Two diastereoisomers. [d] Poorly resolved triplet. [e] Unresolved broad multiplet. [f] Triplet.

For the preparation of unprotected nucleoside 5′-*H*-phosphonothioates **4**, easily accessible nucleoside 9-fluorenemethyl *H*-phosphonothioates[10] of type **2** were used. An attempted deprotection of these compounds with aqueous methylamine produced, however, a mixture of products, most likely due to the competing loss of nucleoside moieties (ca. 25%, ^{31}P NMR). We solved this problem by carrying out the deprotection of nucleoside *H*-phosphonothioates **2** as a two-step "one-pot" reaction. First, the 9-fluorenemethyl group from **2a**−**f** was removed with anhydrous triethylamine (20 min),[10] and the resulting nucleoside *H*-phosphonothioate monoesters without their isolation were treated with aqueous 50% methylamine (RT, 1 h)[23] to remove the sugar and nucleobase protecting groups. The unprotected nucleoside 5′-*H*-phosphonothioates **4a**−**f** were isolated by silica gel column chromatography with excellent yields, and their structures were confirmed by ^{1}H and ^{31}P NMR (Table 1), HRMS, and TLC analysis.

Synthesis of Unprotected Nucleoside 5′-Phosphoromonothioates and Nucleoside 5′-Phosphorodithioates. In contradistinction to phosphite triesters and *H*-phosphonate diesters, the phosphorus center in *H*-phosphonate monoesters is rather resistant to oxidation.[20] However, conversion of these compounds into tervalent silyl derivatives significantly enhanced their susceptibility toward electrophiles and facilitated their oxidative transformations.[24] Inspired by the Hata et al.[25] findings that thymidine bis(trimethylsilyl) phosphite can produce the corresponding phosphorothioate in the reaction with elemental sulfur,[26] we investigated this approach in more detail and developed it as a general procedure for the preparation of various unprotected nucleoside phosphoro*mono*- and phosphoro*di*thioate monoesters.

Chart 1



Preliminary experiments showed that rates of sulfurization of the silylated nucleoside *H*-phosphonates varied significantly with the solvent and the base used for the reaction. We have previously observed the same phenomena during sulfurization of H-phosphonate diesters.[27] These findings suggest that the mechanism involved is similar to that previously found for the sulfurization of tertiary phosphines and phosphite triesters,[28−30] for which second-order kinetics and the opening an octaatomic sulfur ring in the rate-determining step were observed. Since these reactions proceed via a polar transitions state with phosphorus acquiring positive charge, they are sensitive to the polarity of solvents.

Due to significant differences in reactivity of tervalent vs tetracoordinated phosphorous acid esters toward sulfur, first we wanted to assess the extent of their formation during silylation of *H*-phosphonate monoesters under various reaction conditions. Ethyl *H*-phosphonate **7** (δ_P 4.76 ppm, $^1J_{HP}$ = 609.3 Hz, $^3J_{HP}$ = 8.5 Hz) (Chart 1), treated with (TMS)Cl (3 molar equiv) in pyridine produced only the tetracoordinated species, ethyl trimethylsilyl *H*-phosphonate (**8**) (δ_P −3.51 ppm, t, $^1J_{HP}$ = 689.7 Hz, $^3J_{HP}$ = 8.6 Hz), which then reacted with added sulfur (3 molar equiv) with a rate comparable to that of diethyl *H*-phosphonate (a few hours) to produce (after hydrolysis) ethyl phosphorothioate (**10**) (δ_P 43.68 ppm, m, $^3J_{HP}$ = 8.5 Hz).[31] When the silylation of *H*-phosphonate **7** was carried out in pyridine in the presence of triethylamine (20 mol equiv), the putative tervalent species, ethyl bis(trimethylsilyl) phosphite (**9**) (δ_P 117.86, t, $^3J_{HP}$ = 7.3 Hz), was produced as the sole phosphorus-

(23) Similarly to unprotected nucleoside *H*-phosphonates, *H*-phosphonothioates **4a**−**f** were stable under the reaction conditions even during a prolonged (12 h) treatment with aqueous methylamine (^{31}P NMR and TLC analysis).

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(26) The reaction was carried out on a small scale and the product isolated by paper chromatography.

(27) For example, dithymidine (3′-5′) *H*-phosphonate (0.25 mmol/2 mL) in the presence of triethylamine (2 equiv) underwent oxidation with sulfur (2 equiv) to the corresponding dinucleoside phosphorothioate within 1 min in DMF or MeOH, ca. 10 min in pyridine, ca. 40 min in dioxane or toluene, and ca. 90 min in carbon disulfide. When triethylamine was replaced by diisopropylamine or DBU, the reaction in dioxane went to completion within 10 and 1 min, respectively (R. Wallin and J. Stawiński, unpublished results).

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(31) The reaction proceeded slightly faster when 10 molar equiv of (TMS)Cl was used, even though no detectable changes in the composition of the reaction mixture could be observed by ^{31}P NMR spectroscopy.

containing product. This was much more reactive than the monosilyl ester **8** and underwent quantitative sulfurization with added sulfur (3 molar equiv) within 3 min.[32]

Finally we have run the ^{31}P NMR experiment in which nucleoside *H*-phosphonate **3d** (0.1 mol/L) suspended in pyridine was treated with trimethylsilyl chloride ((TMS)-Cl, 5 molar equiv) in the presence of triethylamine (20 molar equiv). This afforded rapidly (<3 min) the corresponding nucleoside bis(trimethylsilyl) phosphite,[33] which upon addition of sulfur (6 molar equiv) was quantitatively converted into nucleoside 5′-phosphorothioate **4d** (for ^{31}P NMR chemical shifts and splitting patterns, see Table 1).

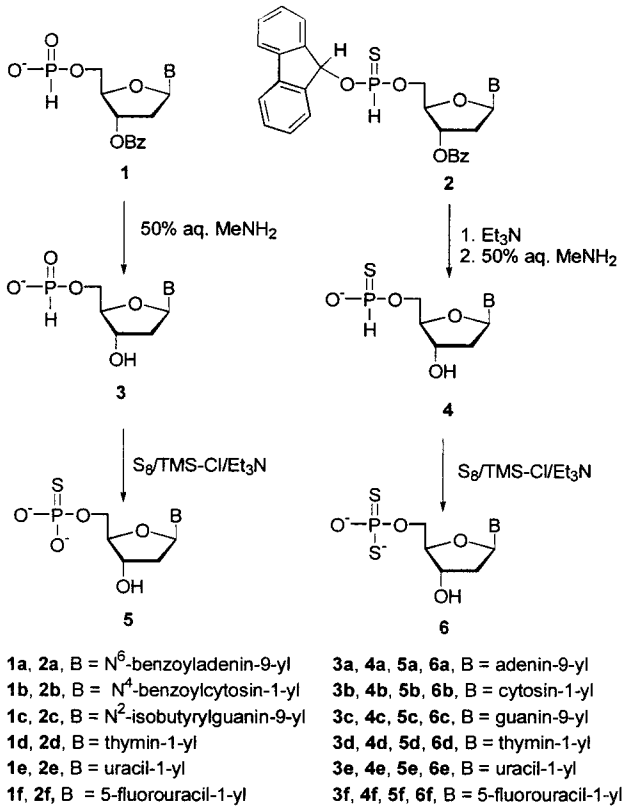
As to a possible role of a base during sulfurization of phosphorus esters, some further observations are pertinent. Triphenyl phosphite (**11**), apparently due to the electron-withdrawing effect of three phenyl groups, is rather resistant to oxidation and does not react with sulfur in toluene[30] or in pyridine within several hours. An analogous reaction when carried out in pyridine in the presence of triethylamine was much faster and went to completion within 30 min, producing triphenyl phosphorothioate (**12**) (δ_P 53.24 ppm). A similar catalytic effect of triethylamine on sulfurization of tertiary phosphines has been previously observed by Bartlett et al.,[29] who assigned it to the conversion of octaatomic sulfur into more reactive polysulfides, mediated by sulfide anion, generated from hydrogen sulfide present in elemental sulfur as impurities. Since we observed that the catalytic effect was more profound in pyridine than in toluene, it is apparent that polar solvents favored this process.[34] Thus, a base during sulfurization of *H*-phosphonate derivatives, most likely plays a dual role: (i) it facilitates abstraction of the P-bound hydrogen to generate the corresponding tervalent species which are more prone to oxidation and (ii) activates sulfur via its conversion to more reactive polysulfides.

On the basis of the above results, we elaborated a general "one-pot" procedure for the synthesis of nucleoside phosphorothioates of type **5** from unprotected nucleoside *H*-phosphonates **3** (Scheme 1). In this approach substrates of type **3** (1 molar equiv) and sulfur (6 molar equiv) suspended in pyridine containing triethylamine (20 molar equiv) were treated with (TMS)Cl (5 molar equiv). After 5 min, the starting material was completely converted (as determined by TLC analysis) to the expected nucleoside phosphorothioates **5**, which after simple workup were isolated in satisfactory yields by means of silica gel flash chromatography. The structure and homogeneity of the obtained final products **5a**−**f** were ascertained on the basis of spectral and chromatographic analysis [^{1}H and ^{31}P NMR (Table 1), HRMS, and TLC].

The same procedure also worked efficiently in the instance of sulfurization of unprotected nucleoside *H*-phosphonothioates **4** to produce quantitatively the corresponding phosphorodithioates **6** in less than 5 min. Although the starting *H*-phosphonothioate monoesters **4** cannot be converted into the corresponding tervalent bis(silyl) phosphites[35] in pyridine, irrespective of excess of (TMS)Cl and triethylamine used, the produced under such conditions tetracoordinated monosilyl derivatives[36] were reactive enough to undergo smooth sulfurization. This is in line with the observed higher susceptibility of *H*-phosphonothioate diesters to oxidation,[37,38] which can be related to higher acidity of the P−H bond in *H*-phosphonothioates vs *H*-phosphonates.[39] We cannot, however, exclude the intermediacy of tervalent silyl species (nucleoside *O*,*S*-disilyl phosphorothioites), which may be formed in small concentrations, below the detection level of ^{31}P NMR spectroscopy. The produced in such a way nucleoside phosphorodithioates **6a**−**f** were isolated essentially as described for *H*-phosphonates of type **3**. One should, however, exercise some caution during the workup procedure, since these compounds became rather unstable at pH below 7.5.[6]

In conclusion, we have developed a simple and efficient method for the synthesis of nucleoside phosphoromonothioates **5** and nucleoside phosphorodithioates **6**, utilizing sulfurization of unprotected nucleoside *H*-phosphonate and nucleoside *H*-phosphonothioates. For the preparation of the latter ones, we designed a simple synthetic

Scheme 1



(32) Triethyl phosphite in methylene chloride or in pyridine undergoes sulfurization to produce triethyl phosphorothioate also in less than 3 min (^{31}P NMR experiment).

(33) Garegg, P. J.; Regberg, T.; Stawiński, J.; Strömberg, R. *J. Chem. Soc., Perkin Trans. 1* **1987**, 1269−1273.

(34) Upon addition of triethylamine to a solution of sulfur in pyridine, a deep yellow color, characteristic for polysulfides, developed. This disappeared during the course of the reaction with phosphite **11**.

(35) Bollmark, M.; Stawiński, J. *Tetrahedron Lett.* **1996**, *37*, 5739−5742.

(36) Incremental addition of (TMS)Cl to **4d** in pyridine (δ_P = 53.7 and 53.1 ppm, $^1J_{HP}$ = 568 and 571 Hz) causes gradual broadening and shifting the signals toward lower field. With 3 equiv of (TMS)Cl the resonances sharpened again, and their chemical shifts and $^1J_{HP}$ coupling constants remained unaffected (δ_P = 57.0 and 56.4 ppm, $^1J_{HP}$ = 659 and 660 Hz) upon further addition of the silylating agent or triethylamine.

(37) Stawiński, J.; Thelin, M.; Zain, R. *Tetrahedron Lett.* **1989**, *30*, 2157−2160.

(38) Stawiński, J.; Strömberg, R.; Szabó, T.; Thelin, M.; Westman, E.; Zain, R. *Nucleic Acids Sym. Ser.* **1991**, *24*, 95−98.

(39) Guthrie, J. P. *Can. J. Chem.* **1979**, *57*, 236−239.

protocol involving phosphonylation or thiophosphonylation of standard nucleosidic precursors, followed by a rapid deprotection with methylamine. The ^{31}P NMR studies on model compounds enabled us to delineate the most crucial parameters affecting the sulfurization reaction. The main advantages of the developed methods for the preparation of nucleoside phosphoro*mono*thioates and nucleoside phosphoro*di*thioates are the following: (i) The phosphorothioate or phosphorodithioate function in nucleotides **5** or **6** is generated in the last synthetic step, and thus a possibility of its degradation is minimized. (ii) The sulfurization is rapid and occurs under mild reaction conditions. (iii) The purification procedure of the products **5** and **6** by silica gel chromatography is simple, fast, and compatible with a multigram scale syntheses. (iv) The starting materials, nucleoside *H*-phosphonates **3** and nucleoside *H*-phosphonothioates **4**, are stable, easy to handle, and readily accessible from standard or commercially available nucleosidic precursors.

Some compounds prepared during the course of these studies (i.e. **1d**−**f**, **5d**−**f**, **6d**−**f**) were preliminarily screened for a biological activity as thymidylate synthase inhibitors.[8] More studies on this subject are in progress.

Experimental Section

^{1}H and ^{31}P NMR spectra were recorded at 300 MHz spectrometer. The ^{31}P NMR experiments were carried out in 5 mm tubes using 0.1 mol/L concentration of phosphorus-containing compound. Mass spectra were recorded with liquid secondary ion mass technique (LSIMS) using Cs^+ (12 keV) for ionization. TLC analyses (Merck silica gel 60 F_{254} precoated plates) were carried out in saturated chambers using the following solvent systems: (A) i-PrOH/H_2O/25% NH_4OH (7:2:1, v/v/v); (B) CH_2Cl_2/MeOH/Et_3N (85:10:5, v/v/v). The $R_{f\mathrm{Thy}}$ values reported are relative to thymidin-5′-yl *H*-phosphonate **3d** and marked as $R_{f\mathrm{Thy}}$. The amount of water in solvents was measured with Karl Fisher coulometric titration. Methylene dichloride was dried over P_2O_5, distilled, and kept over 4 Å molecular sieves until the amount of water was less than 10 ppm. Pyridine was stored over 4 Å molecular sieves until the amount of water was below 20 ppm. Triethylamine was distilled and stored over CaH_2. Trimethylsilyl chloride was distilled before use. Elemental sulfur (pa grade) was used without additional purification. For column chromatography silica gel 60 (Merck) was used. Protected nucleoside 5′-*H*-phosphonates[15] **1a**−**f** and nucleoside 9-fluorenemethyl 5′-*H*-phosphonothioates[10] **2a**−**f** were prepared according to published procedures.

General Procedure for the Synthesis of Nucleoside *H*-Phosphonates 3a−f. A suitably protected nucleoside (3′-*O*-benzoylthymidine, 3′-*O*-benzoyl-2′-deoxyuridine, 3′-*O*-benzoyl-5-fluoro-2′-deoxyuridine, 3′-*O*-benzoyl-N^2-isobutyryl-2′-deoxyguanosine, 3′-*O*,N^4-dibenzoyl-2′-deoxycytidine, or 3′-*O*,N^6-dibenzoyl-2′-deoxyadenosine, 1 mmol) was rendered anhydrous by repeated evaporation of added pyridine (2 × 10 mL), dissolved in the same solvent (10 mL) and treated with diphenyl *H*-phosphonate (3 molar equiv, 15 min). The reaction was quenched by the addition of water−triethylamine (1:1, v/v, 4 mL) and left for 15 min. The mixture was concentrated and the residue treated with 50% aqueous CH_3NH_2 (10 mL) for 60 min. After evaporation of the solvents under vacuum, the oily residue was partitioned between water (10 mL) and CH_2Cl_2 (10 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL) and concentrated, and the residue, after being dissolving in a minimum volume of MeOH, was applied on a silica gel column, equilibrated with CH_2Cl_2−MeOH (95:5, v/v). The column was washed with CH_2Cl_2 containing Et_3N (95:5, v/v), and the unprotected nucleoside *H*-phosphonates **3** were eluted from the gel with methanol. Fractions containing pure products were collected and evaporated to dryness to yield colorless, hygroscopic foams. For the ^{31}P NMR data, see Table 1.

2′-Deoxyadenosin-5′-yl *H*-phosphonate, triethylammonium salt (3a): yield 82%; $R_{f\mathrm{Thy}}$ = 1.22 (A), 0.74 (B); ^{1}H NMR δ_H (D_2O) 1.09 (9H, t, J = 7.6 Hz), 2.21, 2.28 (2H, 2m), 3.15 (6H, q, J = 7.6 Hz), 3.87 (2H, m), 4.05 (1H, m), 4.54 (1H, m), 6.26 (1H, t, J = 6.6 Hz), 6.48 (1H, d, $^1J_{\mathrm{HP}}$ = 639.0 Hz), 7.97, (1H, s), 8.18 (1H, s). HRMS (LSIMS, glycerol) [M$^-$]: *m*/*z* 314.0669; calcd for $[C_{10}H_{13}N_5O_5P]^-$, 314.0654.

2′-Deoxycytidin-5′-yl *H*-phosphonate, triethylammonium salt (3b): yield 84%; $R_{f\mathrm{Thy}}$ = 1.11 (A), 0.42 (B); ^{1}H NMR δ_H (D_2O) 1.07 (9H, t, J = 7.2 Hz), 2.14, 2.20 (2H, 2m), 2.99 (6H, q, J = 7.2 Hz), 3.86 (2H, m), 3.98 (1H, m), 4.32 (1H, m), 5.88 (1H, d, J = 7.6 Hz), 6.11 (1H, t, J = 6.6 Hz), 6.54 (1H, d, $^1J_{\mathrm{HP}}$ = 638.4 Hz), 7.71 (1H, d, J = 7.6 Hz). HRMS (LSIMS, glycerol) [M$^-$]: *m*/*z* 290.0535; calcd for $[C_9H_{13}N_3O_6P]^-$, 290.0542.

2′-Deoxyguanosin-5′-yl *H*-phosphonate, triethylammonium salt (3c): yield 69%; $R_{f\mathrm{Thy}}$ = 1.0 (A), 0.20 (B); ^{1}H NMR δ_H (D_2O) 1.10 (9H, t, J = 7.4 Hz), 2.35, 2.64 (2H, 2m), 3.03 (6H, q, J = 7.4 Hz), 3.75 (2H, m), 4.05 (1H, m), 4.52 (1H, m), 6.12 (1H, t, J = 6.9 Hz), 6.65 (1H, d, $^1J_{\mathrm{HP}}$ = 639.6 Hz), 7.86 (1H, s). HRMS (LSIMS, glycerol) [M$^-$]: *m*/*z* 330.0613; calcd for $[C_{10}H_{13}N_5O_6P]^-$, 330.0603.

Thymidin-5′-yl *H*-phosphonate triethylammonium salt (3d): yield 74%; $R_{f\mathrm{Thy}}$ = 1.0 (A), 1.0 (B); ^{1}H NMR δ_H (D_2O) 1.12 (9H, t, J = 7.5 Hz), 1.75 (3H, m), 2.20, (2H, 2m), 3.03 (6H, q, J = 7.5 Hz), 3.91 (2H, m), 4.01 (1H, m), 4.21 (1H, m), 6.19 (1H, t, J = 6.9 Hz), 6.61 (1H, d, $^1J_{\mathrm{HP}}$ = 637.5 Hz), 7.55 (1H, m). HRMS (LSIMS, glycerol) [M$^-$]: *m*/*z* 305.0545; calcd for $[C_{10}H_{14}N_2O_7P]^-$, 305.0539.

2′-Deoxyuridin-5′-yl *H*-phosphonate, triethylammonium salt (3e): yield 88%; $R_{f\mathrm{Thy}}$ = 0.98 (A), 0.83 (B);^{1}H NMR δ_H (D_2O) 1.06 (9H, t, J = 7.4 Hz), 2.18 (2H, 2m), 2.98 (6H, q, J = 7.4 Hz), 3.85 (2H, m), 3.95 (1H, m), 4.34 (1H, m), 5.70 (1H, d, J = 8.1 Hz), 6.08 (1H, t, J = 6.9 Hz), 6.26 (1H, d, $^1J_{\mathrm{HP}}$ = 638.4 Hz), 7.68 (1H, d, J = 8.0 Hz). HRMS (LSIMS, glycerol) [M$^-$]: *m*/*z* 291.0375; calcd for $[C_9H_{12}N_2O_7P]^-$, 291.0382.

5-Fluoro-2′-deoxyuridin-5′-yl *H*-phosphonate, triethylammonium salt (3f): yield 89%; $R_{f\mathrm{Thy}}$ = 0.94 (A), 0.78 (B); ^{1}H NMR δ_H (D_2O) 1.11 (9H, t, J = 7.6 Hz), 2.16 (2H, 2m), 3.07 (6H, q, J = 7.5 Hz), 3.83 (2H, m), 3.94 (1H, m), 4.32 (1H, m), 6.08 (1H, t, J = 7.2 Hz), 6.55 (1H, d, $^1J_{\mathrm{HP}}$ = 639.0 Hz), 7.83 (1H, d, $^3J_{\mathrm{HF}}$ = 6.3 Hz). HRMS (LSIMS, glycerol) [M$^-$]: *m*/*z* 309.0285; calcd for $[C_9H_{11}FN_2O_7P]^-$, 309.0288.

General Procedure for the Synthesis of Nucleoside 5′-*H*-Phosphonothioates 4a−f. A nucleoside 9-fluorenemethyl *H*-phosphonothioate (**2a**−**f**, 1 mmol) in pyridine (5 mL) was reacted with triethylamine (10 mL) for 20 min to remove the 9-fluorenemethyl group (TLC analysis). The solvent was removed under vacuum and the residue treated with aqueous methylamine (50%, 2 mL) during 1 h. After concentration to a viscous oil, the mixture was dissolved in water (30 mL) and extracted with CH_2Cl_2 (2 × 50 mL). The aqueous phase was concentrated, dissolved in a minimum volume of CH_2Cl_2−MeOH (4:1, v/v), and applied on a silica gel column. The unprotected nucleoside *H*-phosphonothioates **4** were eluted from the column using CH_2Cl_2−MeOH−Et_3N (75:20:5, v/v/v). Fractions containing pure compounds **4** were combined, evaporated, and, after being dissolved in a minimum amount of methanol, freeze-dried from benzene. White, amorphous, hygroscopic powders were obtained. For the ^{31}P NMR data, see Table 1.

2′-Deoxyadenosin-5′-yl *H*-phosphonothioate, triethylammonium salt (4a): yield 82%; $R_{f\mathrm{Thy}}$ = 1.17 (A), 1.36 (B); ^{1}H NMR δ_H (D_2O) 1.09 (9H, t, J = 7.5 Hz), 2.42, 2.67 (2H, 2m), 3.01 (6H, q, J = 7.5 Hz), 3.93 (2H, m), 4.10 (1H, m), 4.55 (1H, m), 6.27 (1H, t, J = 6.9 Hz), 7.60, 7.62 (1H, 2d, $^1J_{\mathrm{HP}}$ = 593.2 Hz, $^1J_{\mathrm{HP}}$ = 594.0 Hz), 7.97, (1H, s), 8.25 (1H, 2s) (multiplicity of some signals is due to the presence of P-diastereoisomers); HRMS (LSIMS, glycerol), [M$^-$] *m*/*z* 330.0428, calcd for $[C_{10}H_{13}N_5O_4PS]^-$ 330.0426.

2′-Deoxycytidin-5′-yl *H*-phosphonothioate, triethylammonium salt (4b): yield 81%; $R_{f\mathrm{Thy}}$ = 1.17 (A), 1.36 (B); ^{1}H

NMR δ_H (D_2O) 1.15 (9H, t, J = 7.5 Hz) 2.20, 2.29 (2H, 2m) 3.06 (6H, q, J = 7.5 Hz), 4.02 (2H, m), 4.10 (1H, m), 4.44 (1H, m), 5.96 (1H, d, J = 7.6 Hz), 6.21 (1H, t, J = 6.9 Hz), 7.76, 7.78 (1H, 2d, $^1J_{PH}$ = 593.1 Hz), 7.83, 7.85 (1H, 2d, J = 7.6 Hz) (multiplicity of some signals is due to the presence of P-diastereoisomers); HRMS (LSIMS, glycerol), [M^-] *m/z* 306.0294, calcd for $[C_9H_{13}N_3O_5PS]^-$ 306.0313.

2′-Deoxyguanosin-5′-yl *H*-phosphonothioate, triethylammonium salt (4c): yield 88%; R_{fThy} = 0.92 (A), 0.44 (B); ^{1}H NMR δ_H (D_2O) 1.17 (9H, t, J = 7.5 Hz), 2.42, 2.71 (2H, 2m), 3.09 (6H, q, J = 7.5 Hz), 4.01 (2H, m), 4.14 (1H, m), 4.61 (1H, m), 6.19 (1H, t, J = 6.9 Hz), 7.69 (1H, d, $^1J_{HP}$ = 594.9 Hz), 7.97, 7.99 (1H, 2s) (multiplicity of some signals is due to the presence of P-diastereoisomers); HRMS (LSIMS, glycerol), [M^-] *m/z* 346.0354, calcd for $[C_{10}H_{13}N_5O_5PS]^-$ 346.0375.

Thymidin-5′-yl *H*-phosphonothioate, triethylammonium salt (4d): yield 81%; R_{fThy} = 1.14 (A), 1.63 (B); ^{1}H NMR δ_H (D_2O) 1.16 (9H, t, J = 7.4 Hz), 1.82 (3H, m), 2.26 (2H, 2m), 3.17 (6H, q, J = 7.4 Hz), 4.03 (2H, m), 4.09 (1H, m), 4.47 (1H, m), 6.23 (1H, t, J = 6.9 Hz), 7.62 (1H, m), 7.77, 7.79 (1H, 2d, $^1J_{HP}$ = 592.5 Hz), (multiplicity of some signals is due to the presence of P-diastereomers); HRMS (LSIMS, glycerol), [M^-] *m/z* 321.0311, calcd for $[C_{10}H_{14}N_2O_6PS]^-$ 321.0310.

2′-Deoxyuridin-5′-yl *H*-phosphonothioate, triethylammonium salt (4e): yield 92%; R_{fThy} = 0.97 (A), 1.50 (B); ^{1}H NMR δ_H (D_2O) 1.11 (9H, t, J = 7.5 Hz), 2.31 (2H, 2m), 3.04 (6H, q, J = 7.5 Hz), 3.91 (2H, m), 4.05 (1H, m), 4.42 (1H, m), 5.74, 5.77 (1H, 2d, J = 8.1 Hz), 6.16 (1H, m), 7.71, 7.72 (1H, 2d, $^1J_{HP}$ = 593.4 and 594.3 Hz) 7.79, 7.82 (1H, 2d, J = 8.1 Hz) (multiplicity of some signals is due to the presence of P-diastereoisomers); HRMS (LSIMS, glycerol), [M^-] *m/z* 307.0129, calcd for $[C_9H_{12}N_2O_6PS]^-$ 307.0154.

5-Fluoro-2′-deoxyuridin-5′-yl *H*-phosphonothioate, triethylammonium salt (4f): yield 83%; R_{fThy} = 0.92 (A), 1.42 (B); ^{1}H NMR δ_H (D_2O) 1.12 (9H, t, J = 7.5 Hz), 2.24 (2H, 2m), 3.03 (6H, q, J = 7.5 Hz), 3.98 (2H, m), 4.04 (1H, m), 4.41 (1H, m), 6.14 (1H, m), 7.72, 7.74 (1H, 2d, $^1J_{HP}$ = 594.0 and 594.6 Hz), 7.92, 7.93 (1H, 2d, $^3J_{HF}$ = 6.6 and 6.3 Hz) (multiplicity of some signals are due to the presence of P-diastereomers); HRMS (LSIMS, glycerol), [M^-] *m/z* 325.0063, calcd for $[C_9H_{11}FN_2O_6PS]^-$ 325.0059.

General Procedure for the Synthesis of Nucleoside Phosphorothioates 5a−f. Nucleoside 5′-*H*-phosphonate **3** (1 mmol, prepared as above) and sulfur (3 molar equiv) were rendered anhydrous by repeated evaporation of added pyridine (2 × 10 mL) and suspended in the same solvent (10 mL) containing triethylamine (20 molar equiv). To this, under stirring, was added trimethylsilyl chloride (5 molar equiv), and after 10 min the reaction mixture was cooled to ca. 0 °C and quenched with 25% aqueous ammonia (1 mL). The reaction mixture was concentrated to a small volume, and the oily residue was partitioned between CH_2Cl_2 (10 mL) and water (10 mL). The aqueous phase was extracted with CH_2Cl_2 (2 × 10 mL), evaporated to dryness, and, after being dissolving in a minimum volume of methanol−25% aqueous ammonia (95:5, v/v), applied on a silica gel column, equilibrated with 2-propanol−concentrated ammonia−water (90:5:5, v/v/v). The column was washed with the same solvent, and the unprotected nucleoside phosphorothioates **5** were eluted from the gel with methanol−25% aqueous ammonia (95:5, v/v). The fractions containing pure products were collected and evaporated to dryness to yield colorless, hygroscopic foams. For the ^{31}P NMR data, see Table 1.

2′-Deoxyadenosin-5′-yl phosphorothioate, ammonium salt (5a): yield 79%; R_{fThy} = 0.81 (A), 0.32 (B); ^{1}H NMR δ_H (D_2O) 2.37, 2.61 (2H, 2m), 3.84 (2H, m), 4.09 (1H, m), 4.53 (1H, m), 6.28 (1H, t, J = 7.2 Hz), 8.00 (1H, s), 8.39 (1H, s). HRMS (LSIMS, glycerol) [M^-]: *m/z* 346.0383; calcd for $[C_{10}H_{13}N_5O_5PS]^-$, 346.0375.

2′-Deoxycytidin-5′-yl phosphorothioate, ammonium salt (5b): yield 86%; R_{fThy} = 0.70 (A), 0.24 (B); ^{1}H NMR δ_H (D_2O) 2.18 (2H, 2m), 3.90 (2H, m), 4.05 (1H, m), 4.42 (1H, m), 5.99 (1H, d, J = 7.5 Hz), 6.16 (1H, t, J = 6.9 Hz), 8.01 (1H, d, J = 7.5 Hz). HRMS (LSIMS, glycerol) [M^-]: *m/z* 322.0290; calcd for $[C_9H_{13}N_3O_6PS]^-$, 322.0263.

2′-Deoxyguanosin-5′-yl phosphorothioate, ammonium salt (5c): yield 54%; R_{fThy} = 0.42 (A), 0.11 (B); ^{1}H NMR δ_H (D_2O) 2.31, 2.53 (2H, 2m), 3.79 (2H, m), 4.02 (1H, m), 4.49 (1H, m), 6.00 (1H, t, J = 6.6 Hz), 7.93 (1H, s). HRMS (LSIMS, glycerol) [M^-]: *m/z* 362.0331; calcd for $[C_{10}H_{13}N_5O_6PS]^-$, 362.0324.

Thymidin-5′-yl phosphorothioate, ammonium salt (5d): yield 72%; R_{fThy} = 0.64 (A), 0.70 (B); ^{1}H NMR δ_H (D_2O) 1.74 (3H, m), 2.17 (2H, 2m), 3.82 (2H, m), 3.97 (1H, m), 4.41 (1H, m), 6.15 (1H, t, J = 6.3 Hz), 7.66 (1H, m). HRMS (LSIMS, glycerol) [M^-]: *m/z* 337.0244; calcd for $[C_{10}H_{14}N_2O_7PS]^-$, 337.0259.

2′-Deoxyuridin-5′-yl phosphorothioate, ammonium salt (5e): yield %; R_{fThy} = 0.61 (A), 0.53 (B); ^{1}H NMR δ_H (D_2O) 2.14 (2H, 2m), 3.76 (2H, m), 3.94 (1H, m), 4.35 (1H, m), 5.70 (1H, d, J = 8.1 Hz), 6.08 (1H, t, J = 6.9 Hz), 7.87 (1H, d, J = 8.1 Hz). HRMS (LSIMS, glycerol) [M^-]: *m/z* 323.0114; calcd for $[C_9H_{12}N_2O_7PS]^-$, 323.0103.

5-Fluoro-2′-deoxyuridin-5′-yl phosphorothioate, ammonium salt (5f): yield 94%; R_{fThy} = 0.58 (A), 0.50 (B); ^{1}H NMR δ_H (D_2O) 2.23 (2H, 2m), 4.01 (2H, m), 4.11 (1H, m), 4.81 (1H, m), 6.21 (1H, m), 8.06 (1H, d, $^3J_{HF}$ = 6.3 Hz). HRMS (LSIMS, glycerol) [M^-]: *m/z* 341.0001; calcd for $[C_9H_{11}FN_2O_7PS]^-$, 341.0009.

General Procedure for the Synthesis of Nucleoside Phosphorodithioates 6a−f. A nucleoside *H*-phosphonothioate (**4a**−**f**, 1 mmol) and sulfur (3 molar equiv) were rendered anhydrous by evaporation of added excess of pyridine (2 × 10 mL) and suspended in pyridine (10 mL) containing triethylamine (20 molar equiv). When the mixture became homogeneous (ca. 10 min), trimethylsilyl chloride (5 molar equiv) was added. After 15 min 0.1 M NaOH (7 molar equiv) was added, and the solvent and excess reagents were evaporated under vacuum. The resulting oily residue was triturated with methanol (10 mL) and centrifuged off the precipitated salts, and the methanolic solution containing **6** was evaporated. Crude nucleoside phosphorodithioates **6** were dissolved in a minimum volume of MeOH−concentrated NH_4OH (9:1, v/v) and applied on a silica gel column, preequilibrated with 2-propanol−H_2O−concentrated NH_4OH (90:5:5, v/v/v). The column was washed out with *i*-PrOH−H_2O−NH_4OH concentrated (85:10:5, v/v/v), and the unprotected nucleoside phosphorodithioates **6** were eluted with MeOH−concentrated NH_4OH (9:1, v/v). The fractions containing pure compounds **6** were collected, evaporated, and freeze-dried from 1 M TEAB buffer (pH 8.5). White, hygroscopic, amorphous powders were obtained. For the ^{31}P NMR data, see Table 1.

2′-Deoxyadenosin-5′-yl phosphorodithioate, ditriethylammonium salt (6a): yield 75%; R_{fThy} = 0.77 (A), 1.22 (B); ^{1}H NMR δ_H (D_2O) 0,87 (18H, t, J = 7.2 Hz) 2.38 (12H, q, J = 7.2 Hz) 2.47, 2.70 (2H, 2m), 3.98 (2H, m), 4.19 (1H, m), 4.65 (1H, m), 6.28 (1H, t, J = 7.2 Hz), 8.10 (1H, s), 8.61 (1H, s). HRMS (LSIMS, glycerol), [M^-]: *m/z* 362.0129; calcd for $[C_{10}H_{13}N_5O_4PS_2]^-$, 362.0147.

2′-Deoxycytidin-5′-yl phosphorothioate, ditriethylammonium salt (6b): yield 81%; R_{fThy} = 0.48 (A), 0.47 (B); ^{1}H NMR δ_H (D_2O) 0.88 (18H, t, J = 7.5 Hz) 2.25, (2H, 2m) 2.39 (12H, q, J = 7.5 Hz), 3.99 (2H, m), 4.10 (1H, m), 4.49 (1H, m), 6.01 (1H, d, J = 7.7 Hz), 6.24 (1H, t, J = 6.9 Hz), 8.12 (1H, d, J = 7.7 Hz). HRMS (LSIMS, glycerol), [M^-]: *m/z* 338.0013; calcd for $[C_9H_{13}N_3O_5PS_2]^-$, 338.0034.

2′-Deoxyguanosin-5′-yl phosphorodithioate, ditriethylammonium salt (6c): yield 77%; R_{fThy} = 0.24 (A), 0.28 (B); ^{1}H NMR δ_H (D_2O) 0.88 (18H, t, J = 7.5 Hz), 1.15, 2.67 (2H, 2m), 2.34 (12H, q, J = 7.5 Hz), 3.97 (2H, m), 4.15 (1H, m), 4.62 (1H, m), 6.21 (1H, m), 8.10 (1H, s). HRMS (LSIMS, glycerol), [M^-]: *m/z* 378.0109; calcd for $[C_{10}H_{13}N_5O_5PS_2]^-$, 378.0096.

Thymidin-5′-yl phosphorodithioate, ditriethylammonium salt (6d): yield 75%. R_{fThy} = 0.44 (A), 1.49 (B); ^{1}H NMR δ_H (D_2O) 0.74 (18H, t, J = 7.3 Hz), 1.67 (3H, m), 2.06 (2H, 2m), 2.25 (12H, q, J = 7.3 Hz), 3.84 (2H, m), 3.90 (1H, m), 4.33 (1H, m), 6.16 (1H, t, J = 6.3 Hz), 7.48 (1H, m). HRMS (LSIMS, glycerol), [M^-]: *m/z* 353.0023; calcd for $[C_{10}H_{14}N_2O_6PS_2]^-$ 353.0031.

2′-Deoxyuridin-5′-yl phosphorodithioate, ditriethylammonium salt (6e): yield 89%; $R_{f\mathrm{Thy}} = 0.35$ (A), 1.40 (B); ^{1}H NMR δ_H (D_2O) 1.14 (18H, t, $J = 7.5$ Hz), 2.26 (2H, 2m), 3.07 (12H, q, $J = 7.5$ Hz), 3.98 (2H, m), 4.11 (1H, m), 4.52 (1H, m), 5.82 (1H, d, $J = 8.2$ Hz), 6.21 (1H, t, $J = 6.6$ Hz), 8.10 (1H, d, $J = 8.2$ Hz); HRMS (LSIMS, glycerol), [M$^-$] m/z 338.9873, calcd for $[C_9H_{12}N_2O_6PS_2]^-$ 338.9875.

5-Fluoro-2′-deoxyuridin-5′-yl phosphorodithioate, ditriethylammonium salt (6f): yield 76%; $R_{f\mathrm{Thy}} = 0.26$ (A), 1.41 (B); ^{1}H NMR δ_H (D_2O) 0.82 (18H, t, $J = 7.2$ Hz), 2.10 (2H, 2m), 2.33 (12H, q, $J = 7.2$ Hz), 3.92 (2H, m), 4.32 (1H, m), 4.76 (1H, m), 6.15 (1H, m), 7.84 (1H, d, $^3J_{\mathrm{HF}} = 6.0$ Hz); HRMS (LSIMS, glycerol), [M$^-$] m/z 356.9800, calcd for $[C_9H_{11}FN_2O_6PS_2]^-$ 356.9780.

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Supporting Information Available: NMR spectra (24 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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