Silylation-Mediated Transesterification of *O*-Phenyl H-Phosphonothioates – A New Entry to Nucleoside H-Phosphonothioate Monoesters

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O-Phenyl H-phosphonothioate undergoes a facile transesterification with suitably protected nucleosides upon in situ silylation with *tert*-butyldiphenylsilyl chloride in pyridine/toluene to produce the corresponding 3′-H-phosphonothioates in good yields.

Introduction

During the last decade we have introduced and have been investigating various aspects of H-phosphonothioates ("H" indicates a P−H bond)[1−5] as a new type of synthetic intermediates for the preparation of biologically active phosphorus compounds based on H-phosphonate methodology. Due to their higher stability, ease of handling, and efficiency in solid-phase synthesis,[6−8] H-phosphonothioate building blocks[1,9] seem to be superior to phosphorothioamidite derivatives[10] in the synthesis of nucleotide analogues with multiple modifications at the phosphorus centre that are difficult to prepare in other ways, for example from nucleoside phosphorodithioates,[6,7,11,12] nucleoside phosphoramidothioates,[13] nucleoside phosphorofluoridothioates,[14] etc.

Despite their usefulness in the synthesis of various types of phosphorus compounds, there are only a handful of synthetic methods available for the preparation of nucleoside H-phosphonothioate monoesters. These compounds can be obtained either by non-oxidative thiation of nucleoside H-phosphonate derivatives (e.g. by sulfhydrolysis of tervalent P^{III} species[3] or from aryl nucleoside H-phosphonate derivatives[15]) or by using various thiophosphonylation protocols.[2,5,16,17] This latter group includes methods based on reactions of suitably protected hydroxylic compounds with triethylammonium phosphinate in the presence of a condensing agent, followed by sulfurization of the phosphinate monoesters formed in situ,[2] sulfhydrolysis of aryl H-phosphonate diesters,[16] or a dedicated H-phosphonothioate group-transfer reagent, *O*-(9-fluorenylmethyl) phosphonothioate.[5,17] Although all these methods have their own merits and usually afford products in good yields, we would like to expand the array of synthetic methods available for the preparation of H-phosphonothioate monoesters by developing an approach based on transesterification of tetracoordinate P^{III} compounds containing an H−P=S function. This would provide additional insight into the reactivity of compounds bearing an H−P=S functionality and, due to the differences in the underlying chemistry and the reaction conditions used, it could expand the range of hydroxylic compounds that can be subjected to H-thiophosphonylation.

In this paper we describe the preparation of *O*-phenyl H-phosphonothioate monoester and its use in a silylation-mediated transesterification reaction to obtain various nucleoside H-phosphonate monoesters.

Results and Discussion

We were looking for a reagent which is stable, easy to prepare, and does not require an additional synthetic step (e.g. a removal of a phosphate protecting group) to convert intermediate compounds into H-phosphonothioate monoesters. An attractive candidate for this purpose seemed to be *O*-phenyl H-phosphonothioate. As we were aware of the high propensity of aryl H-phosphonate diesters to undergo hydrolysis and sulfhydrolysis, it seemed likely that *O*-phenyl H-phosphonothioate could be obtained upon treatment of diphenyl H-phosphonate with hydrogen sulfide. The presence of a negative charge at the phosphorus centre should make this compound stable and resistant to spontaneous air oxidation, but at the same time it would effectively prevent transesterification of the phenoxy group by the nucleo-

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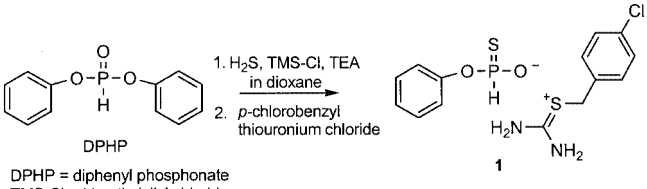
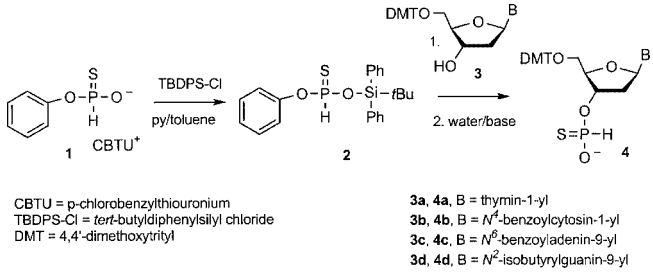
side. As a viable means to overcome the anticipated low reactivity of this reagent, we considered that carrying out a transesterification reaction with nucleoside in the presence of a silylating agent should make the phosphorus center more susceptible to a nucleophilic attack towards oxygen nucleophiles by *O*-silylating the H-phosphonothioate monoester function.[18] This approach would have an additional advantage over the methods based on sulfhydrolysis of reaction intermediates[3,15,16] in that inconveniences connected with the preparation of a stock solution of hydrogen sulfide prior to the reaction would be alleviated.

The synthesis of *O*-phenyl H-phosphonothioate (**1**) by sulfhydrolysis of diphenyl H-phosphonate (DPHP) was investigated first (Scheme 1). The reaction was carried out by adding a saturated solution of hydrogen sulfide (3.3 molar excess), trimethylsilyl chloride (TMSCl, 1.7 equiv.), and triethylamine (TEA, 7 equiv.) to a solution of DPHP in pyridine. TMSCl was used to ensure anhydrous reaction conditions, and TEA to prevent the formation of phenyl H-phosphonodithioate.[16] The reaction proceeded as expected and produced *O*-phenyl H-phosphonothioate (**1**) as the major product along with some small amounts of phenyl H-phosphonate monoester (5−10%).[19] Since the removal of this side product by column chromatography on silica gel was inefficient, and handling of an oily triethylammonium salt of *O*-phenyl H-phosphonothioate (**1**) inconvenient, we tried to convert reagent **1** into a derivative that would permit crystallographic purification of the crude reaction mixture. Among various salts investigated, the (*p*-chlorobenzyl)thiouronium derivative turned out to be crystalline, and provided reagent **1** as a stable solid with good solubility in organic solvents and free of phenyl H-phosphonate monoester.[20]



Scheme 1

We then investigated the reaction conditions most suitable for transferring an H-phosphonothioate moiety into nucleosides **3**. Pyridine, or solvent systems containing pyridine, seemed to be the obvious choice as pyridine should provide efficient nucleophile catalysis during both reaction steps (i.e. during the conversion of **1** into a silyl ester, and during the transesterification reaction of the produced intermediate of type **2** with nucleoside **3**). In addition, the weakly basic properties of pyridine should ensure the stability of the acid-sensitive dimethoxytrityl groups in the protected nucleoside moieties during the course of the reaction. Preliminary screening of various silylating agents [TMSCl, *tert*-butyldimethylsilyl (TBDMS), and *tert*-butyldiphenylsilyl (TBDPS) chlorides] was carried out in neat pyridine, by allowing equimolar amounts of **1**, a silylating agent, and a suitably protected nucleoside **3a** to react (Scheme 2). The reactions were not particularly clean and ^{31}P NMR spectroscopy revealed the formation of several side products. Most notable among these were dinucleoside H-phosphonothioate diesters and silylated H-phosphonothioates, when TMSCl was used as the silylating agent.[21] Although far from satisfactory, the best results were obtained with TBDPSCl. This reagent produced the least number of side products during the reaction in pyridine and, due to its low reactivity towards hydroxy groups in nucleosides **3**, it was used for further optimisation studies.



Scheme 2

We assumed that the source of most side reactions observed was, most likely, a too high reactivity of the reaction system containing pyridine, and thus we searched for a solvent composition that would provide the best compromise between reactivity and selectivity. After extensive experimentation with various organic solvents, we arrived at a solvent system consisting of toluene (4 parts) and pyridine (1 part), which allowed a very clean reaction of **1** with nucleoside **3a** in the presence of TBDPSCl, in a reasonable time period (overnight reaction).[22]

These reaction conditions were then applied for preparative syntheses involving other protected nucleosides **3**, and the desired nucleoside H-phosphonothioates **4** were isolated in 72−77% yield after simple aqueous workup followed by silica-gel column chromatography (Scheme 2). TLC analysis indicated, in all instances, the complete disappearance of the starting material **3**,[23] and ^{31}P NMR spectroscopy confirmed that the only nucleotide-containing material formed was the corresponding H-phosphonothioate **4**.

Conclusions

We have developed a new thiophosphonylating reagent — *O*-phenyl H-phosphonothioate (**1**) — that, in a silylation-mediated transesterification reaction with nucleosides **3** under mild conditions, produces the corresponding nucleoside 3′-H-phosphonothioates **4** in good yield. This reagent is easy to prepare on a large scale from inexpensive, commercially available materials, is crystalline, and can be stored at room temperature for a prolonged period of time (months) without noticeable decomposition. This approach can probably be extended to other hydroxylic compounds and thus expands the array of synthetic methods available for the preparation of H-phosphonothioate monoesters.

Experimental Section

Material and Methods: ^1H and ^{31}P NMR spectra were recorded with a Varian Unity 400 BB VT spectrometer. The ^{31}P NMR spectroscopy 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; 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 a CH_2Cl_2/CH_3OH (9:1, v/v) solvent system. To avoid smearing, the plates were immersed in CH_2Cl_2/CH_3OH (9:1, v/v) containing 1% of triethylamine, before chromatography, and dried. Pyridine (LabScan Ltd.), toluene and anhydrous acetonitrile (LabScan Ltd.) were stored over molecular sieves (4 Å). Diphenyl H-phosphonate, trimethylsilyl chloride, and *tert*-butyldiphenylsilyl chloride were commercial-grade reagents from Aldrich. Triethylamine (Aldrich) was freshly distilled. The protected nucleosides were obtained according to a published procedure.[24] The assignment of signals in the ^{31}P NMR spectra to particular products or intermediates was carried out on the basis of their chemical shifts, multiplicity of the signals in ^1H-coupled and ^1H-decoupled spectra, by spiking the reaction mixtures with appropriate species, and, if possible, by isolation of the compound in question from reaction mixtures. The assignment of the proton and carbon resonances of compounds **4** was carried out on the basis of known or expected chemical shifts in conjunction with ^1H-^1H, ^1H-^{13}C, and DEPT correlated NMR spectroscopy.

Synthesis of (*p*-Chlorobenzyl)thiouronium Chloride: *p*-Chlorobenzyl chloride (161 g, 1.0 mol) and thiourea (76 g, 1.0 mol) were refluxed in ethanol (200 mL) for 2 h. The reaction mixture was cooled down and the product was filtered off, and recrystallized from 36% aq. HCl/water (1:1, v/v). Yield: 209 g (88%). The ^1H NMR spectrum of the product was identical with a commercial sample of (*p*-chlorobenzyl)thiouronium chloride.

Synthesis of *O*-Phenyl H-Phosphonothioate (*p*-Chlorobenzyl)thiouronium Salt 1: A mixture of a freshly prepared solution of H_2S in dioxane (1 M solution, 200 mmol, 200 mL), trimethylsilyl chloride (12.7 mL, 100 mmol), and triethylamine (60 mL, 432 mmol) was added to a stirred solution of diphenyl H-phosphonate (11.48 mL, 60 mmol) in pyridine (100 mL). After 1 h, the reaction solvents were evaporated to dryness, the residue dissolved in ethanol (100 mL), and (*p*-chlorobenzyl)thiouronium chloride (13.3 g, 56.0 mmol) was added. The mixture was gently heated to obtain a homogeneous solution, concentrated, and the residue washed with water (2 × 100 mL). After recrystallization from ethanol, white crystals were obtained (15.75 g, 70% yield). M.p. 134−135 °C. $C_{14}H_{16}ClN_2O_2PS_2$ (374.85): calcd. C 44.86, H 4.30, N 7.47; found C 45.05, H 4.53, N 7.33. HRMS for $C_6H_6O_2PS$ [M − cbtu]$^-$: calcd. 172.9826; found 172.9833. ^1H NMR ([D$_6$]DMSO): δ = 9.32 (s, 4 H, 2 × NH_2), 9.01 (d, $^1J_{P,H}$ = 568.9 Hz, PH), 7.47−7.38 (m, 4 H, H2, H3), 7.25 (3 m, 2 H, H9), 7.09 (2 m, 2 H, H8), 7.01 (2 m 1 H, H10), 4.49 (s, 2 H, H5) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 169.44 (C6), 153.44, 153.32 (C7, $J_{C,P}$ = 8.8 Hz), 135.09 (C4), 133.32 (C1), 131.54 (C3), 129.84 (C9), 129.42 (C2), 123.58 (C10), 121.61, 121.54 (C8, $J_{C,P}$ = 5.4 Hz), 34.03 (C5) ppm. ^{31}P NMR ([D$_6$]DMSO): δ = 49.80 ($^1J_{P,H}$ = 568.9 Hz) ppm.

General Procedure for the Preparation of Protected Nucleoside 3′-H-Phosphonothioates 4 (TEAH$^+$ Salts): A suitably protected nucleoside **3** (1 mmol; 0.55 g **3a**, 0.64 g **3b**, 0.66 g **3c**, 0.63 g **3d**) and *O*-phenyl H-phosphonothioate (*p*-chlorobenzyl)thiouronium salt **1** (0.55 g, 1.5 mmol) were rendered anhydrous by co-evaporation with added pyridine (3 × 20 mL), and dissolved in a pyridine/toluene mixture (1:4, v/v; 50 mL). *tert*-Butyldiphenylsilyl chloride (0.4 mL, 1.5 mmol) was added to this mixture, with vigorous stirring, and it was left overnight. When complete (TLC analysis), the reaction mixture was quenched by addition of pyridine (5 mL), triethylamine (1 mL), and water (0.8 mL), concentrated to dryness, and partitioned between dichloromethane (100 mL) and saturated aq. $NaHCO_3$ (100 mL). The organic layer was washed with saturated aq. $NaHCO_3$ (2 × 100 mL), dried with anhydrous Na_2SO_4, and the solvents were evaporated. The residue was purified by silica-gel column chromatography using a gradient of methanol (6−10%) and triethylamine (0.01−0.5%) in dichloromethane to furnish nucleoside H-phosphonothioates **4** as white, amorphous solids (purity >98% by ^1H NMR spectroscopy).

5′-*O*-Dimethoxytritylthymidine 3′-H-Phosphonothioate (4a): 523 mg (72% yield). R_f = 0.34. ^{31}P NMR ($CDCl_3$): δ = 54.65 and 53.78 (each dd, $^1J_{P,H}$ = 580.0 and 583.9 Hz; $^3J_{P,H}$ = 12.6 and 12.0 Hz) ppm. HRMS for $C_{31}H_{32}N_2O_8PS$ [M − TEAH]$^-$: calcd. 623.1622; found 623.1633. The ^1H and ^{13}C NMR spectroscopic data of the compound were identical to those reported previously.[2]

5′-*O*-Dimethoxytrityl-*N*^{4}-benzoyldeoxycytidine 3′-H-Phosphonothioate (4b): 627 mg (77% yield). R_f = 0.36. ^{31}P NMR ($CDCl_3$): δ = 53.03 and 51.72 (each dd, $^1J_{P,H}$ = 577.2 and 586.5 Hz; $^3J_{P,H}$ = 11.4 and 12.0 Hz) ppm. HRMS for $C_{37}H_{35}N_3O_8PS$ [M − TEAH]$^-$: calcd. 712.1888; found 712.1897. The ^1H and ^{13}C NMR spectroscopic data of the compound were identical to those reported previously.[2]

5′-*O*-Dimethoxytrityl-*N*^{6}-benzoyldeoxyadenosine 3′-H-Phosphonothioate (4c): 609 mg (72% yield). R_f = 0.38. ^{31}P NMR ($CDCl_3$): δ = 54.35 and 54.30 (each dd, $^1J_{P,H}$ = 581.5 and 582.1 Hz; $^3J_{P,H}$ = 10.6 and 11.7 Hz) ppm. HRMS for $C_{38}H_{35}N_5O_7PS$ [M − TEAH]$^-$: calcd. 736.2000; found 736.2010. The ^1H and ^{13}C NMR spectroscopic data of the compound were identical to those reported previously.[2]

5′-*O*-Dimethoxytrityl-*N*^{2}-isobutyryldeoxyguanosine 3′-H-Phosphonothioate (4d): 624 mg (76% yield). R_f = 0.26. ^{31}P NMR ($CDCl_3$): δ = 54.61 and 53.95 (each dd, $^1J_{P,H}$ = 585.4 and 579.0 Hz; $^3J_{P,H}$ = 13.3 and 11.4 Hz) ppm. HRMS for $C_{35}H_{37}N_5O_8PS$ [M − TEAH]$^-$: calcd. 718.2106; found 718.2120. The ^1H and ^{13}C NMR spectroscopic data of the compound were identical to those reported previously.[2]

Acknowledgments

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[18] A similar approach was recently used by Jones et al. for simultaneous phosphonylation and silylation of ribonucleosides by phenyl H-phosphonate and *tert*-butyldimethylsilyl chloride. Unfortunately, due to the low reactivity of phenyl H-phosphonate, the reaction had to be carried out in pyridine for 48 h, and required the presence of a strong base, DBU, to effect the transesterification. Such reaction conditions are not compatible with *N*-acyl-protected nucleosides. See: Q. L. Song, W. M. Wang, A. Fischer, X. H. Zhang, B. L. Gaffney, R. A. Jones, *Tetrahedron Lett.* **1999**, *40*, 4153−4156.
[19] Formation of phenyl H-phosphonate (^{31}P NMR spectroscopy) is probably due to the presence of adventitious water or to partial disproportionation of DPHP under the reaction conditions. See: A. Kers, I. Kers, J. Stawinski, M. Sobkowski, A. Kraszewski, *Tetrahedron* **1996**, *52*, 9931−9944.
[20] Later experiments showed that the presence of phenyl H-phosphonate monoester (5−10%) in the reaction mixture containing **1**, nucleoside **3a**, and TBDPSCl does not lead to any detectable formation of the corresponding nucleoside H-phosphonate derivatives, probably because of the significantly lower reactivity of the silylated phenyl H-phosphonate relative to the phenyl H-phosphonothioate derivative. See ref.[18]
[21] The formation of a dinucleoside H-phosphonothioate diester in this reaction is probably due to the lability of trimethylsilyl esters and the partial generation of a nucleoside H-phosphonothioate monoester from the corresponding silylated species. The produced monoester could then react with the silylated intermediate of type **2** present in the reaction mixture to afford unsymmetrical H-pyrophosphonothioate, from which, upon reaction with nucleoside **3a**, either the desired silylated product **4a** or dinucleoside H-phosphonothioate diester would be formed.
[22] The reactions with added TEA were less efficient and usually occurred to only about 50% completion, most likely due to consumption of the silylating agent by the released phenol (^{31}P NMR experiments). Also, the presence of TEA stimulated silylation of nucleosides **3** (TLC analysis).
[23] Occasionally, some amount of the silylated nucleoside was formed but it did not exceed 5%.
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