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Aryl H-Phosphonates. 8. Simple and Efficient Method for the Preparation of Nucleoside H-Phosphonothioate Monoesters

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Abstract: An efficient method for the preparation of nucleoside 3'-H-phosphonothioate monoesters was developed. It consists of phosphonylation of suitably protected nucleosides with diphenyl H-phosphonate to produce nucleoside phenyl H-phosphonates, followed by their sulfhydrolysis with hydrogen sulfide. © 1999 Elsevier Science Ltd. All rights reserved.

Oligonucleotide analogues are attracting increasing interest due to the possibility of using this type of compound as specific gene inhibitors at the mRNA (antisense oligonucleotides)[1] or the double–stranded DNA level (antigene oligonucleotides).[2] Due to the medical importance of this issue, a great effort has been put into design of various types of antisense and antigene agents to find compounds with optimal chemical, enzymatic and pharmaco-dynamic properties.[3] Among the most promising oligonucleotide analogues, from a therapeutic point of view, are those bearing modifications at the phosphorus center.[4] These, are usually prepared using the phosphoramidite[5] or the H-phosphonate methodology.[6]

Some years ago, we introduced H-phosphonothioates[7] as a new type of synthetic intermediate for the preparation of phosphorothioates and phosphorodithioates[8] or other types of nucleotide analogues, difficult to obtain by other routes, *e.g.* nucleoside phosphorodithioate monoesters,[9] nucleoside phosphoramidothioates,[10] nucleoside phosphorothiofluoridates.[11] As a starting material in all these syntheses the corresponding H-phosphonothioate monoesters are used. In contradistinction to a plethora of methods devised for the preparation of H-phosphonate monoesters, there is in principle only one approach of preparative value for the conversion of alcohols into H-phosphonothioate monoesters. This consists of phosphinylation of a suitably protected hydroxylic component with triethylammonium phosphinate in the presence of a condensing agent, followed by sulfurization.[12]

To facilitate access to H-phosphonothioate monoesters and make up for some shortcomings of the existing method, we searched for a new approach that could be based on another chemical principle, and for this purpose investigated sulfhydrolysis of aryl H-phosphonate diesters (Scheme 1). The rationale behind it was the known efficient formation of nucleoside phenyl H-phosphonates in the reaction of the corresponding nucleosides with diphenyl H-phosphonate and a high susceptibility of aryl H-phosphonate diesters toward nucleophilic

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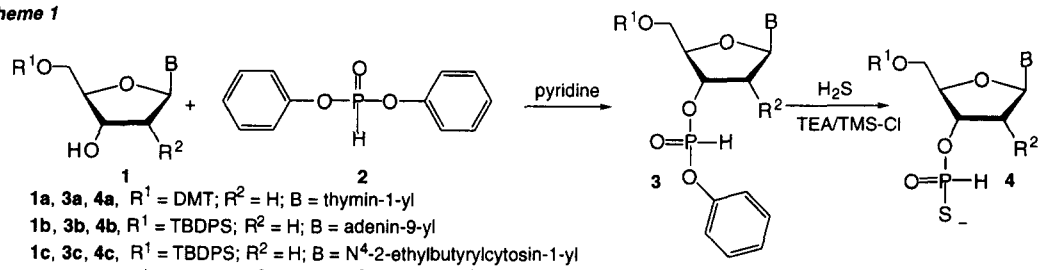
substitution at phosphorus, a phenomenon exploited recently in the synthesis of nucleoside H-phosphonate monoesters.[13]

Unfortunately, attempted synthesis of thymidine H-phosphonothioate **4a** *via* phosphonylation of **1a** in pyridine with diphenyl H-phosphonate **2** (3 equiv.), followed by treatment with H_2S (6 equiv. in dioxane) gave, despite of clean formation of the intermediate **3a** [δ_P = 4.4 ppm, $^1J_{PH}$ = 727 Hz (d)], a mixture of products consisting, *inter alia*, of almost equimolar amounts of nucleoside H-phosphonodithioate **5** [δ_P = 85.0 ppm, $^1J_{PH}$ = 529 Hz (d)] and the desired product **4a** [δ_P = 56.9 & 56.3 ppm, $^1J_{PH}$ = 569 & 571 Hz (d)] (^{31}P NMR). Since the formation of the H-phosphonodithioate **5** was somewhat unexpected, we wanted to get some insight into its origin and investigated as a model reaction, the sulfhydrolysis of diphenyl H-phosphonate **2**.

Scheme 1



1, **2**, **3**, **4**

1a, 3a, 4a, R^1 = DMT; R^2 = H; B = thymin-1-yl
1b, 3b, 4b, R^1 = TBDPS; R^2 = H; B = adenin-9-yl
1c, 3c, 4c, R^1 = TBDPS; R^2 = H; B = N^4-2-ethylbutyrylcytosin-1-yl
1d, 3d, 4d, R^1 = TBDPS; R^2 = H; B = N^2-propionyl-O^6-(N,N-diphenylcarbamoyl)guanin-9-yl
1e, 3e, 4e, R^1 = TBDPS; R^2 = OTBDMS; B = uracil-1-yl

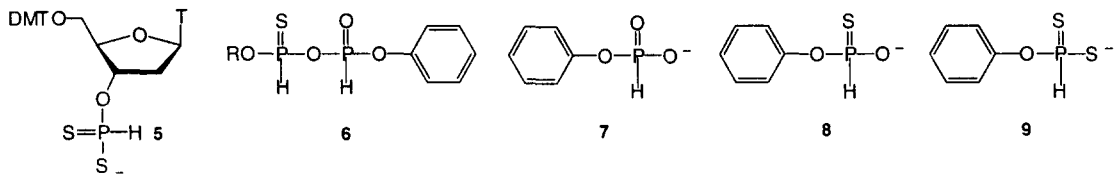
Abbr.: DMT - 4,4'-dimethoxytrityl; TBDPS - *t*-butyldiphenylsilyl; OTBDMS - *t*-butyldimethylsiloxy; TEA - triethylamine; TMS-Cl - trimethylsilyl chloride

In acetonitrile, in the absence of a base, aryl H-phosphonate **2** did not react with H_2S (2 equiv), but upon addition of pyridine [acetonitrile-pyridine 4:1 (v/v)] a slow reaction occurred, which after overnight went almost to completion affording equimolar amounts of phenyl H-phosphonodithioate **9** [δ_P = 85.8 ppm, $^1J_{PH}$ = 546 Hz (d)] and phenyl H-phosphonate **7** [δ_P = -0.67 ppm, $^1J_{PH}$ = 629 Hz (d)]. The expected product, phenyl H-phosphonothioate **8** [δ_P = 53.4 ppm, $^1J_{PH}$ = 580 Hz (d)] was present in the reaction mixture but was less than 5%. This product distribution[14] was rationalized by assuming that the primary product of the reaction, phenyl H-phosphonothioate **8**, reacted with **2** forming an intermediate **6** (R= phenyl), which upon reaction with H_2S afforded equimolar amounts of H-phosphonodithioate **9** and H-phosphonate **7**. This implied (i) that both reactions, the formation of **6** (R = phenyl) and its subsequent sulfhydrolysis, were faster than the generation of **8** from diphenyl H-phosphonate **2**, and (ii) that mixed H-pyrothiophosphonate **6** (R = phenyl) reacted chemoselectively[15] at the thiophosphonyl center. To verify this hypothesis, we investigated the reaction of nucleoside H-phosphonothioate **4a** with diphenyl H-phosphonate **2** (2 equiv.) and H_2S (4 equiv.) in acetonitrile-pyridine (4:1, v/v). In agreement with the postulated reaction pathway, the first ^{31}P NMR spectrum recorded (after ca 5 min) showed a complete disappearance of signals due to **4a** and the formation of H-phosphonodithioate **5**.[16] Within this time, **2** underwent only negligible sulfhydrolysis, as judged from a low intensity of the signal due to **8**.

Having established this plausible source of side-product formation (*i.e.* **5** or **9**) during sulfhydrolysis of aryl H-phosphonates (*e.g.* **2** or **3**), we attempted to suppress it by making the formation of H-phosphonothioates **4** or **8** faster than their subsequent reactions with **2**. To this end, the sulfhydrolysis of **2** was carried out under

analogous conditions as described above [acetonitrile-pyridine 4:1 (v/v) and 4 equiv. of H_2S] but in the presence of triethylamine (2 equiv.).[17] The reaction proved to be significantly faster (completion within a few minutes) than that in the absence of a strong base and this time produced the desired phenyl H-phosphonothioate **8** as the major product (> 95%). Using DBU (2 equiv.) instead of triethylamine completely eliminated the formation of phenyl H-phosphonodithioate **9** (^{31}P NMR spectroscopy).

Chart 1



The efficacy of this approach for the synthesis of nucleoside H-phosphonothioates **4** was evaluated by reacting nucleoside **1a** with diphenyl H-phosphonate **2** in pyridine followed by sulfhydrolysis with 6 equiv. of H_2S in the presence of triethylamine (4 equiv). In this instance the formation of the side product **5** was practically eliminated and the desired product, nucleoside H-phosphonothioate **4a**, was obtained in ca 90% yield after simple work-up and silica gel chromatography.

Although the formation of H-phosphonodithioates had been effectively suppressed, the procedure gave variable yields of the desired products of type **4**. We found that this was due to a competing hydrolysis of the intermediate **3** by spurious water introduced with H_2S, which resulted in the formation of the corresponding H-phosphonate monoesters. To remedy this problem, we carried out the sulfhydrolysis in the presence of trimethylsilyl chloride, added to the stock solution of H_2S. With these changes in the protocol, the synthesis of nucleoside H-phosphonothioates **4a-e** bearing various protecting groups became reproducible, giving consistently high yields of the desired products without concomitant formation of H-phosphonodithioates or H-phosphonate monoesters.

*A typical procedure for the preparation of nucleoside H-phosphonothioates **4a-e***

To the stirred solution of diphenyl H-phosphonate **2** (3 mmol, 0.574 mL) in pyridine (15 mL), nucleoside **1a-e** in pyridine (1 mmol/15 mL) was added dropwise during 30 min. The stirring was continued for another 15 min and then a mixture of H_2S (10 mmol, 10 mL of 1 M stock solution),[18] triethylamine (3 mL) and trimethylsilyl chloride (5 mmol, 0.635 mL) was added. When the sulfhydrolysis was complete (ca 60 min, TLC analysis) the mixture was concentrated, partitioned between methylene chloride (75 mL) and 5% aq. $NaHCO_3$ (50 mL), and the organic layer was successively washed with 5% aq. $NaHCO_3$ (2 x 50 mL) and 1M TEAB buffer (75 mL). After evaporation to dryness, the residue was purified by silica gel chromatography using a shallow stepwise gradient of methanol (3-10%) in methylene chloride containing 0.5% triethylamine. Nucleoside H-phosphonothioates **4** were isolated as triethylammonium salts. Yields: **4a**, 89%; **4b**, 84%; **4c**, 91%; **4d**, 88%; **4e**, 94%.[19]

In conclusion, we have developed a simple, fast, and efficient method for the preparation of nucleoside H-phosphonothioate monoesters using inexpensive, commercially available reagents. The approach is suitable for

the preparation of both deoxyribo- (**4a-d**) and ribonucleotide derivatives (**4e**) and does not require protection of moderately reactive amino functions in a substrate (*e.g.* **4b**). Since phosphonylation with diphenyl H-phosphonate and the subsequent sulfhydrolysis occur under mild reaction conditions, this method should be perceived as a general approach for the introduction of H-phosphonothioate monoester moiety into natural products.

Acknowledgements

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REFERENCES AND NOTES

1. Wagner, R. W. *Nature* **1994**, *372*, 333-335.
2. Thuong, N. T.; Helene, C. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 666-690.
3. Agrawal, S.; Zhao, Q. *Current Opinion Mol. Biol.* **1998**, *2*, 519-528.
4. Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543-584.
5. Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1992**, *48*, 2223-2311.
6. Stawinski, J.; Strömberg, R. *Trends Org. Chem.* **1993**, *4*, 31-67.
7. Stawinski, J.; Thelin, M.; Zain, R. *Tetrahedron Lett.* **1989**, *30*, 2157-2160.
8. Stawinski, J.; Thelin, M. *Tetrahedron Lett.* **1992**, *33*, 3189-3192.
9. Jankowska, J.; Sobkowska, A.; Cieslak, J.; Sobkowski, M.; Kraszewski, A.; Stawinski, J.; Shugar, D. *J. Org. Chem.* **1998**, *63*, 8150-8156.
10. Kers, I.; Stawinski, J.; Kraszewski, A. *Tetrahedron Lett.* **1998**, *39*, 1219-1222.
11. Bollmark, M.; Stawinski, J. *Tetrahedron Lett.* **1996**, *37*, 5739-5742.
12. Stawinski, J.; Thelin, M.; Westman, E.; Zain, R. *J. Org. Chem.* **1990**, *55*, 3503-3506.
13. Jankowska, J.; Sobkowski, M.; Stawinski, J.; Kraszewski, A. *Tetrahedron Lett.* **1994**, *35*, 3355-3358.
14. A similar product distribution we observed previously in the reaction of a nucleoside phosphonic pivalic mixed anhydride with hydrogen sulfide. *See*, Zain, R.; Strömberg, R.; Stawinski, J. *J. Org. Chem.* **1995**, *60*, 8241-8244.
15. The observed chemoselectivity can be explained, *e.g.* by higher acidity of the P-H at the thiophosphonyl center. This should facilitate formation of tervalent species that are expected to be more susceptible to the reaction with soft nucleophiles, *e.g.* sulfide anion, aryloxides.
16. Nucleoside H-phosphonodithioate **5** can also be formed from **6** (R= nucleosid-3'-yl) *via* another pathway, involving intermediacy of the corresponding nucleoside phenyl H-phosphonothioate. In separate experiment we found that such compounds are, indeed, more susceptible to sulfhydrolysis than diphenyl H-phosphonate.
17. We considered two targets for base catalysis in this reaction: H_2S (the increased formation of HS^-) and diphenyl H-phosphonate **2** (the increased formation of tervalent species).
18. 1M stock solution of H_2S in dioxane was prepared by passing H_2S through the solvent until saturation. For the use in the sulfhydrolysis, trimethylsilyl chloride and triethylamine were added to the appropriate volume of the stock solution.
19. Compounds **4a-e** were isolated as mixtures of diastereomers (ratio ca 1:1 for **4a-d** and 4:1 for **4e**) and were of purity >98%. Their identity were confirmed by ^1H, ^{13}C, ^{31}P, and ^1H- ^1H correlated NMR spectroscopy. Some diagnostic spectral data [compound, δ_P ($^1J_{PH}$, $^3J_{PH}$), $\delta_{H1'}$, $\delta_{H3'}$]: **4a**, 54.9 & 54.0 ppm (578 & 583, 11.9 & 12.2 Hz, dd), 6.6, 5.3 ppm; **4b**, 54.5 & 54.4 ppm (579 & 581, 11.6 & 12.2 Hz, dd), 6.5, 5.4 ppm; **4c**, 55.5 & 54.0 ppm (576 & 585, 11.6 & 11.9 Hz, dd), 6.3, 5.2 ppm; **4d**, 54.8 & 54.7 ppm (580 & 583, 12.2 & 11.6 Hz, dd), 6.3, 5.3 ppm; **4e**, 57.0 & 54.3 ppm (585 & 577, 13.2 & 12.9 Hz, dd), 5.7, 4.8 ppm.