Pergamon

Tetrahedron Letters, Vol. 38, No. 11, pp. 2007-2010, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)00218-9

9-Fluorenemethyl H-Phosphonothioate, a Versatile Reagent for the Preparation of H-Phosphonothioate, Phosphorothioate, and Phosphorodithioate Monoesters

Jadwiga Jankowska, Jacek Cieślak, and Adam Kraszewski*

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznań, Poland

Jacek Stawiński*

Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden.

Abstract: Simple and efficient synthesis of a new H-phosphonothionylating reagent, 9-fluorenemethyl H-phosphonothioate, was developed. The synthetic utility of the reagent has been demonstrated in the preparations of nucleoside H-phosphonothioate, nucleoside phosphorothioate, and nucleoside phosphorodithioate monoesters.

Owing to their favourable chemical and enzymatic properties, oligonucleoside phosphorothioate[1] and oligonucleoside phosphorodithioate[2] analogues are in focus of pharmaceutical sciences as potential antisense and antigene therapeutics[2,3].

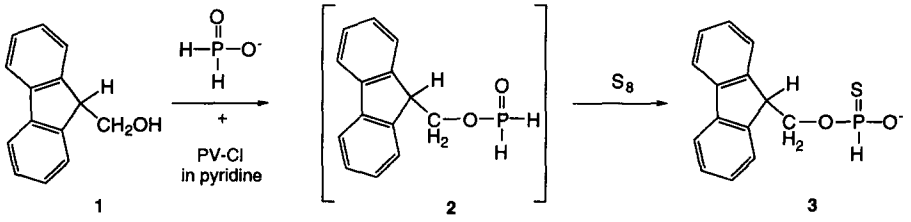
To expand synthetic methodologies available for the preparation of these types of compounds[4-6], we have recently developed an efficient synthesis of nucleoside H-phosphonothioate monoesters[7] and showed, that these compounds, after conversion to H-phosphonothioate diesters in the chlorophosphates promoted condensations[8,9], provide a convenient entry to nucleoside phosphorothioate, phosphorodithioate and phosphoroselenothioate analogues.

Since H-phosphonothioate diesters are useful synthetic intermediates for the preparation of phosphate analogues difficult to obtain by other routes[9-11], we have been searching for a H-phosphonothionyl group-transferring reagent, which would make this methodology even more versatile and efficient. Our primary goal was to develop an alternative method for the synthesis of nucleoside H-phosphonothioates[7] and to provide an easy access to phosphate monoesters analogues, namely, to nucleoside phosphorothioates and nucleoside phosphorodithioates[12]. Having at hand an efficient method for the formation of H-phosphonothioate diesters from the corresponding monoesters[8,9], as a viable approach we considered to use an alkyl H-phosphonothioate monoester with a β-eliminating group as an H-phosphonothionyl transferring reagent. The procedure would thus consist of generating a nucleoside alkyl H-phosphonothioate, from which the corresponding nucleoside H-phosphonothioate, nucleoside phosphorothioate, or nucleoside phosphorodithioate may be produced after further chemical transformations.

Among various alkyl H-phosphonothioate monoesters investigated for this purpose, the most promising appeared to be 9-fluorenemethyl H-phosphonothioate **3**[13]. The 9-fluorenemethyl[12,14,15] offers several advantages as a phosphate (or phosphonate) protecting group. It is a lipophilic, UV_{254} absorbing moiety, that provides a convenient handle for the purification and the detection of corresponding derivatives, and a possibility of its β-elimination removal minimizes "wrong-breakdown" during the final deprotection step.

For the preparation of the reagent **3**, we have designed a synthetic scheme based on the method[7] previously developed in these Laboratories, that makes use of commercial available products (Scheme 1). The efficacy of this approach was evaluated by condensing 9-fluorenemethanol (**1**) (2.0 equiv.) and triethylammonium phosphinate (1.0 equiv.) in the presence of pivaloyl chloride (PV-Cl, 1.5 equiv.) in pyridine. The ^{31}P NMR spectroscopy indicated (spectrum after 3 min.) a smooth formation of the expected intermediate **2** (δ_P = 15.70 ppm, $^1J_{HP}$ = 566.4 Hz, $^3J_{HP}$ = 7.9 Hz, tt), which upon addition of sulfur (3.0 equiv.) produced the H-phosphonothioate **3** (δ_P = 57.44 ppm , $^1J_{HP}$ = 581.1 Hz, $^3J_{HP}$ 9.2 Hz, dt). Since all the steps proceeded very cleanly, we tried to further simplify the preparation of **3** and carried out the synthesis depicted in Scheme 1 as a four-component reaction. To this end 9-fluorenemethanol **1**, triethylammonium phosphinate, and sulfur were allowed to react in the presence of PV-Cl using the above stoichiometry. After ca 10 min the ^{31}P NMR spectrum revealed that H-phosphonothioate **3** was formed in a yield >95%. This procedure was used for the preparation of fluorenemethyl H-phosphonothioate **3**[16] on a large scale (10 mmol). The reagent **3** produced in this way can be stored for several months (in a refrigerator) without noticeable decomposition.

Scheme 1

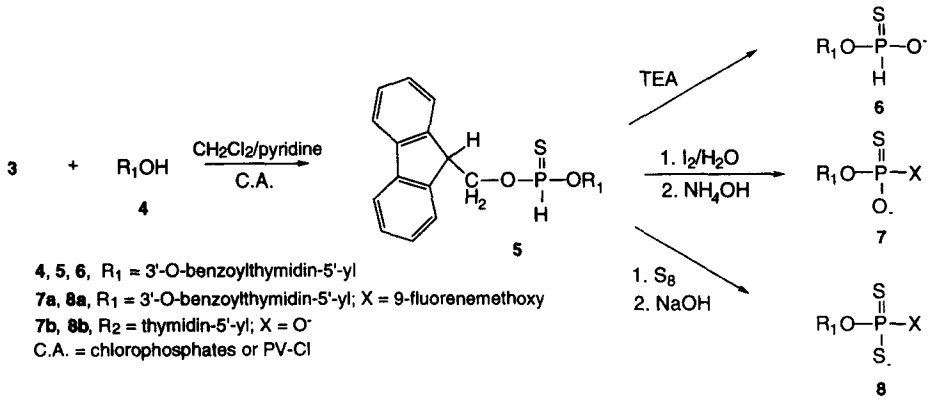


The usefulness of **3** as a H-phosphonothionyl-transferring reagent was assessed in reactions with 3'-O-benzoylthymidine **4** as a representative nucleosidic acceptor (Scheme 2). Since H-phosphonothioate diesters of type **5** in neat pyridine are prone to the ligand exchange process[8], the coupling reactions were performed in methylene dichloride containing a limited amount of pyridine (10 %, v/v). Under these experimental conditions, using diethyl phosphorochloridate (2.5 equiv.) or PV-Cl (1.2 equiv.) as a condensing agent, the reaction of the nucleoside **4** with the reagent **3** (1.2 - 1.5 equiv.) went to completion in less than 3 min to produce the mixed H-phosphonothioate diester **5**[17] (δ_P = 72.12 and 71.36 ppm, $^1J_{HP}$ = 660.4 and 662.9 Hz, $^3J_{HP}$ = 8.6 Hz, dq) as a sole nucleotidic product.

The intermediate **5** can be isolated (if so desired) or may be used *in situ* for further transformations[18]. Depending on the reaction conditions and type of reagents used, the H-phosphonothioate **5** can be converted into other synthetically or biologically useful compounds. Thus, a treatment of **5** with triethylamine in anhydrous acetonitrile (1 : 4, v/v) during 20 min quantitatively produced the nucleoside 5'-H-phosphonothioate **6**[19] (δ_P = 55.15 and 54.94 ppm, $^1J_{HP}$ = 577.4 and 574.4 Hz, $^3J_{HP}$ = 7.3 Hz, dt). Alternatively, oxidation of **5** with I_2 (1.5 equiv) in pyridine-water (98:2, v/v) afforded rapidly (< 3 min) the phosphorothioate diester **7a** (δ_P = 60.58 and 60.18 ppm, $^3J_{HP}$ = 7.4 and 6.5 Hz, q) as the sole product. The 9-fluorenemethyl and benzoyl groups were

removed from **7a** simultaneously by treatment with pyridine - 33% aqueous ammonia (1:1, v/v; RT, 24 h) to produce the thymidine 5'-thiophosphate **7b**[20] (δ_P = 47.77 ppm) in 84% yield.

Scheme 2



4, 5, 6, R_1 = 3'-O-benzoylthymidin-5'-yl
7a, 8a, R_1 = 3'-O-benzoylthymidin-5'-yl; X = 9-fluorenemethoxy
7b, 8b, R_2 = thymidin-5'-yl; X = O^-
C.A. = chlorophosphates or PV-Cl

Treatment of the H-phosphonothioate **5** with sulfur (3 equiv.) in methylene dichloride containing lutidine[21] (10%) furnished a clean formation (1 h) of the phosphorodithioate **8a** (δ_P = 113.80 ppm), which was isolated in 91% yield[22]. Removal of the protecting groups from **8a** with pyridine - 33% aqueous ammonia (1:1, v/v; RT, 24 h) afforded the desired nucleoside phosphorodithioate **8b** (δ_P = 87.86 ppm) as a predominant product (80 %, ^{31}P NMR spectroscopy), but the reaction mixture also contained dithiophosphate, thymidine (TLC), and the nucleoside phosphorothioate **7a**[23]. Since phosphorothioate monoesters are less stable than their oxygen congeners[24,25], we assumed that instability of phosphorodithioate monoesters observed by us and others[12,26] is probably due to the ease of generation of dithiometaphosphate in general acid catalyzed reaction. Indeed, when instead of ammonia 0.1 N NaOH (5 equiv.) was used for the deprotection of **8a**, a considerable improvement in stability of the produced phosphorodithioate **8b** was achieved[27].

In conclusion, the new reagent, 9-fluorenemethyl H-phosphonothioate **3**, provides a new and efficient entry to a synthetically important starting material, nucleoside H-phosphonothioates, and to phosphorothioate monoester analogues, which are difficult to access by other routes. The reagent is a stable solid, with good solubility in organic solvent, and it can be prepared in "one pot" reaction on a large scale from commercially available materials.

Acknowledgements
We are indebted to Prof. Maciej Wiewiórowski and to Prof. Per J. Garegg for their interest and helpful discussions. Financial support from the State Committee for Scientific Research, Republic of Poland, the Swedish Natural Science Research Council, and the Swedish Research Council for Engineering Sciences is gratefully acknowledged.

REFERENCES AND NOTES

1. Stein, C. A.; Cohen, J. S. Phosphorothioate oligodeoxynucleotide analogues. In *Oligodeoxynucleotides-Antisense Inhibitors of Gene Expression;* J. S. Cohen, Ed.; The Macmillan Press Ltd.: New York, 1989; Vol. 12; pp. 97-117.
2. Marshall, W. S.; Caruthers, M. H. *Science* **1993**, *259*, 1564-1569.
3. Matsukura, M.; Zon, G.; Shinozuka, K.; Stein, C. A.; Mitsuya, H.; Cohen, J. S.; Broder, S. *Gene* **1988**, *72*, 343-347.

4. Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *49*, 6123-6194.
5. Stawiński, J.; Strömberg, R. *Trends Org. Chem.* **1993**, *4*, 31-67.
6. Dahl, O. *Sulfur Reports* **1991**, *11*, 167-192.
7. Stawiński, J.; Thelin, M.; Westman, E.; Zain, R. *J. Org. Chem.* **1990**, *55*, 3503-3506.
8. Zain, R.; Stawiński, J. *J. Org. Chem.* **1996**, *61*, 6617-6622.
9. Stawiński, J.; Thelin, M.; Zain, R. *Tetrahedron Lett.* **1989**, *30*, 2157-2160.
10. Bollmark, M.; Zain, R.; Stawiński, J. *Tetrahedron Lett.* **1996**, *37*, 3537-3540.
11. Bollmark, M.; Stawiński, J. *Tetrahedron Lett.* **1996**, *37*, 5739-5742.
12. Seeberger, P. H.; Yau, E.; Caruthers, M. H. *J. Am. Chem. Soc.* **1995**, *117*, 1472-1478.
13. After this work was completed, 9-fluorenemethyl H-phosphonothioate has been advocated by Caruthers *et al.* as a useful reagent for the introduction of the 5'-terminal phosphorodithioate function to oligonucleotides (see, Seeberger, P. H.; Jorgensen, P. N.; Bankaitisdavis, D. M.; Beaton, G.; Caruthers, M. H. *J. Am. Chem. Soc.* **1996**, *118*, 9562-9566). The reported preparation of the reagent was, however, lengthy (a multi-step synthesis) and required several purification steps of the final product.
14. Katagiri, N.; Bahl, C. B.; Itakura, K.; Michniewicz, J.; Narang, S. A. *J. Chem. Soc. Chem. Commun.* **1973**, 803-804.
15. Yang, Z. W.; Xu, Z. S.; Shen, N. Z.; Fang, Z. Q. *Nucleosides & Nucleotides* **1995**, *14*, 167-173.
16. ***The preparation of 3.*** 9-Fluorenemethanol (**1**, 2.0 molar equiv.), triethylammonium phosphinate (1.0 molar equiv.) and sulfur (3.0 molar equiv.) were rendered anhydrous by repeated evaporation of added pyridine. The residue was dissolved in pyridine (1 mL/0.1 mM of triethylammonium phosphinate) and PV-Cl (1.5 molar equiv.) was added. After stirring for 20 min, the solvent was removed under vacuum, the residue dissolved in CH_2Cl_2 and washed with aq. saturated $NaHCO_3$. The organic layer was dried over Na_2SO_4, evaporated and the product **3** was isolated by a silica gel column chromatography using, consecutively, CH_2Cl_2 and CH_2Cl_2 containing 1.5 % of methanol, as eluents. Chromatographically homogeneous H-phosphonothioate **3** (triethylammonium salt) was obtained as microcrystalline solid after precipitation from n-hexane. Yield, 89%. ^1H NMR: δ_H ($CDCl_3$); 1.0 (9H, t, J = 7.2 Hz, CH_2CH_3), 2.73 (6H, q, J = 7.2 Hz, CH_2CH_3), 4.13 (1H, t, J = 6.0 Hz, Flu. H - 9), 4.28 (2H, m, Flu. CH_2), 7.20 - 7.67 (8H, Flu arom. protons), 7.74 (1H, d, $^1J_{HP}$ = 605.9 Hz, H-P).
17. Compound **5** was isolated in 91% yield after a silica gel column chromatography (CH_2Cl_2/iso-PrOH, 99:1, v/v) and its structure was confirmed by ^1H and ^{31}P NMR spectroscopy.
18. For the purpose of the present studies all reactions involving **5** were carried out on the isolated compound.
19. The reaction was exceptionally clean. The nucleoside H-phosphonothioate **6** was obtained as chromatographically homogeneous product in 96% yield after precipitation from diethyl ether/n-hexane (1:1 v/v), and it was found to be identical (^1H and ^{31}P NMR) with an authentic sample obtained on a different way.
20. Silica gel column chromatography using *iso*-PrOH/H_2O/NH_4OH (85 : 10 : 5, v/v/v) followed by MeOH/NH_4OH (9:1, v/v) as eluents. Purity and identity of **7b** (diammonium salt) was confirmed by ^1H and ^{31}P NMR spectroscopy, and by TLC analysis (silica gel, *iso*-PrOH/H_2O/NH_4OH, 85 : 10 : 5, v/v/v).
21. In pyridine, apparently due to extended time of the reaction, the formation of **8a** was accompanied by side products, which most likely resulted from the occurrence of the ligand exchange process in **5** under the reaction conditions.
22. The phosphorodithioate **8a** was isolated by a silica gel column chromatography using gradient of methanol (0 - 20 %) in methylene dichloride. It was characterized by ^1H and ^{31}P NMR spectroscopy, and TLC analysis.
23. After removing the excess of ammonia and pyridine, the amount of side products considerably increased, indicating that the degradation of **8b** also occurred during work-up (*i.e.* during concentration of the reaction mixture).
24. Harnett, S. P.; Lowe, G. *J. Chem. Soc. Chem. Commun.* **1987**, 1416-1418.
25. Cullis, P. M.; Misra, R.; Wilkins, D. J. *J. Chem. Soc. Chem. Commun.* **1987**, 1594-1596.
26. Reese, C. B.; Shek, L. H. K.; Zhao, Z. Y. *Tetrahedron Lett.* **1994**, *35*, 5085-5088.
27. After evaporation of the solvents, **8b** was purified as described for **7b** and was isolated in 76% yield. The structure and purity of **8a** was confirmed by ^1H and ^{31}P NMR spectroscopy, and by TLC analysis.

(*Received in UK* 12 *December* 1996; *revised* 17 *January* 1997; *accepted* 31 *January* 1997)