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An Oxathiaphospholane Approach to One-pot Phosphorothioylation of Isoprenoid Alcohols

Katarzyna Zmudzka,[†] Barbara Nawrot,[†] Tadeusz Chojnacki,[‡] and Wojciech J. Stec*,[†]

Department of Bioorganic Chemistry, Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, 90-363 Lodz, Poland, and Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawinskiego 5a, 02-106 Warszawa, Poland

wjstec@bio.cbmm.lodz.pl

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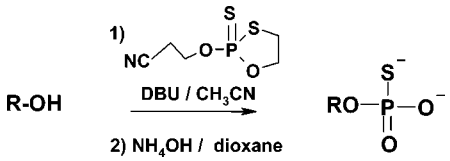
ABSTRACT



Allyl, isopentenyl, geranyl, citronellyl, farnesyl, and phytyl alcohols were transformed in good yield (>60%) into their phosphorothioates via a DBU-assisted 1,3,2-oxathiaphospholane ring-opening condensation with 2-(2-cyanoethoxy)-2-thiono-1,3,2-oxathiaphospholane, a reagent that is stable and easy to handle, followed by subsequent removal of the 2-cyanoethyl group from the intermediate phosphorothioate diester under basic conditions.

Phosphorothioate analogues of biophosphates are indispensable tools for the study of the mechanisms of action of enzymes responsible for their biosynthesis and metabolism.[1,2] In addition, phosphorothioate analogues of oligonucleotides constitute so far the only class of modified oligonucleotides that has found practical therapeutic application in the so-called antisense strategy.[3] Interestingly, in contrast to phosphorylated nucleosides, proteins, inositols, and lipids, one of the most abundant class of biophosphates, namely, prenyl diphosphates modified with a phosphorothioate function, have received relatively little attention. This is because prenyl diphosphates react via allylic carbocations leading to formation of carbon−carbon bonds,[4] with the diphosphate moiety playing the role of a leaving group. Replacement of diphosphate by methanebisphosphonate,[5] phosphonophosphate,[6] phosphonophosphinate,[7] or (1-thio)diphosphate makes it possible to obtain modified substrates useful for the study of the mechanism of action of prenyl transferases. These studies indicate that prenyl transferases readily recognize the diphosphate moiety. Moreover, as demonstrated in the pioneering studies of Poulter et al., the preparation of polyprenyl (1-thio)diphosphates caused problems because of the easy thiono-thiolo rearrangement of the intermediate *O*-allyl-*O*,*O*-dialkyl phosphorothioates.[8] Successful preparation of geranyl (1-thio)diphosphate was achieved via the *O*-geranyl *H*-phosphonate, followed by its sulfurization to give geranyl *O*-phosphorothioate in 46% yield.[9] Here we present an alternative approach to the synthesis of isoprenyl

[†] Centre of Molecular and Macromolecular Studies.
[‡] Institute of Biochemistry and Biophysics.

(1) Verma, S.; Eckstein, F. *Annu. Rev. Biochem.* **1998**, *67*, 99.
(2) Brautigam, C. A.; Steitz, T. A. *J. Mol. Biol.* **1998**, *277*, 363.
(3) *Applied Antisense Oligonucleotide Technology*; Stein, C. A., Krieg, A. M., Eds.; Wiley-Liss: New York, 1999
(4) Lange, B. M.; Rujan, T.; Martin, W.; Croteau, R. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 13172.
(5) Stremler, K. E.; Poulter, C. D. *J. Am. Chem. Soc.* **1987**, *109*, 5542.
(6) Corey, E. J.; Volante, R. R. *J. Am. Chem. Soc.* **1976**, *98*, 1291.
(7) McClard, R. W.; Fujita, T. S.; Stremler, K. E.; Poulter, C. D. *J. Am. Chem. Soc.* **1987**, *109*, 5544.
(8) Poulter, C. D.; Mautz D. S. *J. Am. Chem. Soc.* **1991**, *113*, 4895 and references therein.
(9) Mautz, D. S.; Davidson, V. J.; Poulter, C. D. *Tetrahedron Lett.* **1989**, *30*, 7333.

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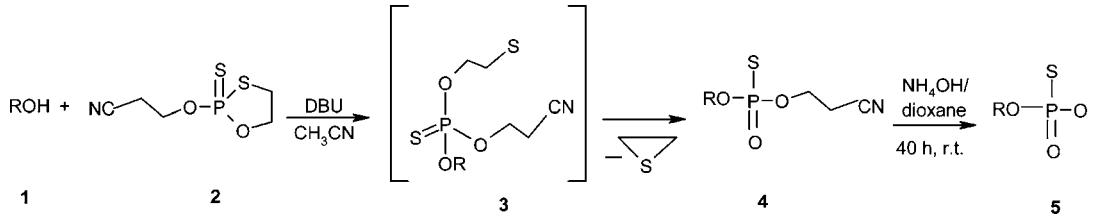
Scheme 1. Synthesis of Phosphorothioates of Isoprenoid Alcohols **1a**−**f**[a]



[a] Via DBU-assisted condensation of 2-(2-cyanoethoxy)-2-thiono-1,3,2-oxathiaphospholane **2** with ethylene episulfide elimination followed by removal of the 2-cyanoethyl protecting group from diester **4**. (Abbreviations are as in Table 1).

phosphorothioates, based on an oxathiaphospholane ring-opening condensation (OTP), developed in this laboratory.[10]

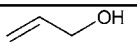
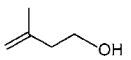
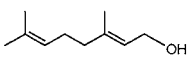
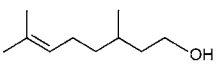
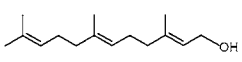
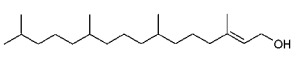
Because the direct oxathiaphosphathioylation of isoprenoid alcohols followed by OTP ring opening with alcohols or water leads to a complicated mixture of products (data not shown), we designed a procedure involving the reaction of 2-(2-cyanoethoxy)-2-thiono-1,3,2-oxathiaphospholane (**2**) with allyl, isopentenyl, geranyl, citronellyl, farnesyl, and phytyl alcohols (**1**) (Scheme 1). These reactions, like other 1,3,2-oxathiaphospholane ring-opening condensations, required the presence of an equimolar amount of DBU and were performed in acetonitrile solution (except **1f**, for which the reaction was carried out in dichloromethane) at 0−5 °C. ^{31}P NMR spectra recorded for the reaction mixtures indicated that, after 30 min, the resonance line at 105.6 ppm, characteristic for **2**, had almost entirely disappeared and a new resonance line at ca. 56 ppm, characteristic for *O*,*O*-dialkyl phosphorothioates **4**, had appeared.

The NMR spectra indicated that the yield of conversion of **2** → **4** was more than 80%. After solvent removal, the residue was treated with a mixture of dioxane and 28% aqueous ammonia (1:1, v/v). The resulting mixture was kept for 48 h at room temperature and concentrated in vacuo, and the residual oil was chromatographed on a silica gel column. Products **5** as $DBUH^+/NH_4^+$ salts were eluted with propan-2-ol/water/ammonia (7:1:1 v/v/v) in the yields listed in Table 1 where their spectral characteristics are also presented.

Isoprenoid alcohols **1e** and **1f** were inseparable mixtures of *cis*- and *trans*-isomers isolated from plants.[11] Their derivatives **4** (diesters) did not show separate resonance lines in their ^{31}P NMR spectra. However, when transformed into monoesters **5e** and **5f**, respectively, both showed two ^{31}P NMR signals. The ratio of upfield to downfield signals for **5e** is 1:2, whereas for **5f** it is 2:1 (Table 1).

It should be emphasized that the oxathiaphospholane method is especially suitable for the one-pot synthesis of *O*-allyl esters of phosphorothioic acid. The OTP ring-opening condensation of reagent **2** with alcohols **1** yields the triester **3**, not prone to the thiono-thiolo rearrangement[8,12,13] otherwise observed for allyl alcohol derivatives. DBU-assisted elimina-

Table 1. Spectral Characteristics of Diesters **4** and Monoesters **5**

alcohol **1**	formula	^{31}P NMR (δ, ppm) of **4** (NMR yield in %)	content of **5**/**4** in crude reaction mixture (NMR yield in %)	^{31}P NMR (δ, ppm) of **5**[c]	calc. MW of **5**	exp.[b] MW of **5**	yield of isolated **5** (%)
a allyl		56.2 (0.5)	64.2/6.5	44.7	152.9775	152.9777	63.0
b isopentenyl		56.0 (80.0)	71.6/0.9	43.4	181.0088	181.0084	68.6
c geranyl		56.6 (88.0)	80.0/3.0	45.9[d]	249.0714	249.0721	65.7
d citronellyl		56.1 (86.1)	75.5/3.4	43.4	251.0871	251.0872	62.0
e farnesyl[a]		56.2 (82.3)	66.7/4.3	43.9; 44.3 (1:2)	317.1340	317.1350	63.0
f phytyl[a]		57.0 (93.5)	63.8/4.9	43.4; 43.7 (2:1)	391.2436	391.2442	62.2

[a] Starting alcohol was used as a mixture of *cis*/*trans* isomers. [b] HRMS data; *m*/*z* of anionic moiety of **5** is given. [c] ^{31}P NMR data for pure compunds **5** isolated by silica gel column chromatography with propan-2-ol/water/ammonia (7:1:1 v/v/v) mixture. [d] 45.2 ppm, according to ref 9.

Table 2. Products of Transformation of **4c** → **5c** in Basic Conditions As Determined by ^{31}P NMR

reaction conditions	substrate **4c**, ~56 ppm (%)	product **5c**, ~45 ppm (%)	side product, ~17 ppm (%)	other products (%)
28% aq NH_4OH, 55 °C, 4 h	10	60	22	8
28% aq NH_4OH/EtOH 1:1, 55 °C, 2 h	16	45	26	13
28% aq NH_4OH/EtOH 1:1, 55 °C, 5 h	10	45	30	15
28% aq NH_4OH, rt, 12 h	40	22	28	10
90% Et_3N, rt, 1 h	100	0	0	0
prolonged **2** → **4** reaction time to 40 h	30	24	34	12
0.3 equiv DBU, rt, 7 days	1	32	57	10
28% aq NH_4OH/dioxane 1:1, rt, 48 h	3	80	15	2

tion of ethylene episulfide provides ionic derivatives **4** that are stable in neutral conditions. However, when treated with various ammonia solutions, compounds **4** are transformed either to their thiolo-isomers [R^1O-P(O)(O^-)-SR^2, R^1 = 2-cyanoethyl, R^2 = isoprenyl residue] or to monoesters **5** (desired products). Both products can be unequivocally distinguished by means of ^{31}P NMR spectroscopy, as the thiolo-esters absorb at ca. 17 ppm, and monoesters **5** at 45 ppm. Transformation of **4** → **5** in basic conditions was optimized using geranyl diester **4c** (Table 2). The best yield of the desired monoester **5c** was obtained when the deprotection was carried out in 28% aqueous NH_4OH/dioxane, 1:1 v/v mixture, for 48 h at room temperature.

The content of desired **5** and the residual **4** in the crude deprotection mixture, obtained in the above optimized conditions, is given in Table 1. The side products exhibiting chemical shift at ca. 17 ppm were unstable under the chromatographic conditions and were not characterized.

Michael-type addition of trisodium phosphorothioate[14] and phosphorothioate monoester[15] to an activated double bond has been reported in the literature. Also, in our previous studies we observed that acrylonitrile may be scavenged by dialkyl phosphorothioates with the formation of *S*-2-cyanoethyl thioloesters,[16] and hence it is conceivable that products with the above chemical shift can originate from the scavenging of released acrylonitrile by **5**, giving [R^2O-P(O)-(O^-)-SR^1, R^1 = 2-cyanoethyl, R^2 = isoprenyl residue]. This is especially relevant for phosphorothioates of nonallyl alcohols **1b** and **1d**.

In conclusion, the direct one-pot phosphorothioylation of alcohols **1**, including the allyl-type molecules, can be accomplished by DBU-assisted OTP ring-opening condensation with the stable and easily accessible reagent **2**. Resulting prenyl phosphorothioates can be efficiently transformed to (1-thio)diphosphates,[9] analogues of substrates for prenyl transferases.

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Supporting Information Available: Detailed procedure for the synthesis of compound **2** and general procedure for the phosphorothioylation of alcohols **1a**−**f**; spectral characteristics of **2** including 2D ^{1}H−^{1}H COSY and ^{1}H−^{13}C HSQC spectra, one-dimensional ^{1}H NMR (with and without phosphorus decoupling), ^{13}C NMR (with and without phosphorus and proton decoupling) spectra, and ^{31}P NMR, ^{1}H NMR, and HRMS spectra of **5a**−**f**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) Olesiak, M.; Krajewska, D.; Wasilewska, E.; Korczynski, D.; Baraniak, J.; Okruszek, A.; Stec, W. J. *Synlett* **2002**, *6*, 967.
(11) Commercially available from Sigma-Aldrich.
(12) Bruzik, K.; Stec, W. J. *J. Org. Chem.* **1981**, *46*, 1618.
(13) Bruzik, K.; Stec, W. J. *J. Org. Chem.* **1981**, *46*, 1625.
(14) Eckstein, F.; Burgers, P. M. J. *Biochemistry* **1979**, *18*, 592.
(15) Alefelder, S.; Patel, B. K.; Eckstein, F. *Nucleic Acids Res.* **1998**, *26*, 4983.
(16) Guga, P.; Maciaszek, A.; Stec, W. J. Unpublished results.