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DBU-assisted 1,3,2-oxathiaphospholane ring-opening condensation with selected *O*-, *S*-, *N*- and *C*-nucleophiles

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The authors dedicate this manuscript to Professor G.M. Blackburn on the occasion of his 70th birthday

Abstract—The reactivity of protected thymidine 3′-*O*- and 5′-*O*-(2-thio-1,3,2-oxathiaphospholanes) towards various nucleophiles in the presence of DBU is presented and mechanistic implications are discussed.

Diastereomers of protected nucleoside 3′-*O*-(2-thio-1,3,2-oxathiaphospholane)s[1] and 3′-*O*-(2-thio-'spiro'-4,4-pentamethylene-1,3,2-oxathiaphospholane)s[2] are used as monomers for the solid-phase synthesis of oligo(nucleoside phosphorothioate)s of pre-determined sense of P-chirality at each internucleotide phosphorothioate linkage. A 1,3,2-oxathiaphospholane ring-opening condensation method was also used for the synthesis of mixed backbone phosphate/phosphorothioate[2] oligonucleotides and [^{18}O]-labelled isotopomeric oligonucleotides.[3] Condensation of 5′-*O*-DMT-thymidine 3′-*O*-(2-seleno-1,3,2-oxathiaphospholane) with 3′-*O*-acetylthymidine in the presence of 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU), followed by removal of protecting groups, provided dithymidyl (3′,5′)phosphoroselenoates that were further converted into their corresponding phosphorofluoridates.[4] The scope and limitations of this oxathiaphospholane methodology have also been demonstrated in the synthesis of other classes of dinucleotides, for example, *ribo*-,[5] 2′,5′-*ribo*,[6] *xylo*-[7] and 'locked'-[8]dinucleoside phosphorothioates, –borane-phosphates,[9] –*N*3′-*O*5′-phosphoramidates and -phosphoramidothioates,[10] and 5′-*O*-phosphorothio conjugates of oligonucleotides with alcohols,[11] carbohydrates[12] and amino acids.[13] Synthetic methods leading to the compounds mentioned above were based upon the use of 5′-*OH*-nucleosides (nucleotides) as nucleophiles attacking the phosphorus atom of the corresponding 1,3,2-oxathiaphospholanes. The other classes of nucleophilic reagents employed in nucleoside oxathiaphospholane chemistry were 3-hydroxypropionitrile,[14] polyols[15] and fluoride anions.[16] Independently, based upon the same concept of ring-opening condensation followed by spontaneous elimination of ethylene sulfide, the corresponding 1,3,2-dithiaphospholanes were used, providing oligo(nucleoside phosphorodithioate)s in high yields.[17] The application of 1,3,2-oxathiaphospholanes in the synthesis of P-chiral analogues of biophosphates has been reviewed recently.[18]

The aim of the present studies was to examine the reactivity of 2-substituted 2-thio-1,3,2-oxathiaphospholanes towards various types of nucleophile such as aliphatic alcohols, phenols, thiols, amines and carbanions. It was also assumed that such an analysis of the scope and limitations of oxathiaphospholane methodology would provide new data allowing for elucidation of the role of DBU in these reactions. Thymidine derivatives **1** and **2** were chosen as simple models for these studies.

An unresolved mixture of diastereomers of 5′-*O*-DMT-thymidine 3′-*O*-(2-thio-1,3,2-oxathiaphospholane)[1] (**1**) reacted smoothly with dry methanol[†] used in a two molar excess and in the presence of an equimolar

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[†] All reagents employed in the studies described here were thoroughly dried by conventional methods and reactions were performed under strictly anhydrous conditions.

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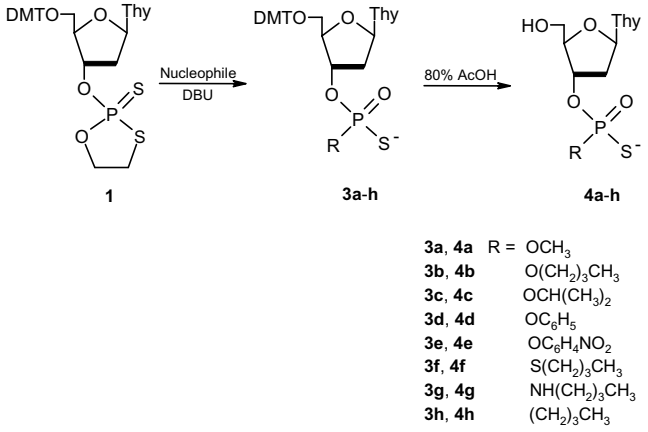
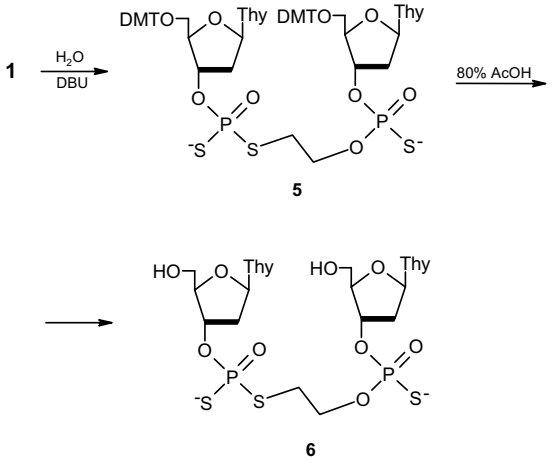


Scheme 1.

amount of DBU. Reactants were dissolved in dry acetonitrile. Even at −40 °C the reaction with MeOH was completed within 10 min (^{31}P NMR assay) yielding the product of ring-opening condensation **3a** as a mixture of diastereomers in an 1:1 ratio (Scheme 1).

No intermediate was observed in this reaction. Interestingly, reactions of **1** with other aliphatic alcohols like *n*-butanol and *iso*-propanol were much slower, requiring 10 and 15 min at room temperature, respectively [TLC and ^{31}P NMR control (disappearance of the resonance signal of **1**)]. Phosphorothioates **3a**–**c** were further 5′-*O*-deprotected (treatment with 80% acetic acid) to provide, after DEAE-Sephadex chromatographic purification, products **4a**–**c**. The yields of products **4a**–**c** and their physico-chemical characteristics are summarized in Table 1. The procedure used for the isolation of **4a**–**c** was not optimized and the yields of products are considerably lower than those present in the reaction mixtures. *n*-Butyl and *iso*-propyl phosphorothioates **4b** and **4c** consisted of a mixture of diastereomers, as proved for methyl ester **4a**, however, in both ^{31}P NMR and HPLC assays, they showed single resonance lines and single peaks, respectively.

Examination of the ^{31}P NMR spectra of the reaction mixtures indicated that in the case of less reactive alcohols like *n*-butanol and *iso*-propanol the desired products **4** were contaminated with a 'dimeric' by-product **5** (δ 70.79, 70.70, 56.93, 56.70). Its formation was attributed to traces of water present in the reaction mixtures. Indeed, for these alcohols the lowest water content that could be achieved by standard drying procedures[19] was 50 ppm. To confirm this hypothesis, oxathiaphospholane **1** was reacted with water under the same conditions as used for its reactions with alcohols and it was found that product **5** was formed exclusively (Scheme 2).



Scheme 2.

Table 1. The yields and physico-chemical characteristics of thymidine 3′-*O*-modified phosphorothioates **4a**–**h**

Nucleophile	Reaction time (min)	Yield of condensation[a]	Product[b]	Yield (%)	t_R [RP-HPLC[c]] (min)	δ_{31P} (D_2O)	*m*/*z* MS [-FAB]
MeOH	<5	96	**4a**	68	11.03	57.13, 57.14	351.4
n-BuOH	10	78	**4b**	37	16.93	55.42	393.2
i-PrOH	15	43	**4c**	24	13.08	54.08	379.2
PhOH	<5	98	**4d**	80	17.29, 17.42	52.34	413.4
p-O_2N–C_6H_4OH	180	78	**4e**	49	19.46, 19.53	51.71	458.4
n-BuSH	<5	96	**4f**	82	19.30	74.90	409.3
n-BuNH_2	180	65	**4g**	40	19.70	59.57	392.1
n-BuLi[d]	30	43	**4h**	19	16.17, 16.35	82.59	377.4

[a] ^{31}P NMR assay; the content of **3a**–**h** in the reaction mixtures obtained. Apart from reactions of **1** with *p*-$O_2NC_6H_4OH$ and *n*-BuLi the only other product observed in reaction mixtures was **5**.

[b] General procedure for the synthesis of **4a**–**h**: To a solution of a mixture of diastereomers of oxathiaphospholane **1** (0.34 g, 5 mmol) in acetonitrile (10 mL) the appropriate nucleophile (10 mmol) followed by DBU (75 μL, 5 mmol) were added with stirring. When the reaction was complete (TLC assay), the solvent was evaporated and the residue was re-dissolved in 80% acetic acid (10 mL) and left for 1 h. The resulting solution was concentrated and re-dissolved in 1 M triethylammonium bicarbonate pH 5 (TEAB) (10 mL) and extracted with diethyl ether (2×5 mL). The aqueous solution obtained was applied on a Sephadex column, which was eluted with a linear gradient (0.02–0.4 M) of TEAB. Appropriate fractions were combined and concentrated. Products **4a**–**h**, which were a mixture of two diastereomers, were obtained in the form of solid glasses.

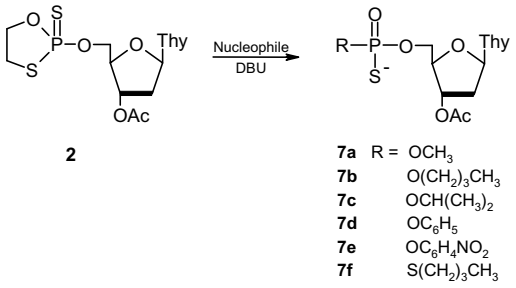
[c] Conditions: C-18 reverse phase column, flow 1 mL/min, λ 260 nm and gradient 0 → 80% B (A, 0.1 M CH_3COONH_4, B, 0.04 M CH_3COONH_4 in 60% acetonitrile) in 20 min.

[d] Reaction was performed in dry THF at −78 °C.

Compound **5** was detritylated to give, after DEAE-Sephadex purification, 'dimeric' product **6** in 47% yield. This result indicates that water effectively competes with the less reactive *n*-butanol and *iso*-propanol for nucleophilic substitution at the phosphorus atom. A similar phenomenon was also observed earlier[20] in a reaction of **1** with 3′-*O*-acetylthymidine. These observations explain why successful solid-phase synthesis of oligonucleotides utilizing oxathiaphospholane methodology requires the use of strictly anhydrous acetonitrile (water content below 20 ppm).

To examine the reactivity of protected nucleoside 2-thio-1,3,2-oxathiaphospholanes towards other classes of nucleophiles, reactions of **1** with phenol, *p*-nitrophenol, *n*-butanethiol, *n*-butylamine and *n*-butyllithium were performed. Each of these nucleophiles, except for *n*-butyllithium, did not react with oxathiaphospholane **1** in the absence of DBU. The yields of products **4d**–**h** obtained and their physico-chemical data are summarized in the Table 1. The lower yield of the ring-opening condensation observed for *n*-butylamine shows that amines are less reactive than alcohols towards oxathiaphospholanes. It was found that reaction of **1** with *n*-butylamine was complete after 180 min (TLC and ^{31}P NMR assay), while in the case of *n*-butanol the time required for the total disappearance of the substrate was only 10 min. In the case of *n*-butyllithium the lower yield of **3h** is probably connected with the high reactivity of this reagent leading to less regioselectivity in the ring-opening process as compared with 100% P–S bond cleavage observed in reactions of **1** with the other nucleophiles examined. An inspection of the ^{31}P NMR spectra of the reaction mixture of oxathiaphospholane **1** with *n*-butyl-lithium revealed the presence of major signals at 80.4 ppm (43% of the total P-containing compounds) and at 72.6 ppm (12%) suggesting, most probably, the formation of a phosphinyl by-product as a result of a subsequent substitution of the thymidyl moiety in compound **3h** by the *n*-butyl carbanion. This process was very recently described by Chen and Wiemer[21] in model studies of the reaction of diethyl phosphite with *n*-butyllithium.

The reactivity of oxathiaphospholane **1** towards some other anionic nucleophiles, for example, carboxylates and dialkyl phosphates in the presence of DBU was also examined, however, these attempts to prepare mixed carboxylic–phosphoric anhydrides or unsymmetrical pyrophosphates were unsuccessful (^{31}P NMR assay).



Scheme 3.

More satisfactory results were obtained in experiments involving 3′-*O*-acetylthymidine 5′-*O*-(2-thio-1,3,2-oxathiaphospholane)[14a] (**2**). Reactions of **2** with methanol, phenol, *p*-nitrophenol and *n*-butylmercaptan gave exclusively products resulting from oxathiaphospholane ring opening by these nucleophiles (Scheme 3).

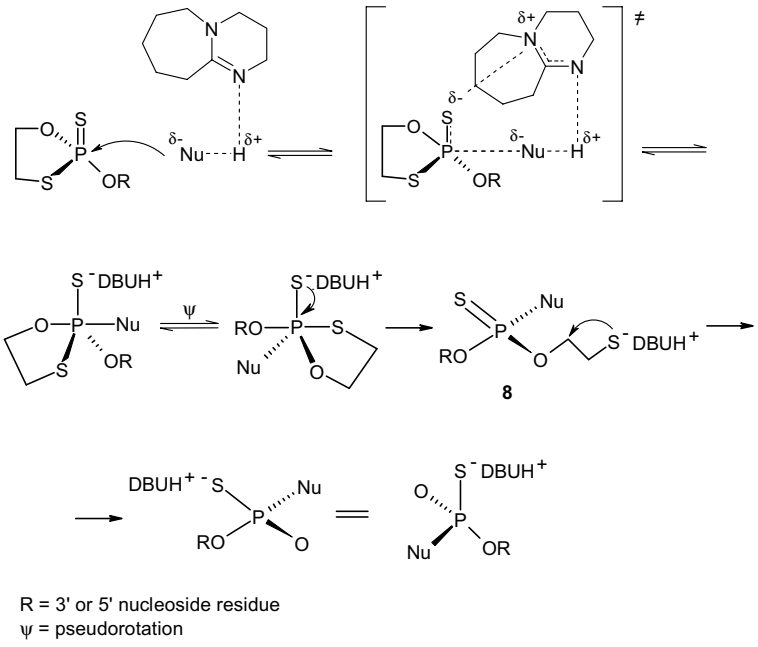
5′-Modified nucleotides **7a**–**f** (Table 2) were obtained in good yields demonstrating that the synthesis of this type of compound can be performed efficiently with the use of oxathiaphospholane chemistry. The higher yields in these ring-opening reactions of 5′-*O*-(2-thio-1,3,2-oxathiaphospholane) **2** as compared with the analogous 3′-*O* compound **1** probably reflects the easier steric accessibility of the phosphorus atom for an attacking nucleophile in **2** than in **1**. An inspection of the results summarized in Table 2 indicates that separation of signals in the ^{31}P NMR spectra, as well as separation of the peaks in HPLC chromatograms for **7a**–**f** were feasible. This observation is intriguing since, in spite of numerous efforts, we were unable to separate, by chromatographic methods, diastereomers of compound **2**, in contrast to the successful separation and isolation of individual diastereomers of compound **1** and other protected nucleoside 3′-*O*-(2-thio-1,3,2-oxathiaphospholanes). Such separation was a corollary to the success of the stereocontrolled synthesis of PS-oligonucleotides.[1,2]

Table 2. The yields and physico-chemical characteristics of 3′-*O*-acetylthymidine 5′-*O*-modified phosphorothioates **7a**–**f**

Nucleophile	Yield of condensation[a]	Product[b]	Yield (%)	t_R [RP-HPLC] (min)	δ_{31P} (CD_3CN)	*m/z* MS [−FAB]
MeOH	100	**7a**	52	12.71, 12.98	58.13, 57.95	391.1
n-BuOH	80	**7b**	72	16.28, 16.55	56.81, 56.59	435.1
i-PrOH	64	**7c**	56	14.47, 14.79	55.55, 55.33	421.1
PhOH	100	**7d**	87	16.00, 16.37	53.21, 52.92	454.9
p-$O_2NC_6H_4OH$	100	**7e**	76	17.80	51.74, 51.60	500.0
n-BuSH	100	**7f**	69	17.89, 18.32	74.85, 74.72	451.1

[a] ^{31}P NMR assay.

[b] General procedure for the synthesis of **7a**–**f**: To a solution of a mixture of diastereomers of oxathiaphospholane **2** (0.21 g, 5 mmol) in acetonitrile (10 mL) the appropriate nucleophile (10 mmol) followed by DBU (75 μL, 5 mmol) were added with stirring. When the reaction was complete (TLC assay), the solvent was evaporated and the residue was re-dissolved in chloroform supplemented with 5% of methanol and this solution was applied to a silica gel column. Compounds **7a**–**f**, which were a mixture of two diastereomers, were eluted with a mixture of chloroform and methanol (4:1, v/v) and were obtained in the form of solid foams.



Scheme 4.

For reactions of protected nucleoside 3′-*O*-(2-thio-1,3,2-oxathiaphospholanes) with alcohols in the presence of DBU two mechanisms have been previously proposed:

1. DBU acts as a base yielding an alkoxide anion, which attacks the phosphorus atom; the resulting P^V intermediate undergoes ligand exchange (*pseudorotation*) leading to a product with retention of configuration.[1,22,23]
2. DBU acts as a nucleophile leading to a P^V intermediate, which reacts further with an alcohol to give a P^{VI} intermediate; its collapse provides the observed product.[24]

The first mechanistic proposal does not explain why bases stronger than DBU like 2-*tert*-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine (BEMP) or potassium *tert*-butoxide did not promote these reactions as efficiently as DBU (as measured by rates of reactions and yields of products). The second mechanistic hypothesis may not adequately explain the earlier observed stereospecificity of condensation of 1,3,2-oxathiaphospholanes with alcohols. To explain the unique properties of DBU in reactions of oxathiaphospholanes with *O*-, *S*-, *N*- and *C*-nucleophiles we propose that this reagent possesses biphilic catalytic properties, acting as a Lewis base activating the corresponding nucleophilic agent and as a Lewis acid, accommodating (neutralizing) the negative charge formed on the exocyclic sulfur atom. Both these simultaneous interactions lead to stabilization of the transition state, which occurs in the first reaction step (Scheme 4).

This hypothesis allows for an explanation of the unique properties of DBU in the reactions of various nucleophiles with 1,3,2-oxathiaphospholanes and the stereospecificity of these transformations. Such a concept is also supported by the fact that imidazole, although a weak base, possesses a similar arrangement of nitrogen atoms as DBU and can also act as promoter in the reaction of oxathiaphospholane **1** with 3′-*O*-acetylthymidine.[25] It was also found that protonation of DBU[‡]completely abolishes its catalytic properties. DBU is generally used as a hindered base or a proton scavenger.[26] In a recent study it was shown that DBU could also play a pivotal role in nucleophilic catalysis during esterification of carboxylic acids.[27] However, to the best of our knowledge, this is the first suggestion that DBU can act as a bifunctional catalyst. This type of catalysis has attracted great attention in recent years[28] mostly due to its ability to promote high stereoselectivity.[29]

The formation of a different type of product in the reaction of water with oxathiaphospholane **1** can be rationalized by an assumption that due to the additional negative charge located at the oxygen atom connected to phosphorus, the elimination of episulfide from intermediate **8** is relatively slow, and the competitive reaction of the thiolate anion with another molecule of **1**, leading to 'dimeric' product **5**, may occur.

The reactivity of various nucleophilic reagents towards oxathiaphospholanes **1** and **2** as shown above suggests the possibility of application of this methodology to the synthesis of other important classes of nucleoside derivatives, like di and triphosphates, and other classes of phosphorylated biomolecules like peptides, sugars, inositols and others. Such studies are now in progress in this Laboratory.

[‡] DBU *p*-toluenesulfonate was used in these studies.

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