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The Case for the Intermediacy of Monomeric Metaphosphate Analogues during Oxidation of *H*-Phosphonothioate, *H*-Phosphonodithioate, and *H*-Phosphonoselenoate Monoesters: Mechanistic and Synthetic Studies

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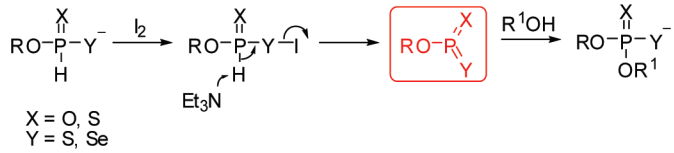


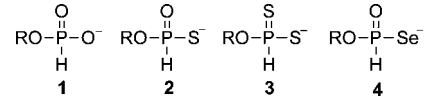
Studies on the reaction of *H*-phosphonothioate, *H*-phosphonodithioate, and *H*-phosphonoselenoate monoesters with iodine in the presence of a base led to identification of a unique oxidation pathway, which consists of the initial oxidation of the sulfur or selenium atom in these compounds, followed by oxidative elimination of hydrogen iodide to generate the corresponding metaphosphate analogues. The intermediacy of the latter species during oxidation of the investigated *H*-phosphonate monoester derivatives with iodine was supported by various diagnostic experiments. The scope and limitation of these oxidative transformations for the purpose of the synthesis of nucleoside phosphorothioate, nucleoside phosphorodithioate, and nucleoside phosphoroselenoate diesters was also investigated.

Introduction

Oxidative transformation of P(III) compounds, e.g., tervalent phosphite triesters ($\lambda^3\sigma^3$),[1] or four-coordinated H-phosphonate derivatives ($\lambda^5\sigma^4$),[2,3] is a powerful synthetic method for the preparation of biologically important phosphorus compounds and their analogues, bearing single or multiple modifications at the phosphorus center.[2,4,5] In contrast to phosphorous acid triesters (phosphites) and diesters (*H*-phosphonates), that undergo oxidation under mild conditions, the corresponding monoesters (*H*-phosphonate monoesters) are more resistant to oxidation[6] and usually require prior conversion into electrically neutral derivatives (e.g., silyl ethers) to react with electrophiles (oxidants).[2,5]

However, during studies on nucleoside *H*-phosphonate monoester analogues it was observed that replacement of one of the nonbridging oxygen atoms in *H*-phosphonate monoesters **1** (Chart 1) by sulfur to form *H*-phosphonothioate monoesters **2** made such derivatives highly susceptible to oxidation by iodine.[7,8] Since other *H*-phosphonate monoester analogues, namely *H*-phosphonodithioate **3** and *H*-phosphonoselenoate **4** monoesters, were found to also undergo rapid oxidation with iodine, we decided to investigate this phenomenon in more detail.

CHART 1



The observation that *H*-phosphonate monoester analogues **2**−**4** undergo easy oxidation with iodine without prior neutral-

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(1) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1992**, *48*, 2223–2311. Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *49*, 6123–6194.
(2) Stawinski, J. In *Handbook of Organophosphorus Chemistry*; Engel, R., Ed.; Marcel Dekker: New York, 1992; pp 377−434.
(3) Stawinski, J.; Strömberg, R. *Trends Org. Chem.* **1993**, *4*, 31–67.
(4) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *49*, 10441–10488. Guga, P.; Okruszek, A.; Stec, W. J. In *New Aspects In Phosphorus Chemistry*; Majoral, J. P., Ed.; Springer: New York, 2002; Vol. 220, pp 169−200; Wozniak, L. A.; Okruszek, A. *Chem. Soc. Rev.* **2003**, *32*, 158–169.
(5) Stawinski, J.; Kraszewski, A. *Acc. Chem. Res.* **2002**, *35*, 952–960.
(6) Nylén, P. *Z. Anorg. Allg. Chem.* **1937**, *230*, 385–404.
(7) Brill, W. K.-D.; Yau, E. K.; Caruthers, M. H. *Tetrahedron Lett.* **1989**, *30*, 6621–6624.
(8) Bollmark, M.; Stawinski, J. *Tetrahedron Lett.* **1996**, *37*, 5739–5742.

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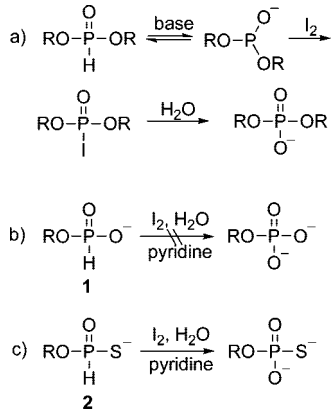
SCHEME 1

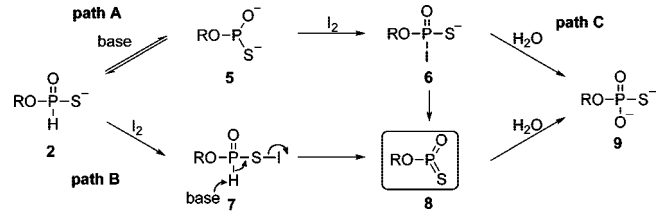


ization of negative charges was significant on three counts. First, it may suggest that, in contradistinction to *H*-phosphonate monoester **1**, for the thio and seleno analogues **2**−**4**, a new, low-energy pathway for the reaction with electrophiles exists. Second, since on theoretical grounds oxidation of **2**−**4** might occur with intermediacy of the corresponding metaphosphate analogues, it was tempting to experimentally verify this assumption. If true, this may provide a convenient way to generate otherwise difficultly accessible metathio-, metadithio-, and metaselenophosphates for mechanistic studies, under exceedingly mild reaction conditions. Third, the thio-, dithio-, and selenometaphosphate analogues were expected to be powerful phosphorylating species, and thus a direct oxidation of **2**−**4** with iodine could be developed into a one-pot preparative oxidative phosphorylation of alcohols, for the synthesis of phosphorothioate, phosphorodithioate, and phosphoroselenoate diesters.

For oxidation of *H*-phosphonate diesters, iodine is commonly used[9] and a mechanism of this reaction was studied in detail (Scheme 1a).[10] In the first step of this reaction, the phosphorus-bound hydrogen is abstracted by a base and the tervalent phosphite anion formed reacts with iodine to produce the corresponding phosphoroiodidate intermediate, which may undergo various S_N2(P)-type reactions. As was mentioned above, in the instance of *H*-phosphonate monoesters (**1**, Scheme 1b) the reaction with iodine does not occur, because abstraction of the proton from the P−H bond, which would lead to a phosphite dianion, is apparently much more difficult to perform due to the presence of negative charge. This problem is usually overcame by a transient conversion of *H*-phosphonate monoesters into electrically neutral species by silylation[2,5] prior to oxidation. In this context, it may come somewhat as a surprise that *H*-phosphonothioate monoesters (**2**, Scheme 1c) are readily oxidized by iodine, without invoking a presilylation step.

On mechanistic grounds, higher susceptibility of *H*-phosphonothioate monoesters **2** vs *H*-phosphonate monoesters **1** to oxidation with iodine could be due to the higher acidity of the P−H bond in the thio analogues **2** relative to their oxygen

SCHEME 2



counterparts **1**,[11] and thus oxidation of *H*-phosphonothioates **2** could proceed via path A as shown in Scheme 2. In this scenario, the initially formed phosphorothioite dianion **5** reacts with iodine to form phosphorothioiodidate **6** which, via elimination of a good leaving group (iodide), collapses to metathiophosphate **8**. Another possibility, although less likely, is that phosphorothioiodidate **6** might undergo direct bimolecular displacement of iodide by a nucleophile to form the final product of the reaction, the corresponding phosphorothioate monoester **9** (Scheme 2, path C).

However, one can also envisage another mechanism that avoids, probably unfavorable, formation of phosphite dianion **5**. Since P(V) compounds with the P−S−X functions (X = Br, Cl)[12] are known, and intermediacy of species containing the P−S−I bond system is assumed in various iodine-promoted desulfurizations of phosphorothioate diesters,[13,14] a mechanism involving initial oxidation of sulfur, to form an intermediate of type **7** containing the S−I bond, appears as a viable alternative (Scheme 2, path B). Since the P−H bond in **7** is likely to be significantly more acidic than that in the starting *H*-phosphonothioate **2**, elimination of hydrogen iodide should easily occur to produce the same reactive intermediate as that formed via path A, metathiophosphate **8**.

In this paper we present our mechanistic and synthetic studies on oxidation of *H*-phosphonate monoester analogues of type **2**−**4** with iodine. Several different techniques, including competition experiments, stereochemical studies, and trapping experiments, were used to provide evidence for the intermediacy of the corresponding metathiophosphates, metadithiophosphates, and metaselenophosphates in these reactions. Also, additional experiments were carried out to elucidate the most probable way in which these intermediates are formed (path A vs path B in Scheme 2). Finally, a number of preparative examples of oxidative couplings of nucleoside *H*-phosphonothioate and its dithio and seleno analogues with hydroxylic compounds are also described.

Results and Discussion

Since the first reports on the possibility of a dissociative mechanism involvement in phosphate monoester hydrolysis,[15]

(9) Garegg, P. J.; Regberg, T.; Stawinski, J.; Strömberg, R. *J. Chem. Soc., Perkin Trans. I* **1987**, *126*, 1269–1273. Garegg, P. J.; Lindh, I.; Regberg, T.; Stawinski, J.; Strömberg, R.; Henrichson, C. *Tetrahedron Lett.* **1986**, *27*, 4051–4054. Garegg, P. J.; Lindh, I.; Regberg, T.; Stawinski, J.; Strömberg, R.; Henrichson, C. *Tetrahedron Lett.* **1986**, *27*, 4055–4058.

(10) Nylén, P. *Z. Anorg. Allg. Chem.* **1938**, *235*, 161–182. Lewis, E. S.; Spears, L. G. *J. Am. Chem. Soc.* **1985**, *107*, 3918–3921.

(11) Guthrie, J. P. *Can. J. Chem.* **1979**, *57*, 236–239. Petrov, E. S.; Terekhova, M. I.; Malakhova, I. G.; Tsvetkov, E. N.; Shatenshtein, A. I.; Kabachnik, M. I. *Zh. Obsh. Khim.* **1979**, *49*, 2410–2414. Matveeva, A. G.; Terekhova, M. I.; Aladzheva, I. M.; Odinets, I. L.; Petrov, E. S.; Mastryukova, T. A.; Kabachnik, M. I. *Zh. Obsh. Khim.* **1993**, *63*, 607–610.

(12) Michalski, J.; Skowronska, A.; Lopusinski, A. *Phosphorus Sulfur Silicon* **1991**, *58*, 61–88. Skowronska, A.; Dembinski, R.; Gwara, J.; Michalski, J. *Phosphorus Sulfur Relat. Elem.* **1988**, *39*, 119–122. Michalski, J.; Potrzebowski, M.; Lopusinski, A. *Angew. Chem., Int. Ed.* **1982**, *21*, 135–136. Lopusinski, A.; Potrzebowski, M. *Phosphorus Sulfur Relat. Elem.* **1987**, *32*, 55–64. Michalski, J.; Mikolajczak, J.; Skowronska, A. *J. Am. Chem. Soc.* **1978**, *100*, 5386–5390.

(13) Connolly, B. A.; Potter, B. V. L.; Eckstein, F.; Pingoud, A.; Grotjahn, L. *Biochemistry* **1984**, *23*, 3443–3453. Wyrzykiewicz, T. W.; Cole, D. L. *Nucleic Acids Res.* **1994**, *22*, 2667–2669. Lowe, G.; Tansley, G.; Cullis, P. M. *J. Chem. Soc., Chem. Commun.* **1982**, 595–598. Sammons, R. D.; Frey, P. A. *J. Biol. Chem.* **1982**, *257*, 1138–1141. Cummins, J. H.; Potter, B. V. L. *J. Chem. Soc., Chem. Commun.* **1985**, 800–802.

(14) Bollmark, M.; Stawinski, J. *Chem. Commun.* **1997**, 991–992.

(15) Butcher, W. W.; Westheimer, F. H. *J. Am. Chem. Soc.* **1955**, *77*, 2420–2424. Barnard, D. W. C.; Bunton, C. A.; Llewellyn, D. R.; Oldham, K. G.; Silver, B. L.; Vernon, C. A. *Chem. Ind. (London, U.K.)* **1955**, 760–763.

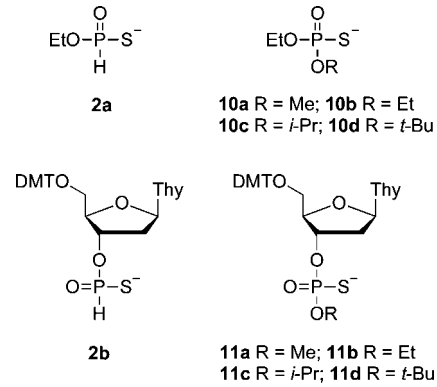
the intermediacy of a monomeric metaphosphate has been implicated in numerous chemical- and enzyme-catalyzed reactions.[16–18] However, despite intense synthetic[16–19] and theoretical[20] studies, the monomeric metaphosphate (a metaphosphate anion or its monoester) remained an elusive intermediate.[21] The dissociative nature of the transition state for the phosphoryl group transfer reactions has been supported by product analysis, kinetic studies, thermodynamic activation parameters, heavy-atom kinetic isotope effects, structure−reactivity correlation, and stereochemical studies.[22,23] At present the consensus probably exists that in protic media monomeric metaphosphate most likely is not formed as a diffusible species (stereochemical studies);[24] however, in nonprotic solvents and under conditions of poor solvation, monomeric metaphosphate can be formed as a kinetically significant entity.[25] Apart from the parent compounds, some of the metaphosphate analogues, e.g., metathio-,[26,27] metadithio-,[23,28] metaamidothiophosphates,[29,30] metaphosphonates,[31] and others,[18] have also been investigated, but to lesser extent.

There have been at least two major driving forces for the continuing interest in monomeric metaphosphate chemistry. As a low-coordination P(V) phosphorus species ($\lambda^5\sigma^3$), monomeric metaphosphate is interesting on theoretical grounds and its postulated intermediacy in many reactions kindled the hope of its isolation and characterization. On the synthetic part, high electrophilicity at the phosphorus center of the monomeric metaphosphate anion and its monoesters stimulated research directed toward development of new phosphorylating agents. Since the reactivity of metaphosphate intermediates can be modulated by Lewis bases,[8,32,33] these species (as well as their analogues) might be convenient phosphate transferring reagents, provided that they could be efficiently generated under mild conditions from easily accessible precursors.

Bellow, we address some mechanistic and synthetic issues of the reactions of *H*-phosphonate monoester analogues **2**−**4** which most likely involve intermediacy of the metathio-, metadithio-, and metaselenophosphates.

Probing the Intermediacy of Metathiophosphate 8 during Oxidation of *H*-Phosphonothioates. To probe the formation of metathiophosphate during oxidation of *H*-phosphonothioate monoesters of type **2** with iodine we have chosen ethyl *H*-phosphonothioate[34] (Chart 2, **2a**, triethylammonium salt) as a model compound. First, the reaction of **2a** with iodine in the presence of different alcohols was examined. When iodine (1.1 equiv) in THF was added to a solution of **2a** containing 10 equiv of an alcohol (MeOH, EtOH, *i*-PrOH, or *t*-BuOH) and Et_3N (3 equiv) in the same solvent, the brown iodine coloration immediately vanished. The ^{31}P NMR spectra of the reaction mixtures showed complete disappearance of the starting material **2a** (55.0 ppm, dt, ^1J = 580 Hz, ^3J = 9.6 Hz) and a clean formation of the corresponding phosphorothioate diesters (Chart 2, **10a**−**d**). Chemical identity of the products formed was additionally confirmed by ESI mass spectrometry (negative mode).[35]

CHART 2



Since it is known that sterically hindered tertiary alcohols cannot act as nucleophiles in a bimolecular $S_N2(P)$ substitution, phosphorylation of *t*-BuOH is usually considered a test for the involvement of three-coordinate metaphosphate species ($\lambda^5\sigma^3$) in the reaction.[36] In light of this, the above experiments indicate that during the oxidation of **2a** with iodine a reaction pathway involving formation of ethyl metathiophosphate (structure of type **8**, Scheme 2) may indeed exist. Although this seems to be the most likely pathway for the formation of the *tert*-butyl ester (**10d**), for 1° and 2° alcohols, the involvement of $S_N2(P)$ reaction (path C, Scheme 2) cannot be ruled out. Since the reactions studied were too fast to be followed by ^{31}P NMR spectroscopy,[37] we addressed this issue by carrying out competition experiments, in which mixtures of primary and secondary alcohols with *t*-BuOH were used.[38] If *t*-BuOH and, for instance, MeOH both

(16) Westheimer, F. H. *Chem. Rev.* **1981**, *81*, 313–326.
(17) Quin, L. D. *Coord. Chem. Rev.* **1994**, *137*, 525–559.
(18) Romanenko, V. D.; Sanchez, M. *Coord. Chem. Rev.* **1997**, *158*, 275–324.
(19) Banks, M. R.; Gosney, I.; Kilgour, D.; Cadogan, J. I. G.; Hodgson, P. K. G. *Heteroatom Chem.* **1996**, *7*, 503–514.
(20) Florian, J.; Warshel, A. *J. Phys. Chem. B* **1998**, *102*, 719–734. Hu, C. H.; Brinck, T. *J. Phys. Chem. A* **1999**, *103*, 5379–5386. SaintMartin, H.; Vicent, L. E. *J. Phys. Chem. A* **1999**, *103*, 6862–6872. Bianciotto, M.; Barthelat, J.-C.; Vigroux, A. *J. Am. Chem. Soc.* **2002**, *124*, 7573–7587.
(21) Hengge, A. C.; Onyido, I. *Curr. Org. Chem.* **2005**, *9*, 61–74.
(22) Kirby, A. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1965**, *87*, 3209–3216. Herschlag, D.; Jencks, W. P. *J. Am. Chem. Soc.* **1986**, *108*, 7938–7946.
(23) Bourne, N.; Williams, A. *J. Am. Chem. Soc.* **1984**, *106*, 7591–7596.
(24) Buchwald, S. L.; Pliura, D. H.; Knowles, J. R. *J. Am. Chem. Soc.* **1984**, *106*, 4916–4922. Blättler, W. A.; Knowles, J. R. *J. Am. Chem. Soc.* **1979**, *101*, 510–511. Admiraal, S. J.; Herschlag, D. *J. Am. Chem. Soc.* **2000**, *122*, 2145–21.
(25) Lowe, G.; Sproat, B. S. *J. Chem. Soc., Chem. Commun.* **1978**, 783–785. Cullis, P. M.; Iagrossi, A.; Rous, A. J. *J. Am. Chem. Soc.* **1986**, *108*, 7869–7870.
(26) Cullis, P. M.; Misra, R.; Wilkins, D. J. *J. Chem. Soc., Chem. Commun.* **1987**, 1594–1596.
(27) Quin, L. D.; Hermann, P.; Jankowski, S. *J. Org. Chem.* **1996**, *61*, 3944–3950.
(28) Yoshifuji, M.; An, D. L.; Toyota, K.; Yasunami, M. *Tetrahedron Lett.* **1994**, *35*, 4379–4382.
(29) Harger, M. J. P.; Pater, C. *Chem. Commun.* **1999**, 931–932.
(30) Harger, M. P. J.; Jones, D. K. *Chem. Commun.* **1999**, 339–340.
(31) Nonas, T.; Jankowski, S. *J. Org. Chem.* **2005**, *70*, 562–566.
(32) Ramirez, F.; Marecek, J. F. *Tetrahedron* **1979**, *35*, 1581–1589. Quin, L. D.; Bourdieu, C.; Quin, G. S. *Tetrahedron Lett.* **1990**, *31*, 6473–6476.
(33) Knorre, D. G.; Lebedev, A. V.; Levina, A. S.; Rezvukhin, A. I.; Zarytova, V. F. *Tetrahedron* **1974**, *30*, 3073–3079.
(34) Michalski, J.; Tulimowski, Z.; Wolf, R. *Chem. Ber.* **1965**, *98*, 3006–3009.
(35) Isolation not attempted. ^{31}P NMR (THF) and MS data: **10a**, δ_P 59.7 ppm, m, HRMS *m/z* 154.9930 ([M−H]$^-$, $C_3H_8O_3PS^-$ calcd 154.9937); **10b**, δ_P 58.2 ppm, qu, ^3J = 8.2 Hz, HRMS *m/z* 169.0100 ([M−H]$^-$, $C_4H_{10}O_3PS^-$ calcd 169.0094); **10c**, δ_P 55.9 ppm, q, ^3J = 8.6 Hz, HRMS *m/z* 183.0270 ([M−H]$^-$, $C_5H_{12}O_3PS^-$ calcd 183.0250); **10d**, δ_P 49.8 ppm, t, ^3J = 8.0 Hz, HRMS *m/z* 197.0401 ([M−H]$^-$, $C_6H_{14}O_3PS^-$ calcd 197.0407).
(36) Ramirez, F.; Marecek, J. F.; Yemul, S. S. *J. Am. Chem. Soc.* **1982**, *104*, 1345–1349.
(37) The most reliable method to check whether more reactive alcohols also choose to react via metaphosphate or not are kinetic studies. Since an alcohol molecule is not involved in the formation of metathiophosphate **8**, neither in path A nor in path B (Scheme 2), the rates of these reactions were expected to be independent of the alcohol concentration. On the other hand, if the bimolecular displacement occurs (Scheme 2, path C) the overall reaction rate should display first-order dependence on the alcohol concentration. See, e.g., ref 31.
(38) Jankowski, S.; Quin, L. D. *J. Am. Chem. Soc.* **1991**, *113*, 7011–7017.

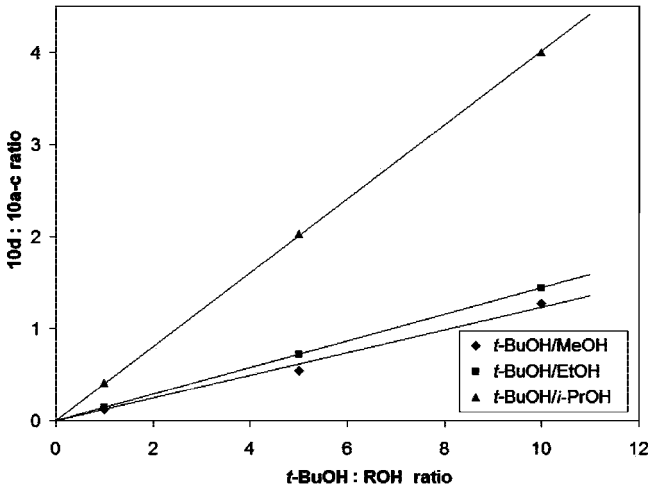


FIGURE 1. Plot of the ratio of phosphorothioate diesters **10d**:**10a**−**c** against the *t*-BuOH/ROH concentration. The ordinate values were obtained from integration of ^{31}P NMR signals originating from the products.

react *only* with the intermediacy of metathiophosphates of type **8**, the relationship between the alcohol composition taken to the reaction and the ratios of the corresponding products formed (**10d** vs **10a**) should be linear. In other words, the selectivity of the reaction in such a case should be independent of the ratio of the alcohols used. If, however, the more reactive alcohol (e.g., MeOH) could form the corresponding phosphorothioate diester also via the $S_N2(P)$ process (path C in Scheme 2), this should be manifested in a nonlinear plot of the products ratio vs alcohols ratio.

The competition experiments were carried out with mixtures of neat alcohols as reaction media, to maximize the probability of the bimolecular substitution (path C, Scheme 2).[27,29,39,40] Negative results of these experiments would then also exclude the possibility of participation of a $S_N2(P)$ pathway in dilute reaction solutions. To this end MeOH, EtOH, and *i*-PrOH were mixed with *t*-BuOH in three different ratios and these mixtures were used as reaction media for oxidative couplings with *H*-phosphonothioate monoester **2a**. The results in Figure 1 clearly show that in all three cases a linear relationship was obeyed. This indicates that more nucleophilic alcohols also reacted via the elimination−addition pathway and the contribution of a direct $S_N2(P)$ reaction was apparently negligible even for MeOH. The inverse of the slopes in Figure 1 represents relative selectivity for a given alcohol vs *t*-BuOH, and the values obtained for the reactions investigated were 8.1, 6.9, and 2.5 for MeOH, EtOH, and *i*-PrOH, respectively. Such small numbers point to small steric effects and are in line with little (if any) involvement of sterically sensitive $S_N2(P)$ processes.[31,41]

To support further the intermediacy of metathiophosphate **8** during oxidation of *H*-phosphonothioates of type **2** with iodine, we carried out some stereochemical studies.

By performing these oxidative phosphorylations with an enantiomerically pure *H*-phosphonothioate monoester, it should be possible to distinguish between bimolecular and unimolecular mechanisms by examining the stereochemistry of the product

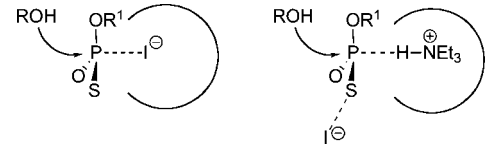


FIGURE 2. Possible explanation for a partial stereospecificity observed during oxidative phosphorylations in neat alcohols.

formed. If a planar metaphosphate structure was involved, racemization of a chiral starting material was expected, while a bimolecular substitution should lead to inversion of configuration at the phosphorus center.[16]

Although separation of ethyl *H*-phosphonothioate **2a** into enantiomers should be a feasible task,[29] we decided to use for our stereochemical studies another model compound, namely nucleoside *H*-phosphonothioate **2b** (Chart 2).[42] Since **2b** contains a chiral D-deoxyribose moiety, the ^{31}P NMR signals originating from its stereoisomers possessing R_P and S_P configurations have distinct chemical shifts and the same is valid for the corresponding phosphorothioate diesters **11a**−**d** (Chart 2). This should enable very convenient analysis of stereochemical outcomes of oxidative phosphorylations of alcohols with *H*-phosphonothioate **2b** with use of ^{31}P NMR spectroscopy.

When pure R_P and S_P diastereoisomers of nucleoside *H*-phosphonothioate **2b** were reacted separately with iodine (1.1 equiv), in presence of Et_3N (3 equiv), in *t*-BuOH as a solvent, the reactions showed only negligible stereospecificity for the formation of phosphorothioate **11d** (ca. 10% de). Analogous experiments carried out in neat MeOH proceeded also with extensive epimerization, but with noticeably higher stereospecificity (**11a**, ca. 25% de).[43] The significant erosion of stereochemistry that accompanied these reactions suggested metathiophosphate of type **8** as a major intermediate during oxidation of **2b** with iodine.[44] However, the observed partial stereospecificity also deserves some comments.

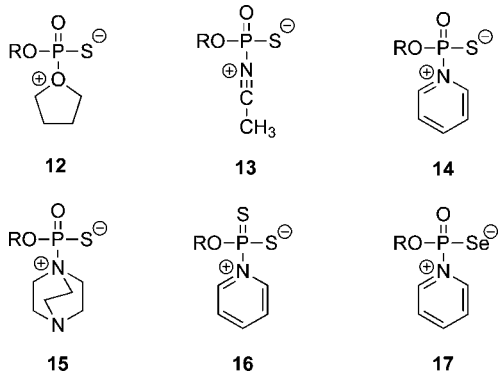
Although a contribution of the $S_N2(P)$ process (path C, Scheme 2) could account for certain de of the product with inverted configuration (at least in the instance of the reaction with MeOH), a bimolecular substitution at the phosphorus center for *t*-BuOH seemed unlikely. A more likely explanation for the residual stereospecificity observed could be that in the presence of a high concentration of nucleophiles (neat alcohols), metathiophosphate intermediates of type **8** were not formed as completely free, diffusible species, but were attacked by nucleophiles also in a solvent cage, on the face opposite to that still shielded by iodide (path A, Scheme 2) or by the triethylammonium cation (path B, Scheme 2) (Figure 2). This interpretation is consistent with the fact that a certain degree of stereospecificity was observed for the reaction in neat *t*-BuOH, and is also in line with the results of the competition experiments that, to high probability, ruled out a direct nucleophilic displacement at the phosphorus center. Since **2b**-R_P and **2b**-S_P are diastereomers, one cannot completely exclude the possibility of a certain amount of stereochemical influence from the chiral nucleoside moiety; however, this phenomenon is usually

(39) Quin, L. D.; Jankowski, S. *J. Org. Chem.* **1994**, *59*, 4402–4409.
(40) Jankowski, S.; Quin, L. D.; Paneth, P.; O'Leary, M. H. *J. Am. Chem. Soc.* **1994**, *116*, 11675–11677. Jankowski, S.; Quin, L. D.; Paneth, P.; O'Leary, M. H. *J. Organomet. Chem.* **1997**, *529*, 23–27.
(41) Harger, M. J. P. *Chem. Commun.* **2004**, *17*, 1952–1953.
(42) Hiresova, R.; Putilova, L.; Kullberg, M.; Stawinski, J. *Collect. Symp. Ser.* **2005**, *7*, 201–205.
(43) ^{31}P NMR data: **11a**, δ_P 58.8, 58.7 (neat MeOH), 59.6, 59.4 (THF), 59.0, 58.8 ppm (MeCN); **11d**, δ_P 51.0, 50.9 (neat *t*-BuOH), 50.7, 50.5 (THF), 51.3, 51.2 ppm (MeCN).
(44) Friedman, J. M.; Freeman, S.; Knowles, J. R. *J. Am. Chem. Soc.* **1988**, *110*, 1268–1275.

negligible for reactions at the phosphorus center for DNA derivatives.[45,46]

It has been postulated on several occasions that highly electrophilic metaphosphate and its analogues should form adducts with Lewis bases, including solvent molecules possessing lone electron pairs.[16,17,44,47,48] Formation of such species, in which fast exchange of the coordinated solvent molecules may occur prior to the reaction with a nucleophile, was advocated as an efficient mechanism for racemization in the reactions involving metaphosphate intermediates.[44] Consistent with these, when oxidative phosphorylation of MeOH or *t*-BuOH (10 equiv) with pure diastereoisomers of nucleoside *H*-phosphonothioate **2b** was carried out in THF or MeCN as solvents, 100% epimerization at the phosphorus center was observed.[43] Structures of such putative adducts of metathiophosphate of type **8** with THF and MeCN are shown in Chart 3 (**12** and **13**, respectively).

CHART 3



Although the above results indicated the formation of structures of type **12** and **13** during the course of the reactions, we were not able to observe these intermediates by ^{31}P NMR spectroscopy, even at −80 °C. In the absence of alcohols, the addition of iodine to a solution of ethyl *H*-phosphonothioate **2a** in THF or MeCN resulted in an intractable mixture of products (^{31}P NMR experiments) suggesting polymerization of the metathiophosphate intermediate.

However, since metaphosphates are known to form stable adducts with tertiary amines,[8,16,17,33,49,50] we attempted to generate analogous species from the putative metathiophosphate **8** and pyridine or 1,4-diazabicyclo[2.2.2]octane (DABCO) (Chart 3, compounds **14** and **15**). To this end, a solution of iodine (1.1 equiv) in pyridine was added to ethyl *H*-phosphonothioate **2a** in the same solvent, at room temperature. The ^{31}P NMR spectrum of the reaction mixture showed an immediate disappearance of the starting material **2a**, and a clean formation of a product resonating at 56.0 ppm, which on the basis of its chemical shift was assigned to the anticipated ethyl metathiophosphate−pyridine adduct **14** (R = Et).[8] To verify this structural assignment, the reaction mixture was analyzed by ESI mass spectrometry, which indeed, among other signals, revealed the expected mass peak of this adduct (HRMS: *m/z* 283.0657; $[M + pyridineH]^+$, $C_{12}H_{16}N_2O_2PS^+$ calcd 283.0665). Compound **14** (R = Et) appeared to be rather resistant toward alcohols, and at least 10 equiv of MeOH, EtOH, or *i*-PrOH had to be used to cleanly transform it into the phosphorothioate diesters **10a**−**c**. The reaction with *t*-BuOH was sluggish and resulted in significant side-product formation. As was reported previously, the pyridine adduct of metathiophosphate **14** reacted efficiently only with good nucleophiles, e.g., fluoride ions[8] or amines.[51]

Ethyl metathiophosphate−DABCO adduct **15** (R = Et) was generated analogously to that of **14**, by reacting *H*-phosphonothioate **2a** with iodine (1.1 equiv) in THF in the presence of DABCO (10 equiv). Adduct **15** (R = Et) was cleanly formed under these conditions, as indicated by the ^{31}P NMR spectra and MS analysis (δ_P = 65.8 ppm; HRMS *m/z* 349.1813 ($[M + DABCOH]^+$, $C_{14}H_{30}N_4O_2PS^+$ calcd 349.1822). Compound **15** (R = Et) was rather stable in the reaction mixture; however, upon treatment with an excess of alcohols (10 equiv), it was readily transformed into the corresponding phosphorothioate diesters (**10a**−**d**).

To sum up this part, the chemical and stereochemical experiments discussed above provided strong support for the intermediacy of monomeric metathiophosphate **8** in the investigated oxidative phosphorylations, and thus the reaction of *H*-phosphonothioate monoesters with iodine may constitute a novel, efficient method for generation of this electrophilic phosphorus species.

Toward the Mechanism of Metathio-, Metadithio-, and Metaselenophosphate Formation. Althoug, the studies described so far provided strong support for the intermediacy of metathiophosphate in the discussed reactions, they did not address the question on which pathways these species were formed. We considered two plausible pathways, path A and path B, that may lead to metathiophosphate **8** (Scheme 2) and in this context two issues arise: (i) why is a particular mechanism followed in preference to the other mechanisms and (ii) what is the nature of the transition from one mechanism to another as reactants or conditions are changed. In our case, the answer to these questions should clarify the issue, why *H*-phosphonate monoesters of type **1** are resistant to oxidation with iodine, while the other chalcogen analogues (compounds of type **2**−**4**) undergo rapid oxidation under such conditions.

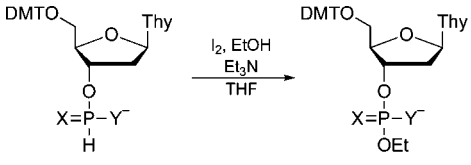
As was mentioned in the Introduction, the affinity of iodine to sulfur is a known phenomenon, which originates from the softness of both atoms.[13,14] This should favor a mechanism involving initial iodination of the sulfur in compound **2** (path B, Scheme 2). On the other hand, increased acidity of the P−H bond in *H*-phosphonothioates, compared to their oxygen counterparts,[11] may indicate a mechanistic route that commences with the generation of phosphite dianion **5**, followed by its reaction with iodine at the phosphorus center (path A, Scheme 2).

(45) Almer, H.; Stawinski, J.; Strömberg, R. *J. Chem. Soc., Chem. Commun.* **1994**, 1459–1460.
(46) Stawinski, J.; Thelin, M. *J. Org. Chem.* **1994**, *59*, 130–136.
(47) Chanley, J. D.; Feageson, E. *J. Am. Chem. Soc.* **1958**, *80*, 2686–2691.
(48) Quin, L. D.; Bourdieu, C.; Quin, G. S. *Tetrahedron Lett.* **1990**, *31*, 6473–6476.
(49) Ramirez, F.; Marecek, J. F. *Tetrahedron* **1979**, *35*, 1581–1589.
(50) Meisel, M.; Grunze, H. *Z. Anorg. Allg. Chem.* **1968**, *360*, 277–283.
(51) Kers, I.; Stawinski, J.; Kraszewski, A. *Tetrahedron Lett.* **1998**, *39*, 1219–1222.
(52) Heath, D. F. *J. Chem. Soc.* **1956**, *379*, 3796–3804. Heath, D. F. *J. Chem. Soc.* **1956**, 3804–3809. Cruden, E. W.; Hudson, R. F. *J. Chem. Soc.* **1962**, 3591–3599. Gerrard, A. F.; Hamer, N. K. *J. Chem. Soc. B* **1967**, 1122–1125. Gerrard, A. F.; Hamer, N. K. *J. Chem. Soc. B* **1968**, 539–543. Maynard, J. A.; Swan, J. M. *Proc. Chem. Soc. London* **1963**, 61–62. Maynard, J. A.; Swan, J. M. *Aust. J. Chem.* **1963**, *16*, 596–608. Kenyon, G. L.; Westheimer, F. H. *J. Am. Chem. Soc.* **1966**, *88*, 3557–3561. Kenyon, G. L.; Westheimer, F. H. *J. Am. Chem. Soc.* **1966**, *88*, 3561–3566. Katzhendler, J.; Karaman, R.; Gibson, D.; Breuer, E.; Leader, H. *J. Chem. Soc., Perkin Trans. 2* **1989**, *2*, 589–594. Banks, M. R.; Cadogan, J. I. G.; Gosney, I.; Hodgson, P. K. G.; Kilgour, D.; Rodger, D. R. *Phosphorus Sulfur Silicon* **1993**, *76*, 37–40. Kehler, J.; Breuer, E. *Chem. Commun.* **1997**, 1751–1752.

SCHEME 3

X = halide, protonated oxygen or nitrogen, carbon
Y, Z = O, S, NR.

SCHEME 4



1a X = O, Y = O
2b X = O, Y = S
3a X = S, Y = S
4a X = O, Y = Se

no reaction for X, Y = O,
11b X = O, Y = S
18 X = S, Y = S
19 X = O, Y = Se

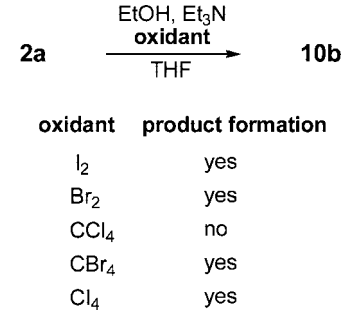
DMT = 4,4'-Dimethoxytrityl; Thy = thymidin-1-yl.

An intriguing feature of path B is that it would constitute a new mechanistic pathway in which metaphosphate analogues could be formed. Usually metaphosphates and their analogues are generated from P(V) derivatives bearing a good leaving group and an internal nucleophile as is shown in Scheme 3.[16,17,27,39,49,52] For cyclic phosphorus compounds, Z and X are part of a ring system, and fragmentation to metaphosphates occurs under pyrolytic conditions.[38,53] Other methods of metaphosphate generation, such as fragmentations of phosphoryl radicals,[54] mixed phosphoric−carboxylic anhydrides[55] or 2,4-bis(4-methoxyphenyl)-2,4-dithioxo-1,3,2,4-dithiadiphosphetane (Lawesson reagent),[38] also have been developed; however, their applicability is limited to specific metaphosphate analogues.

In contradistinction to all these methods, generation of metathiophosphate **8** via path B (Scheme 2) occurs in an oxidation step. Mechanistically, this means that iodine oxidizes first sulfur in *H*-phosphonothioate **2** to form iodosulfenyl derivative **7**, and this P(III) intermediate undergoes internal disproportionation that leads to oxidation of the phosphorus atom and generation of metathiophosphate **8**.

Below we present experiments which, collectively, may lend support for this new mechanism for the generation of metathio-, metadithio-, and metaselenophosphate.

To have uniform experimental data concerning the susceptibility of *H*-phosphonate monoesters of type **1**, and their thio (**2**), dithio (**3**), and seleno (**4**) analogues (Chart 1) to oxidation with iodine, we investigated the reactions of *H*-phosphonate (**1a**),[56] *H*-phosphonothioate (**2b**), *H*-phosphonodithioate (**3a**),[7] and *H*-phosphonoselenoate (**4a**)[57] with EtOH (10 equiv), in the presence of iodine (1.1 equiv) and Et_3N (3 equiv), in THF at room temperature (Scheme 4). For all *H*-phosphonate derivates but **1a**, a rapid (<15 s) and clean transformation into the corresponding phosphodiester analogues (**11b**, **18**, **19**; Scheme 4) was observed (^{31}P NMR experiments)[58] under these reaction conditions. Since *H*-phosphonate **1a** remained unchanged even after prolonged reaction time (1 h), it seems that at least one chalcogen atom, possessing high affinity to a soft electrophile (iodine), i.e., sufur[13,14] or selenium,[14,46] must be attached to the phosphorus center to permit easy oxidation into P(V) compounds.



oxidant	product formation
I_2	yes
Br_2	yes
CCl_4	no
CBr_4	yes
Cl_4	yes

FIGURE 3. Reaction of ethyl *H*-phosphonothioate (**2a**) with EtOH, promoted by different oxidants.

To check if the reactions of *H*-phosphonothioate **2b**, *H*-phosphonodithioate **3a**, and *H*-phosphonoselenoate **4a** with iodine proceeded via the same type of intermediate, i.e., the corresponding metathio-, metadithio-, and metaselenophosphate, in separate experiments we attempted to trap these species in the form of the corresponding pyridine adducts. Indeed, upon addition of iodine into solutions of **2b**, **3a**, and **4a** in pyridine, ^{31}P NMR spectroscopy revealed formation of the expected metaphosphate adducts of type **14** (R = 5′-*O*-dimethoxytritylthymidin-3′-yl, δ_P = 58.7 ppm, t, ^3J = 8.9 Hz), **16** (R = 5′-*O*-dimethoxytritylthymidin-3′-yl, δ_P = 118.5 ppm, t, ^3J = 9.7 Hz), and **17** (R = 5′-*O*-dimethoxytritylthymidin-3′-yl, δ_P = 53.5 ppm, t, ^3J = 9.6 Hz), respectively (Chart 3).

Although these experiments supported, in principle, path B (oxidation of the chalcogen atoms with iodine as an initial reaction step), we could not rule out path A, since the presence of sulfur or selenium at the phosphorus center usually increases the acidity of the P−H protons (vide supra).

To evaluate the importance of the affinity of iodine to sulfur and selenium in these reactions, we turned our attention to other oxidants, the electrophilic centers of which represented a spectrum in terms of hardness and softness (Figure 3). Thus, in addition to bromine, different carbon tetrahalides, which are known as efficient oxidizing agents used in the Atherton−Todd reaction, were tested.[59]

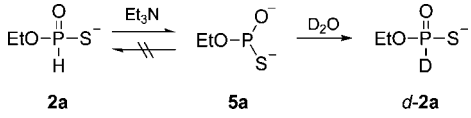
As a model compound, ethyl *H*-phosphonothioate monoester **2a** was chosen, and it was allowed to react with EtOH (10 equiv) in the presence of Et_3N (3 equiv), and the appropriate oxidant (1.1 equiv of X_2 or 3 equiv of CX_4) in THF, at room temperature (Figure 3). Progress of the reactions was followed by ^{31}P NMR spectroscopy.

As is apparent from data in Figure 3, all the oxidants investigated, except CCl_4, successfully promoted oxidative thiophosphorylation of ethanol. CCl_4 is known as a donor of hard, electrophilic chlorine, which can efficiently oxidize *H*-phosphonate diesters.[60] Additionally, it was also reported that

(53) Quin, L. D.; Sadanani, N. D.; Wu, X. P. *J. Am. Chem. Soc.* **1989**, *111*, 6852–6853.
(54) Avila, L. Z.; Forst, J. W. *J. Am. Chem. Soc.* **1988**, *110*, 7904–7906.
(55) Symes, J.; Modro, T. A. *Can. J. Chem.* **1986**, *64*, 1702–1708.
(56) Jankowska, J.; Sobkowski, M.; Stawinski, J.; Kraszewski, A. *Tetrahedron Lett.* **1994**, *35*, 3355–3358.
(57) Bollmark, M.; Kullberg, M.; Stawinski, J. *Tetrahedron Lett.* **2002**, *43*, 515–518. Kullberg, M.; Stawinski, J. *Synthesis* **2005**, 1668–1674.

(58) Isolation not attempted. ^{31}P NMR (THF) and MS data: **11b**, δ_P 57.3, 57.0 ppm, HRMS *m/z* 667.1913 ([M−H]$^-$, $C_{33}H_{36}N_2O_9PS^-$ calcd 667.1885); **18**, δ_P 113.5 ppm, HRMS *m/z* 683.1623 ([M−H]$^-$, $C_{33}H_{36}N_2O_8PS_2^-$ calcd 683.1656); **19**, δ_P 52.4, 52.2 ppm, HRMS *m/z* 715.1278 ([M−H]$^-$, $C_{33}H_{36}N_2O_9PSe^-$ calcd 715.1329).
(59) Atherton, R. F.; Todd, A. R. *J. Chem. Soc.* **1947**, 674–678.
(60) Steinberg, G. M. *J. Org. Chem.* **1950**, *15*, 637–647.

SCHEME 5



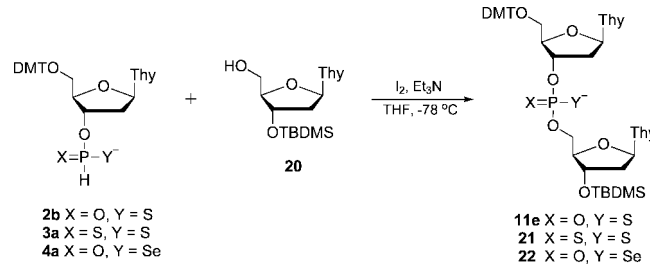
CCl_4 is able to oxidize *H*-phosphonate monoesters of type **1**, provided that the monoalkyl phosphite dianion could be generated (reflux in neat Et_3N).[61] Thus, lack of any reaction of *H*-phosphonothioate **2a** with this oxidant, and a rapid oxidation of **2a** with all soft halogen donors (I_2, Br_2, CBr_4 and CI_4), indicates that sulfur−halogen interaction is probably crucial for the reaction to proceed.

To have some insight into the acidity of the P−H bond of *H*-phosphonothioate monoester **2a**, a simple proton−deuterium exchange experiment in neat D_2O, catalyzed by Et_3N, was carried out (Scheme 5). To this end **2a** and Et_3N (3 equiv) were dissolved D_2O and the proton−deuterium exchange process was followed with ^{31}P NMR spectroscopy.[62] It was expected that in neat D_2O, every ionization of **2a** to form dianion **5a** will result in the incorporation of the deuterium. Since after 1 h only ca. 50% of the starting material **2a** became deuterated, this means that generation of phosphorothioite dianion **5a** was relatively slow. Taking into account that oxidation of *H*-phosphonothioate **2a** with iodine in THF takes a few seconds, it is rather unlikely that it may proceed via path A (Scheme 2), i.e., with the intermediacy of phosphite dianion **5**.

Development of Preparative Protocols for Oxidative Phosphorylations with *H*-Phosphonothioate, *H*-Phosphonodithioate, and *H*-Phosphonoselenoate Monoesters. Having established the mechanistic bases of the investigated reactions, we wanted to determine scope and limitations of these transformations for the purpose of practical, preparative thio-, dithio-, and selenophosphorylation. As was shown in the previous sections, a number of phosphorothioate diesters could be obtained from ethyl *H*-phosphonothioate **2a** (**10a**−**d**; Chart 2) or from nucleoside *H*-phosphonothioate **2b** (**11a**, **11b**, **11d**; Chart 2) via oxidative thiophosphorylation promoted by iodine. Also nucleosides *H*-phosphonodithioate (**3a**) and *H*-phosphonoselenoate (**4a**) were successfully used for oxidative phosphorylation of ethanol, producing the corresponding phosphorodithioate (**18**) and phosphoroselenoate (**19**) diesters (Scheme 4).

Due to the growing interest in nucleotide analogues bearing single or multiple modifications at the phosphorus center (e.g., phosphorothioates, phosphorodithioates, phosphoroselenoates, etc.) as tools in biochemistry and medicinal chemistry,[63] we decided to focus on optimization of the reaction conditions for the preparation of such nucleotide analogues via oxidative phosphorylation. Attractive features of nucleoside *H*-phosphonothioate monoesters of type **2**, *H*-phosphonodithioates **3**, and *H*-phosphonoselenoates **4** (Chart 1) as substrates for oxidative phosphorylation are that these compounds are readily available and, in contrast to *H*-phosphonate diester derivatives, can transfer unprotected thiophosphoryl, dithiophosphoryl, and selenophosphoryl groups to hydroxylic compounds (vide supra).

SCHEME 6



First, using nucleoside *H*-phosphonothioate monoester **2b** as a model compound, different solvents were screened for their efficiency as reaction media for the oxidative phosphorylations investigated. For this purpose, *H*-phosphonothioate **2b** was allowed to react with iodine (1.1 equiv), in the presence of MeOH (10 equiv) and Et_3N (3 equiv), in THF, dioxane, MeCN, pyridine, toluene, and CH_2Cl_2 as solvents. It was found that in the instance of THF, dioxane, MeCN, and pyridine, a clean and rapid formation of phosphorothioate diester **11a** (Chart 2) occurred (^{31}P NMR analysis). In contrast to these, when the reaction was carried out in toluene or CH_2Cl_2, the ^{31}P NMR spectra of the reaction mixtures showed a large number of signals (∼20) of different intensity, indicating polymerization of the generated metathiophosphate and/or its reaction with the thymine nucleobase.[16] On this basis we tentatively concluded that supporting solvents for oxidative phosphorylations in which metaphosphate-type of intermediates are involved must have a Lewis base character (e.g., THF, dioxane, MeCN, pyridine). These solvent are apparently not passive during the course of the reaction, but play an important stabilizing role for the generated metathiophosphate intermediates, forming the corresponding adducts (e.g., **12**, **13**, **14**; Chart 3). If such a stabilization is impossible, which is probably the case for solvents lacking lone electron pairs (e.g., toluene, CH_2Cl_2), the very reactive free metathiophosphate polymerizes before it "finds" a nucleophile.

Since so far all the experiments on oxidative phosphorylations were performed in the presence of 10 equiv or in neat alcohols, we investigated if it was possible to decrease the excess of an alcohol taken to the reaction. We found that in THF as a solvent, nucleoside *H*-phosphonothioate **2b** could be efficiently coupled when 3 equiv of 1° alcohols (MeOH and EtOH), 5 equiv of 2° alcohol (*i*-PrOH), or 10 equiv of 3° alcohol (*t*-BuOH) were used for the reaction, to produce cleanly the corresponding phosphorothioate diesters (**11a**−**d**). If the amount of a given alcohol was lower, it resulted in partial polymerization of the metathiophosphate and side product formation. Applying this procedure on a preparative scale enabled isolation of pure **11a**−**d** in 85−93% yield.

As a final stage of these synthetic studies we attempted to use this newly developed protocol for the preparation of compounds with modified 3′−5′ internucleotide linkages, i.e., dinucleoside phosphorothioate (**11e**), dinucleoside phosphorodithioate (**21**), and dinucleoside phosphoroselenoate (**22**), via oxidative phosphorylation of 3′-*O*-TBDMS-thymidine (**20**) (Scheme 6).

Somewhat surprisingly, when we added iodine (1.1 equiv) to a THF solution containing nucleoside *H*-phosphonothioate

(61) Blackburn, G. M.; Cohen, J. S.; Lord, T. *J. Chem. Soc. (C)* **1966**, 239–245.

(62) ^{31}P NMR data (D_2O): **2a**, δ_P = 50.4 ppm; *d*-**2a**, δ_P = 50.0 ppm, $^1J_{P\text{-}D}$ = 224 Hz.

(63) Eckstein, F. *Annu. Rev. Biochem.* **1985**, *54*, 367–402. Eckstein, F. *Angew. Chem., Int. Ed.* **2003**, *14*, 160–166. Nawrot, B.; Widera, K.; Wojcik, M.; Rebowska, B.; Nowak, G.; Stec, W. J. *FEBS J.* **2007**, *274*, 1062–1072. Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543–584. Kurreck, J. *Eur. J. Biochem.* **2003**, *270*, 1628–1644. Wilson, C.; Keefe, A. D. *Curr. Opin. Chem. Biol.* **2006**, *10*, 607–614. Wilds, C. J.; Pattanayek, R.; Pan, C. L.; Wawrzak, Z.; Egli, M. *J. Am. Chem. Soc.* **2002**, *124*, 14910–14916.

2b, 3′-protected thymidine derivative **20** (3 equiv),[64] and Et_3N (3 equiv), the expected dinucleoside phosphorothioate **11e** was not formed (TLC and ^{31}P NMR analysis). Instead, a familiar pattern of signals, originating from polymerization of the generated metathiophosphate intermediate, could be observed in the ^{31}P NMR spectra of the reaction mixture. Increasing the excess of nucleoside **20** up to 10 equiv resulted in no improvements.

A possible explanation why this reaction failed could be that effective nucleophilicity of nucleoside **20** was too low to secure a successful attack on a short-living metathiophosphate intermediate, even if this was partially stabilized by THF. A large size of the nucleoside molecule implies both low diffusion coefficient (slow diffusion) and long correlation time (slow rotation),[65] which together with a steric hindrance can lower the ability of **20** to capture the metathiophosphate intermediate.

To remedy this problem, we attempted to increase a lifetime of the metathiophosphate adduct by carrying out the reaction in pyridine, which forms much more stable adducts with metathiophosphates (Chart 3, **14**). Under such reaction conditions, dinucleoside phosphorothioate **11e** was indeed formed, but unfortunately, the reaction was slow (ca. 3 h) and resulted in significant side product formation. Using DABCO in THF as the reaction media (intermediate of type **15**; Chart 3) did not improve the outcome of the reaction. These experiments indicated that the reaction proceeding via metaphosphate-type intermediates requires fine-tuning of the reactivity of the metaphosphate adducts formed to achieve satisfactory results. Since metaphosphate adducts of type **14** and **15** were apparently not reactive enough toward nucleoside **20**, we investigated once again the THF adduct of type **12**, but to lower its reactivity,[48] this time we performed the oxidative thiophosphorylation of **20** at −78 °C. It was rewarding to find that under such reaction conditions dinucleoside phosphorothioate **11e** was rapidly formed (ca. 30 s), but for the reaction to proceed cleanly, it was necessary to use 10 equiv of nucleoside **20**. The same protocol was applied to nucleoside *H*-phosphonodithioate monoester **3a** and nucleoside *H*-phosphonoselenoate monoester **4a**, which upon the reaction with nucleoside **20** furnished cleanly dinucleoside phosphorodithioate **21** and dinucleoside phosphoroselenoate **22**, respectively, in good isolated yields.[66]

Conclusions

In summary, the mechanism of oxidation of *H*-phosphonothioate monoesters with iodine was examined in detail. The results of different diagnostic experiments, including reaction with tertiary alcohol, competition experiments, stereochemical studies, and trapping the intermediates in the form of observable adducts with amines, were consistent with the elimination−addition process and supported the notion that these reaction proceeded with the intermediacy of a metathiophosphate monoester. Additional studies have shown that the key step of the transformation was oxidation of the sulfur atom of the *H*-phosphonothioate monoester by iodine to produce a iodosulfenyl intermediate that collapsed to the corresponding metathiophosphate derivatives. A similar behavior of *H*-phosphonodithioate and *H*-phosphonoselenoate monoesters toward iodine pointed to the involvement of analogous species, i.e., the corresponding metadithiophosphate and metaselenophosphate in these reactions. Formation of metathiophosphates, metadithiophosphates, and metaselenophosphates via oxidation of a phosphorus-bound chalcogen atom by iodine constitutes a new mechanistic pathway for generation of these types of tricoordinate P(V) phosphorus species ($\lambda^5\sigma^3$), under exceedingly mild reaction conditions.

The potential of the developed method for generation of different metaphosphate analogues, for preparative phosphorylation of hydroxylic compounds, was demonstrated in the synthesis of various nucleoside phosphorothioate, phosphorodithioate, and phosphoroselenoate diesters. Since *H*-phosphonothioate, *H*-phosphonodithioate, and *H*-phosphonoselenoate monoesters are readily available, this experimentally simple reaction can be of practical use in the synthesis of various phosphate analogues. As these reactions proceed with the intermediacy of planar intermediates, i.e., the corresponding metaphosphate derivatives, they are expected to be inherently nonstereospecific.

Experimental Section

General Procedure for Oxidative Esterification of 5′-*O*-Dimethoxytritylthymidin-3′-yl *H*-Phosphonothioate (2b, Triethylammonium Salt) with Simple Alcohols. To a solution of **2b**[42,67] (145 mg, 0.2 mmol), an alcohol (MeOH: 24 μL, 19 mg, 0.6 mmol; EtOH: 35 μL, 28 mg, 0.6 mmol; *i*-PrOH: 76 μL, 60 mg, 1 mmol; *t*-BuOH: 190 μL, 148 mg, 2 mmol), and Et_3N (83 μL, 61 mg, 0.6 mmol) in THF (3 mL) was added dropwise a solution of iodine (56 mg, 0.22 mmol) in the same solvent (3 mL) with vigorous stirring. After the addition was complete (ca. 1 min), the solvent was evaporated, the residue was partitioned between CH_2Cl_2 and 5% aq $NaHCO_3$, and the aqueous phase was once more extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and evaporated to dryness. The products were purified by silica gel column chromatography, using a stepwise gradient of MeOH (0−5%) in CH_2Cl_2, containing 0.02% Et_3N.

5′-*O*-Dimethoxytritylthymidin-3′-yl Methyl Phosphorothioate, Triethylammonium Salt (11a). White powder, 141 mg (93%), 1:1 mixture of diastereoisomers. ^1H NMR (400 MHz, $CDCl_3$): δ 11.87 (1H, b, $(CH_3CH_2)_3NH^+$), 8.81 and 8.78 (1H, 2 × s, H3), 7.62 and 7.60 (1H, 2 × s, H6), 7.43−6.77 (13H, m, DMT protons), 6.44 (1H, m, H1′), 5.29 (1H, m, H3′), 4.36 and 4.31 (1H, 2 × m, H4′), 3.77 (6H, s, 2 × OCH_3), 3.60 and 3.54 (3H, 2 × d, CH_3OP, $J_{CH3\text{-}P}$ = 13.0 Hz), 3.46−3.29 (2H, m, H5′, H5″), 3.08 (6H, q, $(CH_3CH_2)_3NH^+$, $J_{CH_2\text{-}CH_3}$ = 7.4 Hz), 2.77−2.59 (1H, m, H2′), 2.48−2.23 (1H, m, H2″), 1.36 (3H, s, CH_3), 1.32 (9H, t, $(CH_3CH_2)_3NH^+$, $J_{CH_3\text{-}CH_2}$ = 7.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.8 and 163.7 (C4), 158.6 ($C4_{DMT}$, $C4'_{DMT}$), 150.6 and 150.5 (C2), 144.37 and 144.35 ($C1''_{DMT}$), 135.8, 135.7, 135.6, 135.5, 135.4 and 135.3 (C6, $C1_{DMT}$, $C1'_{DMT}$), 130.1 ($C2_{DMT}$, $C6_{DMT}$, $C2'_{DMT}$, $C6'_{DMT}$), 128.2 and 127.9 ($C2''_{DMT}$, $C3''_{DMT}$, $C5''_{DMT}$, $C6''_{DMT}$), 127.0 ($C4''_{DMT}$), 113.2 ($C3_{DMT}$, $C5_{DMT}$, $C3'_{DMT}$, $C5'_{DMT}$), 111.3 and 111.2 (C5), 87.01 and 86.97 (Ar_3C_{DMT}), 85.5 and 85.1 (2 × d, C4′, $J_{4'\text{-}P}$ = 6.0 Hz), 84.6 and 84.5 (C1′), 76.6 and 76.4 (2 × d, C3′, $J_{3'\text{-}P}$ = 5.8 Hz), 64.1 and 63.8 (C5′), 55.20 and 52.18 (2 × OCH_3), 52.96 and 52.93 (2 × d, CH_3OP, $J_{CH_3\text{-}P}$ = 6.1 Hz), 45.7 ($(CH_3CH_2)_3NH^+$), 39.7 and 39.5 (2 × d, C2′, $J_{2'\text{-}P}$ = 4.2 Hz), 11.6 and 11.5 (CH_3), 8.6 ($(CH_3CH_2)_3NH^+$). ^{31}P NMR (162 MHz, $CDCl_3$): δ 57.3 and 57.1. HRMS: *m/z* 695.2181 ($[M - H]^-$, $C_{35}H_{40}N_2O_9PS^-$ calcd 695.2198).

5′-*O*-Dimethoxytritylthymidin-3′-yl Ethyl Phosphorothioate, Triethylammonium Salt (11b). White powder, 134 mg (87%), 1:1

(64) Boehringer, M. P.; Graff, D.; Caruthers, M. H. *Tetrahedron Lett.* **1993**, *34*, 2723–2726.

(65) Anslyn, E. V.; Dougherty, D. A. *Modern Physical Organic Chemistry*; University Science Books: Sausalito, CA, 2006; pp 155−157.

(66) Excess of nucleosidic component **20** could be efficiently recovered during chromatography (see the Supporting information).

(67) Stawinski, J.; Thelin, M.; Westman, E.; Zain, R. *J. Org. Chem.* **1990**, *55*, 3503–3506.

mixture of diastereoisomers. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.96 (1H, b, H3), 7.61 and 7.60 (1H, 2 × q, H6, $J_{6\text{-}CH_3}$ = 1.1 Hz), 7.32−6.77 (13H, m, DMT protons), 6.44 and 6.43 (1H, 2 × dd, H1′, $J_{1'-2'}$ = 8.1 Hz, $J_{1'-2''}$ = 5.3 Hz), 5.28 (1H, m, H3′), 4.36 and 4.32 (1H, 2 × m, H4′), 4.07−3.84 (2H, m, CH_3CH_2), 3.76 (6H, s, 2 × OCH_3), 3.50 (0.5H, dd, H5′, $J_{5'-5''}$ = 10.5 Hz, $J_{5'-4'}$ = 2.8 Hz), 3.45−3.36 (1.5H, m, H5′, H5″), 2.97 (6H, m, $(CH_3CH_2)_3NH^+$, $J_{CH_2\text{-}CH_3}$ = 7.3 Hz), 2.66 (1H, m, H2′), 2.35 (1H, m, H2″), 1.36 and 1.33 (3H, 2 × d, CH_3, $J_{CH_3\text{-}6}$ = 1.1 Hz), 1.28−1.21 (10.5H, apparent t, $(CH_3CH_2)_3NH^+$ and CH_3CH_2, $J_{(CH_3\text{-}CH_2)_3NH^+}$ = 7.2 Hz), 1.14 (1.5H, CH_3CH_2, $J_{CH_3\text{-}CH_2}$ = 7.1 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.9 (C4), 158.56 and 158.53 ($C4_{DMT}$, $C4'_{DMT}$), 150.45 and 150.41 (C2), 144.33 and 144.32 ($C1''_{DMT}$), 136.75 and 135.73 (C6), 135.50, 135.47, and 135.3 ($C1_{DMT}$, $C1'_{DMT}$), 139.1 ($C2_{DMT}$, $C6_{DMT}$, $C2'_{DMT}$, $C6'_{DMT}$), 128.1 and 127.9 ($C2''_{DMT}$, $C3''_{DMT}$, $C5''_{DMT}$, $C6''_{DMT}$), 126.0 ($C4''_{DMT}$), 113.2 ($C3_{DMT}$, $C5_{DMT}$, $C3'_{DMT}$, $C5'_{DMT}$), 111.11 and 111.06 (C5), 86.9 (Ar_3C_{DMT}), 85.4 and 85.1 (2 × d, C4′, $J_{4'\text{-}P}$ = 5.9 Hz), 84.62 and 84.57 (C1′), 76.6 and 76.3 (2 × d, C3′, $J_{3'\text{-}P}$ = 5.5 Hz), 63.9 (C5′), 61.86 and 61.83 (2 × d, CH_3CH_2, $J_{CH\text{-}P}$ = 5.8 Hz), 55.2 (2 × OCH_3), 45.7 ($(CH_3CH_2)_3NH^+$), 39.7 and 39.5 (2 × d, C2′, $J_{2'\text{-}P}$ = 3.7 Hz), 16.3 and 16.2 (2 × d, CH_3CH, $J_{CH3\text{-}P}$ = 5.9 Hz), 11.52 and 11.46 (CH_3), 9.2 ($(CH_3CH_2)_3NH^+$). ^{31}P NMR (162 MHz, $CDCl_3$): δ 57.3 and 57.1. HRMS: *m*/*z* 667.1913 ([M − H]$^-$, $C_{33}H_{36}N_2O_9PS^-$ calcd 667.1885).

5′-*O*-Dimethoxytritylthymidin-3′-yl Isopropyl Phosphorothioate, Triethylammonium Salt (11c). White powder, 141 mg (90%), 1:1 mixture of diastereoisomers. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.79 and 8.72 (1H, 2 × s, H3), 8.01 (1H, b, $(CH_3CH_2)_3NH^+$), 7.63 and 7.62 (1H, 2 × q, H6, $J_{6\text{-}CH_3}$ = 1.2 Hz), 7.43−6.79 (13H, m, DMT protons), 6.46 and 6.44 (1H, 2 × dd, H1′, $J_{1'-2'}$ = 7.4 Hz, $J_{1'-2''}$ = 5.5 Hz), 5.28 (1H, m, H3′), 4.64 and 4.55 (1H, 2 × dsep, $(CH_3)_2CH$, $J_{CH\text{-}P}$ = 10.5 Hz, $J_{CH\text{-}(CH_3)_2}$ = 6.3 Hz), 4.40 and 4.35 (1H, 2 × m, H4′), 3.77 (6H, s, 2 × OCH_3), 3.52 (0.5H, dd, H5′, $J_{5'-5''}$ = 10.5 Hz, $J_{5'-4'}$ = 2.8 Hz), 3.45−3.36 (1.5H, m, H5′, H5″), 2.91 (6H, q, $(CH_3CH_2)_3NH^+$, $J_{CH_2\text{-}CH_3}$ = 7.2 Hz), 2.71 and 2.67 (1H, 2 × ddd, H2′, $J_{2'-2''}$ = 13.4 Hz, $J_{2'-1'}$ = 5.5 Hz, $J_{2'-3'}$ = 1.1 Hz), 2.35 (1H, m, H2″), 1.34 and 1.33 (3H, 2 × d, CH_3, $J_{CH_3\text{-}6}$ = 1.2 Hz), 1.28 (1.5H, d, $(CH_3)_2CH$, $J_{CH_3\text{-}CH}$ = 6.3 Hz), 1.24 (9H, t, $(CH_3CH_2)_3NH^+$, $J_{CH_3\text{-}CH_2}$ = 7.2 Hz), ∼1.23 (1.5H, $(CH_3)_2CH$), 1.20 (1.5H, d, $(CH_3)_2CH$, $J_{CH_3\text{-}CH}$ = 6.3 Hz), 1.12 (1.5H, d, $(CH_3)_2CH$, $J_{CH_3\text{-}CH}$ = 6.3 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.6 (C4), 158.63 and 158.60 ($C4_{DMT}$, $C4'_{DMT}$), 150.22 and 150.19 (C2), 144.41 and 144.36 ($C1''_{DMT}$), 136.9 and 135.5 (C6), 135.38 and 135.34 ($C1_{DMT}$, $C1'_{DMT}$), 130.1 ($C2_{DMT}$, $C6_{DMT}$, $C2'_{DMT}$, $C6'_{DMT}$), 128.2 and 127.9 ($C2''_{DMT}$, $C3''_{DMT}$, $C5''_{DMT}$, $C6''_{DMT}$), 127.0 ($C4''_{DMT}$), 113.2 ($C3_{DMT}$, $C5_{DMT}$, $C3'_{DMT}$, $C5'_{DMT}$), 111.10 and 111.07 (C5), 87.0 (Ar_3C_{DMT}), 85.6 and 85.3 (2 × d, C4′, $J_{4'\text{-}P}$ = 6.2 Hz), 84.75 and 84.71 (C1′), ∼76.6 (C3′), 70.1 and 69.9 (2 × d, $(CH_3)_2CH$, $J_{CH\text{-}P}$ = 6.0 Hz), 64.09 and 64.03 (C5′), 55.2 (2 × OCH_3), 45.8 ($(CH_3CH_2)_3NH^+$), 39.7 and 39.5 (2 × d, C2′, $J_{2'\text{-}P}$ = 3.9 Hz), 24.1, 23.9, 23.76, and 23.73 (4 × d, $(CH_3)_2CH$, $J_{CH_3\text{-}P}$ = 5.0 Hz), 11.49 and 11.46 (CH_3), 9.5 ($(CH_3CH_2)_3NH^+$). ^{31}P NMR (202 MHz, $CDCl_3$): δ 52.2 and 52.1. HRMS: *m*/*z* 681.1999 ([M − H]$^-$, $C_{34}H_{38}N_2O_9PS^-$ calcd 681.2041).

***tert*-Butyl 5′-*O*-dimethoxytritylthymidin-3′-yl Phosphorothioate, Triethylammonium Salt (11d).** White powder, 136 mg (85%), 1:1 mixture of diastereoisomers. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.96 (1H, b, H3), 7.63 (1H, s, H6), 7.48−6.68 (13H, m, DMT protons), 6.47 (1H, m, H1′), 5.33 (1H, m, H3′), 4.42 (1H, m, H4′), 3.78 (6H, s, 2 × OCH_3), 3.53 (0.5H, dd, H5′, $J_{5'-5''}$ = 10.6 Hz, $J_{5'-5''}$ = 2.9 Hz), 3.46 (0.5H, dd, H5′, $J_{5'-5''}$ = 10.6 Hz, $J_{5'-4'}$ = 2.3 Hz), 3.43−3.38 (1H, m, H5″), 3.06 (6H, q, $(CH_3CH_2)_3NH^+$, $J_{CH_2\text{-}CH_3}$ = 7.3 Hz), 2.72 (1H, dd, H2′, $J_{2'-2''}$ = 13.3 Hz, $J_{2'-1'}$ = 5.6 Hz), 2.36 (1H, m, H2″), 1.50 and 1.40 (9H, 2 × s, $(CH_3)_3C$), 1.36 and 1.34 (3H, 2 × s, CH_3), 1.31 (9H, t, $(CH_3CH_2)_3NH^+$, $J_{CH_3\text{-}CH_2}$ = 7.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.8 (C4), 158.54 and 158.51 ($C4_{DMT}$, $C4'_{DMT}$), 150.4 and 150.3 (C2), 144.39 and 144.35 ($C1''_{DMT}$), 135.90 and 135.85 (C6), 135.57, 135.51, 135.35, and 135.31 ($C1_{DMT}$, $C1'_{DMT}$), 130.1 ($C2_{DMT}$, $C6_{DMT}$, $C2'_{DMT}$, $C6'_{DMT}$), 128.18, 128.13, and 127.9 ($C2''_{DMT}$, $C3''_{DMT}$, $C5''_{DMT}$, $C6''_{DMT}$), 126.9 ($C4''_{DMT}$), 113.2 ($C3_{DMT}$, $C5_{DMT}$, $C3'_{DMT}$, $C5'_{DMT}$), 111.1 and 111.0 (C5), 86.9 (Ar_3C_{DMT}), 85.6 and 85.0 (2 × d, C4′, $J_{4'\text{-}P}$ = 6.7 Hz), 84.72 and 84.68 (C1′), 79.6 and 79.5 (2 × d, $(CH_3)C$, J_{C-P} = 9.2 Hz), ∼76.8 and 76.4 (2 × d, C3′, $J_{3'\text{-}P}$ = 5.3 Hz), 64.2 and 64.1 (C5′), 55.2 (2 × OCH_3), 45.5 ($(CH_3CH_2)_3NH^+$), 39.7 and 39.3 (2 × d, C2′, $J_{2'\text{-}P}$ = 3.8 Hz), 30.22 and 30.15 (2 × d, $(CH_3)_3C$, $J_{CH_3\text{-}P}$ = 4.4 Hz), 11.43 (CH_3), 8.1 ($(CH_3CH_2)_3NH^+$). ^{31}P NMR (162 MHz, $CDCl_3$): δ 51.0 and 50.7. HRMS: *m*/*z* 695.2181 ([M − H]$^-$, $C_{35}H_{40}N_2O_9PS^-$ calcd 695.2198).

General Procedure for Oxidative Esterification of 5′-Dimethoxytritylthymidin-3′-yl *H*-Phosphonothioate (2b), 5′-Dimethoxytritylthymidin-3′-yl *H*-Phosphonodithioate (3a), and 5′-Dimethoxytritylthymidin-3′-yl *H*-Phosphonoselenoate (4a) (All Triethylammonium Salts) with 3′-*O*-(*tert*-Butyldimethylsilyl)thymidine (20). To a solution of **2b**,[42,67] **3a**[7] or **4a**[57] (0.2 mmol), **20**[64] (714 mg, 2 mmol), and Et_3N (83 μL, 61 mg, 0.6 mmol) in THF (3 mL) was added a solution of iodine (56 mg, 0.22 mmol) in the same solvent (3 mL) dropwise, at −78 °C with vigorous stirring. After the addition was complete, the mixture was allowed to warm to room temperature and the solvent was evaporated. The residue was partitioned between CH_2Cl_2 and 5% aq $NaHCO_3$ and the aqueous phase was once more extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and evaporated to dryness. The products were isolated by silica gel column chromatography, using a stepwise gradient of MeOH (0−5%) in CH_2Cl_2, containing 0.02% Et_3N. A yield of 1.3−1.5 mmol (450−550 mg) of 3′-*O*-(*tert*-butyldimethylsilyl)thymidine **20** could be recovered during the chromatography.

3′-*O*-(*tert*-Butyldimethylsilyl)thymidin-5′-yl 5′-*O*-Dimethoxytritylthymidin-3′-yl Phosphorothioate, Triethylammonium Salt (11e). White powder, 175 mg (81%), 1:1 mixture of diastereoisomers. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.89 (1H, b, $(CH_3CH_2)_3NH^+$), 8.90, 8.89, 8.81, and 8.73 (2H, 4 × s, $H3_a$, $H3_b$), 7.78, 7.77, 7.64, and 7.59 (2H, 4 × s, $H6_a$, $H6_b$), 7.45−6.81 (13H, m, DMT prot.), 6.48−6.34 (2H, m, $H1'_a$, $H1'_b$), 5.36 (1H, m, $H3'_a$), 4.62−4.31 (1H, 3 × m, $H3'_b$), 4.39 and 4.31 (1H, 2 × m, $H4'_a$), 4.19−3.88 (3H, m, $H4'_b$ $H5'_b$, $H5''_b$), 3.81, 3.80, and 3.79 (6H, 3 × s, 2 × OCH_3), 3.56−5.36 (2H, m, $H5'_a$, $H5''_a$), 3.10 and 3.09 (6H, 2 × q, $(CH_3CH_2)_3NH^+$, $J_{CH_2\text{-}CH_3}$ = 7.3 Hz), 2.68 and 2.56 (1H, 2 × dd, $H2'_a$, $J_{2'a\text{-}2''a}$ = 13.3 Hz, $J_{2'a\text{-}1'a}$ = 5.3 Hz), 2.43−2.10 (3H, m, $H2''_a$, $H2'_b$, $H2''_b$), 1.98, 1.95, 1.48, and 1.39 (6H, 4 × s, 2 × CH_3), 1.33 (9H, t, $(CH_3CH_2)_3NH^+$, $J_{CH_3\text{-}CH_2}$ = 7.3 Hz), 0.90 and 0.88 (9H, 2 × s, $(CH_3)_3CSi$), 0.09 and 0.06 (6H, 2× s, $(CH_3)_2Si$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.90, 163.85, 163.74, and 163.71 ($C4_a$, $C4_b$), 158.69, 158.66, 158.64, and 158.58 ($C4_{DMT}$, $C4'_{DMT}$), 150.39 and 150.38 ($C2_a$, $C2_b$), 144.32 and 144.30 ($C1''_{DMT}$), 136.4, 135.7, 135.6, 135.5, 135.4, and 135.1 ($C6_a$, $C6_b$, $C1_{DMT}$, $C1'_{DMT}$), 130.1 and 130.0 ($C2_{DMT}$, $C6_{DMT}$, $C2'_{DMT}$, $C6'_{DMT}$), 128.2, 128.1, 128.0, 127.8, and 127.7 ($C2''_{DMT}$, $C3''_{DMT}$, $C5''_{DMT}$, $C6''_{DMT}$), 127.1 and 127.0 ($C4''_{DMT}$), 113.3 and 113.1 ($C3_{DMT}$, $C3'_{DMT}$, $C5_{DMT}$, $C5'_{DMT}$), 111.3, 111.2, 111.1, and 111.0 ($C5_a$, $C5_b$), 87.0 and 86.9 (Ar_3CO_{DMT}), 86.5, 86.4, 86.1, 85.7, 85.6, 83.3, 85.2, 85.1, 85.0, 84.9, 84.7, and 84.6 ($C1'_a$, $C1'_b$, $C4'_a$, $C4'_b$), ∼77.2 ($C3'_a$), 72.84 and 72.80 ($C3'_b$), 65.8 and 65.1 (d, $C5'_b$, $J_{5'\text{-}P}$ = 7.9 Hz), 64.0 and 63.9 ($C5'_a$), 55.2 (2 × OCH_3), 45.6 ($(CH_3CH_2)_3NH^+$), 40.9 and 40.8 ($C2'_b$), 39.8 and 39.1 (d, $C2'_a$, $J_{2'\text{-}P}$ = 3.9 Hz), 25.7 ($(CH_3)_3CSi$), 17.9 ($(CH_3)_3CSi$), 12.45, 12.39, 11.6, and 11.5 (CH_{3a}, CH_{3b}), 8.6 ($(CH_3CH_2)_3NH^+$), −4.68, −4.71, −4.74, and −4.76 ($(CH_3)_2Si$). ^{31}P NMR (202 MHz, $CDCl_3$): δ 57.5 and 57.3. HRMS: *m*/*z* 977.3330 ([M − H]$^-$, $C_{47}H_{58}N_4O_{13}PSSi^-$ calcd 977.3233).

3′-*O*-(*tert*-Butyldimethylsilyl)thymidin-5′-yl 5′-*O*-Dimethoxytritylthymidin-3′-yl Phosphorodithioate, Triethylammonium Salt (21). White powder, 167 mg (76%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.29 (1H, b, $(CH_3CH_2)_3NH^+$), 8.56 and 8.48 (2H, 2 × s, $H3_a$, $H3_b$), 7.83 and 7.59 (2H, 2 × s, $H6_a$, $H6_b$), 7.43−6.88 (13H, m, DMT prot.), 6.43 (1H, dd, $H1'_a$, $J_{1'a\text{-}2'a}$ = 8.6 Hz, $J_{1'a\text{-}2''a}$ = 5.4 Hz), 6.40 (1H, dd, $H1'_b$, $J_{1'b\text{-}2'b}$ = 7.9 Hz, $J_{1'b\text{-}2''b}$ = 6.1 Hz), 5.47 (1H, dd, $H3'_a$, $J_{3'a\text{-}P}$ = 12.5 Hz, $J_{3'a\text{-}2''a}$ = 5.8 Hz), 4.56 (1H, m,

H3′$_b$), 4.40 (1H, m, H4′$_a$), 4.26 (1H, m, H5′$_b$), 4.06 (1H, m, H5″$_b$), 4.03 (1H, m, H4′$_b$), 3.78 (6H, s, 2 × OCH_3), 3.48 (1H, dd, H5′$_a$, $J_{5'a\text{-}5''a}$ = 11.5 Hz, $J_{5'a\text{-}4'a}$ = 2.8 Hz), 3.41 (1H, dd, H5″$_a$, $J_{5''a\text{-}5'a}$ = 11.5 Hz, $J_{5''a\text{-}4'a}$ = 2.4 Hz), 3.19 (6H, q, (CH$_3$CH_2)$_3$NH$^+$, $J_{CH_2\text{-}CH_3}$ = 7.3 Hz), 2.67 (1H, dd, H2″$_a$, $J_{2''a\text{-}2'a}$ = 13.6 Hz, $J_{2''a\text{-}1'a}$ = 5.4 Hz), 2.35 (1H, m, 2′$_a$), 2.20−2.12 (2H, m, H2′$_b$, H2″$_b$), 1.96 and ∼1.35 (6H, 2 × s, 2 × CH_3), 1.37 (9H, t, (CH_3CH$_2$)$_3$NH$^+$, $J_{CH_3\text{-}CH_2}$ = 7.3 Hz), 0.87 (9H, 2 × s, (CH_3)$_3$CSi), 0.07 and 0.06 (6H, 2 × s, (CH_3)$_2$Si). ^{13}C NMR (125 MHz, CDCl$_3$): δ 163.8 and 163.6 (C4$_a$, C4$_b$), 158.64 and 158.60 (C4$_{DMT}$, C4′$_{DMT}$), 150.4 and 150.2 (C2$_a$, C2$_b$), 144.4 (C1″$_{DMT}$), 136.5, 135.7, 135.5, and 135.3 (C6$_a$, C6$_b$, C1$_{DMT}$, C1′$_{DMT}$), 130.16 and 130.12 (C2$_{DMT}$, C6$_{DMT}$, C2′$_{DMT}$, C6′$_{DMT}$), 128.1 and 128.0 (C2″$_{DMT}$, C3″$_{DMT}$, C5″$_{DMT}$, C6″$_{DMT}$), 127.0 (C4″$_{DMT}$), 113.2 and 113.1 (C3$_{DMT}$, C3′$_{DMT}$, C5$_{DMT}$, C5′$_{DMT}$), 111.3 and 111.1 (C5$_a$, C5$_b$), 87.0 (Ar$_3$CO$_{DMT}$), 86.6 (d, C4′$_b$, $J_{4'b\text{-}P}$ = 9.3 Hz), 85.2 (d, C4′$_a$, $J_{4'a\text{-}P}$ = 5.5 Hz), 84.9 and 84.7 (C1′$_a$, C1′$_b$), ∼76.8 (C3′$_a$), 72.2 (C3′$_b$), 65.6 (d, C5′$_b$, $J_{5'\text{-}P}$ = 7.5 Hz), 63.8 (C5′$_a$), 55.2 (2 × OCH$_3$), 46.1 ((CH$_3$CH_2)$_3$NH$^+$), 40.8 (C2′$_b$), 39.5 (d, C2′$_a$, $J_{2'\text{-}P}$ = 3.9 Hz), 25.8 ((CH_3)$_3$CSi), 17.9 ((CH$_3$)$_3$CSi), 12.5 and 11.5 (CH_{3a}, CH_{3b}), 8.6 ((CH_3CH$_2$)$_3$NH$^+$), −4.7 ((CH_3)$_2$Si). ^{31}P NMR (162 MHz, CDCl$_3$): δ 113.0. HRMS: *m*/*z* 993.3116 ([M − H]$^-$, C$_{47}$H$_{58}$N$_4$O$_{12}$PS$_2$Si$^-$ calcd 993.3005).

3′-*O*-(*tert*-Butyldimethylsilyl)thymidin-5′-yl 5′-*O*-Dimethoxytritylthymidin-3′-yl Phosphoroselenoate, Triethylammonium Salt (22). White powder, 174 mg (77%), 1:1 mixture of diastereoisomers. ^{1}H NMR (500 MHz, CDCl$_3$): δ 11.58 (1H, b, (CH$_3$CH$_2$)$_3$N*H*$^+$), 8.62 and 8.49 (2H, 2 × s, H3$_a$, H3$_b$), 7.74 and 7.62 (2H, 2 × s, H6$_a$, H6$_b$), 7.44−6.78 (13H, m, DMT prot.), 6.46−6.32 (2H, m, H1′$_a$, H1′$_b$), 5.43 (1H, m, H3′$_a$), 4.52−4.40 (1H, 2 × m, H3′$_b$), 4.29 (1H, m, H4′$_a$), 4.13 (1H, m, H5′$_b$), 4.06 and 3.98 (1H, 2 × m, H4′$_b$), 3.91 (1H, m, H5″$_b$), 3.78 (6H, s, 2 × OCH_3), 3.58−5.29 (2H, m, H5′$_a$, H5″$_a$), 3.11 (6H, q, (CH$_3$CH_2)$_3$NH$^+$, $J_{CH_2\text{-}CH_3}$ = 7.4 Hz), 2.75−2.00 (4H, m, H2′$_a$, H2″$_a$, H2′$_b$, H2″$_b$), 1.96, 1.95 and 1.38 (6H, 3 × s, 2 × CH_3), 1.32 (9H, t, (CH_3CH$_2$)$_3$NH$^+$, $J_{CH_3\text{-}CH_2}$ = 7.4 Hz), 0.88 and 0.87 (9H, 2 × s, (CH_3)$_3$CSi), 0.08 and 0.05 (6H, 2 × s, (CH_3)$_2$Si). ^{13}C NMR (125 MHz, CDCl$_3$): δ 163.8, 163.7, 163.63 and 163.58 (C4$_a$, C4$_b$), 158.67 and 158.65 (C4$_{DMT}$, C4′$_{DMT}$), 150.3 (C2$_a$, C2$_b$), 144.3 (C1″$_{DMT}$), 136.2 135.7, 135.6, 135.4, 135.4, and 135.2 (C6$_a$, C6$_b$, C1$_{DMT}$, C1′$_{DMT}$), 130.1 (C2$_{DMT}$, C6$_{DMT}$, C2′$_{DMT}$, C6′$_{DMT}$), 128.2 and 128.0 (C2″$_{DMT}$, C3″$_{DMT}$, C5″$_{DMT}$, C6″$_{DMT}$), 127.1 (C4″$_{DMT}$), 113.3 (C3$_{DMT}$, C3′$_{DMT}$, C5$_{DMT}$, C5′$_{DMT}$), 111.2 and 111.1 (C5$_a$, C5$_b$), 87.1 (Ar$_3$CO$_{DMT}$), 86.4 and 86.3 (2 × d, C4′$_b$, $J_{4'b\text{-}P}$ = 8.6 Hz), 85.5, 85.2, 85.06, 85.01, 84.9, 84.7, 84.6 and 84.5 (C1′$_a$, C1′$_b$, C4′$_a$), 77.6 (d, C3′$_a$, $J_{C3'a\text{-}P}$ = 5.2 Hz), 72.9 (C3′$_b$), 66.0 and 65.3 (d, C5′$_b$, $J_{5'\text{-}P}$ = 6.1 Hz), 64.1 and 63.9 (C5′$_a$), 55.2 (2 × OCH_3), 45.8 ((CH$_3$CH_2)$_3$NH$^+$), 40.8 (C2′$_b$), 39.8 and 39.1 (C2′$_a$), 25.7 ((CH_3)$_3$CSi), 17.9 ((CH$_3$)$_3$CSi), 12.52, 12.45, 11.57 and 11.51 (CH_{3a}, CH_{3b}), 8.2 ((CH_3CH$_2$)$_3$NH$^+$), −4.66, −4.68, and −4.71 ((CH_3)$_2$Si). ^{31}P NMR (202 MHz, CDCl$_3$): δ 52.2 and 52.0. HRMS: *m*/*z* 1025.2657 ([M − H]$^-$, C$_{47}$H$_{58}$N$_4$O$_{13}$PSeSi$^-$ calcd 1025.2678).

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Supporting Information Available: NMR spectra of compounds **11a**−**e**, **21**, and **22**. This material is available free of charge via the Internet at http://pubs.acs.org.

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