Nucleoside H-Phosphonates. 14. Synthesis of Nucleoside Phosphoroselenoates and Phosphorothioselenoates *via* Stereospecific Selenization of the Corresponding H-Phosphonate and H-Phosphonothioate Diesters with the Aid of New Selenium-Transfer Reagent, 3*H*-1,2-Benzothiaselenol-3-one

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An efficient conversion of nucleoside H-phosphonate and nucleoside H-phosphonothioate diesters into the corresponding phosphoroselenoates and phosphorothioselenoates was achieved with a new selenium-transferring reagent, 3*H*-1,2-benzothiaselenol-3-one (BTSe, 1). The reagent is soluble in common organic solvents and shows an enhanced rate of selenium transfer compared to elemental selenium or potassium selenocyanate. The reaction was found to be stereospecific and occurs with retention of configuration at the phosphorus center. BTSe also proved to be effective in the conversion of phosphite triesters into the corresponding phosphoroselenoates under reactions conditions which are compatible with both solution- and solid-phase synthesis of oligonucleotides.

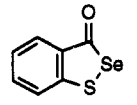
Introduction

During the past few decades extensive studies on the biological role of selenium clearly demonstrated the essentiality of this element for mammals.[1] The metabolic role of selenium is largely determined by the reactions catalyzed by selenium-dependent enzymes in which selenium exists in the form of the amino acid selenocysteine.[2] It is believed that incorporation of selenium into macromolecules requires generation of highly reduced selenium compounds which can also be utilized for the *in vivo* replacement of sulfur by selenium in some modified nucleobases in tRNA.[1,3]

The close resemblance of selenium to the other biologically important element sulfur (actually closer than that of sulfur to oxygen[4]) constitutes a strong rationale for the potential therapeutic importance of selenium. Indeed, several organoselenium compounds show a wide spectrum of biological activity, e.g., anticancer, antihistamine, or anti-inflammatory properties.[4,5] Despite the well-documented biological activity of organophosphorus compounds containing P–Se–C fragments (e.g., potent cholinesterase inhibitors[5]) and an indication that metabolism of selenium-containing compounds may involve P–Se intermediates,[3] phosphoroselenoates have received relatively little attention.[6–9] This can partly be due to the relatively high cytotoxicity of these compounds,[9] some instability (which is sometimes manifested as a slow "washout" of selenium[9]), and lack of efficient and automated synthetic methods for the preparation of phosphoroselenoates.

Analogues of natural products containing P=Se bonds are most conveniently accessible *via* selenization of the corresponding P(III) derivatives.[6,7,9–11] However, the major obstacle which hampers further developments of these synthetic methods was the limited choice of available selenium-transfer reagents. Application of elemental selenium[6] or potassium selenocyanate,[9,11] which have been used almost exclusively as a source of electrophilic selenium in this type of reaction, poses serious experimental problems. Low solubility in organic solvents, low reactivity, and usually heterogeneous reaction conditions are the main disadvantages of these reagents.

In this paper we describe our chemical and stereochemical studies directed toward the development of an efficient method for the conversion of nucleoside H-phosphonate and nucleoside H-phosphonothioate diesters into the corresponding phosphoroselenoate and phosphorothioselenoate diesters using a new selenium-transfer reagent, 3*H*-1,2-benzothiaselenol-3-one (BTSe, 1).[12] Also some



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results concerning selenization of phosphite triesters, which may be relevant to oligonucleoside phosphoroselenoate synthesis *via* the phosphoramidite method,[13] will be discussed.

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Results and Discussion

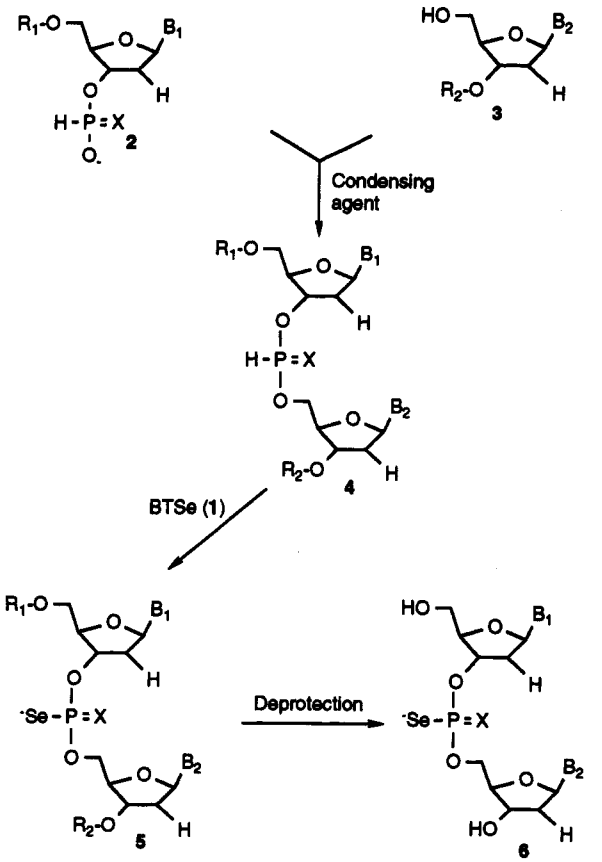
To alleviate problems associated with selenization of P(III) compounds using elemental selenium or potassium selenocyanate, we tried to develop a reagent soluble in common organic solvents and with enhanced rate of selenium transfer. Guided by results from our previous investigations on sulfurizing reagents[14] suitable for H-phosphonate and H-phosphonothioate diesters, we have attempted to evaluate 3*H*-1,2-benzothiaselenol-3-one (1) as a source of electrophilic selenium. For that purpose we have developed a simple one-pot synthesis of 1 starting from commercial available dithiosalicylic acid and triphenylphosphine selenide.[15] Similarly to its thio analogue,[14] the reagent 1 was found to be a stable, crystalline solid, with good solubility in organic solvents (e.g., acetonitrile, methylene chloride, pyridine).

To demonstrate the utility of 1 as a selenium-transferring reagent we have synthesized two dinucleoside H-phosphonate (**4a**, **4b**) and one dinucleoside H-phosphonothioate diesters (**4c**). Various protecting groups (5′- or 3′-*O*-monomethoxytrityl, 5′-*O*-dimethoxytrityl, 3′-*O*- and N^2-benzoyl, N^2-isobutyryl) and heterocyclic bases were chosen to demonstrate feasibility of deprotection of phosphoroselenoates under standard reaction conditions used in oligonucleotide synthesis. The syntheses were carried out by condensation of an appropriate nucleoside 3′-H-phosphonate (**2a**, **2b**) or nucleoside 3′-H-phosphonothioate (**2c**) monoester with a suitable protected nucleosidic component (**3**) in the presence of pivaloyl chloride (for H-phosphonate esters) or diphenyl phosphorochloridate (for H-phosphonothioates) as a coupling agent (Scheme 1). In order to investigate the stereochemical outcome of selenization, all dimers **4** were also separated into R_P and S_P diastereomers using silica gel chromatography.

The efficacy of BTSe (1) was first checked on diastereomeric mixtures of the H-phosphonates **4a** and **4b** and on the H-phosphonothioate diester **4c**. In all instances the reaction of 1 in acetonitrile in the presence of 2 equiv of triethylamine furnished fast and clean conversion of the dimers **4** into the corresponding phosphoroselenoates (**5a**,**b**) or phosphorothioselenoate (**5c**) (^{31}P NMR). As anticipated, the reagent 1 was completely unreactive toward H-phosphonate and H-phosphonothioate diesters in neutral solvents in the absence of an external base.

Since the ratios of diastereomers in the starting H-phosphonate and H-phosphonothioate diesters **4** were preserved in the products **5** (^{31}P NMR), it seemed likely that the mode of selenium transfer in these reactions is stereospecific. This was further substantiated by selenization of separate R_P and S_P diastereomers of **4** (four H-phosphonate and two phosphorothioate dimers). The results of these experiments clearly showed that selenization of H-phosphonate and H-phosphonothioate diesters was completely stereospecific and also that there was no detectable difference in reactivity between the diastereomers. Typical ^{31}P NMR spectra of the crude reaction mixtures obtained after selenization of the R_P diastereomer of the dimer **4a** with BTSe (1) and with KSeCN are presented on Figure 1, parts a and b, respectively. One should note that the selenization reaction with BTSe is much faster and cleaner than that with KSeCN; however,

Scheme 1



2a - B_1=T; X=O; R_1=mmt
2b - B_1=G^{isb}; X=O; R_1=dmt
2c - B_1=T; X=S; R_1=mmt
2d - B_1=T; X=O; R_1=dmt
3a - B_2=T; R_2=mmt
3b - B_2=T^{bz}; R_2=Bz
4a,**5a** - B_1=B_2=T; X=O; R_1=R_2=mmt
4b,**5b** - B_1=G^{isb}; B_2=T^{bz}; X=O; R_1=dmt; R_2=Bz
4c,**5c** - B_1=T; X=S; R_1=dmt

6a - B_1=B_2=T; X=O
6b - B_1=G; B_2=T; X=O
6c - B_1=B_2=T; X=S
mmt - 4-methoxytriphenylmethyl
dmt - 4,4′-dimethoxytriphenylmethyl
Bz - benzoyl
T^{bz} - N^3-benzoylthymidin-1-yl
G^{isb} - N^2-isobutyrylguanin-9-yl
T - thymin-1-yl
G - guanin-9-yl

in both instances the same diastereomer of the phosphoroselenoate **5a** is formed.

If one assumes a mechanism of selenization with BTSe (Scheme 2) to be similar to that of sulfurization with 3*H*-1,2-benzodithiol-3-one[14] the most likely stereochemical outcome of selenium transfer to the dimers **4** should be retention of configuration at the phosphorus center. The absolute configurations of the separate diastereomers of the H-phosphonate diesters **4a** and **4b** were established by their stereospecific sulfurization with elemental sulfur[16,17] followed by enzymatic digestion with SVPD.[18,19] Configurational assignment of the phosphorus diastereomers of the H-phosphonothioate dimer **4c** was done *via* a stereochemical correlation as described elsewhere.[20] In agreement with the previously observed correlation between the ^{31}P NMR chemical shift and the elution order

(14) Stawinski, J.; Thelin, M. *J. Org. Chem.* **1991**, *56*, 5169–5175.
(15) Alternatively, (phenylseleno)phosphonic dichloride can be used as a source of selenium in this reaction; see ref 12.
(16) Seela, F.; Kretschmer, U. *J. Org. Chem* **1991**, *56*, 3861–3869.
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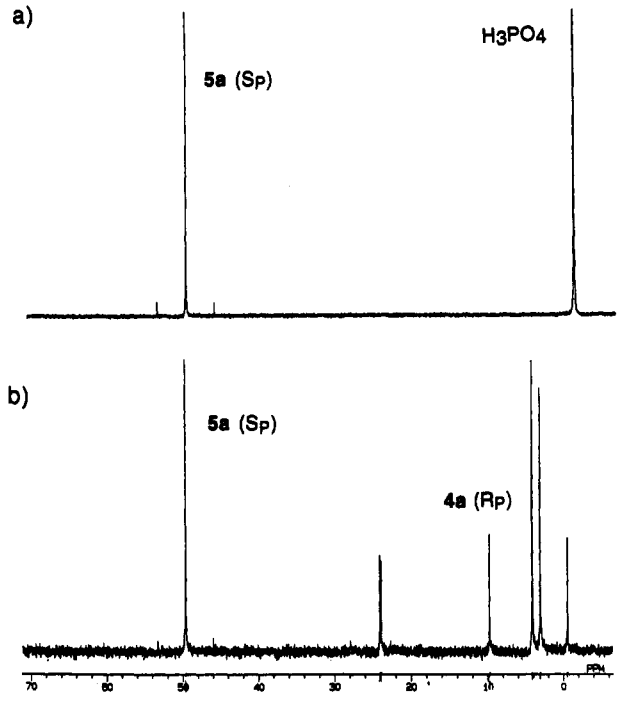


Figure 1. ^{31}P NMR spectra of crude reaction mixtures after selenization of the H-phosphonate diester **4a** (R_P) in pyridine–TEA (95:5, v/v) with 1.5 equiv of the respective selenizing reagents: (a) BTSe (1) (after ~2 min, no signal from the starting material visible); (b) KSeCN (after 4 days).

from the silica gel column with absolute configurations at the phosphorus center of H-phosphonate and H-phosphonothioate diesters,[16,21,22] the "faster" moving diastereomers of **4a–c** (which also resonated at higher field in ^{31}P NMR) were found to have the R_P, while the other ones ("slower" ones which resonated at lower field in ^{31}P NMR), the S_P configurations (see also Table 1).

To designate the absolute configuration at the phosphorus center in the dinucleoside phosphoroselenoates, the four dimers, **6a** (R_P and S_P) and **6b** (R_P and S_P), obtained from selenization and deprotection of separate diastereomers of **4a** and **4b**, were subjected to enzymatic digestion with P1[7] endonuclease. Only two phosphoroselenoates, namely those derived from the R_P ("faster moving") diastereomers of **4a** and **4b** were substrates for the enzyme.[23] This identified their absolute configurations at the phosphorus centers as S_P[7,24] and also, together with the known stereochemistry of the starting dimers **4a** and **4b**, delineated the stereochemical mode of the selenium transfer as occurring with retention of configuration.[25] Since selenization of the H-phosphonates **4** with elemental selenium (and most likely with potassium selenocyanate, see Figure 1) gave identical stereochemical outcomes to reactions with BTSe (1), one can conclude that these reagents also furnish selenization, as expected, with retention of configuration at the phosphorus centers.

The stereochemical outcome of selenization of H-phosphonothioate diesters (R_P and S_P **4c**) could not be established by an enzymatic assay due to a complete resistance of the phosphorothioselenoates **6c** toward nucleases P1 and SVPD. However, since selenization of the separate diastereomers of **4c** with elemental selenium or potassium selenocyanate gave the same stereochemical outcome as the reaction with **1**, and taking into account the relative positions of the resonances of the **6c** diastereomers in the ^{31}P NMR spectra (Table 1), we assumed that selenization of the H-phosphonothioates **4c** also occurred with retention of configuration. Thus, we tentatively assigned the S_P configuration at the phosphorus center to the diastereomer of **6c** derived from R_P **4c** and the R_P configuration to that obtained from the S_P H-phosphonothioate **4c**.

Although the selenization of the dimers **4** with the reagent **1** was fast and clean, the reaction mixtures did not remain homogeneous as the reaction proceeded and formation of a yellow precipitate was observed.[12] This could possibly lead to serious complications during solid-phase synthesis. Further investigation of this problem showed that in basic solvents (e.g., neat pyridine) all reactions were substantially slower than those in acetonitrile in the presence of triethylamine, however, the mixtures remained homogenous throughout the course of the reactions and afterwards. This led us to the development of a solvent system consisting of pyridine–triethylamine which secured homogeneous reaction conditions without compromising stereospecificity and the rate of selenium transfer.[12]

The efficacy of BTSe (1) and the new reaction conditions were evaluated during synthesis of dodecathymidine undecaphosphoroselenoate {[Tp(Se)]$_{11}$T} according to the H-phosphonate methodology.[26–28] After assembly of the desired chain length, the support-bound dodecathymidine undeca-H-phosphonate was converted into the corresponding phosphoroselenoate by treatment with 0.1 M BTSe (1) in pyridine–triethylamine 95:5 (v/v) and then released from the support with aqueous ammonia. The crude reaction mixture was analyzed by HPLC and ^{31}P NMR spectroscopy. The reverse-phase HPLC[9] of the dimethoxytritylated phosphoroselenoate showed two close peaks with longer retention times than those for the corresponding phosphorothioate (data not shown). The phenomenon of elution of full-length oligomers in a form of two peaks during HPLC was previously observed for 5′-O-tritylated phosphorothioates[29] and phosphoroselenoates[9] and it was attributed to a slightly different retention time between oligomers with the opposite stereochemistry at the 5′-terminal internucleotidic bond.[29] Apparently the presence of a trityl group in the 5′-position magnifies this difference since after detritylation the dodecathymidine undecaphosphoroselenoate eluted from

(20) Stereoselective desulfurization with *m*-chloroperoxybenzoic acid followed by stereospecific sulfurization, or stereospecific oxidation with 3*H*-2,1-benzoxathiol-3-one 1-oxide and enzymatic digestion with SVPD. See also refs 22 and 38.

(21) Almer, H.; Stawinski, J.; Strömberg, R.; Thelin, M. *Nucl. Acids Res., Symp. Ser.* **1991**, *24*, 227.

(22) Stawinski, J.; Thelin, M. *Tetrahedron Lett.* **1992**, *33*, 3189–3192.

(23) Enzymatic digestion with SVPD could not be used for unequivocal differentiation between R_P and S_P diastereomers of **6a** and **6b** phosphoroselenoates due to substantially lower rates of hydrolysis than those with endonuclease P1 and also due to occurrence of apparently some side reactions. See also ref 7.

(24) Potter, B. V. L.; Connolly, B. A.; Eckstein, F. *Biochemistry* **1983**, *22*, 1369–1377.

(25) The change in notation from R_P in **4a** and **4b** to S_P in **5a** and **5b** does not indicate a change in stereochemistry and it is solely due to CIP rules of the priority order of substituents.

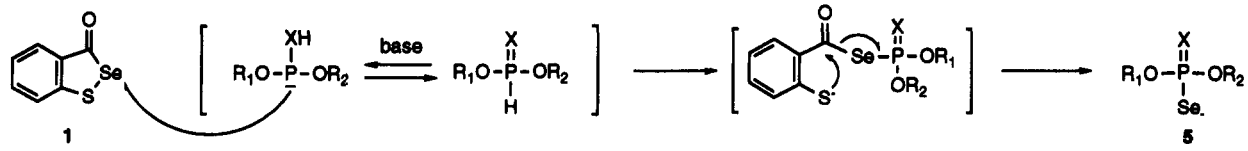
(26) Garegg, P. J.; Regberg, T.; Stawinski, J.; Strömberg, R. *Chem. Scr.* **1985**, *25*, 280–282.

(27) Garegg, P. J.; Lindh, I.; Regberg, T.; Stawinski, J.; Strömberg, R.; Henrichson, C. *Tetrahedron Lett.* **1986**, *27*, 4051–4054.

(28) Froehler, B. C.; Ng, P. G.; Matteucci, M. D. *Nucleic Acids Res.* **1986**, *14*, 5399–5407.

(29) Stec, W. J.; Zon, G.; Uznanski, B. *J. Chromatogr.* **1985**, *326*, 263–280.

Scheme 2



R_1=5'-O-monomethoxytrityithymidin-3'-yl
R_2=3'-O-monomethoxytrityithymidin-5'-yl
X=oxygen or sulfur

Table 1. ^{31}P NMR Chemical Shifts (in ppm)[a] and J Coupling Constants (in Hz) for Compounds 4–6[b]

compound	R_P	S_P
4a	7.88 ($^1J_{PH}$ = 719, $^3J_{PH}$ = 7.3) (faster)[c]	9.79 ($^1J_{PH}$ = 717, $^3J_{PH}$ = 7.4) (slower)
4b	8.36 ($^1J_{PH}$ = 717, $^3J_{PH}$ = 7.5) (faster)	9.57 ($^1J_{PH}$ = 715, $^3J_{PH}$ = 9.2) (slower)
4c	70.1 ($^1J_{PH}$ = 669, $^3J_{PH}$ = 9.8) (faster)	71.4 ($^1J_{PH}$ = 666, $^3J_{PH}$ = 9.9) (slower)
5a	51.8 ($^1J_{PSe}$ = 825)	52.1 ($^1J_{PSe}$ = 824)
5b	50.7 ($^1J_{PSe}$ = 824)	51.9 ($^1J_{PSe}$ = 821)
5c	103.2 ($^1J_{PSe}$ = 771)	103.7 ($^1J_{PSe}$ = 776)
6a	49.4 ($^1J_{PSe}$ = 777)	48.6 ($^1J_{PSe}$ = 774)
6b	49.7 ($^1J_{PSe}$ = 779)	48.5 ($^1J_{PSe}$ = 775)
6c	102.3 ($^1J_{PSe}$ = 754)	100.6 ($^1J_{PSe}$ = 754)

[a] Two percent H_3PO_4 in D_2O as external reference. [b] All spectra recorded in pyridine except for 5a–c where D_2O was used as a solvent. [c] "Faster" and "slower" refer to the elution order of the two diastereomers from a silica gel column (see also Experimental Section).

the reverse-phase column as a single, slightly broad, peak (results not shown).

High lipophilicity of oligonucleoside phosphoroselenoates (as manifested, *inter alia*, by their long retention time during reverse-phase HPLC) and their partial instability posed some analytical problems. Since neither reverse-phase nor regular ion-exchange HPLC were able to distinguish between oligomers of the same chain length but with different ratios of phosphoroselenoate to phosphodiester linkages, we used ^{31}P NMR spectroscopy and strong-anion-exchange chromatography with "soft-base" anionic eluents[30] for characterization of the crude dodecathymidine undecaphosphoroselenoate. The latter technique was demonstrated to be efficient for the separation of the oligonucleotide analogues with all phosphorothioate internucleosidic linkages from those containing a small percentage of phosphodiester bonds.

The ^{31}P NMR spectrum of freshly prepared $[Tp(Se)]_{11}T$ (Figure 2a) showed that the conversion of the H-phosphonate functions into phosphoroselenoate linkages with BTSe (1) at the oligomer level was rather an efficient process as judged from the absence of phosphodiester resonances and the presence of one predominant peak in the HPLC elution profile. However, upon standing at room temperature for 5 days resonances from the phosphodiester linkages became detectable in the ^{31}P NMR spectrum and the new pattern of peaks was observed in the HPLC elution profile (Figure 2b). These changes can most likely be attributed to a slow selenium washout as it was observed previously for other oligonucleoside phosphoroselenoates.[9] To substantiate the assumption that the observed changes in the HPLC pattern and in the ^{31}P NMR spectra were due to the loss of selenium rather than due to accompanied hydrolysis, a sample of $[Tp(Se)]_{11}T$ with the HPLC characteristics as in Figure 2b

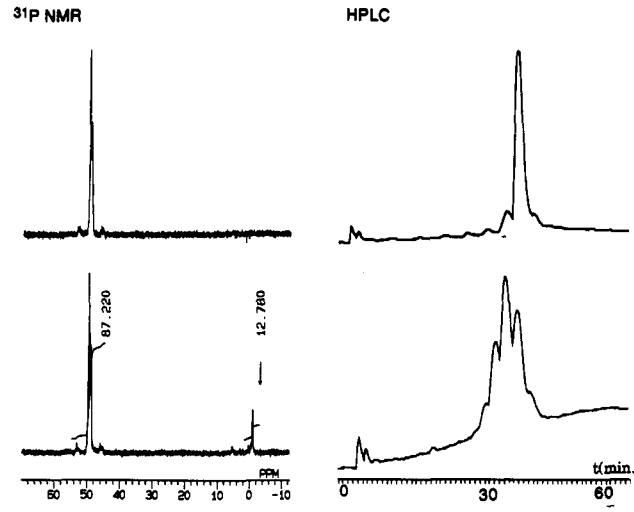


Figure 2. The ^{31}P NMR spectra and the HPLC elution profiles (PL-SAX column, gradient of 1 M KBr in 50 mM K_2HPO_4, water–acetonitrile 9:1, v/v) of dodecathymidine undecaphosphoroselenoate. (a) The crude reaction mixture after selenization of the dodecathymidine undeca-H-phosphonate with BTSe (1) and splitting from the support with aqueous ammonia; (b) the same sample after standing for 5 days at room temperature (arrow in the ^{31}P NMR spectrum indicates resonances from phosphodiesters).

was subjected to treatment with iodine in aqueous pyridine.[31] One major peak corresponding to $(Tp)_{11}T$ (retention time of ~21 min) was observed upon the HPLC analysis which was indicative of the integrity of the oligonucleotidic chain in the oligomer. In agreement with this, a nearly identical HPLC elution profile was obtained when the freshly prepared $[Tp(Se)]_{11}T$ was treated with iodine under the same conditions and also when the crude dodecathymidine undeca-H-phosphonate was oxidized with iodine–water–pyridine[32] to the product $(Tp)_{11}T$ (Figure 3). The latter finding indicates that treatment of the phosphoroselenoates with iodine under these reaction conditions effectively replaces selenium by oxygen without noticeable oligonucleotide bond cleavage. This may be of analytical value for characterization of phosphoroselenoates.

As a final stage of these investigations we evaluated utility of BTSe (1) as a selenium-transfer reagent for phosphite triesters. This can be relevant to selenization of tervalent phosphorus derivatives in the phosphoramidite approach to the synthesis of oligonucleotide analogues. To this end triethyl phosphite was subjected to selenization with 1 under various reaction conditions (Table 2). Progress of the selenization was followed by ^{31}P NMR

(30) Bergot, B. J.; Egan, W. *J. Chromatogr.* **1992**, *599*, 35–42.

(31) Studies on mechanism and kinetics of selenium washout and those of deselenization of phosphorothioselenoates are in progress in this laboratory.

(32) Garegg, P. J.; Regberg, T.; Stawinski, J.; Strömberg, R. *J. Chem. Soc., Perkin Trans. 1* **1987**, 1269–1273.

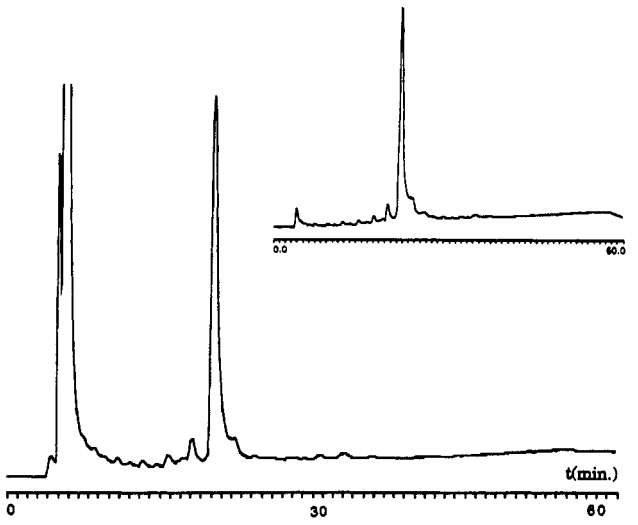


Figure 3. The HPLC chromatogram (PL-SAX column, gradient of 1 M KBr in 50 mM K_2HPO_4) after deselenization of $[Tp(Se)]_{11}T$ with iodine–water–pyridine (the peak at $t_R \sim 21$ min corresponds to $(Tp)_{11}T$ and a strong absorption at $t_R \sim 0$ min was due to non-nucleosidic material). The insert shows the HPLC elution profile of the crude reaction mixture after oxidation of $[Tp(H)]_{11}T$ with iodine–water–pyridine.

Table 2. Reaction Conditions for the Selenization of Triethyl Phosphite with BTSe (1)[a]

entry	solvent	base	time (min)	comments
1	CH_3CN	–	80	precipitation[b]
2	CH_3CN/pyridine, 1:1, v/v	–	160	
3	CH_3CN	$N(Et)_3$ (5%)	5	
4	CH_3CN	DBU (2%)	<5	precipitation[b]
5	pyridine	–	>240	
6	pyridine	$N(Et)_3$ (5%)	100	
7	pyridine	DBU (2%)	<5	precipitation[b]

[a] For reaction conditions, see Experimental Section. [b] Precipitation most likely due to a decomposition of the reagent or due to low solubility of the reaction products from 1 under the reaction conditions.

spectroscopy. In all reactions the disappearance of a signal from triethyl phosphite ($\delta = 139.55$ ppm) was accompanied by formation of a new resonance which on the basis of its chemical shift value, the presence of characteristic satellite signals due to ^{31}P–^{77}Se coupling and multiplicity of signals due to ^{31}P–^{1}H couplings, was assigned to triethyl phosphoroselenoate[33] ($\delta = 71.48$ ppm, $^1J_{PSe} = 941$ Hz, $^3J_{PH} = 9.8$, heptet).

The rate of selenization of triethyl phosphite with BTSe (1) varied depending on the solvent used and the presence of a base in the reaction mixtures. As expected the reaction proceeded with a moderate rate in neat acetonitrile, but it was much faster in the presence of a strong base (Table 2, entries 1 and 3). In contradistinction to H-phosphonate and H-phosphonothioate diesters 4, the selenization of triethyl phosphite with BTSe was considerably slower when acetonitrile was replaced by pyridine (Table 2, entry 6) or when instead of neat acetonitrile its mixture with pyridine was used (Table 2, entries 1 and 2). In the presence of DBU the reaction proceeded at approximately the same rate both in acetonitrile and in pyridine (Table 2, entries 4 and 7). We have noted, however, that DBU promotes decomposition of 1, but this reaction is apparently slower than selenization.[34] Potassium selenocyanate which was previously used for selenization of oligonucleoside phosphite triesters[11] was found to be less reactive than BTSe toward triethyl phosphite under most of the reaction conditions investigated.[35]

Comparing rates of selenization of triethyl phosphite under various reaction conditions and taking into account a compatibility of the latter one with solid-phase synthesis, it seems that the conversion of tervalent phosphorus compounds into the corresponding phosphoroselenoates using BTSe (1) should be most conveniently achieved in acetonitrile in the presence of triethylamine (Table 2, entry 3).

In conclusion, BTSe (1) represents a new source of electrophilic selenium for the conversion of H-phosphonate and H-phosphonothioate diesters into phosphoroselenoates and phosphorothioselenoates, respectively. Phosphite triesters also undergo smoothly selenization with 1. The reagent is stable and easily accessible, and its main advantages are (i) solubility in common organic solvent, (ii) enhanced rate of selenium transfer, (iii) stereospecific mode of selenization (retention of configuration), and (iv) 1 can be used under variety of reaction conditions, also those that are compatible with solid-phase synthesis of oligonucleotide analogues.

Experimental Section

Materials and Methods. Pyridine, acetonitrile, and triethylamine (TEA) were refluxed with CaH_2 and then distilled and stored over molecular sieves (4 Å) or CaH_2 (TEA). 2,2′-Dithiosalicylic acid and triphenylphosphine selenide were commercial grade (Aldrich and Janssen, respectively). TLC analyses were carried out on Merck silica gel 60 F_{254} using chloroform–methanol 9:1 (v/v). Compounds **2a–d** were synthesized according to published procedures.[26,36] Nucleosides and P1 endonuclease were purchased from Sigma. Reactions monitored by ^{31}P NMR were carried out in 10-mm NMR tubes using 25 μmol of phosphorus-containing compounds in 2 mL of an appropriate solvent. HPLC analyses[30] were performed on PL-SAX strong-anion-exchanger column (Polymer Lab) using a gradient of buffer B in buffer A (5–80%, 60 min) with a flow rate of 3 mL/min. Buffer A was 50 mM K_2HPO_4 in water–acetonitrile (9:1, v/v, pH = 6.3) and buffer B, 1 M KBr in 50 mM K_2HPO_4 in water–acetonitrile (7:3, v/v). All nucleosides, nucleoside 3′-H-phosphonates, and nucleoside 3′-H-phosphonothioates were rendered anhydrous by evaporation of added pyridine before used for reactions.

Preparation of 3*H*-1,2-Benzothiaselenol-3-one (1). Method a:[12] To a mixture of 2,2′- dithiosalicylic acid (3.0 g, 10 mmol) in toluene (50 mL) and pyridine (1.6 mL, 20 mmol) was added (phenylseleno)phosphonic dichloride[37] (5.0 mL, 10 mmol). The mixture was refluxed for 2 h and concentrated to an oil, and the residue was purified on a silica gel column using a stepwise gradient of ethyl acetate in petroleum ether (0–10%) as eluent. The appropriate fractions were collected and concentrated, and the solid residue was then recrystallized from ethanol: yield 1.45 g (67%); mp 79 °C.

Method b: 2,2′- Dithiosalicylic acid (1.5 g, 5 mmol) and triphenylphosphine selenide (5.1 g, 15 mmol) were dissolved in dioxane (50 mL) and refluxed for 3 days. The solvent was removed by evaporation and the solid residue was purified as described

(33) Glidewell, C.; Leslie, E. J. *J. Chem. Soc., Dalton Trans.* **1977**, 527–531.

(34) When BTSe (1) was dissolved in pyridine or in acetonitrile containing 2% of DBU, a complete decomposition of the reagent 1 occurred within ~5 min as judged from the formation of a yellow precipitate and a complete loss of selenizing activity of the solution.

(35) Only in pyridine–TEA (Table 2, entry 6) was the rate of selenization with KSeCN close to that with BTSe.

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

(37) Michael, J. P.; Reid, D. H.; Rose, B. G.; Speirs, R. A. *J. Chem. Soc., Chem. Commun.* **1988**, 1494–1496.

above: yield 0.77 g (71%); mp 79 °C; ^{13}C NMR (CD_3CN, 50 °C, δ in ppm) 198.1, 150.5, 134.8, 134.2, 128.1, 127.1, 126.6; ^1H NMR (CD_3CN, δ in ppm) 7.85 (ddd, ^3J = 8.1 Hz, ^4J = 1.5 Hz, ^4J = 0.7 Hz), 7.75 (dt, ^3J = 8.2 Hz, ^4J = 1.1 Hz), 7.68 (ddd, ^3J = 8.2 Hz, ^3J = 6.8 Hz, ^4J = 1.5 Hz), 7.37 (ddd, ^3J = 8.1 Hz, ^3J = 6.8 Hz, ^4J = 1.1 Hz). Anal. Calcd for C_7H_4OSSe: C, 39.1; H, 1.9; S, 14.9; Se, 36.7. Found: C, 39.1; H, 2.0; S, 15.1; Se, 36.9.

5'-*O*-(Monomethoxytrityl)thymidin-3'-yl 3'-*O*-(Monomethoxytrityl)thymidin-5'-yl Phosphonate (4a). The dimer **4a** was synthesized from **2a** (0.748 g, 1.1 mmol) and **3a** (0.514 g, 1.0 mol) and separated into the R_P and S_P diastereomers as described before.[38]

4a (R_P) (faster moving isomer): yield 0.583 g (55%); ^{31}P NMR data, see Table 1; ^1H NMR ($CDCl_3$, δ in ppm) 8.61 and 8.59 (2s, 2 × 1H), 7.5–6.8 (m, 28H) 6.70 (d, $^1J_{PH}$ = 716 Hz, 1H), 6.35 (dd, ^3J = 8.5 and 5.6 Hz, 1H), 6.25 (dd, ^3J = 8.5 and 5.7 Hz, 1H), 5.16 (m, 1H), 4.27 (m, 1H), .4.14 (m, 1H), 4.00 (m, 1H), 3.92 (m, 1H), 3.79 (s, 3H), 3.78 (s, 3H), 3.64 (m, 1H), 3.48 (dd, 1H), 3.33 (dd, 1H), 2.4 (m, 2H), 1.95 (m, 1H), 1.73 (m, 1H), 1.82 and 1.42 (2s, 2 × 3H).

4a (S_P) (slower moving isomer): yield 0.395 g (37%); ^{31}P NMR data, see Table 1; ^1H NMR ($CDCl_3$, δ in ppm, * indicates protons in the thymidin-5'-yl unit of the dimer) 8.58 and 8.47 (2s, 2 × 1H), 7.5–6.8 (m, 28H) 6.77 (d, $^1J_{PH}$ = 714 Hz, 1H), 6.41 (dd, ^3J = 8.8 and 5.5 Hz, 1H), 6.32 (dd, ^3J = 8.8 and 5.5 Hz, 1H), 5.20 (m, 1H), 4.19 (m, 1H, 4.15 (m, 1H), 3.95 (m, 1H), 3.78 (s, 3H), 3.76 (s, 3H), 3.65 (m, 2H), 3.47 (dd, 1H), 3.35 (dd, 1H), 2.45 (m, 2H), 1.94 (m, 1H), 1.65 (m, 1H), 1.85 and 1.40 (2s, 2 × 3H).

5'-*O*-(Dimethoxytrityl)-*N*^{2}-isobutyryldeoxyriboguanosin-3'-yl 3'-*O*,*N*^{3}-Dibenzoylthymidin-5'-yl Phosphonate (4b). The dimer **4b** was synthesized from **2b** (0.172 g, 0.214 mmol) and **3b** (0.098 g, 0.218 mmol) analogously to the described procedure.[38] The R_P and S_P diastereomers of **4b** were separated on a silica gel column using a stepwise gradient of methanol in chloroform (0–10%).

4b (R_P) (faster moving isomer): yield 0.090 g (37%); ^{31}P NMR data, see Table 1; ^1H NMR ($CDCl_3$, δ in ppm) 9.09 (s, 1H), 8.47 (s, 1H), 8.0–6.7 (m, 24H) 7.04 (d, $^1J_{PH}$ = 709 Hz, 1H), 6.50 (dd, ^3J = 8.4 and 5.5 Hz, 1H), 6.13 (dd, ^3J = 8.4 and 5.5 Hz, 1H), 5.50 (m, 1H), 5.35 (m, 1H), 4.70–4.00 (m, 4H), 3.77 (s, 6H), 3.36 (m, 2H), 2.95–2.30 (m, 5H) 2.12 (s, 3H), 1.02 (d, 6H).

4b (S_P) (slower moving isomer): yield 0.103 g (42%); ^{31}P NMR data, see Table 1; ^1H NMR ($CDCl_3$, δ in ppm) 9.11 (s, 1H), 8.0–6.7 (m, 24H) 7.10 (d, $^1J_{PH}$ = 717 Hz, 1H), 6.46 (dd, ^3J = 7.3 and 6.2 Hz, 1H), 6.15 (dd, ^3J = 9.1 and 5.1 Hz), 5.55 (m, 2H), 4.60–4.25 (m, 4H), 3.76 (s, 6H), 3.38 (ddd, 2H), 2.95–2.25 (m, 5H) 2.15 (s, 3H), 1.00 (d, 6H).

5'-*O*-(Monomethoxytrityl)thymidin-3'-yl 3'-*O*-(Monomethoxytrityl)thymidin-5'-yl Phosphonothioate (4c). The dimer **4c** was synthesized from **2c** (0.765 g, 1.1 mmol) and **3a** (0.514 g, 1.0 mol) essentially as described before[38] but using diphenyl phosphorochloridate[39] (3 equiv) as a condensing agent.

4c (R_P) (faster moving isomer): yield 0.468 g (43%); ^{31}P NMR data, see Table 1; ^1H NMR ($CDCl_3$, δ in ppm, * indicates protons in the thymidin-5'-yl unit of the dimer) 8.69 and 8.67 (2s, 2 × 1H), 7.61 (d, $^1J_{PH}$ = 668 Hz, 1H), 7.5–6.8 (m, 28H), 6.34 (dd, ^3J = 8.2 and 5.8 Hz, 1H), 6.25 (dd, ^3J = 8.4 and 5.8 Hz, 1H), 5.44 (m, 1H), 4.27 (m, 1H), 4.14 (m, 1H), 4.00 (m, 2H), 3.79 and 3.78 (2s, 2 × 3H), 3.56 (m, 1H), 3.41 (m, 1H), 3.33 (dd, 1H), 2.3 (m, 2H), 2.00 (m, 1H), 1.75 (m, 1H), 1.86 and 1.47 (2s, 2 × 3H).

4c (S_P) (slower moving isomer): yield 0.573 g (52%); ^{31}P NMR data, see Table 1; ^1H NMR ($CDCl_3$, δ in ppm) 8.68 and 8.53 (2s, 2 × 1H), 7.67 (d, $^1J_{PH}$ = 664 Hz, 1H), 7.5–6.8 (m, 28H), 6.40 (dd, ^3J = 8.8 and 5.5 Hz, 1H), 6.34 (dd, ^3J = 8.8 and 3.3 Hz, 1H), 5.45 (m, 1H), 4.20 (m, 1H), 4.06 (m, 1H), 3.91 (m, 1H), 3.79 and 3.78 (2s, 6H), 3.65 (m, 2H), 3.41 (m, 2H, 2.45 (m, 2H), 1.97 (m, 1H), 1.68 (m, 1H), 1.87 and 1.45 (2s, 2 × 3H).

Thymidin-3'-yl Thymidin-5'-yl Phosphoroselenoate, Sodium Salt (6a). To a solution of 5'-*O*-(monomethoxytrityl)-thymidin-3'-yl 3'-*O*-(monomethoxytrityl)thymidin-5'-yl phosphonate (**4a**) (0.161 g, 0.15 mmol) in pyridine (7.5 mL) was added 3*H*-1,2-benzothiaselenol-3-one (0.043 g, 0.20 mmol) followed by triethylamine (55.6 μL, 0.40 mmol). After 15 min the solvent was removed by evaporation and the residue was purified on a short silica gel column using a stepwise gradient of methanol in chloroform (5–15%, v/v). The purified selenophosphate was detritylated with 80% aqueous acetic acid (2 h) and the solvent was removed by evaporation. The residue was dissolved in water (1 mL) and passed through a short Dowex 50X-2 (H^+) and then through an Amberlyst (Na^+) column. Water was removed by lyophilization, the residue desalted on a Sephadex G 10 column and lyophilized again.

6a (S_P) [from **4a** (R_P)]: yield 0.060 g (63%); ^{31}P NMR data, see Table 1; ^1H NMR (D_2O, δ in ppm) 7.75 and 7.68 (2s, 2 × 1H), 6.33 (t, ^3J = 6.8, 1H), 6.24 (t, ^3J = 6.8, 1H), 5.03 (m, 1H), 4.61 (m, 1H), 4.25–4.15 (m, 4H), 3.85 (m, 4H), 2.60–2.35 (m, 4H), 1.94 and 1.90 (2s, 2 × 3H); HRMS (FAB) calcd for $C_{20}H_{26}N_4O_{11}PSe$ ($M - Na^+$) 609.0502, found ($M - Na^+$) 609.0512. Anal. Calcd for $C_{20}H_{26}N_4O_{11}PSeNa \cdot 1.5H_2O$: C, 36.5; H, 4.4; N, 8.5. Found: C, 36.2; H, 4.2; N, 8.3.

6a (R_P) [from **4a** (S_P)]: yield 0.070 g (74%); ^{31}P NMR data, see Table 1; ^1H NMR (D_2O, δ in ppm) 7.74 and 7.67 (2s, 2 × 1H), 6.32 (t, ^3J = 7.0, 1H), 6.23 (t, ^3J = 6.8, 1H), 5.03 (m, 1H), 4.59 (m, 1H), 4.25–4.18 (m, 4H,), 3.85 (m, 4H), 2.64–2.30 (m, 4H), 1.96 and 1.89 (2s, 2 × 3H); HRMS (FAB) calcd for $C_{20}H_{26}N_4O_{11}PSe$ ($M - Na^+$) 609.0502, found ($M - Na^+$) 609.0507. Anal. Calcd for $C_{20}H_{26}N_4O_{11}PSeNa \cdot 1.5H_2O$: C, 36.5; H, 4.4; N, 8.5. Found: C, 36.5; H, 4.2; N, 8.4.

Deoxyriboguanosin-3'-yl Thymidin-5'-yl Phosphoroselenoate, Sodium Salt (6b). To a solution of 5'-*O*-(dimethoxytrityl)-*N*^{2}-isobutyryldeoxyriboguanosin-3'-yl 3'-*O*,*N*^{3}-dibenzoylthymidin-5'-yl phosphonate (**4b**) (0.050 g, 0.044 mmol) in pyridine (2 mL) was added 3*H*-1,2-benzothiaselenol-3-one (0.019 g, 0.088 mmol) followed by triethylamine (24.5 μL, 0.176 mmol). After 15 min the solvent was removed by evaporation and the residue dissolved in 80% aqueous acetic acid (5 mL). When the detritylation reaction was over (2 h) the solution was evaporated to dryness and the traces of acetic acid were removed by evaporation of added toluene. The solid residue was purified on a short silica gel column using a stepwise gradient of chloroform–methanol (95:5–80:20, v/v). The fractions containing the product were collected and subjected to the ammonia treatment overnight (5 mL of 32% aqueous ammonia–ethanol, 3:1, v/v). The reaction mixture was evaporated to dryness, the residue partitioned between water and diethyl ether, and the aqueous layer evaporated. The conversion of the phosphoroselenoate into sodium salt was carried out as described for **6a**.

6b (S_P) [from **4b** (R_P)]: yield 0.014 g (49%); ^{31}P NMR data, see Table 1; ^1H NMR (D_2O, δ in ppm) 7.95 (s, 1H), 7.61 (s, 1H), 6.28 (t, ^3J = 7.1, 1H), 6.23 (t, ^3J = 6.3, 1H), 5.15 (m, 1H), 4.58 (m, 1H), 4.33 (m, 1H), 4.25–4.10 (m, 3H), 3.86 (m, 2H), 2.75 (m, 2H), 2.34 (m, 2H), 1.81 (s, 3H); HRMS (FAB) calcd for $C_{20}H_{25}N_7O_{10}PSe$ ($M - Na^+$) 634.0567, found ($M - Na^+$) 634.0549.

6b (R_P) [from **4b** (S_P)]: yield 0.015 g (52%); ^{31}P NMR data, see Table 1; ^1H NMR (D_2O, δ in ppm) 7.96 (s, 1H), 7.65 (s, 1H), 6.30 (t, ^3J = 6.8, 1H), 6.23 (t, ^3J = 6.8, 1H), 5.12 (m, 1H), 4.60 (m, 1H), 4.31 (m, 1H), 4.25–4.15 (m, 3H), 3.84 (m, 2H), 2.75 (m, 2H) 2.34 (m, 2H), 1.81 (s, 3H); HRMS (FAB) calcd for $C_{20}H_{25}N_7O_{10}PSe$ ($M - Na^+$) 634.0567, found ($M - Na^+$) 634.0570.

Thymidin-3'-yl Thymidine-5'-yl Phosphorothioselenoate, Sodium Salt (6c). To a solution of 5'-*O*-(monomethoxytrityl)-thymidin-3'-yl 3'-*O*-(monomethoxytrityl)thymidin-5'-yl phosphonothioate (**4c**) (0.164 g, 0.15 mmol) in pyridine (7.5 mL) was added 3*H*-1,2-benzothiaselenol-3-one (0.043 g, 0.20 mmol) followed by triethylamine (55.6 μL, 0.40 mmol). After 15 min the solvent was removed by evaporation and the residue purified on a short silica gel column using a stepwise gradient of methanol in chloroform (5–15%, v/v). The removal of monomethoxytrityl groups and the conversion into sodium salt was carried out as described above for **6a**.

6c (S_P) [from **4c** (R_P)]: yield 0.055 g (57%); ^{31}P NMR data, see Table 1; ^1H NMR (D_2O, δ in ppm) 7.77 and 7.66 (2s, 2 × 1H), 6.33 (t, ^3J = 7.0, 1H), 6.23 (t, ^3J = 6.8, 1H), 5.17 (m, 1H), 4.61 (m, 1H), 4.3–4.1 (m, 4H), 3.90 (dd, 1H), 3.82 (dd, 1H), 2.60 (m, 1H), 2.45–2.30 (m, 3H), 1.96 and 1.88 (2s, 2 × 3H); HRMS (FAB) calcd for $C_{20}H_{26}N_4O_{11}PSSe$ ($M - Na^+$) 625.0273, found ($M - Na^+$) 625.0285.

(38) Stawinski, J.; Strömberg, R.; Zain, R. *Tetrahedron Lett.* **1992**, *33*, 3185–3138.
(39) Stawinski, J.; Thelin, M.; Zain, R. *Tetrahedron Lett.* **1989**, *30*, 2157–2160.

6c (R_P) [from **4a** (S_P)]: yield 0.058 g (60%); ^{31}P NMR data, see Table 1; ^{1}H NMR (D_2O, δ in ppm) 7.77 and 7.66 (2s, 2 × 1H), 6.32 (t, ^3J = 6.8, 1H), 6.23 (t, ^3J = 6.8, 1H), 5.15 (m, 1H), 4.60 (m, 1H), 4.3–4.2 (m, 4H), 3.86 (m, 2H), 2.60 (m, 1H), 2.45–2.30 (m, 3H), 1.96 and 1.87 (2s, 2 × 3H); HRMS (FAB) calcd for $C_{20}H_{28}N_4O_{11}PSSe$ (M − Na^+) 625.0273, found (M − Na^+) 625.0280.

Enzymatic Hydrolysis of 6a–c Using P1 Endonuclease. One milligram of each diastereomer of the phosphorothioselenoates **6a–c** were dissolved in a buffer solution (400 μL, obtained by mixing 380 μL of 30 mM $(NH_4)_2SO_4$ and 20 μL of 10 mM $ZnSO_4$, pH = 5.3) and a stock solution of P1 endonucleases (100 μL, 255 units in 760 μL of 30 mM $(NH_4)_2SO_4$ and 40 μL of 10 mM $ZnSO_4$) was added. The samples were kept at 37 °C overnight. After that time the TLC analysis (silica gel plates, 2-propanol–1 M triethylammonium bicarbonate, 9:1, v/v) revealed that the diastereomers of **6a** and **6b** derived from the faster moving isomers of **4a** and **4b** were substrates for the enzyme while the opposite diastereomers (designated later as (R_P)-**6a** and (R_P)-**6b**), were not. Both diastereomers of the phosphorothioselenoate **6c** were resistant toward P1 endonuclease under the reaction conditions.

Solid-Phase Synthesis of Dodecathymidine Undecaphosphoroselenoate [Tp(Se)]$_{11}$T. The syringe technique was used starting with 0.10 g of the solid support (CPG 500 Å) loaded with 5′-*O*-(dimethoxytrityl)-3′-*O*-succinylthymidine (24 μmol/gram). The reactions were carried out in a Hamilton gas-tight 5-mL syringe equipped with a sinter glass at the base. The standard H-phosphonate method was used for the assembly of the oligonucleotidic chain.[27] The protocol for the synthesis was as follows (cycle name, reagent, time): (i) detritylation, 2% dichloroacetic acid in dichloroethane (4 × 1 mL), 3.5 min; (ii) washing, dichloroethane (3 × 1 mL), pyridine–acetonitrile 1:1 (3 × 1 mL), 2 min; (iii) coupling, **2d** (60 mM in pyridine–acetonitrile 1:1, 600 μL) and pivaloyl chloride (180 mM in pyridine–acetonitrile 1:1, 600 μL), 1.5 min; (iv) washing, pyridine–acetonitrile 1:1 (3 × 1 mL), dichloroethane (3 × 1 mL), 2 min. The coupling yield per step was 98.5–99.5% as judged from the trityl assay. On completion of the cycles, the support-bound oligomer containing H-phosphonate internucleosidic bonds was selenized with 1 (0.1 M in pyridine–triethylamine, 95:5, v/v) for 30 min and then washed successively with pyridine–acetonitrile 1:1 (3 × 1 mL) and dichloroethane (3 × 1 mL). To check the efficiency of assembly of the oligonucleotidic chain, a part of the support-bound oligonucleoside H-phosphonate was oxidized with 2% iodine in pyridine–water 98:2.[27] After selenization or oxidation with iodine, the oligomer was cleaved from the support by treatment with 33% aqueous ammonia–ethanol (3:1, v/v) for 2 h. The purity of the dodecathymidine phosphoroselenoate [Tp(Se)]$_{11}$T was checked by ^{31}P NMR and HPLC.

Deselenization of the Dodecathymidine Undecaphosphoroselenoate {[Tp(Se)]$_{11}$T → (Tp)$_{11}$T}. To a solution of [Tp(Se)]$_{11}$T in water (1 mL, ~20 OD) was added iodine (1 M in pyridine) in small portions until no further decolorization of added iodine solution was observed. Aliquots were taken after 5, 10, 15 and 30 min, evaporated, and then partitioned between water and carbon tetrachloride. The aqueous layer was collected and checked by HPLC. It was found that the conversion of [Tp(Se)]$_{11}$T to (Tp)$_{11}$T was complete within 5 min of iodine treatment (see Figure 3). No further changes in the HPLC elution profiles were observed after the prolonged iodine treatment (30 min).

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