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Diastereomers of Nucleoside 3′-*O*-(2-Thio-1,3,2-oxathia(selena)phospholanes): Building Blocks for Stereocontrolled Synthesis of Oligo(nucleoside phosphorothioate)s†

Wojciech J. Stec,* Andrzej Grajkowski, Anna Kobylańska, Bolesław Karwowski, Maria Koziołkiewicz, Konrad Misiura, Andrzej Okruszek, Andrzej Wilk, Piotr Guga, and Małgorzata Boczkowska

Contribution from the Polish Academy of Sciences, Centre of Molecular and Macromolecular Studies, Department of Bioorganic Chemistry, Sienkiewicza 112, 90-363 Łódź, Poland

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Abstract: Diastereomerically pure 5′-*O*-DMT-nucleoside 3′-*O*-(2-thio-1,3,2−oxathiaphospholanes) (**3**) and their oxathiaphospholane ring-substituted analogues (**20**) were used for the synthesis of stereoregular oligo(nucleoside phosphorothioate)s (S-Oligos). The oxathiaphospholane ring-opening condensation requires the presence of strong organic base, preferably DBU. The yield of a single coupling step is ca. 95% and resulting S-Oligos are free of nucleobase- and sugar-phosphorothioate backbone modifications. The diastereomeric purity of products was estimated on the basis of diastereoselective degradation with Nuclease P1 and a mixture of snake venom phosphodiesterase and *Serratia marcescens* endonuclease. Thermal dissociation studies of heteroduplexes S-Oligos/DNA and S-Oligos/RNA showed that their stability is stereochemistry- and sequence-dependent.

Introduction

Antisense oligonucleotides complementary to selected regions of mRNA may inhibit biosynthesis of those proteins encoded or controlled by genetic information included in these particular parts of mRNA. This principle is the basis for a new strategy in medicinal chemistry to combat diseases caused by biosynthesis of mutant or unwanted proteins. For this application synthetic oligonucleotides (typically bearing 15−30 nucleobases) should be resistant to cellular nucleolytic activities. Therefore, many analogues of oligonucleotides have been synthesized and tested in this "antisense mRNA" strategy.[1] So far, the most promising results[2] have been obtained using oligo(nucleoside phosphorothioate)s (S-Oligos), analogues of natural oligonucleotides bearing a phosphorothioate function at each internucleotide position.

The ability of S-Oligos to inhibit the biosynthesis of proteins *in vitro* has been tested using several model systems, and some encouraging results were reported showing the efficacy of S-Oligos *in vivo*.[3] The reports of successful protection of

† Abbreviations: ATP, adenosine 5′-triphosphate; Bz, benzoyl; DCA, dichloroacetic acid; DMAP, 4-(dimethylamino)pyridine; DMT, dimethoxytrityl; DPC, diphenylcarbamoyl; EI, electron impact; FAB, fast atom bombardment; iBu, isobutyryl; LCA CPG, long chain alkylamino controlled pore glass; MS, mass spectrometry; PAGE, polyacrylamide gel electrophoresis; RP HPLC, reverse phase high performance liquid chromatography; TLC, thin layer chromatography; Tris-Cl, tris(hydroxymethyl)aminomethane hydrochloride.

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animals against several types of cancer *via* inhibition of oncogenes by means of complementary S-Oligos continue to grow.[4] In addition the first clinical trials combating AIDS in humans have commenced.[2,5] These results demonstrate the specific recognition of selected mRNA (or pre-mRNA) by administered S-Oligos, and suppression of the translation process by regioselective cleavage of mRNA (involved in heterodimer mRNA/S-Oligo) by ribonuclease H.[6]

Although the detailed mode of action of S-Oligos is still a matter of dispute, or even controversy,[7] rational drug design based upon blocking biological information included in low-copy number mRNA is appealing and worthy of further study. A still unsolved and often unappreciated problem concerning the use of S-Oligos in the antisense strategy is their polydiastereoisomerism.[8] Replacement of one of two nonbridging oxygens at phosphorus by sulfur induces asymmetry at the phosphorus atom. Current synthetic methods used to prepare S-Oligos are nonstereospecific. Therefore, the product consists of a mixture of 2^n diastereomers (n is the number of internucleotide phosphorothioate functions in an oligonucleotide bearing $n+1$ bases). Because molecular recognition is often dependent on the chirality of the substrate, a hypothesis has been formulated that the biological activity of the S-Oligo diastereomers may be stereodependent. This suggests that for S-Oligos receptor-mediated transport through cell membranes, interaction with intracellular proteins and, finally, recognition of the target mRNA may all be stereoselective processes, which can either counterbalance or enhance biological effects of a diastereomeric pool administered to the biological system.

An effective concentration of S-Oligos (e.g., 25 mer) to confer cellular protection from a lethal pathogen (e.g., virus) is usually within the range of 0.25−25 μM.[3d,e] Since the total number of diastereomers in this case is 2^{14}= 16 384, the effective concentration might be significantly lower if the administered S-Oligos consisted of more active diastereomers. Unfortunately, which one(s) of the 2^n components of the mixture are most effective biologically have yet to be determined. However, even if identified, the desired isomer(s) cannot be isolated from less active components by any known physical method. In this report, we present our efforts toward stereocontrolled synthesis of S-Oligos of medium size suitable for biological activity testing in comparison with "random mixture of diastereomers".[9]

Historically, the first enzymatic synthesis of poly(ribonucleoside phosphorothioate)s described in the literature[10] has proven to be a fully stereoselective process leading to species bearing phosphorothioates of [R_p]-configuration at each internucleotide position. This stereoselectivity was confirmed for all types of polymerases accepting nucleoside 5′-α-thiotriphosphates as substrates.[11] Moreover, the effectiveness of enzymatic synthesis has been so far shown only for polynucleotides.[12] Only recently Dervan et al.,[13a] and independently Agrawal et al.[13b] synthesized [all-R_p]-oligo(nucleoside phosphorothioate)s of predetermined size and base-sequence.

Limited stereoselectivity of the chemical synthesis of dinucleoside-3′,5′-phosphorothioates has been observed since their first preparation.[14] Early attempts at the stereocontrolled synthesis of S-Oligo, starting from diastereomerically pure nucleoside 3′-*O*-(N,N-diisopropyl *O*-methylphosphoroamidites) (**1**), were unsuccessful because tetrazole, the catalyst for the condensation of **1** with 5′-OH-nucleosides, caused rapid epimerization of the P-chiral substrate.[15] Hata's efforts to develop the stereoselective synthesis of dinucleoside-3′,5′-phosphorothioates were only partially successful.[16] Cosstick and Williams obtained similar results by stereoselective activation of pro-P-chiral nucleoside 3′-*O*-(*S*-alkyl phosphorothioates).[17] Lesnikowski et al. performed a successful synthesis based on stereospecific nucleophilic substitution of the p-nitrophenoxyl group in nucleoside 3′-*O*-(S-alkyl-*O*-*p*-nitrophenyl phosphorothioates) (**2**) by 5′-OH-nucleoside activated by *t*-BuMgCl.[18] This approach was used for in solution synthesis of tetra(thymidine phosphorothioate) (all-R_p- and all-S_p-tetramers). However, this methodology could not be adapted to solid phase synthesis, thereby limiting its utility for the preparation of S-Oligos applicable to the antisense-strategy.

Results and Discussion

Ring-Opening Condensation. Our earlier attempts at the stereospecific synthesis of nucleoside 3′-*O*-[^{16}O,^{17}O,^{18}O]-phosphates[19] showed that diastereomerically pure thymidine 3′-*O*-(*O*-*p*-nitrophenyl phosphorothioate) reacted with styrene [^{18}O]oxide in the presence of [^{17}O]water is converted into 5′-thymidine 3′-*O*-[^{16}O,^{17}O,^{18}O]phosphate with a high degree of stereoselectivity. Our hypothesis that in this reaction thymidine 3′-*O*-(2-oxo-1,3,2-oxathiaphospholane) is formed as an intermediate has been further confirmed *via* ^{31}P NMR.[19e] Both oxathiaphospholane formation and subsequent ring opening with [^{17}O]water were stereospecific processes. This observation led us to assume that 5′-*O*-DMT-nucleoside 3′-*O*-(2-thio-1,3,2-oxathiaphospholane) (**3**) may react with alcohols in a stereospecific manner.[20] The synthesis of compound **3** is depicted in

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Scheme 1

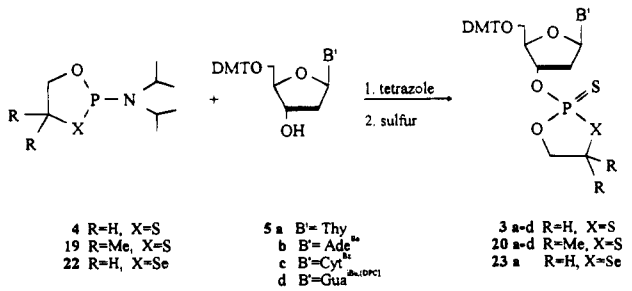

Table 1. Characteristics of Oxathiaphospholane Monomers **3** and **20**

B′	yield (%)	composition (fast:slow)	^{31}P NMR (ppm, CD_3CN)	TLC[a]
3a Thy	80	54:46	105.89 (fast)	0.30 (fast)
			105.95 (slow)	0.26 (slow)
3b Ade^{Bz}	82	49:51	105.58 (fast)	0.43 (fast)
			105.53 (slow)	0.37 (slow)
3c Cyt^{Bz}	82	55:45	105.86 (fast)	0.32 (fast)
			105.81 (slow)	0.28 (slow)
3d Gua^{iBu,DPC}	83	64:36	105.70 (fast)	0.30 (fast)
			105.46 (slow)	0.23 (slow)
20a Thy	82	52:48	108.1 (slow)	0.36 (slow)
			107.7 (fast)	0.41 (fast)
20b Ade^{Bz}	80	48:52	107.7 (slow)	0.43 (slow)
			107.4 (fast)	0.48 (fast)
20c Cyt^{Bz}	82	46:54	108.0 (slow)	0.39 (slow)
			107.6 (fast)	0.45 (fast)
20d Gua^{iBu}	78	47:53	108.1 (slow)	0.60[b]
			107.7 (fast)	(slow + fast)

[a] TLC analysis was performed on HP TLC plates (Merck). Developing systems: for **3** B = Thy, Gua^{iBu,DPC}—butyl acetate; B = Ade^{Bz}, Cyt^{Bz}—ethyl acetate; for **20** B = Thy—butyl acetate/benzene 1:1, B = Ade^{Bz},Cyt^{Bz}—ethyl acetate/benzene 2:1, B = Gua^{iBu}—chloroform/methanol 9:1. [b] Many developing systems were tested, but we were not able to achieve separation of the diastereomers of **20d** on HP TLC.

Scheme 1. Reaction of 2-chloro-1,3,2-oxathiaphospholane[21] with 2 molar equiv of diisopropylamine gave 2-*N*,*N*-diisopropylamino-1,3,2-oxathiaphospholane (**4**), which upon reaction with 5′-*O*-DMT-nucleoside (**5**) in the presence of tetrazole (phosphitylation), followed by addition of sulfur, gave the desired oxathiaphospholane **3**. The compounds **3a**−**d** are formed as mixtures of diastereomers in the ratio ca 55:45 (^{31}P NMR assay). Chromatographic purification on silica gel gave pure **3a**−**d** in 80−83% yield (Table 1).

It appeared that reaction between **3** (B′ = Thy) and 3′-*O*-protected nucleoside **6a** (B′ = Thy, R = Ac) required the presence of a base-catalyst (Scheme 2). However, a stoichiometric amount of triethylamine resulted in only 5% yield of the desired 3′,5′-diprotected dinucleoside-3′,5′-phosphorothioate (**8**) after 0.5 h. We subsequently found that a 5-fold molar excess of 1,4-diazabicyclo[5.4.0]undec-7-ene (DBU) effectively promotes the reaction, and after 5 min compound **8** was obtained in 95% yield.

This result suggested that the ring-opening condensation followed by spontaneous elimination of ethylene episulfide may be useful for generation of internucleotide phosphorothioate linkages under conditions adaptable to the requirements of solid phase synthesis. Compound **3** was reacted with immobilized thymidine (**6b**, B′ = Thy, R = $COCH_2CH_2CO$-LCA-CPG) to give the desired dinucleotide, but a high molar excess of **3** (20-fold) and DBU (220-fold) was necessary to complete the reaction in a reasonable time (<5 min) with sufficient yield

Scheme 2

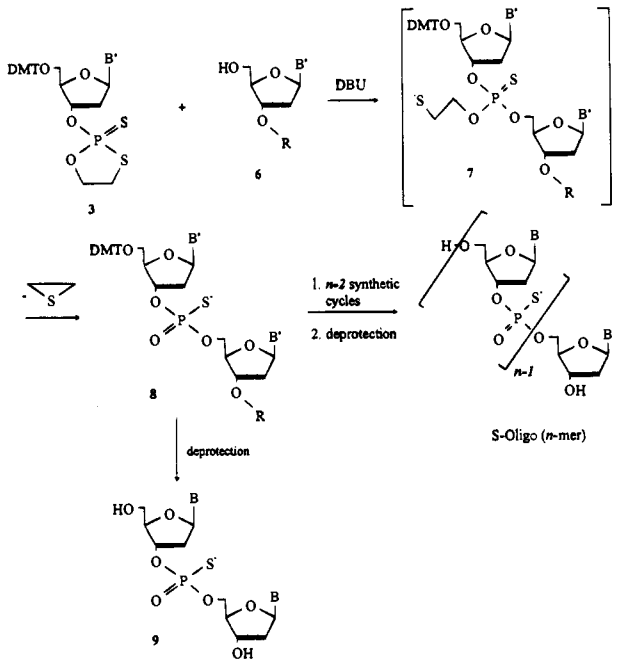

Table 2. S-Oligos Prepared *via* the Oxathiaphospholane Method (Obtained as the "Random Mixture of Diastereomers[1a]")

sequence	DMT$^+$ yield[a]	preparative yield[b] (OD_{260})	purity[c] (%)
10 d[$(T_{PS})_{11}$T][d]	96.6	26.5	>98
d[$(T_{PS})_{11}$T][e]	91.3	32.0	>98
11 d[$(A_{PS})_{11}$A][d]	96.4	16.0	81
d[$(A_{PS})_{11}$A][e]	95.0	16.0	84
12 d[$G_{PS}G_{PS}G_{PS}A_{PS}A_{PS}T_{PS}T_{PS}C_{PS}C_{PS}$C][d]	92.0	9.7	92
13 d[$(T_{PS}T_{PS}G_{PS}G_{PS})_2$]$T_{PS}T_{PS}G_{PS}$G[d]	93.0	6.0	80
14 d[$(C_{PS})_{27}$C][d]	ND	7.8	82[f]

[a] Average repetitive yield calculated on the basis of dimethoxytrityl cation absorbance decay measured at 504 nm. [b] 1 μmol scale of synthesis. [c] Determined from 20% PAGE analysis of samples labeled with ^{32}P at the 5′-end of the corresponding S-Oligo (after double HPLC purification). [d] Synthesized from **3**. [e] Synthesized from **20**. [f] Determined from 20% PAGE analysis of the crude sample labeled with ^{32}P at the 5′-end.

(ca. 95%). The resulting diastereomeric mixture of dinucleoside-3′,5′-phosphorothioate (**9**, B = Thy, R_p:S_p ratio = 55:45) was analyzed by RP-HPLC and proved to be identical with T_{PS}T prepared by the conventional method. As described by Brown et al.,[22] DBU partially cleaves the standard linker (−$COCH_2CH_2CO$-LCA−) and releases the dinucleotide from the solid support. Therefore, all four 5′-*O*-DMT-nucleosides (N-protected if necessary) were immobilized on controlled pore glass *via* a DBU-resistant sarcosinyl-succinoyl linker to give **6c** (R = $COCH_2CH_2CON(Me)CH_2CO$-LCA-CPG).[22] Compounds **8** (B′ = Thy, Ade^{Bz}, Cyt^{Bz}, or Gua^{iBu,DPC}) derived from **6c** were successively detritylated and reacted with appropriate monomers **3**, and after *n*−2 synthetic cycles, followed by final deprotection, corresponding *n*-mer S-Oligos were obtained (see Table 2). The protocol of synthesis is presented in Table 3. The methylene chloride wash following the coupling step was found to be

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Table 3. Synthetic Protocol for the Automated Solid Phase Synthesis of S-Oligos Using Monomers **3** or **20**

step	reagent or solvent	vol. (mL)	purpose	time (s)
1	a) dichloroacetic acid in CH_2Cl_2	2.3	detritylation	50
	(b) acetonitrile	7.0		150
2	(a) activated **3** or **20** in CH_3CN[a]	0.6	coupling	220[b]
	(b) methylene chloride	3.33	wash	200
	(c) acetonitrile	7.0	wash	150
3	(a) DMAP/Ac_2O/lutidine in THF	0.33	capping	20
	(b) acetonitrile	7.0	wash	150

[a] For the 1 μmol scale, a mixture of 450 μL of 0.5 M DBU in CH_3CN and 150 μL of 0.1 M **3** or **20** in CH_3CN was used. [b] In the case of **20** the coupling time was 320 s.

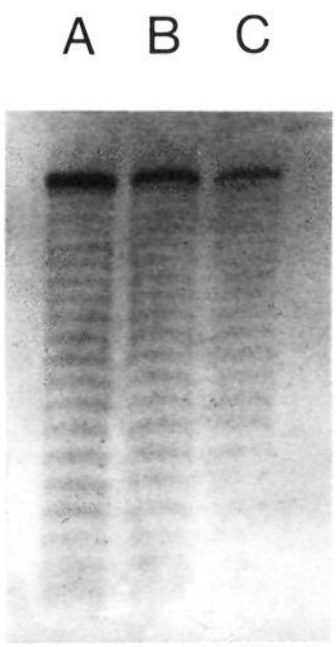

Figure 1. Electrophoretic analysis of crude d[$(C_{PS})_{27}$C] (**14**). Visualization of the bands was done with "Stains-all" (Fluka BioChemica). Consecutive lanes contained 1.2, 0.8, and 0.4 OD units of **14**.

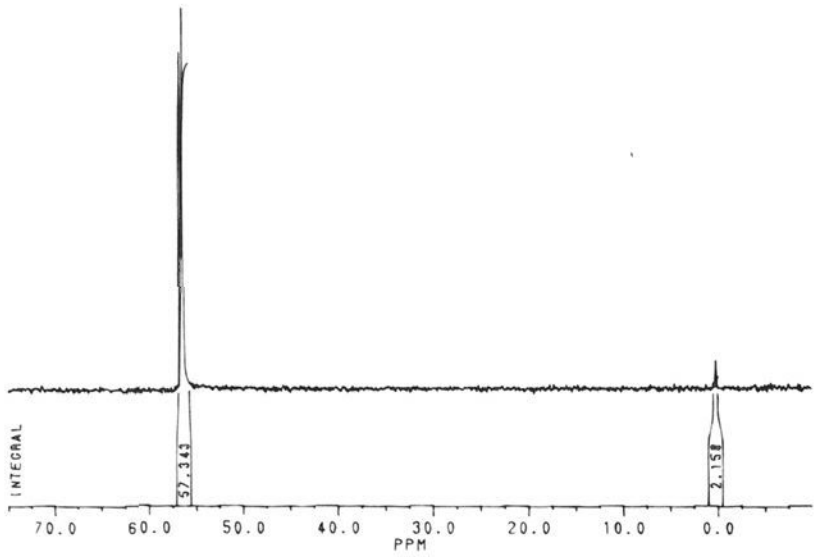

Figure 2. ^{31}P NMR (81 MHz for ^{31}P) spectrum of crude d[$(C_{PS})_{27}$C] in CD_3CN. Parameters: aquisition time 0.803 s, sweep width 10 200 Hz, time domain 16K, Fourier transformation in 16K, 4174 scans, line broadening 0.5 Hz.

necessary to avoid clogging of the lines by a deposit formed from some decomposition of monomer **3** and DBU, which are premixed in the delivery line. The efficiency of this method was shown by the synthesis of d[$(C_{PS})_{27}$C], which was obtained in a 3.8% yield with a purity of 82% (defined as the content of 28 mer). Examination of the ^{31}P NMR spectra of crude d[$(C_{PS})_{27}$C] (Figure 2) revealed that in addition to the expected phosphorothioate resonances, the product also contained ca. 3.5% phosphodiester resonances found at δ 0.11 ppm. The mechanism for contaminant formation remains unknown, but it has been found that careful distillation of dichloroacetic acid (a detritylating reagent) to a certain extent prevents this process. The goal of our research was to develop a stereocontrolled

Table 4. Diastereomeric Purity (DP) of Dinucleoside Phosphorothioates ($N_{PS}N'$, **9**) Prepared by the Oxathiaphospholane Method

		DP of **9**[b] (%)			
N	DP of monomers **3**[a]	N′= A	N′ = G	N′ = C	N′ = T
A	B′ =Ade^{Bz}				
	99.5% fast	99.5 (S_p)	99.5 (S_p)	99.6 (S_p)	99.5 (S_p)
	99.0% slow	99.4 (R_p)	99.3 (R_p)	99.0 (R_p)	99.4 (R_p)
G	B′ = G^{iBu,DPC}				
	99.5% fast	99.8 (S_p)	99.7 (S_p)	100.0 (S_p)	99.8 (S_p)
	98.1% slow	98.2 (R_p)	98.5 (R_p)	98.6 (R_p)	96.6 (R_p)
C	B′ = C^{Bz}				
	99.5% fast	99.8 (S_p)	100.0 (S_p)	100.0 (S_p)	99.8 (S_p)
	98.5% slow	98.7 (R_p)	98.8 (R_p)	98.8 (R_p)	98.8 (R_p)
T	B′ = T				
	99.5% fast	99.0 (S_p)	98.4 (S_p)	99.3 (S_p)	99.1 (S_p)
	99.5% slow	98.2 (R_p)	97.5 (R_p)	98.0 (R_p)	98.1 (R_p)

[a] DP of **3** estimated from integration of ^{31}P NMR signals. [b] DP estimated from integration of RP-HPLC peaks; column ODS Hypersil (5 μm) 250 × 4.6 mm, buffer A: 0.1 M triethylammonium bicarbonate (TEAB), buffer B: 40% CH_3CN in 0.1 M TEAB, gradient 3% of B/min over 5 min, then 1% of B/min.

method for S-Oligos synthesis. Therefore, compounds **3a−d** were chromatographically separated into their individual diastereomers. Column chromatography was performed on silica gel (Merck 60H) with ethyl acetate (for B′ = Ade^{Bz}, C^{Bz}) or butyl acetate (for B′ = T, G^{iBu,DPC}) as eluents. The separation was laborious. For example, only 200 mg of "fast"-eluting and 150 mg of "slow"-eluting pure diastereomers of **3b** (B′ = Ade^{Bz}) were obtained from 1 g of the mixture after a 5-fold run of each partially enriched fraction through the column. The diastereomeric purity of the separated monomers was determined by ^{31}P NMR. Condensation of pure diastereomers **3a−d** with 3′-*O*-immobilized nucleosides **6c** gave the corresponding dinucleotides **9**. Their diastereomeric purity, determined by RP HPLC after ammoniolytic cleavage from the support and base deprotection, proved the coupling process was 98+% stereoselective (see Table 4).

The "fast"-eluting diastereomers of **3a−d** are precursors for [S_p]-dinucleoside-3′,5′-phosphorothioates, while "slow"-eluting diastereomers of **3** are precursors for the [Rp]-counterparts, as verified by chromatographic and spectroscopic comparison of obtained products with genuine samples of dinucleoside 3′,5′-phosphorothioates and also by enzymatic digestions with svPDE (S_p-protective)[23] and nuclease P1 (R_p-protective).[24]

Starting from pure diastereomers of **3**, several "stereoregular" oligonucleotides have been synthesized (Table 5). These oligonucleotides were purified *via* a two-step RP-HPLC chromatography (DMT-on and DMT-off). The chain length integrity was assessed by polyacrylamide gel electrophoresis (see Figure 3). From the data in Table 5 as well as from other results[25] it has been concluded that in the case of medium size S-oligos (e.g., 15-mers) the final yield was *ca.* three times lower than that of oligomers produced by the phosphoramidite/sulfurization process, which is not stereospecific.

Attempted Improvements of the Oxathiaphospholane Method. The synthesis of a dodecamer with a repetitive yield of 90% gives a final product yield of only 31% ($0.9^{11} = 0.31$). Furthermore, if the monomers **3** are only 95% diastereomerically pure, the diastereopurity of the resulting dodecamer (defined

(23) (a) Eckstein, F.; Burgers, P. M. J.; Hunneman, D. H. *J. Biol. Chem.* **1979**, *254*, 7476−7478. (b) Eckstein, F.; Burgers, P. M. J.; Sathyanarayana, B. K.; Saenger, W. *Eur. J. Biochem.* **1979**, *100*, 585−591. (c) Benkovic, S. J.; Bryant, F. R. *Biochemistry* **1979**, *18*, 2825−2828.

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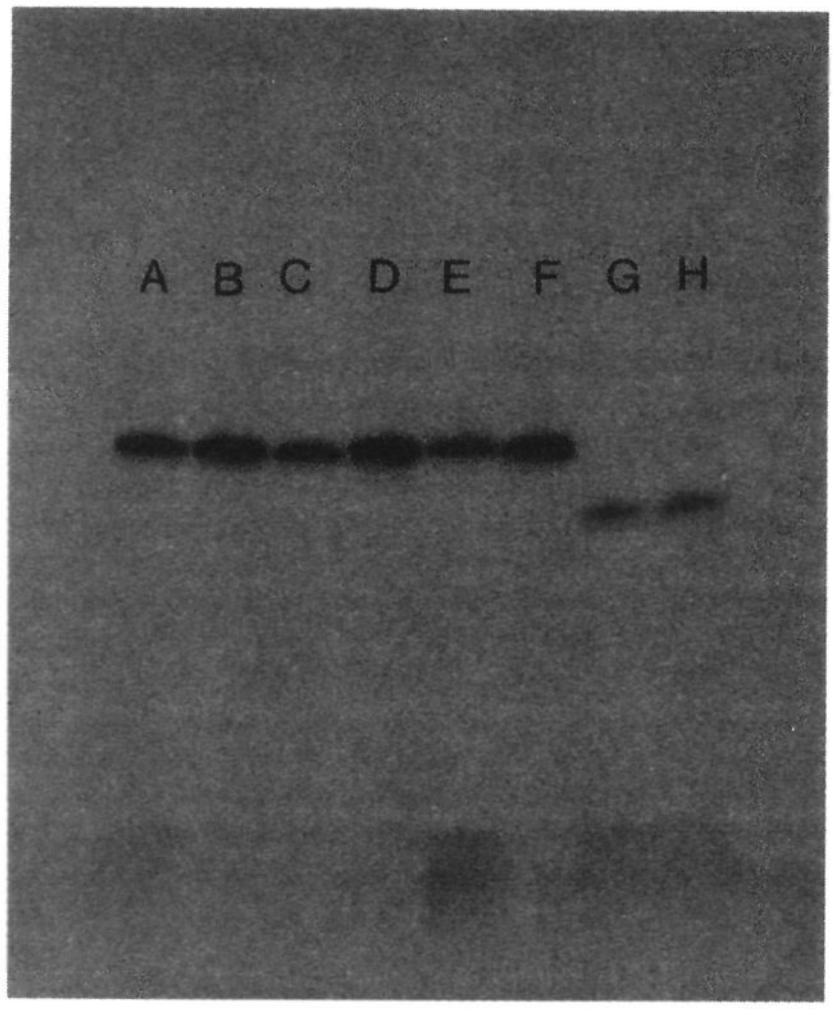


Figure 3. Electrophoretic analysis of purified (double RP-HPLC) ^{32}P-5′-end labeled d[$(T_{PS})_{11}$T] (**10**) synthesized from mix-**3a** (lane A), fast-**3a** (B), slow-**3a** (C), mix-**20a** (D), slow-**20a** (E), fast-**20a** (F), and d[$(C_{PS})_9$C] (**17** and **18**) (lanes *G* and *H*).

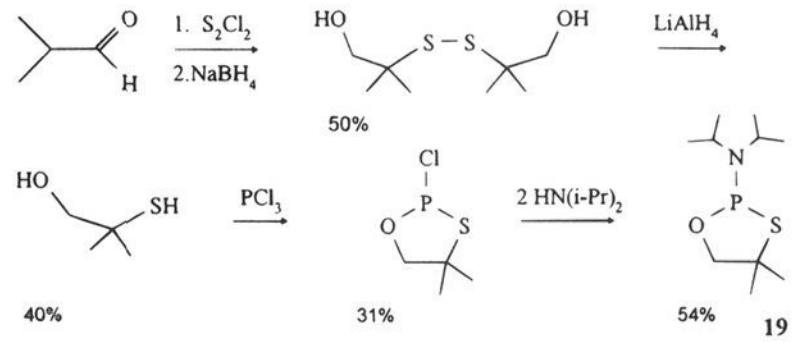
Table 5. Yield and Purity of Stereoregular S-Oligos Synthesized by the Oxathiaphospholane Method and Isolated by RP HPLC (1 μmol Scale)

sequence[a]	DMT$^+$ yield[b] (%)	preparative yield (OD_{260})	purity[c] (%)
10 d[$(T_{PS})_{11}$T]			
all-R_p from slow-**3a**	90.5	8.5	>98
all-S_p from fast-**3a**	95.8	19.0	>98
all-R_p from fast-**20a**	95.7	23.0	>98
all-S_p from slow-**20a**	88.6	12.5	>98
11 d[$(A_{PS})_{11}$A]			
all-R_p from slow-**3b**	98.3	26.0	84
all-S_p from fast-**3b**	94.9	26.0	93
all-R_p from fast-**20b**	98.4	20.0	85
all-S_p from slow-**20b**	96.5	20.0	88
12 d[$G_{PS}G_{PS}G_{PS}A_{PS}A_{PS}T_{PS}T_{PS}C_{PS}C_{PS}$C]			
all-R_p from fast-**20a-c**[d]	92.5	10.0	90
all-S_p from fast-**3a-d**	88.0	5.0	95
15 d[$A_RC_SA_RC_SA_RC_SA_RC_SA_R$C]			
from fast-**20b** and slow-**20c**	88.0	9.0	85
16 d[$C_SC_SC_SC_RC_RC_SC_SC_SC_S$C]			
from fast-**3c** and slow-**3c**	94.2	19.0	>98
17 d[$C_SC_SC_SC_SC_SC_SC_RC_RC_S$C]			
from fast-**3c** and slow-**3c**	93.5	18.0	>98
18 d[$C_SC_SC_SC_SC_SC_RC_RC_SC_S$C]			
from fast-**3c** and slow-**3c**	94.5	19.0	>98

[a] In compounds **15**−**18** subscripts R and S refer to the absolute configuration of corresponding phosphorothioate centers. [b] Average step-yield calculated from dimethoxytrityl cation absorbance decay measured at 504 nm. [c] Determined from 20% PAGE analysis of samples labeled with ^{32}P at 5′-end of the corresponding S-Oligo after double HPLC purification. [d] Because of the difficult separation of **20d** into diastereomers, slow-**3d** was used in the synthesis of [all-R_p]-**12**.

as a content of the desired diastereoisomer in the product) will be only 57% ($0.95^{11} = 0.57$), provided that all coupling steps are fully stereospecific. It should be emphasized that the crude product will contain only 18% (0.31×0.57) of the desired S-Oligo (with respect to both chain length and diastereopurity). Therefore, efforts were undertaken to improve the coupling efficiency as well as separation of the monomers.

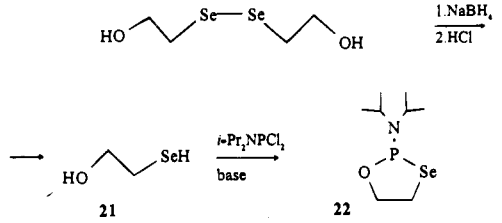
Scheme 3



5′-*O*-DMT-Nucleoside-3′-*O*-(2-thio-4,4-dimethyl-1,3,2-oxathiaphospholanes). Difficulties experienced during chromatographic separation of diastereomers of **3** forced us to prepare congeners which may possess better chromatographic properties. We have examined an approach based on enhanced "asymmetry" of the oxathiaphospholane part of the molecule. Stronger differentiation was achieved by substitution at position 4 of the oxathiaphospholane ring with two methyl groups. This modification does not create any new centers of chirality within the substrate molecule and also was not expected to hamper the elimination of the corresponding episulfide, since after ring-opening the anionic sulfur atom would attack the unsubstituted carbon atom at the β-position. The desired phosphitylating reagent **19** was obtained by the sequence of reactions depicted in Scheme 3. Ring substituted oxathiaphospholanes **20** were synthesized according to the procedure outlined in Scheme 1 and the diastereomers **20a**−**c** were separated by column chromatography into "fast"- and "slow"-eluting species. We have found this separation to be more efficient compared to the separation of **3** (see Experimental Section). Unfortunately, the separation of **20d** (B′ = G^{iBu}) into diastereomers was more difficult than separation of **3d**. The characteristics of the pure diastereomers are presented in Table 1. As previously described, diastereomeric purity of the separated isomers was assigned on the basis of two criteria: (1) integration of the corresponding signals in their ^{31}P NMR spectra and (2) DBU-assisted coupling of pure diastereomers with nucleoside bound to the solid support (**6c**), followed by RP-HPLC analysis of the diastereomeric composition of resulting dinucleoside-3′,5′-phosphorothioates **9**. Both analytical methods gave almost identical results which indicated that (1) the condensation process is stereospecific, and (2) contrary to the "fast"-eluting isomers of **3**, all four "fast"-eluting diastereomers of **20** are precursors of the internucleotide phosphorothioate of Rp configuration. Several S-Oligos were synthesized starting from diastereomeric mixtures of **20** (Table 2), "fast"-eluting **20** and "slow"-eluting **20** (Table 5). Their chain length homogeneity was checked by electrophoretic analysis of ^{32}P-5′-end labeled samples (Figure 3). It should be emphasized that our early preparations gave products **10** and **11** contaminated with S-Oligos longer than dodecamers (up to total 4% of 13-, 14-, 15-, or 16-mers). Careful examination of substrates **3** and **20** revealed that upon storage they undergo some decomposition leading to partial 5′-*O*-detritylation. Obviously, 5′-detritylated **3** and **20** during the condensation process may undergo uncontrolled chain elongation to give the undesired "long-mers". To minimize potential decomposition, isolated pure monomers were twice co-evaporated with dry toluene (to remove residual water and pyridine) and stored in tightly closed vessels at −20 °C.

In conclusion, we acknowledge the advantage of the easier separation of **20a**−**c** compared to that of **3a**−**c**, despite the more difficult preparation of the corresponding mercaptoalcohol (see Scheme 3). The still unsatisfactory repetitive yield which influences the amount of S-Oligos obtained requires further investigation.

Scheme 4



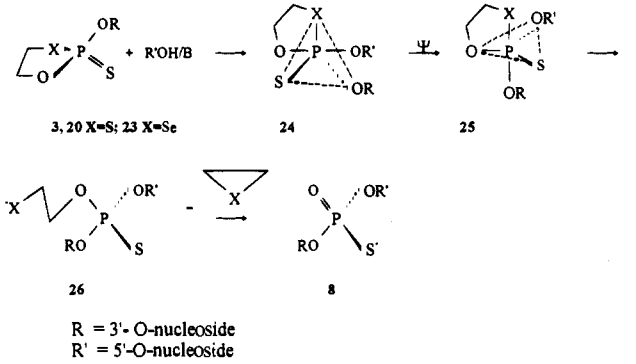
Nucleoside 3′-*O*-(2-Thio-1,3,2-oxaselenaphospholanes).[26] Many examples in the literature show that the cleavage of the phosphorus−selenium bond (P−SeR) occurs much faster than cleavage of the phosphorus−sulfur bond (P−SR). For example, Krzyżanowska et al. have proved[27] that, under controlled time conditions, silver nitrate-assisted ethanolysis of O,S,Se-trimethyl phosphoroselenothioate occurs with exclusive replacement of the MeSe− group. Higher lability of the P−Se bond vs the P−S bond in nucleophilic substitution at phosphorus may affect the coupling yield. We examined nucleoside 3′-*O*-(2-thio-1,3,2-oxaselenaphospholanes) as potential monomers in stereocontrolled synthesis of S-Oligos. 2-Hydroxyethyl selenol (**21**) upon condensation with dichloro-*N*,*N*-diisopropylaminophosphine gave 2-*N*,*N*-diisopropylamino-1,3,2-oxaselenaphospholane (**22**) (see Scheme 4).

Compound **22** was then condensed with 5′-*O*-DMT-thymidine in the presence of 1*H*-tetrazole and the resulting 5′-*O*-DMT-thymidine 3′-*O*-(1,3,2-oxaselenaphospholane) was, without isolation, sulfurized with elemental sulfur to give 5′-*O*-DMT-thymidine 3′-*O*-(2-thio-1,3,2-oxaselenaphospholane) (**23a**) (see Scheme 1). The product formed was a mixture of two diastereomers in *ca.* 1:1 ratio (^{31}P NMR assay). Condensation of **23a** in solution with an equimolar amount of 3′-acetyl thymidine (**6a**, B′ = Thy) in the presence of 2 equiv of DBU gave dinucleotide **8** in 85% yield (^{31}P NMR assay). The monomer **23a** (B′ = Thy) was then condensed with thymidine bound to a solid support. After deprotection, $T_{PS}T$ was obtained in a 98% yield (RP-HPLC assay). However, chromatographic separation of **23a** into diastereomerically pure species was even more difficult than separation of **3** or **20**. When partially enriched **23a** ("fast":"slow" ratio: 77:23) was used as a substrate in the manual solid phase synthesis (*vide supra*), a mixture of R_p- and S_p-**9** was obtained in a 25:75 ratio as measured by RP-HPLC. This indicates that the ring opening condensation is highly stereoselective, and the "fast"-eluting diastereomer **23** is the precursor of [S_p]-**9**.

Despite the encouraging yields of the prepared dimers, the tedious preparation of **23**, the unpleasant nature of the starting material (due to the odor of organoselenium byproducts) and the difficulties experienced during the separation of the diastereomers forced us to abandon oxaselenaphospholane chemistry.

Mechanism of Ring-Opening Condensation. The method described here for the stereocontrolled synthesis of S-Oligos is based upon the concept of nucleophilic substitution at the phosphorus of the 2-thio-1,3,2-oxathia(selena)phospholane ring system. The 5′-OH-3′-*O*-protected nucleoside **6** activated by a strong base, preferably DBU, attacks the phosphorus atom from the side opposite to the endocyclic P−O bond leading to P^V intermediate **24**, which before collapse must undergo intramolecular rearrangement (pseudorotation) to form **25**. The cleavage of the P−X bond (X = S, Se) is followed by the kinetically fast elimination of ethylene episulfide or ethylene episelenide

Scheme 5



from **26**, leading to **8**. This mechanism, derived from our earlier studies,[28] seems to be the most rational explanation for the high chemoselectivity and stereospecificity of the oxathia(selena)-phospholane ring-opening condensation. The role played by DBU in the ring opening condensation remains obscure. DBU has long been considered to be a strong non-nucleophilic base;[29] however, DBU possesses strong nucleophilic activity toward P^{III}-coordinate species.[30] Also, methanolysis of 2-α-naphthyl-ethylamino-2-thio-1,3,2-oxathiaphospholane performed in the presence of *the equimolar* amount of DBU was effective for chemoselective and stereospecific ring-opening process.[28] Thus, the necessity for a large molar excess of DBU for effective condensation using our method remains unclear.

Moreover, other organic bases such as *N*-methylimidazole, 4-(dimethylamino)pyridine, and 2-*tert*-butylimino-2-(diethylamino)-1,3-dimethylperhydro-1,3,2-diazaphosphorine were less effective compared to DBU. However, if DBU acts as a nucleophile in this reaction, DBU-ammonium ligand−ligand exchange should occur and would lead to P-epimerization. The high stereospecificity of the single condensation step, as indicated in Table 4, suggests this is not the case.

Possible Side Reactions. Since chemical synthesis of oligonucleotides involves multiple repetition of a synthetic cycle, conditions applied during the whole process of chain elongation should not be harmful to any part of the growing oligonucleotide. Because the process of ring-opening condensation is accompanied by elimination of ethylene episulfide, which should be considered to be a potential modifier of nucleobases, the integrity of Ade, Gua, Cyt, and Thy in S-Oligos obtained via the OTP method has been checked. Using monomers **3** and the standard synthetic protocol (Table 3) two independent syntheses of decamer d[$G_{PS}G_{PS}G_{PS}A_{PS}A_{PS}T_{PS}T_{PS}C_{PS}C_{PS}C$] (**12**) were performed. The only difference between those syntheses was that guanine residue in **3** either was or was not protected at O-6 site with the DPC group. After release from the solid support and base deprotection (ammoniolysis) the crude products **12** (approximately 1 OD unit of each) were treated with formic acid. Under the same conditions, samples of **12** obtained after first HPLC purification (DMT-ON, two samples) and after the second one (DMT-OFF, two samples) were hydrolyzed. The resulting hydrolysates were dried in the stream of argon and analyzed using RP-HPLC. Typical chromatograms showed the presence of three minor peaks (total area <3%) in addition to four peaks of cytosine, guanine, thymine, and adenine. Two of those minor peaks were eluted just before and after cytosine,

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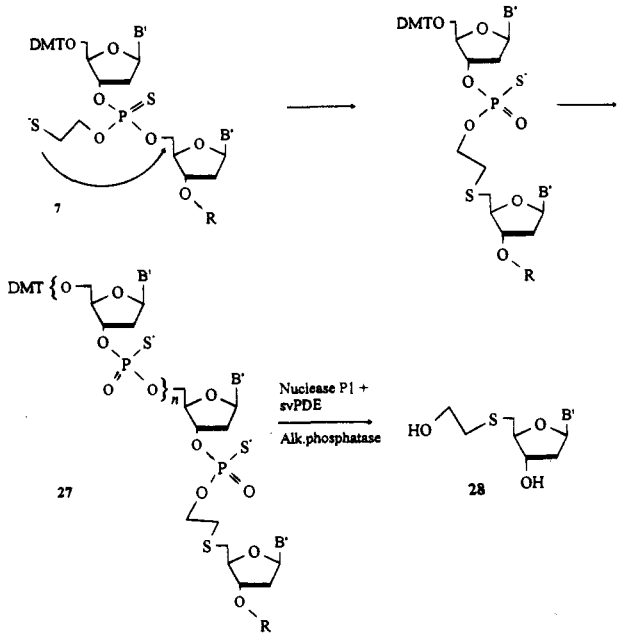
(27) Krzyżanowska, B.; Stec, W. J.; Wieczorek, M. W.; Błaszczyk, J. *Heteroatom Chem.* **1991**, *2*, 123−139.

(28) Uznański, B.; Grajkowski, A.; Krzyżanowska, B.; Kaźmierkowska, A.; Stec, W. J.; Wieczorek, M. W.; Błaszczyk, J. *J. Am. Chem. Soc.* **1992**, *114*, 10197−10202.

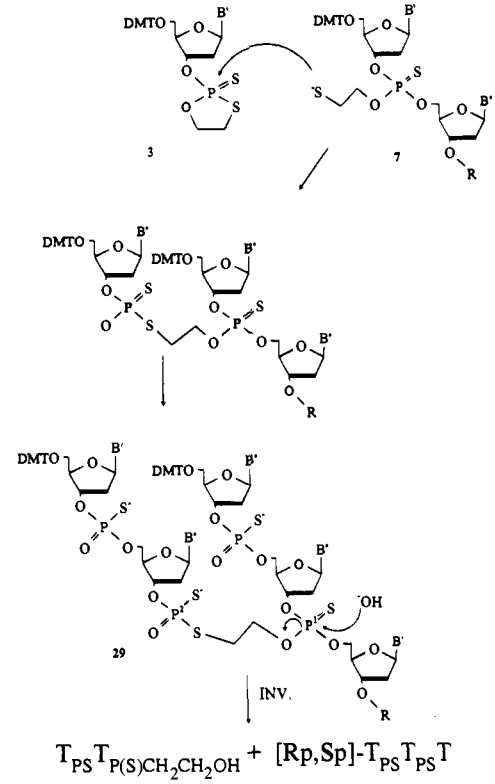
(29) Oediger, H.; Moller, F.; Eiter, K. *Synthesis* **1972**, 591.

(30) (a) Reed, R.; Reau, R.; Dahan, F.; Bertrand, G. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 399−401. (b) Leśnikowski, Z. J.; Zabawska, D.; Jaworska-Maślanka, M. M.; Schinazi, R. F.; Stec, W. J. *New J. Chem.* **1994**, *18*, 1197−1204.

Scheme 6



Scheme 7



while the third one was eluted well after adenine. In the control experiment unmodified oligonucleotide d[GGGAATTCCC] (prepared via phosphoramidite approach) was also degraded by formic acid. Analysis of this last hydrolysate showed the presence of the first and second of the aforementioned extra peaks in the similar ratio. Since the intensity of the third peak (not found in the control experiment) did not exceed 0.7%, we have come to the conclusion that the oxathiaphospholane method produces S-Oligos virtually free of base modification.

Next, we investigated the inertness of the growing S-Oligo (which formally is an *O,O*-dialkyl phosphorothioate polyanion counterbalanced by protonated DBU molecules) to the conditions of the OTP method. A sample of dithymidyl 3′,5′-phosphorothioate was exposed to the capping solution (acetic anhydride/DMAP) for the time equivalent to 20 capping steps. The ^{31}P NMR spectrum recorded after this experiment did not show any O- or S-acetylated products. Similarly, 5′,3′-diprotected dithymididyl 3′,5′-phosphorothioate was exposed to DBU and a diastereomeric mixture of **3**. After waiting the time equivalent to 12 coupling steps (3600 s), no indication of branching through the phosphorothioate group was detected (no signals corresponding to pyrophosphorodithioates were found in the ^{31}P NMR). Then we focused our attention on a possible side reaction involving the intermediate **7** (Schemes 6 and 7). If elimination of episulfide from **7** is slow, an attack of the sulfur atom of the 2-mercaptoethyl group on the electrophilic center nearby must be considered. It may occur either at the 5′-carbon atom of the downstream nucleoside (an intramolecular attack, Scheme 6) or at the phosphorus atom of another molecule of **3** (an intermolecular attack, Scheme 7). The intramolecular attack should give construct **27**, possessing a modified 5′-deoxy-5′-(2-hydroxyethylthio)nucleotide. This type of side reaction would be especially difficult to detect by ^{31}P NMR or PAGE. To determine if this is a problem in our syntheses the combined crude $(T_{PS})_{11}T$ from several preparations using the oxathiaphospholane method was hydrolyzed using a "cocktail" composed of nuclease P1 and svPDE, followed by further treatment of the resulting mononucleotides with alkaline phosphatase to remove the 5′-phosphorothioate groups. The enzymatic cleavage of **27** should release 5′-deoxy-5′-(2-hydroxyethylthio)-thymidine (**28**). For the purpose of chromatographic detection compound **28** was independently synthesized by reaction of 5′-deoxy-5′-bromothymidine with 2-mercaptoethanol.[31] The product of the enzymatic digestion was analyzed by HPLC, and no trace of 5′-deoxy-5′-(2-hydroxyethylthio)thymidine was detected. On the other hand we know that the oligomer possessing butanediol residue instead of deoxyribose ring was efficiently hydrolyzed enzymatically.[32] Alternatively, if intermolecular attack takes place (Scheme 7), the synthesis of [R_p,R_p]-($T_{PS}T_{PS}T$) starting from diastereomerically pure **3** (B′ = Thy) should give [R_p,S_p]-$T_{PS}T_{PS}T$ and $T_{PS}T_{P(S)SCH_2CH_2OH}$, as depicted in Scheme 7. This inversion of configuration at phosphorus P^1 would result from the attack of hydroxide ion at the P^1 atom in **29** during ammoniolytic release from the solid support. We saw no evidence for this side reaction in our model experiments. The hydroxide ion may also attack the P^2 atom leading to the cleavage of the P^2–S bond, with subsequent elimination of ethylene episulfide. In this case the trimer is indistinguishable from the compound being synthesized. However, this direction of attack is less likely because hydrolytic decomposition of a triester (attack at P^1) is much more favored than attack at the charged P^2 atom.

Thus, merely satisfactory repetitive yield is caused by low coupling step efficiency (not exceeding 96%, as measured by trityl cation assay), rather than by possible side reactions. Nonetheless, the method of synthesis presented in this paper is effective for the preparation of medium-size S-Oligos, although the overall yield of oligonucleotide is significantly lower than that of S-Oligos prepared by the nonstereoselective phosphoroamidite/stepwise sulfurization method.

Diastereomeric Purity Assessments. A stereocontrolled method for the synthesis of S-Oligos should be accompanied by a complementary analytical method to determine the diastereomeric purity of the products. As previously described, the diastereomeric purity of **3**, **20**, and **23** was determined by

(31) Hacia, J. G.; Dervan, P. B.; Wold, B. J. *Biochemistry* **1994**, *33*, 6192.

(32) Wilk, A.; Koziolkiewicz, M.; Grajkowski, A.; Uznanski, B.; Stec, W. J. *Nucleic Acids Res.* **1990**, *18*, 2065–2068.

^{31}P NMR, while the diastereomeric composition of dimers **8** and **9** was derived from HPLC chromatograms. Direct assignment of stereochemistry is possible for phosphorothioate dimers and sometimes trimers, where HPLC separation of the two (or four) possible diastereomers is reliable, and the diastereomers of opposite configuration from expected are detectable. However, assignment of diastereomeric purity of constructs longer than trimers using either ^{31}P NMR or HPLC techniques is problematic due to lack of resolution of the individual isomers. A more reliable method seems to be the diastereoselective hydrolysis of S-Oligos by enzymes such as nuclease P1 and, to some extent, snake venom phosphodiesterase (svPDE). In the late 1970s Eckstein[23a] and Benkovic[23b] found that dinucleoside (3′,5′)phosphorothioates undergo hydrolysis by svPDE in a stereodependent manner. Under all conditions tested to date, svPDE recognizes and hydrolyzes dinucleoside phosphorothioates of R_p-configurations. Conversely, nuclease P1 (a Zn-dependent *endo*nuclease) assists the hydrolysis of internucleotide S_p-phosphorothioates, while internucleotide R_p-phosphorothioates are inert.[24] Thus, treatment of stereoregular S-Oligos with svPDE, and independently with nuclease P1, may be used to determine the diastereomeric purity of given construct. We have examined the stereospecificity and activity of these enzymes toward decamers **15**–**18**. Nuclease P1, possessing both endo- and exonucleolytic activity, was found to degrade S_p phosphorothioate linkages independent of their content and position within the oligonucleotide chain. Alternatively, the proof of diastereomeric purity of S-Oligos using svPDE, which is known to act as a 3′-*exo*nuclease, was much more difficult. The exonucleolytic activity of this enzyme allows for the cleavage of [R_p]-phosphorothioates only from the 3′-end of S-Oligo, while those located "inside" the oligomer chain often remain intact. However, effective degradation of **15** (containing phosphorothioate moieties of both R_p and S_p configuration) by svPDE indicates that this enzyme also possesses some endonucleolytic activity.[9b] Using S-Oligos **16**–**18** with a defined absolute configuration at each phosphorus atom, we found that svPDE is able to degrade a single R_p-phosphorothioate bond located between [all-S_p] parts of S-Oligos.

Because of the limited ability of svPDE to degrade R_p linkages located within the oligonucleotide chain, a search for other R_p-specific endonuclease was undertaken. We have found that the endonuclease from *Serratia marcescens (Sma E)*[33] accepts S-Oligos longer than octamers as substrates and cleaves phosphorothioate linkages with the R_p-configuration. Therefore, we used a *mixture* of svPDE and Sma E and, independently, nuclease P1 for assignment of the diastereomeric purity of stereoregular S-Oligos. It should be emphasized that conditions for complete degradation of the corresponding MIX-constructs must be found before analysis of the stereoregular isomers, based on the assumption that reaction conditions (temperature, enzyme concentration, incubation time) valid for 100% degradation of MIX substrates would be appropriate for degradation of all phosphorothioate bonds of the "wrong" configuration contaminating stereoregular S-Oligos. However, the reaction conditions are dependent upon the length and sequence of the S-Oligo and therefore must be optimized for each compound. We hydrolyzed ^{32}P-labeled oligomers **10** and **11** using optimized conditions and analyzed the enzymic hydrolysates by PAGE followed by densitometric analysis of autoradiograms. The relevant data are collected in Table 6. The S-Oligo [all-R_p]-**10** prepared from slow-**20** with a diastereomeric purity of 98% has been partially digested with nuclease P1 and the content of "undigested" [all-R_p]-**10** (75% of the total product) corresponded well with the calculated diastereomeric purity $(0.98)^{11} = 80\%$. Similar results were obtained with digestion of [all-R_p]-**11** which was synthesized from slow-**3** (B′ = Ade^{Bz}) with a diastereomeric purity of 99.5%. After a 24 h incubation, 92% of the substrate remained intact agreeing well with the calculated diastereomeric purity of 95% ($0.995^{11} = 0.95$). Independently, using the combination of R_p-specific enzymes (svPDE and Sma E) showed that 80% of the oligomer [all-S_p]-**11** and 68% of the oligomer [all-S_p]-**10** (with a calculated diastereomeric purity of 95% and 89.5%, respectively) remained full length. These values are somewhat lower than expected. However, it is known[34] that svPDE is not fully stereoselective enzyme and to the limited extent hydrolyzes also dinucleoside phosphorothioates of S_p configuration. Furthermore, the nucleases exhibit reduced discrimination toward phosphorothioate linkages in oligonucleotides as compared to that in dinucleotides.[35] One has to realize that high concentration of the enzymes (see Experimental Section) as well as long total incubation time (24 h) promote additional degradation.

Table 6. Enzymatic Assignment of Diastereomeric Purity of S-Oligos—the Percent of Undigested S-Oligos After Incubation with nP1 or svPDE/Sma E for a Given Period of Time[a]

		time of incubation (h)				
substrate, calc. DP	enzyme(s)	0	4	8	16	24
MIX-**10**	svPDE/Sma E	100	45.9	23.1	5.9	2.2
	nP1	100	5.2	2.5	0	0
[all-R_p]-**10** 80%	svPDE/Sma E	100	0	0	0	0
	nP1	100	79.3	78.4	78.0	74.5
[all-S_p]-**10** 89.5%	svPDE/Sma E	100	84.2	83.4	73.4	68.0
	nP1	100	0	0	0	0
MIX-**11**	svPDE/Sma E	100	8.0	0.5	0	0
	nP1	100	2.5	0	0	0
[all-R_p]-**11** 95%	svPDE/Sma E	100	0	0	0	0
	nP1	100	91.6	90.0	95.0	91.6
[all-S_p]-**11** 95%	svPDE/Sma E	100	93.4	90.8	83.4	78.8
	nP1	100	0	0	0	0

[a] Derived from PAGE analysis followed by densitometry.

Thermal Dissociation Studies (T_m). The ability of S-Oligos to bind RNA sequence-specifically and also to induce cleavage by RNase H is important for their use as "antisense" agents. Higher thermodynamic stability of certain S-Oligo/RNA duplexes, expressed by their melting temperature, may suggest more promising efficacy for those S-Oligos as potential therapeutics. However, this prediction alone may not be an adequate indicator of an S-Oligos *in vivo* activity, which also may be dependent on cellular uptake, exo- and endonuclease resistance, specific interactions with proteins, pharmacokinetics, etc.

Theoretical considerations[36] suggest that double stranded S-Oligo/DNA helices (presumably existing in B conformation) involving [all-S_p]-S-Oligos should be more stable than those with [all-R_p] chirality. This prediction has been rationalized in terms of destabilizing interactions caused by the higher steric demands of the sulfur atom (compared to the oxygen atom) directed "inward" in the double helix with the R_p S-Oligo.[37] Furthermore, if the negative charge in the phosphorothioate anion is localized on the sulfur atom,[38] its "inward" orientation may further destabilize the duplex by causing stronger repulsion

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Table 7. Melting Temperatures for Duplexes Formed with MIX- or Stereoregular S-Oligos and DNA or RNA Templates

duplex	T_m (°C)	duplex	T_m (°C)
dT_{12}/dA_{12}	36	dT_{12}/rA_{10}	23
[all-R_p]-**10**/dA_{12}	18	[all-R_p]-**10**/rA_{10}	6
MIX-**10**/dA_{12}	17	MIX-**10**/rA_{10}	–
[all-S_p]-**10**/dA_{12}	14	[all-S_p]-**10**/rA_{10}	–
dA_{12}/dT_{12}	36	dA_{12}/rU_{12}	23
[all-R_p]-**11**/dT_{12}	29	[all-R_p]-**11**/rU_{12}	37
MIX-**11**/dT_{12}	31	MIX-**11**/rU_{12}	29
[all-S_p]-**11**/dT_{12}	34	[all-S_p]-**11**/rU_{12}	18

[a] The melting profiles were recorded at 260 nm in 10 mM sodium cacodylate (pH 7.4), containing 10 mM $MgCl_2$ and 70 mM NaCl, with oligomer concentration 2.4 μM. The temperature gradient was 0.2 °C/min. The optical pathlength, 10 mm.

of negative charges on both complementary strands. In order to investigate the influence of chirality of S-Oligos on the stability of their corresponding duplexes, melting temperatures for dA_{12}/dT_{12}, MIX-**11**/dT_{12}, [all-R_p]-**11**/dT_{12}, [all-S_p]-**11**/dT_{12}, dA_{12}/U_{12}, MIX-**11**/U_{12}, [all-R_p]-**11**/U_{12}, [all-S_p]-**11**/U_{12} and MIX-**10**/dA_{12}, [all-R_p]-**10**/dA_{12}, [all-S_p]-**10**/dA_{12}, MIX-**10**/rA_{10}, [all-R_p]-**10**/rA_{10}, and [all-S_p]-**10**/rA_{10} were determined. The melting curves obtained were cooperative, and melting temperatures were calculated using the first order derivative method. Hysteresis, when comparing forward and reverse curves, did not exceed 2 °C. We found (see Table 7) that, as expected, the duplex formed with [all-S_p]-**11** and dT_{12} is more stable (T_m = 34 °C) than either duplex MIX-**11**/dT_{12} (T_m = 31 °C) or [all-R_p]-**11**/dT_{12} (T_m = 29 °C). However, the duplex consisting of [all-S_p]-**10** and dA_{12} is, surprisingly, somewhat less stable than duplexes [all-R_p]-**10**/dA_{12} and MIX-**10**/dA_{12}. It should be emphasized that within the pairs of corresponding duplexes (e.g., [all-S_p]-**11**/dT_{12} and [all-S_p]-**10**/dA12, which differ only in that which strand contains phosphorothioates) the number and nature of hydrogen bonds involved in the duplex structure as well as the orientation of the sulfur atoms within the grooves of the helix are the same, while there is a significant difference in duplex stability. Obviously, there must be some other factors influencing the stability of these duplexes[39] and these phenomena require further investigation.

Conclusions. Among the several attempts at stereocontrolled synthesis of S-Oligos, only the oxathiaphospholane method, based on DBU-assisted nucleophilic attack of 5′-OH-nucleosi(ti)des on the phosphorus atom of diastereomerically pure nucleoside-3′-*O*-(2-thio-1,3,2-oxathiaphospholane), is adaptable to automated solid phase synthesis. Reasonable coupling times (*ca.* 300 s), about a 94% step-yield, and the high stereospecificity of the condensation (>98%) allowed us to synthesize medium size stereoregular S-Oligos on an automated DNA synthesizer. The quality of S-Oligos prepared by OTP method in respect to the chain integrity (contamination by short- and long-mers) and to the level of contamination with phosphates, is comparable with that of S-Oligos prepared by the phosphoroamidite/sulfurization method. The most limiting factor in the OTP methodology is laborious separation of oxathiaphospholanes **3**. Attempts at modification of the oxathiaphospholane skeleton and trials with such monomers as **20** and **23** did not improve the yield or quality of the products enough to compensate for their laborious preparation. In the control experiment it has been proven that the S-Oligo constructs did not contain detectable amount of modified bases. The diastereomeric purity of S-Oligos prepared by the OTP method can be assigned by enzymatic digestion. Nuclease P1 is the enzyme of choice for determining the diastereomeric purity of [all-R_p]-constructs, because all phosphorothioates of [S_p]-configuration undergo hydrolysis. The amount of undigested [all-R_p]-constructs remaining after treatment with nuclease P1 reflects its diastereomeric purity. The determination of the D.P. of [all-S_p]-S-Oligos is more complicated. We have demonstrated that the endonuclease from *Serratia marcescens* accepts S-Oligos longer than 8-mers as substrates and cleaves phosphorothioate internucleotide linkages of R_p-configuration. Therefore, the combination of Sma E with 3′-exonuclease svPDE gives a new tool for the determination of diastereomeric purity of [all-S_p]-S-Oligos.

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Experimental Section

The nuclear magnetic resonance spectra were recorded on a Bruker AC-200 instrument (200 MHz, TMS internal standard for ^1H and 85% H_3PO_4 as the external standard for ^{31}P). The FAB-MS spectra (13 keV, CS^+) were recorded on a Finnigan MAT 95 spectrometer. Ultraviolet (UV) spectra and melting profiles were recorded on a GBC 916 spectrophotometer equipped with a Thermocell unit. Densitometry of autoradiograms was performed on a LKB Ultroscan XL densitometer. HPLC analyses were performed on a LDC Analytical system (pumps CM3500 and CM3200, SpectroMonitor SM4100). The elemental analysis was performed by the Laboratory of Microanalysis of this Centre. Evaporations were carried out at 40 °C (or lower) using an aspirator or oil pump vacuum. Deoxyribonucleosides were purchased from Pharma Waldhof (FRG). 1H-Tetrazole and dicyclohexylcarbodiimide (DCC) were purchased from Janssen Chimica (Belgium). Acetonitrile and 1,4-diazabicyclo[5.4.0]undec-7-ene (DBU) were supplied by Merck (FRG). Diisopropylamine, β-cyanoethanol, and succinic anhydride were purchased from Aldrich (USA). Fmoc-Sarcosine was purchased from Bachem (Switzerland). Long chain alkylamine controlled pore glass (500 A, mesh 80–120) was supplied by Sigma (USA). Phosphorus trichloride, ethyl acetate, and butyl acetate were purchased from POCH (Poland).

Snake venom phosphodiesterase (svPDE, EC 3.1.15.1) was obtained from Boehringer Mannheim (FRG). Nuclease P1 (nP1, EC 3.1.30.1) and nuclease from Serratia marcescens (Sma E, EC 3.1.30.2) were purchased from Sigma. T4 polynucleotide kinase (EC 2.7.1.78) was obtained from Amersham (USA). [γ-^{32}P]ATP was synthesized by Dr.A. Plucienniczak of the Centre of Microbiology and Virology of the Polish Academy of Sciences (Lodz, Poland).

2,2′-Dithiobis(2-methylpropionaldehyde). This compound was synthesized from isobutyraldehyde as described by K. Hayashi. The product was distilled under reduced pressure and a fraction boiling at 93–94 °C/0.01 mmHg was collected.

2,2′-Dithiobis(2-mercapto-2-methylpropan-1-ol). Into a suspension of $NaBH_4$ (20.3 g, 0.54 mol) in 300 mL of isopropyl alcohol was added dropwise 2,2′-dithiobis(2-methylpropionaldehyde) (37.7 g, 0.18 mol). The mixture was refluxed for 8 h and then evaporated, and a 1.5 M solution of sodium hydroxide (200 mL) was added. The mixture was cautiously neutralized with concentrated hydrochloric acid and extracted with chloroform (2 × 150 mL). The organic layer was dried with magnesium sulfate, and the solvent was evaporated. The residue was dissolved in dry benzene (100 mL), and the solvent was evaporated with exclusion of moisture. The crystalline product was precipitated from diethyl ether/hexane. White crystals (28.8 g) were collected (75% yield, mp 56–58 °C).

2-Mercapto-2-methylpropan-1-ol. Into a suspension of lithium aluminum hydride (0.76 g, 0.02 mol) in 100 mL of dry diethyl ether (atmosphere of dry argon, two necked flask equipped with a condenser and a funnel) was added, with magnetic stirring, dropwise over 60 min a solution of 2,2′-dithiobis(2-mercapto-2-methylpropan-1-ol) (4.29 g,

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(41) Bis-(2-hydroxyethyldiselenide), prepared according to published method [Jakiwczyk, O. M.; Kristoff, E. M.; McPhee, D. J. *Synth. Commun.* **1993**, *23*, 195–199], was contaminated with considerable amounts of corresponding mono- and triselenide influencing the yield of 2-hydroxyethyl selenol.

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0.02 mol) in 40 mL of diethyl ether. The reaction was exothermic, and mild reflux occurred. The stirring was continued for 1 h, and excess of reducing agent was cautiously decomposed with ethyl acetate, followed by THF containing traces of moisture, and finally 10% water/THF. Inorganic salt was filtered, and the filtrate was dried with magnesium sulfate. The solvents were evaporated, and the residue was distilled under reduced pressure to give 1.73 g (40% yield) of a colorless oil (bp 70 °C/30 mmHg, n^{D}_{20} 1.469).

2-Chloro-1,3,2-oxathiaphospholane. Into a mixture of pyridine (79.1 g, 1.0 mol) and benzene (400 mL) were added with stirring at room temperature 2-mercaptoethanol (39.1 g, 0.5 mol) and phosphorus trichloride (68.7 g, 0.5 mol). Stirring was continued for 0.5 h, pyridinium chloride was filtered off, and the filtrate was condensed under reduced pressure. The crude product was purified *via* distillation under reduced pressure, and a fraction boiling at 70−72 °C/20 mm Hg was collected: ^{31}P NMR δ 205.0 ppm (benzene); yield 72% lit.[21] bp 84−85 °C/12 mmHg.

2-Chloro-4,4-dimethyl-1,3,2-oxathiaphospholane. Dry benzene (150 mL) was poured into a flask equipped with a thermometer and two separatory funnels. The flask was cooled to 5 °C, and a solution of PCl_3 (42 mmol, 5.82 g) in 10 mL of benzene, and, simultaneously, a solution of 2-mercapto-2-methylpropan-1-ol (28.3 mmol, 3.0 g) and triethylamine (56.5 mmol, 5.7 g) in 10 mL of benzene were added dropwise with magnetic stirring. The temperature of the reaction mixture was kept below 10 °C. Stirring was continued at room temperature for 30 min, and triethylamine hydrochloride was filtered off. After evaporation of the solvent under reduced pressure the product was distilled to give 1.0 g of a colorless liquid: 20.8% yield; bp 40 °C/0.01 mmHg; δ ^{31}P NMR 222.3 ppm; EI-MS (70 eV) *m/z* 170, M^+, 100%, *m/z* 135, $[M - Cl]^+$, 63%.

2-*N*,*N*-Diisopropylamino-1,3,2-oxathiaphospholane. Into a solution of 2-chloro-1,3,2-oxathiaphospholane (28.5 g, 0.2 mol) in *n*-pentane (300 mL) was added dropwise with stirring at room temperature diisopropylamine (40.5 g, 0.4 mol). After 0.5 h diisopropylamine hydrochloride was removed by filtration, the solvent was evaporated, and the product was distilled under reduced pressure to give 29 g of colorless liquid: 70% yield; bp 70 °C/0.1 mmHg; δ ^{31}P NMR 147.8 ppm; MS *m/z* 207, M^+.

2-*N*,*N*-Diisopropylamino-4,4-dimethyl-1,3,2-oxathiaphospholane. To a solution of 2-chloro-4,4-dimethyl-1,3,2-oxathiaphospholane (16.5 mmol, 2.8 g) in benzene (5 mL) and petroleum ether (20 mL) was added dropwise with stirring while keeping the temperature of the reaction mixture below 5 °C a solution of diisopropylamine (33 mmol, 3.145 g) in petroleum ether (5 mL) . Stirring was continued at room temperature for 1 h, and diisopropylamine hydrochloride was filtered off. The solvent was evaporated, and the product was distilled under reduced pressure to give 2.2 g of colorless liquid: 53% yield; bp 53−55 °C/0.01 mmHg; δ ^{31}P NMR 154.1 ppm; MS *m/z* 253, M^+, 42%, *m/z* 135, $[M - NiPr_2]^+$, 100%.

Phosphitylation of Protected Nucleosides with 2-*N*,*N*-Diisopropylamino-1,3,2-Oxathiaphospholane—General Procedure. Appropriately protected deoxyribonucleoside (10 mmol) (A, G, T, or C) and 11 mmol of 1*H*-tetrazole (DNA synthesis grade) mixed together in a 50 mL flask were dried under high vacuum for 12 h and then dissolved in 25 mL of dry methylene chloride. To the magnetically stirred solution was added dropwise at room temperature 11 mmol of 2-*N*,*N*-diisopropylamino-1,3,2-oxathiaphospholane. The reaction was complete in 2 h (by TLC), and then elemental sulfur (15 mmol) was added. Stirring was continued for 12 h, and then the excess sulfur was filtered off. After evaporation of the solvent the residue was dissolved in 5−6 mL of chloroform (distilled with pyridine) and applied to a 230−400 mesh silica gel column (6 × 30 cm, 170 g). The column was eluted with chloroform (200 mL) and then chloroform−methanol 97:3. Appropriate fractions were combined and evaporated under reduced pressure to give desired compounds **3a−c** and a precursor of **3d** (5′-*O*-DMT-N-2-iBu-guanosine-3′-*O*-(2-thio-1,3,2-oxathiaphospholane)) in 80−83% yield. The diastereomeric composition, chemical shifts in ^{31}P NMR and TLC parameters of compounds **3a−c** are given in Table 1.

Phosphitylation of Protected Nucleosides with 2-*N*,*N*-Diisopropylamino-4,4-dimethyl-1,3,2-Oxathiaphospholane—General Procedure. Appropriately protected deoxyribonucleoside (4.3 mmol) (A, G, T, or C) and 5 mmol of 1*H*-tetrazole (DNA synthesis grade) mixed together in a 25 mL flask were dried under high vacuum for 12 h and then dissolved in 8 mL of dry methylene chloride. To the magnetically stirred solution, 4.5−5.0 mmol of 2-*N*,*N*-diisopropylamino-4,4-dimethyl-1,3,2-oxathiaphospholane was added at room temperature. The reaction was complete in 2 h (by TLC), and then elemental sulfur (4.5−5.0 mmol) was added. Stirring was continued for 12 h, and the mixture was concentrated to 1/3 the initial volume. Acetonitrile (2 mL) was added, and the excess sulfur was filtered off. After solvent evaporation the residue was dissolved in 5−6 mL of chloroform (distilled with pyridine) and applied to a silica gel column (3 × 20 cm). The column was eluted with chloroform (chloroform−methanol 100:3 in the case of the G derivative). Appropriate fractions were combined and evaporated under reduced pressure to give desired compounds **20a−d** in 78−82% yield. The products were stored at −20 °C. Their diastereomeric composition, chemical shifts in ^{31}P NMR, and TLC parameters are given in Table 1: MS (+FAB) **20a**: *m/z* 710.3 (M^+, 2.16%), 711.4 ($M^+ + 1$, 1.77%), 303.2 (DMT^+, 100%); **20b**: *m/z* 824.0 ((M^+, 3.58%), 825.0 ($M^+ + 1$, 1.69%), 303.1 (DMT^+, 100%); **20c**: *m/z* 840.3 ($M + K^+$, 2.03%), 841.3 ($M + K + 1^+$, 0.77%), 303.2 (DMT^+, 100%); **20d**: *m/z* 806.3 (M^+, 2.08%), 807.3 ($M^+ + 1$, 0.78%), 303.2 (DMT^+, 100%); ^{1}H NMR (CD_3CN) **20a**: δ 1.44−1.57 (pair of doublets, 6H), 2.25−2.31 (m, 1H), 2.43−2.51 (m, 1H), 3.26−3.40 (m, 2H), 3.76 (s, 6H), 3.97−4.26 (m, 4H), 5.35−5.45 (m, 1H), 6.21−6.29 (m, 1H), 6.83−6.90 (m, 4H), 7.10−7.53 (m, 10H), 8.92 (s, 1H); **20b**: 1.54−1.61 (pair of doublets, 6H), 2.67−2.79 (m, 1H), 3.17−3.37 (m + m, 1H + 2H), 3.73 (s, 6H), 4.15−4.27 (m, 2H), 4.32−4.38 (m, 1H), 5.53−5.62 (m, 1H), 6.41−6.48 (t, 1H), 6.75−6.85 (m, 4H), 7.08−7.30 (m, 9H), 7.36−7.41 (m, 2H), 7.49−7.68 (m, 3H), 7.96−8.02 (m, 2H), 8.23−8.24 (d, 1H), 8.54−8.55 (d, 1H); **20c**: 1.51−1.58 (pair of doublets, 6H), 2.34−2.48 (m, 1H), 2.68−2.81 (m, 1H), 3.40−3.42 (d, 2H), 3.76 (s, 6H), 4.28−4.35 (dt, 1H), 5.24−5.37 (m, 1H), 6.12−6.18 (t, 1H), 6.85−6.90 (d, 4H), 7.10−7.67 (m, 114H), 7.91−7.96 (m, 2H), 8.11−8.15 (d, 1H), 9.10 (a broad singlet, 1H).

Synthesis of 3d. Protection of 5′-*O*-DMT-N-2-iBu-guanosine-3′-*O*-(2-thio-1,3,2-oxathiaphospholane) at the O-6 Site with Diphenylcarbamoyl Chloride. To a solution of 5′-*O*-DMT-N-2-iBu-guanosine-3′-*O*-(2-thio-1,3,2-oxathiaphospholane) (5.0 g, 6.5 mmol) in pyridine (70 mL) were added, with stirring, at room temperature diisopropylethylamine (1.69 mL, 9.75 mmol) and diphenylcarbamoyl chloride (2.99 g, 13 mmol). The mixture was stirred for 1 h, concentrated to 1/2 of the initial volume, and filtrated through silica gel (ca. 20 g). The silica gel was washed with chloroform (300 mL), and the filtrate was concentrated to dryness. The residue was dissolved in 3 mL of a mixture chloroform/hexane/benzene (5:1:2, v/v/v) containing 0.2% of pyridine and applied onto a column (30 × 6 cm) containing ca. 100 g of the silica gel H60. The column was eluted with the same mixture of solvents. Appropriate fractions (TLC control; silica gel plates, R_f 0.68, chloroform/methanol 20:1) were collected and evaporated under reduced pressure to give a pale yellow oil. The pure product **3d** (5.61 g, 89% yield) was dissolved in dry toluene, concentrated again, and stored in tightly closed vessel at −20 °C. Its diastereomeric composition, chemical shifts in ^{31}P NMR and TLC parameters are given in Table 1: MS (+FAB) *m/z* 972.2 (M^+, 0.75%), 973.2 ($M^+ + 1$, 1.59%), 303.2 (DMT^+, 100%); ^{1}H NMR (CD_3CN) δ 1.09−1.13 (pair of doublets, 6H), 2.63−2.76 (m, 2H), 3.15−3.35 (m, 2H), 3.42−3.53 (m, 3H), 3.68−3.69 (pair of singlets, 6H), 4.22−4.55 (m, 3H), 5.51−5.61 (m, 1H), 6.3−6.37 (t, 1H), 6.66−6.77 (m, 4H), 7.12−7.52 (m, 18H), 8.15 (s, 1H), 8.59 (s, 1H).

Separation of the Diastereomers of Oxathiaphospholane Monomers 3a−d and 20a−d. A solution of 1 g of monomer **3** (or **20**) in 3 mL of ethyl or butyl acetate was applied onto a column (30 × 6 cm) containing 100 g of silica gel (Merck 60H). The column was eluted with 700−800 mL of appropriate eluent (see note to Table 1), and the fractions of 10−12 mL were collected. TLC control of the eluate was performed on HP-TLC plates. Enriched fractions were combined, concentrated, and rechromatographed. Typically, only 200 mg of "fast"-migrating and 150 mg of "slow"-migrating pure diastereomers of **3** were obtained after a 5-fold run of each partially enriched fraction through the column. The amount of silica gel used for the last two (of the five) separations was reduced to 50−60 g. Separation of monomers **20b** and **20c** onto isomers was more efficient and amounts of pure isomers comparable to those of **3** were obtained after only 3-fold

chromatography. As mentioned earlier, separation of **20d** was more difficult than the separation of **3d**. All separated isomers were stored at −20 °C.

Automated Solid-Phase Synthesis of S-Oligos. The synthesis of S-Oligos was performed on a ABI 391 synthesizer (Applied Biosystems, Inc., Foster City, CA). Standard solutions of DCA in methylene chloride and DMAP/Ac_2O/lutidine in THF were used for detritylation and capping steps, respectively. The 0.5 M solution of DBU in CH_3-CN was delivered from the position of 1*H*-tetrazole. The crucial parameters of the protocol are shown in Table 3. The detailed protocol for the synthesis is available on request.

5′-End Labeling of S-Oligos by [γ-^{32}P]ATP. S-Oligo (0.1 A_{260} unit, 3−6 μg, 0.6−1.0 nmol) was dissolved in 20 μL of a buffer containing 10 mM Tris-Cl (pH 8.5), 10 mM $MgCl_2$, and 7 mM 2-mercaptoethanol. Then [γ-^{32}P]ATP solution (1 μL 10 μCi) and T4 polynucleotide kinase (5 U) were added. Labeling was completed after 3 h at 37 °C. The purity of the oligomers was evaluated by running ^{32}P labeled samples on a 20% polyacrylamide/7 M urea gel, followed by densitometric analysis of the autoradiograms.

Proof of Diastereomeric Purity of S-Oligos. (1) The diastereomeric purity of [all-S_p] S-Oligos was determined using two [Rp]-specific nucleases: snake venom phosphodiesterase (svPDE) and endonuclease from *Serratia marcescens* (Sma E). The samples of oligonucleotides [Mix; all-R_p and all-S_p] were incubated with svPDE and Sma E at 37 °C for 24 h. In a typical experiment the reaction mixture (60 μL) contained 600 units of Sma E, 3 μg of svPDE, 100 mM Tris-Cl (pH 8.5), 15 mM $MgCl_2$, and 1−2 μg (0.2−0.3 nmol) of S-Oligo labeled at the 5′-end with ^{32}P. Aliquots (10 μL), taken after 0, 2, 4, 8, 16, and 24 h, were heat-denaturated and analyzed by 20% polyacrylamide/7 M urea gel electrophoresis.

(2) The diastereomeric purity of [all-R_p] constructs was assessed by means of [S_p]-specific nuclease P1. The samples of oligonucleotides [Mix, all-R_p and all-S_p] were incubated with this enzyme at 21 °C for 24 h. In a typical experiment the reaction mixture (60 μL) contained 0.05−0.3 μg of nuclease P1, 100 mM Tris-Cl (pH 7.2), 1 mM $ZnCl_2$, and 1−2 μg (0.2−0.3 nmol) of 5′-^{32}P labeled oligonucleotide. Aliquots (10 μL), taken after 0, 2, 4, 8, 16, and 24 h, were heat-denaturated and analyzed by 20% polyacrylamide/7 M urea gel electrophoresis.

5′-Deoxy-5′-(2-hydroxyethylmercapto)thymidine. To a solution of 5′-deoxy-5′-bromo-3′-*O*-acetylthymidine (36.3 mg, 0.1 mmol) in 400 μL of anhydrous ethanol was added at room temperature, 250 μL of a 1 M ethanol solution of sodium 2-hydroxyethylmercaptide. The reaction mixture was kept at 80 °C for 15 min and then cooled, and the solvent was evaporated. The crude product was extracted from the residue with chloroform−methanol (10:1 v/v, 3 mL) and purified by column chromatography on silica gel (230−400 mesh) using chloroform−methanol (gradient from 20:1 to 10:1 v/v) as eluent. Fractions containing desired product (TLC control, chloroform−methanol 10:1, R_f 0.2) were combined and concentrated to give 18 mg of 5′-deoxy-5′-(2-hydroxyethylmercapto)thymidine as an amorphous powder: ^{1}H NMR (CD_3CN) δ 1.835 (d, 3H, 1.2Hz, CH_3); δ 2.132−2.195 (m, 2H, H2′); δ 2.818 (d, 2H, 6.0 Hz, H5′); δ 3.633 (t, 2H, 6.4 Hz, CH_2O); δ 3.910 (t of d, 1H, 4.0 Hz, 6.0 Hz, H4′); δ 4.266 (q, 1H, 4.0 Hz, H3′); δ 6.174 (t, 1H, 6.9 Hz, H1′); δ 7.357 (q, 1H, 1.2 Hz, H6); MS FAB (LSIMS) *m/z* 303 $[M + 1]^+$.

2-Hydroxyethyl Selenol. Bis-(2-hydroxyethyl) diselenide[41] (19.84 g, 80 mmol) was dissolved in 200 mL of ethanol and cooled in a water/ice bath. To the stirred solution was added (in several portions) under dry argon 1.81 g (48 mmol) of $NaBH_4$. Stirring was continued at room temperature for 1 h, and then concentrated hydrochloric acid (24 mL) was added dropwise with stirring. The resultant suspension was filtered, and the filtrate was concentrated under reduced pressure. The crude product (20.08 g) was distilled under reduced pressure to give 7.72 g of 2-hydroxyethyl selenol (31%) as a colorless oil: bp 64−66 °C/12 mmHg; lit.[42] 75 °C/16 mmHg); ^{1}H NMR (200 MHz, $CDCl_3$) δ −0.66 (t, J = 7.5 Hz, 1H); δ 2.37 (br s, 1H); δ2.75 (dt, J = 7.5 Hz, J = 6.3 Hz, 2H); δ 3.77 (br t, J = 5.2 Hz, 2H). MS (EI), *m/z* 125 ($M^{\bullet+}$). Attention: 2-hydroxyethyl selenol requires handling in an argon atmosphere because air oxidizes it rapidly to diselenide.

2-(*N*,*N*-Diisopropylamino)-1,3,2-oxaselenaphospholane. A solution of 2-hydroxyethyl selenol (5.0 g, 40 mmol) in dry methylene chloride (40 mL) was added dropwise to a stirred and cooled (−30 °C) solution of dichloro-*N*,*N*-diisopropyloaminophosphine[43] (8.1 g, 40 mmol) and dry triethylamine (12.2 mL, 88 mmol) in 160 mL of dry methylene chloride. Stirring at −30 °C was continued for 15 min and then at room temperature for 1 h. The reaction mixture was filtered, and the solvent was evaporated. The residue was shaken with hexane (80 mL), and the mixture was filtered and concentrated. The crude product (10.6 g) was distilled under reduced pressure to give 2-(*N*,*N*-diisopropylamino)-1,3,2-oxaselenaphospholane as a colorless oil: 6.70 g, 66%, bp 83−84 °C/0.5 mmHg; ^{1}H NMR (C_6D_6) δ 1.11 (dd, J = 11.7 Hz, J = 6.8 Hz, 12H); δ 2.48−2.59 (m, 1H); δ 2.63−2.76 (m, 1H); δ 3.35−3.64 (m, 3H), δ 3.93−4.18 (m, 1H); ^{31}P NMR (C_6D_6) δ 157.0 ($^1J_{P-Se}$ = 222 Hz); MS (EI) *m/z* 255 ($M^{\bullet+}$).

5′-*O*-Dimethoxytritylthymidine-3′-*O*-(2-thio-1,3,2-oxaselenaphospholane). To a suspension of 5′-*O*-dimethoxytritylthymidine (1.64 g, 3 mmol) and 1H-tetrazole (234 mg, 3.3 mmol) in dry methylene chloride (15 mL) was added dropwise with stirring neat 2-(*N*,*N*-diisopropylamino)-1,3,2-oxaselenaphospholane (0.84 g, 3.3 mmol). After 2 h elemental sulfur (144 mg, 4.5 mmol) was added, and stirring was continued for 16 h. The suspension was filtered, and the filtrate was extracted with water (15 mL). The organic layer was dried with magnesium sulfate and concentrated to give a foam (2.94 g). The crude product was purified by column chromatography on silica gel (50 g) with a mixture of methylene chloride/methanol 39:1 (v/v) and then methylene chloride/methanol 19:1 as the eluents. Appropriate fractions (TLC control: methylene chloride/methanol 19:1, R_f = 0.38) were collected and evaporated to dryness to give the product (1.15 g, 53%) as a solid foam: ^{1}H NMR ($CDCl_3$) δ 1.42−1.44 (m, 3H), 2.30−2.69 (m, 2H), 3.38−3.51 (m, 2H), 3.56−3.71 (m, 2H), 3.79 (s, 6H), 4.29−4.31 (m, 1H) 4.38−4.54 (m, 2H), 5.48−5.57 (m, 1H), 6.42−6.49 (m, 1H), 6.79−6.88 (m, 4H), 7.15−7.42 (m, 9H), 7.59−7.62 (m, 1H), 8.30 (s, 1H); ^{31}P NMR (C_6D_6) δ 93.2 and 93.7 ppm ($^1J_{P-Se}$ = 462 Hz for both isomers); MS (+FAB) *m/z* 730.4 ($M^+ + 1$); elemental analysis for $C_{33}H_{35}N_2O_8PSSe$: calc./found C 54.32/54.20, H 4.83/5.10, N 3.84/3.55, P 4.25/5.18, S 4.40/4.56.

A sample of the product (120 mg) was then chromatographed on silica gel 60H (12 g, MERCK) using butyl acetate as a eluent. "Fast"-enriched and "slow"-enriched fractions (ratio 77:23, 41 mg and 30:70, 68 mg, respectively) were obtained. The ratio of isomers was determined by ^{31}P NMR.

Solid Phase Synthesis of $T_{PS}T$ Using Oxaselenaphospholane Monomer. 5′-*O*-DMT-thymidine (1 μmol) bound to the standard LCA CPG solid support was detritylated with a 2% solution of DCA in methylene chloride, then washed thoroughly with 10 mL of dry acetonitrile, and dried. For the coupling step a solution of 5′-*O*-dimethoxytritylthymidine-3′-*O*-(2-thio-1,3,2-oxaselenaphospholane (**29**, 29 mg, 40 μmol) and DBU (15 μL, 100 μmol) in dry acetonitrile (150 μL) was prepared and instantly introduced into the column. After 10 min the column was washed with dry acetonitrile (10 mL), and the product was detritylated. The column was washed again, and the dinucleotide was released from the support by treatment with concentrated ammonia (1 mL) for 1 h. The solution was evaporated to dryness, and the residue was dissolved in water (1 mL) and filtered. RP-HPLC analysis of the crude product showed the presence of $T_{PS}T$ (98%) and unreacted thymidine (2%). Similar syntheses were performed using "fast-" and "slow-enriched monomers **29**.

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