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**THE SYNTHESIS OF 5'-O-DMT-THYMIDINE  
3'-O-(2-THIO-1,3,2-OXASELENAPHOSPHOLANE)  
AND ITS POSSIBLE APPLICATION IN STEREOCONTROLLED SYNTHESIS  
OF OLIGO(NUCLEOSIDE PHOSPHOROTHIOATE)S**

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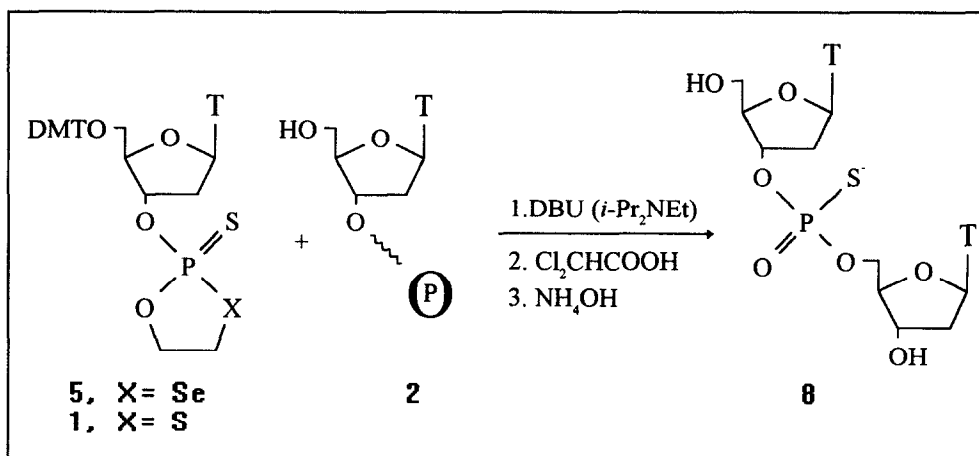
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**Abstract.** 5'-O-DMT-Thymidine 3'-O-(2-thio-1,3,2-oxaselenaphospholane) has been obtained and employed in the reaction with 3'-protected thymidine in the presence of base-catalysts. If DBU was used, dithymidyl(3',5')phosphorothioate was formed in 98% yield with high stereospecificity. This result kindled new hopes for efficient stereocontrolled solid-phase synthesis of oligo(nucleoside phosphorothioate)s.

The stereocontrolled synthesis of P-chiral analogues of oligonucleotides poses one of the most challenging problems in oligonucleotide chemistry.<sup>1,2</sup> Routine methods of synthesis of oligo(nucleoside phosphorothioate)s (Oligo-S), one of the most promising classes of "antisense" agents,<sup>3,4</sup> all lead to the mixture of 2<sup>n</sup> diastereomers, where *n* means the number of internucleotide phosphorothioate functions. Each component of this mixture constitutes an individual compound characterized by its unique chirality, and therefore is able to interact with other biomolecules by its own right. Therefore, the results of all biological tests utilizing the so-called "random mixture of diastereomers" have to be taken with caution. Cellular uptake, intracellular compartmentalization, stability against cellular nucleases, avidity towards target RNA, and specific or nonspecific interactions with cellular proteins can be stereodependent towards individual diastereomers or populations thereof characterized by similar chirality. Recently we have demonstrated that 5'-O-DMT-nucleoside-3'-O-(2-thio-1,3,2-oxathiaphospholane)s (**1**) undergo 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)-assisted reaction with nucleosides 3'-O-anchored to solid support (**2**).<sup>5</sup> This reaction occurs with cleavage of the endocyclic P-S bond and is followed by base-catalyzed elimination of ethylene episulphide.

The resulting product has been identified, after cleavage from the solid support and removal of protective groups, as dinucleoside(3',5')phosphorothioate (**3**). Most important, the separation of **1** into diastereomeric species allowed to conclude that DBU-assisted reaction of **1** with **2** occurs with full stereospecificity. However, the application of oxathiaphospholanes **1** to the preparation of diastereomerically pure oligo(nucleoside phosphorothioate)s (**4**) of predetermined sense of chirality at phosphorus of each internucleotide phosphorothioate function has serious limitations, since under conditions of solid phase synthesis the step-yield is ca. only 90-95%.

Therefore, the resulting **4** are formed in unsatisfactory yield and are contaminated with shorter sequences; the most effective method for purification is isolation of **4** by means of preparative electrophoresis and gel extraction. To avoid these inconveniences we speculated that the replacement of **1** by corresponding nucleoside-3'-O-(2-thio-1,3,2-oxaselenaphospholanes) (**5**) might bring an improvement in step yield because of the known higher reactivity of the P-Se bond as compared with the P-S bond.<sup>6</sup> We expected that condensation of **5** with **2** would require less basic catalyst than DBU which is known to be responsible for several unwanted side reactions. 5'-O-DMT-Thymidine-3'-O-(2-thio-1,3,2-oxaselenaphospholane) (**5**) was obtained as the mixture of two diastereomers in the ratio 55:45<sup>7</sup> in tetrazole-assisted reaction of 5'-O-DMT-thymidine with N,N-diisopropylamino-1,3,2-oxaselenaphospholane (**6**)<sup>8</sup>, followed by sulphurization with elemental sulphur. Its reaction with 3'-O-acetyl-thymidine in the presence of diisopropylethylamine was, on the contrary to our expectations, very slow, and after 5h gave 5'-O-DMT-thymidylyl-3'-O-acetylthymidylyl(3',5')phosphorothioate (**7**) in only 10% yield. Also the use of LiCl, which is known to enhance the amine-basicity,<sup>9</sup> did not bring any substantial improvement in the yield of the expected product (equimolar amounts of nucleosidyl components, 10-fold molar excess of diisopropylethylamine and 5-fold molar excess of LiCl in DMF solution), which was 18%. However, this reaction occurs with 95% yield in 5 min. in acetonitrile solution, if a 2-fold molar excess of DBU is used instead of diisopropylethylamine. Similar results were obtained under conditions of solid-phase synthesis.



After acid-detritylation of 3'-O-bound to solid-support 5'-O-DMT-thymidine (1  $\mu$ mole), a 0.2 M solution of **5** and a 1 M solution of diisopropylethylamine or a 0.5 M solution of DBU in acetonitrile, respectively, were applied to the column. After 10 min. the column content was washed with acetonitrile and after detritylation dithymidylyl(3',5')phosphorothioate (**8**) was released from the solid support by ammoniolysis. The yield of **8** was 2% in the case of diisopropylethylamine and 98% in the case of DBU.

The ratio of [Rp]:[Sp] diastereomers within **8** was 47:53 and 43:57, respectively.<sup>10</sup> Within the following set of experiments the separation of **5** into diastereomeric species was undertaken to check the stereospecificity of oxaselenaphospholane ring opening process. The single run of **5** through a silica-gel column (Kieselgel 60H, Merck, ratio **5** to silica-gel 1:100) with butyl acetate as an eluent allowed collection of fractions significantly enriched with "fast"-eluted **5** (diastereomeric ratio 77:23; <sup>31</sup>P-NMR:  $\delta$  93.92, 93.72 ppm, respectively). When used for the solid-phase synthesis of **8** under conditions of DBU catalysis (time and concentrations as above), the ratio of diastereomers was [Rp]:[Sp]=25:75. "Slow"-eluted **5** (diastereomeric ratio 70:30), gave diastereomers **8** with the ratio [Rp]:[Sp]= 62:38. These results demonstrated that the formation of a phosphorothioate dimer occurs with full stereospecificity and that the "fast"-eluted **5** is the substrate for [Sp]-**8**, while for the preparation of [Rp]-**8** "slow"-eluted **5** has to be used. Both, high stereospecificity of the reaction under investigation and the satisfactory yield of **8** are encouraging and kindled hope for improvement in the stereocontrolled synthesis of oligo(nucleoside phosphorothioate)s (**4**), although we realize that an access to substrates **5** is more troublesome than the preparation of corresponding oxathiaphospholanes.<sup>5</sup> Moreover, the separation of **5** into diastereomeric species also may pose a serious problem. Work on this aspect is in progress.

The most unexpected result noticed within the course of the above studies is the relatively low reactivity of oxaselenaphospholanes in the presence of catalysts of lower basicity than DBU. This may indicate that the ring opening process does not depend upon the liability of the bond between phosphorus and the leaving ligand, but rather upon the concentration of nucleophile resulting from ionization of attacking 5'-hydroxyl group. However, the use of bases even stronger than DBU, such as 2-*tert*-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine,<sup>11</sup> does not improve the coupling efficiency. Therefore, this observation suggests that the function of the base is not limited to the enhancement of nucleophilicity of 5'-oxygen of nucleoside and may indicate the intermediacy of species resulting from interactions of oxathiaphospholanes or oxaselenaphospholanes with DBU, generally considered as a non-nucleophilic base. Studies on interactions between **5** and DBU are in progress and results will be published in due course.

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## References and Notes

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4. Milligan, J.F.; Matteucci, M.D.; Martin, J.C. *J.Med.Chem.* 1993, 36, 1923.
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6. Lower stability of a phosphorus-selenium bond as compared to the corresponding phosphorus-sulphur bond has been noticed in our earlier studies. We have observed that silver-promoted ethanolysis of S,Se-dimethylphosphoroselenothioamides occurs predominately with replacement of the MeSe-ligand (Krzyżanowska, B.; Stec, W.J. *Heteroatom Chem.* 1991, 2, 123) and that under conditions of catalyzed solvolysis of selenoesters corresponding thioesters remain practically intact (Woźniak, L.A.; Krzyżanowska, B.; Stec, W.J. *J.Org.Chem.* 1992, 57, 6057).
7. The ratio of diastereomers of **5** was determined by  $^{31}\text{P}$ -NMR:  $\delta$  93.2, 93.7 ppm,  $^1J_{31\text{P}-77\text{Se}}=462$  Hz (for both diastereomers). Spectra were recorded for  $d_6$ -benzene solutions on a Bruker AC 200 with spectral width 2000 Hz. Careful analysis of several batches of **5** revealed the presence of 3÷5% of contaminant, which has been identified as 5'-O-DMT-thymidine-3'-O-(2-seleno-1,3,2-oxaselenaphospholane) [**9**,  $^{31}\text{P}$  NMR:  $\delta$  85.3, 85.0 ppm,  $^1J_{31\text{P}-77\text{Se}} = 948$  Hz (P=Se) and 469 Hz (P-Se)] (Stec, W.J., Okruszek, A., Uznański, B., Michalski, J., *Phosphorus*, 1972, 2, 97). **9** was also obtained on independent way in reaction of 5'-O-DMT-thymidine with **6** in the presence of tetrazole, and subsequent selenization with elemental selenium. Studies on the elucidation of the mechanism of formation of **9** accompanying the synthesis of **5** are in progress.
8. N,N-Diisopropylamino-1,3,2-oxaselenaphospholane [**6**,  $^{31}\text{P}$ -NMR,  $\delta$  157.0 ( $^1J_{31\text{P}-77\text{Se}} = 222$  Hz), ( $d_6$ -benzene)] was obtained by the reaction of 2-hydroxyethylselenol [prepared by the  $\text{NaBH}_4$  reduction of appropriate diselenide (Jakiwicz, O.M.; Kristoff, E.M.; McPhee, D.J. *Synth. Commun.* 1993, 23, 195)] with dichloro-N,N-diisopropylaminophosphine in the presence of triethylamine. The identity of **6** was confirmed by  $^1\text{H}$ -,  $^{13}\text{C}$ -NMR and MS.
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10. The yield of **8** and the ratio of diastereomers were assigned by reverse-phase HPLC (Stec, W.J.; Zon, G.; Uznański, B. *J.Chromatogr.* 1985, 326, 263). The identity of **8** was confirmed by co-injection with an authentic sample synthesized by the phosphoramidite method with bis(O,O-diisopropoxy phosphinothioyl)disulfide as a sulphurizing reagent (Stec, W.J.; Uznański, B.; Wilk, A.; Hirschbein, B.L.; Fearon, K.L.; Bergot, B.J. *Tetrahedron Lett.* 1993, 34, 5317). Minute amounts (ca 4%) of the contaminant with longer retention times these that for **8** were found to accompany every preparation of **8**, which was identified as dithymidylyl(3',5')-phosphoroselenoate (**10**) (Stec, W.J., Zon, G., Egan, W., Stec, B., *J.Am.Chem.Soc.*, 1984, 106, 6077). **10** results from the reaction of **9** (see ref.7) with **2**, as proved in the independent experiment. Studies on the utilisation of **9** for the synthesis of oligo(nucleoside phosphoroselenoate)s are in progress.
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