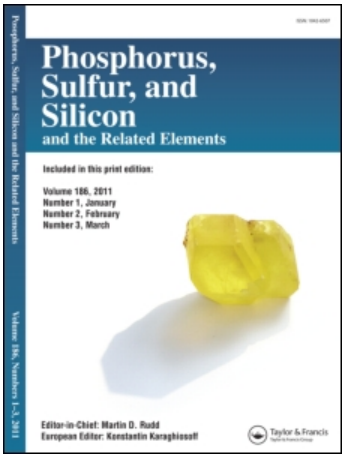

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Nucleoside-*O*-(2-Thiono-1,3,2-Oxathiaphospholane)S-Versatile Tools in the Synthesis of Oligonucleotide Analogues

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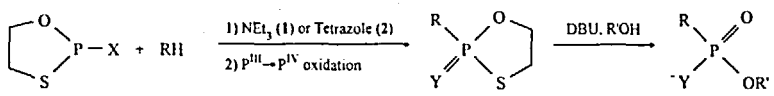
The scope and limitations of 1,3,2-oxathiaphospholane ring opening condensation process are discussed with the emphasis paid to internucleotide bond formation and stereocontrolled synthesis of P-chiral phosphorothioate analogues of oligonucleotides.

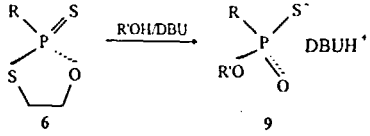
Keywords: Oligonucleotides; phosphorothioates; 1,3,2-oxathiaphospholanes; ring-opening condensation

The designed in this laboratory approach to *phosphylation* of alcohols and amines relies upon condensation of either 2-chloro- or 2-*N,N*-diisopropylamino-1,3,2-oxathiaphospholanes (**1**, **2**) with alcohols[1] or amines[2] (**3**, **4**) followed by oxidation of intermediary phosphite or phosphoramidite. Resulting 2-alkoxy- or 2-alkylamino-2-(oxo-, thiono-, or seleno)-1,3,2-oxathiaphospholanes (**5**, **6**, **7**) under DBU-assisted treatment with alcohols undergo regioselective oxathiaphospholane ring-opening followed by fast elimination of ethylene sulphide, providing corresponding diesters or amidoesters of phosphoric (**8**)[3,4], phosphorothioic (**9**)[4], or phosphoroselenoic (**10**)[5] acids.



1. X = Cl
2. X = $NiPr_2$
3. R = AlkO
4. R = AlkNH
5. X = O
6. X = S
7. X = Se
8. X = O
9. X = S
10. X = Se

Compounds **5** upon treatment with fluoride ion provide *N*-alkyl phosphoramidofluoridates[3] or *O*-alkyl phosphorofluoridates.[6] If R constitutes chiral auxiliary, compounds **6** can be separated into diastereomerically pure species. Their conversion to **9** occurs with full stereospecificity (>99%) and net *retention* of configuration at phosphorus atom. The yield of this ring opening condensation is higher than 95% under conditions of solid phase oligonucleotide synthesis.[4]



6 **9**

Results of *ab initio* calculations[7] indicate that 1,3,2-oxathiaphospholane ring opening condensation occurs *via* TBP-intermediate with apical positions occupied by *endocyclic* oxathiaphospholane oxygen and attacking alkoxide (R'O) groups, respectively. Such intermediate undergoes pseudorotation accompanied by the cleavage of P-S bond followed by ethylene sulphide elimination, violating the Westheimer Rule: *apical entry-apical departure*[8].

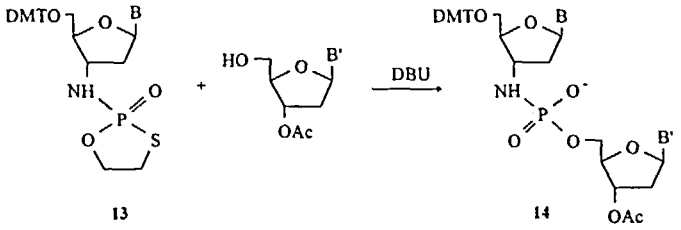
Diastereomerically pure nucleoside 3'-*O*-(2-thiono-1,3,2-oxathiaphospholane)s (**6**, R=3'-nucleoside) appeared to be most reliable, so far, substrates for the stereocontrolled synthesis of P-chiral oligo(nucleoside phosphorothioate)s **(11)**[4]. While enzymatic synthesis of **11** provides PS-oligos of [All-R_P]-configuration[9], oxathiaphospholane (OTP) method allows for the synthesis of phosphorothioate analogues of oligonucleotides with predetermined sense of chirality at each internucleotide phosphorothioate linkage.

In the light of our observation that 3'-exonucleases present in cellular media are not able to cleave internucleotide phosphorothioates of [S_P]-configuration[10], stereocontrolled synthesis of PS-oligos is of special importance. OTP method allows for the synthesis of PS-oligos or PO/PS chimeric oligonucleotides[11] (mixed backbone oligonucleotides) protected at flanking 3'- and/or 5'-internucleotide with [S_P]-phosphorothioates from exonucleolytic degradation. Therefore, PS-oligo constructs, broadly used in *antisense strategy of suppression of translation process*[12], if

appropriately protected can keep their structural integrity. Interference of biological activity of PO-oligos with products of their enzymatic degradation, namely nucleoside 5'-*O*-phosphates, has been recently demonstrated.[13] Moreover, short-mers resulting from degradation of PS-oligos may be responsible for the side-effects accompanying antisense experiment. We demonstrated that nucleoside 3'-*O*-(2-oxo-1,3,2-oxathiaphospholanes) (**5**, R=3'-nucleoside; for P^{III}-P^{IV} conversion SeO_2 is the reagent of choice) can be used for the synthesis of short PO-oligonucleotides (e.g. dodecamers), or, if used in combination of **5** and **6**, for the synthesis of PO/PS-chimeric molecules, where PS-internucleotide linkages posses predetermined sense of chirality.[11]

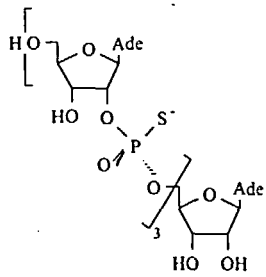
Recently, new generation of oligonucleotide constructs, bearing attached to antisense oligonucleotide at 5'-end tetra(adenosine 2',5'-phosphorothioate)s, has been described.[14] $_{PS}$A2',5'$_{PS}$A2',5'$_{PS}$A2',5'$_{PS}$A plays an important role of activation of RNase L, an ubiquitous enzyme responsible for cleavage of mRNA complementary to *antisense* oligonucleotide. We demonstrated that 5'-*O*-DMT-N^6-benzoyladenosine 3'-*O*-*t*-butyldimethylsilyl-2'-*O*-(2-thiono-1,3,2-oxathiaphospholane), if separated into pure diastereomers, allows for the first solid-phase, stereocontrolled synthesis of [All-R_P]- and [All-S_P] tetra-adenosine (2',5') phosphorothioates (**12**).[15]

The synthesis of corresponding dodecamers is in progress.

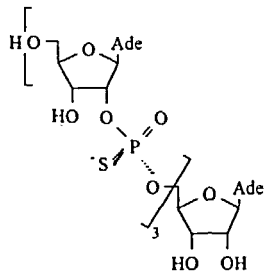


Somehow disappointing were the results on application of OTP method to the synthesis of oligo(nucleoside phosphoramidate)s[16]. Although nucleoside 3'-deoxy-3'-amino-3'-*N*-(2-oxo-1,3,2-oxathiaphospholane) (**13**) react with 5'-OH nucleoside providing in >95% yield dinucleoside phosphoramidate (**14**), solid-phase synthesis of oligo(nucleoside phosphoramidate)s can not be achieved because of poor solubility

of anchored to solid support **14** in non-hydroxylic media[3]. Studies on „in-solution" synthesis of **10** and the ring-opening polymerization of **6** leading to P-homochiral poly(nucleotide phosphorothioate)s are in progress[17].



All-R_P-12



All-S_P-12

References

[1] W.J. Stec, A. Grajkowski, M. Koziołkiewicz, and B. Uznański, *Nucleic Acids Res.*, **19**, 5883 (1991).

[2] B. Uznanski, A. Grajkowski, B. Krzyżanowska, A. Kaźmierkowska, W.J. Stec, M.W. Wieczorek, and J. Błaszczyk, *J.Am.Chem.Soc.*, **114**, 10197 (1992).

[3] J. Baraniak, and D. Korczyński, unpublished results.

[4] W.J. Stec, A. Grajkowski, B. Karwowski, A. Kobylańska, M. Koziołkiewicz, K. Misiura, A. Okruszek, A. Wilk, P. Guga, and M. Boczkowska, *J.Am.Chem.Soc.*, **117**, 12019 (1995).

[5] K. Misiura, and W.J. Stec, *Bioorg. Med. Chem. Let.*, **4**, 1037 (1994).

[6] K. Misiura, D. Szymanowicz, and W.J. Stec, *Chem.Commun.*, **1998**, 515.

[7] T. Uchimaru, W.J. Stec, and K. Taira, *J.Org.Chem.*, **62**, 5793 (1997).

[8] R.S. Westheimer, Acc.Chem.Res., **1**, 70 (1968); G.R.J. Thatcher, and R. Kluger, *Adv.Phys.Org.Chem.*, **25**, 99 (1989).

[9] J.G. Hacia, B.J. Wold, and P.B. Dervan, *Biochemistry*, **33**, 5367 (1994); J. Tang, A. Roskey, Y. Li, and S. Agrawal, *Nucleosides Nucleotides*, **14**, 985 (1995).

[10] M. Koziołkiewicz, M. Wójcik, A. Kobylańska, B. Karwowski, B. Rębowska, P. Guga, and W.J. Stec, *Antisense Nucl. Acids Drug Dev.*, **7**, 43 (1997).

[11] W.J. Stec, B. Karwowski, P. Guga, M. Koziołkiewicz, M. Boczkowska, M.W. Wieczorek, and J. Błaszczyk, *J.Am.Chem.Soc.* – in press.

[12] E. Uhlmann, and A. Peyman, *Chem.Rev.*, **90**, 544 (1990).

[13] J.L. Vaerman, P. Moureau, F. Deldime, P. Lewalle, C. Lammineur, F. Morschhauser, and P. Martiat, *Blood*, **90**, 331 (1997).

[14] W. Xiao, G. Li, R.K. Maitra, A. Maran, R.H. Silverman, and P.F. Torrence, *Bioconjugate Chem.*, **4**, 467 (1993).

[15] Xian-Bin Yang, A. Sierzchała, K. Misiura, W. Niewiarowski, M. Sochacki, W.J. Stec, and M.W. Wieczorek, *J.Org.Chem.* – in press.

[16] S.M. Gryaznov, and J. Chen, *J.Am.Chem.Soc.*, **116**, 3143 (1994).

[17] B. Karwowski, and P. Guga, unpublished results.