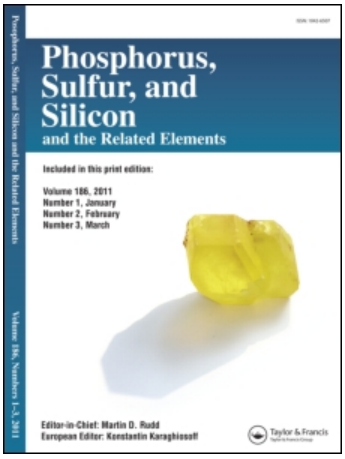


Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

Synthesis of P-Chiral [18 O]-Labeled Isotopomeric Oligo(deoxyribonucleoside Phosphorothioate)s and Phosphates

Piotr Guga[a]; Krzysztof Domański[a]; Maria Koziołkiewicz[a]; Alina Owczarek[a]; Wojciech J. Stec[a]
[a] Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Łódź, Poland

Online publication date: 27 October 2010

To cite this Article Guga, Piotr , Domański, Krzysztof , Koziołkiewicz, Maria , Owczarek, Alina and Stec, Wojciech J.(2002) 'Synthesis of P-Chiral [18 O]-Labeled Isotopomeric Oligo(deoxyribonucleoside Phosphorothioate)s and Phosphates', Phosphorus, Sulfur, and Silicon and the Related Elements, 177: 6, 1601 — 1604
To link to this Article: DOI: 10.1080/10426500212288
URL: http://dx.doi.org/10.1080/10426500212288

Phosphorus, Sulfur and Silicon, 2002, Vol. 177:1601–1604

1042-6507/02 $12.00 + .00
DOI: 10.1080/10426500290093135

SYNTHESIS OF P-CHIRAL [^{18}O]-LABELED ISOTOPOMERIC OLIGO(DEOXYRIBONUCLEOSIDE PHOSPHOROTHIOATE)S AND PHOSPHATES

Piotr Guga, Krzysztof Domański, Maria Koziołkiewicz, Alina Owczarek, and Wojciech J. Stec
Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, 90-363 Łódź, Poland

(Received July 30, 2001; accepted December 25, 2001)

Diastereomerically pure, isotopically labeled 5′-O-DMT-nucleoside-3′-O-(2-thio-4,4-"spiro"-pentamethylene-1,3,2-[^{18}O]oxathiaphospholane)s and -(2-oxo-4,4-"spiro"-pentamethylene-1,3,2-[^{18}O]oxathiaphospholane)s suitable for stereocontrolled synthesis of P-chiral oligonucleotide [^{18}O]phosphorothioates and [^{18}O]phosphates were synthesized. Obtained in that way "chimeric" oligonucleotide d[$A_{PO}A_{PS\text{-}Rp,Sp}A_{PS\text{-}Rp,Sp}A_{PS\text{-}Rp}C_{PS(18O)\text{-}Rp}G_{PS\text{-}Rp}T_{PS\text{-}Rp}T_{PS\text{-}Rp,Sp}T_{PO}T$] was used for determination of the stereochemistry of hydrolysis by endonuclease from Serratia marcescens.

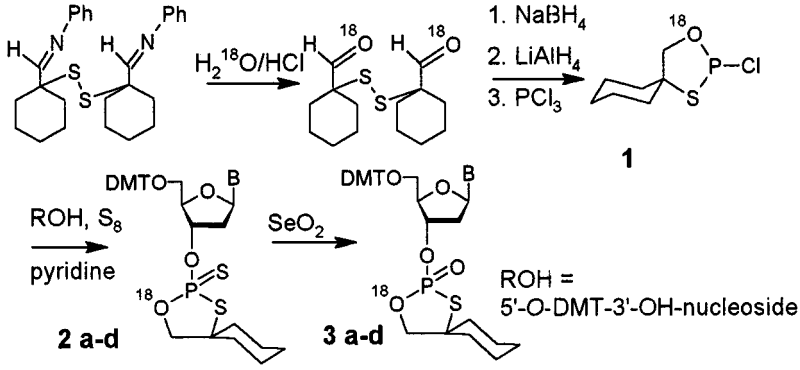
Keywords: DNA probes; isotopic labeling; nucleases

In studies on the mechanism of action of nucleases and phosphotransferases, configurational analysis of the products of enzymatic degradation of stereodefined P-chiral substrates (e.g., oligo(deoxyribonucleoside phosphorothioate)s (PS-Oligos) or [^{18}O]phosphates (P^{18}O-Oligos)) in isotopically labeled water allow one to distinguish between *one-step* and *two-step* modes of enzyme action, with enzyme-assistance in nucleophilic substitution at P atom or involvement of covalent enzyme-substrate intermediate, respectively.[1] Standard chemical methods provide PS-Oligos as a mixture of 2^n diastereoisomers, where *n* is a number of phosphorothioate linkages.[2] The first method for their stereocontrolled synthesis, so called oxathiaphospholane approach, was elaborated in this laboratory,[3] and stereodefined PS-Oligos were used for biochemical and biophysical studies, including stability

Address correspondence to P. Guga. E-mail: pguga@bio.cbmm.lodz.pl

against nucleases and thermal stability of duplexes or other high-order structures.[4–7] Since the presence of a sulfur atom changes the properties of internucleotide bonds,[8] certain properties of PS-Oligos are different from those of natural oligonucleotides. These inconveniences can be avoided by use of P-chiral $P^{18}O$-Oligos for hydrolysis in [^{17}O]water.

The oxathiaphospholane method has been applied for the synthesis of stereodefined $PS^{18}O$-Oligos and $P^{18}O$-Oligos. The synthesis of labeled phosphitylating reagent 2-chloro-"spiro"-4,4-pentamethylene-1,3,2-[^{18}O]oxathiaphospholane (**1**) is depicted on Scheme 1. Attempts



SCHEME 1

at acid-catalyzed hydrolysis of cyclohexanecarbo-*N*-phenyl-imine to introduce the isotope label yielded the crystalline cyclic trioxane (resulting from trimerization of cyclohexanecarbaldehyde) instead of the desired labeled aldehyde. For the trimeric product, the m.p. 173–179°C was found, and its ^{1}H NMR contained characteristic doublet at δ 4.49 ppm assigned to O—C<u>H</u>C_6H_{11}—O. The isotope label was introduced by hydrolysis of 2,2′-dithiobis(cyclohexanecarbo-*N*-phenyl-imine) upon treatment with anhydrous HCl in the presence of 1.1 molar equivalent of [^{18}O]water (95 at.% ^{18}O). After two-step reduction of the aldehyde group and disulfide bond followed by reaction of resulting (1-sulfanyl- cyclohexyl)methan[^{18}O]ol with PCl_3, the phosphitylating reagent **1** was obtained with isotope enrichment of 87 atom % ^{18}O. Phosphitylation of 5′-*O*-DMT-nucleosides with **1**, followed by sulfurization, gave monomers **2a–d** (B = Ade^{Bz}, Cyt^{Bz}, Gua^{iBu}, Thy), which subsequently were separated chromatographically into pure P-diastereomers of R_P and S_P absolute configuration, the precursors of [^{18}O]phosphorothioate dinucleotides of the R_P and S_P absolute configuration, respectively.

To obtain monomers for direct synthesis of stereodefined $P^{18}O$-Oligos, diastereomerically pure monomers **2a–d** were stereospecifically

(as judged by ^{31}P NMR spectroscopy) and quantitatively oxidized with SeO_2 yielding 5′-*O*-DMT-nucleoside-3′-*O*-(2-oxo-"spiro"-4,4-pentamethylene-1,3,2-[^{18}O]oxathiaphospholane)s (**3a–d**), further characterized by FAB MS and ^{31}P NMR. Chemical correlation proved that a sequence of reactions: *slow*-**2**→**3**→P^{18}O-Oligo yields the [^{18}O]phosphate internucleotide bond of R_P absolute configuration. The ^{18}O-labeled cytidyl monomer **2b** and appropriate 2-thio and 2-oxo unlabeled oxathiaphospholane monomers were used for the synthesis of oligonucleotide d[$A_{PO}A_{PS\text{-}Rp,Sp}A_{PS\text{-}Rp,Sp}A_{PS\text{-}Rp}C_{PS(18O)\text{-}Rp}G_{PS\text{-}Rp}T_{PS\text{-}Rp}T_{PS\text{-}Rp,Sp}T_{PO}T$]. Its structure was based on the results of precise studies on the stereochemical and sequence preferences of the otherwise sequence nonspecific *Serratia marcescens* nuclease. Therefore, the hydrolysis (in unlabeled water) by the enzyme produced the ^{18}O-labeled oligomer d[$_{PS(18O)}G_{PS\text{-}Rp}T_{PS\text{-}Rp}T_{PS\text{-}Rp}T_{PO}T$] almost quantitatively. It was isolated by RP-HPLC and ligated by T4 DNA ligase to the 3′-end of a 44-base long "hairpin" oligonucleotide template 5′-d[CGCATCTCAAAAAAACAGAAGAGGGCCCTTTTGGG CCCTCTTCT]-3′. According to Mizuuchi, the ligation of a 5′-*O*-phosphorothioylated DNA is stereoselective and results in formation of an R_P-internucleotide phosphorothioate bond.[9] The ligation product was treated with nuclease P1 and resulting d[$_{PO}T_{PS(18O)\text{-}Rp}G_{PS\text{-}Rp}T_{PS\text{-}Rp}T_{PS\text{-}Rp}T$] was analyzed by MALDI TOF mass spectrometry, which confirmed the retention of ^{18}O-oxygen. This result indicates that the *Serratia* endonuclease cleaves the internucleotide phosphorothioate with inversion of configuration via *in-line* mechanism.

ACKNOWLEDGMENTS

The financial support of this work by The State Committee for Scientific Research (KBN), Poland, is gratefully acknowledged.

REFERENCES

[1] J. A. Gerlt, in *Nucleases, 2nd Edition*, Cold Spring Harbor Laboratory Press, 1–34 (1993).

[2] W. J. Stec and A. Wilk, *Angew. Chem.*, **106,** 747–761 (1994); *Angew. Chem. Int. Ed. Engl.*, **33,** 709–722 (1994).

[3] P. Guga, M. Koziołkiewicz, A. Okruszek, and W. J. Stec, *Applied Antisense Oligonucleotide Technology* (Eds.: C. Stein, A. Krieg), John Wiley and Sons, Inc., New York, 23–50 (1998).

[4] C. A. Brautigam and T. A. Steitz, *J. Mol. Biol.*, **277,** 363–377 (1998).

[5] M. Koziołkiewicz, A. Krakowiak, M. Kwinkowski, M. Boczkowska, and W. J. Stec, *Nucleic Acids Res.*, **23,** 5000–5005 (1995).
[6] M. Koziołkiewicz, A. Krakowiak, A. Owczarek, and M. Boczkowska, *RNA Biochemistry and Biotechnology* (Eds.: J. Barciszewski, B. Clark), Kluwer Academic Publishers, Dodrecht/Boston/London, 315–324 (1999).
[7] W. J. Stec, B. Karwowski, M. Boczkowska, P. Guga, M. Koziołkiewicz, M. Sochacki, M. Wieczorek, and J. Błaszczyk, *J. Am. Chem. Soc.*, **120,** 7156–7167 (1998).
[8] P. A. Frey and R. D. Sammons, *Science*, **228,** 541–545 (1985).
[9] K. Mizuuchi, T. J. Nobbs, S. E. Halford, K. Adzuma, and Qin, J. *Biochemistry*, **38,** 4640 (1999).