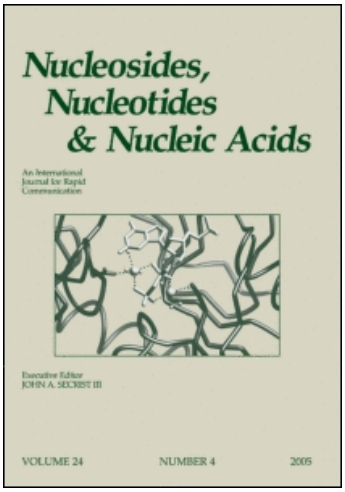

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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Online publication date: 09 August 2003

To cite this Article Owczarek, Alina , Kaczmarek, Renata , Mikołajczyk, Barbara , Wasilewska, Ewa , Korczyński, Dariusz , Baraniak, Janina , Koziołkiewicz, Maria , Stec, Wojciech J. and Brenner, Charles(2003) 'Stereochemical Analysis of Diastereomeric 1,3-bis(Adenosine-5'-O-phosphorothioyl)glycerols', Nucleosides, Nucleotides and Nucleic Acids, 22: 5, 797 — 799
To link to this Article: DOI: 10.1081/NCN-120022637
URL: http://dx.doi.org/10.1081/NCN-120022637

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, Nos. 5–8, pp. 797–799, 2003

Stereochemical Analysis of Diastereomeric 1,3-bis(Adenosine-5′-O-phosphorothioyl)glycerols

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INTRODUCTION

We have published recently[1] that 1,3-bis(adenosine-5′-phosphorothioyl)glycerol (**1**), prepared according to the oxathiaphospholane methodology[2] possesses stronger inhibitory activity towards FHIT protein, as compared with all known inhibitors.[3] Fhit protein is the Ap_3A hydrolase which binds and cleaves diadenosine polyphosphates and acts as a tumor suppressor.[4] Loss of Fhit protein is among the earliest known events in the development of a variety of the most common and lethal human malignancies. Function of Fhit in tumor suppression does not require diadenosine polyphosphates cleavage but correlates with the ability to form enzyme-substrate complexes. If Fhit-substrate complexes promote tumor suppression by stimulating a pro-apoptotic effector,[5] then Fhit inhibitors, that resemble natural substrates, may promote or antagonize Fhit function, depending on their features, in Fhit + cells.

DOI: 10.1081/NCN-120022637
1525-7770 (Print); 1532-2335 (Online)

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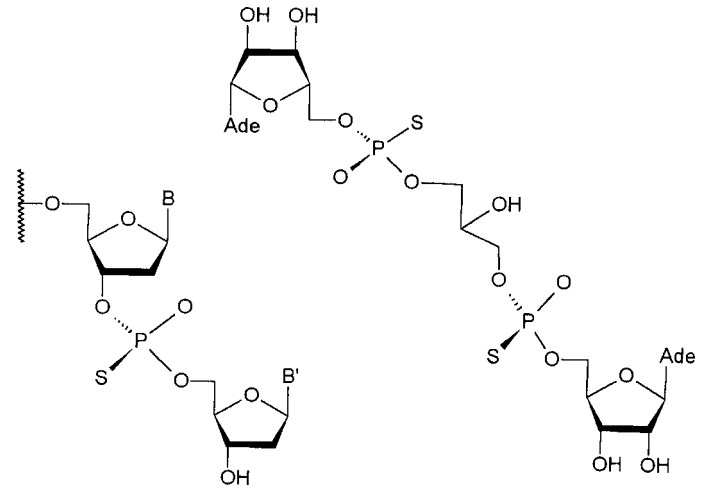
RESULTS AND DISCUSSION

In this communication we present preliminary studies concerning stereochemical analysis of compound **1**, prepared[2] as the mixture of eight diastereoisomers (2^3, two chiral centers at phosphorus and one chiral/pseudochiral center at C-2 atoms of glycerol moiety). Since interaction of **1** with proteins may be *a priori* considered as stereodependent event, attempts at separation of **1** into individual diastereoisomers by means of RP-HPLC technique were undertaken. These efforts were partially successful, and only one isomer assigned as **1a** was isolated as pure specimen; inhibitory effect of this individual isomer towards Fhit is under investigation. Independently, experiments towards assignment of absolute configuration at P atom in **1a** were performed. Diastereoisomer **1a** appeared to be resistant towards *snake venom phosphodiesterase* (*sv*PDE) which is known to hydrolyse stereoselectively internucleotide 3′,5′-phosphorothioates of R_P-configuration.[6,7]

Topological analysis indicates (Sch. 1) that *sv*PDE-resistant S_P-dinucleoside 3′,5′-phosphorothioate, due to Cahn-Ingold-Prelog rules, corresponds to *sv*PDE-resistant R_P,R_P-isomer of **1**. Such assignment is validated by observation, that diastereoisomeric mixture of **1** under treatment with *sv*PDE undergoes degradation, rendering intact **1a**. From the resulting mixture besides two compounds corresponding to mono-(adenosine-5′-O-phosphorothioyl)glycerol of R_C,R_P- and S_C,R_P-configuration, adenosine-5′-O-phosphorothioate has been isolated (HPLC coinjection, MALDI-TOF MS analysis). Compound **1** (as the mixture of all possible diastereoisomers) was digested with *sv*PDE in the buffer containing [^{18}O] water, and resulting adenosine-5′-O-[^{18}O]phosphorothioate was isolated by means of RP-HPLC. Its stereochemical analysis was performed according to the methodology developed recently in this laboratory.[8] Taking into account that *sv*PDE cleaves internucleotide



Scheme 1.

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phosphorothioate of R_P-configuration with retention (involvement of covalent enzyme-substrate complex[9]) we were able to prove that undigested by *sv*PDE isomer **1a** possesses R_P-configuration (data not shown). Studies upon inhibitory activity of **1a** towards Fhit protein are in progress and results will be published in due course.

ACKNOWLEDGMENT

This work was financially assisted by the State Committee for Scientific Research (KBN), grant no. Z-KBN K005/T09 (to W.J.S.).

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