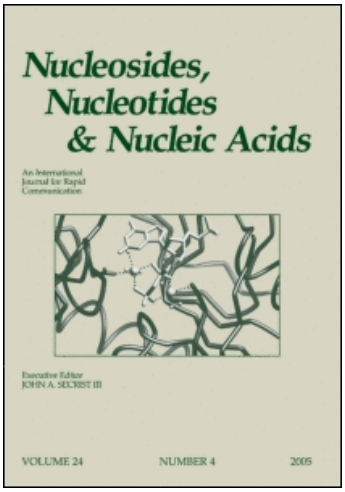

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Determination of the Absolute P-Configuration of a Phthalidyl-Phosphonate Thymidine-Thymidine Dimer

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Online publication date: 09 August 2003

To cite this Article Fendrich, W. and Meier, C.(2003) 'Determination of the Absolute P-Configuration of a Phthalidyl-Phosphonate Thymidine-Thymidine Dimer', Nucleosides, Nucleotides and Nucleic Acids, 22: 5, 1127 — 1129
To link to this Article: DOI: 10.1081/NCN-120022817
URL: http://dx.doi.org/10.1081/NCN-120022817

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, Nos. 5–8, pp. 1127–1129, 2003

Determination of the Absolute P-Configuration of a Phthalidyl-Phosphonate Thymidine-Thymidine Dimer

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ABSTRACT

Phthalidyl modified oligonucleotide thymidine-thymidine dimer building blocks were synthesized via the H-phosphonate-method. The compounds which are diastereomeric at the phosphorus atom were separated by chromatography and the absolute configuration at the phosphorus atoms was determined using ROE-experiments using the corresponding methyl-phosphonates.

Key Words: Pro-oligonucleotide; Phthalidylphosphonate; P-configuration determination; Antisense.

INTRODUCTION

For the use of oligonucleotides (ODN) as antisense therapeutics,[1] the ODN's have to be modified to afford certain stability against degradation with exo-nucleases as well as the capability to undergo a passive membrane transport. In the pro-ODN concept the loss of a non-toxic masking group would release an unmodified antisense-ODN. Therefore, we developed the α-hydroxyphosphonate-masking

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DOI: 10.1081/NCN-120022817
1525-7770 (Print); 1532-2335 (Online)

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group,[2] which can undergo a phosphonate-phosphate rearrangement. To stabilize the α-hydroxy group we use a lactone as protection group. To introduce this modification into ODN's via the phosphoramidite method, we synthesized thymidine-thymidine dimer-building blocks.[2]

RESULTS

Starting from the thymidine-thymidine-H-phosphonate, we have introduced the phthalidyl group into this dimer building block by deprotonation of the H-phosphonate with triethylamine and reaction with 2-carboxylbenzaldehyde followed by lactonisation with DCC (Fig. 1, left).

To prevent the phosphonate-phosphate rearrangement, the reaction has to be carried out under dry conditions. In the synthesis of the H-phosphonate, a racemic mixture of both diastereomers at the phosphorus atom is formed which can be separated by chromatography ("fast" and "slow" isomer). To attribute the absolute configuration at the phosphorus atom we used ROE-experiments and the methylphosphonates[3] prepared from the H-phosphonate (Fig. 1, right). Both the phthalidyl-phosphonate and the methyl-phosphonate can be synthesized under retention of configuration.[4] Assuming the shown conformation (Fig. 2, top), were

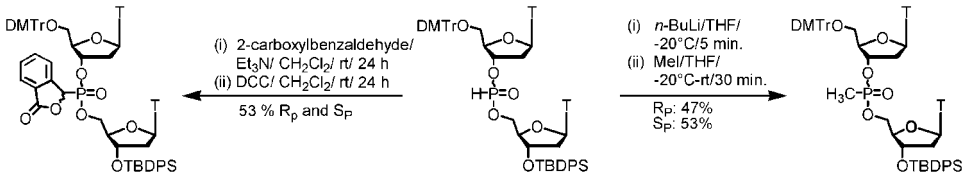

Figure 1. Synthesis of the phthalidyl- and the methyl-phosphonates.

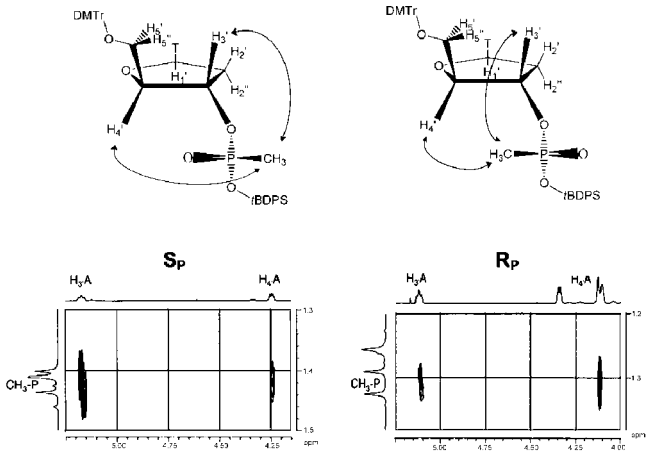

Figure 2. ROE-experiments at the S_p- and R_p-methylphosphonates.

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both thymine are staggered like in a DNA-helix, the S_p-diastereomer should show a greater ROE-signal between the methyl group and the H3′-proton as for the signal between the methyl group and the H4′-proton. For the R_p-diastereomer the opposite should be found (Fig. 2, bottom). To fix this conformation we used the sterically demanding protecting group *t*-butyldiphenylsilyl-group for the 3′-hydroxy group.

From these experiments the "fast"-isomer has R_P-configuration at the phosphorus atom and the "slow"-isomer S_P-configuration.

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