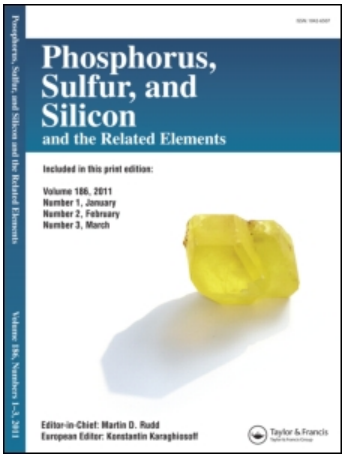

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Phosphorothioate Analogues : Probes for Studying DNA Conformation

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PHOSPHOROTHIOATE ANALOGUES : PROBES FOR STUDYING DNA CONFORMATION.

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Abstract

Oligonucleotides containing stereospecifically incorporated phosphorothioate residues have been synthesised and their conformation studied using CD spectroscopy.

INTRODUCTION

Phosphorothioate analogues of nucleotides have been applied to a wide variety of biochemical investigations; and in particular the study of enzyme, catalysed nucleotidyl and phosphoryl transfer processes.[1] More surprisingly, however, these analogues have found utility as probes in the study of DNA conformation. Although the classical right-handed double-helical form of DNA (B-DNA) has been known for over 30 years it is only since 1979 that a left-handed form of DNA (Z-DNA) has been shown to exist. The Z-form was found to occur in crystals of oligonucleotides with alternating d(C-G) sequences.[2] The two forms are in equilibrium, the position of which is determined by environmental factors such as salt concentration.

In an attept to understand the mechanism of the B-Z transition a variety of nucleotide analogues have been incorporated into polynucleotides in order to establish the effect that a particular modification has on the equilibrium. Particularly interesting are those studies performed on poly d(pCp(S)G) and

poly d(pGp(S)C) two polymers in which a sulphur atom stereospecifically replaces one of two non-bridging oxygen atoms in the phosphodiester bonds.[3] Poly d(pGp(S)C) having the sulphur substitution in the d(GpC) linkage readily undergoes the salt induced B-Z transition, at a salt concentration very much lower than that required to induce the transition in poly d(G-C). However, in poly d(pCp(S)G) having the substitution in the d(CpG) phosphodiester bond, the transition is completely blocked.[3] These polymers were prepared enzymatically and as a consequence contain phosphorothioate groups of the Rp configuration.[1]

In order to examine the role of the phosphorothioate group more closely in the B-Z transition, and particularly the effect that a phosphorothioate group of the Sp configuration might have on the equilibrium, the following four phosphorothioate-containing octamers have been synthesized chemically. (Rp)- and (Sp)- d[G(p(S)CpG)$_3$p(S)C] and (Rp)- and (Sp)- d[C(p(S)GpC)$_3$p(S)G] [4]. The use of Rp and Sp denotes that all the phosphorothioate residues in the oligomer and of that particular configuration.

Synthesis of Oligonucleotides

The dinucleoside phosphorothioates were prepared by condensing the appropriate deoxynucleoside 3'-(methoxymorpholinophosphite) and 3'-(methoxyacetyl)-2'-deoxynucleoside derivative as shown in figure 1. After removal of the 3'methoxyacetyl group the two diastereomers were separated by silica gel chromatography. The absolute configuration of the dinucleoside phosphorothioates was assigned using established criteria.[5]

The synthesis of the oligonucleotides was achieved by coupling the 3'-O-(morpholinomethoxyphosphite) derivative of the protected dinucleoside phosphorothioate to a solid phase support. The synthesis cycle was repeated a further three times to obtain the octamers.

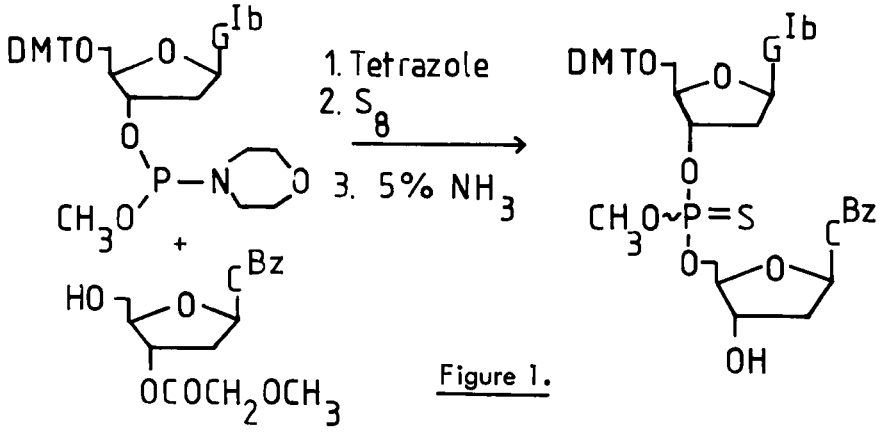

Figure 1.

The oligomers were assembled and the phosphate and base moieties deprotected using the standard phosphite methodology developed by Caruthers.[6]

Characterization and Conformational Studies on the Oligonucleotides

The purity of the oligonucleotides was established by HPLC. Characterization was obtained through enzymatic digestion followed by HPLC analysis of the digest produced, and by ^{31}P NMR. The ability of each oligonucleotide to adopt a left-handed conformation under high salt concentration was screened for using circular dichroism.[7] Both (Rp)-d[G(p(S)CpG)$_3$p(S)C] and (Sp)-d[C(p(S)GpC)$_3$p(S)G] are able to form Z-type structures at high concentrations of NaCl. In the case of the former where a phosphorothioate linkage of the Rp configuration occurs 5' to a deoxycytidine residue, the transition to the Z-form is potentiated in comparison to the unmodified oligomer. (Sp)-d[G(p(S)CpG)$_3$p(S)C]and (Rp)-d[C(p(S)GpC)$_3$p(S)G] retain the B-conformation even at high NaCl concentrations.

The possible influences that the phosphorothioate groups can exert on the B-Z equilibrium are complex since both forms have to be considered. The X-ray structural analysis of the 2-conformation in four crystal forms of d(C-G)$_3^8$ suggests that the sulphur atoms in Rp-d[G(p(S)CpG)$_3$p(S)C]

may be involved in a hydrogen bonding interaction with the N2 atom of guanine.

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