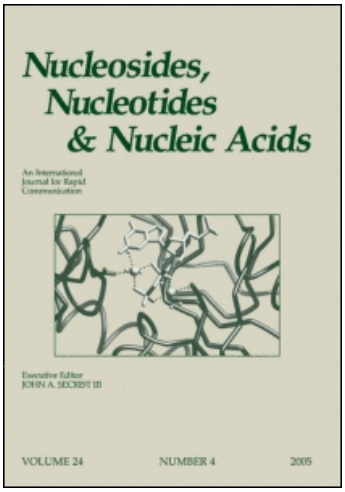

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Richard Cosstick[a]; Joanne Buckingham[a]; John Brazier[a]; Julie Fisher[b]
[a] Department of Chemistry, University of Liverpool, Liverpool, United Kingdom [b] Department of Chemistry, University of Leeds, Leeds, United Kingdom

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STABILIZATION OF MULTI-STRANDED NUCLEIC ACID STRUCTURES USING 3′-S-PHOSPHOROTHIOLATE LINKAGES

Richard Cosstick, Joanne Buckingham, and John Brazier □ *Department of Chemistry, University of Liverpool, Liverpool, United Kingdom*

Julie Fisher □ *Department of Chemistry, University of Leeds, Leeds, United Kingdom*

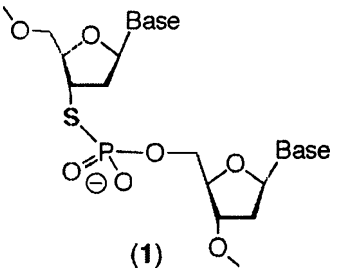
□ *3′-S-Phosphorothiolate linkages incorporated into an oligodeoxynucleotide have been shown to stabilise duplex formation with a complementary RNA strand, but destabilise a duplex formed with a complementary DNA strand. The four-stranded i-motif structure is also stabilised this modification.*

Keywords Oligonucleotide; phosphorothiolate; i-motif

INTRODUCTION

In recent years, DNA and RNA analogues have been investigated extensively as potential chemotherapeutic agents and have become essential tools for probing structural and mechanistic aspects of nucleic acid biochemistry. Nucleic acid analogues in which one bridging phosphoryl oxygen atoms are replaced with a heteroatom are particularly attractive since they are achiral and are closely related structurally, to the natural system. Of particular note in this class are 3′ − *N*-phosphoramidate[1] and the 3′ − *S*-phosphorothiolate linkages.[2] Over the last 10–15 years oligonucleotides containing a 3′ − *S*-phosphorothiolate linkage (**1**) have become valuable tools for investigating the role of metal ions in enzyme-catalysed nucleic acid cleavage processes.[3] More recently, NMR studies on oligodeoxynucleotides containing a 3′-*S*-phosphorothiolate linkage have shown that 3'–sulfur substitution results in a sugar conformation that is predominately 3'–*C*–*endo*.[4] In addition, to the conformational studies, thermal melting experiments have revealed, that when one or two phosphorothiolate linkages are placed in a DNA strand, this linkage stabilises duplex formation with complementary RNA, by up to about 2°C per modification.[4]

Address correspondence to Richard Cosstick, Department of Chemistry, University of Liverpool Liverpool, L69 7ZD, UK. E-mail: rcosstic@liv.ac.uk



STRUCTURE 1

We now review the synthesis oligonucleotides containing 3′ – *S*-phosphorothiolate linkages and the effect of this linkage on the stability of duplex and intercalating (i-motif) structures.

SYNTHESIS

Oligonucleotides containing 3′-*S*-phosphorothiolate linkage associated with either a cytosine or thymine base were prepared by automated phosphoramidite chemistry as previously described (Figure 1).[4] The best results for coupling the 3′ – *S*-phosphorothioamidite (2; Figure 1) were achieved using a 1 molar solution of either 5-(ethylthio)tetrazole (ETT) or *N*-(phenyl)imidazolium triflate (PIT) as the activator and 0.02 M iodine solution as the oxidant. Although the average coupling yields with PIT were marginally higher (93%) than those obtained with ETT (92%), the latter was routinely used as it is commercially available. Using this protocol

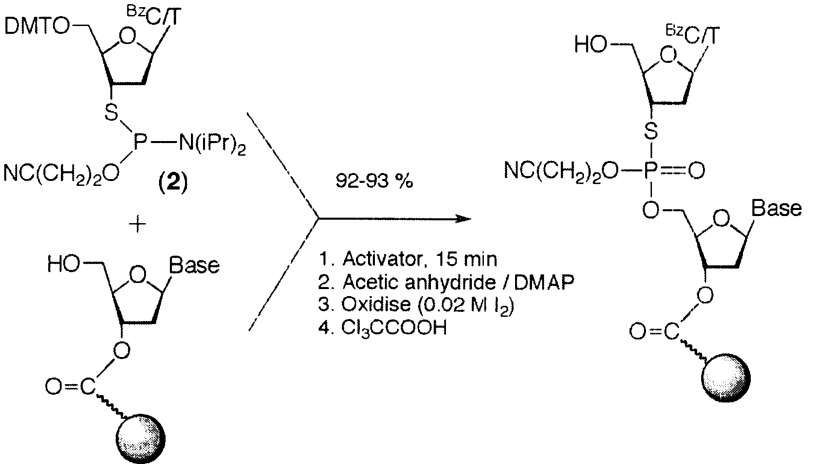


FIGURE 1 Solid-phase synthesis of oligodeoxynucleotides containing 3′ – *S*-phosphorothiolate linkages.

oligonucleotides containing up to 5 phosphorothiolate linkages could be prepared and isolated in a pure form.

Effect of 3′-*S*-phosphorothiolate Linkages on Duplex Stability

Using the sequence 5′-d(GCGTTTTTTTTTTGCG), up to 5 phosphorothiolate linkages were incorporated into the central T_{10} tract. The thermal melting temperatures (Tms) of duplexes formed between the phosphorothiolate-modified oligodeoxynucleotides and the complementary RNA strand revealed an increase in the Tm of about 1.4°C per modification. Conversely, the Tms of duplexes formed between the phosphorothiolate substituted DNA oligodeoxynucleotides and the complementary DNA strand were reduced by about 1°C per modification. The greater duplex stability of duplexes formed with the complementary RNA can be explained by the effect that the phosphorothiolate linkage has on the conformation of the sugar. The 3′-sulfur atom induces a 3′–*C*–*endo* or north conformation in the sugar of the nucleotide to which it is directly attached. This is the same conformation that ribose sugars adopt in RNA and thus preorganises the oligodeoxynucleotides containing the 3′ − *S*-phosphorothiolate linkages for duplex formation with RNA.

Effect of 3′-*S*-phosphorothiolate Linkages on the Stability of the i-motif

The i-motif is a 4-stranded intercalated DNA structure that has hemiprotonated cytosine•cytosine base pairs and as a result is most stable at low pH (∼4.5). Detailed structural studies have revealed that the deoxyribose sugars adopt the 3′–*C*–*endo* or north conformation, although interestingly ribose sugars destabilise the i-motif. It, thus, appeared that the phosphorothiolate modification could possibly stabilise the i-motif structure and to investigate this possibility several i-motif sequences were prepared containing this modification. The sequences prepared are shown in Table 1 together with their melting temperatures.

TABLE 1 UV T_m data for oligonucleotide sequences; **Cs** represents the deoxycytidine phosphorothiolate analogue

Entry number	Sequence	T_m/°C[a]
1	d(TCCCCC)	46.0
2	d(TCC**Cs**CC)	48.5
3	d(TCC**CsCs**C)	53.0
4	d(TC**CsCs**CC)	54.5

[a]50 mM sodium citrate buffer, pH 4.6, 5 μM strand concentration.

The i-motif with one modified linkage (entry 2) shows a modest 2.5°C increase, in Tm in comparison to the unmodified sequence. However, the i-motif sequences with 2 modifications (entries 3 and 4) show substantial increases (≥7°C) in the Tm values. These results strongly suggest that the i-motif is stabilised by deoxyribose sugars that are able to adopt a 3′–*C*–*endo* conformation. This is to our knowledge, the first example of a deoxycytidine analogue that stabilises the i-motif structure.[5]

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