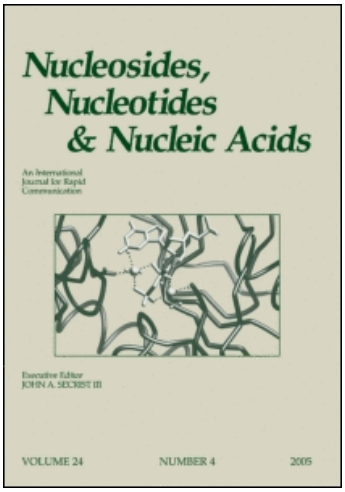

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DINUCLEOSIDE MONOPHOSPHATE ANALOGUES CONTAINING DISULFIDE LINKAGES

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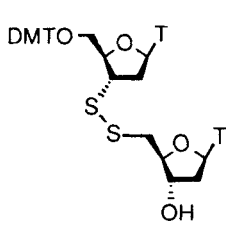
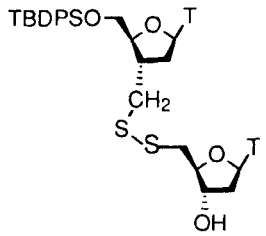
ABSTRACT. Two dinucleoside monophosphate analogues containing disulfide linkages (**1** and **2**) have been prepared for incorporation into oligonucleotides. The modified oligomers will be tested for their potential as antisense agents.

INTRODUCTION Synthetic oligonucleotides with favourable antisense properties are few in number. Whilst many nuclease-resistant analogues have been prepared, relatively few of these have displayed favourable hybridization properties with their target RNA. Recently, backbone modifications which confer structural rigidity have attracted interest as they may provide the necessary preorganization requirements for optimal duplex formation. Solution conformation studies have shown that disulfide bonds exist as rigid systems which are conformationally restricted.[1] Thus oligonucleotides in which the phosphodiester bonds are replaced with neutral, achiral disulfide linkages (**1** and **2**) are attractive antisense candidates since they are not only expected to be nuclease resistant but they may also display good hybridization properties.

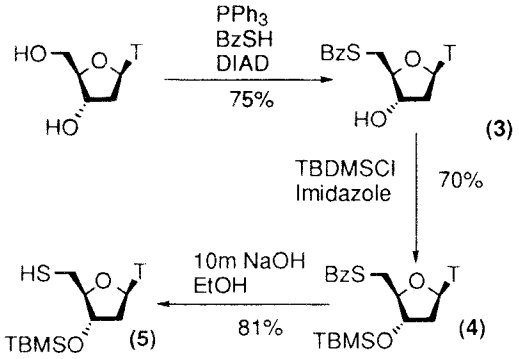


(1)



(2)

RESULTS AND DISCUSSION For both thymidine dimers it was believed that the disulfide bond would be formed through an exchange reaction between an activated *S*-nucleosidyl-*S*-aryldisulfide and 5'-thiothymidine. A standard Mitsonobu reaction on thymidine using thiobenzoic acid afforded the thiobenzoate **3** (scheme 1). Conversion to



Scheme 1

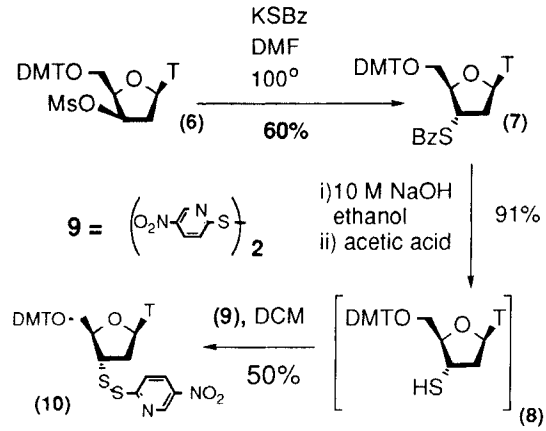
the tertiarybutyltrimethylsilyl (TBDMS) ether **4** and subsequent hydrolysis gave the target precursor **5** in excellent yields.

3'-Deoxy-5'-dimethoxytrityl-3'-5-(5-nitropyridyl-2-disulfanyl)thymidine **10** was selected as a disulfide precursor to compound **1** and prepared according to scheme 2.[2] The 3'-thiol was not isolated due to its tendency to undergo oxidative dimerization; the crude reaction mixture was precipitated from hexane and reacted directly with the sulfenylating agent.

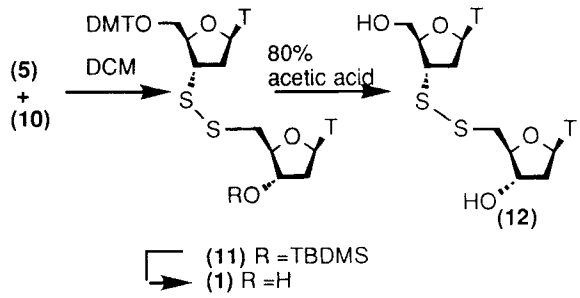
The exchange reaction between **10** and 1.5 equivalents **5** in DCM yielded the fully protected dimer (**11**, scheme 3) in 68% yield. Deprotection with triethylamine trihydrofluoride yielded the tritylated dimer **1** which is an immediate precursor to the dinucleoside synthon for oligonucleotide synthesis. Dimer **1** was treated with 80% acetic acid to yield the fully deprotected dimer **12**. The 400MHz ^{1}H NMR spectrum of **12** showed characteristic shifts for the H3' proton (4.50 ppm) and the H1' (pseudo triplet, 6.24ppm, j=7.1 Hz) of the 5'-thio sugar and for the H3' (3.83 ppm) and the H1' (doublet of doublets 6.16 ppm, j=4.7, 6.7 Hz) of the 3'-thio sugar. **12** is stable to an iodine/water/triethylamine oxidising solution over a period of 6 hours.

Synthesis of the top half of dimer **2** started from the previously reported aldehyde **13** [3](scheme 4). Quantitative reduction to the hydroxymethyl nucleoside **14** followed by mesylation gave compound **15** in excellent yield. Reaction of **15** with the sodium salt of 4-methoxybenzenemethanethiol in *N,N*-dimethylacetamide (DMA) yielded **16**. Sulfenylation with 2-nitrophenylsulfenylchloride in acetic acid and DCM afforded the disulfide **18.**

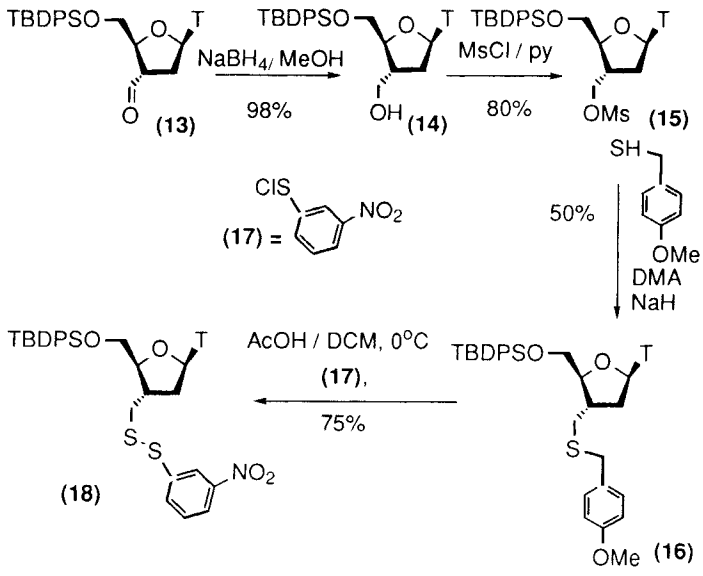
The use of an unprotected thiol in the exchange reaction was now investigated. Hence compound **3** was hydrolysed directly to afford the thiol **19** which was then coupled to the disulfide **18** in MeOH/DCM to yield the dimer **2** in poor yield. This is probably due



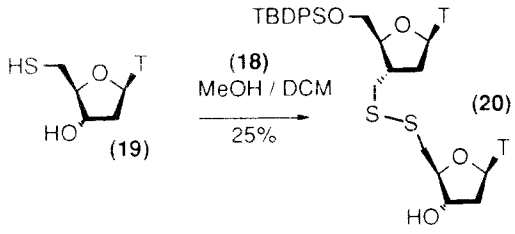
Scheme 2



Scheme 3



Scheme 4



Scheme 5

to the poor solubility of **19** and thus a more efficient route to this dimer will be from the protected thiol **15** . The 400 MHz ^{1}H spectrum of (**20**) showed characteristic shifts for the H3' (4.46 ppm) and H1' (pseudo triplet, 6.25 ppm, j=6.7 Hz) of the 5'-thio sugar and for the H3' (2.81ppm) and H1' (pseudo triplet, 6.17 ppm, j=6.05 Hz) for the 3'-thio sugar. The TBDPS group was removed cleanly on a TLC level using triethylamine trihydrofluoride to afford dimer **2**. The linkage is stable to acetic acid and the iodine/water/triethylamine oxidising solution.

CONCLUSIONS Two novel dinucleosides with modified internucleosidic linkages have been synthesized. Following their incorporation into oligonucleotides, the thermal melting temperature ("Tm") values of the modified oligomers and their resistance to nucleases will be assessed.

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REFERENCES

1. Pappalardo G.C., Ronsivalli G., *Tetrahedron* 1972, **28,** 4147
2. Cosstick R., Taylor R. A., Baxter A.D., Earnshaw D.J, Scott G.K., Higson A.P., *Tetrahedron*, 1996, **52** , 1027
3. Sanghvi Y.S., Bharadwaj R., De Mesmaeker A., *Synthesis*, 1994, *1163-1166*