Site-specific Disulfide Bridges in Oligodeoxyribonucleotide Duplexes containing 6-Mercaptopurine and 4-Thiothymine bases

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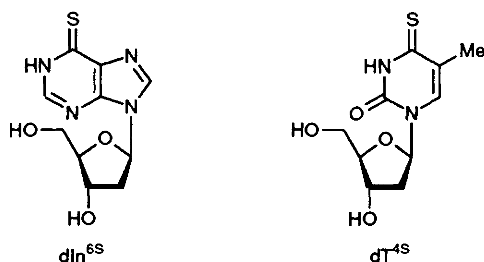
A self complementary oligodeoxynucleotide duplex containing 6-thio-2′-deoxyinosine and 4-thio-2′-deoxythymidine undergoes cross-linking to form interstrand disulfide bridges under aerobic conditions; the reaction is reversed by dithiothreitol.

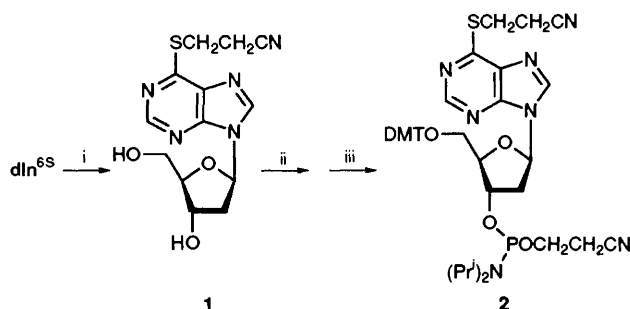
It has previously been proposed that oligonucleotides containing sequence-specific interstrand cross-links are potentially useful tools for the study of fundamental biological processes.[1–4] Whilst it is possible to obtain oligonucleotides cross-linked at a single site using bis-electrophiles,[5] this type of approach generally produces a heterogeneous mixture of monoadducts and cross-links. However, several oligonucleotide analogues have been prepared that allow the introduction of sequence-specific cross-links. Those that have proved most successful involve the introduction of reactive aziridine nucleoside analogues into oligodeoxyribonucleotides by either chemical[6] or enzymatic[3] procedures and photoinduced cross-linking[7] using a psoralen-modified oligodeoxyribonucleotide. Very recently it has been demonstrated that oligodeoxyribonucleotide duplexes containing N^6-thioalkyl derivatives of 2′-deoxyadenosine located in consecutive base pairs in opposite strands are able to cross-link through the formation of a disulfide bridge.[4]

We now report that complementary DNA duplexes containing 4-thio-2′-deoxythymidine and 6-thio-2′-deoxyinosine in direct opposition undergo site-specific cross-linking resulting in the formation of a disulfide bridge directly between the pyrimidine and purine bases.

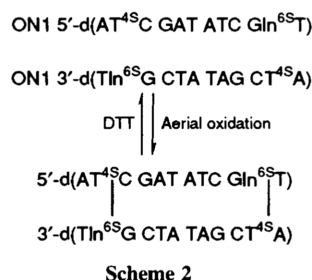
6-Thio-2′-deoxyinosine (dIn^{6S}) was prepared from 2′-deoxyinosine according to the procedure of Robins and Basom.[8] It was apparent that a successful strategy for the incorporation of dIn^{6S} into oligonucleotides *via* the phosphoramidite approach would require protection of the sulfur atom to mask its nucleophilicity and prevent oxidation to dimeric disulfides. Treatment of dIn^{6S} with an excess of 3-bromopropionitrile and potassium carbonate in dimethylformamide (DMF) under anhydrous conditions gave 6-*S*-(2-cyanoethyl)thio-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine **1** in 93% yield (Scheme 1). UV Spectrophotometry readily distinguished this *S*-alkylated product [λ_{max} (MeOH) 277 nm] from the *N*-alkylated isomer [λ_{max} (MeOH) 318 nm] which we had previously isolated as a minor product when the alkylation was performed with acrylonitrile. Model studies demonstrated that the cyanoethyl group could be quantitatively removed with concentrated ammonia solution under conditions used to deprotect the heterocyclic bases.



Conversion of **1** into the fully protected phosphoramidite **2** was achieved in two steps[9] with an overall yield of 72%. The phosphoramidite was used in automated DNA synthesis and



Scheme 1 *Reagents and conditions*: i, $BrCH_2CH_2CN$, K_2CO_3, DMF, room temp.; ii, 4,4′-dimethoxytrityl (DMT) chloride, 4-(dimethylamino)pyridine, NEt_3, pyridine; iii, 2-cyanoethyl-*N*,*N*-diisopropylaminochlorophosphite, *N*,*N*-diisopropylethylamine, CH_2Cl_2



Scheme 2

sequences containing 4-thio-2′-deoxythymidine were prepared and deprotected as described.[10] All oligonucleotides were characterised by enzymatic digestion to the constituent deoxynucleosides and composition analysis by HPLC.[11] The interstrand disulfide bridge formation was most easily demonstrated in the self-complementary duplex (ON1) (Scheme 2). Under aerobic conditions an aqueous solution of ON1 in 50 mmol dm^{-3} sodium hydrogen carbonate and stored at 5 °C underwent slow oxidation that could be monitored by reversed-phase HPLC (linear gradient of 6–15% acetonitrile in 100 mmol dm^{-3} triethylammonium acetate pH 6.5 over 25 min). The oxidation was essentially complete after five days with ON1 [retention time (t_r) 20.1 min] being converted into one major product (t_r 28.3 min) which could be reduced back to ON1 by treatment with dithiothreitol (DTT). Isolation of this oxidation product followed by enzymatic digestion and composition analysis by HPLC (Fig. 1) demonstrated the presence of the mixed disulfide formed between dT^{4S} and dIn^{6S}. The fact that the digestion reveals the presence of very little, if any, of the dimeric disulfides of dT^{4S} and dIn^{6S} (t_r 30 and 16 min, respectively), demonstrates that the disulfide bridges are being formed with a very high degree of sequence specificity and do not result from the random association of the two oligonucleotide strands. Direct evidence for cross-linking has come from the analysis of the oxidised duplex by electrophoresis on a 15% polyacrylamide gel run under denaturing conditions. Examination of the gel by UV shadowing showed that in comparison to series of single-stranded markers ON1 ran as a standard 12-mer whilst cross-linked ON1 ran much more slowly with a mobility in between that of a 25- and 26-mer.†

In conclusion, the results presented here are consistent with the reversible formation of an interstrand disulfide bridge directly between dIn^{6S} and dT^{4S} residue. Whilst the aerobic oxidation is slow there is obviously potential to increase the rate of disulfide formation by addition of a mild oxidant. The effect of the interstrand cross-links on the oligonucleotide stability and conformation is currently under investigation.

† It should be noted that we have been unable to obtain ^{32}P labelled autoradiographs of the polyacrylamide gels, because reducing agents present in the commercial polynucleotide kinase preparations reduce the interstrand disulfide bridges.

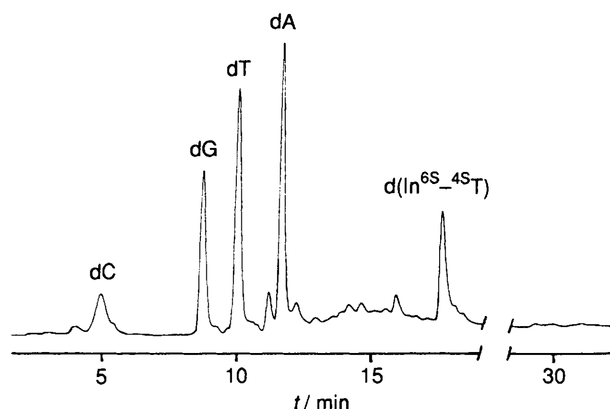


Fig. 1 HPLC analysis of oxidised ON1 after digestion with snake venom phosphodiesterase and alkaline phosphatase on C_{18} reversed-phase silica using a gradient of 2–16% acetonitrile in 50 mmol dm^{-3} KH_2PO_4 pH 6.0. The gradient was run over 30 min and then held at the final conditions for a further 5 min; detection was monitored at 260 nm. The unsymmetrical disulfide $dIn^{6S-4S}dT$ was identified by co-elution with an authentic sample.

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