Duplex Cross-Links

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# Selective Platination of Modified Oligonucleotides and Duplex Cross-Links**

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Since synthetic oligonucleotides complementary to mRNA sequences were shown to inhibit Rous sarcoma virus replication,[1] oligonucleotide chemists have synthesized all types of analogues to render oligonucleotides suitable as antisense agents. In this context, platinated oligonucleotides have been

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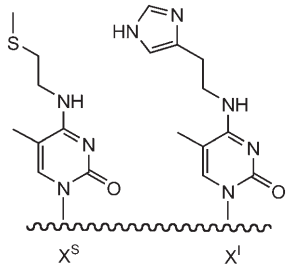
evaluated as potential drugs that may promote the formation of interchain cross-links and strengthen hybridization with the target RNA.

Duplexes with platinum cross-links have been obtained by annealing oligonucleotide strands either to platinated oligonucleotides with one labile bond[2] or to chains with *trans*-$Pt(NH_3)_2$–GNG adducts (N = any nucleoside).[3] Biological assays have proven that GNG-platinated oligoribonucleotides are able to modulate gene expression.[4] Alternatively, by taking advantage of the high affinity of phosphorothioate groups for platinum(II) complexes,[5] oligonucleotides containing a single phosphorothioate modification have been cross-linked to other oligonucleotide chains or to proteins.[6] Nevertheless, the scope of applications of platinated oligonucleotides has been limited because the preference of platinum for guanine rather than the other nucleobases restricts the position and number of guanines in the chain designed to first react with the metal.[2,3]

We have found that end-modified oligonucleotides with thioether and imidazole groups appended to 5-methylcytosines in neighboring positions (Scheme 1, $X^S$ and $X^I$ denote both the nucleobase and the nucleoside) react with transplatin to give chelates in which the platinum coordinates to the thioether and imidazole groups even in chains containing various guanines.[7] Hence, we have exploited such modified oligonucleotides to prepare duplexes with *trans*-$Pt(NH_3)_2$ cross-links without sequence restriction, either by using preplatinated oligonucleotides or by mixing all the components in a one-pot reaction. The results of this study are reported here.




***Scheme 1.*** Structures of the modified nucleobases containing the thioether and imidazole groups.

Platinated oligonucleotides $\mathbf{X^IX^S}$ACGTTGAG (**3**) and $\mathbf{X^SX^I}$ACGTTGAG (**4**; the coordination positions are highlighted in bold) were obtained by the reaction of transplatin with the corresponding modified oligonucleotides, $X^IX^S$ACGTTGAG (**1**) and $X^SX^I$ACGTTGAG (**2**).[7] Then, **3** (or **4**) was annealed to an equimolar amount of the complementary chain $^{5'}$dCTCAACGTGTTTG (**5**), and ligand-exchange substitution afforded exclusively the interchain-cross-linked product **6** (or **7**; Figure 1 A).

The formation of **6** or **7** in the reaction mixture was detected both by anion exchange HPLC and polyacrylamide gel electrophoresis (PAGE; see the Supporting Information).

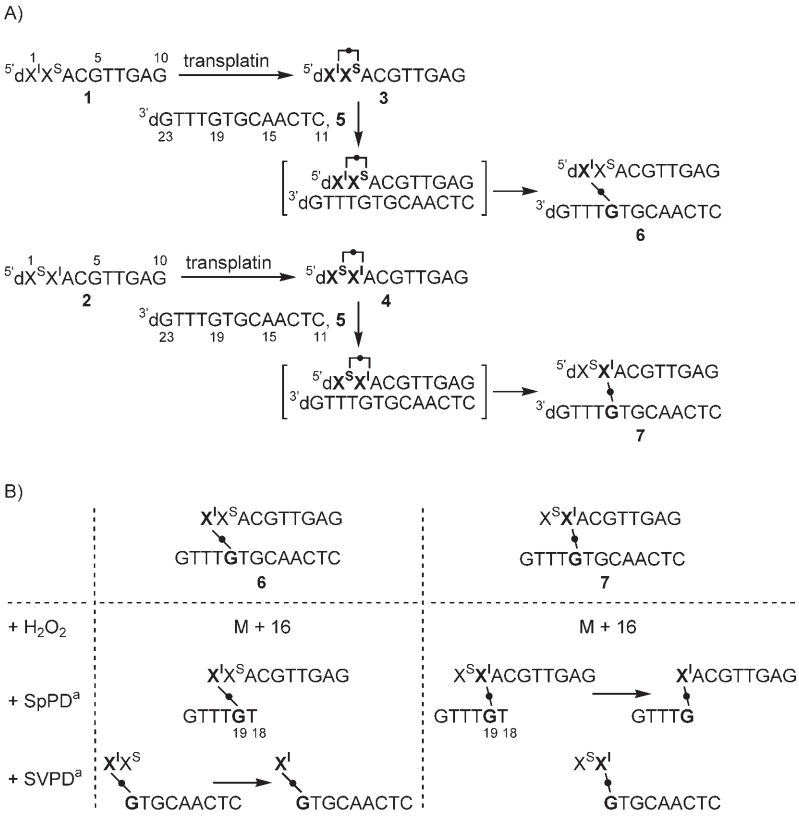



***Figure 1.*** A) Reaction of the platinated oligonucleotides **3** and **4** with **5**. B) Characterization of the adducts formed. [a]SpPD = calf spleen phosphodiesterase (5′-exonuclease); SVPD = snake venom phosphodiesterase (3′-exonuclease).

Yet the presence of **3** and **4** could only be assessed by anion exchange HPLC, a result showing that the N–Pt–S adducts are not stable in the electrophoresis analysis conditions.

Cross-linked duplexes **6** and **7** were isolated by anion exchange HPLC, and their structures were inferred from MALDI-TOF mass spectrometry analysis before (see the Supporting Information) and after chemical and enzymatic reactions (Figure 1 B).[8] The isolated products contained two chains and the *trans*-$Pt(NH_3)_2$ moiety, and their oxidation by $H_2O_2$ (indicated by $m/z$ ratios that were 16 units higher, the result of thioether to sulfoxide conversion) indicated that $X^S$ was not linked to the metal. Platinum coordination to $X^I$ of chain **1** and G19 of chain **5** (base numbers are shown in Figure 1 A) was surmised from the results of the digestions with the two exonucleases. The enzymatic elimination (3′-exonuclease) of $X^S$, placed next to platinated $X^I$, was slower than that of the other nucleosides. It was also found that digestion of chain **5** with the 5′-exonuclease did not stop at the platinated nucleobase but at the previous one. In the case of **7**, T18 was removed after longer digestion times, thereby confirming coordination to G19. In the case of **6**, coordination to G19 could be presumed from the well-known platinum preference for guanine rather than thymine and it was definitely established by NMR spectroscopy (see below).

The possibility of obtaining (*trans*-$Pt(NH_3)_2$)-cross-linked duplexes without previous platination of one of the two strands was then explored. One-pot cross-linking experiments were carried out by mixing equimolar amounts of the

modified oligonucleotide (**1** or **2**), the complementary chain **5**, and transplatin in duplex-forming conditions. In both cases, the same cross-linked duplexes as in the previous experiments (**6** and **7**) were formed (Figure 2).

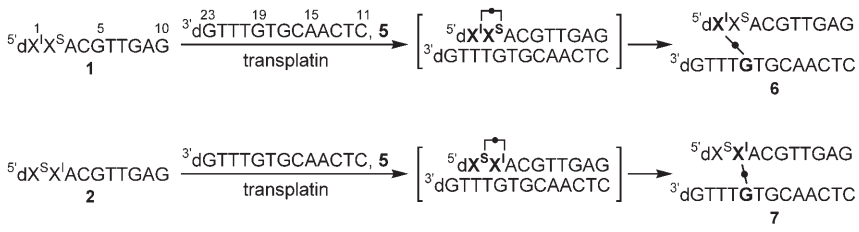



***Figure 2.*** Synthesis of the cross-linked duplexes in a one-pot reaction.

Anion exchange HPLC monitoring of the evolution of the reactions allowed chelates **3** and **4** to be detected, which indicates that platination of the modified strand in the duplex precedes the rearrangement that yields the cross-link; this probably accounts for the slower formation of the cross-linked duplexes in these one-pot reactions.

To get further insight into the platination sites in **6** and the possible distortions produced by the cross-link in the structure of the duplex, the reaction between **1**, **5**, and transplatin was repeated on a larger scale, and an NMR spectroscopy study of compound **6** was carried out (the duplex formed by **1** and **5** was used as a control). Most of the $^1$H signals could be assigned by following standard procedures (see the Supporting Information). Significant chemical-shift differences between the two duplexes were observed in the protons of residues $X^I$, $X^S$, A3, and C4, as well as in T18, G19, and T20. The largest difference was observed in the signal for H8 of G19, which was downfield shifted by 0.7 ppm in **6** (see the Supporting Information). This was a clear indication of platination at position *N*7 of this guanine. Other residues, including the remaining guanines, were mainly unaffected. Interprotonic distances derived from NOE cross-peaks indicated that the duplex was not much distorted by the cross-link.

In many of the residues close to the cross-link, two sets of signals were observed (see the Supporting Information). This effect was not the consequence of a chemical-exchange process, since no signal coalescence was observed upon a rise in temperature. The NMR spectra were consistent with the formation of two isomers, which result from platinum coordination to either of the two nitrogen atoms of the imidazole ring.[8]

UV-monitored thermal-denaturation experiments showed that the metal linkage between the two strands was highly stabilizing. The melting temperature ($T_m$) of the control duplex (**1** + **5**) was 32 °C, while that of **6** was 72 °C ($\Delta T_m = 40$ °C). Duplex stabilization has also been found in other *trans*-Pt($NH_3$)$_2$ cross-linked chains,[9] but not to such an extent. The higher stability of **6** as compared with other cross-linked duplexes may be because the metal is not directly linked to the *N*4 atom of the cytosine; this probably decreases the distortion created by the platinum cross-link.

To assess the effect of the presence and position of guanine residues in the overhang of the complementary chain, different oligonucleotides (**8**–**10**) were synthesized and treated with **1** and transplatin in duplex-forming conditions (Figure 3). Isolation and characterization of the cross-linked duplexes showed that, in all cases, the *trans*-Pt($NH_3$)$_2$ unit was linked to $X^I$ and the guanine facing $X^S$ in the complementary strand. This result proves that the regioselectivity of the rearrangement is very high and shows that, even when there are two guanines close to each other (as in chain **10**), the platinum prefers to migrate to the guanine opposite $X^S$ rather than to its neighbor. This finding also agrees with the fact that formation of cross-linked duplex **7**, which involved platinum migration from $X^S$ to the guanine opposite $X^I$, was slower than formation of cross-linked duplex **6** regardless of the synthetic procedure.

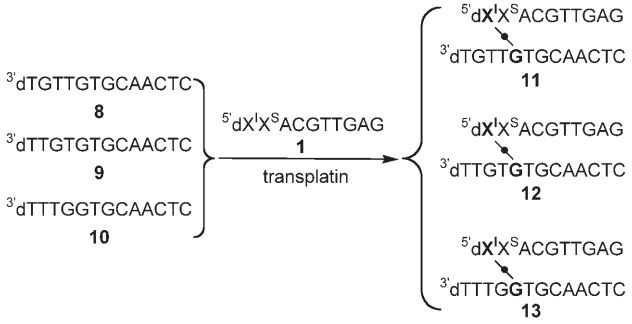



***Figure 3.*** Results of the reactions of transplatin with **1** and complementary strands with different sequences.

Finally, transplatin was treated with **1** and three strands of different length that were noncomplementary to **1** ($^{5'}$dGATCTAAAAGACTTT, $^{5'}$dCGGATATACCG, and $^{5'}$dACCATTCGTAGC). Chelate **3** was the only platinated product found in the reaction mixtures. Hence, hybridization is required for platinum migration, and non-complementary strands do not become cross-linked.

In summary, the reaction between transplatin and oligonucleotides with thioether and imidazole groups appended to neighboring nucleobases afforded thioether–Pt–imidazole chelates that, upon hybridization with a complementary strand, underwent a rearrangement process that yielded duplexes with $X^I$–guanine *trans*-Pt($NH_3$)$_2$ cross-links. The cross-link did not interfere with base-pairing and greatly stabilized the duplex.

Exactly the same cross-linked products were obtained by mixing equimolar amounts of transplatin and the two oligonucleotide chains in duplex-forming conditions, a result proving that previous isolation of the platinated modified oligonucleotide is not necessary. However, the thioether–Pt–imidazole chelates were always detected in these one-pot reaction mixtures, which suggests that their formation preceded that of the cross-linked duplexes. The fact that no other chelates were detected confirms our previous finding that the high affinity of sulfur for the metal drives the formation of the intrachain thioether–Pt–imidazole adducts[7] and prevents other oligonucleotide sequences from being platinated, even if they contain various guanine residues.

The ligand-exchange substitution process was found to be highly regioselective, as platinum migration from the thioether to the guanine opposite $X^S$ was preferred over migration to the guanine opposite $X^I$, and the former was more rapid than the latter. Cross-linking processes were rather slow, but, based on findings previously reported by Leng and coworkers,[3b] the replacement of 2′-deoxyoligonucleotides by oligoribonucleotides is expected to increase the rate of the rearrangement that yields the cross-linked duplex.

Finally, it is important to point out that cross-linking is the result of an annealing-promoted rearrangement. In other words, the cross-linking process is also sequence selective, since it only takes place if the two chains are complementary.

To the best of our knowledge, the goal of targeting and cross-linking complementary oligonucleotides without sequence restriction has been achieved here for the first time. Work is in progress to examine the potential therapeutic use of thioether- and imidazole-modified oligonucleotides.

## Experimental Section

Cross-linking reaction between platinated oligonucleotides **3** or **4** and the complementary chain **5**: Modified oligonucleotide **1** (or **2**; optical density units at 260 nm ($OD_{260}$ units) = 3.0) was treated with transplatin (3.2 μL of a 10 mM aqueous solution) in perchlorate buffer (3.16 mL; final oligonucleotide concentration = 10 μM) for 24 h at room temperature. Platinated oligonucleotides were isolated by reversed-phase HPLC.[7] **3** (or **4**; $OD_{260}$ units = 0.45) and an equimolar amount of **5** was dissolved in phosphate buffer (0.47 mL). The mixture (duplex concentration = 10 μM; pH 6.8–6.9) was stirred at 16 °C for 12 days, and the target duplex **6** (or **7**) was isolated by anion exchange HPLC.

One-pot cross-linking reactions: Modified oligonucleotide **1** (or **2**; $OD_{260}$ units = 1.0) and either the complementary (**5**, **8**, **9**, or **10**) or non-complementary chain (1 equiv) were dissolved in phosphate buffer (1.05 mL). The solution was heated to 90 °C and allowed to cool down slowly (overnight) to room temperature to favor duplex formation. After addition of transplatin (1 equiv; 1.1 μL of a 10 mM aqueous solution), the mixture (final duplex concentration = 10 μM; pH 6.8–6.9) was stirred in a bath at 16 °C for 12–19 days. The progress of the reaction was monitored by MALDI-TOF MS, anion exchange HPLC, and PAGE (20% acrylamide, 7 M urea). The cross-linked duplex was isolated by anion exchange HPLC and desalted by elution through a Sep-Pak column.

Spectroscopic studies: Thermal denaturation and NMR experiments were carried out in phosphate buffer, with 2 and 200 μM solutions of duplex, respectively (see the Supporting Information for additional details).

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