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Factors Affecting DNA–DNA Interstrand Cross-Links in the Antiparallel 3'–3' Sense: A Comparison with the 5'–5' Directional Isomer

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Abstract

Reported herein is a study of the unusual 3'–3' 1,4-GG interstrand cross-link (IXL) formation in duplex DNA by a series of polynuclear platinum anticancer complexes. To examine the effect of possible preassociation through charge and hydrogen-bonding effects the closely related compounds [*trans*-PtCl(NH₃)₂]₂(μ-*trans*-Pt(NH₃)₂{NH₂(CH₂)₆NH₂})₂]⁴⁺ (BBR3464, **1**), [*trans*-PtCl(NH₃)₂]₂(μ-NH₂(CH₂)₆-NH₂)₂]²⁺ (BBR3005, **2**), [*trans*-PtCl(NH₃)₂]₂(μ-H₂N(CH₂)₃NH₂(CH₂)₄)₂]³⁺ (BBR3571, **3**) and [*trans*-PtCl(NH₃)₂]₂(μ-H₂N(CH₂)₃-N(COCF₃)(CH₂)₄)₂]²⁺ (BBR3571-COCF₃, **4**) were studied. Two different molecular biology approaches were used to investigate the effect of DNA template upon IXL formation in synthetic 20-base-pair duplexes. In the “hybridisation directed” method the monofunctionally adducted top strands were hybridised with their complementary 5'-end labelled strands; after 24 h the efficiency of interstrand cross-linking in the 5'–5' direction was slightly higher than in the 3'–3' direction. The second method involved “postsynthetic modification” of the intact duplex; significantly less cross-linking was observed, but again a slight preference for the 5'–5' duplex was present. 2D [¹H,¹⁵N] HSQC NMR spectroscopy studies of the reaction of [¹⁵N]-**1** with the sequence 5'-d{TATAC-ATGTATA}₂ allowed direct comparison of the stepwise formation of the 3'–3' IXL with the previously studied 5'–5' IXL on the analogous sequence 5'-d(ATATGTACATAT)₂. Whereas the preassociation and aquation steps were similar, differences were evident at the monofunctional binding step. The reaction did not yield a single distinct 3'–3' 1,4-GG IXL, but numerous cross-linked adducts formed. Similar results were found for the reaction with the dinuclear [¹⁵N]-**2**. Molecular dynamics simulations for the 3'–3' IXLs formed by both **1** and **2** showed a highly distorted structure with evident fraying of the end base pairs and considerable widening of the minor groove.

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Introduction

DNA-modifying agents have found considerable use as anti-cancer chemotherapeutic agents. Of the various structurally distinct lesions formed on DNA, bifunctional interstrand cross-links (IXLs) are considered especially effective in inhibiting transcription and replication.[1,2] Further, IXLs are difficult to repair because damage on both strands must be excised with the possibility of loss of template DNA for subsequent replication.[1,3] Polynuclear platinum compounds in which two or three platinum coordination units are linked in a linear fashion, are a structurally distinct class of platinum-based anticancer drugs.[4] Their DNA-binding modes are distinct from those of cisplatin and oxaliplatin, and the consequences in terms of downstream effects such as protein recognition and DNA repair are also clearly distinct.[5,6] Whereas the *cis*-[PtX₂(amine)₂] family produces predominantly the well-studied 1,2-GG intrastrand cross-link,[7,8] polynuclear platinum drugs produce long-range intra- and interstrand cross-links in which the sites of platination can be separated by up to four intervening base pairs.[4–6] Thus, the cytotoxic effects of active polynuclear compounds might be due to a cumulative effect of the intra- and interstrand adducts produced by these drugs, but the role of structurally unique IXLs in their antitumour effects may predominate.[5]

The trinuclear platinum complex BBR3464 ($[\{trans\text{-PtCl}(\text{NH}_3)_2\}_2(\mu\text{-trans-Pt}(\text{NH}_3)_2\{\text{NH}_2(\text{CH}_2)_6\text{NH}_2\}_2)]^{4+}$; **1**, Figure 1a) has undergone phase II clinical trials.[9–11] Designed synthesis of polyamine backbones gives a series of exceptionally potent compounds that mimic successfully the biological activity of **1**. [12,13]

A distinct feature of highly active polynuclear compounds is the presence of a central moiety (platinum–tetraam(m)ine coordination sphere or the polyamine nitrogen atom) capable of preassociation or “non-covalent” (hydrogen-bonding and electrostatic) interactions with biomolecular targets. Polyamine-bridged dinuclear platinum compounds are viable “second-generation” analogues of **1** and activity within this series is affected both by the overall charge and chain length of the polyamine linker.[12] In comparison to dinuclear compounds with straight-chain alkanediamine (e.g., hexanediamine) linkers cytotoxicity, antitumour activity and cellular accumulation are all enhanced by the presence of the “central” preassociation motif.[14,15] Further, the rate and extent of cross-link formation is dependent on the charge. [16] Molecular biology studies have shown that the formation of long-range IXLs of **1** occurs in both the 5′–5′ and 3′–3′ sense, forming “directional isomers”. [5] The directionality is dependent on the nature of the cross-link. The 1,2-IXL forms in preferentially the 3′–3′ direction, the 1,4-IXL forms in both directions in approximately equal proportions, whereas the 1,6-IXL forms in preferentially the 5′–5′ direction. By monitoring the annealing of a “bottom” to a “top” strand containing a discrete monofunctional platinated site, the kinetics of formation of 1,2- and 1,4-IXLs were found to be similar and faster than that for the analogous 1,6-IXL.[5] The structures of the 3′–3′ and the 5′–5′ 1,4-GG directional isomers are similar but distinct, and the distortions induced are summarised in Figure 2.

Chemical probes such as KMnO₄, diethylpyrocarbonate (DEPC) and KBr/KHSO₅, which are base-specific (T, A/G, and C, respectively), give useful information on local conformational perturbation upon DNA adduct formation.[17–21] The major conformational changes of the 5′–5′ IXLs occurred 5′ to the binding site, with somewhat weaker distortions on T residues within the binding site.[5] In contrast, the 3′–3′ IXL showed the most reactivity with the two central AA–TT base pairs separating the platinated bases.[5]

Comparison of cleavage patterns by DNase I footprinting in modified and non-modified DNA gives information on the structural perturbation caused by any adduct. Structural perturbations induced by platinum binding in the major groove translate into the minor groove, which allows their detection by DNase I with very high sensitivity. The 3'-3' IXL of **1** induces the larger conformational alteration in DNA but the 5'-5' IXL distortions extended over a wider range of nucleotides.[22] Thus, the delocalised and distinct nature of these adducts is confirmed in the presence of a biologically relevant protein.

NMR spectroscopic structural studies of the 5'-5' 1,4-IXL of **1** confirm the conformational flexibility and delocalised nature of the lesion. An NMR spectroscopic analysis of the 1,4-GG IXL that is formed in the 8-mer duplex 5'-d(ATG*-TACAT)₂ showed evidence for conformational changes (*anti* to high *anti/syn*) for the adenines outside the binding site; this suggests a delocalised lesion in which the conformational changes are transmitted beyond the actual site of platination.[23] In contrast, cisplatin-induced rigid bending of DNA maintains the B-form in solution.[24,25] The kinetics of the 5'-5' 1,4-GG IXL formation by **1** and the dinuclear analogue BBR3005 (**2**) were followed by [¹H, ¹⁵N] HSQC NMR spectroscopy by using the self-complementary duplex 5'-d(ATATGTACATAT)₂ (duplex **IV**, Figure 1b).[26, 27] The stepwise formation of the bifunctional cross-link through preassociation, aquation of the Pt-Cl bonds, monofunctional binding and cross-link closure through bifunctional binding were easily monitored. NMR spectroscopic evidence further showed the presence of two distinct conformers for the 1,4-IXL, whereas there is only one conformer for the 1,6-IXL. [26,27]

The 3'-3' 1,4-GG IXL might be intuitively more distorting because it is formed in an antiparallel manner, and it is therefore of interest to examine its formation and structure. The fact that dinuclear **2** only forms 5'-5' IXLs, whereas **1** forms 1,4-IXLs in both directions, suggested that the directionality of the IXL formed might be related to the preassociation of the charged central linker in the DNA minor groove.[26] It was therefore of interest to compare the IXL preference in the closely related pair **3** and **4** (Figure 1a) in which the only difference is the charge and the nature of the central nitrogen atom (NH₂⁺ or NHCOCF₃). Thus, this pair of compounds makes an excellent set for comparison with **1** and **2**. Further, because dinuclear platinum-polyamine compounds might be "second-generation" clinical candidates, it is of interest to confirm that the DNA-binding profile is similar. We have therefore used [¹H, ¹⁵N] NMR spectroscopy to examine the stepwise formation of the 3'-3' IXL by reaction of **1** and **2** with the self-complementary duplex 5'-d{TATA-CATGTATA}₂ (duplex **III**). The sequence allows a direct comparison with the formation of the 5'-5' 1,4-GG IXL with duplex **IV**, discussed above in which the presence of conformers of the final bifunctional adduct is clearly seen.

This paper reports on the generality of directional isomer IXL formation by polynuclear platinum compounds and examines the effect of the DNA template (single-stranded oligonucleotide versus duplex) on the extent of IXL formation. The overall results suggest that formation of directional isomers (5'-5' and 3'-3') is a common feature, but the 3'-3' IXL, once formed, might be highly distorting. Molecular models suggest reasons why and allow comparison with the 5'-5' isomer.

Results

To examine the effect of possible preassociation through charge and hydrogen-bonding effects we first examined the closely related compounds **3** and **4**. Previous studies[28] have shown that the extent of interstrand cross-linking and half-life of the reaction with DNA depend on the nature of the central nitrogen atom. The sequence specificity of interstrand cross-linking for these two compounds has already been demonstrated and DNA-mapping experiments

confirmed G platination as well as the formation of long-range cross-links.[16,28] IXLs, by definition, must be formed through monofunctional platination on one strand followed by cross-link closure (bifunctional platination step) on the second strand. In the first binding step the kinetic preferences are similar to those of mononuclear species. The array of possible adducts becomes, however, different upon the coordination of the second platinum unit. With this consideration we designed the synthetic 20-base-pair (bp) oligodeoxyribonucleotide duplexes **I** and **II** (Figure 1b). The pyrimidine-rich top strands of these duplexes contain only one G in the centre (printed in bold in Figure 1b). These top single strands were modified at this central G residue by reaction with **3** or **4**. The bottom complementary strands contain Gs in different positions to the single central C (complementary to the platinated G in the top strand) so that the IXLs can form either on the 3'-side (duplex **I**) or the 5'-side (duplex **II**) of the central C residue of the bottom strand, that is, in the 3'-3' or 5'-5' direction. The monofunctionally complexed top strands of the duplexes were hybridised with their complementary 5'-end-labelled strands. The hybrids were incubated in 0.1M NaClO₄ at 37°C, aliquots were withdrawn at various time intervals and analysed by gel electrophoresis under denaturing conditions.

Figures 3 and 4 show the subsequent incubation results seen through new bands migrating at markedly slower rates. Their intensity increased with the incubation time with a concomitant decrease in the intensity of the band corresponding to the non-cross-linked duplex, an indication that IXLs were formed. From the ratio of the intensities of the bands corresponding to cross-linked duplexes and the sum of intensities of all bands, the percentage of IXLs was calculated (Table 1). For the duplex **II** after 24 h the efficiency of interstrand cross-linking in the 5'-5' direction was about 68 and 90% for complex **3** and **4**, respectively. Results obtained for the duplex **I** show that after 24 h cross-linking efficiency in the 3'-3' direction for **3** and **4** was slightly lower: 61 and 73%, respectively. The relative cross-linking efficiency of **1** for formation of the 3'-3' and 5'-5' cross-links was approximately 50%.[5] Thus a small but significant influence of the central linker on IXL preference may be inferred.

The residues involved in the 5'-5' and 3'-3' IXLs of **3** and **4** formed in the duplexes **I** or **II** were identified as previously.[5] After a 24 h reaction period, the bands corresponding to the interstrand cross-linked duplexes were cut from the gel, the duplexes were isolated and further characterised by Maxam–Gilbert footprinting. The interstrand cross-linked duplexes were reacted with dimethyl sulfate (DMS), which does not react with platinated G because the N7 position is no longer accessible.[29] These samples were then treated with NaCN to remove the adducts and finally also with piperidine. The treatment with piperidine of the control, unplatinated duplex resulted in the cleavage at all G sites in the bottom strand. If the cross-linked duplexes treated with DMS and subsequently NaCN were cleaved, the bands corresponding to all G residues were observed except those platinated and involved in the interstrand cross-link. These observations allow the unambiguous assignment of the platinated G in the bottom strand by **3** or **4** and that the IXLs formed by both dinuclear platinum complexes were 1,4-IXLs either in 5'-5' or in 3'-3' direction (not shown).

Careful examination of the gels shown in Figures 3 and 4 indicates that after a 24 h reaction period the cross-linking reactions of **3** or **4**, in both the duplexes **I** and **II**, resulted in the formation of new bands (designated as *) migrating more slowly than unmodified single-stranded oligonucleotide, but much faster than the interstrand cross-linked duplexes. Maxam–Gilbert (DMS) footprinting confirmed the bands as emanating from long-range intrastrand cross-links. The rearrangement resulting in the formation of intrastrand cross-links in the bottom strand of duplex **I** proceeds faster than in duplex **II**; after 24 h, the percentage of intrastrand cross-links formed by **3** and **4** with duplex **I** was 6.2% and 2.7%, respectively, whereas only 2.0% and an unobservable amount were formed for duplex **II** with **3** and **4**,

respectively. Thus, the IXLs formed by **4** are apparently more stable than those of **3**, which is consistent with the higher efficiency of **4** to form IXLs in plasmid DNA[28] (see Table 1).

Effect of DNA template on IXL formation

The strategy of first forming a monofunctional adduct on one strand, purification followed by annealing of the second complementary strand is an example of “hybridisation-directed IXL formation”. [30] To examine the effects of the nature of the DNA template on IXL formation, similar experiments were carried out for complexes **1**, **3** and **4** on the intact duplexes **VI** and **VII** (for their sequences see Supporting Information, Figure S1a) involving “postsynthetic modification” of the intact duplex. Analysis of cross-linking was carried out as above (Figure S1 and S2 in the Supporting Information) and the results are summarised in Table 1.

Surprisingly, significantly less cross-linking was observed under these conditions, but there was again a slight preference for the 5′–5′ duplex. A lower interstrand cross-linking frequency is related to the experimental conditions. The lower yield could be markedly affected, in contrast to the “hybridisation-directed IXL formation” experiments, by the rate of the binding resulting in the monofunctional adducts. Further, to keep the duplex double-stranded, rather high concentrations of NaClO₄ (0.1M) and lowered temperature (15°C) are used; in this case the rate of binding and subsequent closure of monofunctional adducts to IXLs is apparently decreased. Thus, direct comparison of the yield of interstrand cross-linking in “hybridisation-directed IXL formation” versus “postsynthetic modification” is not strictly possible. Nevertheless, the preference for 3′–3′ or 5′–5′ orientation of IXLs should not be affected.

[¹H,¹⁵N] NMR spectroscopic studies

To examine the mechanistic details of the formation of the 3′–3′ 1,4-GG IXL, platination reactions of the self-complementary 12 bp duplex **III** (Figure 1b) with [¹⁵N]-**1** and [¹⁵N]-**2** were followed by using 1D ¹H and 2D [¹H,¹⁵N] HSQC NMR spectroscopy. (Data for [¹⁵N]-**2** are provided in the Supporting Information.) The sequence of duplex **III** was chosen in direct analogy to the sequence **IV**, in which a 5′–5′ IXL is formed. NMR spectroscopic studies that follow the kinetics of formation of the 5′–5′ IXL, by both [¹⁵N]-**1** and [¹⁵N]-**2**, have been reported elsewhere.[26,27] A pH of 5.3 was chosen to allow direct comparison with these studies. The general scheme for formation of the two possible 1,4-GG IXL (5′–5′ and 3′–3′) is shown in Scheme 1.

The methodology follows the previous studies carried out with the duplex **IV**. [26] As well as the recording of sequential [¹H,¹⁵N] HSQC NMR spectra, ¹H NMR spectra were recorded to monitor oligonucleotide base pairing and to follow changes in oligonucleotide chemical shifts on binding of the platinum complex. ¹H NMR resonances for duplex **III** were assigned from 2D ¹H NOESY NMR spectroscopic data, which allowed identification of specific changes that occurred immediately upon addition of [¹⁵N]-**1**. At pH 5.3 the Pt–NH₃ peaks of the monoaqua monochloro species are well separated in the 2D [¹H,¹⁵N] HSQC NMR spectra. As the reaction proceeds, however, the Pt–NH₃ peaks become overlapped as a result of several non-specific adducts forming (Figure 5). Because there was significant overlap in this region, identifying all species proved to be rather difficult. In the Pt–NH₂ region, there was also significant overlap as well as (for the linker Pt–NH₂ groups) suppression of cross-peaks in close proximity to the ¹H₂O resonance.

Analysis of reaction of [¹⁵N]-**1** with duplex **III**

To examine precovalent binding interactions, ¹H and [¹H,¹⁵N] HSQC NMR spectra were recorded immediately upon addition of [¹⁵N]-**1** to duplex **III**. Table 2 lists the chemical shifts in the ¹H and ¹⁵N dimensions of the Pt–am(m)ine groups of [¹⁵N]-**1** on addition to the duplex **III**, in comparison with the corresponding data for duplex **IV**. [26] The similarity of the ¹H and ¹⁵N chemical shifts for preassociated **1** indicate that a similar electrostatic interaction with

the DNA occurs in both cases. Compared to the shifts of [^{15}N]-**1** in the absence of DNA, there is a significant deshielding in the ^1H dimension of the NH_3 groups in the terminal $\{\text{PtN}_3\text{Cl}\}$ moiety ($\Delta\delta = 0.05$ ppm) and a more pronounced deshielding ($\Delta\delta = 0.12$ ppm) for the NH_3 groups in the central $\{\text{PtN}_4\}$ linker, as expected due to the higher charge (+2) of this group. As was observed for duplex **IV**, [26] there is evidence for minor-groove binding of the linker of **1** with duplex **III**, based on significant changes in chemical shifts detected for the adenine H2 protons of residues A4 ($\Delta\delta = 0.09$ ppm) and A6 ($\Delta\delta = 0.09$ ppm). No significant changes in shifts are observed for the H6/H8 protons other than for A10 H8 ($\Delta\delta = 0.05$ ppm) and T7 H6 ($\Delta\delta = 0.05$ ppm) (Figure 6). No significant changes are observed for the imino or the H1'/H2'/H2&Prime protons of **III**.

After ≈ 1 h the monoaqua monochloro species accounts for approximately 3% of the total species in solution. All ^1H , ^{15}N resonances of the $\{\text{PtN}_4\}$ linker group as well as the signals for the $\{\text{PtN}_3\text{Cl}\}$ moiety are assumed to be concealed underneath the resonances for the crosspeaks of the dichloro species (**1**). The ^1H and ^{15}N chemical shifts of the Pt-NH_3 resonance of the $\{\text{PtN}_3\text{O}\}$ group ($\delta = 4.21\text{--}61.9$ ppm, Figure 5) are very similar to that observed in the reaction with duplex **IV**, showing a pronounced downfield shift ($\Delta\delta\ ^1\text{H} = 0.21$ ppm, $\Delta\delta\ ^{15}\text{N} = 0.6$ ppm) compared to that of a control sample at identical pH. The stronger electrostatic interaction with the duplex (than occurs for the dichloro species) is a consequence of the increased charge (+2).

Thus far the kinetic profile for [^{15}N]-**1** follows what has been delineated for other sequences: preassociation followed by aquation. Significantly different behaviour is observed, however, for the subsequent monofunctional binding step. In all previous [^1H , ^{15}N] HSQC NMR studies to date in which we have followed formation of IXLs in the 5'-5' direction with either [^{15}N]-**1**, [26] or the dinuclear [^{15}N]-**2**, [27,31] the monofunctional adduct has been characterised by a $^1\text{H}/^{15}\text{N}$ cross-peak near $\delta = 4.30\text{--}61.0$ ppm, assigned to the Pt-NH_3 group bound to guanine N7, with the partner peak for the unbound $\{\text{PtN}_3\text{Cl}\}$ end distinctly deshielded ($\Delta\delta \approx 0.02$ ppm) in the ^1H dimension from that of the parent dichloro species. This deshielding has been attributed to hydrogen-bonding interactions between the ammine groups and the DNA phosphate backbone that are seen in molecular models of the monofunctional adduct. [27] In the present case the first evidence for a monofunctionally bound species is seen approximately 30 min after the addition of [^{15}N]-**1** to duplex **III**. A $^1\text{H}/^{15}\text{N}$ cross-peak at $\delta = 4.26\text{--}61.0$ ppm has similar chemical shifts to that observed upon reaction with [^{15}N]-**1** duplex **IV** ($\delta = 4.29\text{--}60.6$ ppm). [26] However, a number of other broadened peaks also appear in this region (Figure 5) suggesting that there might be more than one monofunctionally bound species present. No distinct peak is observed that is assignable to the Pt-NH_3 group at the unbound $\{\text{PtN}_3\text{Cl}\}$ end of these monofunctional adducts, and it is assumed that these peaks are concealed beneath that of the dichloro species (**1**). As the reaction proceeds, no distinct cross-peaks corresponding to major bifunctional products appear in the [^1H , ^{15}N] HSQC NMR spectra, but rather a series of peaks is distributed over a large area ($\delta = 4.12\text{--}62.3$ to $4.48\text{--}60.1$ ppm, Figure 5). This dispersion suggests the formation of more than one species, precluding detailed analysis. Besides the expected guanine binding, adenine platination is an obvious possibility. Platination at the N7 of guanine residues is expected to result in a significant downfield shift of the G8 H8 signal and in all previous studies we have observed the appearance of peaks at $\delta = 8.5\text{--}8.6$ ppm indicative of G N7-platinated duplex in both monofunctional and bifunctional adducts. Over time broad peaks are seen at $\delta \approx 8.6$ ppm and also in the $8.9\text{--}9.2$ ppm range assignable to monofunctional adducts (Supporting Information, Figure S3); this is consistent with binding to adenine as well as guanine. [32] It is also possible that some intrastrand cross-link formation could occur, either directly or through rearrangement of IXLs, as noted above.

The reaction of [^{15}N]-**2** with duplex **III** follows closely that which is observed for [^{15}N]-**1**. No preassociation is observed however because the dinuclear **2** has no charged central linker. The reaction proceeds through the formation of the monoaquated species $\{\text{PtN}_3\text{O}\}$ followed by the

formation of several monofunctionally bound species. No distinct 3'-3' 1,4-GG IXL is observed on completion of the reaction, and both ^1H and $[\text{H},^{15}\text{N}]$ HSQC NMR spectra show that more than one cross-linked adduct forms, probably involving binding at both guanine and adenine residues (Supporting Information, Figure S4 and S5).

Influence of central base pairs and sequence length

Based on the NMR spectroscopic studies (above), $[\text{N}]-\mathbf{1}$ forms multiple bifunctional cross-links with the self-complementary 12-mer sequence, 5'-(TATACATGTATA)₂ (duplex **III**). No major changes in reaction profile were observed in similar NMR spectroscopic studies by using a modified duplex 5'-(TATACAAGTATA)-5'-(TATACTTGTATA; duplex **Vb**) in which the "central" base pairs within the designed 1,4-GG cross-link are AA-TT rather than AT-TA (Supporting Information, Figure S6). Further, incubation of $[\text{N}]-\mathbf{1}$ with the 8-mer sequence designed to form a 3'-3' IXL (and an analogue of the 5'-5' sequence previously studied)[23] also gave multiple products that precluded isolation (data not shown). The sequences used in NMR spectroscopic studies are generally shorter than those used in molecular biology experiments. To examine the effect of sequence length, single-stranded oligonucleotide, 5'-(CCTTCTTGTTTT; top strand of duplex **Va**), was incubated with a twofold excess of **1** at 37°C for 1 h. The platinated single strand was purified by ion-exchange fast protein liquid chromatography (FPLC) then annealed with the complementary strand 5'-(GGAAGAACAAAA; bottom strand of duplex **Va**; by heating to 70°C), and incubated at 37°C for a further 24 h. After incubation the reaction product, along with the top strand of duplex **Va** and platinated top strand of duplex **Va**, were 5'-end labelled with $\gamma\text{-}^{32}\text{P}$ and analysed on a 20% denaturing polyacrylamide gel, which showed evidence for the formation of a 3'-3' IXL (data not shown). Thus, sequence length per se does not affect the results.

Molecular modelling

To examine the nature of the final bifunctional IXL a 10 ns molecular dynamics (MD) simulation was used to model the interaction of **1** with the 3'-3' sequence **III** in comparison with the analogous 5'-5' sequence **IV**. The model was generated by binding the two end Pt moieties of **1** to the N7 of the guanine residues. The structures were then minimised by using molecular mechanics followed by a MD simulation. The simulation for the a 3'-3' IXL showed a highly distorted structure with evident fraying of the end base pairs and considerable widening of the minor groove (Figure 7). These results, along with the NMR spectroscopic studies, indicate that a 3'-3' 1,4-GG IXL is likely to be a highly distorted adduct. In contrast the 5'-5' 1,4-GG IXL shows minimal distortion to the duplex. A similar highly distorted structure was observed in the model of the 3'-3' 1,4-GG IXL of the dinuclear **2** (Supporting Information, Figure S7).

Discussion

Firstly, the formation of directional isomers in 1,4-IXLs is a general one for polynuclear platinum compounds. The formation of the 3'-3' IXL for **4** contrasts with little formation for the dinuclear complex **2**. It is possible that preassociation could still occur for **4**, but the central nitrogen atom could be a hydrogen-bonding acceptor rather than a donor, as in **3**. It is also possible that a small amount of hydrolysis products gave the charged species, under the conditions of these experiments. The greater efficiency of "hybridisation-directed cross-link" over the "post-synthetic modification" method in forming site-specific modified duplexes has been previously noted.[30] In the former, platination of the "top" strand of the DNA is followed by heating and annealing, which might relieve some of the energy constraints in bifunctional cross-link formation. Of interest is that the efficiency of IXL formation in short (20-mer) duplexes is somewhat less than that in plasmid DNA,[28] which might reflect a template effect of the larger oligonucleotide.

Secondly, the NMR spectroscopic analysis along with molecular biology experiments (such as those using chemical probes of DNA conformation, DNA bending/unwinding and DNase I footprinting[5,22]) suggest a highly distorted structure for the 3'–3' IXL in contrast to the 5'–5' case. Interestingly, some evidence exists in the literature for conformational flexibility in closely related systems. By using a N4C-ethyl-N4C cross-link to mimic the IXL of alkylating agents, Noll et al. constructed plasmid DNAs in which modified cytosines are incorporated into single CpG or GpC steps in small duplexes and then incorporated into plasmid DNA. [33] Both sequences were substrates for DNA repair enzymes but with a fourfold difference in NER efficiency between the two, the CpG step being more robust. NMR spectroscopic structures of decamers containing the CpG (5'–5') IXL showed a well-defined structure whereas disruptions caused by the CpG (3'–3') produced considerable conformational flexibility; this precluded structural analysis by NMR spectroscopy. Interestingly, atomic force microscopy (AFM) imaging of plasmid DNA containing the site-specific cross-links showed that the increased flexibility extended to the much larger DNA fragments.[33] Thus, the effect of conformational changes upon differences in orientation of the interstrand cross-link might be a general phenomenon. In the specific case of platinum-induced cross-links, this might hold only for the 1,4-IXL case.

There are significant differences in experimental procedure between molecular biology and solution studies. The NMR spectroscopy experiments run with both the 3'–3' 1,4-GG (duplex **III**) as well as the modified 3'–3' 1,4-GG sequence (duplex **Vb**) showed evidence for preassociation, aquation and monofunctional binding comparable to the experiments that were carried out with the 5'–5' 1,4-GG sequence.[26] This suggests that initially the complexes interact with the DNA similarly as for the formation of the 5'–5' IXLs. However, due to structural reasons a single 3'–3' 1,4-GG IXL does not form under the conditions of these solution experiments. The molecular model showed that platination of the two guanine residues, separated by two residues (-AT-), would result in severe distortion of the duplex. The models showed major opening of the major groove with severe fraying of the central base pairs. These results might reflect the chemical probe and DNase I results in which strong alterations of the central base pairs are observed (Figure 2).[5,22] Note also that altered conformational changes in the central AT base pairs is a significant feature of the 5'–5' 1,4-GG IXL formed in the 8-mer and that distortions occur beyond the binding site.[23,34] Thus, it is reasonable to expect similar features in the present case.

Conclusions

In conclusion, unusual antiparallel 3'–3' 1,4-interstrand cross-linking is a general feature of polynuclear platinum compounds and will contribute to the genotoxic insult preferred to the cell treated with agents such as **1** and **3**. NMR spectroscopic studies and molecular dynamics indicate that this unusual lesion will be conformationally flexible and considerably distorting to the DNA. The results further confirm that cross-link orientation may have significantly different conformational consequences in specific DNA regions. These considerations apply only to the 1,4-case; 1,2 IXLs form readily in short sequences (unpublished data). The results do emphasise, however, that the multiplicity of DNA–DNA cross-linked adducts formed by flexible polynuclear platinum compounds might present significant challenges to cellular repair processes and the specifics of DNA repair might vary within the family of cross-links.

Experimental Section

Chemicals

The acetate salts of the oligonucleotides 5'-d(TATACATG-TATA) (duplex **III**), 5'-(TATACAAGTATA; **Vb** bottom strand) and 5'-d(TATACTTGTATA) (**Vb**, top strand) were purchased from Gene-Works (Hindmarsh, SA, Australia). 5'-d(CCTTCTTGT TTT) (**Va** top

strand) and 5'-d(AAAACAAGAAGG) (**Va** bottom strand) were purchased from Integrated DNA Technologies, (Coralville, IA, USA) and contained no salts. The nitrate salts of fully [^{15}N]-labelled [$\{\text{trans-PtCl}(\text{NH}_3)_2\}_2(\mu\text{-trans-Pt}(\text{NH}_3)_2\{\text{NH}_2(\text{CH}_2)_6\text{NH}_2\}_2)\}^{4+}$ (BBR3464, [^{15}N]-**1**) and [$\{\text{trans-PtCl}(\text{NH}_3)_2\}_2(\mu\text{-NH}_2(\text{CH}_2)_6\text{NH}_2)\}^{2+}$ (BBR3005, [^{15}N]-**2**) were prepared as described elsewhere.[26,27] The syntheses of [$\{\text{trans-PtCl}(\text{NH}_3)_2\}_2(\mu\text{-H}_2\text{N}(\text{CH}_2)_3\text{NH}_2(\text{CH}_2)_4)\}^{3+}$ (BBR3571, **3**) and [$\{\text{trans-PtCl}(\text{NH}_3)_2\}_2\{\mu\text{-H}_2\text{N}(\text{CH}_2)_3\text{N}(\text{COCF}_3)(\text{CH}_2)_4\}\}^{2+}$ (BBR3571-COCF₃, **4**) have been described elsewhere.[28]

Molecular biology

The synthetic oligodeoxyribonucleotides for gel electrophoresis experiments were synthesised and purified as described previously.[5] T4 polynucleotide kinase was purchased from New England Biolabs Inc (Beverly, MA). Acrylamide, bisacrylamide, and urea were from Merck (Darmstadt, Germany). DMS was from Sigma (Prague, Czech Republic). [$\gamma\text{-}^{32}\text{P}$]ATP was from Amersham Biosciences. ATP was from Roche Molecular Biochemicals (Mannheim, Germany). Platination of oligonucleotides in “hybridisation-directed IXL formation” experiments was performed in the following way: The single-stranded oligonucleotides [the top strands of the duplexes **I** or **II** (Figure 1b)] were treated in stoichiometric amounts with the platinum compounds **3** or **4**. The platinated oligonucleotides were repurified by ion-exchange HPLC. We verified that the modified oligonucleotides contained two platinum atoms by platinum flameless atomic absorption spectrophotometry (FAAS) and by measurement of the optical density. By using DMS footprinting of platinum on DNA,[29] we also verified that in the platinated top strands of all duplexes, N7 of a single guanine (G) residue was not accessible for reaction with DMS. These results indicate that one molecule of **3** or **4** was coordinated to a single G residue in the top strands of all duplexes. The platinated top strands were mixed with 5'-end-labelled unplatinated complementary strands (the bottom strands of the duplexes **I** and **II**) in 0.1 M NaCl, the mixtures were briefly heated to 50°C (for 5 min), cooled on an ice bath, precipitated by EtOH, dissolved in 0.1 M NaClO₄, and further incubated at 37°C (for 24 h). Aliquots were withdrawn at various time intervals and the products were separated on denaturing 24% PAA/8 M urea gel; the bands were analysed by densitometry or were cut off from the gel, eluted, precipitated by EtOH, dissolved in 50 mM NaCl and further characterised by Maxam–Gilbert (DMS) footprinting.

Platination of intact double-helical duplexes was performed by using self-complementary 16 bp duplexes **VI** and **VII** (see Figure S1 in the Supporting Information for their sequences). These duplexes were designed in such a way that they contained G residues in different positions to the single cytosine (C) so that the IXLs could close to Gs oriented to either the 5' side (in duplex **VII**) or the 3' side (in duplex **VI**) of the central C residue of the bottom strand, that is, in the 3'–3' or 5'–5' direction, respectively. The oligonucleotides were 5'-end labelled by T4 polynucleotide kinase and [$\gamma\text{-}^{32}\text{P}$]ATP, allowed to hybridise in 0.1 M NaClO₄ at 4°C, and the resulting duplexes were incubated in 0.1 M NaClO₄ at 15°C with equimolar amount of **1**, **3** or **4**. It was verified by native PAA gel electrophoresis that under these conditions more than 98% of the oligonucleotide strands were in the double-stranded form. The aliquots were withdrawn at various time intervals and analysed by gel electrophoresis under denaturing conditions.

Absorption spectra were measured with a Varian Cary 4000 UV/vis spectrophotometer equipped with a thermoelectrically controlled cell holder and quartz cells with a path length of 1 cm. Purification of duplexes with the aid of HPLC was carried out on a Waters HPLC system consisting of Waters 262 pump, Waters 2487 UV detector, and Waters 600S controller with MonoQ HR 5/5 column. The FAAS measurements were carried out on a Varian AA240Z Zeeman atomic absorption spectrometer equipped with a GTA 120 graphite tube atomiser. The gels were visualised by using a BAS 2500 FUJIFILM bioimaging analyser, and the

radioactivity associated with bands was quantitated with the AIDA image analyser software (Raytest, Germany).

NMR spectroscopy

The NMR spectra were recorded on Bruker AVANCE 600 MHz spectrometer (^1H , 599.92 MHz; ^{15}N , 60.79 MHz; ^{31}P , 242.94 MHz). The ^1H spectra were internally referenced to TSP (sodium 3-trimethylsilyl-D₄-propionate) and the ^{15}N chemical shifts were externally referenced to $^{15}\text{NH}_4\text{Cl}$ (1.0_M in 1.0_M HCl in 5% D₂O in H₂O). The ^1H spectra were acquired with water suppression by using the WATERGATE pulse sequence.[35] The 2D [^1H , ^{15}N] HSQC (heteronuclear single-quantum coherence) NMR spectra (decoupled by irradiation with the GARP-1 sequence during acquisition) optimised for $^1J(^{15}\text{N}, ^1\text{H}) = 72$ Hz were recorded by using the standard Bruker phase-sensitive HSQC pulse sequence.[36] ^{31}P NMR spectra were referenced externally to phosphoric acid and acquired with a *zgig* pulse sequence.[37] The samples were not spun during acquisition. Typically for 1D ^1H spectra, 32 transients were recorded with spectral width of 12 kHz and a relaxation delay of 1.5 s. For 2D [^1H , ^{15}N] HSQC NMR spectra eight transients were collected for 128 increments of t_1 with an acquisition time 0.152 s and spectral widths of 2 kHz in f_2 (^1H) and 1.8 kHz in f_2 (^{15}N). 2D spectra were completed in 14 min and were processed by using Gaussian weighting functions in both dimensions. For ^{31}P NMR spectra, 128 transients were recorded with a spectra width of 4.8 kHz, a relaxation delay of 0.25 s and an acquisition time of 3.38 s. 64k data points were used for processing. The pH of the solutions were measured by using a Shindengen ISFET (semiconductor) pH meter (pH Boy-KS723; SU-26F) and calibrated against buffers of pH 4.0 and 6.9.

DNA preparation for NMR spectroscopic studies

Duplex III—A 9.6 mM solution of duplex **III** (0.1 mL) was desalted by means of a NAP-25 column. All solutions used for the desalting process were filtered through 2.5 μm filters. The sample was made up to 2.5 mL by using MilliQ H₂O. The NAP-25 column was first rinsed with MilliQ H₂O (25 mL) then the sample was loaded onto the column and eluted with 3.5 mL ($\times 4$) of MilliQ H₂O. The DNA sample was collected in the first 2.5 mL to elute out of the column and then freeze-dried. Duplex **III** was dissolved in a mixture of 23.23 mM Na₃PO₄ (310 μL , pH 5.4) and 0.384_M NaClO₄ (100 μL). D₂O (24 μL) and TSP (2 μL , 13.3 mM) were also added to the NMR tube. This gave a final concentration of 15 mM Na₃PO₄ and 100 mM NaClO₄. To anneal duplex **III**, the sample was heated in a water bath to 90°C and then allowed to cool to RT over a few hours. The concentration of duplex **III** was estimated by using UV spectrophotometry and ^{31}P NMR spectroscopy (relative to a known concentration of PO_4^{3-}) to be ≈ 1 mM.

Duplex Vb—5'-d(TATACAAGTATA) (**Vb** bottom strand) and 5'-d(TA-TACTTGTATA) (**Vb**, top strand) were each desalted separately by using NAP-25 columns. UV spectrophotometry was used to estimate the molar quantity of each strand after desalting, as described above, after which each solution was freeze-dried. The “bottom” strand of duplex **Vb** was reconstituted in filtered MilliQ H₂O (70 μL) and the “top” strand of duplex **Vb** was reconstituted in 73 μL such that in a 480 μL sample the annealed duplex **Vb**, would have a final concentration of 1 mM (confirmed by using ^{31}P NMR spectroscopy). TSP (2 μL , 13.3 mM), D₂O (24 μL , 5%), Na₃PO₄ buffer (50 μL , 144 mM, pH 5.4) and NaClO₄ (58 μL , 0.662_M) were added to the solution. This gave a final concentration of 15 mM Na₃PO₄ and 100 mM NaClO₄. The solution was annealed by heating at 90°C for 30 min before cooling to RT over a few hours. 2D ^1H NOESY NMR spectra were recorded at 298 K to confirm that the oligonucleotide strands were in duplex form.

Reaction of [¹⁵N]-**1** with duplexes **III** and **Vb**

A freshly prepared solution of [¹⁵N]-**1** was dissolved in MilliQ H₂O, such that the final concentration in the NMR sample (300 μL) was ≈ 1 mM. To start the reaction, [¹⁵N]-**1** was added to the sample of the duplex (preparation as described above). The pH of the sample was adjusted to 5.3 (duplex **III**) or 5.4 (duplex **Vb**) by using 0.2, 0.01, and 0.005_M solutions of HClO₄ and NaOH, in 95% H₂O/5% D₂O. All aliquots that were removed for pH measurement were not returned to the sample due to chloride leaching. The reactions at 298 K were followed by 1D ¹H and 2D [¹H,¹⁵N] HSQC NMR spectroscopy to completion.

Molecular modelling

The molecular model of the 3'–3' 1,4-GG interstrand cross-link of **1** bifunctionally bound to duplex **III** was created in Amber 9[38] with periodic boundary conditions by using a TIP3P waterbox (10.0 Å). The molecular mechanics parameters for the Pt ion were based on calculations reported previously,[39,40] and the partial atomic charges of **1** with two chloro ligands removed have been previously developed in our laboratory.[41,42] A 10 ns molecular dynamics production was run by using supercomputers at the Australian Partnership for Advanced Computer (APAC). 3DNA[43] was used to generate Cartesian coordinate files for each molecular dynamics step. These files were then used to generate images in Swiss PDB Viewer 3.7,[44] which were rendered in the ray tracing program POV-Ray 3.5.[45] Similar protocols were followed for the model of the 5'–5' cross-link of **1** with duplex **IV** and the 3'–3' 1,4-GG interstrand cross-link of **2** and duplex **III** (data shown in Supporting Information).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Noll DM, Mason TM, Miller PS. Chem Rev 2006;106:277–301. [PubMed: 16464006]
2. McHugh PJ, Spanswick VJ, Hartley JA. Lancet Oncol 2001;2:483–490. [PubMed: 11905724]
3. Huang JC, Zamble DB, Reardon JT, Lippard SJ, Sancar A. Proc Natl Acad Sci USA 1994;91:10394–10398. [PubMed: 7937961]
4. Farrell N. Met Ions Biol Syst 2004;42:251–296. [PubMed: 15206105]
5. Kasparkova J, Zehnulova J, Farrell N, Brabec V. J Biol Chem 2002;277:48076–48086. [PubMed: 12226099]
6. Brabec V, Kasparkova J, Vrana O, Novakova O, Cox JW, Qu Y, Farrell N. Biochemistry 1999;38:6781–6790. [PubMed: 10346899]
7. Jung Y, Lippard SJ. Chem Rev 2007;107:1387–1407. [PubMed: 17455916]
8. Takahara PM, Rosenzweig AC, Frederick CA, Lippard SJ. Nature 1995;377:649–652. [PubMed: 7566180]
9. Jodrell DI, Evans TRJ, Steward W, Cameron D, Prendiville J, Aschele C, Noberasco C, Lind M, Carmichael J, Dobbs N, Camboni G, Gatti B, De Braud F. Eur J Cancer 2004;40:1872–1877. [PubMed: 15288289]

10. Hensing TA, Hanna NH, Gillenwater HH, Camboni MG, Allievi C, Socinski MA. *Anti-Cancer Drugs* 2006;17:697–704. [PubMed: 16917215]
11. Calvert AH, Thomas H, Colombo N, Gore M, Earl H, Sena L, Camboni G, Liati P, Sessa C. *Eur J Cancer* 2001;37:965.
12. Farrell, N. *Platinum-Based Drugs in Cancer Therapy*. Kelland, LR.; Farrell, N., editors. Humana Press; Totowa: 2000. p. 321–338.
13. Rauter H, Di Domenico R, Menta E, Oliva A, Qu Y, Farrell N. *Inorg Chem* 1997;36:3919–3927.
14. Montero EI, Benedetti BT, Mangrum JB, Oehlsen MJ, Qu Y, Farrell NP. *Dalton Trans* 2007:4938–4942. [PubMed: 17992278]
15. Hegmans A, Qu Y, Kelland LR, Roberts JD, Farrell N. *Inorg Chem* 2001;40:6108–6114. [PubMed: 11703107]
16. McGregor TD, Hegmans A, Kasparkova J, Nepelchova K, Novakova O, Penazova H, Vrana O, Brabec V, Farrell N. *J Biol Inorg Chem* 2002;7:397–404. [PubMed: 11941497]
17. Nielsen PE. *J Mol Recognit* 1990;3:1–24. [PubMed: 2191698]
18. Brabec V, Sip M, Leng M. *Biochemistry* 1993;32:11676–11681. [PubMed: 8218235]
19. Bailly C, Gentle D, Hamy F, Purcell M, Waring MJ. *Biochem J* 1994;300:165–173. [PubMed: 8198530]
20. Ross SA, Burrows CJ. *Nucleic Acids Res* 1996;24:5062–5063. [PubMed: 9016685]
21. Bailly, C.; Waring, MJ. *Methods Mol Biol*. Fox, KR., editor. Vol. 90. Humana Press; Totowa: 1997. p. 51–79.
22. Chvalova K, Kaspárková J, Farrell N, Brabec V. *FEBS J* 2006;273:3467–3478. [PubMed: 16824042]
23. Qu Y, Scarsdale NJ, Tran MC, Farrell NP. *J Biol Inorg Chem* 2003;8:19–28. [PubMed: 12459895]
24. Teuben JM, Bauer C, Wang AH, Reedijk J. *Biochemistry* 1999;38:12305–12312. [PubMed: 10493798]
25. Gelasco A, Lippard SJ. *Biochemistry* 1998;37:9230–9239. [PubMed: 9649303]
26. Hegmans A, Berners-Price SJ, Davies MS, Thomas DS, Humphreys AS, Farrell N. *J Am Chem Soc* 2004;126:2166–2180. [PubMed: 14971952]
27. Cox JW, Berners-Price SJ, Davies MS, Qu Y, Farrell N. *J Am Chem Soc* 2001;123:1316–1326. [PubMed: 11456703]
28. Hegmans A, Kasparkova J, Vrana O, Kelland LR, Brabec V, Farrell NP. *J Med Chem* 2008;51:2254–2260. [PubMed: 18338842]
29. Kaspárková J, Mellish KJ, Qu Y, Brabec V, Farrell N. *Biochemistry* 1996;35:16705–16713. [PubMed: 8988007]
30. Noll DM, Noronha AM, Wilds CJ, Miller PS. *Front Biosci* 2004;9:421–437. [PubMed: 14766379]
31. Berners-Price SJ, Davies MS, Cox JW, Thomas DS, Farrell N. *Chem Eur J* 2003;9:713–725.
32. Lemaire D, Fouchet HH, Kozelka J. *J Inorg Biochem* 1994;53:261–271.
33. Noll DM, da Silva M, Webba, Noronha AM, Wilds CJ, Colvin OM, Gamcsik MP, Miller PS. *Biochemistry* 2005;44:6764–6775. [PubMed: 15865422]
34. Qu Y, Scarsdale NJ, Tran MC, Farrell N. *J Inorg Biochem* 2004;98:1585–1590. [PubMed: 15458820]
35. Piotto M, Saudek V, Sklenar V. *J Biomol NMR* 1992;2:661–665. [PubMed: 1490109]
36. Palmer AGI, Cavanagh J, Wright PE, Rance M. *J Magn Reson* 1991;93:151–170.
37. McCoy MA, Mueller L. *J Magn Reson Ser A* 1993;101:122–130.
38. Case, DA.; Darden, TA.; Cheatham, TE., III; Simmerling, CL.; Wang, J.; Duke, R.; Lou, R.; Merz, KM.; Pearlman, DA.; Crowley, M.; Walker, RC.; Zhang, W.; Wang, B.; Hayik, S.; Roitberg, A.; Seabra, G.; Wong, KF.; Paesani, F.; Wu, X.; Brozell, S.; Tsui, V.; Gohlke, H.; Yang, L.; Tan, C.; Mongan, J.; Hornak, V.; Cui, G.; Beroza, P.; Mathews, DH.; Schafmeister, WS.; Ross, WS.; Kollman, PA. *Amber 9, Assisted Model Building with Energy Refinement*. University of California; San Francisco: 2006.
39. Yao S, Plastaras JP, Marzilli LG. *Inorg Chem* 1994;33:6061–6067.
40. Scheeff ED, Briggs JM, Howell SB. *Mol Pharm* 1999;56:633–643.
41. Thomas, DS. PhD Thesis. The University of Western Australia; Australia: 2006.
42. Moniodis, JJ. PhD Thesis. The University of Western Australia; Australia: 2006.

43. Lu XJ, Shakked Z, Olson WK. J Mol Biol 2000;300:819–840. [PubMed: 10891271]
44. Guex N, Peitsch MC. Electrophoresis 1997;18:2714–2723. [PubMed: 9504803]
45. Cason, C. POV-Ray rendering engine for Windows v3.5. <http://www.povray.org/>

Figure 2.

A schematic diagram demonstrating conformational alterations induced in DNA duplexes by a single, site-specific 1,4-GG IXLs of **1** in the 3'-3' and 5'-5' direction determined by molecular biology techniques. Full and open spots indicate strong and weak reactivity of chemical probes of DNA conformation. Adapted from reference [5].

Figure 3.

Kinetics of the IXL formation in the duplex **II** by **3** and **4**. A) Autoradiogram of a 24% polyacrylamide/8_M urea gel of the duplexes the bottom strand of which were ³²P-end-labelled. Lanes: 1, 9–0 min; 2, 10–30 min; 3, 11–1.5 h; 4, 12–2.5 h; 5, 13–4 h; 6, 14–6 h; 7, 15–8 h; 8, 16–24 h. B) Percentage of interstrand cross-linking reaction calculated from the ratio of the intensity of the corresponding fragment to the sum of intensities of all bands. Symbols: ▲, IXL (bands designated as IXL); ■, nonmodified single-stranded oligonucleotide (bands designated as ss); ▼, single-stranded oligonucleotide containing intrastrand adduct (bands designated as *), see the text for details.

Figure 4.

Kinetics of the IXL formation in the duplex **I** by **3** and **4**. A) Autoradiogram of a 24% polyacrylamide/8M urea gel of the duplexes whose bottom strand were ^{32}P -end labelled. Lanes: 1, 9–0 min; 2, 10–30 min; 3, 11–1.5 h; 4, 12–2.5 h; 5, 13–4 h; 6, 14–6 h; 7, 15–8 h; 8, 16–24 h. B) Percentage of interstrand cross-linking reaction calculated from the ratio of the intensity of the corresponding fragment to the sum of intensities of all bands. Symbols: $\blacktriangle$, IXL (bands designated as IXL); $\blacksquare$, nonmodified single-stranded oligonucleotide (bands designated as ss); $\blacktriangledown$, single-stranded oligonucleotide containing intrastrand adduct (bands designated as *), see the text for details.

Figure 5. 2D [^1H , ^{15}N] HSQC NMR (600 MHz) spectra at 298 K of duplex **III** after reaction with [^{15}N]-**1** for the times shown. Peaks have been assigned to the Pt-NH₃ end and linker groups for the intermediate species seen in the reaction prior to the formation of bifunctionally cross-linked adducts (see Table 2). After 54 h there is no distinguishable 3'-3' 1,4-GG interstrand cross-link, but numerous cross-linked adducts that cannot be assigned.

Figure 6. ^1H NMR chemical shift changes ($\Delta\delta = \delta(\text{duplex III-1}) - \delta(\text{duplex III})$) seen after the addition of $[\text{^{15}N}]\text{-1}$ to duplex III. Key: ●, H2 protons; □, aromatic H8 protons; ◇ aromatic H6 protons; × H42 protons.

Figure 7.

Snapshots of the molecular dynamics simulations run on the 5'-5' 1,4-GG (A) and 3'-3' 1,4-GG (B) cross-links formed by BBR3464 (**1**). The 3'-3' 1,4-GG IXL is highly distorted with obvious widening of the minor groove and fraying of the base pairs. The 5'-5' 1,4-GG IXL is much less distorting with the duplex maintaining its integrity.

Scheme 1.

A schematic showing the directionality of the two possible DNA-DNA interstrand cross-links (3'-3' and 5'-5') that multinuclear platinum complexes can form.

Table 1

Effect of DNA template and platinum compound on directional isomer formation.

	1 (BBR3464)		3 (BBR3571)		4 (BBR3571-COCE ₃)	
	[%]	Incubation time [h]	[%]	Incubation time [h]	[%]	Incubation time [h]
ssDNA 5'-5' <i>[a]</i>	≈50 <i>[c]</i>	24	68	24	90	24
ssDNA 3'-3' <i>[a]</i>	≈50 <i>[c]</i>	24	61	24	73	24
duplex 5'-5' (VI) <i>[b]</i>	22	75	14	72	17	72
duplex 3'-3' (VII) <i>[b]</i>	11	75	6	72	7	72
plasmid DNA <i>[d]</i>	20	24	33	24	90	24

[a] Hybridisation-directed IXL formation, see Figure 1 for the sequence.

[b] Post-synthetic modification, see Supporting Information, Figure S1 for the sequence.

[c] Percentages refer to relative formation of specific cross-links, see Figure 2; from ref. [5].

[d] From ref. [28].

Table 2

^1H and ^{15}N NMR chemical shifts for the terminal Pt–NH₃ groups of intermediate species observed in the reaction of BBR3464 ([^{15}N]-**1**) with DNA duplexes **III** and **IV**, in 15 mM phosphate buffer at pH 5.3.^[a]

species L/L'	Duplex III (3'–3') $\delta^1\text{H}/^{15}\text{N}$ [ppm]	Duplex IV (5'–5') $\delta^1\text{H}/^{15}\text{N}$ [^b] [ppm]
Cl/Cl (1) ^[c]	3.91/–64.1	3.91/–64.2
Cl/H ₂ O	concealed ^[d]	concealed ^[d]
Cl/H ₂ O	4.21/–61.9	4.19/–61.8
Cl/GN7	concealed ^[d]	3.93/–64.2
Cl/GN7	[^e]	4.29/–60.6

^[a] ^1H referenced to TSP; ^{15}N referenced to $^{15}\text{NH}_4\text{Cl}$ (external).

^[b] From ref. [26].

^[c] The $^1\text{H}/^{15}\text{N}$ shifts of the Pt–NH₃ groups of the central linker are also similar in the reactions with duplex **III** ($\delta = 4.26/–63.3$ ppm) and duplex **IV** ($\delta = 4.27/–63.3$ ppm).[26]

^[d] Assumed to be concealed underneath the cross-peak for the Cl/Cl species (**1**).

^[e] In contrast to the reaction with duplex **IV** there are several cross-peaks assignable to monofunctional adducts (possibly bound to A and G N7, see text and Figure 5); one peak at $\delta = 4.30/–60.7$ ppm has similar chemical shift to that of the GN7 bound adduct in the reaction with duplex **IV**