Comments on Inorganic Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455155

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To cite this Article Hambley, Trevor W.(1992) 'What Can Be Learnt from Computer-Generated Models of Interactions Between DNA and Pt(II) Based Anti-Cancer Drugs?', Comments on Inorganic Chemistry, 14: 1, 1 — 26
To link to this Article: DOI: 10.1080/02603599208048655
URL: http://dx.doi.org/10.1080/02603599208048655

What Can Be Learnt from Computer-Generated Models of Interactions Between DNA and Pt(II) Based Anti-Cancer Drugs?

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Received February 21, 1992

The use of computer-generated models to study the interactions between Pt(II) based anti-cancer drugs and their putative target, DNA, is described and critically analyzed. Computer-generated models have been used to gain insight into the factors which may mediate binding and influence anti-cancer activity. The emphasis has been on the distortions the binding of *cisplatin* causes in the DNA structure and on the hydrogen bonding and non-bonding interactions between the bound *cisplatin* moiety and the DNA. Computer-generated models have also been used to investigate why *cisplatin* does not bind to certain base sequences and hence have led to the design of compounds with potentially different binding specificities to those of *cisplatin*. Possible interactions between the new bisplatinum class of drugs and DNA have been modeled to determine how these drugs might bind.

The use of modeling to design new Pt(II)-based drugs is at present in its infancy. Strategies for the design of new compounds which might prove fruitful are outlined. Limitations to modeling and design approaches are discussed as is their use in conjunction with structural data from NMR spectra.

Key Words: *Pt anti-cancer drugs, cisplatin, molecular modeling, molecular mechanics*

Comments Inorg. Chem.
1992, Vol. 14, No. 1, pp. 1–26
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INTRODUCTION

The cytotoxic action of *cisplatin* (*cis*-$Pt(NH_3)_2Cl_2$) was discovered by Rosenberg and co-workers in the late 1960's while studying the effect of electric fields on cell growth.[1,2] *Cisplatin* entered clinical trials in the early 1970's and was found to be a highly successful treatment for some cancers, in particular for testicular cancers, where cure rates of up to 90% were achieved.[3] In recent years, the platinum-based drugs, *cisplatin* and its close relative *carboplatin*, have been the most heavily used of all anti-cancer drugs in the USA.[4]

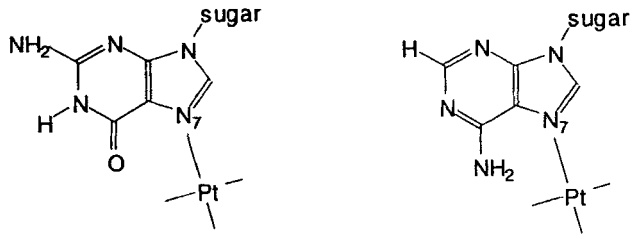
Despite the success of *cisplatin*, new drugs are desirable because although *cisplatin* is highly active against some tumours, it is almost totally inactive against others (natural resistance) and some tumours become desensitized to *cisplatin* following treatment (acquired resistance). In addition *cisplatin* has a number of severe side effects which can limit its use. This has prompted an enormous research effort aimed at determining the mechanism of action of *cisplatin* and at developing new platinum-based anti-cancer drugs. Despite these efforts, the details of the mechanism of action are yet to be unequivocally established and very few new drugs are in clinical use.

Rational drug design based on a knowledge, at the atomic level, of drug/substrate interactions is one of the central techniques of modern drug design. The three dimensional structures of drug/substrate complexes are sometimes available from crystallographic or NMR studies but are more often produced by theoretical methods such as molecular mechanics. Our group has been using these techniques, initially to aid in the elucidation of the mechanism of action of platinum-based drugs and ultimately to aid in the design of new drugs. Our approach has been to use computer-generated models of the putative drug/substrate adducts to design new compounds which can be used to test the proposed mechanisms of action and which might eventually be of use as new anti-cancer drugs.[5-8]

For computer-generated models to have veracity and be useful they must be consistent with what is known about the mechanism of action and be consistent with empirically established structure-activity relationships. Much is known about the way *cisplatin* acts

to cause cell death. On entering the cell, one or both of the chloro ligands are lost and the resultant complex binds to DNA via one or both of the available coordination sites.[9] The preferred sites of attachment for Pt are the N7 atoms of guanine and adenine (Fig. 1).[9] However, the presence of two available sites on the Pt leads to a number of bidentate (or bifunctional) complexes between it and the DNA (Fig. 2)[9] and it is these which are believed to interfere with DNA replication and so lead to cell death. Three of these adducts are intrastrand, to adjacent purine bases (guanine–guanine, GpG 60% (Fig. 2(a)), adenine–guanine, ApG 25% (Fig. 2(b))) and to two bases separated by a third (GpNpG <10% (Fig. 2(c))).[10] The fourth is an interstrand adduct linking guanine bases (<10% (Fig. 2(d)).[10]

Clearly, if one is to rationally design new anti-cancer drugs, it



Guanine Adenine

FIGURE 1 The preferred mode of binding of Pt(II) to guanine (a) and adenine (b).

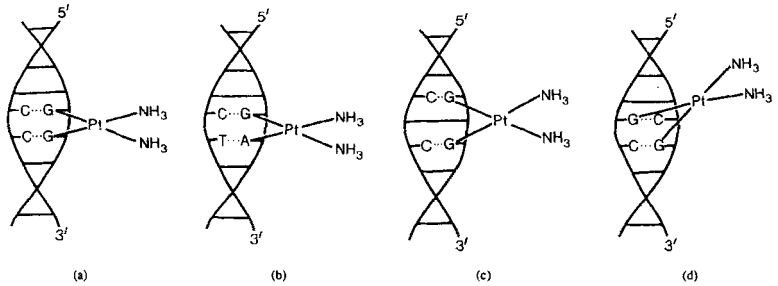


FIGURE 2 The major bifunctional adducts formed between *cisplatin* and DNA; (a) intrastrand GpG, (b) intrastrand ApG, (c) intrastrand GpNpG, (d) interstrand.

is essential to know which of these adducts leads to the death of tumour cells. It is difficult to establish this directly, as reactions between *cisplatin* and DNA produce all types of adducts. However, it has been shown that treatment at a level at which only intrastrand adducts should be present is sufficient to interfere with DNA replication,[11] suggesting that these may be the critical adducts, but it is not clear whether this interference is sufficient or responsible for the killing of tumour cells. More recently it has been shown that selective removal of intrastrand, but not interstrand, adducts does not prevent the cytotoxic effects of *cisplatin*,[12] suggesting that the interstrand adducts are important. Thus, the direct evidence is inconclusive and perhaps contradictory at present.

Indirect evidence is also available in the form of empirical structure/activity relationships. Specifically, active compounds have *cis* leaving groups and *cis* am(m)ine non-leaving groups each with at least one hydrogen atom.[13,14] Thus, the *trans* isomer of *cisplatin* is inactive and complexes in which all am(m)ine hydrogens have been replaced by alkyl groups are inactive. The majority of active complexes are square planar; some octahedral Pt(IV) complexes are active but it is probable that these are reduced to square planar Pt(II) on entering the cell.[15] Analogous Pd(II) complexes are inactive,[13,14] probably indicating that the greater kinetic inertness of Pt(II) is important for effecting anti-cancer activity. This indirect evidence cannot be used to deduce which adduct is responsible for activity. However, if a model of a given adduct displays features that are consistent with structure/activity relationships, then it is more likely to be an adduct responsible for the cytotoxic activity. The possibility that two or more of the adduct types are involved in the mechanism of action cannot be ruled out. Given the present uncertainty as to the exact mechanism of action of *cisplatin* it is both prudent and valuable to consider each of the adduct types. As mentioned, rational drug design requires structural knowledge at the atomic level of the details of the important drug/substrate interaction. However, in the case of *cisplatin* there is only limited information of this type available because, to date, no *cisplatin*/duplex DNA complex containing one of the bifunctional adducts described above has been crystallographically characterized. Crystal structures of *cisplatin* with the short single strand fragments of DNA pG*pG* and pCpG*pG* have been reported[16,17] and these

show that the Pt binds to the N7 atoms of the guanines. However, the secondary interactions, which may influence binding and activity, will clearly depend upon the conformation of the oligonucleotide and this will be different in single-stranded and double-stranded DNA. The crystal structure of *cisplatin* bound to a dodecamer duplex has been determined, but in this case it is bound monofunctionally,[18] and it is believed that such adducts are not responsible for cytotoxic activity.[19] Also, disorder or partial occupancy of the Pt atom limited the reliability of the information on secondary interactions of the DNA which could be derived from this structure.[18]

NMR studies have also provided some evidence on the nature of the interactions. They show, for example, that at least some of the interstrand hydrogen bonds involving the bases coordinated to Pt atom persist, that the DNA adopts a B type conformation and that the sugar on the 5′ side of the adduct adopts an N type (or C_2'-*endo*) conformation.[20,21] However, NMR data alone is not able to provide an unequivocal picture of the binding site. The paucity of experimental data, particularly data relevant to the minor interactions, has prompted the use of molecular modeling methods to investigate further the drug/DNA interactions. What is readily available are crystal structures of small DNA molecules and of *cisplatin*/nucleotide complexes. These structures can be used to guide the development of putative models of the *cisplatin*/DNA interactions. Computer-generated models of the various adducts reveal hydrogen-bonding and non-bonding interactions between the drug and the DNA and drug/DNA interactions that may mediate activity. Calculations also make it possible to access which adduct is most consistent with the observed structure–activity relationships.

THE METHODS OF MOLECULAR MODELING

Prior to the development of modern computational methods, the only way drug/substrate interactions could be modeled was by using physical models such as CPK kits. Now it is relatively easy to generate models of numerous possible interactions and then to select those which are most consistent with the data available.

These computational methods facilitate the design of compounds that test the veracity of the model and/or are expected to be more active based on the assumption that the interaction modeled is responsible for the activity. In order to obtain further details on the interactions between *cisplatin* and DNA, we and others have produced computer-generated models and used computer graphics to generate and/or analyze these models. The technique used most frequently for the generation of such models is molecular mechanics, which is now briefly described.

The molecular mechanics method is based on the principle that the energy costs associated with the distortion of internal coordinates, such as bond length and angles, and with interatomic interactions can be estimated with simple expressions. For example, a bond is treated as a spring with "strength" k_b and the energy cost, E_b, of distorting the bond from its ideal length, r_0, to a length r_{ij} is calculated using a standard Hooke's Law function as shown in Eq. (1):

$$E_b = 1/2k_b(r_0 - r_{ij})^2. \quad (1)$$

The collection of force constants, such as the "strength" of the spring k_b and the ideal bond length r_0, is called the force field. Force fields have been developed for organic compounds,[22] metal complexes,[23,24] and, more recently, for proteins and nucleic acids.[25]

The total strain energy is calculated by summing over all interaction types and all interactions as in Eq. (2):

$$U_{\text{total}} = \Sigma E_b + \Sigma E_\theta + \Sigma E_\varphi + \Sigma E_\delta + \Sigma E_{\text{nb}} + \Sigma E_\epsilon + \Sigma E_{\text{hb}}. \quad (2)$$

The interaction types and the way they are calculated vary from force field to force field. However, for macromolecules such as proteins or DNA, bond length (ΣE_b), bond angle (ΣE_θ), torsion angle (ΣE_φ) and out-of-plane (ΣE_δ) distortions and non-bonded (ΣE_{nb}), electrostatic (ΣE_ϵ) and hydrogen bonded (ΣE_{hb}) interatomic interactions are included, and relatively simple expressions are used to calculate the energies.

Given the model for a molecule, the aim is to find the molecular geometry or geometries which have the global or local minimum

in strain energy. A number of procedures have been developed for strain energy minimization and these vary in their computational requirements and sophistication. The methods currently used can be categorized as either first or second derivative. First derivative methods and their variants such as the conjugate gradients method have modest computational requirements, and each cycle of the iterative procedure requires little computer time. Therefore, they are generally the method of choice for macromolecules.[25] However, many cycles are required to reach convergence and a minimum in energy. Also, it is not possible to verify that a true energy minimum has been reached. Second derivative methods such as the Newton–Raphson method are more mathematically precise and reach a verifiable energy minimum in a small number of cycles. However, each cycle takes a long time to complete and memory requirements are proportional to the square of the number of atoms. Therefore, they are more commonly used for small molecules. The Newton–Raphson method has been the method of choice for most molecular mechanics studies of metal complexes.

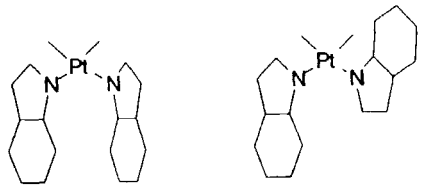
In all of the calculations we have performed, we have used the Newton–Raphson method for energy minimization. A primary reason for adopting this approach is that the force fields for modeling metal complexes have nearly all been developed using Newton–Raphson minimization and therefore extension of the methods to model Pt–nucleotide interactions is facilitated. Also, mathematical constraints on internal coordinates such as torsion angles are easily applied when using Newton–Raphson minimization. Thus it is possible to precisely investigate potential energy surfaces and barriers to rotation.

As a first step in modeling Pt–DNA interactions, we developed a force field for modeling small bis(nucleobase)diam(m)ineplatinum(II) complexes (e.g., $[Pt(G)_2(NH_3)_2]$.[26] This was necessary to develop the force constants for the interactions between Pt and nucleic acids. Crystal structures of numerous such complexes are available and the ability to reproduce these structures was used as a test of the force constants. We used this model to investigate the isomeric preferences of these complexes; the nucleobases can be disposed toward the same side of the coordination plane (head-to-head, HTH) or toward opposite sides (head-to-tail, HTT) (Fig. 3). We were able to show that the preferred arrange-

ment depended on whether the nucleobase was adenine or guanine, whether it was a simple nucleobase (e.g., 9-ethylguanine), a nucleoside or a nucleotide, and also depended on the nature and geometry of the am(m)ine ligand(s). Making use of the ability to apply constraints, we also investigated the barrier to interconversion between the HTH and HTT isomers.[26] The barriers also depend on whether the nucleobase is adenine or guanine and on the am(m)ine ligands and vary primarily as a result of interactions between the groups in the exocyclic 6-position of the nucleobase and the substituents on the am(m)ine nitrogen atoms. The calculated results were all in good agreement with experiment and proved the usefulness of molecular mechanics methods in modeling Pt-nucleotide interactions. They also emphasized the importance of secondary interactions between the am(m)ine ligands and the nucleotides to which the Pt was bound.

MODELING THE BINDING OF *CISPLATIN* TO DNA

A number of *cisplatin*/DNA binding modes have been observed[9,10] and some or all of these may play a role in the cytotoxic action of *cisplatin*. Thus, models of each of these binding modes are of potential significance in gaining an understanding of the factors that mediate activity. Also, modeling may be able to aid in the elucidation of the reasons some binding modes do not occur.



HTH HTT

FIGURE 3 The head-to-head (HTH) and head-to-tail (HTT) isomers of [Pt(base)$_2$(diam(m)ine)] complexes.

Intrastrand Binding of *Cisplatin* to GpG and ApG Sequences

A number of groups have produced computer-generated models of *cisplatin*/DNA interactions.[7,8,27-32] The most frequently studied model is of the intrastrand adduct to adjacent guanine bases, G*pG* (Fig. 2(a)), and a number of observations have been made with respect to most of these models: (i) The binding of *cisplatin* probably causes a kink in the helical DNA structure.[27-29] (ii) The binding causes a tilting of the two coordinated guanine bases toward one another.[7,8,27-32] (iii) When *cisplatin* binds to B-DNA it frequently causes a change in the conformation of the sugar of the guanosine of the 5′ side of the adduct.[8,27-32] (iv) The two ammine ligands each form a hydrogen bond to the DNA, one to a backbone phosphate oxygen and the other to O6 of the coordinated guanine on the 3′ side.[8,27-32]

There is also considerable variation in the details of these different models. This is not surprising, given that DNA adopts a continuum of conformations depending on the base sequence and on the environment. Equally, there are a multitude of ways in which DNA might deform to accommodate a coordinated $Pt(NH_3)_2^{2+}$ moiety. Thus, there are fundamental problems in accounting for the flexibility of DNA, either with or without Pt binding. A number of possible geometries have been considered by different groups in which some of the features listed above differ substantially. Kozelka *et al.* have produced kinked and unkinked models and models with the base pairing intact or disrupted.[27-29] McCarthy *et al.* have considered models with different hydrogen-bonding schemes in which both of the ammine groups are hydrogen bonded to both phosphate oxygen atoms and exocyclic oxygen atoms of guanine.[30,31] They have also considered models in which the ammine groups are not directly hydrogen bonded to the phosphate oxygen atoms, but rather are hydrogen bonded to water molecules which are in turn hydrogen bonded to the phosphate oxygen atoms. Herman *et al.* developed a series of models taking into account data from NMR measurements on a decanucleotide.[32] They concluded that the duplex was both kinked and unwound, that the conformation of nearby sugar rings was affected and that the stacking of bases and base-pair hydrogen bonding were disrupted and in a state of flux.

None of these models can be clearly distinguished as being more likely than the others on the basis of calculations alone and it is likely that many of these, as well as others, as yet unconsidered, contribute to the family of available local energy minima. Thus, each of the models has to be viewed as just one possible representation of the *cisplatin*/DNA adduct. Nevertheless, as mentioned above, there are some common features and most of these are consistent with experimental observations.

In nearly all of the studies, each of the ammine groups was found to be involved in at least one hydrogen-bond and this accords with one of the best established structure-activity relationships. That is, replacement of the H(ammine) atoms with alkyl groups reduces the activity of the complexes and replacement of all such atoms leads to a total loss of activity.[13,14,33] This has led to the suggestion that the ability to form these hydrogen bonds is an essential prerequisite for anti-cancer activity.[33] However, it should be noted that a number of active Pt compounds with no H(am(m)ine) atoms have now been reported. In all of these the nitrogen donor groups are trigonal planar and are consequently less sterically demanding than the tetrahedral am(m)ine groups.[34,35] Thus, while the hydrogen bonds may facilitate and strengthen binding, their replacement by alkyl groups probably decreases activity more as a consequence of the increased steric interactions rather than the energy lost by the inability to hydrogen bond.

We have also modeled the binding of *cisplatin* to ApG sequences (Fig. 2(b)), and the interaction is essentially identical to that observed for *cisplatin* bound to a GpG sequence. A view of the energy minimized geometry obtained for *cisplatin* bound to the ApG sequence of GAGG : CCTC[8] is shown in Fig. 4. The Pt atom is forced into close proximity with the exocyclic NH_2 group of the adenine and this may account for the reduced preference for binding to the ApG sequence (ca. 25%) compared to the GpG sequence (ca. 60%). However, the *two* hydrogen bonds are observed, again consistent with the structure/activity relationship described above and its current interpretation.

Intrastrand Binding to GpA Sequences

Cisplatin binds intrastrand to GpG and ApG sequences; however, it does not bind to GpA sequences of duplex DNA.[10,36,37] Dewan

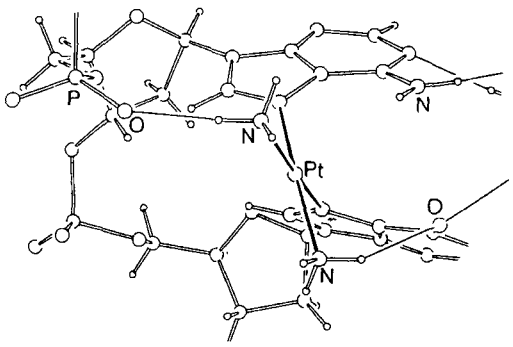


FIGURE 4 A molecular model of *cisplatin* bound intrastrand to an ApG sequence.

was the first to propose an explanation for this curious sequence specificity.[38] He noted that when *cisplatin* binds to a guanine base of a B-DNA structure, probably the first step in bifunctional adduct formation, the distance to N7 of a purine on the 5′ side is ~3 Å and that to one on the 3′ side is ~5 Å and postulated that this greater distance in the latter case led to non-formation of the GpA adduct.[38] However, we felt that this explanation was unlikely to be correct, particularly given that adducts of the type GpNpG do form and that for these to form a Pt . . . N7 distance of ~7 Å must be bridged. We have therefore used computer-generated models to investigate the non-formation of the *cisplatin*/GpA adduct.[7,8]

As described above, our models show that when *cisplatin* binds to GpG and ApG sequences the NH_3 groups make two hydrogen bonds, one to a backbone phosphate oxygen atom and one to the exocyclic oxygen of the guanine on the 3′ side. In the case of the GpA sequence there is no guanine on the 3′ side; rather there is an adenine, and the equivalent exocyclic site is occupied by an NH_2 group. A model of the GpA adduct (Fig. 5) shows that the NH_3 ligand adjacent to the 3′ purine makes a highly unfavourable contact with this NH_2 group. Coordination of the platinum to the adenine greatly restricts the ability of the NH_2 groups to avoid the contact and in the energy minimized structure it remains highly unfavourable. The binding of *cisplatin* is believed to be kinetically controlled[39] and therefore we have also modeled a putative five-coordinate transition state. In this the NH_3 and NH_2 groups are forced even closer together by the more crowded coordination

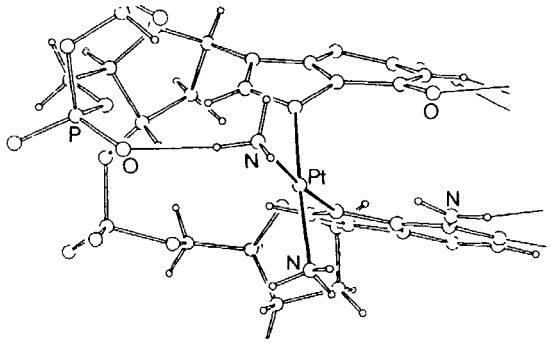


FIGURE 5 A molecular model of *cisplatin* bound intrastrand to a GpA sequence.

environment.[8] We have therefore concluded that this interaction destabilizes the GpA interaction sufficiently to prevent its formation. The models that led us to this postulate also led us to the design of compounds which would test the postulate by binding to GpA sequences. The design philosophy is that if an unfavourable contact between an NH_3 ligand and an NH_2 group is inhibiting binding then replacement of the NH_3 ligand by a group with the potential to hydrogen bond to the NH_2 group should lead to a complex able to bind to GpA sequences. The first series of compounds prepared to test the postulate have a bidentate amine-sulfoxide ligand replacing the two ammine ligands.[40] The sulfoxide group readily hydrogen bonds through the oxygen atom to acidic H atoms. A model of such a complex bound to a GpA sequence is shown in Fig. 6.

We have used plasmid binding studies to show that these compounds do bind to DNA. They have low *in vitro* and *in vivo* cytotoxic activities; this is probably a consequence of the increased kinetic lability of the site *trans* to the sulfoxide group compared to that *trans* to an am(m)ine group. Studies are underway to determine whether these compounds do indeed bind to GpA sequences. A second series, of aminooxime complexes has been prepared and similar testing is underway.[41]

Modeling the binding of *cisplatin* to the ApG and GpA sequences has led us to an explanation for an observed sequence specificity and aided in the design of compounds to test the hypothesis.

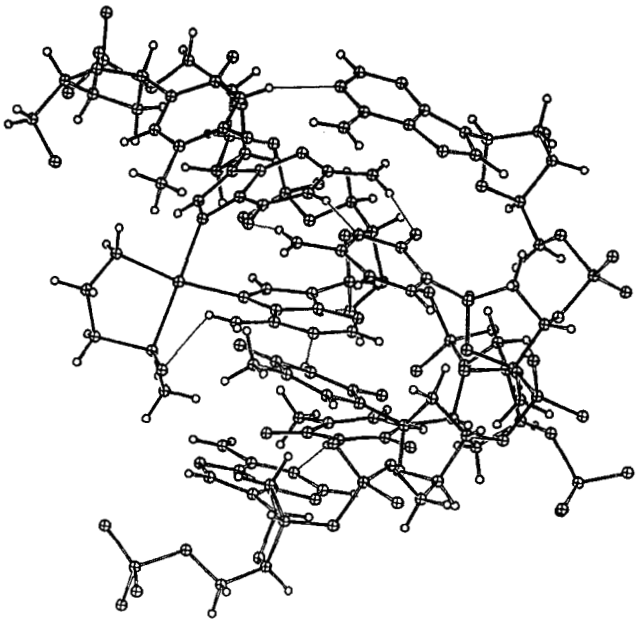


FIGURE 6 A molecular model of $[Pt(O{=}S(CH_3)CH_2CH_2NH_2)]^{2+}$ bound intrastrand to a GpA sequence.

INTERSTRAND BINDING OF *CISPLATIN*

Only a small portion (<10%) of the *cisplatin* that binds to DNA binds to an interstrand manner.[9,10] However, there have long been suggestions that these interactions are the ones responsible for the cytotoxic action of *cisplatin*,[15] and recently correlations have been reported between cellular resistance to *cisplatin* and the ability to selectively repair the interstrand adduct.[12] It is easier to conceptualize how an interstrand adduct would interfere with DNA replication than would one of the intrastrand adducts since the former links the two chains together with coordinate bonds. Given this interest, we undertook a modeling study of the interstrand adduct. All reported [9,10,42] interstrand adducts link two guanine bases and therefore we modeled the interactions with the sequences $(5'\text{-GpG-}3')_2$ and $(5'\text{-CpG-}3')_2$. In the former case the models were established with surprising ease, but in the latter case no interstrand adduct could be produced without severely disrupting the duplex

structure with an associated high energy cost. Why this is so is most easily visualized by considering as a first step hypothetical models with separate Pt atoms coordinated to each of the two guanine bases. The formation of the interstrand adduct can then be pictured as the two Pt atoms coming together. This approach is more informative than considering a Pt atom bound to one guanine N7 and then calculating the non-bonded Pt . . . N7 distance because it takes account of the orientation of the lone pairs on the two N7 atoms. In the case of the GpC adduct the initial separation is 6.3 Å and the Pt atoms can be "merged" relatively easily. Conversely, in the case of the CpG adduct, the initial distance is 10.9 Å, and merging results in destruction of the helix structure which would have a high energy cost. Leng and co-workers have shown that interstrand adducts form only with (5'-GpC-3') sequences which accords with our observations.[42]

A view of the model for *cisplatin* bound to this sequence is shown in Fig. 7. The adduct causes a closing of the major groove but, aside from this, the effects on the DNA structure are minor. The planar $Pt(NH_3)_2(-N7-)_2$ moiety lies perpendicular to the axis of the DNA duplex. The complex has C_2 symmetry with the axis passing through the Pt atom. Of significance is the observation that each of the NH_3 ligands makes a hydrogen bond with a backbone phosphate oxygen atom. Thus, the model suggests that the interstrand adduct, like the intrastand adduct, is consistent with the structure/activity relationship which establishes the need for two H(amine) atoms for cytotoxic activity.

MODELING THE BINDING OF OTHER *CIS*-DIAMINEPLATINUM(II) COMPLEXES

The anti-cancer activity of many *cis*-diamineplatinum(II) complexes with substituted amine ligands has been tested but to date none have been found which are more active than *cisplatin*.[33] The prevalence of hydrogen bonds in the models described above suggests that this may be a consequence of superior hydrogen bonding to the H atoms of the NH_3 ligands compared to those of substituted amines. It may also be due to the lower steric requirements of the NH_3 ligands. Models of platinum(II) complexes with ligands other

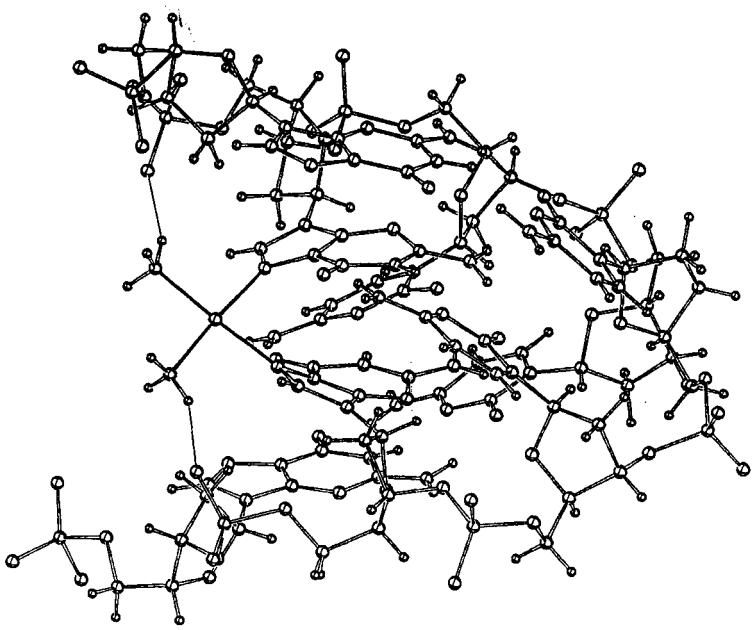


FIGURE 7 A molecular model of *cisplatin* bound interstrand to a GpC:GpC sequence.

than NH_3 bound to DNA have the potential to establish whether steric interactions between the substituents on the amine ligands and the DNA do influence binding. However, there have been few such studies carried out to date.

McCarthy *et al.* have modeled the binding of the series $Pt(NH_2R)_2^{2+}$, where R is H, methyl, cyclopropyl, cyclobutyl and cyclopentyl, and have concluded that a correlation exists between a calculated "binding energy" and the anti-cancer activity of each member of the series.[30] However, they observed only a slight variation in the steric contribution to the binding energy across the series and it is not clear whether this variation is sufficient to account for the variation in activity.

We have modeled the binding of the *meso*, *R*,*R*, and *S*,*S* isomers of cyclohexane-1,2-diamineplatinum(II) to DNA.[5] It has been reported that each of these has a different anti-cancer activity.[43,44] For each of the isomers, orientation of the H atoms involved in

hydrogen bonding to the DNA is different, that for the *S,S* enanitomer allowing the strongest hydrogen bonding.[5] However, the differences are small and since the relative activities of the complexes depend on the system used for testing it is impossible to conclude that a correlation exists. It is clear from these models[5] that the cyclohexane group does not interact significantly with the DNA and therefore is not an impediment to the binding of the complex. This is in accord with the good *in vitro* and *in vivo* anti-cancer activity of these complexes. The lower anti-cancer activity compared to that of *cisplatin* may be a consequence of the restrictions the cyclohexane ring places on the disposition of the H atoms involved in hydrogen bonding or to a poorer intrinsic hydrogen bonding capacity of H(amine) atoms of substituted amine ligands. It may also be due to other factors entirely, such as the different solubility of the complexes and their undoubtedly different transport properties.

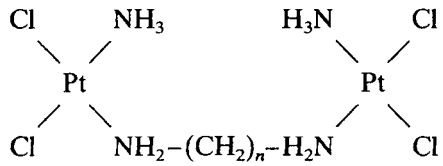
MODELING THE BINDING OF *TRANS*-DDP TO DNA

Trans-diamminedichloroplatinum(II), *trans*-DDP, the *trans* isomer of *cisplatin*, is almost totally inactive as an anti-cancer agent.[9,13,14,33] It has been suggested that this inactivity is a consequence of *trans*-DDP being unable to form intrastrand adducts with adjacent residues.[9] Instead the *trans* isomer most probably binds to GpXpG sequences and in so doing would be likely to produce large disruptions to the DNA structure which would be readily recognized by repair enzymes.[9] Molecular modeling obviously has potential to show whether such a distortion does indeed occur. Only one such study has been reported; Lepre *et al.* used molecular dynamics to model the binding of *trans*-DDP to a G*pApG* sequence of a dodecanucleotide.[45] They found that a local disruption of the base-pairing and base-stacking occurred and only a small distortion of the phosphate backbone took place. It is not known whether these disruptions are more likely to be detected by damage recognition enzymes; however, there is certainly more disruption than is observed in the intrastrand binding of *cisplatin*. Hydrogen bonding of the NH_3 groups to phosphate and guanine

oxygen atoms of one strand and to a sugar oxygen atom of the other was also observed.

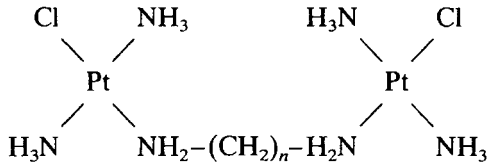
BISPLATINUM COMPLEXES

Nearly all of the active Pt complexes reported have been monomeric; however, recently Farrell and co-workers in Vermont have developed a novel series of dimeric platinum complexes.[46-48] These all contain two Pt atoms linked by diaminealkanes ($NH_2-(CH_2)_n-H_2N$) of varying length but fall into two classes. In the first of these classes, each of the Pt atoms has two leaving groups (e.g., Cl^-), one NH_3 ligand and the fourth site is occupied by the linking diaminoalkane:



abbreviated 2,2-*c,c*.

These complexes are made up of to two *cisplatin*-like units, and therefore it is not surprising that they have similar anti-cancer properties to those of *cisplatin*. In the second class each of the Pt atoms is coordinated to one leaving group, two NH_3 ligands and the bridging ligand:



abbreviated as 1,1-*t,t*.

The presence of only one leaving group means that each Pt atom can bind only monofunctionally to DNA, and therefore these represent a new class of cytotoxic drug. Monoplatinum analogues such as $[Pt(dien)Cl]^+$ are inactive,[19,33] and therefore these bisplatinum

complexes evidently derive their cytotoxicity from a different mechanism than that of *cisplatin*, presumably involving the binding of both Pt atoms.

At present, little is known about how either of these classes of biplatinum complex interacts with DNA. We have produced and analyzed computer-generated models for these complexes bound to DNA in order to seek answers to the following questions: do the complexes bind intrastrand or interstrand, what is the ideal size for the linking group, what interactions does the linking chain make with the DNA, and what sequences do the complexes prefer to bind to?

2.2-*c,c*-Bisplatinum Complexes

As described, these complexes consist of two *cisplatin* like groups linked by an aliphatic chain. Models were established by simulating *cisplatin* binding to both strands of DNA containing the sequence -GGCC-. When this is done, two of the ammine ligands are in close proximity and insertion of the linking chain is easily accomplished. This, of course, does not represent the binding process but the final model is a probable mode for binding. It is clear from the models investigated that interstrand binding, in which the Pt atoms are bound to different strands, is preferred over intrastrand binding. Not surprisingly, each of the binding sites resembles *cisplatin* binding to DNA with hydrogen bonds between the am(m)ine ligands and phosphate and exocyclic guanine O6 atoms (Fig. 8). The linking group lies in the major groove and makes close contacts with the edges of exposed bases. It is clear that much of the longer linking groups (n = 5,6) is redundant and that a chain with 4 or fewer carbon atoms should be able to link adjacent GpG sequences. This is consistent with the observation that of those tested, the compound with n = 4 is the most active; compounds with longer chains would have to overcome an increased entropic factor. Experiments are underway to establish whether compounds with shorter chains are indeed active. The possibility of the complexes with longer chains linking sequences separated by one or more bases cannot be ruled out but has not yet been explicitly investigated.

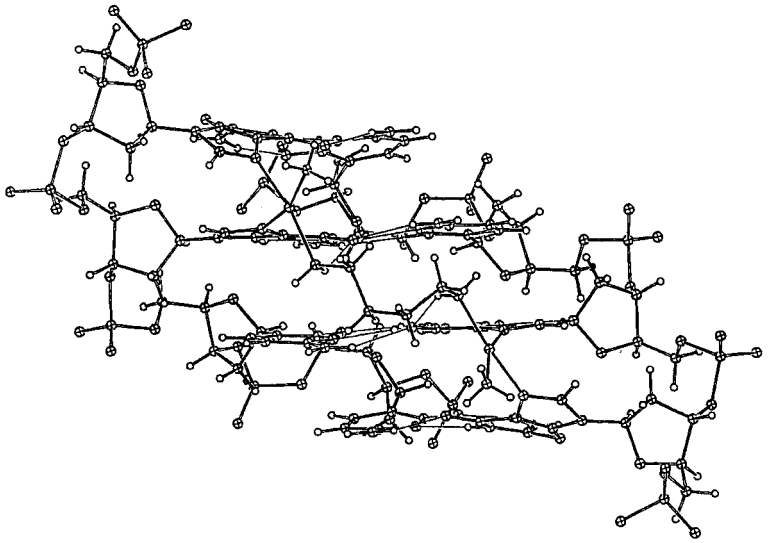


FIGURE 8 A molecular model of $(Cl)_2NH_3PtNH_2(CH_2)_4NH_2PtNH_3(Cl)_2$ cross-linking the two strands of duplex DNA.

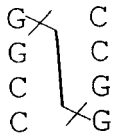


FIGURE 9 Schematic view of the binding of 1,1-*t,t* to duplex DNA.

1,1-*t,t*-Bisplatinum Complexes

These complexes consist of two platinum units, each of which can bind only monofunctionally to DNA. Thus, the binding of these complexes can be expected to be very different from that of *cis-platin*. In order to investigate how these might bind to DNA we first modeled the binding of $Pt(NH_3)_3$ units at a number of sites in sequences containing -GGCC-. It was found that when these units were bound to G_1 and G_4 (Fig. 9), they were ideally disposed for a linking group to be able to join them. As in the case of the 2,2-*c,c* compounds, the shorter linking groups are adequate to bridge the two platinum moieties bound in this way. The linking groups again make a number of close contacts with the exposed

edges of the bases and the NH_3 ligands hydrogen bond to phosphate and guanine O atoms (Fig. 10).

DESIGN OF NEW DRUGS

The design of drugs based on the three-dimensional structures of substrates is an active and growing area at present. It is a motivation for the determination of structures by crystallographic, NMR and theoretical methods, but is at present still in its infancy. There has been little done in the way of design of new Pt(II) based anticancer drugs. This is perhaps not surprising because what the models described above suggest that *cisplatin* is probably in some respects the ideal compound, since it has excellent hydrogen bonding prop-

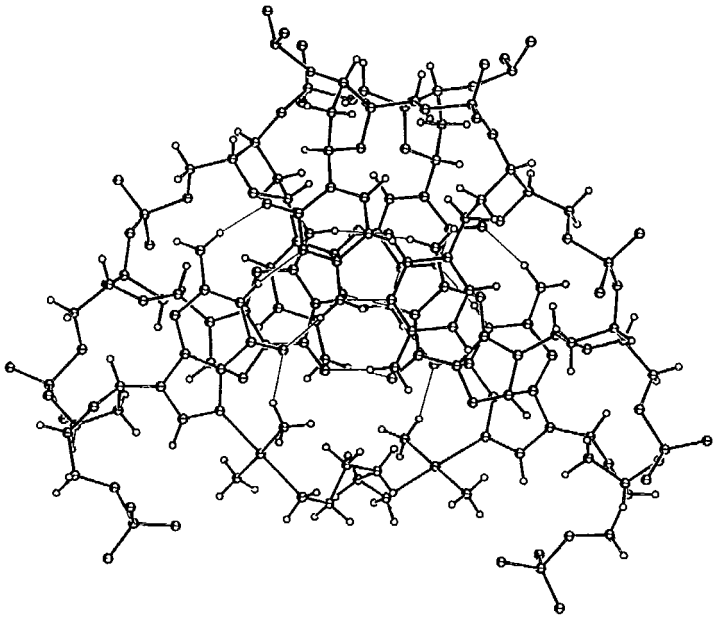


FIGURE 10 A molecular model of $Cl(NH_3)_2PtNH_2(CH_2)_4NH_2Pt(NH_3)_2Cl$ cross-linking the two strands of duplex DNA showing how the linking group lies in the major groove.

erties and its small size means that unfavourable steric interactions with the DNA are not an impediment to binding. Any modification of the complex by substitution of the NH_3 ligand with amine ligands must reduce the rotational freedom of the ligand, and so perhaps reduce the potential for hydrogen bonding, and must also increase the steric bulk of the ligand, potentially interfering with the binding. However, the modeling studies do suggest a number of approaches to drug design.

Our modeling has suggested that the reason *cisplatin* does not bind to GpA sequences is an unfavourable interaction between an NH_3 ligand and the exocyclic NH_2 group of the adenine. This led us to the idea that replacement of one of the NH_3 ligands with a group capable of hydrogen bonding to the adenine NH_2 group might bind to GpA sequences and thus have different properties than those of *cisplatin*.[8] In order to test this idea we designed two classes of complexes, one with aminesulfoxide ligands,[40] the other with aminooxime ligands.[41] We have shown that both classes of complex bind to DNA but have only modest anti-cancer activity. Studies are underway to determine whether these compounds do indeed bind preferentially to GpA sequences.

The new bis-platinum complexes developed by Farrell and coworkers[46-48] are an obvious target for drug design. We have already shown that complexes with shorter linking groups than those currently tested should be able to bind to DNA, and experiments are underway to test the hypothesis. Furthermore, the models have shown that the linking groups lie in the major groove of DNA and make close contacts with the exposed edges of bases. There is the potential to modify the linkers so that they make specific interactions with the bases and thus, to design sequence specificity.

Modeling of the binding of the $Pt(chxn)^{2+}$ moiety to DNA shows that atoms linking the two amine donor groups lie in the major groove and are not a major impediment to binding.[5] Thus, complexes can be modified along this backbone by addition of groups such as carrier molecules or molecules preferentially taken up by tumour cells, without these added molecules interfering with binding. Equally, it is clear from the models that bulky groups added directly to the amine donor atoms are more likely to be an impediment to binding.

LIMITATIONS OF THE MODELING APPROACH

It is clear from the foregoing that molecular modeling has provided significant insight into the details of the interactions between Pt(II)-based drugs and DNA, insight which has not been available from any other source. However, it must be kept in mind that these models are derived using approximate and empirically developed methods. The models that are produced depend on the force field, the collection of parameters that define the energy costs of the deformation of internal coordinates. In the limited number of studies described above at least four different force fields have been used and this is undoubtedly reflected in differences in the results obtained; for instance, in the number of hydrogen bonds involving the am(m)ine ligands and in the way the DNA is predicted to deform to accommodate the Pt complex.

The second, and perhaps more important, qualification is that each model represents but one possible picture of the drug/DNA adduct. For example, Kozelka *et al.*[27-29] have investigated a number of possible geometries for a single *cisplatin*/oligonucleotide complex and concluded that it is not possible to say that one model is clearly more "correct" than the others. DNA is a flexible molecule which exhibits extensive conformational variation at primary, secondary and tertiary levels; for example, in sugar puckering, helix conformation and super-helix coiling. Generally DNA adopts the classical B form in solution but the conformation is sequence dependent and some sections will adopt more A-like forms. We have shown that there are differences in the binding of *cisplatin* to A- and B-forms.[8]

Finally, any model is only the sum of the assumptions made in creating it. Therefore, modeling is unlikely to produce a radically new idea. The value of modeling comes when the ideas generated are tested by experiment. In order to overcome these limitations it is important to use as much experimental evidence as possible when establishing models. In this context NMR data, particularly NOE interactions from 2D experiments, are proving important in refining and testing models. It is also important to test experimentally any predictions that arise from the models, as for example we have attempted to do in order to determine why *cisplatin* does not bind to GpA sequences.

CONCLUSIONS

The bulk of molecular modeling of Pt/DNA interactions has related to the intrastrand adduct of *cisplatin* with adjacent guanine bases. These models have facilitated an increased understanding of empirical structure/activity relationships. Specifically, the observation that each of the NH_3 ligands is involved in hydrogen bonds with the DNA suggests an explanation for the requirement that each am(m)ine group have at least one H atom for the complex to have anti-cancer activity.

The potential of molecular modeling to contribute to the design of new platinum-based drugs is as yet largely unfulfilled. We have used modeling to design compounds that test the importance of various adduct types in effecting the anti-cancer activity of *cisplatin*-like drugs and to test the hypotheses on the factors which mediate binding to DNA and anti-cancer activity. However, as far as we can determine there has been no success in the design of new drugs with increased anti-cancer activity or more desirable properties. Modeling shows that this may be expected since *cisplatin* is in some respects perhaps the ideal compound. However, we believe there is potential to design sequence specific drugs and to design drugs with carrier groups attached in ways that do not interfere with binding and do not reduce anti-cancer activity.

Molecular modeling of Pt/DNA interactions has been motivated in part by a lack of structural information from experimental work. Improvements in the quality and the analysis of NMR data[49] means that increasingly structural details of *cisplatin*/DNA interactions are being elucidated by these methods.[20,50–56] However, NMR often does not provide sufficient information to unequivocally establish the three-dimensional structure. Thus, molecular modeling and information from NMR spectra are complementary; the modeling can be used to produce structures consistent with the NMR data, and the NMR data can be used to choose between various molecular models. It is therefore probable that future modeling work will be closely linked with NMR studies.

Acknowledgments

We wish to acknowledge the financial support of the Australian Research Council and the Sydney University Cancer Research Fund and the use of the equipment

of the Sydney University Biomolecular Graphics Facility. We also wish to thank Dr. J. Mitchell Guss for helpful discussions and comments.

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