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Supplementary Material Available: ^{13}C NMR spectral parameters for 7 and 8 (Table III) and selected nonbonding distances between ring-A atoms and the midpoint of ring C, nonbonding angular deviation from the plane of ring C, a torsion angle deviation from the plane of ring C, and the corresponding values for 11 (X-ray) (Table IV) (3 pages). Ordering information is given on any current masthead page.

Binding of *cis*-(1,2-Diaminocyclohexane)platinum(II) and Its Derivatives to Duplex DNA

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A theoretical study is presented for the binding of *RR*, *SS*, *SR*, and *RS* isomers 1,2-diaminocyclohexane (DAC) or *cis*-Pt^{II}(DAC) to DNA. *cis*-Pt^{II}(DAC) is ligated to N7(G) on two adjacent intrastrand guanine bases in a kinked pentamer duplex of DNA (AT, CG*, CG*, GC, AT). The relative stability of the complexes is determined by calculating the relative conformational energy of the *cis*-Pt^{II}(DAC)(DNA) complexes with molecular mechanics (MM) and the intrinsic binding or ligation energy with quantum mechanics (QM). The results suggest that the *RR* and *SS* isomers of Pt^{II}(DAC) adducts with DNA are more stable than the *SR*/*RS* isomer by 1.7 kcal/mol relative to the *cis*-Pt^{II}-$(DAC(H_2O)_2$ aquated species. Calculations on the overall stability of these isomers show that the *SS* and *RR* isomers are 6.5–8.2 kcal/mol more stable than the *SR*/*RS* isomers when bound to DNA, and this is attributed to differences in the strain energy in the DAC rings. The theoretical analyses of these compounds correlate a small differential activity with the trend in intrinsic binding energies. The *RR* isomer is more active in B16 melanoma cells, and the *SS* is most active in L1210 leukemia, and in general the *RR* and *SS* isomers are more active than the *SR* and *RS* in most cell types. The fact that the activity is DNA dependent suggests that excision or repair mechanisms may be taking place and that additional mechanistic steps beyond molecular modeling and quantum mechanical calculations are required to fully understand the activity. These studies of molecular fit of *cis*-Pt^{II}(DAC) to DNA are used to suggest substituted DAC compounds that may yield similar binding characteristics. Modifications to yield DAC derivatives are recommended in anticipation that they may also exhibit activity.

Since the discovery of the activity of *cis*-diamminedichloroplatinum(II) in 1969 by Rosenberg,[1] a search has been made for other compounds that will exhibit antitumor activity. For the parent *cis*-$Pt^{II}(NH_3)_2$ compound, the active site is bidentate addition of platinum to N7 atoms of adjacent guanine moieties within the DNA molecule.[2,3] This knowledge was utilized in preparation of the isomeric series of 1,2-diaminocyclohexane (DAC) compounds. The DAC compounds exhibit lower toxicity and a lack of cross resistance over the parent compound while maintaining good activity.[4-6] These compounds exist in more than one isomeric form: as the *SS*, *RR*, and the equivalent *SR*/*RS* forms of the cyclohexane ring in its attachment through the amines to Pt(II), as shown in Figure 1. In different tumor cell types, these isomeric compounds exhibit varying levels of antitumor activity. The entire series exhibits antitumor activity, but the *RR* and *SS* forms are, in general, more active than the *SR* and *RS* isomers. This variance in activity is demonstrated by the following: the *RR* isomer is most active in B16 melanoma cells, while the *SS* isomer shows the greater activity in L1210 leukemia.[7,8] In an attempt to understand the difference in activity shown for these isomers, the *cis*-Pt^{II}(DAC) and *cis*-Pt^{II}(DAC)-(DNA) adducts are studied with molecular mechanics (MM) to examine the conformational effects, and with quantum mechanics (QM) to analyze the ligand binding energy of these complexes.

The relative binding energies of these compounds to DNA were investigated as one possible factor in the activity of these compounds and the molecular fit into the DNA as a second. We previously introduced a pentamer duplex model to account for the molecular fit of a series of substituted Pt(II) diammines.[9,10] This intermediate conformation or IC pentamer duplex (AT, CG*, CG*, GC, AT) allowed for an approximately square-planar complex. The success of the IC (intermediate conformation) kinked

Table I. Relative Molecular Mechanics Energies of Pt^{II}(DAC) Bound to Conformation IC in Duplex DNA[a]

data	*RR*	*SS*	*SR*	*RS*
TE	-2006	-2006	-2006	-2006
U	-489	-497	-495	-487
Q	-1945	-1943	-1941	-1941
T	172	173	175	173
R	24	24	23	23
A	232	237	231	226

[a] The total energy (TE) is the sum of steric (*U*), Coulombic (*Q*), torsional (*T*), stretching (*R*), and bending (*A*) contributions.

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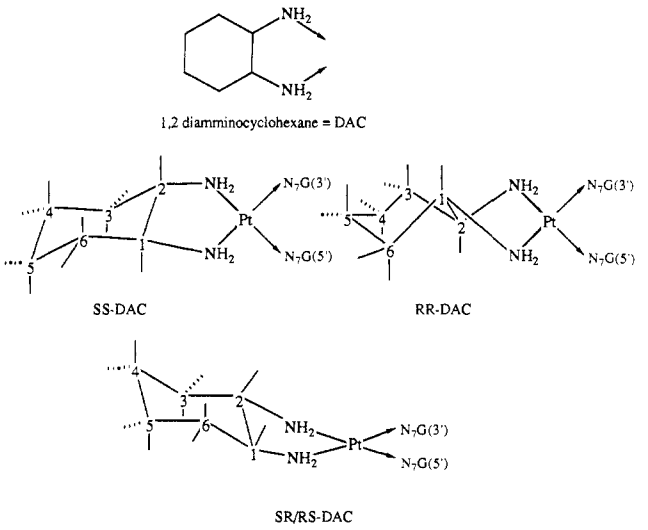

Figure 1. Structures of the isomeric DAC diammines.

receptor site in modeling the substituted $Pt^{II}(NH_2R)_2$ compounds, where R = CH_3, cyclopropyl, cyclobutyl, and cyclopentyl, suggested the use of this site[9,10] with each of the *cis*-Pt^{II}(DAC) isomers attached to the DNA and extending into the major groove.

Conformational and Intrinisic Binding Energies of the Pt^{II}(DAC)(DNA) Adducts. First, the energy of these complexes was optimized with MM procedures developed by our group. The results are reported in Table I. The total energies of all complexes are substantially the same. A balance among the steric, electrostatic, stretching, and bending energies is achieved. All orientations of each Pt^{II}(DAC) isomer at the N7(G) atoms were examined, and the most energetically favored ones are reported. The conformational energies of the Pt^{II}(DAC)(DNA) complexes suggest that DNA accommodates all isomers equally well. In the present MM procedure, van der Waals–repulsive (U), Coulombic (Q), stretching (R), bending (A), and torsional (T) energy terms are used with previously developed sets of force constants, equilibrium separations, barriers to rotations, and van der Waals radii discussed in detail elsewhere.[9-12] The geometrical parameters for the optimized DAC isomers in the IC model are essentially the same as those presented in detail for the *cis*-$Pt^{II}(NH_3)_2$ in Tables IV and V of ref 9 as well as in Tables I and II for the substituted *cis*-$Pt^{II}(NH_2R)_2$ in ref 10. At the site of platination, the platinum–nitrogen bond lengths are within 0.07 Å of the equilibrium value of 2.00 Å, and the angles around the platinum deviate by 5° from right angles and 26° from linearity, indicating a slight puckering of the platinated site. The helical angles and intra- and interbase C1′ to C1′ distances as well as the intra-base-pair orientations and local kink angles are within 10° and 0.42 Å of those reported.[9,10] The hydrogen bonding of the OII(5′) and O4(T) to the ammines is maintained. The N–H··· O6(G) distance is 1.88 Å, and this bond is bent at an angle of 113°.

The conformational characteristics of these isomeric structures are the same as those for the *cis*-$Pt^{II}(NH_3)_2$. Only a slight modification of the DNA is required to fit the *cis*-Pt^{II}(DAC) isomers into the IC receptor site. Stereographic projections of the *SS*, *RR*, *SR*, and *SR* substituted diammines in duplex DNA are shown in Figures 2–5.

The intrinsic binding of the ligand or the energy that is required to remove the water ligands and bind the guanine ligands to the Pt^{II}(DAC) isomers was also determined. These energies were evaluated quantum mechanically.[13] In a previous study of $Pt^{II}(NH_2R_2)_2$ binding to NH_3, imidazole, and guanine, the trends in binding of each of these substituents yielded the same trends.[9,10] To save computation time in the present investigation, we used NH_3 to represent the binding to nitrogen as an analogue of ligation to the nitrogen atom in guanine.

The binding of the isomers is seen as a stepwise process in which the H_2O of $Pt^{II}(DAC)(H_2O)_2$ undergoes replacement as binding to DNA occurs. In the absence of DNA, the results for the Pt^{II}(DAC) are identical for pairs of isomers: the *SS* and *RR* isomers because they are mirror images, and the *RS* and *SR*, which are related by a C_2 symmetry operation. Therefore, the ligand binding energies of the *SS* and *SR* isomers of the free DAC are measured as being representative of the *SS*/*RR* and *SR*/*RS* couples.

Binding energies of the *SS* isomer exceed the *SR* by the amounts shown:

$Pt^{II}(SS)(H_2O)_2$	E_0	$Pt^{II}(SS)(H_2O)G$	E_1	$Pt^{II}(SS)G_2$	E_2
$Pt^{II}(SR)(H_2O)_2$	$E_0 + 6.5$	$Pt^{II}(SR)(H_2O)G$	$E_1 + 7.0$	$Pt^{II}(SR)G_2$	$E_2 + 8.2$

where E_0, E_1, and E_2, are the quantum mechanical energies of the *SS* (*RR*) isomer. The differential energy of the constrained DAC ring increases with the number of guanine molecules. For the *SS* and *RR* (and in parentheses *SR* and *RS*), the energy required to replace 1 water molecule with G is calculated for the reaction

$$Pt^{II}(DAC)(H_2O)_2 + G \rightarrow Pt(DAC)(H_2O)G + H_2O \quad \Delta E_{01} = -7.4\ (-6.9)\ \text{kcal/mol} \quad (1)$$

followed by a reaction to replace a second water molecule with G

$$Pt^{II}(DAC)(H_2O)G + G \rightarrow Pt(DAC)G_2 + H_2O \quad \Delta E_{12} = -9.8\ (-8.6)\ \text{kcal/mol} \quad (2)$$

to yield the combined overall reaction

$$Pt^{II}(DAC)(H_2O)_2 + 2G \rightarrow Pt(DAC)G_2 + 2H_2O \quad \Delta E_{02} = -17.2\ (-15.5)\ \text{kcal/mol} \quad (3)$$

We conclude that the intrinsic reaction energy for replacing 2 waters by 2 guanines is 1.7 kcal/mol more exothermic for the *SS* isomer than the *SR* isomer.

Design of Platinum Diammine Chemotherapeutic Agents. Substituents that enhance the solubility and enhance the van der Waals contacts without disrupting the receptor site in the DNA were considered. Visual (see Figures 2–5) and energetic evaluation of the DAC substituted ammines reveals that space is available toward the 3′ end of complex to permit substituent replacement of the hydrogens on the cyclohexane ring. Substituents can be selected to enhance solubility and activity without substantially interfering with the receptor site in anticipation that these compounds may exhibit activity. Substitution of representative groups on the ring was studied without additional optimization. The change in the steric energy, ΔU, reported in Table II is used as an indicator of mo-

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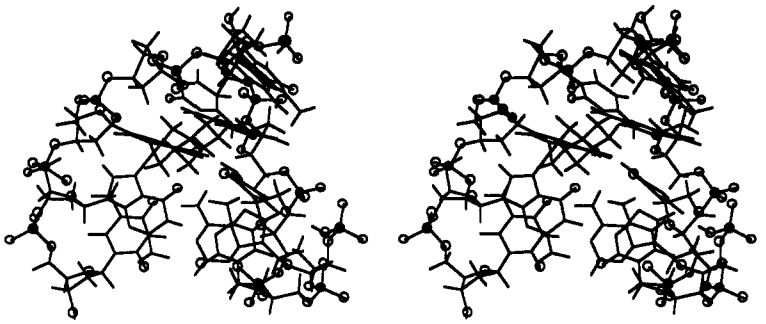


Figure 2. *cis*-Pt^{II}(*SS*)(DAC) adduct to a pentamer duplex of DNA.

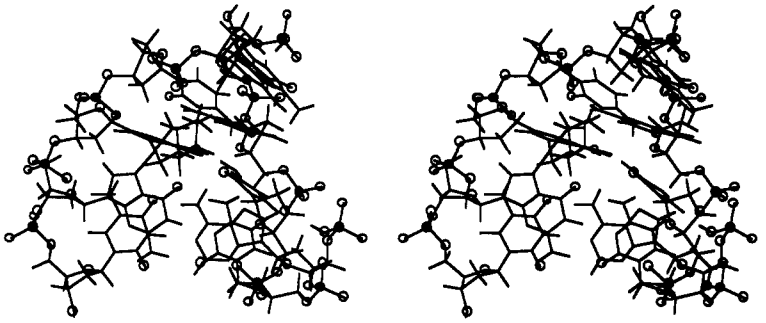


Figure 3. *cis*-Pt^{II}(*RR*)(DAC) adduct to a pentamer duplex of DNA.

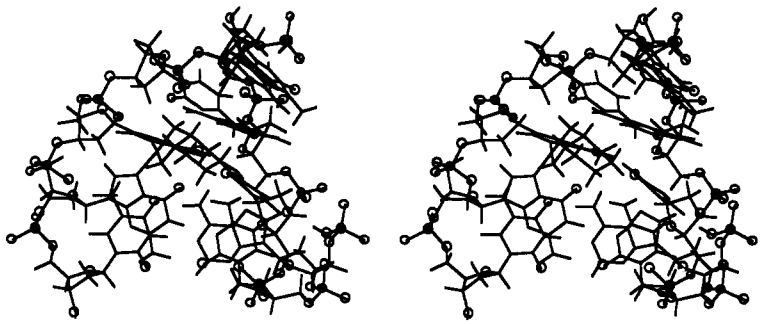


Figure 4. *cis*-Pt^{II}(*SR*)(DAC) adduct to a pentamer duplex of DNA.

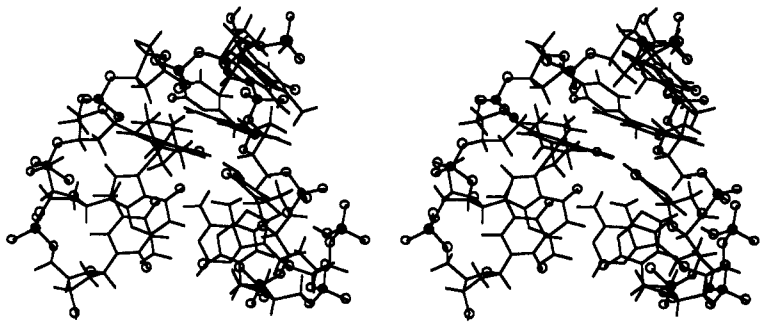


Figure 5. *cis*-Pt^{II}(*RS*)(DAC) adduct to a pentamer duplex of DNA.

lecular fit of these substituted DAC compounds. Specifically, methyl, ethyl, and isopropyl groups were considered as prototypes for one, two, and three heavy atom analogues and classified as follows: the small substituents, $-CH_3$, $-NH_2$, $-OH$; the intermediate substituents, $-CH_2CH_3$, $-CH_2NH_2$, $-CH_2OH$, and $-NHCH_3$; and the bulky substituents, $-CONH_2$, $-CHR_1R_2$, and $-NHR_1R_2$, where R_1 and R_2 are $-CH_3$, $-NH_2$, and $-OH$. These groups occupy a volume comparable to their carbon analogues. The polar $-NH_2$ and $-OH$ groups also enhance solubility. The structures, numbering, and conformations of DAC are illustrated in Figure 1. The 1-, 2-, and 6-positions are inaccessible for substitution. Axial (a) and equatorial (e) substitution can occur at the 3-, 4-, and 5-positions, depending upon substituent size. Small changes in ΔU suggest that the substituted DAC analogues will fit into the DNA and they may be candidates as antitumor agents.

Small-sized substituents, such as $-CH_3$, $-OH$, and $-NH_2$, can be monosubstituted at axial or equatorial positions 3, 4, and 5 for the *RR* and *SS* DAC isomers without substantially changing either the energetics or conformation of the site, except for substitutions at the 3(a)-position of the *SS* and the 5(a)-position of the *RR*, which show conformational interference. For the *SR*/*RS* compounds, substitution occurs best at the 3(e)-, 3(a)-, 4(e)-, and 5-(a)-positions of the ring and the 5(e)-position for the *RS* isomer. Intermediate-sized substituents fit the same pattern as the small substituents, where the *SS* and *RR*

Table II. ΔU upon Substituent Replacement at Various Sites on the DAC Ring[a]

	3e	3a	4e	4a	5e	5a
Monosubstituted Small Substituents, $-CH_3$ Type[b]						
RR	-3	3	-3	-3	-4	496
SS	-3	$>10^4$	-3	-3	-3	-2
SR	-3	-3	-2	1776	202	-3
RS	+3	-2	-2	286	-3	-3
Monosubstituted Intermediate Substituents, $-CH_2CH_3$ Type[c]						
RR	-5	-3	-3	-5	26	2677
SS	-2	$>10^4$	0	-3	1	-6
SR	-4	-4	-4	4037	535	-4
RS	-4	-5	-3	1055	-4	19
Monosubstituted Bulky Substituents, $-CH(CH_3)_2$ Type[d]						
RR	-4	288	-5	1	7	$>10^5$
SS	0	$>10^5$	-4	437	-3	-3
SR	1	$>10^4$	0	$>10^5$	361	16
RS	4	$>10^5$	+4	$>10^5$	-7	$>10^4$
Bisubstitution, $-CH_3$ (3e), $-CH_3$ (Other)[e]						
RR	*e*		-3	-1	-4	496
SS	*e*		-3	-3	-4	-4
SR	*e*		-2	1778	198	3
RS	*e*		3	292	2	3

[a] ΔU is the change in van der Waals–repulsive interactions upon substitution of H. [b] Small-sized substituents refer to analogues of $-CH_3$ such as $-NH_2$ and -OH. [c] Intermediate-sized substituents refer to analogues of $-CH_2CH_3$ such as $-CH_2NH_2$, $-CH_2OH$, and $-NHCH_3$. [d] Bulky substituents refer to analogues of the isopropyl group such as $-CONH_2$, $-CHR_1R_2$, and $-NHR_1R_2$, where R_1 and R_2 are $-CH_3$, $-NH_2$, and -OH. [e] Bisubstitution occurs at site 3e and one of the others indicated.

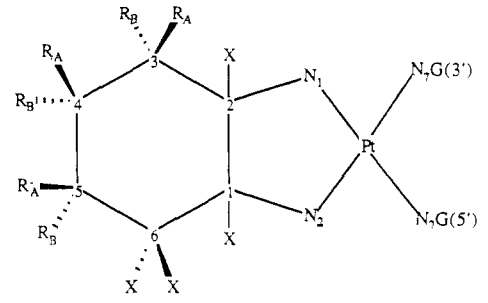
isomers give favorable results for all positions except the (3a) for the *SS* and the 5(a) for the *RR*. The *SR* and *RS* show favorable possibilities at the 3(e)-, 3(a)-, 4(e)-, and 5(a)-positions with conformational exceptions arising at 4(a) for both isomers and 5(e) for the *SR* isomer.

It is possible to substitute at two positions on the ring. Bisubstitution on the 3(e)-position of the ring to the 4- and 5-positions yields candidates that fit well into the DNA. For the *RR* and *SS* isomers bisubstitution occurs to any positions without substantially interfering with the energetics of the site, except the 5(a)-position of the *RR* analogue. For the *SR*/*RS* isomers this substitution best occurs at the equatorial position of atom 3 and the equatorial position of atom 4 for both isomers while the *RS* shows favorable substitution at the 5(e)-position. The 4(a)-position is energetically inaccessible for substitution for *SR*/*RS* isomers together with the 5(e)-position of the *SR* isomer.

It is also possible to monosubstitute larger substituents of the type $-CH(CH_3)_2$, onto the equatorial positions of the rings on the 3-, 4-, and 5-positions of the ring (shown in Figure 1 by dotted lines) without major conformational or energetic interference, with the possible exception of the 5(e)-position of the *SR* ring. Axial substitution in all isomers shows strong interactions which would reduce the binding except the 5(a)-position of the *SS* and *SR* isomers.

In general, ΔU is an indicator of the conformational result of substitution. $\Delta U \approx 0$ suggests that the position is available for substitution by the indicated model group. The possibilities for synthesis of derivatives are summarized in Table III, suggesting a starting point on which the chemist can make newer analogues of hopefully increased solubility and activity.

Table III. Recommended Sites of Substitution on the DAC Ring[a]



	SS	*RR*	*SR*	*RS*
R_{A3}	S, I, L	S, I	S, I, L	S, I, L
R_{B3}		S, I, L	S, I	S, I
R_{A4}	S, I	S, I, L		
R_{B4}	S, I, L	S, I, L	S, I, L	S, I, L
R_{A5}	S, I, L			S, I, L
R_{B5}	S, I, L	S, I, L	S, I, L	S, I

[a] R_A and R_B indicate groups placed above (A) and below (B) the ring as drawn. (N_1, N_2) are (N_A, N_B) for *SS*, (N_B, N_A) for *RR*, (N_A, N_A) for *SR*, and (N_B, N_B) for *RS*. S = small substituents: $-CH_3$, $-NH_2$, and -OH. I = intermediate substituents: $-CH_2CH_3$, $-CH_2NH_2$, $-CH_2OH$, and $-NHCH_3$. L = large substituents: $-CONH_2$, $-CHR_1R_2$, and $-NHR_1R_2$, where R_1 and R_2 are $-CH_3$, $-NH_2$, and -OH.

Discussion and Conclusion

The study of the DAC isomers by molecular mechanics demonstrates that these compounds fit into the active site equally well. Quantum mechanical calculations of the intrinsic ligand binding energies of these compounds demonstrate that the *SS* and *RR* isomers bind better to DNA by 1.7 kcal/mol than the *SR* and *RS* isomers. The slight preference for the *SS* and *RR* compounds is consistent with the differences shown in the observed experimental activity, which indicates a higher activity for the *SS*/*RR* isomers relative to the *SR*/*RS* isomers.[7,8] However, the experimental data demonstrate that these compounds, the *SS*/*RR* isomers themselves, exhibit varying levels of activity within different cell tumor types.[7,8] The observed dependence of activity on the DNA suggests that mechanisms beyond the adduct formation to DNA should be considered in the analysis of activity.

On the basis of the IC model receptor site, additional substituted DAC compounds with enhanced solubility and molecular fit are suggested for synthesis. They include groups with one, two or three heavy atoms, as well as bisubstitution of these groups. The positions that permit conformational accessibility to the DNA active site are those with negligible changes in steric repulsions, or $\Delta U \approx 0$, reported in Table II, and compounds suggested for synthesis are listed in Table III. This will hopefully provide a foundation for design on which the synthetic chemist can construct new analogues.

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