Conformational/Configurational Analysis of All the Binding Geometries of Cobalt(III) Bleomycin

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No crystal structure of metallobleomycin (BLM) exists, and the exact coordination mode of the ligand is unknown. To date, spectroscopic investigations of BLM complexes and crystal structures of BLM models have been used to propose its metal coordination sites. This has led to contradictory interpretations of the metal coordination sphere in BLM. Inorganic molecular mechanics and configurational/conformational searches were used to analyze HOO-CoBLM A2, H_2O-CoBLM A2, and HOO-CoPEP with commonly proposed binding geometries. The lowest energy binding geometry found was one with the mannose carbamoyl bound to the cobalt ion. The Monte Carlo dihedral and translational variational searches were able to find most of the configurations available to cobalt(III) bleomycin in the three binding geometries examined.

Introduction

The bleomycins are a family of glycopeptide-derived antibiotics, which are used in the treatment of Hodgkin's lymphoma, carcinomas of the skin, head and neck, and tumors of the testis.[1] The chemistry and antineoplastic properties of bleomycins have been described in a number of recent reviews.[2] The structures of the two major components of the clinically used mixture, known as Blenoxane, are shown in Figure 1. The mechanism of action of bleomycin (BLM) involves DNA strand scission, which requires oxygen and a metal ion. DNA cleavage is initiated through the abstraction of the deoxyribose C4′ hydrogen, mainly in 5′-GC and 5′-GT sequences.

Cobalt-BLM has been used as a model for FeBLM. It reacts with dioxygen forming a number of products. These are a superoxo−Co(III) complex,[3] a dinuclear μ-peroxo complex,[4] a hydroperoxide complex (green),[5] and an aquo species (brown).[6] CoBLM A2 green is an excellent analogue for the activated HOO-FeBLM species, which is thought to be the species that cleaves DNA. Although the Co-BLM's bind DNA, they do not activate oxygen and thus do not cleave DNA under normal conditions.[7] However, illumination of Co^{III}BLM with UV or visible light results in DNA strand scission.[8] This cleavage is insensitive to molecular oxygen.

BLM Binding Geometry. No crystal structure of BLM exists, and the exact coordination mode of the ligand is unknown. It is very important to know the details of the metal environment because coordinating ligands modify the characteristics of the metal (e.g., redox potential), and the way the bleomycin wraps around the metal also determines BLM's shape and therefore the site and selectivity of DNA binding.[2,9] To date, spectroscopic investigations of BLM complexes and crystal structures of BLM analogues have been used to propose metal coordination sites.[10] This has led to contradictory interpretations of the metal coordination sphere in BLM.

Model Compounds. The first BLM model system for which a crystal structure was determined was CuBLM P3A, a BLM analogue that lacks the two carbohydrates.[11] The copper was coordinated to the primary amine of the β-aminoalanine, the secondary amine of the β-aminoalanine, the pyrimidine, the histidine amide, and the δ-imidazole nitrogen. On the basis of this structure it was proposed that bleomycin binds through sites 59, 5, 12, 14, and 66 in Figure 1. This will be referred to as binding geometry **1**. All crystal structures of the model systems studied to date have lacked both the bithiazole and sugar residues, and have bound copper,[12,13] cobalt,[14,15] and zinc[16] with binding geometry **1**.

FeBLM Compounds. Two different binding geometries have been proposed on the basis of NMR analyses of CO-Fe(II) bleomycin, a diamagnetic low-spin complex. Hilbers[17,18] has proposed that BLM binds the metal through positions 33, 5, 12, 14, and 66; in other words the metal ion binds through the carbohydrate moiety and not the primary amine of the β-aminoalanine. This will be referred to as binding geometry **2**.

Oppenheimer and Hecht proposed that the carbamoyl group in the mannose replaces the amide nitrogen of histidine and

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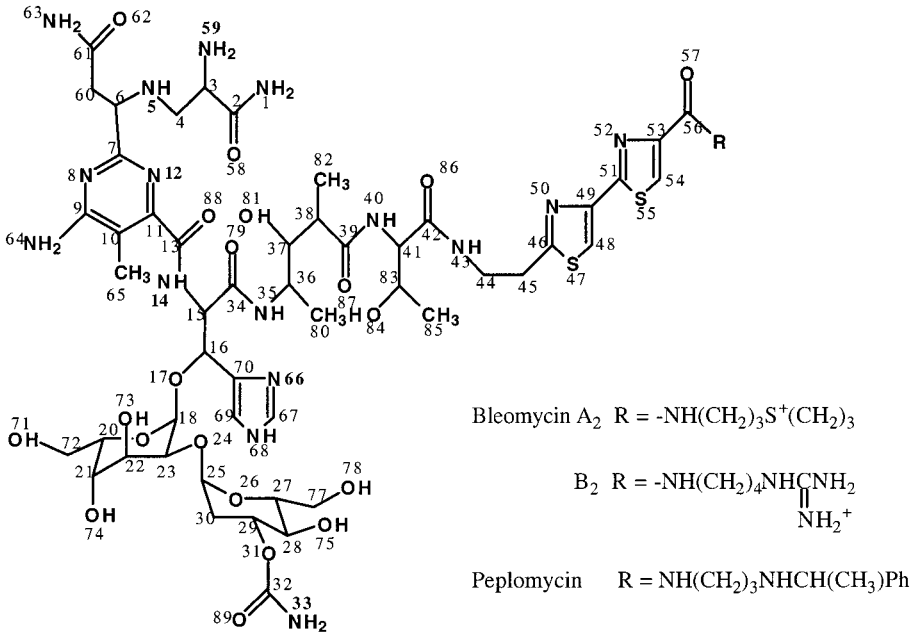

Figure 1. The structure of bleomycin A_2, B_2, and peplomycin. Binding geometry **1** has the cobalt binding through atoms 5, 12, 14, 59, and 66; binding geometry **2** has the cobalt binding through atoms 5, 12, 14, 33, and 66; binding geometry **3** refers to structures in which the cobalt binds atoms 5, 12, 33, 59, and 66.

not the primary amine of the aminoalanine;[19,20] the iron therefore binds at sites 5, 12, 33, 59, and 66 (see Figure 1), i.e., geometry **3**.

EPR studies of nitrosyl iron bleomycin complexes have been interpreted using binding geometry **1**,[21] and recently binding geometry **1** was also used to assign all the peaks in a one- and two-dimensional NMR analysis of paramagnetic Fe^{II}BLM.[22] An optical absorption, circular dichroism, and magnetic circular dichroism spectroscopic investigation of FeBLM and FeBLM derivatives revealed that changing either the mannose carbamoyl or the primary amine of the aminoalanine affected the coordination environment of the iron.[23] A dynamic 6-coordinate structure in which the mannose carbamoyl is displaced by a solvent molecule was proposed to account for these observations.[23] No differences in the coordination geometry of iron(II) bleomycin and iron(II) peplomycin compounds were reported in this study. Peplomycin is a BLM derivative, see Figure 1. It has stronger activity and less pulmonary toxicity than BLM.[24]

CoBLM Compounds. NMR and molecular modeling analyses of the green and brown forms of CoBLM A2, with and without oligonucleotides, have been interpreted using cobalt with binding geometry **1**.[25−28] NMR studies of cobalt(III)−peplomycin (CoPEP) and cobalt(III)−deglycopeplomycin (CodPEP) indicated that PEP coordinates the cobalt(III) ion through the carbamoyl nitrogen (binding geometry **2**) and that the primary aminoalanine nitrogen only binds for dPEP, which has no mannose.[29]

ZnBLM Compounds. Zn^{II}BLM does not cleave DNA, but has been used as a structural model for active forms of metallobleomycins. Although an early 1D NMR study of ZnBLM proposed a tetrahedral zinc complex,[30] and the crystal structure of a ZnBLM model compound has a trigonal-bipyramidal coordination geometry,[16] most proposed geometries have a four-coordinate square-planar zinc. An octahedral model binding through sites 5, 12, 33, 59, 66, and 87 was reported for ZnBLM-A2 on the basis of an early ^{1}H NMR study.[31] Zinc tallysomycin, a bleomycin relative with an additional sugar moiety, has been studied by 2D NMR and restrained molecular mechanics/dynamics.[32] All its possible configurations and conformations were examined, and it was proposed that tallysomycin and, by analogy, bleomycin bind zinc in a five-coordinate square-pyramidal fashion with binding geometry **1**. The mannose covers the sixth site without binding to it.

In this paper, inorganic molecular mechanics[33] and a new inorganic configurational/conformational search method were used to analyze[34] HOO-CoBLM A2, H_2O-CoBLM A2, and HOO-CoPEP with binding geometry **1**, **2**, and **3**.

Configurational/Conformational Searching. Conformational searches of transition metal compounds are complicated by the facts that (1) inorganic complexes are often composed of multidentate ligands, which result in many ring systems joined

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at the metal ion; (2) metal ions can be found in a variety of coordination geometries (e.g., octahedral, trigonal bipyramidal, square pyramidal, square planar, tetrahedral, etc.); (3) and transition metal compounds often adopt geometrical isomers that are not available to organic compounds.[35]

To date, most inorganic MM calculations published either have ignored all the isomers, except the one of interest, or have entered all the possible isomers graphically and minimized them individually.[33]

Molecular mechanics has commonly been used to analyze cobalt(III) coordination[36,37] and bioinorganic[38] compounds. DFT and other quantum mechanical methods might be more accurate,[39] but conformational analyses of molecules with the conformational freedom that CoBLM exhibits are prohibitively time-consuming even for molecular mechanics methods and impossible to carry out at a higher level.

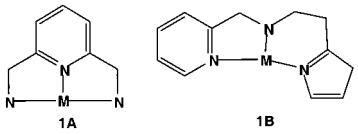
We have previously used solid state structure intrapolation techniques to establish a modified MM2* parameter set for modeling CoBLM.[40] These parameters were used to examine binding geometries **1** and **2** of CoBLM;[41] however, they did not examine the configurational isomers available to each binding geometry, the effect of the axial ligand, nor the difference between BLM and PEP.

Experimental Section

The Cambridge Structural Database (CSD) V 5.18 was searched for all cobalt(III) BLM fragments described in Results and Discussion. Version 5.18 of the CSD was released in October of 1999 and is composed of 207 500 crystal structures.

Conformational searches were conducted using the Monte Carlo (MC) dihedral and molecular position variation method[42,43] with the MM2* force field in MacroModel[44] v7.0. The cobalt(III) parameters were identical to those used previously,[40] except that the ligand−metal−ligand bond angles were replaced with 1,3-nonbonded interactions between the coordinating atoms during MC searches.[33,45,46] This approach has the advantage of being able to model all coordination geometries and configurations with one set of parameters. All the flexible external torsion angles (i.e., dihedrals not involving the metal ion) were varied between 0° and 180°. The cobalt ion, BLM, PEP, OOH, and H_2O were translated by between 0 and 3 Å. Ring closure atoms were defined with default constraints, and all bonds to the metal were broken to generate the BLM, metal, and monodentate ligand fragments that were translated. All multidentate ligand (BLM and PEP) and monodentate ligand (OOH, H_2O) fragments were randomly rotated by between 0° and 180° in each Monte Carlo step. Fifty thousand MC steps were taken during each search. All chiral centers, excepting those generated by metal coordination, were constrained in order to retain the same stereochemistry throughout the search. During the search procedure minimization continued until convergence was reached or until 500 iterations had been performed. The Polak−Ribiere conjugate gradient minimization mode was used "in vacuo" with a derivative convergence criterion of 0.05 kJ/mol. Structures within 100 kJ/mol of the lowest energy minimum were kept for all the HOO-CoBLM A2 structures, and all the conformations within 50 kJ/mol of the lowest energy H_2O-CoBLM and HOO-CoPEP structures were kept. A usage-directed method selected structures for subsequent MC steps. Structures found in the conformational search were considered unique if the least squared superimposition of equivalent non-hydrogen atoms found one or more pairs separated by 0.25 Å or more.

Chart 1



All the structures obtained in the conformational searches were separated into different groups based on their configurations. The xCluster program[47] was employed for this cluster analysis. Proximity matrices were obtained by determining the pairwise distances between structures, using the rms displacement between pairs of atoms in the first coordination sphere after optimal rigid-body superposition.[48] The Multc function in MacroModel 7.0 was used to minimize all the structures with the same configuration (in the same cluster) using a ligand−metal−ligand function in place of the 1−3 nonbonded interaction used in the conformational search.

Results and Discussion

In binding geometries **1** and **2** the cobalt ion is coordinated by the secondary amine of the β-aminoalanine, the pyrimidine nitrogen, and the histidine amide nitrogen. A search of the CSD for the substructure, shown in Chart 1A, which is an approximation of the three binding sites described above, found 137 transition metal complexes with the substructure. They were all planar and had an average N−M−N angle of 157°. This is significantly lower than 180°, but it is not unexpected due to the smaller bite size of planar five-membered rings. Fifty-nine structures were found with the substructure shown in Chart 1B, which is an approximation of the pyrimidine nitrogen, histidine amide nitrogen, and δ-histidine nitrogen binding sites of bleomycin. Most of the substructures were planar; however, five structures had N−M−N angles of less than 120°.

On the basis of the database analysis described above, one would expect the secondary amine of the β-aminoalanine, the pyrimidine nitrogen, and the histidine amide nitrogen to be in the same plane, and if coordinated the δ-histidine nitrogen would most likely also be in that plane, although it could be axially ligated. If the assumptions made above are correct, then metallobleomycins with binding geometries **1**, **2**, or **3** can adopt 34 different binding/configurational isomers (Figures 2 and S1, Supporting Information). Twelve of the 34 isomers are formed by the cobalt ion coordinating to a secondary amine thereby creating a new stereocenter.

In order to conduct a molecular mechanics configurational/conformational search of all the possible isomers of CoBLM a points on a sphere (POS) model had to be used to calculate the

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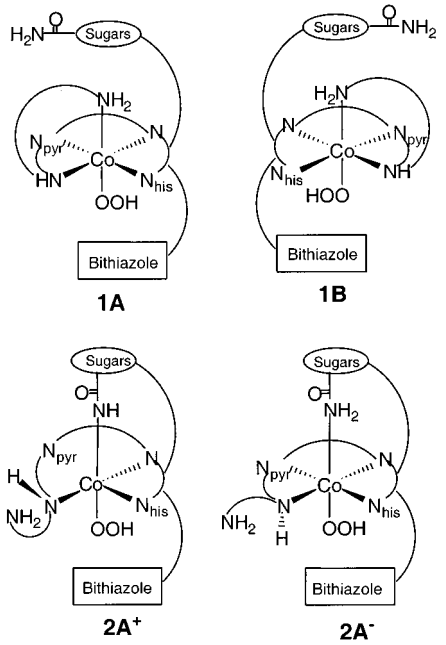

Figure 2. The H_2O-CoBLM A2, HOO-CoPEP, and HOO-CoBLM A2 structures with the lowest strain energy all have configuration $2A^+$. The lowest energy configuration with binding geometry **1** is 1B.

strain energy due to the bond angle deformations around the cobalt ion. The more commonly used valence bond angle model requires a separate set of parameters for each different geometry and set of geometric isomers; therefore, interconversion between geometric isomers is impossible with a valence force field description.[34] Unfortunately, the POS model did not give the same quality of results as the valence bond angles we had used in our initial studies.[40] Therefore, a POS model was used during the conformational search, all the different configurational isomers were separated by cluster analysis of the cobalt ion and the atoms directly bound to it, and then our previously used valence bond angle parameters were used to minimize all the structures contained in each cluster. In each of the systems studied the clustering level with the highest separation ratio efficiently separated all the different configurations from each other. In configurations in which ligation of the sp^3 secondary β-aminoalanine nitrogen produced a chiral nitrogen, cluster analysis of the chiral nitrogen and its substituents was used to separate the two stereoisomers.

A 50 000 MC step conformational search of HOO-CoBLM A2 with binding geometry **1** found 3744 different conformations within 100 kJ/mol of the lowest energy conformation. This search is discussed in detail as it is similar to all nine conformational searches conducted. Four hundred fifty-five conformations were within 50 kJ/mol of the lowest energy conformation. The highest separation ratio (=7.95) for a cluster analysis, obtained by determining the pairwise distances between structures, using the rms displacement between pairs of atoms in the first coordination sphere of the cobalt ion after optimal rigid-body superposition, was at clustering level 452. At this clustering level there are 4 different clusters. All the structures within each cluster had the same configuration, and each cluster had a different configuration. The best clustering level for all 3744 conformations was level 3736 (separation ratio = 1.85). Four of the clusters obtained are the same as those found for the 455 low-energy structures, while the other five clusters do not contain any members that are within 50 kJ/mol of the lowest energy configuration. Minimization of all the POS preminimized structures with the valence bond angle approach resulted in two sets of clusters fusing into each other. The configurations of the structures in each cluster and the energies of the lowest energy conformation within each configuration are listed in Table S1, in the Supporting Information. Identical searches were conducted for HOO-CoBLM A2 with binding geometries **2** and **3**, and H_2O-CoBLM A2 and HOO-CoPEP with binding geometries **1**, **2**, and **3**. (The results are listed in Table S1, Supporting Information.) The table lists more structures for HOO-CoBLM A2 than H_2O-CoBLM A2 and HOO-CoPEP. This is because all structures within 100 kJ/mol of the lowest energy conformation found for the HOO-CoBLM A2 MC search were kept for further minimization with the valence angle approach, while the searches for the H_2O-CoBLM A2 and HOO-CoPEP complexes kept only conformations within 50 kJ/mol. Therefore the H_2O-CoBLM A2 and HOO-CoPEP searches did not find any high-energy configurations.

The H_2O-CoBLM A2, HOO-CoPEP, and HOO-CoBLM A2 structures with the lowest strain energy all have binding geometry **2**. This is the binding geometry with the mannose carbamoyl bound to the cobalt. The lowest energy configuration ($2A^+$ in Figure 2) with this binding geometry is the same as that found in the NMR structure of CoPEP[29] and in our earlier MM studies.[41] Upon binding cobalt, the secondary amine of the β-aminoalanine becomes chiral. There is a significant energy difference between the two stereoisomers: the *R* isomer ($2A^+$ in Figure 2) is at least 40 kJ/mol less sterically strained than the *S* isomer ($2A^-$ in Figure 2). The chirality of the secondary amine of the β-aminoalanine has not been previously mentioned in the CoBLM literature. It was, however, considered in the analysis of the square pyramidal zinc tallysomycin.[32] The lowest energy conformations of H_2O-CoBLM A2, HOO-CoPEP, and HOO-CoBLM A2 with configuration $2A^+$ (Figures 2 and 3) have the following hydrogen bonds in common (the numbers in parentheses refer to atom numbers in Figure 1): the methylvalerate carbonyl oxygen (87)—the hydrogen of the water or hydrogen peroxide; the secondary amine hydrogen of β-aminoalanine (5)—the carbonyl oxygen of β-aminoalanine (58); the carbonyl oxygen of pyrimidinyl propionamide (62)—the mannose carbamoyl hydrogen (33); and the hydrogen of the amide group of methylvalerate (35)—the carbonyl oxygen of the amide group of pyrimidinyl propionamide (88).

The lowest energy configuration found for cobalt bleomycins with binding geometry **1** was configuration 1B, Figure 2. It is identical to that proposed by the Stubbe[27,28] and Petering[25,26] groups on the basis of their NMR studies (configuration 1A in Figure 2), but it has a different screw-sense. It has approximately the same strain energy as the *S* isomer ($2A^-$) of the conformation discussed in the preceding paragraph and is about 40 kJ/mol more strained than the lowest energy conformation of the most favored configuration (configuration $2A^+$ in Figures 2 and 3). According to our calculations the change in screw-sense results in a difference of approximately 20 kJ/mol in strain (configurations 1A and 1B in Figure 2). The lowest energy conformations of H_2O-CoBLM A2, HOO-CoPEP, and HOO-CoBLM A2, configuration 1B in Figures 2 and 3, have the following hydrogen bonds in common (the numbers in parentheses refer to atom numbers in Figure 1): the hydroxide hydrogen of methylvalerate (81)—the carbonyl oxygen of beta- aminoalanine (58); the secondary amine hydrogen of β-aminoalanine (5)—the carbonyl oxygen of pyrimidinyl propionamide (62); the hydroxide hydrogen of threonine (84)—the carbonyl oxygen of threonine (86); and the hydrogen of the amide group of methylvalerate (35)—the carbonyl oxygen of the amide group of pyrimidinyl propionamide (88).

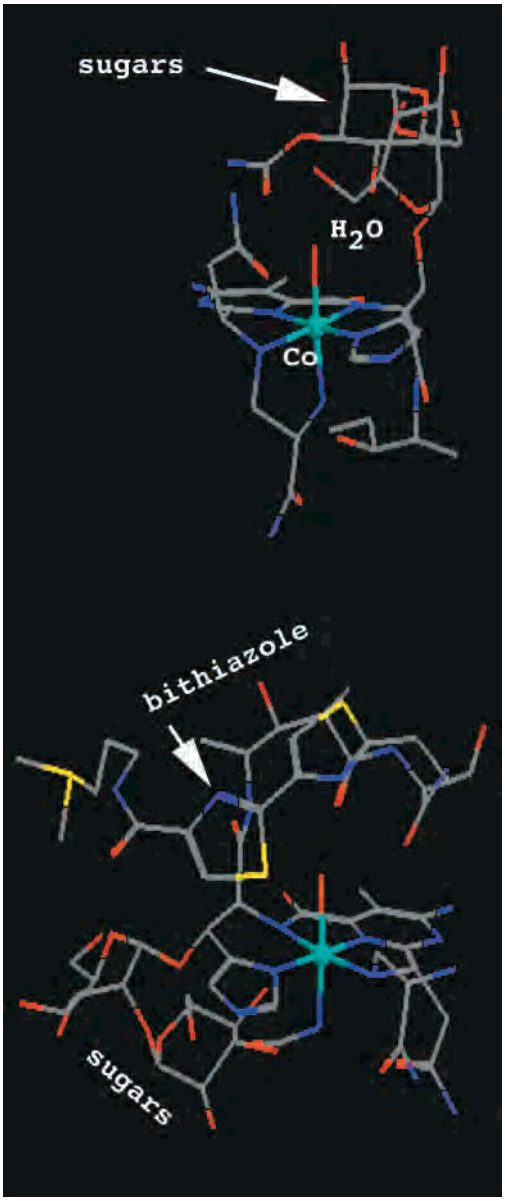

Figure 3. Top: The lowest energy conformation of H_2O-CoBLM A2 with configuration 1B (bithiazole tail has been omitted for clarity). Bottom: The lowest energy conformation of H_2O-CoBLM A2; it has configuration $2A^+$.

The calculations and Table S1 also shows that the lowest energy configuration of binding geometry **1** does not depend on the identity of the coordinating ligand. The HOO and H_2O complexes have the same screw-sense, as reported in the literature.[28]

The force field parameters involving the cobalt ion used in these calculations were derived by solid state structure intrapolation techniques.[33,40] They were calibrated using crystal structures in the Cambridge Structural Database and should be accurate at calculating the structure of cobalt bleomycin as it would be found in the crystal structure. However, no thermodynamic data was used in the derivation of the inorganic parameters, nor was any effort made to model solvation effects, therefore the relative energies calculated and listed in Table S1 are not very accurate.

The conformational search methods employed in this paper found the same low-energy conformations for all the structures examined, except for configurations $2F^-$ and 3J (Figure S1, Supporting Information). The 50 000 MC step conformational search of HOO-CoPEP with binding geometry **2** did not find any low-energy conformations for configuration $2F^-$, although a low-energy $2F^-$ conformation was found for HOO-CoBLM. Furthermore, the conformational search for HOO-CoPEP and H_2O-CoBLM in binding geometry **3** did not find the low-energy conformations for configurations $3J^+$ and $3J^-$ (Figure S1, Supporting Information).

Conclusion

The aim of the work presented here was to extend our earlier studies on conformational searching of transition metal compounds[34] to larger and more complex compounds like cobalt bleomycin, and to examine the binding geometries and configurations available to cobalt bleomycin. Figure S1 shows all the different configurations available to cobalt bleomycin in the three proposed binding geometries. Most of these configurations have not been examined in previous NMR/MD studies.

The lowest energy binding geometry/configuration found had binding geometry **2** with the same configuration as found in the NMR structure of CoPEP[29] and in our earlier MM studies.[41] Neither of these reports considered the *S* isomer, which is at least 40 kJ/mol more sterically strained than the *R* isomer. CoBLM might adopt a different binding geometry when it is solvated or when it is bound to DNA.

The energy difference between the lowest energy configurations of binding geometries **1** and **2** and binding geometry **3** is large enough that it can be assumed that binding geometry **3** is highly unlikely.

The results presented here clearly show that the Monte Carlo dihedral and translational variational search is a significant improvement on the current inorganic conformational search method in which all the possible configurational isomers of inorganic complexes are entered graphically and minimized individually. However, some caution has to be exercised as even 50 000 MC steps failed to find the same low-energy conformations for all the binding geometries and configurations studied. The conformational search methods described here and found in MacroModel 7.0 allow the inorganic molecular mechanics practitioner to use a one-step procedure to find all the low-energy conformations as well as all the structural isomers. These techniques are much quicker at finding low-energy conformations and structural isomers than the commonly used manual method. Furthermore, the Monte Carlo dihedral and translational variational method is capable of finding conformations that are far from ideal conformations which would not be found if all the possible ideal isomers were entered and minimized individually.

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Supporting Information Available: Figure S1, the 34 possible configurations available to CoBLM, and Table S1, the relative strain energies of all the lowest energy conformations found for each possible configuration with the three commonly reported binding geometries. This material is available free of charge via the Internet at http://pubs.acs.org.

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