Binding Geometry of Cobalt Bleomycin, An Empirical Force-field Analysis

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Empirical force-field calculations have shown that the co-ordination geometry **1** of bleomycin (BLM) binding to cobalt proposed by Umezawa and that, **2**, proposed by Hilbers is sterically possible. In the calculations it was found that changing the charge on the metal ion has a significant effect on the BLM conformation, however the differences in conformation between the two binding geometries are much larger than those due to charge differences. Based on BLM–DNA docking studies, it is suggested that both the bithiazole tail and the metal-binding region of BLM with binding geometry **2** could be interacting with the minor groove of DNA.

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 tumours of the testis.[1a,b] Recently they have been proven to be active against AIDS-associated mucocutaneous Kaposi's sarcoma.[1c,d]

The structures of the two major components of the clinically used mixture are shown in Fig. 1. The mechanism of action of bleomycin (BLM) is thought to involve DNA strand scission which requires oxygen and a metal ion.[4] *In vivo* it is proposed that Fe(BLM) is the species responsible for strand scission, though other metallobleomycins can inflict DNA damage *in vitro*.[5] The Fe(BLM) complex binds oxygen and is oxidized to form the 'active' hypervalent oxo-iron species.[4,6] A series of subsequent reactions leads to single- and double-strand DNA breaks[7] with release of free nucleic acid in an oxygen-independent reaction,[8] and to base propenal in an oxygen-dependent reaction.[9] There is some recent evidence that the oxygen-rebound mechanism operative in cytochrome P450 is not the mechanism by which the oxygen-independent BLM-mediated oxidation occurs.[8a,c] The 'activated' BLM has been shown to transfer oxygen to substrates such as cyclohexane and *cis*-stilbene.[10] The presence of a similar hypervalent oxo-iron species has also been implicated in haem[11] and non-haem iron[12] oxo-transfer reactions. Molecular mechanics calculations on Fe(BLM) model systems indicate that activation of Fe(BLM) by O_2 or H_2O_2 may involve a geometrical change to achieve internal hydrogen bonding.[13]

The cobalt(II) complex of BLM has been used as a model for Fe(BLM). It reacts with dioxygen forming a number of products, a brown hyperoxo-cobalt(III) complex,[14] a dinuclear μ-peroxo complex,[15] a green hydroperoxide complex[16] and a brown aqua species.[17] These reactions can be summarized as in Scheme 1.[5c] The complex Co^{II}(BLM) binds to DNA and reacts with oxygen as summarized[5c] in Scheme 2. Although Co(BLM) binds DNA in the same way as does Fe(BLM), it does not activate oxygen and thus does not cleave DNA under normal conditions.[18] Illumination of Co^{III}(BLM) with UV[19] or visible light[20] results in DNA strand scission. This cleavage is insensitive to molecular oxygen.[17]

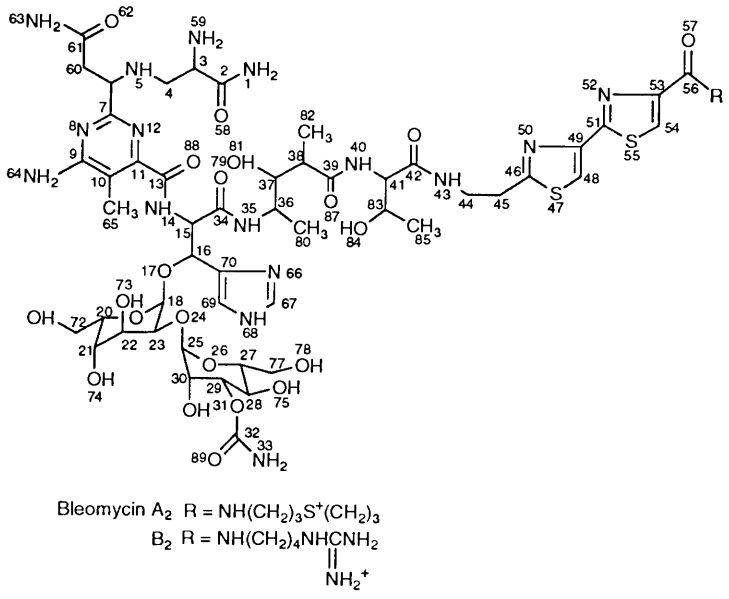
No crystal structure for BLM exists and its exact co-ordination mode is unknown. It is very important to know the details of the metal environment because co-ordinating ligands modify the characteristics of the metal (*e.g.* redox potential), and the way BLM wraps around the metal also determines the shape of BLM and thus the site and selectivity of DNA binding.[21] To date, spectroscopic investigations of BLM[3,9] and crystal structures of BLM analogues have been used to propose metal co-ordination sites.[22] This has led to contradictory interpretations of the metal co-ordination sphere in BLM. The crystal structure of Cu(BLM P3A), a BLM analogue which lacks the two carbohydrates, seems to suggest metal co-ordination through sites 5, 12, 14, 59 and 66 (see Fig. 1).[2,22a] This will be referred to as binding geometry **1**. The structure of cobalt(III) pseudotetrapeptide A has also been solved:[23] it also shows the metal ion binding to the primary amine, secondary amine, pyrimidine, amide and imidazole which occurs in **1**. However, like Cu(BLM P3A), this model system lacks both the bithiazole and sugar residues. On the basis of a recent NMR analysis, Hilbers and co-workers[3c,d] proposed that BLM binds the metal through positions 5, 12, 14, 33 and 66; thus the metal ion binds through the carbohydrate moiety and not the primary amine. This will be referred to as binding geometry **2**. An earlier NMR study[3a] proposed sites 5, 12, 33, 59 and 66 (see Fig. 1) as metal binding sites, *i.e.* geometry **3**.

Since models have shown that Co(BLM) is a very compact complex which should be very strained, we have used inorganic empirical force-field calculations to determine the strain energy in cobalt co-ordinated to bleomycin, with both the co-ordination geometry **1** proposed by Umezawa and co-workers[22a] and that, **2**, proposed by Hilbers and co-workers.[3c,d] We have focused on geometries **1** and **2**, in favour of **3**, because they incorporate binding through the amide (atom 14 in Fig. 1), which can be deprotonated and thus can stabilize the probable hypervalent oxo metal intermediate. These calculations have been computationally intensive and we intend to examine binding geometry **3** next.

Inorganic molecular mechanics (MM) calculations are being used with increasing frequency. A number of parameter sets have been established to analyse inorganic compounds such as metalloporphrins,[24] ferrocenes,[25] crown ethers,[26] platinum nucleotide complexes,[27] lacunar cobalt dioxygen carriers,[28] metalloproteins (zinc in human carbonic anhydrase),[29] organometallic germanium, tin and lead,[30] macrocyclic complexes[31] and nickel(II) in factor F430.[32] A general force field for modelling complexes of Cu^{II}, Ni^{II} ($S = 1$), Co^{III}, Fe^{III}, Cr^{III}, Zn^{II} and Rh^{III} with amine, carboxylate, pyridine, and thioether ligands has been reported by Bernhardt and Comba.[33]

There are two approaches to modelling metal–ligand interactions in MM calculations. The bonded approach is the more common. In this the metal–ligand bond is treated as covalent with an ideal bond length and angle and its appropriate force constant. In the non-bonded approach the metal–ligand interactions are treated with electrostatic and van der Waals forces.[34] A brief discussion of the pros and cons of both methods is given by Hancock and co-workers.[24a]

In our calculations we have used the MM2(87) force field as



Bleomycin A_2 R = $NH(CH_2)_3S^+(CH_2)_3$
B_2 R = $NH(CH_2)_4NHCNH_2$ (with NH_2^+)

Fig. 1 The structure of bleomycin and proposed potential binding sites. Binding geometry **1**[2] involves atoms 5, 12, 14, 59 and 66 while **2** involves 5, 12, 14, 33 and 66[3c,d]

$$Co^{II}(BLM) + O_2 \longleftrightarrow Co\text{–}O_2\text{–}BLM$$

$$2Co\text{–}O_2\text{–}BLM \longleftrightarrow (BLM)Co\text{–}O_2\text{–}Co(BLM)$$

$$(BLM)Co\text{–}O_2\text{–}Co(BLM) + H^+ \longrightarrow HO_2\text{–}Co^{III}(BLM) + Co^{III}(BLM)$$

Scheme 1

$$Co^{II}(BLM)\cdot DNA + O_2 \longleftrightarrow O_2\text{–}Co(BLM)\cdot DNA$$

$$2O_2\text{–}Co(BLM)\cdot DNA \longleftrightarrow DNA\cdot(BLM)Co\text{–}O_2\text{–}Co(BLM)\cdot DNA$$

Scheme 2

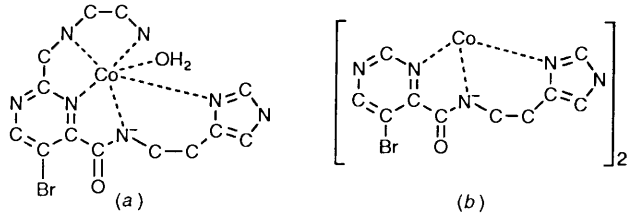


Fig. 2 Structure of (*a*) $[CoL^1(H_2O)][NO_3]_2$ and (*b*) $[Co(L^2)_2]ClO_4$; Co(BLM) analogues used to test the modified force field. Hydrogen atoms are omitted for clarity

modified and implemented in MacroModel v3.1X, with the metal–ligand bond treated as covalent with an ideal bond length and angle. For this analysis of Co(BLM) we determined additional parameters which were added to the MM2(87) forcefield, Table 1, and have been published elsewhere.[35] These parameters were developed specifically for the purpose of analysing the strain in cobalt bleomycin complexes and they are not a general cobalt parameter set.

We decided to analyse the binding energy of Co(BLM A_2) as opposed to Fe(BLM) because the best developed force fields for metal complexes are those for cobalt(III) complexes[37] and because a number of Co(BLM) model systems[38] have been crystallized which can be used to test the force field, Fig. 2. Using the parameters given in Table 1 the minimum-energy conformation of CoL^1 was calculated and superimposed upon the crystal structure of CoL^1;[35] the root-mean-square (r.m.s.) deviation of the superimposition of all non-hydrogen atoms was 0.101 Å. For $Co(L^2)_2$ it was 0.180 Å.

Most inorganic complexes are rigid molecules, where the number of conformations is severely limited by co-ordination to the metal ion, thus finding the global minimum is generally

Table 1 Force constants[a] added to the MM2* force field present in MacroModel v3.1X[35]

Stretching interaction	Bond length/Å	Force constant[b]/ mdyn Å^{-1}
Co–N^2	1.930	2.00
Co–N^3	1.934	2.00
C^2=O^2 (amide)	1.238	9.50
C^2(=O^2)–N^2 (amide)	1.340	6.60
Bending interactions	**Angle/°**	**Bending constant/ mdyn rad^{-2}**
N^0–Co–N^0	90[c]	0.498
C^0–N^2–Co	120	0.516
C^0–N^3–Co	109	0.285
Torsional interactions for C^0–N^0–Co–N^0		
V_1, V_2, V_3		0.000 kJ mol^{-1}
Van der Waals interactions for cobalt		
r = 2.40 Å		ε = 1.339 kJ mol^{-1}

[a] N^2 refers to an sp^2 nitrogen, N^3 to an sp^3 nitrogen, N^0 to any nitrogen; all other atoms are labelled in the same way. [b] dyn = 10^{-5} N. [c] N^0–Co–N^0 angles of 180° were modelled with the same force constants as 90° using the substructure option.[36]

straightforward. The multiple-minimum problem is much greater in organic and especially in biopolymeric molecules where conformational searches are required in most molecular mechanics analyses. Even though the conformational space of cobalt bleomycin is restricted by metal co-ordination and the presence of nine chiral centres, bleomycins are large flexible molecules which can adopt a very large number of conformations, thus we have had to conduct extensive conformational searches in this work.

Conformational searches can be divided into deterministic and stochastic methods. Deterministic or grid searches are usually conducted by systematically varying some or all of the torsional bonds and generating a new geometry after each iteration. These new starting geometries are then minimized and compared to all previously found conformers. The quality of the search depends on the step size of the torsional variation. Though all conformational space can be searched with these

methods they are very time intensive.[39] Stochastic or Monte Carlo searches generate starting geometries by randomly varying selected dihedral angles[40] or atomic positions.[41] These methods are not completely random and are generally complemented by a set of criteria which eliminate searches of chemically unreasonable structures. Molecular dynamics[42] is a method particularly good at finding local minima. While molecular dynamics simulations for a few hundred picoseconds at room temperature generally lead to at most a small number of local minima, at high temperature the kinetic energy is sufficient to pass over energy barriers and a number of new minima can be generated.[43] A comparison of a number of methods of conformational searching has been published.[39] We have used both molecular dynamics and Monte Carlo dihedral searches to find the energy minima for Co(BLM).

The use of conformational analysis in inorganic molecular mechanics calculations has been limited to examining the conformational changes associated with varying the N–Ni–N bond angle in nickel ethylenediamine,[44] the mechanism of conformational interconversion in five-membered diamine chelate rings,[45] studies of ferrocenophanes,[25] factor F430[32a] and copper(II) *N*,*N*-diethylalanine.[46]

Experimental

All calculations were performed using MacroModel v3.1X[36] with BatchMin v3.1 on Silicon Graphics Indigo workstations. The MM2* option of MacroModel was chosen which uses the authentic MM2 force-field equations[47] with three minor changes: (*a*) MM2* mimics electrostatic interactions with partial charges and Coulomb's law, while MM2 uses bond dipoles and the Jeans equation; (*b*) for out-of-plane bending MM2* uses an improper torsion while MM2 uses a pyramidalized distance; and (*c*) MM2* uses specific V_1, V_2 and V_3 torsional terms for conjugated systems whereas MM2 uses an self-consistent field (SCF) π calculation.

The parameters used in our calculations are those given in the mm2.fld file of MacroModel v3.1X, which is an extended version of the MM2(87) parameter set. In order to model the metal–ligand interactions additional parameters previously determined and discussed[35] were added, Table 1. The *trans* N–Co–N angles were differentiated from the *cis* using the substructure function.

Conjugate-gradient minimization using the Polak–Ribiere first-derivative method with restarts every 3*N* iterations was used to obtain an r.m.s. gradient < 1.0 kJ Å^{-1} mol^{-1}. Final minimization to a gradient < 0.1 kJ Å^{-1} mol^{-1} was carried out by a full-matrix Newton–Raphson minimization.

Conformational searching was done by both a torsional Monte Carlo multiple-minimum search[40] and by molecular dynamics. The Monte Carlo (MC) multiple-minimum searches were conducted by randomly changing all the torsional angles in cobalt bleomycin, except those involved in cyclic systems, those that would make no difference (*e.g.* rotating methyl groups) and planar moieties (*e.g.* amides). In binding geometry **2** the ring-closure atoms were specified in order to vary the 14-membered ring formed by co-ordination of atom 33 (Fig. 1) to the cobalt ion. Due to the high flexibility of the bithiazole tail and its relatively minor effect on the metal binding region, no torsional angles in the tail (*i.e.* from atom 40 in Fig. 1) were varied. The searches were set up so that between 2 and 20 different torsional angles were varied between 0 and 180° in each MC step. The least-used structure which was within 50 kJ mol^{-1} of the minimum-energy conformation was selected as the starting geometry for each MC step. The structures generated in this way were minimized by the Polak–Ribiere conjugate-gradient method with no line searching, for a maximum of 500 iterations or till a gradient < 0.1 kJ Å mol^{-1} was obtained. Molecular dynamics simulations were run at 500 K with 2 fs time steps and constrained hydrogen bonds. Structures were sampled every picosecond and then minimized with the multiconformer mode.[48]

Conformational analyses were considered complete when, after at least 500 MC steps and a subsequent 200 ps molecular dynamics run at 500 K, no new conformations were found which were within 20 kJ mol^{-1} of the lowest-energy conformation.

In order to print structures, files were transported to MacroModel v3.5, which was used to create postscript files or saved in Chem-3D format and down loaded onto a Mac.

Results

Low-energy Conformations.—Despite having done extensive conformational searches, the possibility of having missed the lowest-energy conformation is present. However since the bithiazole tail is very flexible, and our conformational searches focused on the sugars and metal binding areas, if any low-energy conformations have been missed the only conformational difference will be in the tail (*i.e.* from atom 40 in Fig. 1). The flexibility of the bithiazole tail has been shown by (*i*) watching molecular dynamics movies, (*ii*) by Fig. 3 and other such overlaps, which show that the major conformational differences between all the conformations found within 20 kJ mol^{-1} of the lowest-energy conformation involve the bithiazole tail, and (*iii*)

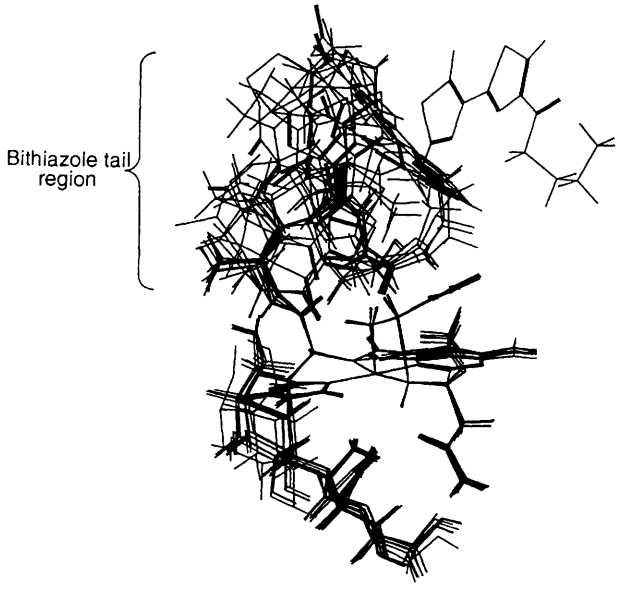


Fig. 3 Superimposition of some of the conformations found within 20 kJ mol^{-1} of the lowest-energy conformation of cobalt(III) bleomycin with co-ordination geometry **1**. All the hydrogens were removed for clarity

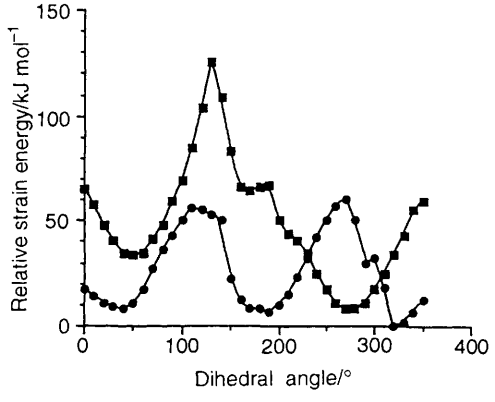


Fig. 4 Plot of the strain energy of Co^{III}(BLM) with binding geometry **1** *vs.* the dihedral angles of atoms 35–36–37–38 ■ and 43–44–45–46 (●) (labelled according to Fig. 1). The multiple minima reflect the flexibility of the BLM tail

by Ramachandran-type plots of dihedral angles in the bithiazole tail which have multiple minima, for example Fig. 4.

Charge on Cobalt.—The use of formal charges for metal ions has been shown to be unrealistic as the charge is partially spread out to the neighbouring ligands.[49] A number of approaches to overcome this problem and to model the transition metal–ligand electron distribution have been used. These range from setting the metal–ligand dipole as zero with no charge to arbitrarily chosen parameters.[24c,31d,e,37a,c,50] It has been shown that in several cases small variations or neglect of electrostatic interactions result in no differences or at most in minor differences in structure.[26c,51]

The MM2* option uses partial charges with Coulomb's law to mimic the metal–ligand non-bonded interactions, thus the potential is inversely proportional to the distance between the two atoms. In our calculations on copper macrocyles,[52] cobalt bleomycin analogues[35] and nickel-containing Factor F430,[32a] which used MM2*, varying the partial charge on the metal had a negligible effect.

Changing the formal charge on the cobalt atom had no effect on the geometry of the cobalt bleomycin analogues CoL^1 and $Co(L^2)_2$. The former minimized when a conformation with cobalt having a formal charge of +3 was superimposed with the conformation obtained using a formal charge of +1 and a root-mean-square deviation for superimposition of all non-hydrogen atoms[53] of 0.017 Å was obtained. In contrast the strain energy and conformation of Co(BLM) is strongly influenced by the charge on cobalt. In order to evaluate the effect of charge on Co(BLM) we performed the calculations for co-ordination geometries **1** and **2** with a formal charge of +1 and +3 on the cobalt atom. Presumably the actual charge on cobalt in Co(BLM) is somewhere between +1 and +3. Fig. 5(*a*) shows the overlap of the minimum-energy conformations obtained for binding geometry **1** with the hypothetical cobalt-(I) and -(III), and Fig. 5(*b*) the corresponding overlap for binding geometry **2**. No matter what the binding geometry is, the metal charge has a significant effect on the conformation of Co(BLM) and thus on its DNA-binding selectivity.

Although the conformation of Co(BLM) in both binding geometries changes substantially with charge, the conformational differences between the two different binding geometries are much greater than those due to charge. For both Co^{III} and Co^{I}, binding geometry **1** has a much more extended structure while **2** is more compact, Fig. 6. A comparison of the strain imposed upon the bleomycin by cobalt in binding geometries is inconclusive. The Co^{III}(BLM) complex with binding geometry **1** is less strained than with geometry **2**. This difference is mainly due to electrostatic interactions between the ligand and the metal, which become less significant in Co^{I}(BLM).

It is known that cobalt, nickel and iron bleomycins all cleave RNA at different places[54] and that this difference is probably due to differences in the shape of the BLM. It has also been shown that extending the length of the bithiazole tail by adding glycine linker units between the metal and the metal-binding region of bleomycin does not change the DNA-cleavage sites of Fe(BLM),[21] thus indicating that the metal-binding region and not the bithiazole tail is of crucial importance in DNA binding and sequence recognition. Furthermore experimental evidence suggests that iron and cobalt bleomycins bind in the minor groove of B-DNA.[55] Up to this point in time it has been suggested that it is only the bithiazole which binds in the minor groove. Considering the aforementioned importance of the metal co-ordination region in DNA binding,[21] we were interested if the structural differences associated with the two binding geometries could result in differing DNA–BLM interactions. Thus we docked the global-minimum structures of both Co(BLM) binding geometries with an idealized B-DNA strand. We found that the bithiazole tail can bind to the minor groove of DNA, irrespective of binding geometry. Fig. 7(*a*) shows that the metal-binding portion of BLM with geometry **1**

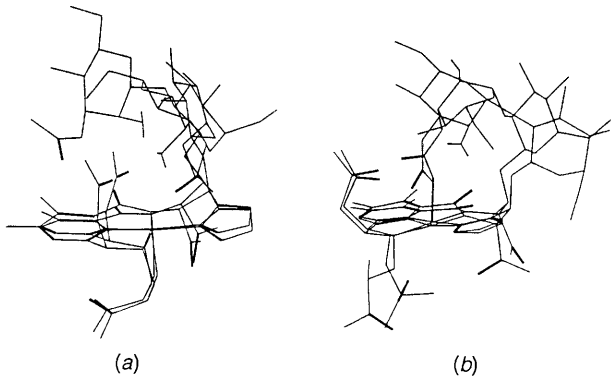


Fig. 5(*a*) Minimum-energy conformation obtained for Co(BLM) with binding geometry **1** and cobalt with a formal charge of +1, overlapped with the corresponding Co(BLM) with a formal charge of +3. (*b*) Overlap of Co^{I}-(BLM) with Co^{III}(BLM) both with binding geometry **2**. In order to simplify the comparison the bithiazole tail which had been present in the minimization was clipped off at atom 38 (Fig. 1) and the metal-binding regions were graphically aligned rather than using the superimposition algorithm to superimpose all non-hydrogen atoms

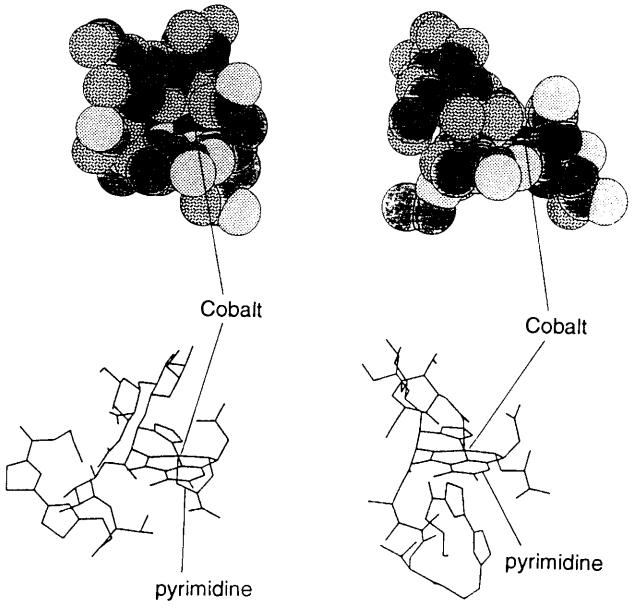


Fig. 6 Lowest-energy conformations of Co(BLM) with a formal charge of +1. The bottom shows a wire-frame skeleton of Co(BLM), the top is a Corey–Pauling–Kolton model from the same perspective with the bithiazole tail removed for clarity. Binding geometry **1** is on the left and **2** on the right

is incapable of binding to the minor groove of B-DNA, given a typical minor groove width of 6.94 Å (P to P distance less 5.8 Å to compensate for the van der Waals radii of the two phosphate groups). On the other hand Figs. 7(*b*) and 8 show that the metal-binding region of BLM with co-ordination geometry **2** is capable of fitting into the minor groove of DNA, thus enhancing the BLM–DNA interaction. The amine (atom 64 in Fig. 1) on the pyrimidine is deep inside the minor groove and could be partially responsible for the selectivity Co(BLM).

We are currently investigating other cobalt bleomycin binding geometries and by molecular dynamics extending our investigation of the Co–BLM–DNA interactions.

Conclusion

We have attempted to use empirical force-field calculations to establish whether geometry **1** or **2** can be eliminated on the basis of an unreasonably high strain energy. This was not possible. However in the course of our calculations we checked the effect of charge on the conformation of Co(BLM). As our two

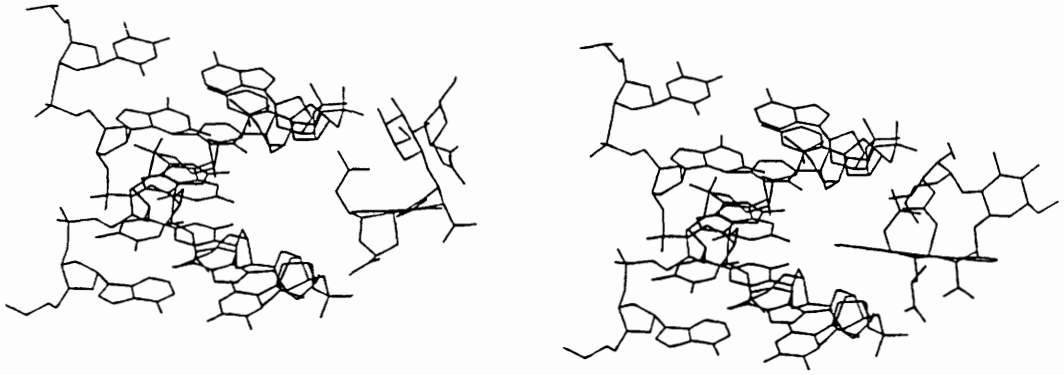


Fig. 7 Docking of Co^{I}(BLM) with binding geometry **1**, left, and with co-ordination mode **2**, right, into the minor groove of self-complementary d(A-T-G-C-C-A)$_2$. The bumpcheck mode was used to prevent any interatomic distances smaller than 70% of the sum of the van der Waals radii. The complex Co(BLM) with geometry **2** is better able to enter the minor groove than is **1**, and BLM N(64) (for numbering see Fig. 1) is within 3.40 Å of guanosine [N(2)] and 2.95 Å of cytidine [O(6)], while the Co is an unobstructed 5.42 Å from adenosine [C(4')]. Bithiazole and hydrogens are removed for clarity

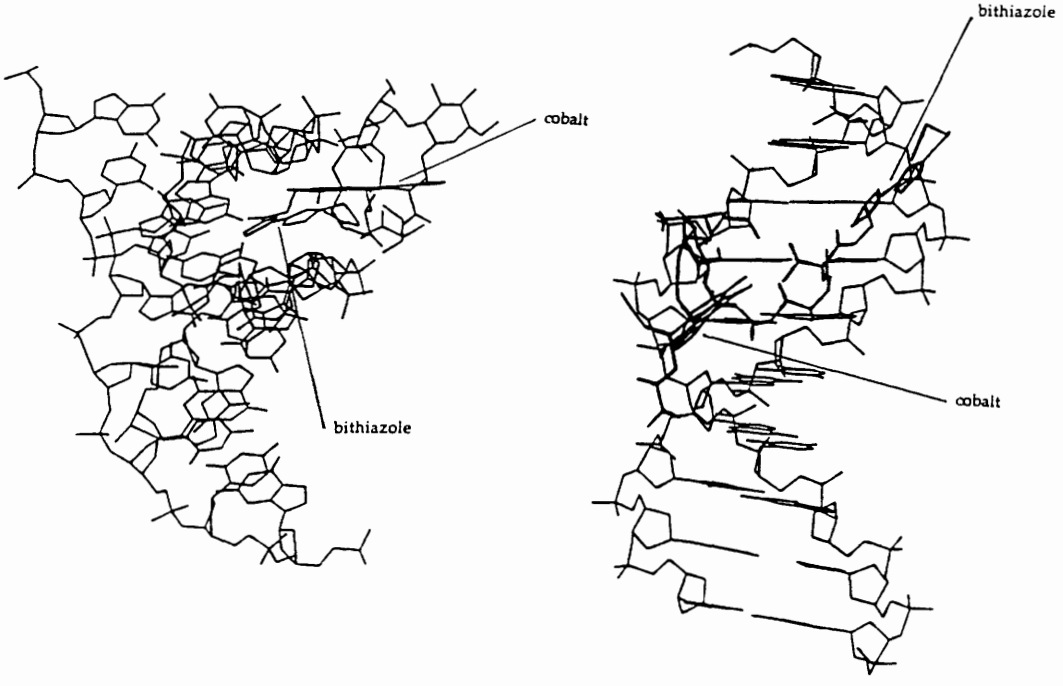


Fig. 8 Two views of Co^{I}(BLM) binding to the minor groove of self-complementary d(G-C-G-C-G-C-G-C-G-C)$_2$. The Figure illustrates the possibility that both the metal-binding region and the bithiazole moiety can be bound to the DNA minor groove

extremes we used cobalt with a formal charge of +3 and +1; in both cases the force field used was that derived for cobalt BLM analogues.[35] Changing the charge on the metal ion has a significant effect on the BLM conformation (Fig. 5), however the differences in conformation between the two binding geometries are much larger (Fig. 6) than those due to charge differences. Based on BLM–DNA docking studies we suggest that both the bithiazole tail and the metal-binding region of BLM with geometry **2** could be bound and wound around the minor groove of DNA.

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References

1 (*a*) R. H. Blum, S. D. Carter and K. Agre, *Cancer*, 1973, **31**, 903; (*b*) D. H. Petering, R. W. Byrnes and W. E. Antholine, *Chem.-Biol. Interact.*, 1990, **73**, 133; (*c*) E. Caumes, G. Guermonprez, C. Katlama and M. Gentilini, *AIDS*, 1992, **6**, 1483; (*d*) K. Lassoued, J. P. Claudel, C. Katlama, M. Janier, C. Picard and S. Matheron, *Cancer*, 1990, **66**, 1869.
2 T. Takita, Y. Muraoka, T. Nakatani, A. Fuji, Y. Iitaka and H. Umezawa, *J. Antibiot.*, 1978, **31**, 1073.
3 (*a*) N. J. Oppenheimer, L. O. Rodriguez, S. M. Hecht, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 5616; (*b*) M. Tsukayama, C. R. Randall, F. S. Santillo and J. C. Dabrowiak, *J. Am. Chem. Soc.*, 1981, **103**, 458; (*c*) M. A. J. Akkerman, E. W. J. F. Neijman, S. S. Wijmenga, C. W. Hilbers and W. Bermel, *J. Am. Chem. Soc.*, 1990, **112**, 7462; (*d*) M. A. J. Akkerman, C. A. G. Haasnoot, U. K. Pandit and C. W. Hilbers, *Magn. Reson. Chem.*, 1988, **26**, 793.
4 J. Stubbe and J. W. Kozarich, *Chem. Rev.*, 1987, **87**, 1107; S. M. Hecht, *Acc. Chem. Res.*, 1986, **19**, 383; Y. Sugiura, T. Takita and H. Umezawa, *Met. Ions Biol. Syst.*, 1985, **19**, 81.
5 (*a*) C. H. Chang and C. F. Meares, *Biochemistry*, 1984, **23**, 2268; (*b*) R. Xu, W. E. Antholine and D. H. Petering, *J. Biol. Chem.*, 1992, **267**, 950; (*c*) Y. Sugiura, *J. Am. Chem. Soc.*, 1980, **102**, 5216.
6 R. M. Burger, J. Peisach and S. B. Horwitz, *J. Biol. Chem.*, 1981, **256**, 11636.
7 V. Murray and R. F. Martin, *J. Biol. Chem.*, 1985, **260**, 10389.
8 (*a*) L. E. Rabow, J. Stubbe and J. W. Kozarich, *J. Am. Chem. Soc.*,

1990, **112**, 3196; (*b*) H. Sugiyama, C. Xu, N. Murugesan, S. M. Hecht, G. A. van der Marcel and J. H. van Boom, *Biochemistry*, 1988, **27**, 58; (*c*) J. W. Sam and J. Peisach, *Biochemistry*, 1993, **32**, 1488.
9 L. Giloni, M. Takeshita, F. Johnson, C. Iden and A. P. Grollman, *J. Biol. Chem.* 1981, **256**, 8608; G. H. McGall, L. E. Rabow, G. W. Ashley, S. H. Wu, J. W. Kozarich and J. Stubbe, *J. Am. Chem. Soc.*, 1992, **114**, 4958.
10 M. Girardet and B. Meunier, *Tetrahedron Lett.*, 1987, **28**, 2955; D. C. Heimbrook, S. A. Carr, M. A. Mentzer, E. C. Long and S. M. Hecht, *Inorg. Chem.*, 1987, **26**, 3835; D. C. Heimbrook, R. L. Mulholland and S. M. Hecht, *J. Am. Chem. Soc.*, 1986, **108**, 7840; N. Murugesan and S. M. Hecht, *J. Am. Chem. Soc.*, 1985, **107**, 493.
11 L.-C. Yuan and T. C. Bruice, *J. Am. Chem. Soc.*, 1985, **107**, 512; T. G. Traylor, W.-P. Fann and D. Bandyopadhyay, *J. Am. Chem. Soc.*, 1989, **111**, 8009; T. G. Traylor and J. P. Ciccone, *J. Am. Chem. Soc.*, 1989, **111**, 8413.
12 R. A. Leising, B. A. Brennan, L. Jr. Que, B. G. Fox and E. Muenck, *J. Am. Chem. Soc.*, 1991, **113**, 3988; Y. Zang, T. Elgren, Y. Dong and L. Jr. Que, *J. Am. Chem. Soc.*, 1993, **115**, 811.
13 Y.-D. Wu, K. N. Houk, J. S. Valentine and W. Nan, *Inorg. Chem.*, 1992, **31**, 720.
14 Y. Suguira, *J. Am. Chem. Soc.*, 1980, **102**, 5216; C. M. Vos and G. Westera, *J. Inorg. Biochem.*, 1981, **15**, 253.
15 J. P. Albertinin and A. Garnier-Suillerot, *Biochemistry*, 1982, **21**, 6777.
16 C.-H. Chang and C. F. Meares, *Biochem. Biophys. Res. Commun.*, 1983, **110**, 959.
17 C.-H. Chang and C. F. Meares, *Biochemistry*, 1984, **23**, 2268.
18 L. H. DeRiemer, C. F. Meares, D. A. Goodwin and C. I. Diamanti, *J. Med. Chem.*, 1979, **22**, 1019.
19 I. Saito, T. Morii, H. Sugiyama, T. Matsuura, C. F. Meares and S. M. Hecht, *J. Am. Chem. Soc.*, 1989, **11**, 2307; C.-H. Chang and C. F. Meares, *Biochemistry*, 1982, **21**, 6332; T. Suzuki, J. Kuwahara, M. Goto and Y. Sugiura, *Biochim. Biophys. Acta*, 1985, **824**, 330; T. Suzuki, J. Kuwahara and Y. Sugiura, *Nucleic Acids Res.*, 1984, **15**, 161.
20 R. Subramanian and C. F. Meares, *J. Am. Chem. Soc.*, 1986, **108**, 6427.
21 B. J. Carter, V. S. Murty, K. S. Reddy, S.-N. Wang and S. M. Hecht, *J. Biol. Chem.*, 1990, **265**, 4193.
22 (*a*) Y. Iitaka, H. Nakamura, T. Nakatani, Y. Muraoka, A. Fujii, T. Takita and H. Umezawa, *J. Antibiot.*, 1978, **31**, 1070; (*b*) T. J. Lomis, M. G. Elliot, S. Siddiqui, M. Moyer, R. R. Koepsel and R. E. Shepard, *Inorg. Chem.*, 1989, **28**, 2369; (*c*) J. D. Tan, S. E. Hudson, S. J. Brown, M. M. Olmstead and P. K. Mascharak, *J. Am. Chem. Soc.*, 1992, **114**, 3841; (*d*) L. Scheich, P. Gosling, S. J. Brown, M. M. Olmstead and P. K. Mascharak, *Inorg. Chem.*, 1991, **30**, 1677; (*e*) S. J. Brown, S. E. Hudson, P. K. Mascharak and M. M. Olmstead, *J. Am. Chem. Soc.*, 1989, **111**, 6446; (*f*) D. T. Dawson, S. E. Hudson and P. K. Mascharak, *J. Inorg. Biochem.*, 1992, **47**, 109; (*g*) E. Kimura, H. Kurosaki, Y. Kurogi, M. Shionoya and M. Shiro, *Inorg. Chem.*, 1992, **31**, 4314.
23 J. C. Dabrowiak and M. Tsukayama, *J. Am. Chem. Soc.*, 1981, **103**, 7543.
24 (*a*) O. Q. Munro, J. C. Bradley, R. D. Hancock, H. M. Marques, F. Marsicano and P. W. Wade, *J. Am. Chem. Soc.*, 1992, **114**, 7218; (*b*) M. A. Lopez and P. A. Kollman, *J. Am. Chem. Soc.*, 1989, **111**, 6212; (*c*) J. A. Shelnutt, C. J. Medforth, M. D. Berber, K. M. Barkigia and K. M. Smith, *J. Am. Chem. Soc.*, 1991, **113**, 4077.
25 J. M. Rudzinski and E. Osawa, *J. Phys. Org. Chem.*, 1992, **5**, 382.
26 (*a*) G. Wipff, P. Weiner and P. A. Kollman, *J. Am. Chem. Soc.*, 1982, **104**, 3249; (*b*) P. A. Kollman, G. Wipff and U. C. Singh, *J. Am. Chem. Soc.*, 1985, **107**, 2212; (*c*) K. Damu, R. D. Hancock, P. W. Wade, J. C. A. Boeyens, D. G. Billing and S. M. Dobson, *J. Chem. Soc., Dalton Trans.*, 1991, 293.
27 M. D. Riely, T. W. Hambley and L. G. Marzilli, *J. Am. Chem. Soc.*, 1988, **110**, 2999.
28 W.-K. Lin, N. W. Alcock and D. H. Busch, *J. Am. Chem. Soc.*, 1991, **113**, 7603.
29 S. C. Hoops, K. W. Anderson and K. M. Merz, *J. Am. Chem. Soc.*, 1991, **113**, 8262.
30 N. L. Allinger, M. I. Quinn, K. Chen, B. Thompson and M. R. Frierson, *J. Mol. Struct.*, 1989, **194**, 1.
31 (*a*) L. Y. Martin, L. De Hayes, L. Zompa and D. H. Busch, *J. Am. Chem. Soc.*, 1974, **96**, 4047; (*b*) Y. Hung, L. Martin, S. C. Jackels, A. M. Tait and D. H. Busch, *J. Am. Chem. Soc.*, 1977, **99**, 4029; (*c*) Y. Hung and D. H. Busch, *J. Am. Chem. Soc.*, 1977, **99**, 4977; (*d*) G. J. McDougall, R. D. Hancock and J. C. A. Boeyens, *J. Chem. Soc., Dalton Trans.*, 1978, 1438; (*e*) M. G. B. Drew and P. C. Yates, *J. Chem. Soc., Dalton Trans.*, 1987, 2563.
32 (*a*) M. Zimmer and R. H. Crabtree, *J. Am. Chem. Soc.*, 1990, **112**, 1062; (*b*) W. A. Kaplan, K. S. Suslick and R. A. Scott, *J. Am. Chem. Soc.*, 1991, **113**, 9824.
33 P. V. Bernhardt and P. Comba, *Inorg. Chem.*, 1992, **31**, 2638.
34 A. Vedani and D. W. Huhta, *J. Am. Chem. Soc.*, 1990, **112**, 4795.
35 R. Charles, M. Ganly-Cunningham, R. Warren and M. Zimmer, *J. Mol. Struct.*, 1992, **265**, 385.
36 F. Mohamadi, N. G. F. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson and W. C. Still, *J. Comput. Chem.*, 1989, **111**, 4379.
37 (*a*) G. R. Brubaker and D. W. Johnson, *Coord. Chem. Rev.*, 1984, **53**, 1; (*b*) T. W. Hambley, *Acta Crystallogr., Sect. B*, 1988, **44**, 601; (*c*) J. F. Endicott, K. Kumar, C. L. Schwartz, M. W. Perkovic and W.-K. Lin, *J. Am. Chem. Soc.*, 1989, **111**, 7411; (*d*) R. Cini, G. Giorgi, F. Laschi, C. Rossi and L. Marzilli, *J. Mol. Biomol. Struct. Dynamics*, 1990, **7**, 859; (*e*) T. Mali, P. W. Wade and R. D. Hancock, *J. Chem. Soc., Dalton Trans.*, 1992, 68; (*f*) I. M. Atkinson, F. R. Keene, J. M. Gulbis, G. H. Searle and E. R. T. Tielink, *J. Mol. Struct.*, 1992, **265**, 189.
38 M. Mutterties, P. K. Mascharak, M. B. Cox and S. K. Arora, *Inorg. Chim. Acta*, 1989, **160**, 123; S. J. Brown, S. E. Hudson, P. K. Mascharak and M. M. Olmstead, *J. Am. Chem. Soc.*, 1989, **111**, 6446.
39 M. Saunders, K. N. Houk, Y. D. Wu, W. C. Still, M. Lipton, G. Chang and W. C. Guida, *J. Am. Chem. Soc.*, 1990, **112**, 1419.
40 G. Chang, W. C. Guida and W. C. Still, *J. Am. Chem. Soc.*, 1989, **11**, 4379.
41 M. Saunders, *J. Comput. Chem.*, 1989, **10**, 203.
42 M. Levitt, *J. Mol. Biol.*, 1983, **168**, 595; J. A. McCammon and M. Karplus, *Acc. Chem. Res.*, 1983, **16**, 187.
43 P. Auffinger and G. Wipff, *J. Comput. Chem.*, 1990, **11**, 19.
44 G. J. McDougall and R. D. Hancock, *J. Chem. Soc., Dalton Trans.*, 1980, 654.
45 T. W. Hambley, *J. Comput. Chem.*, 1987, **8**, 651.
46 B. Kaitner, D. M. Ferguson, N. Paulic and N. Raos, *J. Coord. Chem.*, 1992, **26**, 105.
47 N. L. Allinger, *J. Am. Chem. Soc.*, 1977, **99**, 8127.
48 M. Lipton and W. C. Still, *J. Comput. Chem.*, 1988, **9**, 343.
49 S. C. Hoops, W. A. Kenneth and K. M. Jr. Merz, *J. Am. Chem. Soc.*, 1991, **113**, 8262; L. Banci, I. Bertini, P. Carloni, C. Luchinat and P. L. Orioli, *J. Am. Chem. Soc.*, 1992, **114**, 10684.
50 C. L. Schwartz and J. F. Endicott, *Inorg. Chem.*, 1989, **28**, 4011; K. R. Adam, M. Antolovich, L. G. Brigden and L. F. Lindoy, *J. Am. Chem. Soc.*, 1991, **113**, 3346; D. M. Ferguson and D. J. Raber, *J. Comput. Chem.*, 1990, **11**, 1061; V. J. Thoem, C. C. Fox, J. C. A. Boeyens and R. D. Hancock, *J. Am. Chem. Soc.*, 1984, **106**, 5947; S. C. Mackie and M. C. Baird, *Organometallics*, 1992, **11**, 3712.
51 M. Ragazzi, D. R. Ferro and A. Provasoli, *J. Comput.*, 1986, **7**, 105; M. Clark, R. D. III Cramer and N. Van Opdenbosch, *J. Comput. Chem.*, 1989, **10**, 982.
52 C. R. Canales and M. Zimmer, *J. Mol. Struct.*, 1991, **245**, 341.
53 W. Kabsch, *Acta Crystallogr., Sect. A*, 1976, **32**, 922; 1978, **34**, 827.
54 L. L. Guan, R. Totsuka, J. Kuwahara, M. Otsuka and Y. Sugiura, *Biochem. Biophys. Res. Commun.*, 1993, **191**, 1338.
55 J. Kuwahara and Y. Sugiura, *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 2459.

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