A Designed Synthetic Analogue of Co(III)-Bleomycin with Enhanced DNA-Binding and Photocleaving Activity[1]

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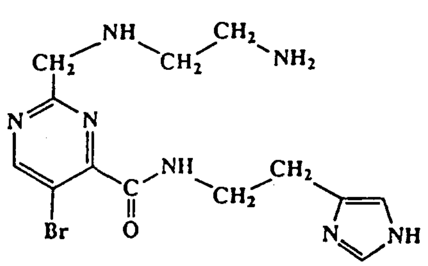
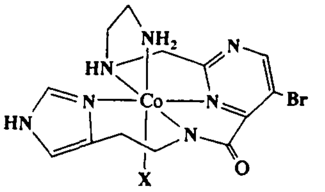
Recent studies have indicated that the Co(III) chelates of the antitumor antibiotic bleomycin (BLM) cleave DNA under UV or visible light and that this photoinduced damage of DNA is insensitive to the presence of dioxygen.[2–4] As part of our efforts toward elucidation of (a) the structures of metallobleomycins (M-BLMs) and (b) the mechanisms of DNA strand scission by them via the synthetic analogue approach,[5–7] we have isolated the Co(III) complexes of the designed ligand PMAH (**1**, H is the dissociable amide H) that resembles the proposed metal-chelating portion of BLM.[8] In a recent account,[9] we have reported the syntheses and structures of three analogues of the compositions $[Co(PMA)(H_2O)](NO_3)_2$ (**2**), [Co(PMA)Cl]Cl (**3**), and [Co(PMA)(*N*-MeIm)]$(NO_3)_2$ (*N*-MeIm = *N*-methylimidazole, **4**) which are good models for the socalled brown, green, and orange forms of Co(III)-BLM.[10] Our results have shown that upon UV illumination, a transient radical is formed in the ligand framework of **2**–**4** that rapidly generates •OH radical in aqueous solutions and causes DNA strand scission. However, since these positively charged model complexes bind to DNA solely through electrostatic attraction, they cleave DNA less efficiently compared to Co(III)-BLMs, which exhibit strong DNA binding due to intercalation of the bithiazole moiety of the drug.[8]



PMAH (1)



$[Co(PMA)X]^{n+}$
2: X=H_2O, n=2;
3: X=Cl, n=1;
4: X=N-MeIm, n=2;
6: X=Int-A, n=2

It has been shown that covalent attachment of DNA intercalators enhances the extent of DNA damage by several metal complexes including Fe-EDTA.[11–13] The possibility of augmentation of the DNA cleavage activity of analogues like **2**–**4** by such a procedure prompted us to synthesize $[Co(PMA)(Int\text{-}A)]Cl_2$ (**6**) where Int-A = acridine-9-carboxamido-*N'*-(3-propyl)imidazole (**5**). Acridine was chosen as the intercalator because (a) its properties as an intercalator are well-established, (b) its DNA binding constant is high, and (c) the binding constant does not change with the nature of the 9-substituents.[14] We report here the synthesis, structure, and spectroscopic properties of **6** and show its high capacity for DNA cleavage under UV illumination.

A mixture of 1 g (3.6 mmol) of 9-chlorocarbonylacridine hydrochloride[15] and 1.48 mL (12.4 mmol) of 1-(3-aminopropyl)-imidazole (Aldrich) in 40 mL of chloroform was heated under reflux for 10 h, and the resulting residue was filtered off. The mother liquor containing Int-A (**5**) was evaporated to dryness, redissolved in methanol, and purified on a silica gel column using methanol as the eluent. Next, 0.1 g (0.17 mmol) of **2** was dissolved in 25 mL of acetone and heated to reflux for 1 h. To the brown-green solution was then added dropwise a solution of 62 mg (0.19 mmol) of **5** in 10 mL of ethanol when the color turned orange. The mixture was heated for 12 h and filtered, and the filtrate was evaporated to dryness. The solid thus obtained was dissolved in 5 mL of water, loaded on a SP-C50-120 Sephadex (Sigma) column, and eluted with 0.1 N KCl solution. The first orange band was collected and desalted by extracting with ethanol. The complex **6** was obtained as an orange microcrystalline solid (yield = 45% on the basis of **2**).[16]

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[1] Dedicated to Richard H. Holm on the occasion of his 60th birthday.

[2] (a) Chang, C. H.; Meares, C. F. *Biochemistry* **1984**, *23*, 2268. (b) Chang, C. H.; Meares, C. F. *Biochemistry* **1982**, *21*, 6332.

[3] Subramanian, R.; Meares, C. F. *J. Am. Chem. Soc.* **1986**, *108*, 6427.

[4] (a) Suzuki, T.; Kuwahara, J.; Goto, M.; Sugiura, Y. *Biochim. Biophys. Acta* **1985**, *824*, 330. (b) Suzuki, T.; Kuwahara, J.; Sugiura, Y. *Nucl. Acids Res.* **1984**, *15*, 161.

[5] (a) Scheich, L. A.; Gosling, P.; Brown, S. J.; Olmstead, M. M.; Mascharak, P. K. *Inorg. Chem.* **1991**, *30*, 1677. (b) Hudson, S. E.; Mascharak, P. K. *Chem. Res. Toxicol.* **1989**, *2*, 411. (c) Brown, S. J.; Hudson, S. E.; Stephan, D. W.; Mascharak, P. K. *Inorg. Chem.* **1989**, *28*, 468. (d) Brown, S. J.; Stephan, D. W.; Mascharak, P. K. *J. Am. Chem. Soc.* **1988**, *110*, 1996. (e) Brown, S. J.; Tao, X.; Stephan, D. W.; Mascharak, P. K. *Inorg. Chem.* **1986**, *25*, 3377.

[6] (a) Brown, S. J.; Olmstead, M. M.; Mascharak, P. K. *Inorg. Chem.* **1989**, *28*, 3720. (b) Muetterties, M.; Cox, M. B.; Arora, S. K.; Mascharak, P. K. *Inorg. Chim. Acta* **1989**, *160*, 123. (c) Brown, S. J.; Hudson, S. E.; Olmstead, M. M.; Mascharak, P. K. *J. Am. Chem. Soc.* **1989**, *111*, 6446. (d) Delany, K.; Arora, S. K.; Mascharak, P. K. *Inorg. Chem.* **1988**, *27*, 705.

[7] (a) Guajardo, R. J.; Hudson, S. E.; Brown, S. J.; Mascharak, P. K. *J. Am. Chem. Soc.*, submitted for publication. (b) Dawson, D. Y.; Hudson, S. E.; Mascharak, P. K. *J. Inorg. Biochem.* **1992**, *47*, 109. (c) Brown, S. J.; Olmstead, M. M.; Mascharak, P. K. *Inorg. Chem.* **1990**, *29*, 3229. (d) Tao, X.; Stephan, D. W.; Mascharak, P. K. *Inorg. Chem.* **1987**, *26*, 754.

[8] (a) Petering, D. H.; Byrnes, R. W.; Antholine, W. E. *Chem. Biol. Interact.* **1990**, *73*, 133. (b) Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107.

[9] Tan, J. D.; Hudson, S. E.; Brown, S. J.; Olmstead, M. M.; Mascharak, P. K. *J. Am. Chem. Soc.* **1992**, *114*, 3841.

[10] (a) Chang, C. H.; Meares, C. F. *Biochem. Biophys. Res. Commun.* **1983**, *110*, 959. (b) DeRiemer, L. H.; Meares, C. F.; Goodwin, D. A.; Diamanti, C. I. *J. Med. Chem.* **1979**, *22*, 1019.

[11] (a) Bailly, C.; Sun, J.-S.; Colson, P.; Houssier, C.; Helene, C.; Waring, M. J.; Henichart, J.-P. *Bioconjugate Chem.* **1992**, *3*, 100. (b) Bailly, C.; Henichart, J.-P. *Bioconjugate Chem.* **1991**, *2*, 379. (c) Bailly, C.; Kenani, A.; Helbecque, N.; Bernier, J. L.; Houssin, R.; Henichart, J.-P. *Biochem. Biophys. Res. Commun.* **1988**, *152*, 695.

[12] (a) Lown, J. W.; Sondhi, S. M.; Ong, C. W.; Skorobogaty, A.; Kishikawa, H.; Dabrowiak, J. C. *Biochemistry* **1986**, *25*, 5111. (b) Hashimoto, Y.; Iijima, H.; Nozaki, Y.; Shudo, K. *Biochemistry* **1986**, *25*, 5103. (c) Lown, J. W.; Joshua, A. V. *J. Chem. Soc., Chem. Commun.* **1982**, 1298.

[13] (a) Dervan, P. B. *Science (Washington, D.C.)* **1986**, *232*, 464. (b) Hertzberg, R. P.; Dervan, P. B. *Biochemistry* **1984**, *23*, 3934. (c) Hertzberg, R. P.; Dervan, P. B. *J. Am. Chem. Soc.* **1982**, *104*, 313.

[14] (a) Drummond, D. S.; Simpson-Gildemeister, V. F. W.; Peacocke, A. R. *Biopolymers* **1965**, *3*, 135. (b) Peacocke, A. R.; Skerrett, J. N. H. *Trans. Faraday Soc.* **1956**, *52*, 261.

[15] White, E. H.; Roswell, D. F.; Dupont, A. C.; Wilson, A. A. *J. Am. Chem. Soc.* **1987**, *109*, 5189.

[16] Selected IR bands (KBr pellet, cm^{-1}): 3390 (s), 3090 (s), 2905 (s), 1630 (vs, ν_{CO}), 1560 (s), 1440 (s), 1345 (m), 1270 (m), 1095 (s), 760 (s). Electronic absorption spectrum (λ_{max}, nm; ϵ, M^{-1} cm^{-1}) in DMSO: 520 sh (140), 385 (6050), 360 (12 800), 350 (10 000).

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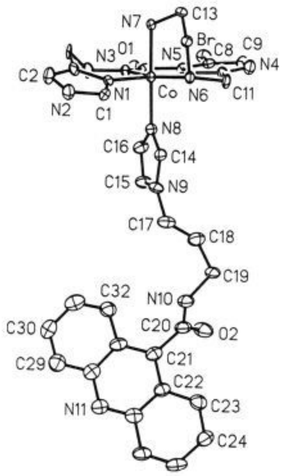

Figure 1. Thermal ellipsoid plot (50% probability level) of the cation of **6**. Hydrogen atoms are omitted for clarity. Selected bond distances (in Å): Co–N(1), 1.923(6); Co–N(3), 1.915(5); Co–N(5), 1.863(6); Co–N(6), 2.004(5); Co–N(7), 1.957(5); Co–N(8), 1.972(5); C(17)–N(9), 1.455(8); C(20)–O(2), 1.229(11); C(21)–C(22), 1.395(10); C(29)–C(30), 1.369(11). Selected bond angles (in deg): N(1)–Co–N(3), 95.9(2); N(3)–Co–N(5), 82.0(2); N(1)–Co–N(6), 97.6(2); N(5)–Co–N(6), 84.4(2); N(1)–Co–N(7), 89.5(2); N(3)–Co–N(7), 89.4(2); N(1)–Co–N(8), 90.4(2); N(7)–Co–N(8), 179.3(2); O(2)–C(20)–C(21), 120.1(7); C(20)–C(21)–C(22), 119.8(8).

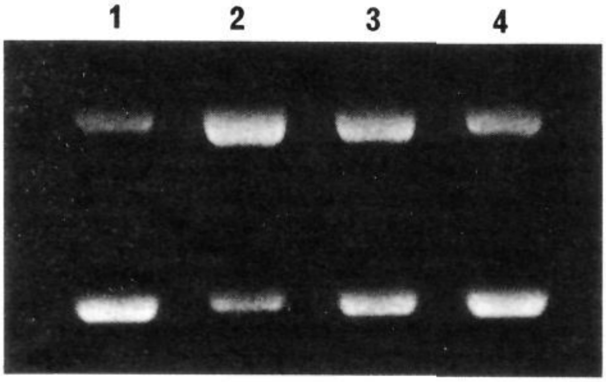

Figure 2. Light-induced DNA (ϕX174 (RF)) cleavage experiments with [Co(PMA)(Int-A)]Cl_2 (**6**) in 25 mM Tris borate, 190 μM EDTA, pH 8.3 buffer at 298 K. DNA concentration: 2 μg/50 μL. Lane 1, DNA only; lane 2, DNA + **6** (10 μM); lane 3, DNA + **4** (500 μM); lane 4, DNA + **5** (10 μM).

The structure[17] of the cation of **6** is shown in Figure 1. The coordination geometry around cobalt is octahedral, and the metric parameters are comparable to those observed with **4**.[9] In addition to the characteristic electronic absorption bands of the acridine moiety,[16] **6** exhibits a shoulder at 520 nm (ϵ = 140 M^{-1} cm^{-1}) due to the presence of the CoN_6 chromophore.[9] That the structure of **6** is retained in solution is readily established by its NMR spectrum (supplementary material).

Binding of **6** to calf thymus DNA (Sigma) was studied spectrophotometrically,[14] and the binding constant was found to be of the order of 10^6 M^{-1}. This value is considerably higher than the binding constant of **5** ($<10^5$ M^{-1}) due to additional electrostatic association of the $[Co(PMA)]^{2+}$ moiety with DNA. For Co(III)-BLMs, the binding constants are in the range of 10^6–10^7 M^{-1}.[2a,18] Clearly, the designed analogue **6** binds to DNA much like the Co(III)-BLMs. This fact permits the use of much lower concentrations of **6** (compared to **2**–**4**) in carrying out a significant extent of light-induced DNA damage. This is shown in Figure 2.[19] Irradiation of supercoiled covalently closed circular (form I) ϕX174 DNA (Bethesda Research Lab) in the presence of 10 μM **6** results in the appearance of nicked circular (form II) DNA (lane 2). A similar extent of DNA strand scission is observed with 500 μM **4** (lane 3),[20] while **5** induces little DNA damage below 50 μM concentration (lane 4). The presence of the intercalator also permits $[Co(PMA)(Int\text{-}A)]^{2+}$ to remain associated with the DNA and cleave it under high salt conditions (50 mM or more of Na_2SO_4 in the incubation medium). The simpler cationic analogues **2**–**4** fail to bind to DNA under such conditions, and hence no photonicking is observed.[9]

In conclusion, the structure of the first model of Co(III)-BLM with a DNA-binding group, namely [Co(PMA)(Int-A)]Cl_2 (**6**), has been determined. To date, no other DNA-cleaving agent of the "hybrid" type has been characterized by X-ray crystallography. **6** binds to DNA very strongly and causes light-induced DNA strand scission at micromolar concentrations. The results clearly show that the intrinsic chemistry of simpler model complexes of M-BLMs can be delivered to DNA efficiently if one attaches a DNA-binding group to them. This in turn indicates the role of the bithiazole group in BLM. Use of **6** as a footprinting agent that gets "turned on" under illumination is also anticipated. At the present time, more NMR and computer-aided modeling studies are in progress in this laboratory to establish the details of the binding of **6** to oligonucleotides, and the results will appear elsewhere in due time.

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Supplementary Material Available: ^{13}C NMR spectra of **4**, **5**, and **6**; crystal structure data for **6** including tables of atomic coordinates (Table S1), complete bond distances (Table S2) and angles (Table S3), anisotropic thermal parameters (Table S4), and H-atom coordinates (Table S5) (9 pages); table of observed and calculated structure factors (Table S6) (16 pages). Ordering information is given on any current masthead page.

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[17] X-ray analysis, orange plates of **6**. 2.0 H_2O, 2.75 CH_3OH from aqueous methanol, $CoC_{35.75}H_{50}N_{11}O_{6.75}BrCl_2$ (**6**), triclinic space group $P\bar{1}$, a = 9.821(2) Å, b = 10.600(2) Å, c = 21.101 Å, α = 89.67(2)°, β = 89.24(2)°, γ = 70.51(2)°, V = 2069.2(16) Å^3, Z = 2, d_{calc} = 1.527 g/cm^3, R = 4.48%, R_w = 4.53%. The structure was solved by direct methods (SHELXTL PLUS, version 4.2).

[18] Kakinuma, J.; Orii, H. *Nuklearmedizin (Stuttgart)* **1982**, *21*, 232.

[19] The samples were placed into a multiple-well tissue culture cluster plate (volume of wells = 90 μL) and covered with a quartz plate and a 320-nm filter (Corning 5950). A transilluminator (UVP-TM-36, λ_{max} = 302 nm, intensity 7 mW/cm^2) was placed face-down over the plate. The distance between the light source and the samples was 4 cm. At the end of 3 h, the samples were loaded onto 1% agarose gel (TAE running buffer) and electrophoresed for 3 h at 100 V.

[20] Result of densitometric measurement on the negative of the gel picture shown in Figure 2: lane 1, 20% open circular form (ocf), 80% supercoiled form (sf); lane 2, 70% ocf, 30% sf; lane 3, 60% ocf, 40% sf; lane 4, 30% ocf, 70% sf.