Structures of Fe(II) Complexes with *N,N,N′*-Tris(2-pyridylmethyl)ethane-1,2-diamine Type Ligands. Bleomycin-like DNA Cleavage and Enhancement by an Alkylammonium Substituent on the N′ Atom of the Ligand

Pierre Mialane,[1a] Alexander Nivorojkine,[1a] Geneviève Pratviel,[1c] Laurent Azéma,[1c] Michaela Slany,[1c] Frédéric Godde,[1c] Ariane Simaan,[1a] Frédéric Banse,[1a] Taraneh Kargar-Grisel,[1d] Guy Bouchoux,[1d] Joelle Sainton,[1a] Olivier Horner,[1a] Jean Guilhem,[1b] Lyuba Tchertanova,[1b] Bernard Meunier,[1c] and Jean-Jacques Girerd*,[1a]

Laboratoire de Chimie Inorganique, URA CNRS 420, Institut de Chimie Moléculaire d'Orsay, Université Paris-Sud, 91405 Orsay, France, Institut de Chimie des Substances Naturelles, UPR CNRS 2301, 91198 Gif-sur-Yvette, France, Laboratoire de Chimie de Coordination du CNRS, 31077 Toulouse, France, and Laboratoire des Mécanismes Réactionnels, UMR CNRS 7651, Ecole Polytechnique, 91128 Palaiseau, France

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The complexes $[L_5Fe^{II}Cl]BPh_4$ and $[L_5Fe^{II}(H_2O)](BPh_4)_2$ (L_5 = *N,N,N′*-tris(2-pyridylmethyl)-*N′*-methyl-ethane-1,2-diamine) have been isolated. Bernal et al. (Bernal, J.; et al. *J. Chem. Soc., Dalton Trans.* **1995**, 3667−3675) have prepared this ligand and the corresponding complex $[L_5Fe^{II}Cl]PF_6$. We obtained the structural data of $[L_5Fe^{II}Cl]BPh_4$ by X-ray diffraction. It crystallizes in the orthorhombic space group $P2_12_12_1$ with $a = 17.645(7)$ Å, $b = 16.077(6)$ Å, $c = 13.934(5)$ Å, $V = 3953(3)$ Å^3, and $Z = 4$. It presents Fe(II)−N bond lengths close to 2.2 Å, typical of high-spin Fe(II). In solution the $[L_5Fe^{II}(H_2O)](BPh_4)_2$ complex showed a dependence of spin state upon the nature of the solvent. It was high spin in acetone and changed to low spin in acetonitrile. This was detected by UV−vis spectroscopy and by ^{1}H NMR. Bernal et al. (ibidem) showed that these complexes in the presence of an excess of H_2O_2 give a purple species, very likely the $[L_5Fe^{III}(OOH)]^{2+}$ derivative, with spectroscopic signatures analogous to those of "activated bleomycin". The formation of $[L_5Fe^{III}(OOH)]^{2+}$ is confirmed here by electrospray ionization mass spectrometry. We found that a L_5/Fe system gave single-strand breaks on plasmid DNA in the presence of either a reducing agent (ascorbate) and air or oxidants (H_2O_2, $KHSO_5$, MMPP) at 0.1 μM concentration. The methyl group in L_5 was substituted by a $(CH_2)_5N(CH_3)_3^+$ group in order to get higher affinity with DNA. The corresponding ligand L_5^+ was used to prepare the complexes $[L_5^+Fe^{II}Cl]Y_2$ (Y = BPh_4^-, PF_6^-, ClO_4^-) and $[L_5^+Fe^{II}Br](PF_6)_2$. The crystal structure of $[L_5^+Fe^{II}Cl](ClO_4)_2$ was solved. It crystallizes in the monoclinic space group $P2_1/a$ with $a = 14.691(2)$ Å, $b = 13.545(2)$ Å, $c = 17.430(2)$ Å, $\beta = 93.43(1)°$, $V = 3462(1)$ Å^3, and $Z = 4$. The Fe(II)−ligand distances are similar to those of $[L_5Fe^{II}Cl]BPh_4$. At the relatively low concentration of 0.01 μM, $[L_5^+Fe^{II}Br]^{2+}$ promoted DNA breaks. The reaction was not inhibited by hydroxyl radical scavengers. The reaction might involve a nondiffusible oxygen reactive species, either a coordinated hydroperoxide or a high-valent metal−oxo entity.

Introduction

In 1981, Burger et al.[2] suggested that "activated bleomycin", an intermediate involved in DNA oxidation by anticancer iron-containing glycopeptide bleomycin (BLM),[3,4] contained an Fe^{III}-OOH group. This attribution has recently been confirmed.[5,6] The group of Mascharak has arrived at the same identification from the study of chemical models.[7] To our knowledge four Fe^{III}-OOH-containing artificial complexes have been spectroscopically identified.[8−11] These artificial $Fe^{III}OOH$ units have also been implied in alkane hydroxylation.[9,10] An $Fe^{III}OOH$ intermediate occurs too in the mechanism of CytP450.[12] Thus the $Fe^{III}OOH$ unit may be considered as a ubiquitous species in oxygen activation.

(1) (a) Université Paris-Sud. (b) Institut de Chimie des Substances Naturelles. (c) Laboratoire de Chimie de Coordination. (d) Laboratoire des Mécanismes Réactionnels.
(2) Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1981**, *256*, 11636.
(3) Burger, R. M.; Kent, T. A.; Horwitz, S. B.; Münck, E.; Peisach, J. *J. Biol. Chem.* **1983**, *258*, 1559.
(4) Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107.
(5) Sam, J. W.; Tang, X.-J.; Peisach, J. *J. Am. Chem. Soc.* **1994**, *116*, 5250.
(6) Westre, T. E.; Loeb, K. E.; Zaleski, J. M.; Hedman, B.; Hogson, K. O.; Solomon, E. I. *J. Am. Chem. Soc.* **1995**, *117*, 1309.
(7) Guajardo, R. J.; Hudson, S. E.; Brown, S. J.; Mascharak, P. *J. Am. Chem. Soc.* **1993**, *115*, 7971.
(8) Bernal, I.; Jensen, I. M.; Jensen, K. B.; McKenzie, C. J.; Toftlund, H.; Tuchagues, J. P. *J. Chem. Soc., Dalton Trans.* **1995**, 3667.
(9) Lubben, M.; Meetsma, A.; Wilkinson, E. C.; Feringa, B.; Que, L., Jr. *Ang. Chem., Int. Ed. Engl.* **1995**, *34*, 1512.
(10) Nguyen, C.; Guajardo, R. J.; Mascharak, P. K. *Inorg. Chem.* **1996**, *35*, 6273.
(11) de Vries, M. E.; La Crois, R. M.; Roelfes, G.; Kooijman, H.; Spek, A. L.; Hage, R.; Feringa, B. L. *Chem. Commun.* **1997**, 1549.
(12) Sono, M.; Roach, M. P.; Coulter, E. D.; Dawson, J. H. *Chem. Rev.* **1996**, *96*, 2841 and references therein. See also: Benson, D. E.; Suslick, K. S.; Sligar, S. G. *Biochemistry* **1997**, *36*, 5104 and references therein.

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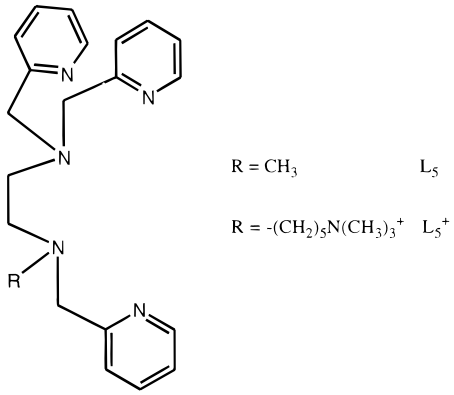
The Fe atom in BLM is believed to be bonded to five nitrogen atoms.[6] This coordination has been established for a Co^{III}(BLM) from solution NMR studies.[13] A very realistic chemical model of the active site of BLM has been prepared by the group of Mascharak.[7] This model and BLM have been compared spectroscopically.[14] In the perspective of industrial applications we decided to synthesize a very simple model of this active site. We selected ligands of the *N,N,N′*-tris(2-pyridylmethyl)-ethane-1,2-diamine type.



R = CH_3 L_5

R = $-(CH_2)_5N(CH_3)_3^+$ L_5^+

We found that indeed the Fe complexes of L_5 catalyze the oxidation of alkanes and polyaromatic compounds by H_2O_2.[15] These complexes have been prepared independently by Bernal et al.[8] Ligand L_5 has been shown to be able to stabilize Fe−O−Fe units.[16]

The group of Toftlund[8] showed that $[L_5Fe^{II}Cl]^+$ in MeOH in the presence of an excess of H_2O_2 gives a purple species (λ_{max} = 537 nm, ϵ = 750 M^{-1} cm^{-1}) with an almost axial X band EPR spectrum ($g_1 = 2.19$, $g_2 = 2.12$, $g_3 = 1.95$). They proposed that this species was an $Fe^{III}OOH$ derivative. The possibility of preparing this hydroperoxo form similar to "activated bleomycin" incited us to test if these complexes have a BLM-like activity on DNA.

We confirm in this paper the spectroscopic observations of the group of Toftlund, and indeed we detected the $[L_5Fe^{III}OOH]^{2+}$ entity by mass spectrometry. We report here on the BLM-like activity on DNA of L_5/Fe systems and the enhancement of this activity by substituting a $(CH_2)_5N(CH_3)_3^+$ group for the methyl of L_5. The ligand so obtained will be noted L_5^+. The motive for this substitution is the research of an electrostatic attraction between the ammonium group and a DNA phosphate group. We report here also the structural data for $[L_5Fe^{II}Cl]^+$ and $[L_5^+Fe^{II}Cl]^{2+}$ cations.

Experimental Section

Materials. All chemical syntheses were performed under argon using Aldrich starting materials as received. Solvents were dried before use. NMR spectra were recorded on a Bruker AC250 spectrometer. For electrochemical experiments, tetrabutylammonium perchlorate (TBAP, Fluka, puriss) was used as supporting electrolyte. Acetonitrile was distilled on CaH_2 under argon. Electrophoresis grade agarose, ascorbate, superoxide dismutase, and catalase were purchased from Sigma. Potassium monopersulfate and magnesium monoperphthalate (MMPP) were from Interox, and hydrogen peroxide was from Janssen. Iron(III) perchlorate was from Aldrich. Iron(III) chloride was from Merck, and bleomycin was a gift from Roger Bellon (Paris). ΦX174 DNA was purchased from Gibco BRL and eluted through a Sephadex G 10 column (Pharmacia) to remove the storage buffer EDTA that otherwise would have been at a 5 μM concentration in plasmid DNA cleavage media. *N,N,N′*-Tris(2-pyridylmethyl)-*N′*-methyl-ethane-1,2-diamine (ligand L_5) was metalated as described below. *N,N,N′*-Tris(2-pyridylmethyl)-*N′*-trismethylammoniumpentyl-ethane-1,2-diamine (ligand L_5^+) was directly provided as a Fe(II) complex, $[L_5^+Fe^{II}Br]^{2+}$ being water-soluble.

***N,N,N′*-Tris(2-pyridylmethyl)-*N′*-methyl-ethane-1,2-diamine (L_5).** This ligand was synthesized following ref 16.

***N,N,N′*-Tris(2-pyridylmethyl)-*N′*-trismethylammoniumpentyl-ethane-1,2-diamine Bromide (L_5^+Br).** This was a multistep synthesis.

(a) *N,N′*-Bis(2-pyridylmethyl)-ethane-1,2-diamine (bispicen).[17] To a solution of 2.6 mL of ethane-1,2-diamine (38 mmol) in 20 mL of methanol was added 7.4 mL (76 mmol) of 2-pyridinecarboxaldehyde. After reflux the solution was cooled to room temperature. $NaBH_4$ (4 g; 106 mmol) in 10 mL of methanol was added. The solution was refluxed overnight. Methanol was evaporated under vacuum. The oily residue was dissolved in water. The aqueous solution was extracted with $CHCl_3$ to bispicen as an oil; yield, 10 g, 86%.

(b) Synthesis of the Aminal. A solution of 1.07 g (4.4 mmol) of bispicen and 0.53 mL (4.4 mmol) of 2-pyridinecarboxaldehyde in 20 mL of ethyl ether was stirred at room temperature on 4 Å molecular sieves with $CaCl_2$ protection. A white precipitate quickly appeared. The solution was stirred overnight. The solid was recovered after filtration and washing with ethyl ether; yield, 1.3 g, 85%. ^{1}H NMR ($CDCl_3$): δ (ppm) 8.55 (d, 1H, H-py), 8.45 (d, 2H, H-py), 7.9 (d, 1H, H-py), 7.7 (t, 1H, H-py), 7.6 (t, 2H, H-py), 7.35 (d, 2H, H-py), 7.2 (t, 1H, H-py), 7.1 (t, 2H, H-py), 4.25 (s, 1H, CH), 3.95 (d, 2H, CH_2-py), 3.75 (d, 2H, CH_2-py), 3.3 (q, 2H, CH_2N), 2.75 (q, 2H, CH_2-N). MS data found for $C_{20}H_{21}N_5$: m/e = 331.423.

(c) *N,N,N′*-Tris(2-pyridylmethyl)-ethane-1,2-diamine. In a 250 mL flask 2.16 g of the aminal (6.5 mmol) was dissolved in 65 mL of methanol. To this solution was added 0.41 g (6.5 mmol) of $NaBH_3CN$ dissolved in 5 mL of methanol and 1 mL (13 mmol) of CF_3CO_2H. The solution was stirred at room temperature with $CaCl_2$ protection for 8 h. A 15% NaOH solution (65 mL) was added. The solution was stirred for 6 h and then extracted with CH_2Cl_2. After drying with Na_2SO_4 and evaporation under vacuum, *N,N,N′*-tris(2-pyridylmethyl)-ethane-1,2-diamine was recovered as a yellow oil; yield, 1.84 g, 85%. ^{1}H NMR ($CDCl_3$): δ (ppm) 8.5 (d, 3H, H-py), 7.55 (m, 6H, H-py), 7.1 (td, 3H, H-py), 3.8 (s, 6H, CH_2-py), 2.75 (s, 4H, CH_2CH_2N), 2.4 (s, 1H, HN).

(d) *N,N,N′*-Tris(2-pyridylmethyl)-*N′*-trismethylammoniumpentyl-ethane-1,2-diamine Bromide (L_5^+Br). *N,N,N′*-Tris(2-pyridylmethyl)-ethane-1,2-diamine (666 mg; 2 mmol) was added to a solution of 376 mg (1.8 mmol) of 1-bromo-5-trimethylammoniumpentane[18] in 100 mL of acetonitrile containing 2 g of Celite/KF.[19] The mixture was refluxed overnight. After cooling at room temperature the solution was filtrated. Acetonitrile was evaporated under vacuum. The oily residue was dissolved in water. The aqueous solution was extracted with CH_2Cl_2 to eliminate the unreacted *N,N,N′*-tris(2-pyridylmethyl)-ethane-1,2-diamine. The aqueous phase was evaporated to dryness.The oil residue was dissolved in hot ethyl acetate and filtrated without cooling. After cooling to room temperature the solvent was evaporated under vacuum. *N,N,N′*-Tris(2-pyridylmethyl)-*N′*-trismethylammoniumpentyl-ethane-1,2-diamine bromide (L_5^+Br) was obtained as a yellow oil; yield, 0.86 g, 50%. ^{1}H NMR ($CDCl_3$): δ (ppm) 8.45 (d, 2H, H-py), 8.4 (d, 1H, H-py), 7.6 (qd, 3H, H-py), 7.4 (d, 2H, H-py), 7.25 (d, 1H; H-py), 7.15 (d, 2H, H-py), 7.05 (d, 1H, H-py), 3.8 (s, 4H, py-CH_2N), 3.6 (s, 2H, py-CH_2N), 3.45 (m, 2H, CH_2N^+), 3.35 (s, 9H, $N^+(CH_3)_3$), 2.6 (s, 4H, NCH_2CH_2N), 2.4 (t, 2H, NCH_2), 1.65, 1.45, 1.25 (m, 6H, CH_2CH_2-CH_2).

(13) Stubbe, J.; Kozarich, J. W.; Wu, W.; Vanderwall, D. E. *Acc. Chem. Res.* **1996**, *29*, 322.
(14) Loeb, K. E.; Zaleski, J. M.; Westre, T. E.; Guajardo, R. J.; Mascharak, P.; Hedman, B.; Hodgson, K.; Solomon, E. I. *J. Am. Chem. Soc.* **1995**, *117*, 4545.
(15) Fraisse, L.; Girerd, J.-J.; Perie, F.; Rabion, A.; Tetard, D.; Verlhac, J.-B.; Nivorozhkin, A. French Patent 95 13580, 1995.
(16) Nivorozhkin, A. L.; Anxolabéhère-Mallart, E.; Mialane, P.; Davydov, R.; Guilhem, J.; Cesario, M.; Audière, J.-P.; Girerd, J.-J.; Styring, S.; Schussler, L.; Seris, J.-L. *Inorg. Chem.* **1997**, *36*, 846.
(17) Heinrichs, M. A.; Hodgson, D. J.; Michelsen, K.; Pedersen, E. *Inorg. Chem.* **1984**, *23*, 3174 and references therein.
(18) Thanei-Wyss, P.; Waser, P. G. *Helv. Chim. Acta* **1983**, *66*, 2198.
(19) Ando, T.; Yamawaki, J. *Chem. Lett.* **1979**, 45. Efficient application of the KF/Al_2O_3 reagent for the N-alkylation was also recently shown: Thangaraj, K.; Morgan, L. R. *Synth. Commun.* **1994**, *24*, 2063.

Table 1. Crystallographic Data for $[L_5Fe^{II}Cl]BPh_4$ and $[L_5{}^+Fe^{II}Cl](ClO_4)_2$

	$[L_5Fe^{II}Cl]BPh_4$	$[L_5{}^+Fe^{II}Cl](ClO_4)_2$
formula	$C_{45}H_{45}BClFeN_5$	$C_{28}H_{41}Cl_3FeN_6O_8$
a, Å	17.645(7)	14.691(2)
b, Å	16.077(6)	13.545(2)
c, Å	13.934(5)	17.430(2)
β, deg	90.0	93.43(1)
V, Å^3	3953(3)	3462(1)
Z	4	4
fw	757.97	751.87
temp, K	294	294
space group	$P2_12_12_1$	$P2_1/a$
λ, Å	0.710 73	0.710 73
μ, mm^{-1}	0.488	0.722
R	5.0	5.2
R_w	7.6	13.7

$[L_5Fe^{II}(H_2O)](BPh_4)_2$. To a solution of 347 mg (1 mmol) of L_5 in 10 mL of methanol under argon was added 338 mg (1 mmol) of $Fe^{II}(BF_4)_2 \cdot 6H_2O$ in 10 mL of methanol. The intense yellow solution was stirred for 30 min. Sodium tetraphenylborate (684 mg; 2 mmol) was added. A yellow precipitate was immediately obtained.

$[L_5Fe^{II}Cl]BPh_4$. To a solution of 347 mg (1 mmol) of L_5 in 10 mL of methanol under argon was added 198 mg (1 mmol) of $Fe^{II}Cl_2 \cdot 4H_2O$ in 10 mL of methanol. The intense yellow solution was stirred for 30 min. Sodium tetraphenylborate (342 mg; 1 mmol) was added. A yellow precipitate was immediately obtained. Large single crystals were obtained after 1 week by slow evaporation of an acetonitrile solution in a glovebox.

$[L_5Fe^{II}Cl]Cl$. To a solution of 347 mg (1 mmol) of L_5 in 10 mL of absolute ethanol under argon was added 200 mg (1 mmol) of $FeCl_2 \cdot 4H_2O$ in 10 mL of absolute ethanol. A yellow powder precipitated and was collected by filtration (yield = 50%).

$[L_5{}^+Fe^{II}Cl](BPh_4)_2$. To a solution of 540 mg (1 mmol) of $L_5{}^+Br$ in 10 mL of methanol under argon was added 198 mg (1 mmol) of $Fe^{II}Cl_2 \cdot 4H_2O$ in 10 mL of methanol. The intense yellow solution was stirred for 30 min. Sodium tetraphenylborate (684 mg; 2 mmol) was added. A yellow precipitate was immediately obtained.

$[L_5{}^+Fe^{II}Cl](PF_6)_2$. Using NH_4PF_6 instead of $NaBPh_4$, it was possible to prepare $L_5{}^+Fe^{II}Cl \cdot (PF_6)_2$. By letting the methanol solution stand at −15 °C overnight, a yellow powder was recovered.

$[L_5{}^+Fe^{II}Br](PF_6)_2$. To a solution of 540 mg (1 mmol) of $L_5{}^+Br$ in 10 mL of methanol under argon was added 338 mg (1 mmol) of $Fe^{II}(BF_4)_2 \cdot 6H_2O$ in 10 mL of methanol. The intense yellow solution was stirred for 30 min. NH_4PF_6 (326 mg; 2 mmol) was added. By letting the methanol solution stand at −15 °C overnight, a yellow powder was recovered.

$[L_5{}^+Fe^{II}Cl](ClO_4)_2$. To a solution of 540 mg (1 mmol) of $L_5{}^+Br$ in 10 mL of acetone under argon was added 198 mg (1 mmol) of $Fe^{II}Cl_2 \cdot 4H_2O$ in 10 mL of acetone. A concentrated acetone solution of $NaClO_4$ was added. The required volume of ethyl acetate to attain a 1/1 mixture was added. After 1 week of evaporation under a weak argon flow, single crystals were obtained.

Crystallographic Data Collection. The crystal data of $[L_5Fe^{II}Cl]BPh_4$ and $[L_5{}^+Fe^{II}Cl](ClO_4)_2$ and the parameters of data collections are summarized in Table 1. Prismatic yellow crystals of $[L_5Fe^{II}Cl]BPh_4$ with the dimensions 0.5 × 0.5 × 0.5 mm and of $[L_5{}^+Fe^{II}Cl](ClO_4)_2$ with the dimensions 0.75 × 0.25 × 0.20 mm were chosen for X-ray diffraction experiments. The unit-cell and intensity data were measured with an Enraf-Nonius CAD-4 diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.710 73 Å). The cell constants were obtained by least-squares procedures based upon the 2θ values of 25 reflections measured in the ranges $20.4° < 2\theta < 24.18°$ for $[L_5Fe^{II}Cl]BPh_4$ and $20.1° < 2\theta < 22.64°$ for $[L_5{}^+Fe^{II}Cl](ClO_4)_2$ at ambient temperature. All reflection intensities were collected in the range $4° < 2\theta < 56°$ within $[-23 < h < 23, 0 < k < 21, 0 < l < 18]$ for $[L_5Fe^{II}Cl]BPh_4$ and in the range $4.1° < 2\theta < 50°$ within $[-17 < h < 17, -16 < k < 16, 0 < l < 20]$ for $[L_5{}^+Fe^{II}Cl](ClO_4)_2$. The total number of collected independent reflections was 9559 for $[L_5Fe^{II}Cl]BPh_4$ and 6078 for $[L_5{}^+Fe^{II}Cl](ClO_4)_2$.

Table 2. Fractional Coordinates for $[L_5Fe^{II}Cl]^+$

	x	*y*	*z*
Fe	4568(1)	7070(1)	9812(1)
Cl	5668(1)	7860(1)	9642(1)
N(1)	3392(2)	6494(2)	9876(2)
C(1)	3118(2)	6476(3)	10886(3)
C(2)	3770(3)	6405(3)	11576(3)
N(2)	4344(2)	7061(2)	11400(2)
C(3)	4105(3)	7858(3)	11835(3)
N(11)	5227(2)	5969(2)	10400(2)
C(21)	5492(2)	5291(2)	9951(3)
C(31)	5904(2)	4682(2)	10400(4)
C(41)	6054(2)	4771(3)	11364(4)
C(51)	5804(2)	5462(3)	11834(3)
C(61)	5385(2)	6055(2)	11345(2)
C(71)	5084(3)	6818(3)	11825(3)
N(12)	3795(1)	8120(2)	9554(2)
C(22)	3983(2)	8919(2)	9693(3)
C(32)	3454(2)	9532(2)	9805(4)
C(42)	2704(2)	9329(3)	9783(4)
C(52)	2496(2)	8519(3)	9609(3)
C(62)	3055(2)	7927(2)	9492(2)
C(72)	2893(2)	7030(3)	9284(3)
N(13)	4451(2)	6340(2)	8510(2)
C(23)	4909(2)	6396(2)	7742(3)
C(33)	4900(3)	5800(3)	7018(3)
C(43)	4434(3)	5139(3)	7089(3)
C(53)	3956(3)	5083(3)	7866(3)
C(63)	3970(2)	5692(2)	8564(3)
C(73)	3478(2)	5657(2)	9452(3)

Solution and Refinement of the Structures. Both structures were solved by direct methods with the program SHELXS86[20] and refined by using the SHELXL93[21] programs. The drawings were prepared with ORTEPII.[22]

Structure of $[L_5Fe^{II}Cl]BPh_4$. The complex crystallizes in the orthorhombic noncentrosymmetrical $P2_12_12_1$ space group with mononuclear iron cation and tetraphenylborate molecule.

Structure of $[L_5{}^+Fe^{II}Cl](ClO_4)_2$. This complex belongs to the monoclinic $P2_1/a$ space group, and the structure consists of a molecular cation and two perchlorate anions, one of them being disordered over two sites with nonidentical occupations (0.75 and 0.25). Particular disorder is observed in the cation molecule (two orientations of the methyl groups at the nitrogen atom of trimethylammonium are realized with site populations of 0.80 and 0.20).

Both structures were refined by full-matrix least-squares approximation based on F^2. Refinement was anisotropic for non-disordered and major components of disordered non-hydrogen atoms. All hydrogen atoms were localized in a difference Fourier synthesis. These were included in the final cycles of refinement with variable isotropic thermal parameters. The hydrogen atoms of most populated site of disordered methyl groups were included in the refinement cycles with fixed isotropic thermal parameters. Final refinement of this model was continued until convergence when $R_1 = 0.050$ for $F^2 > 2\sigma(F^2)$ and $R_w = 0.076$ for $[L_5Fe^{II}Cl]BPh_4$ and $R_1 = 0.052$ for $F^2 > 2\sigma(F^2)$ and $R_w = 0.137$ for $[L_5{}^+Fe^{II}Cl](ClO_4)_2$ were reached. The final difference map showed the largest residual peaks of 0.195 and −0.192 e Å^{-3} for $[L_5Fe^{II}Cl]BPh_4$ and 0.558 and −0.329 e Å^{-3} for $[L_5{}^+Fe^{II}Cl](ClO_4)_2$. The final atomic coordinates are given in Tables 2 and 4; selected bond distances and angles are listed in Tables 3 and 5. A list of all bond distances and angles and a list of anisotropic thermal parameters are available as Supporting Information.

EPR Spectra. EPR spectra were recorded on Bruker ER 200 D and 300 spectrometers at the X-band. For low-temperature studies, an Oxford Instruments continuous flow liquid helium cryostat and a

(20) Sheldrick, G. M. *SHELXS86. Program for the solution of crystal structures*; University of Göttigen: Göttingen, Germany, 1986.
(21) Sheldrick, G.M. *SHELXL-93. Program for the refinement of crystal structures*; University of Göttingen: Göttingen, Germany, 1993.
(22) Johnson, C. K. ORTEPII. Report ORNL-5138; Oak Ridge National Laboratory: Oak Ridge, TN, 1976.

Table 3. Selected Bond Lengths (Å) and Angles (deg) for $[L_5Fe^{II}Cl]^+$

Fe−N(13)	2.171(3)	Fe−N(11)	2.270(3)
Fe−N(12)	2.200(3)	Fe−N(1)	2.274(3)
Fe−N(2)	2.248(3)	Fe−Cl	2.330(1)
N(13)−Fe−N(12)	102.63(10)	N(2)−Fe−N(1)	78.36(11)
N(13)−Fe−N(2)	143.35(11)	N(11)−Fe−N(1)	97.77(10)
N(12)−Fe−N(2)	93.26(10)	N(13)−Fe−Cl	106.79(8)
N(13)−Fe−N(11)	85.92(10)	N(12)−Fe−Cl	94.70(8)
N(12)−Fe−N(11)	167.04(10)	N(2)−Fe−Cl	104.51(8)
N(2)−Fe−N(11)	74.32(10)	N(11)−Fe−Cl	92.05(8)
N(13)−Fe−N(1)	74.05(11)	N(1)−Fe−Cl	170.18(8)
N(12)−Fe−N(1)	75.67(10)		

Table 4. Fractional Coordinates for $[L_5{}^+Fe^{II}Cl]^{2+}$

	x	*y*	*z*
Fe	3896(1)	1727(1)	1889(1)
Cl(1)	2545(1)	801(1)	1829(1)
N(11)	4716(3)	672(3)	1284(2)
C(21)	4492(4)	−281(4)	1162(3)
C(31)	5102(5)	−966(5)	922(4)
C(41)	5975(5)	−661(5)	798(4)
C(51)	6213(4)	297(5)	929(4)
C(61)	5564(4)	962(4)	1156(3)
C(71)	5762(4)	2050(4)	1234(3)
N(12)	3662(3)	2694(3)	891(3)
C(22)	2989(4)	2611(5)	339(3)
C(32)	2829(5)	3300(6)	−239(4)
C(42)	3367(5)	4128(6)	−230(4)
C(52)	4065(5)	4224(5)	327(4)
C(62)	4211(4)	3490(4)	872(3)
C(72)	4970(4)	3558(4)	1496(4)
N(13)	3483(3)	2878(3)	2684(3)
C(23)	3072(4)	3737(4)	2495(4)
C(33)	2936(5)	4471(5)	3012(5)
C(43)	3262(7)	4349(6)	3748(6)
C(53)	3701(6)	3481(6)	3961(4)
C(63)	3784(4)	2756(4)	3418(3)
C(73)	4193(5)	1764(5)	3636(3)
N(1)	5211(3)	2566(3)	1785(3)
C(1)	5674(4)	2603(4)	2564(3)
C(2)	5617(4)	1619(4)	2965(3)
N(2)	4658(3)	1278(3)	3015(2)
C(3)	4649(4)	189(4)	3136(3)
C(4)	5114(6)	−188(5)	3884(4)
C(5)	5096(7)	−1300(6)	3939(4)
C(6)	5747(5)	−1832(5)	3446(4)
C(7)	5658(8)	−2920(6)	3523(6)
N(3)	6348(4)	−3562(4)	3189(3)
C(8)	6430(8)	−3328(8)	2350(6)
C(9)	5953(8)	−4614(7)	3174(7)
C(10)	7160(2)	−3569(4)	3633(2)
C(11)	7220(1)	−3093(9)	2802(1)
C(12)	6804(4)	−4415(3)	3617(6)
C(13)	5813(4)	−3884(8)	2614(5)

Table 5. Selected Bond Lengths (Å) and Angles (deg) for $[L_5{}^+Fe^{II}Cl]^{2+}$

Fe−N(11)	2.181(4)	Fe−N(1)	2.258(4)
Fe−N(12)	2.188(4)	Fe−N(2)	2.283(4)
Fe−N(13)	2.196(4)	Fe−Cl(1)	2.345(1)
N(11)−Fe−N(12)	94.2(2)	N(13)−Fe−N(2)	77.6(2)
N(11)−Fe−N(13)	162.2(2)	N(1)−Fe−N(2)	79.9(2)
N(12)−Fe−N(13)	92.3(2)	N(11)−Fe−Cl(1)	96.82(12)
N(11)−Fe−N(1)	78.0(2)	N(12)−Fe−Cl(1)	101.12(12)
N(12)−Fe−N(1)	74.3(2)	N(13)−Fe−Cl(1)	98.19(12)
N(13)−Fe−N(1)	87.9(2)	N(1)−Fe−Cl(1)	172.63(12)
N(11)−Fe−N(2)	89.1(2)	N(2)−Fe−Cl(1)	105.42(12)
N(12)−Fe−N(2)	152.6(2)		

temperature control system were used. X-band parallel-mode EPR experiments were performed by using a Bruker ER4116DM dual-mode cavity.

Electrospray Ionization Mass Spectrometry. Mass spectrometry experiments were performed on a Quattro II (Micromass, Manchester, U.K.) triple quadrupole electrospray mass spectrometer. Typical optimized values for the source parameters were as follows: capillary 2.5 kV, counter electrode 0.4 kV, source temperature 80 °C, RF lens 0.7 V, skimmer lens offset 5 V. All mass spectra were obtained at a cone voltage of 62 V. Collisional activation experiments were carried out by selecting the parent ion with the first quadrupole and allowing its dissociation in the second quadrupole (RF-only hexapole) cell, using a static pressure of argon of 5×10^{-4} mbar. The daughter ion spectra were obtained by scanning the third quadrupole.

Metalation of Ligands for DNA Cleavage Experiments. The different iron complexes were obtained by mixing at room temperature an equimolar ratio of the desired ligand and iron(III) salt in water (Milli-Q grade). Further dilutions of these initial solutions were done in water in order to add 2 μL of complex solution at the different desired concentrations to DNA solutions. Metalation of L_5 ligands: 1 mL of a 1 mM solution of L_5 in H_2O was added to 10 μL of a 100 mM water solution of $Fe^{III}(ClO_4)_3$. The resulting iron complex was formally written as L_5Fe^{III}. Metalation of bleomycin (BLM): 1 mL of a 50 μM BLM solution in H_2O was added to 5 μL of 10 mM water solution of $Fe^{III}(ClO_4)_3$.

DNA Cleavage Experiments. Cleavage of DNA was carried out in a final volume of 10 μL containing 125 ng of ΦX174 supercoiled DNA (3.5 nM as plasmid final concentration corresponding to 19 μM in base pairs) in 10 mM Tris/HCl buffer pH 7 or 8. Plasmid−DNA cleavage reactions were performed at ambient temperature or at 30 °C and lasted respectively 60 or 30 min without preincubation of iron complexes with DNA. The nuclease activity of the different iron complexes was tested in the presence of air and a reducing agent (ascorbate) or after activation by an oxidizing agent (H_2O_2, $KHSO_5$ or MMPP). The final addition of one of these reagents was considered to start the reaction. Variations of reagent concentrations and experimental conditions are indicated in figure legends. At the end of reaction, 5 μL of a loading buffer [glycerol and 10 mM Tris/HCl buffer at pH 8 (50/50, v/v) and a droplet of bromophenol blue] was added and the samples were immediately deposited and run on a 0.8% agarose slab gel containing 1 μg/mL of ethidium bromide. The electrophoretic migration was at constant current (25 mA) overnight in a 89 mM Tris/borate buffer at pH 8.3 with 2.5 mM EDTA. Fluorescent bands of DNA were then photographed under UV light.

Results and Discussion

Structures. $[L_5Fe^{II}Cl]BPh_4$. The structure of the $[L_5Fe^{II}Cl]^+$ cation is represented in Figure 1. The Fe(II) ion is in a pseudooctahedral environment with the L_5 ligand wrapped around. The sixth position is occupied by the chloride ion. Of the several isomers formally possible, that which is observed has the *N*,*N*′-bis(2-pyridylmethyl)-ethane-1,2-diamine fragment in a cis α configuration around the metal ion. This cis α configuration is always that favored with an ethane-1,2-diamine unit (ref 16 and references therein). A similar structure has been identified by Bernal et al.[8] for the complex $[L_1Fe^{II}(H_2O)]^{2+}$ (L_1 = *N*,*N*′-bis(6-methyl-2-pyridylmethyl)-*N*,*N*′-bis(2-pyridylmethyl)ethane-1,2-diamine). L_1 is a potentially hexadentate ligand, but in that structure it is only pentadentate. The distances around the Fe(II) ion in $[L_5Fe^{II}Cl]^+$ are given in Table 3. As expected, the largest bond distance is the Fe−Cl (2.330(1) Å). The Fe−N distances are similar to those observed for $[L_1Fe^{II}(H_2O)]^{2+}$.[8] They are close to 2.2 Å, which is characteristic of high-spin Fe(II). On the other hand, when Fe(II) is low spin, the Fe−N distances are 0.2 Å shorter, as observed, for instance, in $[L_2Fe^{II}(CH_3CN)]^{2+}$ (L_2 = *N*,*N*-bis(2-pyridylmethyl)-*N*,*N*′-bis(2-pyridyl)methylamine)[9] and $[(TPEN)Fe^{II}]^{2+}$ (TPEN = *N*,*N*,*N*′,*N*′-tetrakis(2-pyridylmethyl)ethane-1,2-diamine).[23]

(23) Chang, H.-R.; McCusker, J. K.; Toftlund, H.; Wilson, S. R.; Trautwein, A. X.; Winkler, H.; Hendrikson, D. N. *J. Am. Chem. Soc.* **1990**, *112*, 6814.

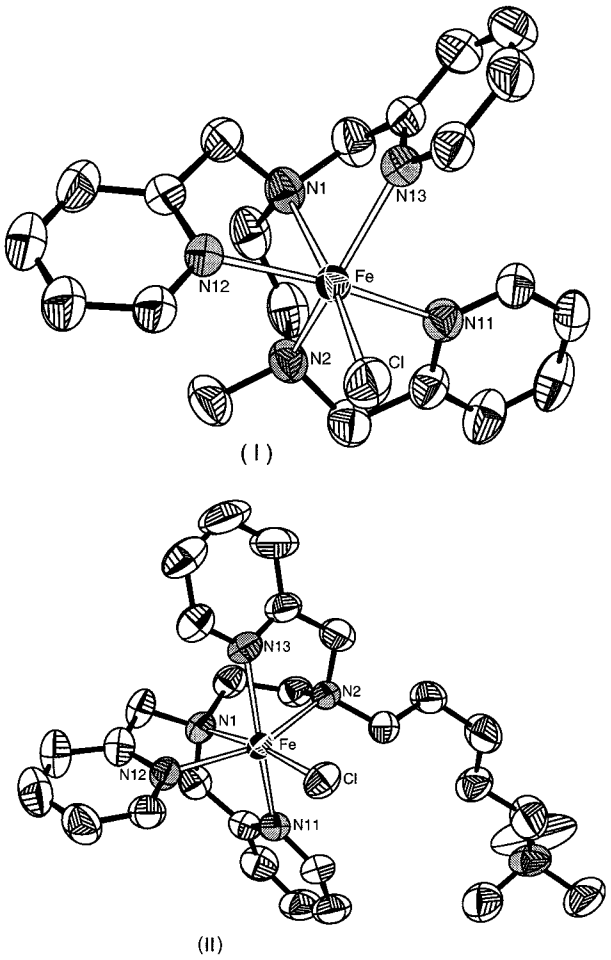


Figure 1. ORTEP diagrams of $[L_5Fe^{II}Cl]^+$ (**I**) and $[L_5{}^+Fe^{II}Cl]^{2+}$ (**II**) with thermal ellipsoids at 50% probability. In $[L_5{}^+Fe^{II}Cl]^{2+}$ only the major part of the disordered methyl groups is shown.

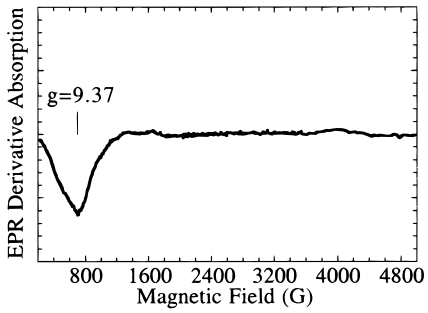


Figure 2. EPR spectrum of a powder sample of [$L_5Fe^{II}(H_2O)$](BPh_4)$_2$ at 4.2 K with a parallel mode cavity. $\nu = 9.3266$ GHz.

[$L_5{}^+Fe^{II}Cl$](ClO_4)$_2$. The structure of the $[L_5{}^+Fe^{II}Cl]^{2+}$ dication is represented in Figure 1. It is similar to the previous one. As expected, the Fe−N distances are typical of high-spin Fe(II) (see Table 5).

EPR Spectroscopy. The EPR spectrum of a powder sample of [$L_5Fe^{II}(H_2O)$](BPh_4)$_2$ at 4.2 K with a parallel-mode cavity is represented in Figure 2. The g value has been found equal to 9.37. This value is very close to those observed for $Fe^{II}SO_4 \cdot 6H_2O$ in frozen aqueous solution ($g = 9.36$) and for the [Fe^{II}-(2-methylimidazole)(mesotetraphenylporphyrin)]$^{2+}$ complex in powder.[24] This transition has been assigned to a resonance inside the $|\pm 2'\rangle$ doublet. We propose the same origin for the signal observed in [$L_5Fe^{II}(H_2O)$](BPh_4)$_2$. The large intensity of the observed resonance suggests that D is negative. This also shows that this compound remains high spin even at 4.2 K.

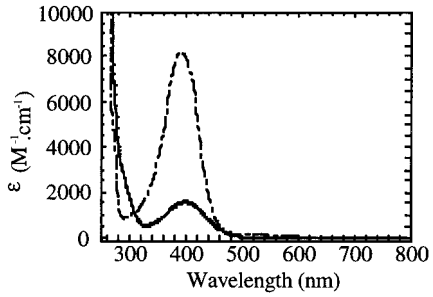


Figure 3. Comparison of the UV−vis spectra of [$L_5Fe^{II}(H_2O)$](BPh_4)$_2$ in acetonitrile (−•−) and acetone (−).

UV−Visible Spectroscopy. The UV−vis spectra in acetone and acetonitrile of [$L_5Fe^{II}(H_2O)$](BPh_4)$_2$ are represented in Figure 3. They are dominated by a LMCT band around 400 nm, the intensity of which is strongly solvent dependent. In acetonitrile the band is intense (389 nm, 8000 M^{-1} cm^{-1}). The large intensity of such a band has already been observed for low-spin Fe(II) complexes.[8] We propose that acetonitrile substitutes for water in $[L_5Fe^{II}(H_2O)]^{2+}$, driving the Fe(II) ion to a low-spin state. In acetone the same band occurs at 397 nm (1600 M^{-1} cm^{-1}). The drop in intensity of that band has already been demonstrated for high-spin Fe(II) complexes.[8] This suggests that either the acetone molecule does not displace the water molecule or, if it substitutes, it does not exert a ligand field effect large enough to make the Fe(II) low spin.

The increase in intensity of a similar band in the spin transition system $[(TPEN)Fe^{II}]^{2+}$ when the complex goes low spin upon cooling has already been studied by Chang et al.[23]

These observations are in keeping with the description of this 400 nm band as arising from MLCT between the Fe(II) "t_{2g}" orbitals and the π^* pyridine orbitals based on overlap consideration. First, the relative insensitivity of the energy of this MLCT transition to the spin state of Fe(II) is in agreement with the "t_{2g}" nature of the d orbitals implied. In fact the disappearance of the putative contribution of the "e_g" orbitals when the metal ion goes from high spin to low spin would expectedly be accompanied by an increase in energy of that transition. Second, the increase in intensity of this MLCT band in going from high-spin Fe(II) $t_{2g}{}^4e_g{}^2$ to low-spin Fe(II) $t_{2g}{}^6$ is related to the increase in the number of "t_{2g}" electrons.

UV−vis spectra of [$L_5Fe^{II}Cl$](BPh_4) have been recorded in acetone and acetonitrile. The MLCT band is observed at 400 nm. This slight red shift upon substitution of water by chloride in similar complexes has already been reported.[8] The intensity is now insensitive to the solvent and stays close to $\epsilon = 1500$ M^{-1} cm^{-1}. It appears that the acetonitrile is not able to displace the chloride anion and that the complex stays high spin. The corresponding observations have been made using ^1H NMR spectroscopy. The spectrum of [$L_5Fe^{II}(H_2O)$](BPh_4)$_2$ in CD_3COCD_3 shows resonances typical of a high-spin complex in the range 2−94 ppm. In CD_3CN the spectrum corresponds to a diamagnetic species. This preliminary observation confirms the ability of CH_3CN to make the Fe(II) species low spin. This is in full agreement with the study by Zang et al. of [(TPA)-$Fe^{II}(CH_3CN)_2]^{2+}$ [25] (low spin) and $[(TPA)_2Fe^{II}{}_2Cl_2]^{2+}$ [26] (high spin).

(24) Hendrich, M. P.; Debrunner, P. G. *J. Magn. Reson.* **1988**, *78*, 133.

(25) Zang, Y.; Kim, J.; Dong, Y.; Wilkinson, E. C.; Appelman, E. H.; Que, L., Jr. *J. Am. Chem. Soc.* **1997**, *119*, 4197.

(26) Zang, Y.; Jang, H. G.; Chiou, Y. M.; Hendrich, M. P.; Que, L., Jr. *Inorg. Chim. Acta* **1993**, *213*, 41.

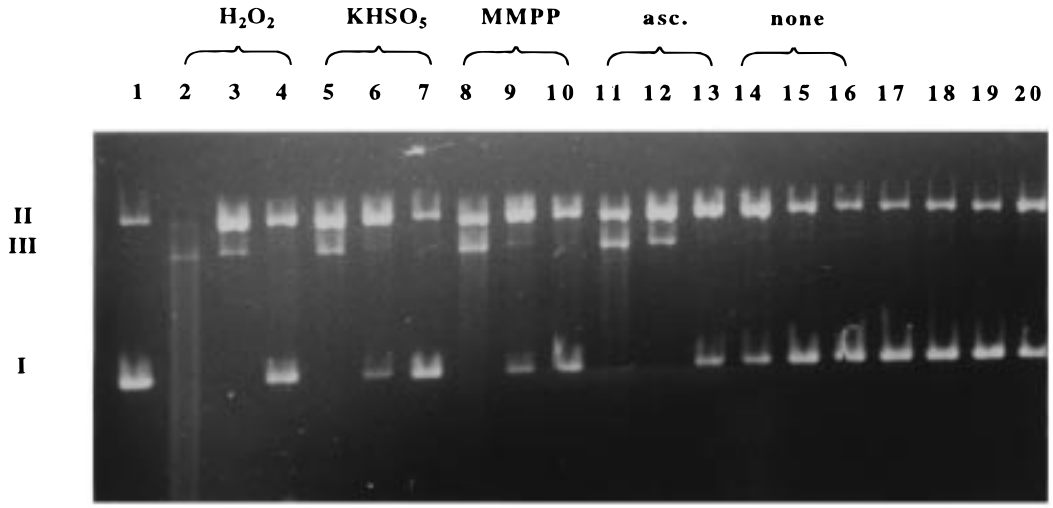


Figure 4. DNA cleavage by L_5Fe^{III} complex in the presence of various cofactors. L_5Fe^{III} was incubated with ΦX174 supercoiled DNA in the presence of 1 mM H_2O_2 (lanes 2−4), 1 mM $KHSO_5$ (lanes 5−7), 0.1 mM MMPP (lanes 8−10), or 1 mM ascorbate (lanes 11−13) or without any added cofactor (lanes 14−16). In each type of cleavage condition, three concentrations of L_5Fe^{III} were assayed: 10, 1, and 0.1 μM from left to right of the figure. Controls: ΦX174 DNA alone (lane 1), or incubated only in the presence of 1 mM H_2O_2 (lane 17), 1 mM $KHSO_5$ (lane 18), 0.1 mM MMPP (lane 19), or 1 mM ascorbate (lane 20) as for assays. Cleavage reactions were performed at room temperature in Tris/HCl buffer (pH 8) for 60 min.

The dication $[L_5{}^+Fe^{II}Cl]^{2+}$ in acetonitrile exhibits the MLCT band at 398 nm (1600 M^{-1} cm^{-1}). The halogenated forms being the only forms available, we did not conduct the same study as above.

Formation of the Peroxo Form. The group of Toftlund[8] showed that $[L_5Fe^{II}Cl](PF_6)$ in MeOH in the presence of an excess of H_2O_2 gives a purple species (λ_{max} = 537 nm, ϵ = 750 M^{-1} cm^{-1}) with an almost axial X-band EPR spectrum (g_1 = 2.19, g_2 = 2.12, g_3 = 1.95). They proposed that this species was an $Fe^{III}OOH$ derivative. A 1 mM solution of $[L_5Fe^{II}Cl](PF_6)$ in MeOH in the presence of 100 equiv of H_2O_2 gives the purple hydroperoxo species with λ_{max} = 537 nm and ϵ = 1000 M^{-1} cm^{-1} (slightly larger than in ref 8) and the same EPR spectrum as that reported by Bernal et al. These spectroscopic data are very similar to those observed by Lubben et al.[9] and de Vries et al.[11] with other pentadentate polypyridine ligands. With 0.5 equiv of H_2O_2, a UV−vis spectrum different from that of $[L_5Fe^{II}Cl]^+$ is obtained. This solution exhibits a rhombic EPR X-band spectrum with g_1 = 2.30, g_2 = 2.12, g_3 = 1.92. This corresponds thus to a low-spin Fe(III) species, which we tentatively describe as $[L_5Fe^{III}Cl]^{2+}$. A similar species has been obtained by Bernal et al.[8] using bromine as an oxidant. The optical density at 537 nm then increased with the further addition of H_2O_2, with an isosbestic point at 465 nm. We needed 100 equiv of H_2O_2 to get the maximum optical density at 537 nm. The experiment was done at 0 °C. An equilibrium must be involved such as

$$[L_5Fe^{III}Cl]^{2+} + H_2O_2 \rightleftharpoons [L_5Fe^{III}OOH]^{2+} + H^+ + Cl^-$$

At room temperature, the UV−vis and EPR spectra change slowly (half-time at room temperature in MeOH: 1 h) into those of an Fe(III) species similar to $[L_5Fe^{III}Cl]^{2+}$.

Electrospray ionization mass spectrometry experiments were carried out on the purple species prepared by addition of 100, 200 or 400 equiv of H_2O_2 to a methanol solution of $[L_5Fe^{II}Cl]Cl$. The spectrum of the latter complex was dominated by the peak at *m/z* 438 corresponding to $[L_5Fe^{II}Cl]^+$. After the addition of 400 equiv of H_2O_2, a new peak at *m/z* 218 appeared which corresponds to the entity $[L_5Fe^{III}OOH]^{2+}$. The spectrum shows other peaks, among them one at *m/z* 438 which is due to $[L_5Fe^{II}Cl]^+$ and one at *m/z* 345, attributed to $[L_4Fe^{II}Cl]^+$ where L_4 is obtained from L_5 by loss of a pyridylmethyl arm. Both species are probably formed by the reduction under the acceleration field of the Fe^{III} corresponding species. The $[L_4Fe^{II}Cl]^+$ species was also observed when $[L_5Fe^{II}Cl]^+$ ions were selected by the first quadrupole and fragmented by collision with argon in the RF-only hexapole.

When a $[L_5Fe^{II}Cl]PF_6$ solution was used to prepare the purple species, peaks at *m/z* 422 and 311 were observed. Those peaks correspond to $[L_5Fe^{II}F]^+$ and $[L_4Fe^{II}F]^+$ ions, which indeed disappeared by using $[L_5Fe^{II}Cl]Cl$. In fact, hydrolysis of the PF_6^- anions under an excess of hydrogen peroxide led to F^- anions which coordinated to iron.

DNA Cleavage. Supercoiled plasmids are sensitive tools to detect and to quantify a low level of single-strand DNA breaks (see refs 28−30 and references therein). The nuclease activity of the iron complexes of L_5 and $L_5{}^+$, L_5Fe^{III} and $[L_5{}^+Fe^{II}Br]^{2+}$, respectively, was studied by using different modes of activation: a reducing agent (ascorbate) in the presence of air to mimic the in vivo activation mode of bleomycin[4,31,32] or an oxidant like hydrogen peroxide,[2,33] potassium monopersulfate,[28,34,35] or magnesium monoperphthalate.[36,37] L_5Fe^{III} showed significant DNA cleavage reactivity in the 0.1−10 μM concentration range (Figure 4). The amount of DNA breaks increased as a function of the iron complex concentration as evidenced by the progressive transformation of plasmid form I to form II and then to

(27) This footnote was deleted on revision.
(28) Silver, G. C.; Trogler, W. C. *J. Am. Chem. Soc.* **1995**, *117*, 3983.
(29) Bernadou, J.; Pratviel, G.; Bennis, F.; Girardet, M.; Meunier, B. *Biochemistry* **1989**, *28*, 7268.
(30) Hertzberg, R. P.; Dervan, P. B. *Biochemistry* **1984**, *23*, 3934.
(31) Hecht, S. M. *Bioconjugate Chem.* **1994**, *5*, 513.
(32) Pratviel, G.; Bernadou, J.; Meunier, B. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 746.
(33) Sigman, D. S.; Bruice, T.W.; Mazumder, A.; Sutton, C. L. *Acc. Chem. Res.* **1993**, *26*, 98.
(34) Pitie, M.; Bernadou, J.; Meunier, B. *J. Am. Chem. Soc.* **1995**, *117*, 2935.
(35) Chen, X.; Rokita, S. E.; Burrows, C. J. *J. Am. Chem. Soc.* **1991**, *113*, 5884.
(36) Pratviel, G.; Bernadou, J.; Ricci, M.; Meunier, B. *Biochem. Biophys. Res. Commun.* **1989**, *160*, 1212.
(37) Mack, D. P.; Dervan, P. B. *Biochemistry* **1992**, *31*, 9399.

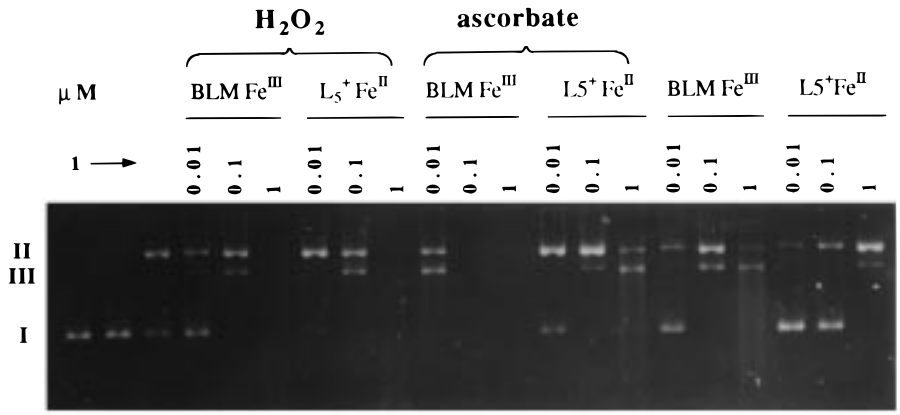


Figure 5. Comparison of the cleavage efficiency of iron complexes of L_5^+ and BLM. The numbering of lanes is from left to right as indicated by the arrow. The iron complexes were activated for DNA cleavage by 1 mM H_2O_2 (lanes 4−9) or by 1 mM ascorbate (lanes 10−15) or incubated with DNA without addition of cofactors (lanes 16−21). The concentration of $[L_5^+Fe^{II}Br]^{2+}$ complex varied from 10 nM (lanes 7, 13, and 19) to 100 nM (lanes 8, 14, and 20) to 1 μM (lanes 9, 15, and 21). The concentration of $(BLM)Fe^{III}$ varied similarly from 10 nM (lanes 4, 10, and 16) to 100 nM (lanes 5, 11, and 17) to 1 μM (lanes 6, 12, and 18). Controls include ΦX174 supercoiled DNA (lane 1), incubated in the presence 1 mM H_2O_2 (lane 2) or 1 mM ascorbate (lane 3) or incubated in the presence of metallocomplexes only: 10 nM, 100 nM, and 1 μM $(BLM)Fe^{III}$ (lanes 16−18, respectively); 10 nM, 100 nM, and 1 μM $[L_5^+Fe^{II}Br]^{2+}$ (lanes 19−21). The reaction was carried out for 60 min in 10 mM Tris/HCl buffer pH 7.5 at 30 °C.

form III from the right to the left in each triad corresponding to various cleaving conditions. Single-strand breaks can be observed at a concentration of L_5Fe^{III} of 0.1 mM in lane 4 (1 mM H_2O_2), lane 10 (0.1 mM MMPP), or lane 13 (1 mM ascorbate). The cleavage efficiency of L_5Fe^{III} was about the same with all these cofactors. A better activity was observed in the presence of H_2O_2 (lanes 2−4). When DNA was incubated with L_5Fe^{III} alone, some breaks were observed when the complex concentration was increased (lanes 14−16). This phenomenon was probably due to the presence of reducing agents in the reaction mixture, but the amount of breaks was significantly below the level of the corresponding assays. We also checked that control experiments with iron salts without ligand in the presence of reducing or oxidizing agents did not show any DNA breaks at the concentrations used in the present study.

The iron complex of L_5^+ ligand showed a better efficiency than the L_5 one due to the additional positive charge on the structure. In the same conditions as described for L_5Fe^{III} we noted that it was about 10 times more active with all different cofactors used to mediate DNA cleavage. A better efficiency was also observed with $[L_5^+Fe^{II}Br]^{2+}$ when H_2O_2 was used. The DNA cleavage by this complex is illustrated in Figure 5, where it was compared to $(BLM)Fe^{III}$. $[L_5^+Fe^{II}Br]^{2+}$ exhibited the same efficiency as $(BLM)Fe^{III}$ in promoting DNA cleavage in the presence of H_2O_2 (lanes 4−6 for BLM and 7−9 for L_5^+), but its activity was below that of bleomycin when the activating was performed with ascorbate (compare lanes 10−12 and 13−15 for BLM and L_5^+, respectively). As noted in Figure 4, the controls obtained when incubating plasmid DNA with the iron complexes alone (lanes 16−21) indicated some DNA breaks. In the case of $[L_5^+Fe^{II}Br]^{2+}$ the amounts of breaks in the controls were significantly lower than those observed in the corresponding assays. For iron−BLM, the control patterns were equivalent to the assays in the presence of H_2O_2. We tentatively attributed these DNA breaks to traces of reducing agents that may be present in the reaction medium or in agarose gels considering the high potency of iron−BLM to induce DNA breaks in reducing conditions (for similar observations, see refs 38 and 39).

In order to obtain additional information regarding the mechanism of DNA cleavage, radical scavengers (ethanol, DMSO), superoxide dismutase (SOD), and catalase were included in the investigation as potential inhibitors of the strand scission activity.[38,40,41] DNA breaks mediated by L_5Fe^{III} and $[L_5^+Fe^{II}Br]^{2+}$ were not affected by the presence of 10% ethanol, 0.4 M DMSO, or glycerol, suggesting that diffusible hydroxyl radicals were not involved in complex-mediated DNA damages with all the different activating agents. When DNA cleavage was performed with the $[L_5^+Fe^{II}Br]^{2+}$/ascorbate system in the presence of SOD (0.1 mg/mL) or catalase (0.1 mg/mL), 30−40% inhibition or 80−100% inhibition was respectively observed. These data indicate that, at least with ascorbate, a reductive dioxygen activation cascade is mainly responsible for DNA cleavage. In particular, H_2O_2 is clearly involved in the mechanism of the reaction as it has been shown previously for iron−methidiumpropyl EDTA[39] or for copper−orthophenanthroline complexes.[40] However, it should be noted that the direct activation of these iron complexes by hydrogen peroxide was not inhibited by hydroxyl radical scavengers (ethanol, DMSO, and glycerol), suggesting that the formation of hydroperoxo−iron complexes might be responsible for DNA cleavage without formation of freely diffusible hydroxyl radicals.

Conclusion

The complexes $[L_5Fe^{II}Cl]BPh_4$ and $[L_5Fe^{II}(H_2O)](BPh_4)_2$ have been isolated. Bernal et al.[8] have prepared the complex $[L_5Fe^{II}Cl]PF_6$. We confirmed the result of Bernal et al.[8] that these complexes in the presence of an excess of H_2O_2 give a purple species with spectroscopic signatures analogous to those of "activated bleomycin", which is very likely the $[L_5Fe^{III}(OOH)]^{2+}$ derivative. Indeed, using $[L_5Fe^{II}Cl]Cl$ we identified, by electrospray ionization mass spectrometry, the $[L_5Fe^{III}(OOH)]^{2+}$ species. In fact, a L_5/Fe system gave single-strand breaks on plasmid DNA in the presence of either a reducing agent (ascorbate) and air or oxidants (H_2O_2, $KHSO_5$, MMPP) at 0.1 μM concentration.

(38) Pratviel, G.; Bernadou, J.; Ricci, M.; Meunier, B. *Biochem. Biophys. Res. Commun.* **1986**, *136*, 1013.
(39) Byrnes, R. W.; Fiel, R. J.; Datta-Gupta, N. *Chem.-Biol. Interactions* **1988**, *67*, 225.
(40) Hertzberg, R. P.; Dervan, P. B. *J. Am. Chem. Soc.* **1982**, *104*, 313.
(41) Marshall, L. E.; Graham, D. R.; Reich, K. A.; Sigman, D. S. *Biochemistry* **1981**, *20*, 244.

In order to increase the affinity of this type of complex for DNA, we substituted a $(CH_2)_5N(CH_3)_3^+$ group for the methyl group of L_5 to get the ligand L_5^+. The complex $[L_5^+Fe^{II}Br]^{2+}$ is an efficient DNA cleaver, especially when activated by H_2O_2. The presence of the cationic charge on the ligand provided the necessary increase in DNA affinity for the complex in order to reduce the distance between the activated metal center and the DNA targets. The absence of inhibition by hydroxyl radical scavengers when these iron(III) complexes were activated directly by hydrogen peroxide suggested that nondiffusible metal−peroxo or metal−oxo species might be responsible for DNA cleavage. On the other hand, the observed inhibition of DNA breaks by SOD and catalase when these iron complexes were activated by reducting agents in the presence of air suggested that the generation of reduced oxygen species was involved in these latter conditions.

The efficient synthesis of *N*,*N*,*N*′-tris(2-pyridylmethyl)-ethane-1,2-diamine with its secondary amine group at the periphery of the ligand will facilitate the preparation of future analogues of this ligand with different vectors (minor groove binders, oligonucleotides, ...) in order to target the nuclease activity of these iron(III) complexes.

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Supporting Information Available: Tables of crystal data, atomic coordinates, selected bond lengths and angles, bond lengths and angles, thermal parameters, and H-atom coordinates for $[L_5Fe^{II}Cl]BPh_4$ and $[L_5^+Fe^{II}Cl](ClO_4)_2$. This material is available free of charge via the Internet at http://pubs.acs.org.

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