Notes

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Metal Complexes with Biologically Important Ligands. 62.[1] Platinum(II) Complexes of 3-(2-Aminoethoxy)estrone and -estradiol

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Introduction

cis-$[PtCl_2(NH_3)_2]$ and second-generation analogues are used as anticancer agents.[2] Biologically active components such as hormones[3] or 1,2-diphenyl-substituted ethylenediamines acting as estrogen-receptor binding molecules[4] have been introduced into Pt(II) complexes in order to facilitate the selective transport into cancerous cells. Platinum(II)–*O*-catecholato complexes have been bound to steroids.[5] Chelating diamine functions have been introduced into the ring A or D of steroids and coordinated to Pt(II).[6] Kidani recently reported Pt(II) complexes in which the hormone is bound to Pt(II) by oxygen functions on the ring D.[7] Gandolfi incorporated hormone molecules into the malonate moiety as the leaving group.[8] We have attached hormones to Pt(II) complexes through coordinated amino acids as esters or amides.[9] The amides were prepared from amino-derivatized estrone and estradiol.

Several neutral mixed amine compounds *cis*-$[PtCl_2(NH_3)(am)]$ show antitumor activity.[10] It has been shown that cationic mixed-amine complexes *cis*-$[PtCl(NH_3)_2(am)]^+$[11] and related cationic species,[12] the nonclassical analogues of cisplatin, are active against several types of tumors; also, negatively charged compounds $[PtCl_3(am)]^-$ (am = NH_3 or caffeine)[13a] and $[Pt(am)_2$(phosphono carboxylate$]^-$[13b] proved to be active. For this reason we have prepared *cis*-$[PtCl_2(NH_3)(am)]$ and *cis*-$[PtCl(NH_3)_2$(am)]Cl complexes with 3-(2-aminoethoxy)estrone (**1a**) and 3-(2-aminoethoxy)estradiol (**1b**) as amines.

Experimental Section

Starting Materials and Physical Methods. The complex $K[PtCl_3(NH_3)]$ was prepared according to Elleman[14] and Dhara,[15] *cis*-$[PtCl_2(NH_3)_2]$ was obtained from Prof. B. Lippert, Dortmund University, and $K_2[PtCl_4]$ was obtained from Degussa AG, Wolfgang. Melting points were determined on a Melt-Temp apparatus and are uncorrected. IR spectra were performed on a Perkin-Elmer Model 325 spectrometer (4000–200 cm^{-1}) in KBr pellets. ^1H NMR spectra were recorded on a JEOL GSX-270 instrument in DMF-d_7; ^{195}Pt NMR spectra were obtained on a AC 200 Bruker spectrometer in DMF-d_7 solutions. Conductivity was measured on a Hanna Instrument HI 8733 conductivity meter in DMF solutions. All reactions were carried out under nitrogen in absolute solvents.

Preparation of Complexes. ***cis*-$[PtCl_2(NH_3)(am)]$ (2a).** 3-(2-Aminoethoxy)estrone (**1a**)[9] (81.4 mg), (0.26 mmol) dissolved in DMF (2 mL) was slowly added to a solution of $K[PtCl_3(NH_3)]$ (94 mg, 0.26 mmol) in DMF (5 mL) at 60 °C. The mixture was heated for 3 h at 70 °C and for 1 h at 80 °C. To monitor the reaction, a drop of solution was evaporated and chromatographed on TLC (aluminum oxide, neutral, Merck, Type E, 60 F_{254}) using $CHCl_3$–EtOH (9.5:0.5) as eluent. When the free hormone had disappeared on TLC, the solution was cooled and filtered from KCl, concentrated to dryness, and triturated once with water and twice with ethanol. An analytical sample was prepared by redissolving the yellow product in a minimum volume of DMF and precipitating it with EtOAc: Yield 124 mg (80%); mp 190 °C dec. Anal. Calcd for $C_{20}H_{30}Cl_2N_2O_2Pt$: C, 40.28; H, 5.07; N, 4.70. Found: C, 40.17; H, 5.18; N, 4.71.

2b was prepared by the same procedure, which gave 122 mg (78%) of product, mp 194 °C dec. Anal. Calcd for $C_{20}H_{32}Cl_2N_2O_2Pt$: C, 40.14; H, 5.39; N, 4.68. Found: C, 40.48; H, 5.17; N, 4.41.

$[PtCl(NH_3)_2$(am)]Cl (3a) and *trans*-$(PtCl_2(NH_3)$(am)] (4a). The hormone (**1a**) (520 mg, 1.66 mmol) was dissolved in DMF (10 mL) and slowly added to a solution of *cis*-$[PtCl_2(NH_3)_2]$ (500 mg, 1.66 mmol) in DMF (20 mL, 65 °C). The reaction was stirred for 3 h at 75 °C and for 5 h at 85 °C and monitored by TLC. After cooling, a small amount of unsoluble white $[Pt(NH_3)_3(am)]Cl_2$ (**5a**) was removed by centrifu-

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gation; the solvent was evaporated and the residue triturated with EtOAc (5 × 50 mL), giving light cream-colored **3a** (789 mg, 78%). Anal. Calcd for $C_{20}H_{33}Cl_2N_3O_2Pt$: C, 39.16; H, 5.42; N, 6.85. Found: C, 39.11; H, 5.74; N, 6.71.

The ethyl acetate solution from triturations was concentrated. The yellow residue of *trans*-$[PtCl_2(NH_3)(am)]$ (**4a**) was triturated with ether (50 mg, 5%). Anal. Calcd for $C_{20}H_{30}Cl_2N_2O_2Pt$: C, 40.28; H, 5.07; N, 4.70. Found: C, 40.53; H, 5.36; N, 4.82.

The insoluble tetraamine complex $[Pt(NH_3)_3(am)]Cl_2$ (**5a**) could not be purified. Anal. Calcd for $C_{20}H_{36}Cl_2N_4O_2Pt$: C, 38.10; H, 5.76; N, 8.89. Found: C, 35.59; H, 5.75; N, 9.10.

$[PtCl(NH_3)_2(am)]Cl$ (3b) and *trans*-$[PtCl_2(NH_3)(am)]$ (4b) were prepared as described for **3a** and **4a** using ligand **1b**. Compound **3b** was obtained in 80% yield and dried for 48 h in high vacuum at 60 °C and 8 h at 70 °C. Anal. Calcd for $C_{20}H_{35}Cl_2N_3O_2Pt \cdot {}^1/_4CH_3CO_2C_2H_5$: C, 39.56; H, 5.85; N, 6.59. Found: C, 39.30; H, 6.02; N, 6.38. Complex **4b** was isolated from ethyl acetate solution. Anal. Calcd for $C_{20}H_{32}Cl_2N_2O_2Pt$: C, 40.14; H, 5.39; N, 4.68. Found: C, 39.68; H, 5.61; N, 4.77.

3b was also prepared by stirring *cis*-$PtCl_2(NH_3)_2$ (60 mg, 0.2 mmol) with $AgNO_3$ (33.9 mg, 0.2 mmol) in dry DMF (20 mL) at ambient temperature in darkness for 15 h. After removal of AgCl by centrifugation, **1b** (63 mg, 0.2 mmol) was added and the reaction mixture left for 48 h under N_2. The solvent was removed in vacuum and the residue triturated with ethyl acetate (5 × 20 mL), redissolved in minimum DMF, and precipitated with ethyl acetate, yielding **3b**, 99 mg, 80%.

In Vitro Chemosensitivity Testing. Cell Line and Culture Conditions. The hormone sensitive MCF-7 (ATCC No. HTB 22) human breast cancer cell line[16] was obtained from the American Type Culture Collection (ATCC) (Rockville, MD). Cell line banking and quality control was performed according to the "seed stock concept" reviewed by Hay.[17] The cells were routinely maintained in Eagle's medium (Sigma, München, FRG) containing L-glutamine, $NaHCO_3$ (2.2 g/L), sodium pyruvate (110 mg/L), 50 mg/L gentamycin (Sebio, Walchsing, FRG), and 10% fetal calf serum (Gibco, Eggenheim, FRG). The cells were serially passaged weekly after trypsinization with trypsin (0.05%)/EDTA (0.02%) (Boehringer, Mannheim, FRG) and cultured in a water-saturated atmosphere of 95% air and 5% carbon dioxide at 37 °C in 75-cm^3 flasks (Falcon Plastics 3023, Heidelberg, FRG). Cells were routinely monitored for, and shown to be free of, Mycoplasma contamination.[18]

Drugs Used as Positive Controls. Cisplatinum (Gold Label) was obtained from Aldrich (Steinheim, FRG) and Tamoxifen (citrate salt) was purchased from Sigma (München, FRG).

Kinetic Crystal Violet Assay. The detailed procedure has been described and evaluated elsewhere.[19] Briefly, the cells (passage 187 from origin) were seeded (100 μL/well) in 96-well flat-bottomed microtitration plates (Falcon Plastics 3075, Heidelberg, FRG) at an appropriate density of ca. 15 cells per microscopic field (Leitz, Diavert, 320×). After 48 h, the medium was carefully removed by suction and replaced by fresh medium (200 μL/well) containing drug (drugs were added as a 1000-fold concentrated stock solution) or pure solvent. The platinum complexes were dissolved in DMF; the stock solutions of Tamoxifen were prepared with 70% (v/v) EtOH. On every plate, rows 5 and 6 (n = 16) served as controls (containing the appropriate solvent), whereas two vertical rows (n = 16) per drug concentration were used. After various times of incubation, the culture medium was shaken off and the cells were fixed with 100 μL of 1% glutardialdehyde in PBS per well for 15 min. The fixative was replaced by 150 μL of PBS per well and the plates were stored in the refrigerator (4 °C). At the end of the experiment all trays were stained simultaneously with 0.02% aqueous crystal violet solution (100 μL/well) for 30 min. Excess dye was removed by rinsing the trays with water for 15 min. The stain bound by the cells was redissolved in 70% ethanol (180 μL/well). Absorbance was measured at 578 nm by using a BIOTEK 309 Autoreader (Tecnomara, Fernwald, FRG).

Drug Action. Drug effects were expressed as corrected T/C values for each group according to

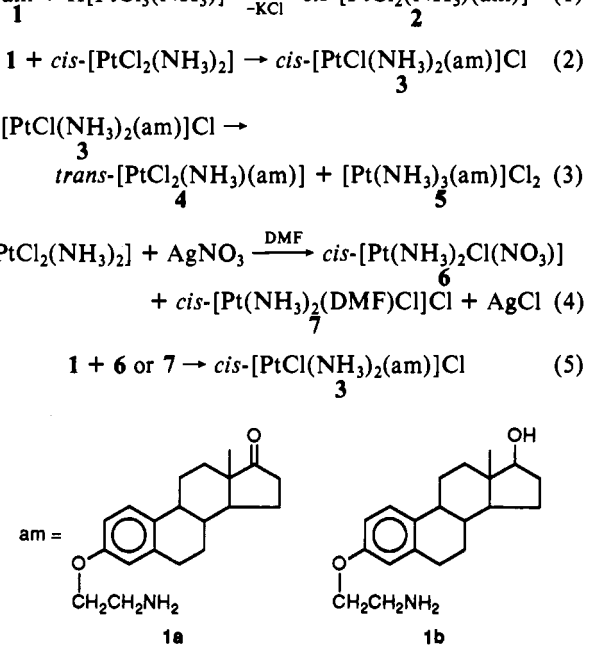
$$(T/C)_{cor} = (T - C_0)/(C - C_0) \times 100\ [\%]$$

where T is the mean absorbance of the treated cells, C is the mean absorbance of the controls, and C_0 is the mean absorbance at the time (t = 0) when drug was added. The calculated experimental errors for T/C_{cor} (according to the Gaussian formula) amounted to about 10% after prolonged times of incubation.

Results and Discussion

3-(2-Aminoethoxy)estrone (**1a**) and 3-(2-aminoethoxy)estradiol (**1b**)[9] react with $K[PtCl_3(NH_3)]$ in DMF solutions at 70–80 °C over 4 h to form the yellow neutral mixed-amine complexes *cis*-$[PtCl_2(NH_3)(am)]$ (**2**) (eq 1). The reaction of **1** with *cis*-$[PtCl_2(NH_3)_2]$ to yield the cationic complexes *cis*-$[PtCl(NH_3)_2(am)]Cl$ (**3**) (eq 2) requires a longer reaction period and is accompanied by formation of *trans*-$[PtCl_2(NH_3)(am)]$ (**4**) and the insoluble tetraamine complex $[Pt(NH_3)_3(am)]Cl_2$ (**5**), probably via a bimolecular reaction of **3** (eq 3), which may explain the trans configuration of **4** (see Scheme I). Higher temperatures or a long reaction period favor this disproportionation. Lippert has found that the cationic complex *cis*-$[PtCl(NH_3)_2L]Cl$ (L = 1-methylcytosine) releases ammonia at room temperature in aqueous solutions with formation of *trans*-$[PtCl_2(NH_3)L]$.[20] This reaction was first observed in mass spectroscopic studies.[21] Lippert has shown that the release of ammonia is not restricted to nucleobase complexes; *trans*-$[PtCl_2(NH_3)_2]$ is formed as well from aqueous solutions of $[PtCl(NH_3)_3]Cl$.[11a]

Scheme I



1a **1b**

We chose DMF as solvent, which, according to Kong and Rochon's suggestion, improves solubility and affects favorably the desired equilibria.[22] By using DMF as a solvent, Passini improved the yield of anionic complexes.[10c] **3b** was also prepared by a two-step reaction sequence: *cis*-$[PtCl_2(NH_3)_2]$ was treated with 1 equiv of $AgNO_3$ in DMF to form, according to Hollis,[11b] a mixture of active species *cis*-$[Pt(NH_3)_2(DMF)Cl]^+$ and *cis*-$[Pt(NH_3)_2(NO_3)Cl]$, which subsequently react with **1b**. Traces of **4b** were detected as well.

The separation of **3** and **5** is easy since the tetraamine complex **5** is insoluble in any organic solvent, whereas the less polar trans isomer **4**, unlike the cis isomer **2**, is soluble in EtOAc.

All complexes exhibit in their IR spectra NH stretching absorptions at 3400–3100 cm^{-1}, aliphatic CH bands of hormone skeleton at 2920 and 2850 cm^{-1}, the C=O stretching of estrone

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Table I. Selected Spectroscopic Data for 3-(2-Aminoethoxy)estrogens and Their Platinum(II) Complexes

compound	no.	$\delta(^1H)$,[a] ppm 18-CH	benzylic	CH_2N	CH_2O	aromatic			$\delta(^{195}Pt)$,[b] ppm	ν(Pt–Cl),[c] cm^{-1}
am	**1a**	0.82	2.80	2.80	3.93	6.63	6.73	7.18		
am	**1b**	0.74	2.78	2.90	3.93	6.63	6.73	7.18		
cis-$[PtCl_2(NH_3)(am)]$	**2a**	0.82	2.76	3.22	4.25	6.69	6.75	7.21		322 m
cis-$[PtCl_2(NH_3)_2(am)]$	**2b**	0.75	2.78	3.14	4.29	6.61	6.78	7.22	−2171	322 m
cis-$[PtCl(NH_3)_2(am)]Cl$	**3a**	0.82	2.80	3.20	4.20	6.65	6.70	7.18	−2023	326 w, br
cis-$[PtCl(NH_3)_2(am)]Cl$	**3b**	0.75	2.80	3.18	4.27	6.69	6.78	7.22		326 w, br
trans-$[PtCl_2(NH_3)(am)]$	**4a**	0.88	2.84	3.09	4.28	6.71	6.79	7.21		330 w, br
trans-$[PtCl_2(NH_3)(am)]$	**4b**	0.75	2.75	3.08	4.20	6.32	6.69	7.18	−2178	330 w, br

[a] NMR spectra were recorded in DMF-d_7; standard TMS. [b] External standard: $\delta(^{195}Pt)$ of K_2PtCl_6 in D_2O.[26] [c] IR spectra taken as KBr disks.

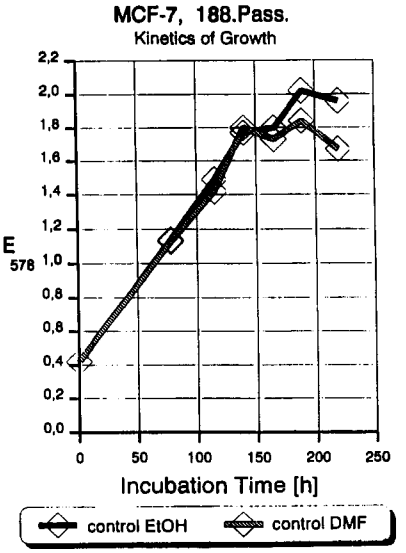

Figure 1. Growth curves of MCF-7 cells (in passage 188) used as "untreated" controls in the chemosensitivity assay. The medium contained no drug but contained the solvents EtOH or DMF.

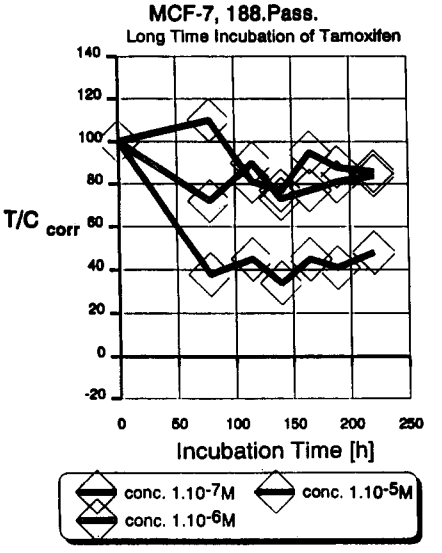

Figure 2. Effect of Tamoxifen on the hormone-sensitive human breast cancer cell line MCF-7. Plots are of corrected T/C values versus time of drug exposure.

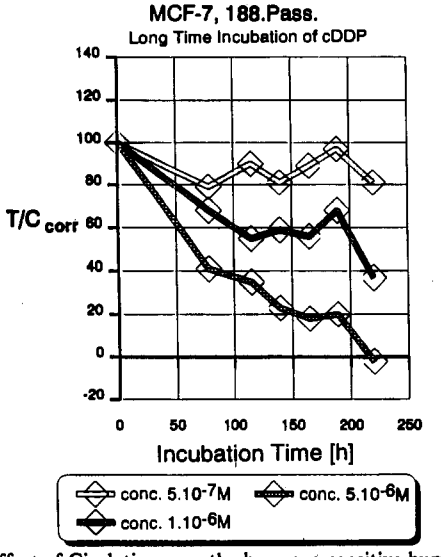

Figure 3. Effect of Cisplatinum on the hormone-sensitive human breast cancer cell line MCF-7 plotted as in Figure 2.

at 1735 cm^{-1}, and aromatic C=C bands at 1605 cm^{-1}. The isomers **2a,b** and **4a,b** differ in their Pt–Cl absorptions. The Pt–Cl band of the cis isomers **2a,b** appears at 322 cm^{-1} whereas that of the *trans*-**4a,b** is observed at 330 cm^{-1}. A similar difference has been found for *cis*- and *trans*-$PtCl_2(NH_3)_2$.[23,24] In the ^1H NMR spectra (Table I) of the complexes as in the parent hormones, 18-CH_3 appears at 0.82 ppm (am = **1a**) and at 0.75 ppm (am = **1b**), respectively. The skeleton protons are spread between 1.1 and 2.4 ppm. Benzylic protons at 2.7–2.8 ppm partially overlap with those of DMF-d_7. Aromatic protons 1-CH and 2-CH form a AB pattern (J = 8.5 Hz); 2-CH has a long-range coupling with 4-CH (J = 1.5 Hz) (see Table I). These similarities rule out a metal-catalyzed rearrangement of the ligand during formation of the complexes. The main differences are observed in the chemical shifts of the aminoethoxy group. In the free ligand, CH_2N appears as a quartet around 2.90 ppm and CH_2O appears as a triplet at 3.90 ppm (J = 5.5 ppm), whereas in the complexes CH_2N protons are shifted to 3.10–3.20 ppm and become very broad. CH_2O protons are shifted to the 4.10–4.20 ppm region and overlap with a part of the Pt–NH protons.

The ^{13}C NMR spectra of the complexes are similar to those of the corresponding ligands. The assignment of all carbons is not possible owing to the overlap with DMF-d_7 carbons, and information about the difference between the coordinated and uncoordinated CH_2NH_2 group is not available.

In the ^{195}Pt NMR spectra, the difference in chemical shifts of cis and trans isomers is 7 ppm. Farrell and Qu[25] observed 4 ppm difference between binuclear diamine cis and trans complexes (in DMF). A 148 ppm difference is observed between the neutral **2b** and the positively charged **3a**, as compared to 200 ppm for $[PtCl(NH_3)_2(pyridine)]^+$.[11b]

Conductivity of the ionic complexes **3a** and **3b** in DMF was 57 and 54 μS respectively, as compared with 2–5 μS for the free ligands and for **2a**, **2b**, **4a**, and **4b**.

Antitumor Tests. Figure 1 illustrates the effect of the solvents DMF and EtOH (used as vehicles for Cisplatin, the complexes **2b** and **3b**, and the partial antiestrogen Tamoxifen) on the pro-

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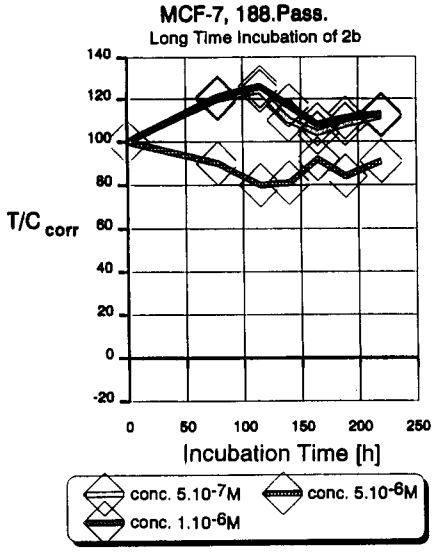

Figure 4. Effect of **2b** on the hormone-sensitive human breast cancer cell line MCF-7 plotted as in Figure 2.

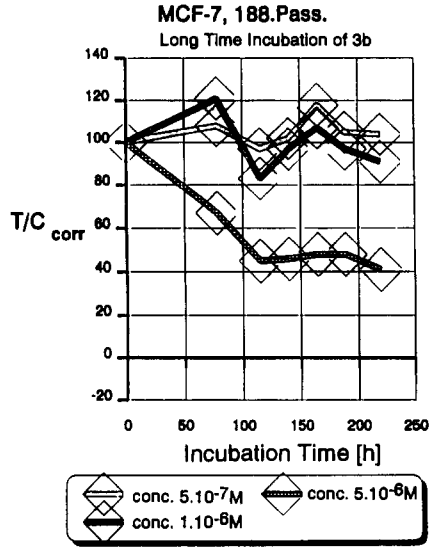

Figure 5. Effect of **3b** on the hormone-sensitive human breast cancer cell line MCF-7 plotted as in Figure 2.

liferation kinetics of MCF-7. Cell proliferation is not significantly affected by the nature of the organic solvent. The results of the in vitro chemosensitivity tests against MCF-7 are summarized in Figures 2–5. Figure 2 demonstrates that cell proliferation is inhibited by 10^{-5} M Tamoxifen, a clinically established drug in the hormonal treatment of breast cancer. The hormone-sensitive cell line was also inhibited by Cisplatinum (Figure 3). Within the selected concentration range (5×10^{-7}, 1×10^{-6}, and 5×10^{-6} M) inhibition was clearly dose-related. No effect was observed for the neutral Pt complex $[PtCl_2(NH_3)(am)]$ (**2b**) (Figure 4), whereas the cationic compound *cis*-$[PtCl(NH_3)_2(am)]Cl$ (**3b**) was cytostatic in a concentration of 5×10^{-6} M with a final T/C_{cor} value of around 40% (Figure 5). The extent of inhibition is comparable with the effect of Tamoxifen. Although we observed a marked antiproliferative effect on the estrogen receptor positive cell line MCF-7, a direct hormonal action seems unlikely because of the cytotoxic potential of **3b** against the L 1210 leukemia of the mouse. According to the generally accepted mechanism of action for platinum anticancer drugs, the positively charged complex must have crossed the cell membrane prior to cross-link formation with the bases of the DNA. Both compounds **2b** and **3b** showed activity against the L 1210 cell line of the mouse (EC 90 = 0.3 μg/mL, 6 days exposure). Complex **3b** was also active in vivo (P388; 46.4 mg/kg: T/C = 150%; LD_{50} = 158 mg/kg). Compound **3a** showed ED 90 > 1 μg/mL (L 1210).

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EPR Spectrum and Metal–Ligand Bonding Parameters of a Low-Spin (Hexaamine)iron(III) Complex

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Introduction

Comparatively few detailed investigations of the EPR spectra of low-spin iron(III) complexes have been undertaken,[1,2] and for those compounds which have been studied, the interpretation of their spectra has been complicated by the fact that the g values are very sensitive to the splitting of the $^2T_{2g}$ ground state. This may be difficult to estimate, as the $^2T_{2g}$ state is both Jahn–Teller unstable and strongly influenced by the π-bonding properties of the ligands. Past studies have sometimes required orbital reduction factors greater than unity, which is chemically unreasonable unless the parameters are modified to include the effects of configuration interaction.[3]

Recently, the preparation of a novel complex of iron(III) with the sexidentate ligand 6,13-dimethyl-1,4,8,11-tetraazacyclotetradecane-6,13-diamine (diammac), was reported.[4] The geometry of the cation $[Fe(diammac)](ClO_4)_3$ is shown in Figure 1. This provides an ideal system to investigate the relationship between the g tensor and the metal–ligand bonding parameters of a low-spin iron(III) complex, since simple bonding considerations suggest that π-bonding interactions with a saturated amine of this kind should be negligible. Moreover, the unit cell of $[Fe(diammac)](ClO_4)_3$ contains two complex cations in the asymmetric unit, possessing slightly different geometries,[4] which allows the influence of changes in the stereochemistry upon the EPR spectrum to be probed. In contrast to previous studies in which perturbation formulas have been used to relate the g values to the energies of higher states,[1] in the current work the **g** tensors are interpreted by carrying out calculations using the computer program CAMMAG, extended to treat the complete d^5 basis set. This program, developed in its original version by Gerloch and co-workers,[5] has recently been used to successfully interpret the energy levels and g values of a wide range of transition-metal complexes within the framework of the angular overlap model (AOM).[6] The present paper reports the single-crystal EPR spectrum of $[Fe(diammac)](ClO_4)_3$. The **g** tensors of the two $[Fe(diammac)]^{3+}$ units present in the complex are interpreted and compared with those reported for other polyamine complexes of Fe(III).

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