place while the sample is heated to 600 °C.

The X-ray diffraction pattern of **6** clearly displays all the peaks that characterize the $Fe_3O_4$ spinel structure.[23] Before it was heated, **5** appeared somewhat amorphous, displaying only hints of the peaks that were observed in the crystalline particles of **6**.

The crystals of **5** gave good single-crystal electron diffraction patterns[24] but were not stable in the electron beam. In this respect they are quite unlike $Fe_3O_4$ itself. Electron diffraction on the crushed material revealed several cleavage planes, in particular the [100] and [$\bar{1}$10] zones. The selected area diffraction pattern corresponding to the [$\bar{1}$10] zone is given in Figure 5. All patterns are entirely consistent with a fundamental $Fe_3O_4$ lattice, with in some cases powder rings arising due to decomposition of the material under the electron beam.

The organic molecules in the magnetic products described above are thought to be directly bonded to the inorganic material via the pyridoxylidene moieties. Chart I delineates possible structures for the small particles of **6** as well as for **5** after due adjustments. The pyridoxylidene:[$Fe_3O_4$] ratios vary from 1:0.77 in **5** to 1:1.17 in **6**, to 1:1.34 in **3b**, to 1:2 in **8**, and to 1:3 in **7**. When it is heated to 600 °C, a dimer of **6** loses formally one unit of $L_AFe_2$ to yield **8**, in which the stoichiometric ratio of 1:12 for ligand:Fe appears to be most favorable for the highest magnetic ordering. The alignment of the organic molecules within the inorganic lattice structure is currently under study.

(22) Sample **7** was obtained from sample **5** after heating to 570 °C. Its analysis corresponded to the composition [$Fe_3O_4$]$_3$(pyridoxylidene)· 1.5$H_2O$, implying a loss of the labile dihydrazinophthalazine moiety from sample **5**. Anal. Calcd for $C_8H_{12}NO_{15.5}Fe_9$: C, 10.98; H, 1.37; N, 1.60. Found: C, 10.81; H, 1.29; N, 1.32.

(23) The *d* values and the relative intensities of peaks are as follows: 2.960 A [220], 30%; 2.529 Å [311], 100%; 2.094 Å [400], 20%; 1.610 Å [511], 30%; 1.482 [440], 40%. The pattern contains also some undefined peaks. The *a* lattice parameter was 8.376 Å.

(24) Single crystals of **5** were examined by using a JEOL JEM 2000FX electron microscope in the conventional manner. The largest crystals were ca. 1 $\mu$m in length.

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to the d–d state limits the photostability of the complex. Ligand dissociation from the distorted d–d state occurs with significant quantum yields, particularly in solvents of low dielectric constant and in the presence of coordinating ions ($Cl^-$, $Br^-$, $NCS^-$).[1,2]

Recent efforts to design $Ru(bpy)_3^{2+}$ type complexes (bpy is 2,2'-bipyridine) that are stable toward ligand photosubstitution have utilized three approaches: (1) synthesis of mixed-ligand complexes of the type $Ru(bpy)_2L_2$, where L is a nonchromophoric ligand with stronger $\sigma$-donor or $\pi$-acceptor properties, thus increasing the energy of the d–d state,[3] (2) synthesis of mixed-chelate $Ru(bpy)_2L$ complexes, where L is a modified chromophoric ligand with a lower lying $\pi^*$ level, thus decreasing the $^3$MLCT energy,[4] and (3) synthesis of cage-type polypyridine complexes in which the three bipyridine ligands are covalently linked and provide an octahedral coordination environment in which the metal is encapsulated.[5,6a] The first two of these approaches involve increasing the energy barrier between the $^3$MLCT and d–d states, whereas the third approach involves prevention of ligand dissociation. In addition, caging is expected to change the shape of the excited-state potential energy wells, especially for large nuclear displacements. Therefore, changes in the rates of radiationless decay processes, particularly those involving extensive nuclear distortions, are expected as a consequence of caging.[7]

As a step toward the design of this class of cage complexes, a series of bipyridine and phenanthroline macrobicyclic ligands has been reported.[8] Ruthenium complexes of these have not been prepared, however, possibly due to the insufficient size of the "cavity" provided. Recently a closed-cage amide-linked tris(bipyridine) complex, prepared with use of the Ru(II) ion as a template, was reported.[6b] When compared to $Ru(bpy)_3^{2+}$, this cage complex exhibited an increased excited-state lifetime at room temperature, as well as a dramatically decreased quantum yield for photosubstitution.[6a]

We report here the preparation of a novel ligand, (Mebpy)$_3$Bz, consisting of three 5,5'-dimethyl-2,2'-bipyridine (5,5'-dmb) groups linked to a central benzene ring via only methylene groups. The



(Mebpy)$_3$Bz
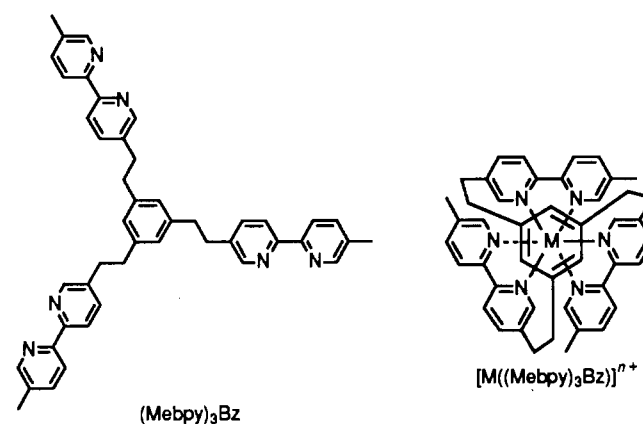
[M((Mebpy)$_3$Bz)]$^{n+}$

synthesis of (Mebpy)$_3$Bz involves the reaction of the carbanion of 5,5'-dmb with 1,3,5-tris(bromomethyl)benzene in a 3:1 ratio.[9]

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## Ground- and Excited-State Properties of a Photostable Hemicage Ruthenium(II) Polypyridine Complex

The properties of the long-lived, luminescent, metal-to-ligand charge-transfer (MLCT) state characteristic of (polypyridine)-ruthenium(II) complexes enable these compounds to sensitize photoinduced electron-transfer and energy-transfer processes. One limitation to the usefulness of these complexes as photosensitizers arises from the existence of a metal-centered (d–d) excited state that can be thermally populated from the $^3$MLCT state. In addition to compromising the excited-state lifetime by providing an additional pathway for decay of the $^3$MLCT state, crossover

**Table I.** Absorption and Redox Properties

| | absorption[a] | | redox potentials ($E_{1/2}$, V vs SCE)[b] | | | |
| compd | $\lambda_{max}$ | $\epsilon$ | $Ru^{3+/2+}$ | $Ru^{2+/+}$ | $Ru^{+/0}$ | $Ru^{0/-}$ |
|---|---|---|---|---|---|---|
| [Ru(5,5'-dmb)$_3$](PF$_6$)$_2$ | 444 | 14 000 | +1.18 | −1.49 | −1.68 | −1.94 |
| [Ru(Mebpy)$_3$)Bz)](PF$_6$)$_2$ | 450 | 14 000 | +1.21 | −1.46 | −1.67 | −1.96 |

[a] Measured in acetonitrile. [b] Measured in acetonitrile containing 0.1 M N(Bu)$_4$PF$_6$; Pt electrode.

**Table II.** Photochemical and Photophysical Properties

| compd | $\lambda_{max}$, nm[a] | $\Phi_{em}$[a] | $\tau$, ns[b] | $\Phi_p$[c] |
|---|---|---|---|---|
| [Ru(bpy)$_3$](PF$_6$)$_2$ | 626 | 0.062[d] | 1060[e] | 0.029[f] |
| [Ru(5,5'-dmb)$_3$](PF$_6$)$_2$ | 611 | 0.032 | 215 | 0.024 |
| [Ru((Mebpy)$_3$Bz)](PF$_6$)$_2$ | 604 | 0.17 | 2400 | <1 × 10$^{-6}$ |

[a] Corrected emission spectra measured in acetonitrile at 25 °C ($\lambda_{ex}$ = 450 nm); quantum yield measured relative to [Ru(bpy)$_3$](PF$_6$)$_2$ (estimated uncertainty ±15%). [b] Measured in acetonitrile by laser flash photolysis following excitation at 450 nm (estimated uncertainty ±5%). [c] Measured in acetonitrile containing 2 mM [N(C$_4$H$_9$)]$_4$Cl by monitoring the decrease in absorbance at $\lambda_{max}$ upon irradiation at 450 nm; calculated relative to the yield for [Ru(bpy)$_3$](PF$_6$)$_2$ (estimated uncertainty ±15%). [d] Reference 10. [e] Reference 1. [f] Reference 4.

The three bipyridine "arms" of this trimer are in a suitable conformation to coordinate simultaneously to a single metal ion, producing the singly capped, hemicaged structure [M((Mebpy)$_3$Bz)]$^{n+}$. The ruthenium(II) complex of this ligand is readily prepared by the reaction of equimolar amounts of (Mebpy)$_3$Bz and Ru(DMSO)$_4$Cl$_2$ in refluxing ethanol and is isolated by column chromatography.

The redox and absorption properties of [Ru((Mebpy)$_3$Bz)](PF$_6$)$_2$ are very similar to those of the parent compound, [Ru(5,5'-dmb)$_3$](PF$_6$)$_2$, as shown in Table I. In the hemicage complex, the absorption $\lambda_{max}$ is shifted slightly to lower energy and, in agreement with this observation, $E_{1/2}$ for the first ligand reduction (Ru$^{2+/+}$ couple) occurs approximately 30 mV lower than that of the parent complex. Thus, it is evident that the linking (Ph–CH$_2$–CH$_2$–) substituents have somewhat less electron-donating characteristics than a methyl group in the 5-position, but this appears to be the only effect of caging on ground-state properties.

In contrast to this, the two complexes have dramatically different photochemical and photophysical properties, as summarized in Table II. Data are presented for Ru(bpy)$_3^{2+}$ for purposes of comparison. Although the emission energies in acetonitrile are similar for [Ru((Mebpy)$_3$Bz)](PF$_6$)$_2$ and [Ru(5,5'-dmb)$_3$](PF$_6$)$_2$, the room-temperature lifetime and emission quantum yield of the hemicage complex are increased by a factor of 10 relative to those of the parent complex. In fact, the emission lifetime of [Ru((Mebpy)$_3$Bz)](PF$_6$)$_2$, 2400 ns, is among the longest reported for Ru(II) complexes with three identical bipyridine ligands. The room-temperature lifetime of (polypyridine)ruthenium(II) complexes is affected by the rate at which the $^3$MLCT state thermally populates the d–d state and therefore by the energy barrier between these two states. When unsubstituted bipyridine ligands are replaced by 5,5'-dimethylbipyridine ligands, the energy of the $^3$MLCT state of the complex is increased. This and other factors result in a high quantum yield for population of the d–d state ($\Phi_{d-d}$ state = 0.93)[11] and therefore a short luminescence lifetime for Ru(5,5'-dmb)$_3^{2+}$. The fact that the lifetime of Ru((Mebpy)$_3$Bz)$^{2+}$

is 1 order of magnitude longer than that of Ru(5,5'-dmb)$_3^{2+}$ implies that, at room temperature, population of the d–d state is a relatively inaccessible pathway for deactivation of the $^3$MLCT state of the hemicage complex. Since emission energies (and therefore $^3$MLCT levels) for the two complexes are similar, an increase in the $^3$MLCT to d–d thermal activation energy for the hemicage complex is implied. This is verified by preliminary temperature-dependent lifetime data obtained for [Ru((Mebpy)$_3$Bz)](PF$_6$)$_2$ in the region 200–300 K. The activation energy for crossover to the d–d state ($\Delta E'$) calculated for the hemicage complex is indeed larger than that reported for the parent complex.[12]

The absence of ligand photodissociation for Ru((Mebpy)$_3$Bz)$^{2+}$ can also be explained on the basis of decreased d–d state accessibility. The quantum yield for photosubstitution ($\Phi_p$) in acetonitrile containing 2 mM [N(C$_4$H$_9$)$_4$]Cl is decreased by a factor of 10$^4$ or greater for Ru((Mebpy)$_3$Bz)$^{2+}$ relative to Ru(5,5'-dmb)$_3^{2+}$. The increased photostability of the hemicage complex is consistent with that reported by Balzani et al. for the amide-linked closed-cage Ru(II) complex.[6a] Other examples of cage-type complexes that exhibit decreased quantum yields for photodecomposition relative to the yields for the corresponding "open" complexes include cobalt(III) and chromium(III) sepulchrate complexes.[7,13,14] In both the sepulchrate complexes and the closed-cage bipyridine complex, the metal ion is completely encapsulated; loss of a ligand should be prohibited by the constraints of these cages. This is not the case for Ru((Mebpy)$_3$Bz)$^{2+}$, however, since the bipyridines are effectively linked only on one side of the complex. Formation of a monodentate bipyridine intermediate, known to be a step in the photosubstitution of Ru(bpy)$_3^{2+}$ complexes, and subsequent dissociation of one bipyridine group, should not be completely prevented in this complex. The increased photochemical stability of Ru((Mebpy)$_3$Bz)$^{2+}$ can therefore be attributed to two factors: (1) the increase in the thermal energy barrier for crossover from the $^3$MLCT state to the d–d state responsible for ligand dissociation and (2) the enhanced ability of the complex to undergo "self-annealing", since a dissociated bipyridine ligand would be held in close proximity for reassociation. Efforts to more fully characterize the excited-state properties of this interesting complex by determining the effects of solvent and temperature on emission energy, quantum yield, and lifetime are in progress.

Finally, the formation of hemicage complexes of (Mebpy)$_3$Bz with other metal ions is possible and may provide a route to exceptionally stable complexes of unusual oxidation states. Fe((Mebpy)$_3$Bz)$^{2+}$ has been prepared and was shown to be extremely stable to decomposition by EDTA and to acid oxidation, unlike its parent complex, Fe(5,5'-dmb)$_3^{2+}$. Studies are in progress to investigate the use of (Mebpy)$_3$Bz as an encapsulating ligand with other metal ions and as a bridging ligand for the formation of bimetallic complexes.

(9) In a typical synthesis, 10.9 mmol of 5,5'-dimethyl-2,2'-bipyridine was treated with 5.6 mmol of lithium diisopropylamide in dry THF at −78 °C. The reaction mixture was stirred for 1.5 h, and a solution of 1.7 mmol of 1,3,5,-tris(bromomethyl)benzene in THF was added. This mixture was warmed to room temperature and allowed to sit for 1 h. Water was added, and THF was removed by rotary evaporation, leaving a cloudy aqueous solution and solid residue. Bipyridine products were extracted with methylene chloride, and (Mebpy)$_3$Bz was isolated by MPLC on silica gel. Anal. Calcd for C$_{45}$H$_{43}$N$_6$: C, 81.05; H, 6.35. Found: C, 80.20; H, 6.39.

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(12) Preliminary data were fit to the equation [$\tau(T)$]$^{-1}$ = $k°$ + $k'°$ exp[−$\Delta E'/kT$].[1] For [Ru((Mebpy)$_3$Bz)](PF$_6$)$_2$, $\Delta E'$ = 5200 cm$^{-1}$, $k°$ = 5.6 × 10$^5$ s$^{-1}$, and $k'°$ = 1.3 × 10$^{13}$ s$^{-1}$ (estimated uncertainty ±20%). For [Ru(5,5'-dmb)$_3$](PF$_6$)$_2$, $\Delta E'$ = 3700 cm$^{-1}$, $k°$ = 2.7 × 10$^5$ s$^{-1}$, and $k'°$ = 2.4 × 10$^{14}$ s$^{-1}$.[11] A more detailed study of the temperature-dependent lifetime of the hemicage complex is underway and will be discussed in a subsequent paper.

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## Protein Site Recognition by Chromium(III) Complexes Probed through NOE Quenching

We have recently become interested in the general principles behind the binding of inorganic substrates to protein molecules as a complement to ongoing work in this laboratory examining the interactions between transition-metal coordination complexes and DNA.[1] An elucidation of the principles of site recognition is the first step toward the rational design of both inorganic drugs as well as site-specific transition-metal labels and probes for proteins. Inorganic complexes may be particularly well suited to such investigations owing to their well-defined coordination geometries and the richness in spectroscopic probes that may be applied. Accordingly, we have synthesized a series of transition-metal-centered molecules containing both hydrogen-bonding moieties and pendant amino acid side chains. We have chosen to study their binding with hen egg white (HEW) lysozyme due to its well-characterized structure and complete NMR assignment.[2] We have discovered that selective paramagnetic quenching of nuclear Overhauser effects (NOE's) provides a sensitive means to map binding sites and to discriminate among binding interactions.

The quenching of NOE's between nuclei by paramagnets is described in both the experimental[3] and theoretical[4] literature. The coupling of unpaired electron spins to proton populations perturbed from spin equilibrium in the NOE experiment efficiently relaxes the system to equilibrium, circumventing the cross-relaxation pathways crucial to the observation of the NOE. As paramagnetic effects are distance dependent, only NOE's to protons in close proximity to the paramagnetic center are affected, allowing selective reduction of NOE signals to protons in the binding region. Hence, binding of a small complex containing a paramagnetic transition-metal center should cause diminution of the intensity of Overhauser effects to protons only in the binding site.

Surprisingly, NOE quenching has not been exploited as a binding site mapping technique on macromolecules.[5,6] We report here the use of this method to map specifically binding interactions between two related inorganic complexes containing pendant amino acid side chains and HEW lysozyme.

Cr(NTA)(Im)₂ (**1**) and Cr(1-PDA)(Im)₂ (**2**)[7] were synthesized and characterized by FABMS, IR spectroscopy, and elemental analyses, and the structure of **2** was determined by X-ray crys-

Cr(NTA)(Im)₂ (**1**)          Cr(1-PDA)(Im)₂ (**2**)
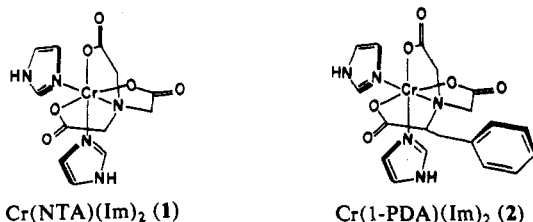
tallography.[8]    Chromium(III), which is one of the classic "shiftless" relaxation agents,[5b] is expected to have the same

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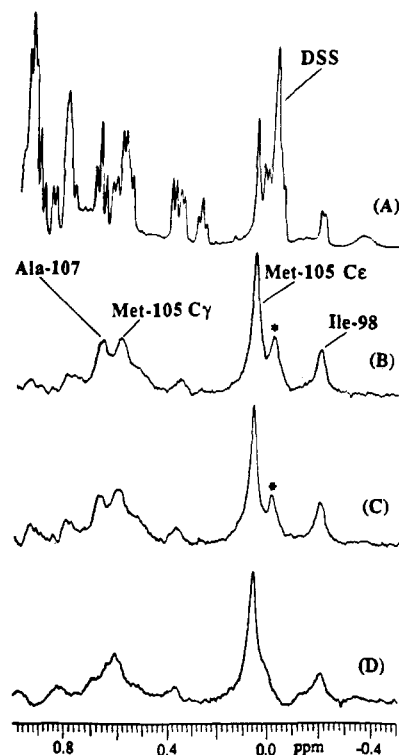
**Figure 1.** (A) ¹H resolution-enhanced 400-MHz NMR spectrum of HEW lysozyme for comparison to NOE spectra. (B) NOE difference spectrum of lysozyme obtained by irradiating Trp-105 C5H at 6.5 ppm in the absence of metal complex. The sign of the NOE's is negative. (C) NOE difference spectrum as in part B but in the presence of a 5:1 ratio of protein to complex **1**. (D) NOE difference spectrum as in part B but in the presence of a 5:1 ratio of protein to complex **2**. All samples were 3 mM in lysozyme in D₂O at pH 3.8, 35 °C. The starred peak is a subtraction artifact due to the DSS standard resonance. Line broadening of 2 Hz has been applied. Note that the NOE to Ala-107 is weakly quenched for **1** and dramatically quenched for **2**. NOE's to Ile-98 also show quenching while no quenching is evident of NOE's to Met-105.

magnetic properties in both **1** and **2**, given that the primary coordination sphere is identical in each case. Under the conditions of these experiments, aquation of the complexes, with loss of imidazole, occurs.[9] Protein dialysis experiments indicate that direct coordination of the complex to the protein is unlikely.

Binding site mapping experiments were conducted following paramagnetic difference[10] (PD) spectroscopy, since it was necessary first to establish which lysozyme ¹H resonances were strongly affected by metal binding and which were unaffected.[11]

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(6) A recent report utilized NOE quenching to study the binding of a paramagnetic ion to an organic molecule (Gaggelli, E.; Tiezzi, E.; Valensin, G. *J. Chem. Soc., Faraday Trans. 2* **1988**, *84*, 141–147).
(7) Abbreviations: NTA = nitrilotriacetate trianion; 1-PDA = 1-phenylalanine-*N,N*-diacetic acid trianion; Im = imidazole; DSS = 2,2-dimethyl-2-silapentane-5-sulfonate.
(8) FABMS (nitrobenzyl alcohol matrix): (**1**) M + H = 377; (**2**) M + H = 467. Anal. Calcd for (**1**) Cr(NTA)(Im)₂·3H₂O: C, 33.49; H, 4.68; N, 16.28. Found: C, 33.55; H, 4.62; N, 16.28. Calcd for (**2**) Cr(1-PDA)(Im)₂·CH₃OH·¹/₂C₇H₈ (from crystal structure): C, 51.84; H, 5.18; N, 12.86. Found: C, 51.00; H, 5.32; N, 13.15. IR (KBr pellet): (**1**) νcₒₒ(asym) = 1661, 1639 cm⁻¹; (**2**) νcₒₒ(asym) = 1641 cm⁻¹. The synthesis, full characterization, crystal and molecular structure will be the subject of a future publication.
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