anation in the solid state of $[Co(NH_3)_4(^{15}NH_3)OH_2]Cl_3$ (8%), however, demonstrate that back-side entry of Cl^- and front-side expulsion of coordinated OH_2 are more likely in the crystalline lattice than in aqueous solution.

Summary

The following represents a summary of the findings of this study: (1) Small, but real, stereochemical change occurs in the $CoOH_2^{3+}$ products derived from the Hg^{2+}- and Ag^+-induced reactions (2-3%). (2) Probably no stereochemical change occurs during production of $CoONO_2^{2+}$ in the same reactions. (3) No stereochemical change occurs in the formation of $CoCl^{2+}$ by $Cl_2(g)$ oxidation of $CoBr^{2+}$. (4) Some stereochemical change (1.4%) accompanies water exchange in $CoOH_2^{3+}$. (5) No significant stereochemical change accompanies anation (by Cl^- or Br^-) of $CoOH_2^{3+}$ in acidic solution, but this result is less certain than the others due to process 4 occurring at the same time.

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A Water-Stable Gadolinium(III) Complex Derived from a New Pentadentate "Expanded Porphyrin" Ligand

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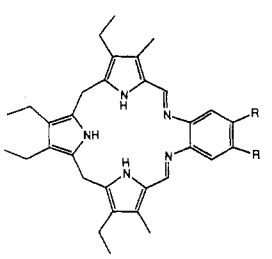
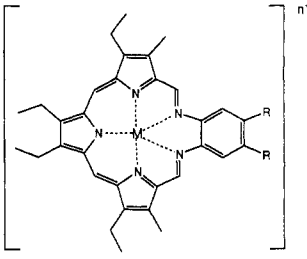
Gadolinium(III) complexes derived from strongly binding anionic ligands, such as diethylenetriaminepentaacetic acid (DTPA),[1-3] 1,4,7,10-tetraazacyclododecane-*N*,*N'*,*N''*,*N'''*-tetraacetic acid (DOTA),[1,4,5] and 1,10-diaza-4,7,13,16-tetraoxacyclooctadecane-*N*,*N'*-diacetic acid (dacda),[1,6] are among the most promising of the paramagnetic contrast agents currently being developed for use in magnetic resonance imaging (MRI).[1] Indeed, $[Gd(DTPA)]^-$ is now being used clinically in the United States in certain enhanced tumor detection protocols. Nonetheless, the synthesis of other gadolinium(III) complexes remains of interest, since such systems might have greater kinetic stability, superior relaxivity, or better biodistribution properties than the existing carboxylate-based contrast agents. One approach currently being pursued is based on using water-soluble porphyrin derivatives, such as tetrakis(4-sulfonatophenyl)porphyrin (TPPS) or tetrakis(*N*-methyl-4-pyridiniumyl)porphyrin (TMPyP).[7-9] Unfortunately, the large gadolinium(III) cation cannot be accommodated completely[10] within the relatively small porphyrin binding core ($r \simeq 2.0$ Å[11]), and as a consequence, gadolinium porphyrin complexes are invariably hydrolytically unstable.[7,8,12,13] Larger porphyrin-like ligands, however, might offer a means of circumventing this problem.[14-22]

We have recently reported[23] the synthesis of a novel "expanded porphyrin" system, **1**,[24] and the structure of the bis(pyridine) adduct of its cadmium(II) complex **2**. The presence in this



10 R = H
11 R = CH_3



1	M = H,	R = H,	n = 0
2	M = Cd,	R = H,	n = 1
3	M = Nd,	R = H,	n = 2
4	M = Sm,	R = H,	n = 2
5	M = Eu,	R = H,	n = 2
6	M = H,	R = CH_3,	n = 0
7	M = Gd,	R = CH_3,	n = 2
8	M = Eu,	R = CH_3,	n = 2
9	M = Sm,	R = CH_3,	n = 2

structure of a near circular, pentadentate binding core, which is roughly 20% larger than that of the porphyrins,[23] coupled with the realization that almost identical ionic radii pertain for hexacoordinate Cd^{2+} ($r = 0.92$ Å) and Gd^{3+} ($r = 0.94$ Å),[25] prompted us to explore the general lanthanide-binding properties of this new monoanionic porphyrin-like ligand. We report here the synthesis and characterization of a water-stable gadolinium(III) complex (**7**) derived formally from a new 16,17-dimethyl-substituted analogue (**6**)[24] of our original "expanded porphyrin" system, as well as the preparation and characterization of the corresponding europium(III) and samarium(III) complexes **8** and **9**.

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Experimental Section

General Information. Electronic spectra were recorded on a Beckman DU-7 spectrophotometer. IR spectra were recorded, as KBr pellets, from 4000 to 600 cm^{-1} on a Perkin-Elmer 1320 spectrometer. Low-resolution fast atom bombardment mass spectrometry (FAB MS) was performed at Austin with a Finnigan-MAT TSQ-70 instrument and either 3-nitrobenzyl alcohol or glycerol/oxalic acid as the matrix; high-resolution FAB MS analyses (HRMS) were performed at the Midwest Center for Mass Spectrometry with CsI as a standard. Elementary analyses were performed by Galbraith Laboratories.

Materials. All solvents and reagents were of reagent grade quality, purchased commercially, and used as received (no special purification or drying was required or used). Sigma lipophilic Sephadex (LH-20-100) and Merck Type 60 (230–400 mesh) silica gel were used for column chromatography.

Preparation of Nd Complex 3. The reduced form of ligand **1** (compound **10**)[26] (50 mg, 0.1 mmol) was stirred with neodymium nitrate pentahydrate (63 mg, 0.15 mmol) and Proton Sponge (64 mg, 0.3 mmol) in chloroform/methanol (150 mL, v/v 1/2) for 1 day. The dark green reaction mixture was poured onto ice/water/ammonium chloride and extracted with chloroform. The organic layer was washed with aqueous ammonium chloride and concentrated under reduced pressure. The complex was chromatographed through Sephadex by using neat chloroform, chloroform/methanol (10/1), methanol, and water, respectively, as eluents. The dark green band collected from methanol was concentrated and recrystallized from chloroform/methanol/*n*-hexane (ratio of chloroform to methanol is 1/2) to yield 13 mg of **3** (18%). For **3**: UV/vis (CH_3OH) λ_{max} (ϵ) 330.5 (33 096), 432.5 (85 762), 710.5 (10 724), 774.5 nm (38 668); FAB MS (glycerol matrix) *m/e* (relative intensity) 631 (^{142}Nd, 95), 633 (^{144}Nd, 100), 635 (^{146}Nd, 77); IR (KBr) ν 3360, 2965, 2930, 2870, 1610, 1560, 1450, 1400, 1350, 1250, 1205, 1135, 1080, 1050, 980, 940, 905, 755 cm^{-1}.

Preparation of Sm Complex 4. Compound **10**[26] (40 mg, 0.08 mmol) was stirred with platinum oxide (18 mg, 0.08 mmol) and samarium acetate hydrate (69 mg, 0.2 mmol) under reflux in benzene/methanol (50 mL, v/v 1/1). After 2 h the reaction mixture was filtered through Celite and concentrated under reduced pressure. The concentrate was purified by chromatography through Sephadex using only chloroform as the eluent. After a red band was discarded,[27] a green band was collected, concentrated in vacuo, and recrystallized from chloroform/*n*-hexane to give 0.8 mg of **4** (ca. 1%). For **4**: UV/vis λ_{max} 438, 706.5, 769 nm; FAB MS (3-nitrobenzyl alcohol matrix) *m/e* (relative intensity) 635 (^{147}Sm, 78), 636 (^{148}Sm, 72), 637 (^{149}Sm, 73), 640 (^{152}Sm, 100), 642 (^{154}Sm, 55).

Preparation of Eu Complex 5. Compound **10**[26] (50 mg, 0.1 mmol) was stirred with europium acetate hydrate (34 mg, 0.1 mmol) and Proton Sponge (64 mg, 0.3 mmol) in chloroform/methanol (150 mL, v/v 1/2) for 1 day. The reaction mixture was poured onto ice/water, and this mixture was extracted with chloroform. The organic layer was washed with aqueous ammonium chloride and then concentrated and recrystallized from chloroform/*n*-hexane. The recrystallized solid was purified by column chromatography through Sephadex using neat chloroform and neat methanol as eluents. The dark green band collected in methanol was concentrated to yield a small amount of a dark green solid (<1%). For **5**: UV/vis λ_{max} 438, 700, 765 nm; FAB MS (3-nitrobenzyl alcohol matrix) *m/e* (relative intensity) 639 (^{151}Eu, 94), 641 (^{153}Eu, 100).

4,5,9,24-Tetraethyl-10,16,17,23-tetramethyl-13,20,25,26,27-pentaazapentacyclo[20.2.1.1^{3,6}.1^{8,11}.0^{14,19}]heptacosa-3,5,8,10,12,14(19),15,17,20,22,24-undecene (11). This macrocycle was prepared in ca. 90% yield from 1,2-diamino-3,4-dimethylbenzene and 2,5-bis((3-ethyl-5-formyl-4-methylpyrrol-2-yl)methyl)-3,4-diethylpyrrole[26] by using the acid-catalyzed procedure reported earlier for the preparation of **10**.[26] For **11**: mp 200 °C dec; ^{1}H NMR δ 1.06 (6 H, t, CH_2CH_3), 1.13 (6 H, t, CH_2CH_3), 2.15 (6 H, s, Ph–CH_3), 2.22 (6 H, s, pyrr–CH_3), 2.38 (4 H, q, CH_2CH_3), 2.50 (4 H, q, CH_2CH_3), 3.96 (4 H, s, (pyrr)$_2$–CH_2), 7.19 (2 H, s, aromatic), 8.10 (2 H, s, CHN), 11.12 (1 H, s, NH), 12.48 (2 H, s, NH); ^{13}C NMR δ 9.49, 15.33, 16.47, 17.22, 17.71, 19.52, 22.41, 117.84, 120.40, 120.75, 125.11, 125.57, 134.95, 135.91, 141.63; UV/vis λ_{max} 367 nm; FAB MS M$^+$ 522; HRMS M$^+$ 521.350 45 (calcd for $C_{34}H_{43}N_5$ 521.351 85).

Preparation of Gd Complex 7. The reduced form of ligand **6** (compound **11**) (42 mg, 0.08 mmol) was stirred with gadolinium acetate tetrahydrate (122 mg, 0.3 mmol) and Proton Sponge (54 mg, 0.25 mmol) in chloroform/methanol (150 mL, v/v 1/2) for 1 day. The dark green reaction mixture was concentrated under reduced pressure and chromatographed through silica gel (25 cm × 1.5 cm) that was pretreated with chloroform/triethylamine (50 mL, v/v 25/1). Chloroform/triethylamine (25/1) and chloroform/methanol/triethylamine (v/v/v 25/2.5/1) were used as eluents. A dark red band was first collected followed by two green bands.[27] The second of these green bands, which showed a clear aromatic pattern by UV/vis, was concentrated and recrystallized from chloroform/*n*-hexane to give 14 mg (22%) of the Gd complex **7**. For **7**: FAB MS (methanol/oxalic acid/glycerol matrix) *m/e* (relative intensity) 671 (^{155}Gd, 58), 672 (^{156}Gd, 78), 673 (^{157}Gd, 94), 674 (^{158}Gd, 100), 676 (^{160}Gd, 64); HRMS M$^+$ 674.2366 (calcd for $C_{34}H_{38}N_5{}^{158}Gd$ 674.2368); UV/vis ($CHCl_3$) λ_{max} (ϵ) 339.5 (14 850), 450.5 (36 350), 694.5 (6757), 758.0 nm (23 767); IR (KBr) ν 2990, 2960, 2900, 2830, 2765, 2700, 2620, 2515, 1710, 1550, 1440, 1410, 1395, 1365, 1265, 1220, 1180, 1150, 1105, 1090, 1060, 1045, 1015, 680 cm^{-1}. Anal. Calcd for $[C_{34}H_{38}N_5Gd](OH)_2 \cdot 2H_2O$: C, 54.89; H, 5.96; N, 9.41. Found: C, 54.49; H, 5.95; N, 8.97.

Preparation of Eu Complex 8. Compound **11** (53 mg, 0.1 mmol) was stirred with europium acetate hydrate (105 mg, 0.3 mmol) and Proton Sponge (64 mg, 0.3 mmol) in chloroform/methanol (150 mL, v/v 1/2) for 6 h. The dark green reaction mixture was concentrated under reduced pressure and purified as described above with the exception that chloroform/triethylamine (25/1) and chloroform/methanol/triethylamine (25/5/1) were used as eluents. The green complex **8** was recrystallized from chloroform/*n*-hexane to yield 26 mg of product (33%). For **8**: UV/vis ($CHCl_3$) λ_{max} (ϵ) 339.5 (24 570), 450.5 (63 913), 696.0 (10 527), 759.0 nm (40 907); FAB MS (methanol/oxalic acid/glycerol matrix) *m/e* (relative intensity) 667 (^{151}Eu, 79), 669 (^{153}Eu, 100); HRMS M$^+$ 669.2336 (calcd for $C_{34}H_{38}N_5{}^{153}Eu$: 669.2340); IR (KBr) ν 2970, 2930, 2870, 2740, 2680, 2600, 2500, 1700, 1535, 1430, 1350, 1255, 1205, 1165, 1135, 1095, 1075, 1050, 1030, 980, 900 cm^{-1}. Anal. Calcd for $[C_{34}H_{38}N_5Eu](OH)_2 \cdot H_2O$: C, 56.66; H, 5.87; N, 9.72. Found: C, 55.92; H, 5.47; N, 9.95.

Preparation of Sm Complex 9. Compound **11** (52 mg, 0.1 mmol) was stirred with samarium acetate hydrate (103.5 mg, 0.3 mmol) and Proton Sponge (64 mg, 0.3 mmol) in chloroform/methanol (150 mL, v/v 1/2) for 1 day. The dark green reaction mixture was concentrated and purified by silica gel chromatography as described above. The resulting crude material was then recrystallized from chloroform/*n*-hexane to give 29 mg of **9** (37% yield). For **9**: UV/vis ($CHCl_3$) λ_{max} (ϵ) 339.5 (21 617), 451.0 (56 350), 695.5 (9393), 760.0 nm (35 360); FAB MS (3-nitrobenzyl alcohol) *m/e* (relative intensity) 663 (^{147}Sm, 74.8), 664 (^{148}Sm, 82.3), 665 (^{149}Sm, 84.58), 668 (^{152}Sm, 100), 670 (^{154}Sm, 78.5); HRMS M$^+$ 668.2300 (calcd for $C_{34}H_{38}N_5{}^{152}Sm$ 668.2322); IR (KBr) ν 2990, 2950, 2890, 2760, 2700, 2620, 2520, 1720, 1620, 1550, 1440, 1360, 1265, 1215, 1175, 1145, 1105, 1085, 1060, 995, 945, 910, 680 cm^{-1}. Anal. Calcd for $[C_{34}H_{38}N_5Sm](OH)_2 \cdot 3H_2O$: C, 54.08; H, 6.14; N, 9.27. Found: C, 54.30; H, 5.66; N, 9.06.

Results

As reported earlier,[23] treatment of the methylene-bridged, or reduced, form of the "expanded porphyrin" **1** (compound **10**) with Cd(II) salts in air-saturated methanol/chloroform at ambient temperature leads to the formation of the green Cd(II) complex **2** in roughly 25% yield, with both metal insertion and oxidation taking place concurrently under the reaction conditions. When a similar procedure was carried out by using a variety of trivalent lanthanide salts [i.e. $Ce(OTf)_3$, $Pr(OAc)_3$, $Nd(NO_3)_3$, $Sm(OAc)_3$, $Eu(OAc)_3$, $Gd(OAc)_3$, $Dy(OTf)_3$, $TbCl_3$, $Er(OTf)_3$, $Tm(NO_3)_3$, and $Yb(NO_3)_3$] no metal complexes of **1** (or **10**) were obtained (as judged by the absence of changes in the UV/vis spectrum). If, however, *N,N',N'',N'''*-tetramethyl-1,8-diaminonaphthalene (Proton Sponge) was added to the various reaction mixtures, the high-energy, low-intensity band of **10** at λ_{max} = 365 nm disappeared over the course of several hours to several days (depending on the salt in question) and was replaced by two strong transitions in the 435–455-nm (Soret) and 760–800-nm (Q-band) regions, suggesting that ligand oxidation and metal binding had occurred.[28] Unfortunately, isolation of these putative metal-containing products proved problematic: Direct chromatography on either silica gel or lipophilic sephadex in general gave only small quantities of metal-free oxidized ligand **1** and essentially none of the desired metalated material. Indeed, only in the case of the

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(27) Faster moving red and, in some cases, green bands were observed in the course of chromatographic isolations. As judged by UV/vis and NMR analyses, these fractions are tentatively assigned as corresponding to partially oxidized and free-base forms of the ligand, respectively.

(28) The relation between the optical bands (nm) observed just after the reaction and the trivalent lanthanide cation employed are as follows: Ce 453, 782; Pr 437, 797; Nd 439, 786; Sm 438, 769; Eu 438, 765; Gd 438, 765; Tb 439, 764; Dy 438, 765; Tm 437, 765; Yb 437, 764.

samarium(III) acetate salt did it prove possible to isolate a trace quantity (ca. 1% yield) of the desired complex (**4**) by chromatography on Sephadex. We were intrigued to find, however, that a dark green neodymium(III) complex **3** could be obtained in almost 20% yield by quenching the reaction mixture with ice water, extracting repeatedly with chloroform, washing with aqueous ammonium chloride, purifying by chromatography on Sephadex, and recrystallizing from chloroform/methanol/*n*-hexane. Unfortunately this workup procedure proved ineffective in the case of the other putative lanthanide complexes (including, unfortunately, that derived from Gd^{3+}), although it did prove possible to obtain trace quantities of the europium(III) complex (**5**) by using this procedure.

Since spectral evidence suggested that metal uptake and ligand oxidation were occurring when macrocycle **10** was treated with numerous other Ln^{3+} salts, we considered it most puzzling that we were only able to isolate the neodymium(III) complex (**3**) in reasonable yield. A careful analysis led us to believe that, in certain instances, notably Sm^{3+}, Eu^{3+}, and Gd^{3+}, the problem was not due to hydrolytic instability. *Rather, it derived from the very high water solubility of the lanthanide complexes that precluded reextraction back into organic solvents following the initial aqueous washes!*[29] This observation led us to consider that more hydrophobic expanded porphyrin analogues would prove valuable in the preparation and isolation of expanded porphyrin lanthanide complexes.

In an effort to test the above assumption, we prepared a simple dimethylated analogue (**11**) of our original reduced expanded porphyrin ligand **10**. This new, more hydrophobic, reduced expanded porphyrin system was obtained in ca. 90% yield by condensing 1,2-diamino-4,5-dimethylbenzene with 2,5-bis((3-ethyl-5-formyl-4-methylpyrrol-2-yl)methyl)-3,4-diethylpyrrole[26] under acid-catalyzed conditions identical with those used to prepare **10**.[26] Treatment of this expanded porphyrin precursor with $Gd(OAc)_3$, $Eu(OAc)_3$, and $Sm(OAc)_3$, under reaction and workup conditions similar to those used to obtain **3**, then gave the cationic complexes **7–9**, as their dihydroxide adducts,[30] in 22%, 33%, and 37% yields, respectively: As near as we have been able to determine, these increased yields derive directly from the increased hydrophobicity of the new dimethyl-substituted expanded porphyrin ligand system (**6**). Apparently, this increased hydrophobicity serves to improve both the initial extractive isolation of the complexes and the subsequent chromatographic purification.

Discussion

The new lanthanide complexes reported here are unique in several ways. For instance, as judged by fast atom bombardment mass spectrometric (FAB MS) analysis, complexes **3–5** and **7–9** are mononuclear 1/1 species, a conclusion that is further supported, in the case of compounds **7–9**, by both high-resolution FAB MS accurate molecular weight determinations and combustion analysis.[32] In other words, we have found no evidence of 1/2 metal to ligand "sandwich" systems, or higher order combinations as are often found in the case of the better studied lanthanide porphyrins.[33]

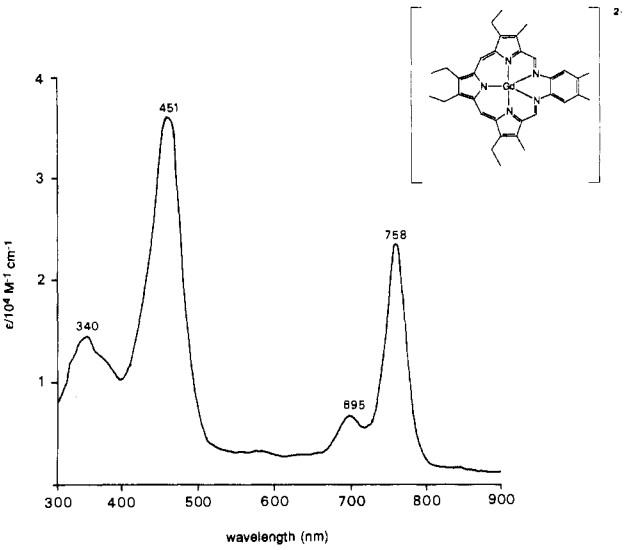

Figure 1. Electronic spectrum of [**7**](OH)$_2$ in $CHCl_3$.

The electronic spectra represent a second remarkable feature of these new materials: All six lanthanide complexes isolated to date display a dominant Soret-like transition in the 435–455-nm region that is considerably less intense than that observed in the corresponding metalloporphyrins (cf. Figure 1)[7] and show a prominent low-energy Q-type band in the 760–800-nm region. This latter feature is diagnostic of this class of 22 π-electron expanded porphyrins[23] and is both considerably more intense and substantially red-shifted (by ca. 200 nm!) as compared to the corresponding transitions in suitable reference lanthanide porphyrins (e.g., $[Gd(TPPS)]^+$, $\lambda_{max} \cong 575$ nm[7]). Within the context of these general observations, it is interesting to note that complexes derived from the somewhat more electron-rich ligand **6** all display Q-type bands that are blue-shifted by ca. 5–15 nm as compared to those obtained from the original expanded porphyrin **1**.

A third notable property of complexes **7–9** is their high solubility in both chloroform and methanol. We find of particular interest the fact that these three complexes are also moderately soluble (to roughly 10^{-3} M concentrations) in 1/1 (v/v) methanol/water mixtures. Thus, although they are sufficiently nonpolar to allow for the initial extractive isolation, these new expanded porphyrin complexes appear sufficiently soluble in polar media so as to allow for their subsequent manipulation and analysis (a consideration that is of obvious importance in the context of designing new MRI contrast agents). Moreover, as initially suggested on the basis of the preliminary studies with **3–5** discussed above, *these materials are stable to these solvent conditions.* For instance, a 3.5×10^{-5} M solution of the gadolinium complex **7** in 1/1 (v/v) methanol/water at ambient temperature shows a slightly greater than 10% bleaching of the Soret and Q-type bands when monitored spectroscopically over the course of 1 week; detailed kinetic studies carried out under these conditions indicate that the half-life for decomplexation and/or decomposition of this complex is 37 days. Of these two bleaching mechanisms, we currently consider the latter decomposition pathway to be the more likely: Although the Q-type transitions of the free base **6** falls ca. 20 nm to the blue of that of **7**,[31] under the conditions of the experiment described above, no detectable shifts in the position of the Q-type band are

(29) Qualitative extractive and spectroscopic analyses of the aqueous washes were performed in the case of the Gd^{3+} complex of **1** and served to indicate that the desired complex was formed and was present in the water layer. Efforts to isolate the complex directly by chromatography on silica gel (without an aqueous wash), however, failed to give isolable quantities of product.

(30) As judged by the IR and microanalytical data, under the reaction and workup conditions, hydroxide anions serve to displace the acetate ligands presumably present following the initial metal insertion procedure. Similar exchanges have also been observed in the case of the cadmium complex **2** (prepared from $Cd(OAc)_2$) where ^1H NMR analyses can be made with ease.[31]

(31) Murai, T.; Hemmi, G.; Sessler, J. L. Unpublished results.

(32) Although these analyses rule out formulations involving 2/1 ligand-to-metal stochiometries, they are not in and of themselves sufficient to rule out structural assignments in terms of 1/1 ligand-to-metal dimeric species (e.g. μ-oxo dimers). Unfortunately, efforts to obtain solution-phase molecular weights were precluded by the relatively low solubilities of the complexes in appropriate solvents (e.g. MeOH, $CHCl_3$). Thus, in the absence of X-ray structural information, formulations in terms of μ-oxo dimers (or related structures) cannot be rigorously excluded. The clean nature of the FAB MS spectra and the lack of discernible shifts in the UV/vis bands as a function of pH, however, lead us to discount this possibility.

(33) (a) Buchler, J. W.; Cian, A. D.; Fischer, J.; Kihn-Botulinski, M.; Paulus, H.; Weiss, R. *J. Am. Chem. Soc.* **1986**, *108*, 3652–3659. (b) Buchler, J. W.; Cian, A. D.; Fischer, J.; Kihn-Botulinski, M.; Weiss, R. *Inorg. Chem.* **1988**, *27*, 339–345. (c) Buchler, J. W.; Scharbert, B. *J. Am. Chem. Soc.* **1988**, *110*, 4272–4276. (d) Buchler, J. W.; Kapellmann, H.-G.; Knoff, M.; Lay, K.-L.; Pfeifer, S. *Z. Naturforsch.* **1983**, *38B*, 1339–1345.

observed, while such bathochromic shifts would be expected if simple demetalation were the dominant pathway.

We feel it is important to stress that the strong hydrolytic stability of complexes **7–9** is in marked contrast to that observed for simple, water-soluble gadolinium porphyrins (e.g. $[Gd(TPPS)]^+$ or $[Gd(TMPyP)]^{5+}$), which undergo water-induced demetalation in the course of several days when exposed to an aqueous environment[7,8] or when "challenged" by treatment with EDTA[34] (conditions under which complexes **7–9** are stable[35]). It thus appears possible that gadolinium(III) complexes derived from the new expanded porphyrin ligand **6**, or its analogues, could provide the basis for developing new paramagnetic contrast reagents for use in MRI applications. In addition, the ease of preparation and stable mononuclear nature of complexes **7–9** suggest that such expanded porphyrin ligands might provide the basis for extending further the relatively underdeveloped coordination chemistry of the lanthanides. We are currently exploring both of these exciting possibilities.

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(35) A full kinetic analysis of the rate of decomplexation of **7** in the presence of EDTA and other chelating agents will be presented elsewhere.[31] Preliminary qualitative tests, however, have been carried out and indicate that the rate of demetalation is *not* appreciable at room temperature.

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Protein Coats of Ferritin Can Sequester Large Amounts of Ferrous Iron

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Maintaining iron in a soluble form compatible with life appears to have been a problem since the accumulation of dioxygen in the atmosphere began about 2.5 billion years ago.[1] Iron proteins participate in a wide variety of crucial reactions during, e.g., DNA synthesis, respiration, photosynthesis, and nitrogen fixation. Contemporary organisms solve the problem of managing iron with a protein, ferritin, that stores iron as hydrous ferric oxide inside a coat of protein (ref 2 is a review). The protein coat of ferritin is a highly conserved, spherical assemblage, 12 nm in diameter, constructed from 24 similar or identical polypeptides. In spite of the features common to all ferritins, both the protein coat and the iron core can vary. In vertebrates, for example, the protein coat of ferritin is encoded in a family of genes, used differentially by specific cell types within an organism, that is regulated by one of the more complex series of reactions to be characterized. With regard to the iron core of ferritin, variations occur in the number of Fe atoms (1–4500), the degree of order (microcrystallinity), and the ratio of P:Fe (<0.05–0.8).

Table I. Accessibility of Fe(II) to *o*-Phenanthroline during Reconstitution of Horse Spleen Ferritin from Protein Coats and $FeSO_4$[a]

time after mixing, h	% added Fe(II) native protein[b]	% added Fe(II) denatured protein[c]
0.25	52	95
0.75	45	83
2	32	77
12	3.9[d]	44

[a] Values are the means of three to seven experiments. The standard deviation is 1–7% of the mean for denatured protein and 10–14% for the native protein (Experimental Section); the percentage of Fe(II) added to buffer that was soluble without protein was 55% at 7 min, 37% at 0.25 h, 20% at 1 h, 15% at 4 h, and 0.5% at 17 h. [b] Native protein—protein in 0.15 M HepesNa, pH 7.0; see Methods. [c] Measured as the percent of total iron that formed the Fe(II)–*o*-phenanthroline complex after removing an aliquot of the Fe/protein mixture and denaturing the protein by heating in 1 N HCl at 100 °C for 30 min; other denaturants were not tested; up to 0.1 μmol of Fe was added to a solution containing 3.78 μmol of *o*-phenanthroline. Analysis of synthetic mixtures of $Fe^{III}(NO_3)_3$ and $Fe^{II}SO_4$ of 1:9, 3:7, and 1:1, analyzed under the same conditions used to denature ferritin, yielded the predicted values (within 10%) for Fe(II) (measured with *o*-phenanthroline) and Fe(III) (measured with hydroxylamine followed by *o*-phenanthroline). For commercial horse spleen ferritin, 16 ± 3% of the iron reacted as Fe(II) after denaturation, suggesting the possibility of reduction of small amounts of Fe during denaturation. [d] One experiment only.

The mechanism of ferritin iron core formation is not well understood. It is known, however, that ferritin can be reconstituted from solutions of simple Fe(II) salts and protein coats; reconstituted iron cores are like native cores by a variety of criteria (for a recent study see ref 3). Note that only small numbers of Fe(II) atoms (8–12) bind to the protein.[10] Larger numbers apparently enter the hollow center of the protein coat. Ferritin cannot be reconstituted from Fe(III) and protein coats.[4] Thus, formation of ferritin iron cores is accompanied by the release of large numbers of electrons and protons, while dissolution of the iron core could require the consumption of electrons and protons. In a cell such as a macrophage, which processes ca. 5×10^8 Fe atoms from an effete red cell in 24 h,[5] with many of the Fe atoms passing in and out of ferritin, the electron and proton flux appears to be considerable.

Recently we showed quantitatively that the complete oxidation of the bulk of Fe(II) added to protein coats of horse spleen ferritin

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