Effects of Multidrug Resistance (*MDR1*) P-Glycoprotein Expression Levels and Coordination Metal on the Cytotoxic Potency of Multidentate (N_4O_2) (Ethylenediamine)bis[propyl(R-benzylimino)]metal(III) Cations

Vijay Sharma, Carolyn L. Crankshaw, and David Piwnica-Worms*

Laboratory of Molecular Radiopharmacology, Mallinckrodt Institute of Radiology, and Department of Molecular Biology and Pharmacology, Washington University Medical School, St. Louis, Missouri 63110

Received November 8, 1995[⊗]

Enhanced mitochondrial transmembrane potentials in tumor cells have been proposed to confer tumor-selective-targeting properties to modestly lipophilic monocationic compounds. To explore the potential cytotoxic activity of lipophilic cationic metallopharmaceuticals containing a highly flexible hexadentate N_4O_2 Schiff-base phenolic ligand, we first synthesized precursors H_3Mabi (**1**) and H_3DMabi (**2**) by condensation of an appropriate linear tetraamine with substituted salicylaldehydes. The desired N_4O_2 ligands, (ethylenediamine)-*N*,*N*′-bis[propyl[(2-hydroxy-3-methoxybenzyl)imino]] and (ethylenediamine)-*N*,*N*′-bis[propyl[(2-hydroxy-4,6-dimethoxybenzyl)-imino]] (R-ENBPI), were obtained by cleavage of the imidazolidine ring, and their corresponding monocationic complexes were produced by reaction with appropriate hydrated salts or acetylacetonates of Al(III), Fe(III), Ga(III), and In(III). All complexes were stable to neutral hydrolysis. In human epidermal carcinoma KB-3-1 cells, cytotoxic potencies of racemic mixtures of these complexes were in the low micromolar range and, for a given ligand, depended on the identity of the coordinating central metal. The active 4,6-dimethoxy-ENBPI complexes were more potent than their 3-methoxy analogs, while the free ligands and metal(III) ions showed little or no cytotoxic activity. Furthermore, in colchicine-selected KB-8-5 multidrug resistant (MDR) cells, modest cellular expression of human *MDR1* P-glycoprotein conferred protection from the cytotoxic activities of Al(III), Fe(III), and Ga(III) R-ENBPI complexes indicating that these complexes were recognized as transport substrates by the P-glycoprotein efflux transporter. However, the cytotoxic activities of the corresponding In(III) complexes, while among the lowest in potencies, were also not altered by expression of *MDR1* P-glycoprotein. Thus, for the Group III elements, human cells were capable of distinguishing R-ENBPI complexes formed of the same ligands with different metals. Furthermore, selected R-ENBPI metal(III) complexes may be useful as novel anticancer metallopharmaceuticals.

Introduction

Selected organic lipophilic monocations, exemplified by tetraphenyl phosphonium,[1] rhodamine 123,[2,3] and 1-methyl-4-phenylpyridinium,[4,5] and selected organometallic lipophilic cations, exemplified by hexakis(2-methoxyisobutylisonitrile)technetium(I) (Tc-SESTAMIBI),[6−9] accumulate within mitochondria of living cells. Characterized by modest hydrophobicity, enabling permeation of biological membrane bilayers, and a delocalized cationic charge, enabling sequestration into compartments generating negative transmembrane potentials, these agents concentrate up to 1000-fold within the mitochondrial matrix. Exploiting this biological targeting property, the compounds have proven utility as biophysical probes of membrane function,[10,11] as models of neurological damage,[12] and in medical imaging.[13] Furthermore, a putative increase in mitochondrial transmembrane potential in cancer cells has been proposed as a potential mechanism confering tumor-specific cytotoxicity to selected lipophilic cations, such as rhodamine 123 and analogs.[3,14,15] Recently, carrier-added ^{99}Tc-SESTAMIBI was shown to possess potent cytotoxic activity against human Alexander hepatocellular carcinoma cells with a half-maximal lethal concentration (LC_{50}) of ∼9 μM, putatively by disrupting mitochondrial function.[16] However, regarding further development as therapeutic metallopharmaceuticals, ^{99}Tc(I) or isostructural rhenium(I) analogs are less than optimal because of the inherent low-energy β-emissions and sluggish coordination reaction kinetics, respectively, of these metals. To further explore alternative scaffolds as potential targeted anticancer metallopharmaceuticals, we sought multidentate ligands capable of conveniently chelating a variety of metals while retaining the biologically desirable lipophilic monocationic characteristics for conferring intracellular targeting.

A variety of multidentate ligands and their corresponding metal complexes have been explored as therapeutic chelating agents for the treatment of metal intoxication,[17,18] as diagnostic radiopharmaceuticals,[19] as paramagnetic contrast agents in magnetic resonance imaging,[20−22] as therapeutic medicinals,[23] to perform synthetic transformations (e.g., asymmetric epoxidations of unfunctionalized olefins[24]), and to study guest−host interactions of various ligands and metals. Among multidentate ligands, Schiff-base ligands[25−27] with an N_4O_2 donor core are well known. The coordination chemistry of these ligands with various transition and main group metals[28−30] and structural aspects of various metal chelates having N_4O_2 and N_4O_3 donor cores with main group metals and lanthanides have been

* Address correspondence: Mallinckrodt Institute of Radiology, Washington University Medical School, Box 8225, 510 S. Kingshighway Blvd., St. Louis, MO 63110. Tel: 314-362-9356. Fax: 314-362-0152. E-mail: piwnica-worms@mirlink.wustl.edu.

⊗ Abstract published in *Advance ACS Abstracts,* August 1, 1996.

S0022-2623(95)00823-5 CCC: $12.00

Chart 1. General Structure of Hexadentate R-ENBPI Ligands

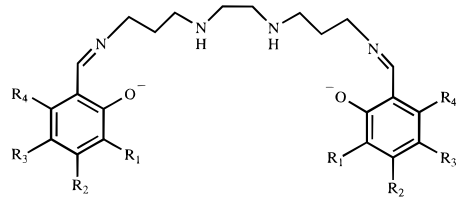
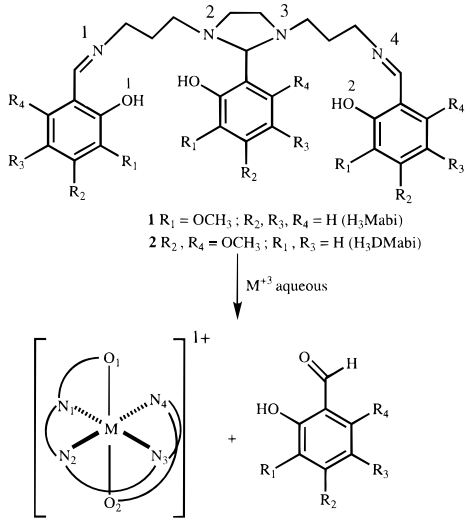


Chart 2. Pathway for the Synthesis of R-ENBPI Metal Complexes



reported.[31−33] Schiff-base N_4O_2 metal complexes have also been reported as potential positron-emitting radiopharmaceuticals for use in myocardial perfusion imaging.[34−36] As potential cytotoxic agents, these hexadentate Schiff-base ligands with the general structure shown in Chart 1 offer tremendous flexibility, since their binding affinities can be varied by inserting appropriate donor atoms to match the requirements of various incoming metals. In addition, while maintaining the inner coordination sphere, the lipophilicity and molecular shape of the resulting complexes can be altered by variation of the substituents on the aromatic rings and hydrocarbon backbone independently. We report here the synthesis of N_4O_2 Schiff-base phenolic complexes of Al(III), Fe(III), Ga(III), and In(III) and characterization of their cytotoxic potency in human epidermal carcinoma KB-3-1 cells and the colchicine-selected multidrug resistant derivative KB-8-5 cells. For these Schiff-base ligands, the cytotoxic potency of their corresponding metallopharmaceuticals depended strongly on the identity of the coordinating central metal. Furthermore, modest expression of the human multidrug resistance (*MDR1*) gene product, P-glycoprotein, conferred protection from the cytotoxic activity of potent Al(III), Fe(III), and Ga(III) complexes.

Results and Discussion

Chemistry. Heptadentate compounds **1** and **2**, precursors of the desired hexadentate ligands, were synthesized by condensation of the appropriate amine and 3 equiv of substituted salicylaldehyde in ethanol (Chart 2). Alternatively, condensation of the amine with 3 equiv of substituted salicylaldehyde in dry methylene chloride in the presence of activated molecular sieves provided spectroscopically identical compounds. Infrared spectra of precursors **1** (H_3Mabi) and **2** (H_3DMabi) showed strong peaks at 1640 and 1625 cm^{-1}, respectively, characteristic of imine C=N bonds. Formation of a five-membered imidazolidine ring due to reaction of a third aldehyde with the two inner adjacent secondary amine nitrogens of *N*,*N*′-bis(aminopropyl)ethylenediamine conferred unique characteristics on the middle ring compared to the two outer aromatic rings. The ^{1}H NMR spectrum of **1** in $CDCl_3$ showed the characteristic imine proton at δ 8.12, multiplets for aromatic protons at δ 7.00−6.55, methoxy substituents at δ 3.88 and 3.86 for the outer and middle rings, respectively, and hydrocarbon protons in the form of overlapping multiplets at δ 3.65, 3.40, 2.80−2.28, and 1.82. Proton-decoupled ^{13}C NMR spectrum showed 20 resonance signals (Figure 1, see Supporting Information), including the characteristic carbon resonance at δ 165.2 for the imine carbon and δ 89.3 for the benzylic carbon, consistent with the proposed structure for precursor **1**. The ^{1}H NMR of precursor **2** in $CDCl_3$ also showed the characteristic imine proton singlet at δ 8.25, aromatic protons at δ 5.99, 5.91, 5.84, and 5.56 in a ratio of 1:1:2:2, for the middle and outer aromatic rings, respectively, a proton assigned to the benzylic position at δ 4.32, different methoxy groups at δ 3.77, 3.76, 3.71, and 3.70 in a ratio of 2:2:1:1, respectively, and protons for the hydrocarbon backbone in the form of a series of multiplets at δ 3.52, 3.35, 2.75−2.30, and 1.80. Proton-decoupled ^{13}C NMR spectrum of precursor **2** in $CDCl_3$ showed 20 resonance signals instead of 22 (Figure 1, Supporting Information), presumably because of overlapping methoxy signals.

When treated with trivalent metals in aqueous conditions, the heptadentate precursors, whether isolated as **1** and **2** or formed as Schiff-base byproducts during condensation reactions of appropriate salicylaldehydes and amines, split off the middle ring, thereby decreasing steric demands on the resultant ligands and providing an N_4O_2 hexadentate donor core.[28] An appealing property of these ligands (R-ENBPI; Chart 1) that we hoped to exploit was their ability to efficiently chelate a wide range of metal ions. Specifically, we synthesized complexes with Fe(III), Ga(III), and In(III) from the reactions of precursors **1** and **2** with corresponding metal(III) acetylacetonates in refluxing ethanol (Chart 2). Alternatively, Al(III) and In(III) complexes were isolated by treatment of precursors **1** and **2** with their appropriate hydrated salts in the presence of base. Formation of metal complexes with the ligands (Chart 1) derived from precursors **1** and **2** and main group metal cations should be governed by the electronic and steric demands of the incoming metals relative to the flexibility of the donor core. Due to the nearly identical six-coordinate ionic radii of Fe^{3+} and Ga^{3+} (0.65 and 0.62 Å, respectively[37]), ligands with high affinity for Fe^{3+} tend to have high affinity for Ga^{3+}, often resulting in similar coordination chemistry. In contrast, Al^{3+} (0.51Å) and In^{3+} (0.81Å) were selected as two extreme limits to analyze the impact of the central metal core on the organic scaffold and to evaluate their overall effect on biological activity. In all cases, complexation of heptadentate compounds **1** and **2** with their corresponding trivalent metal ions led to cleavage of the five-membered imidazolidine ring, thereby exposing the inner secondary amine nitrogens for coordination to the metals. This imparted some degree of preorganization to the N_4O_2 donor core to meet

the demands of the incoming metal ion as the ligands wrapped around the coordination sphere. The ^{1}H NMR spectra of metal(III) complexes recorded in dimethyl sulfoxide-d_6 revealed the absence of benzylic protons and relative simplicity in the aromatic proton region. Because synthesis of the complexes was carried out under aqueous conditions, their ^{1}H NMR spectra in dry dimethyl sulfoxide-d_6 all showed a strong signal around δ 3.25−3.45 tentatively assigned to weakly coordinated water due to hydrogen bonding with the metal complexes. To test this hypothesis, exchange experiments were performed by adding traces of D_2O which produced disappearance of this signal, thereby confirming the presence of coordinated water and, furthermore, allowing proton signals arising from the hydrocarbon backbone underneath this signal to be assigned. Additionally, as expected, proton signals attributed to N−H around δ 4.80−5.22 either disappeared or broadened and moved upfield, whereas the rest of the signals remained unaltered in the D_2O exchange experiment.

Infrared (IR) spectra as KBr pellets of Al(III), Fe(III), Ga(III), and In(III) metal complexes were essentially identical, with only marginal differences in a few selected bands. Broad bands between 3250−3100 and 1550−1560 cm^{-1} were assigned to stretching and bending modes, respectively, of the coordinated amine nitrogens. All complexes, except **1b**, showed a broad band between 3600 and 3300 cm^{-1} which we attributed to O−H stretchings in the metal complexes, suggestive of weakly coordinated water held through hydrogen bonding. In addition, a characteristic band corresponding to υ(C═N) underwent a shift of 5−20 cm^{-1}, confirming coordination of the metals to the imine nitrogens of the ligands. Furthermore, normal bands at 3000−2600 and at 1600, 1480, and 1440 cm^{-1} were observed and assigned to υ(C−H) and υ(C═C), respectively. The appearance of bands below 600 cm^{-1} present in the spectra of complexes, but not in those of the precursors themselves, was probably indicative of υ(M−O) and υ(M−N). Thus, the ^{1}H NMR and IR spectral data were consistent with the proposed structures of the intact complexes in solution and solid state, respectively. Mass spectra (FAB) of all complexes done as 3-nitrobenzyl alcohol or thioglycerol matrices demonstrated mass ion peaks corresponding to the metal ligand cations [(ML)$^+$, M = metal, L = ligand]. Furthermore, absence of peaks in the region of higher atomic mass indicated the absence of dimeric species and in turn supported the proposed monomeric cationic complexes. Molar conductance (κ) measurements for the complexes in acetonitrile at room temperature showed values in the range of 110−174 Ω^{-1} mol^{-1} cm^2, also consistent with a 1:1 electrolyte (monocationic metal complexation).[38] Furthermore, complexes were stable to neutral hydrolysis. ^{1}H NMR and UV/vis spectra of complexes were superimposible before and after incubation in water for 72 h (37 °C, pH 7.5).

The R-ENBPI derivatives of precursors **1** and **2**, containing an N_4O_2 donor core with the general structure shown in Chart 1, wrapped around the central metal ion to provide four six-membered rings and one five-membered ring. In contrast, two six-membered and three five-membered rings are created by compounds containing bis ethylene groups[28] instead of bis propylene groups. As a result of the steric requirements of the hydrocarbon chain, the propylene backbone links adjacent donor atoms so that they must be placed cis to each other, therefore generating angles between adjacent donor atoms close to 90°. This has been reported earlier in the case of [1,10-bis(2-hydroxybenzamido)-3,6-diazadecan]manganese(IV).[39] On the basis of the crystal structures of analogous R-ENBPI complexes of Fe(III) and Ga(III),[36,40] we propose that our R-ENBPI complexes of Al(III), Fe(III), and Ga(III) likely have structures in which the two phenolate oxygens are coordinated trans to one another and cis to the corresponding amine and imine nitrogens, yielding an overall octahedral geometry as shown in Chart 2 or the corresponding enantiomers. However, in the case of the In(III) complexes, the increase in ionic size obtained by moving down the periodic table within the main group metals would likely lead to increased steric constraints on the hydrocarbon backbone. Therefore, we postulate that upon coordination of In(III) a significant change in structure to overcome these constraints, perhaps to a cis arrangement for the phenolic oxygens, may occur. Indeed, preliminary molecular modeling data using the CAChe worksystem correctly predicts the trans arrangement of phenolic oxygens around the central metal core for R-ENBPI Al(III), Fe(III), and Ga(III) complexes but infers the cis configuration for In(III) complexes.[41]

Cytotoxicity. P-Glycoprotein, encoded by the human multidrug resistance (*MDR1*) gene, is an integral plasma membrane transporter which renders tumors resistant to chemotherapy by transporting chemotherapeutic agents out of cells.[42−44] Overexpression of P-glycoprotein is thought to be one prominent mechanism of cancer chemotherapeutic failure in patients. Many drug substrates and modulators (inhibitors) of P-glycoprotein are lipophilic cationic compounds,[45] suggesting that the relationship between cellular cytotoxicity of racemic mixtures of the R-ENBPI metal(III) complexes and expression levels of P-glycoprotein was important to explore. Parental human epidermal carcinoma KB-3-1 cells and the derivative colchicine-selected KB-8-5 multidrug resistant cells (46) express no immunodetectable and modest levels of *MDR1* P-glycoprotein, respectively, as determined by immunoblots of plasma membrane preparations with the anti-P-glycoprotein monoclonal antibodies C219[47] and C494 (D.P.W., unpublished observations). These cells therefore provided a convenient in vitro system for quantitative cytotoxicity assays. Cells in monolayer culture in 96-well plates were exposed to novel R-ENBPI metal complexes over a range of pharmacologically relevant concentrations and cell survival was determined by the SRB method after 3 days in culture.[48] Cells grown in the presence of drug vehicle alone served as control preparations, while cells grown in the presence of high concentrations of the chemotherapeutic agent colchicine (25 μM) documented the effects of maximal cytotoxic activity. Cytotoxic potency was determined by computer fitting of survival curves and determination of an LC_{50}.

In drug-sensitive KB-3-1 cells, (3-MeO-ENBPI)Fe(III) (**1b**; see the Experimental Section) demonstrated an LC_{50} of 9 μM, similar to the cytotoxic activity of Al(III) and In(III) 3-MeO-ENBPI complexes (Figure 2A−D). Ga(III) complexes of 3-MeO-ENBPI were least potent (Table 1). Relative to KB-3-1 cells, expression of *MDR1* P-glycoprotein in KB-8-5 tumor cells conferred dramatic

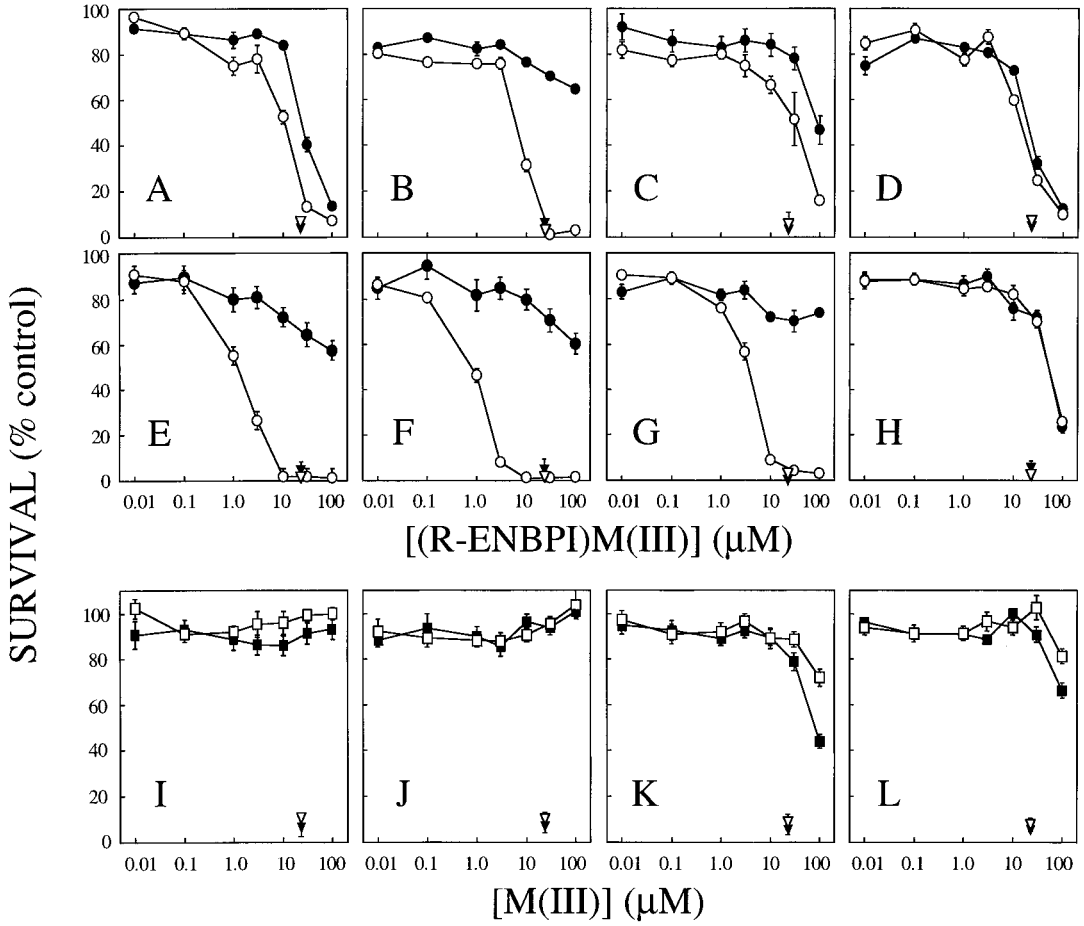


Figure 2. Cell survival studies and LC_{50} determination. Survival of parental KB-3-1 (○, ▽, □) and multidrug resistant KB-8-5 (●, ▼, ■) cells in increasing concentrations of R-ENBPI metal(III) complexes (○, ●), 25 μM colchicine (▽, ▼), or metal(III) ions (□, ■). Panels represent Al(III), Fe(III), Ga(III), and In(III) complexes of 3-MeO-ENBPI (A−D), complexes of 4,6-diMeO-ENBPI (E−H), and the free metals (I−L), respectively. Each point represents the mean of triplicate determinations; bars represent ±SEM when larger then symbol; solid lines are a spline presentation of the data.

Table 1. Cytotoxic LC50 Values for R-ENBPI Metal(III) Complexes in Human Drug-Sensitive (KB-3-1) and Multidrug Resistant (KB-8-5) Cells[a]

		KB-3-1				KB-8-5			
ligand	M(III)	LC_{50} (μM)	SEM	CV%	dependency	LC_{50} (μM)	SEM	CV%	dependency
3-MeO-ENBPI	Al	11.4	3.0	26.2	0.46	27.5[c]	2.7	9.9	0.33
	Fe	9.0	0.5	5.3	0.68	≫100[c]	NA	NA	NA
	Ga	42.6[b]	5.2	12.3	0.45	110[c]	12.6	11.5	0.39
	In	16.6	3.1	18.7	0.41	24.2	3.8	15.8	0.36
4,6-diMeO-ENBPI	Al	1.4[d]	0.1	8.2	0.39	≫100[c]	NA	NA	NA
	Fe	1.1[e]	0.05	4.6	0.23	≫100[c]	NA	NA	NA
	Ga	3.7[f]	0.4	11.2	0.34	≫100[c]	NA	NA	NA
	In	63.8	3.5	5.5	0.38	61.8	9.0	14.5	0.38

[a] LC_{50} values (μM) were determined from sigmoidal curve fittings of the cytotoxic concentration−effect data shown in Figure 2. When cell survival data indicated LC_{50} values greater than 100 μM (the highest drug concentration tested), reliable computer fittings were not possible, and low-potency cytotoxic activities are represented by entries of ≫100 μM; corresponding statistical parameters, no longer meaningful, are not applicable (NA). M(III), coordination metal; LC_{50}, half-maximal lethal concentration; SEM, standard error of mean; CV%, coefficient of variation percent. [b] $p < 0.01$, within KB-3-1, Ga(III) vs Al(III), Fe(III), or In(III) 3-MeO complexes. [c] $p < 0.005$, KB-8-5 vs KB-3-1 with the same M(III) complex. [d] $p < 0.05$, within KB-3-1, Al(III) vs Fe(III), Ga(III), or In(III) 4,6-diMeO complexes. [e] $p < 0.005$, within KB-3-1, Fe(III) vs Ga(III) or In(III) 4,6-diMeO complexes. [f] $p < 0.001$, within KB-3-1, Ga(III) vs In(III) 4,6-diMeO complex.

protection from the cytotoxic action of (3-MeO-ENBPI)-Fe(III) (**1b**), with concentrations of **1b** as high as 100 μM demonstrating no significant cytotoxicity. Additionally, *MDR1* P-glycoprotein conferred modest (2−3-fold) but statistically significant protection from the cytotoxic action of **1a**,**c** while conferring no significant protection from **1d**. In KB-3-1 cells, metal(III) complexes of 4,6-diMeO-ENBPI showed a rank order cytotoxic potency of Fe(III) > Al(III) > Ga(III) ≫ In(III) (Figure 2E−H and Table 1). Overall, the active 4,6-diMeO analogs were more potent than the 3-MeO analogs. Again, expression of modest levels of *MDR1* P-glycoprotein in the KB-8-5 cells conferred robust protection from the cytotoxic action of the (4,6-diMeO-ENBPI)Fe(III) analog (**2b**) and in the same fashion from (4,6-diMeO-ENBPI)-Al(III) (**2a**) and (4,6-diMeO-ENBPI)Ga(III) (**2c**) but conferred no significant modulation of the cytotoxicity of the In(III) analog **2d**. These data would be consistent with Al(III), Fe(III), and Ga(III) complexes being recognized as transport substrates by the human *MDR1*

P-glycoprotein and thereby extruded from the cells and sequestered away from their cytotoxic targets.

Interestingly, there appeared to be a correlation between cytotoxic potency and P-glycoprotein transport activity. As a group, the active 4,6-diMeO-ENBPI analogs were more potent and showed a more robust P-glycoprotein transport profile than the 3-MeO-ENBPI analogs (Figure 2). In(III) complexes **1d** and **2d** were conspicuously lower in cytotoxic potency and devoid of a P-glycoprotein transport profile**.** In addition, cytotoxic activities of nitrate salts of Al(III), Fe(III), Ga(III), and In(III) were evaluated in KB-3-1 and drug resistant KB-8-5 cells (Figure 2I−L). In contrast to their corresponding R-ENBPI complexes, Al(III) and Fe(III) ions did not show any evidence of cytotoxicity at any concentration up to 100 μM nor any differential effect between drug-sensitive and MDR cells. Ga(III) and In(III) salts demonstrated modest cytotoxic activity at 100 μM with a trend toward increased potency in the drug resistant cell line, opposite to effects observed with the metal complexes. Furthermore, precursors **1** and **2**, when added to the aqueous buffer (thereby likely providing the free hexadentate ligand or a hydrolysis product), demonstrated no significant cytotoxic activity except at the highest test concentration of 100 μM where modest activity was observed equally in both cell lines (data not shown). Overall, when combined with the demonstrated stability of the complexes to neutral hydrolysis, these data indicated that the transport and cytotoxic potency of the Al(III), Fe(III), and Ga(III) complexes could likely be attributed to pharmacological effects of the intact complex *per se* within cellular compartments.

The observation that Al(III), Fe(III), and Ga(III) but not In(III) 4,6-diMeO-ENBPI complexes provided the desired cytotoxic properties further argued that the proposed molecular shape (trans phenolic configuration) and charge distribution of the final complexes, not metal-specific properties, generally conferred the pharmacological actions. The stability of the complexes to neutral hydrolysis and lack of activity of the free metal(III) ions would tend to exclude rapid extracellular demetalation reactions, transmetalation reactions with serum metal-binding proteins such as transferrin, or chelation reactions of ligands in neutral intracellular compartments as contributors to their mechanism of action. It would appear that coordination of In(III) distorted or destabilized the complex sufficiently to alter its capacity to accumulate at its cytotoxic target (putative electronegative compartments) or to be recognized by the MDR1 transporter. We speculate that the preferential potency of the Fe(III) complexes may be attributable to specific conformational dimensions of the complex but cannot exclude the possibility of acid hydrolysis of these agents within subcellular organelles such as lysosomes. While these metallopharmaceuticals were designed to exploit electronegative compartments such as mitochondria as a cytotoxic target, further experiments are required to explore the pharmacological sites, mechanism(s) of action, stereochemistry of drug activity, and impact of coadministration of MDR modulators on their anticancer potency.

Conclusions

Monocationic Al(III), Fe(III), Ga(III), and In(III) complexes of substituted Schiff-base N_4O_2 phenol ligands (R-ENBPI) were synthesized and their pharmacological properties explored as a potential class of cytotoxic metallopharmaceuticals. These complexes combine linear ligand flexibility enabling the coordination of a variety of core metals with the lipophilic cationic characteristics of potential-dependent tumor-selective pharmaceuticals. Results showed that the cytotoxic potency of racemic mixtures of these metallopharmaceuticals depended strongly on the identity of the coordinating central metal. In addition, the active 4,6-dimethoxy-substituted ENBPI complexes were more potent than the corresponding 3-methoxy analogs. Furthermore, modest cellular expression of the human *MDR1* P-glycoprotein conferred protection from the cytotoxic activity of Al(III), Fe(III), and Ga(III) R-ENBPI complexes but not In(III) complexes. Remarkably, among the Group III elements, human cells were capable of distinguishing R-ENBPI complexes formed of the same ligands with different metals. A trans configuration of the phenolic moieties around the central coordination sphere of Al(III), Fe(III), and Ga(III) R-ENBPI complexes along with differential stabilities as compared with In(III) complexes may impart these biological activities. However, further studies are needed to evaluate mechanism(s) of action and structure−function relationships of these cytotoxic metal complexes.

Experimental Section

General Methods. Bis(*N*,*N*′-aminopropyl)ethylenediamine, 4,6-dimethoxysalicylaldehyde, and *o*-vanillin (3-methoxysalicylaldehyde) were obtained from Aldrich Chemical Co. Al(III), Fe(III), Ga(III), and In(III) acetylacetonates and their hydrated salts were purchased from Mathey-Johnson/Alfa Chemical Co. and Aldrich. The ^{1}H and ^{13}C NMR spectra were recorded on a GEMINI 300 HHz spectrometer; chemical shifts are reported in δ (ppm) with reference to TMS. IR spectra were recorded on a Perkin-Elmer 1710 Fourier transform spectrophotometer. Mass spectra (LR and HRMS) were obtained from the Washington University Resource for Biomedical and Bioorganic Mass Spectrometry with 3-nitrobenzyl alcohol or thioglycerol as a matrix. Elemental analyses (C, H, N) were performed by Galbraith Laboratories, Knoxville, TN. Molar conductance (κ, Ω^{-1} mol^{-1} cm^2) was determined with a portable conductivity meter (Orion Research, model 120) at 25 °C in acetonitrile with 0.37 mM solutions of each complex. Aqueous stabilities of the complexes were determined by suspending 5−10 mg of sample in 2 mL of H_2O (pH 7.5) in a sealed vial. Samples were stirred for 72 h (37 °C) and evaporated, and residue was analyzed by UV/vis (Beckman 620) or ^{1}H NMR spectroscopy in DMSO-d_6.

Synthesis of Ligands. 2-(2′-Hydroxy-3′-methoxyphenyl)-1,3-bis[4-aza-5-(2″-hydroxy-3″-methoxyphenyl)but-4′-en-1′-yl]-1,3-imidazolidine (H_3Mabi) (1). *N*,*N*′-Bis(aminopropyl)ethylenediamine (286 mg, 1.64 mmol) dissolved in ethanol (5 mL) was added to a stirred solution of *o*-vanillin (748 mg, 4.92 mmol) dissolved in ethanol (10 mL). The reaction mixture was heated to reflux for 3 h. After cooling to room temperature, volatiles were removed through rotatory evaporation, and the residue was dried under reduced pressure for 2 days to yield a bright yellow solid **1** (900 mg, 1.56 mmol, 95% yield). Alternatively, treatment of *N*,*N*′-bis(aminopropyl)-ethylenediamine with 3 equiv of *o*-vanillin in dry methylene chloride in the presence of activated molecular sieves at room temperature stirred for overnight and filtration followed by its subsequent evaporation gave a spectroscopically identical compound: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.82 (m, 4H), 2.28−2.80 (m, 6H), 3.40 (m, 4H), 3.65 (m, 3H), 3.86 (s, 3H), 3.88 (s, 6H), 6.55 (dd, 1H), 6.64 (t, 1H), 6.7−7.0 (m, 7H), 8.12 (s, 2H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 28.7, 49.4, 49.6, 55.6, 55.7, 55.8, 89.3, 111.9, 113.4, 117.1, 117.9, 118.1, 121.2, 122.7, 122.8, 147.2, 147.7, 148.3, 152.4, 165.2; IR (CH_2Cl_2, cm^{-1}) 1640 υ-

(C═N); FAB-HRMS calcd for $C_{32}H_{41}N_4O_6$ $(M + H)^+$ 577.3025, found 577.3020.

2-(2′-Hydroxy-4′,6′-dimethoxyphenyl)-1,3-bis[4-aza-5-(2″-hydroxy-4″,6″-dimethoxyphenyl)but-4‴-en-1′-yl]-1,3-imidazolidine (H_3DMabi) (2). A similar reaction of *N*,*N*′-bis(aminopropyl)ethylenediamine (130 mg, 0.74 mmol) and 4,6-dimethoxysalicylaldehyde (407 mg, 2.24 mmol) produced a pale yellow solid H_3DMabi, **2** (400 mg, 0.60 mmol, 80%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.80 (m, 4H), 2.30−2.75 (m, 6H), 3.35 (m, 4H), 3.52 (m, 2H), 3.70, 3.71 (s, 6H), 3.77, 3.76 (s, 12H), 4.32 (s, 1H), 5.56 (d, 2H), 5.84 (d, 2H), 5.91 (d, 1H), 5.99 (d, 1H), 8.25 (s, 2H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 28.9, 49.2, 49.5, 50.8, 54.9, 55.1, 80.6, 87.2, 89.4, 94.2, 95.2, 101.1, 102.0, 158.1, 160.1, 160.5, 160.6, 161.4, 166.8, 176.0; IR (CH_2Cl_2, cm^{-1}) 1625 υ(C═N); FAB-HRMS calcd for $C_{35}H_{47}N_4O_9$ $(M + H)^+$ 667.3342, found 667.3338.

Synthesis of Metal(III) Complexes. Method A: Appropriate ligand was dissolved in methanol (3 mL), treated dropwise with potassium hydroxide, and heated to reflux for 20 min. While hot, the appropriate hydrated salt dissolved in methanol (5 mL) containing water (2 mL) was added, refluxed for 1 h, cooled, and filtered. The filtrate was slowly evaporated at room temperature to yield a microcrystalline solid, which was separated, washed with water followed by cold methanol and then ether, and dried under reduced pressure overnight.

Method B: Another method involved mixing equimolar quantities of the appropriate ligand and metal acetylacetonates in ethanol. Contents were refluxed for 1 h; then the reaction mixture was treated while hot with an equimolar amount of KI dissolved in water and then refluxed for an additional 5−10 min. The resulting mixture was cooled to room temperature, and a microcrystalline compound precipitated out. This was separated, washed with cold ethanol and then ether, and dried under reduced pressure.

[(3-OMe-ENBPI)Al]$^+$NO$_3^-$ (1a). Using method A involving H_3Mabi ligand (**1**) (160 mg, 0.27 mmol), potassium hydroxide (271 μL, 3 M), and $Al(NO_3)_3$·$9H_20$ (100 mg, 0.27 mmol), washings with ether containing10% MeOH and finally with ether, and drying under vacuum provided **1a** (130 mg, 0.24 mmol, 87%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.50 (m, 2H), 1.85 (m, 2H), 2.68 (m, 2H), 2.88 (m, 4H), 3.15 (m, 2H), 3.38 (m, 4H), 3.68 (s, 6H, OCH_3), 4.90 (bs, 2H, NH), 6.65 (t, 2H, Ar-H), 6.90 (d, 2H, Ar-H), 7.05 (d, 2H, Ar-H), 8.30 (s, 2H, CH═N); IR (KBr, cm^{-1}) 1625 υ(C═N), 1360, 1265 υ(NO_3^-) ; κ (Ω^{-1} mol^{-1} cm^2) 117; MS (FAB) for $[C_{24}H_{32}N_4O_4Al]^+$ m/z = 467.1. Anal. ($C_{24}H_{32}N_5O_7Al$·H_2O) C, H, N.

[(4,6-DiOMe-ENBPI)Al]$^+$NO$_3^-$ (2a). Using method A involving H_3DMabi ligand (**2**) (66 mg, 0.09 mmol), potassium hydroxide (3 M, 98 μL), and $Al(NO_3)_3$·$9H_2O$ (37 mg, 0.09 mmol) yielded **2a** (50 mg, 0.08 mmol, 86%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.50 (m, 2H), 1.90 (m, 2H), 2.70 (m, 2H), 2.92 (m, 4H), 3.15−3.65 (m, 6H), 3.78 (s, 6H, OCH_3), 3.81 (s, 6H, OCH_3), 4.80 (bs, 2H, NH), 5.94 (s, 2H, Ar-H), 6.02 (s, 2H, Ar-H), 8.22 (s, 2H, CH═N); IR (KBr, cm^{-1}) 1620 υ(C═N), 1280b υ(NO_3^-); κ (Ω^{-1} mol^{-1} cm^2) 158; MS (FAB) for $[C_{26}H_{36}N_4O_6Al]^+$ m/z = 527.2. Anal. ($C_{26}H_{36}N_5O_9Al$·H_2O) C, H, N.

[(3-OMe-ENBPI)Fe]$^+$I$^-$ (1b). Using method B involving H_3Mabi ligand (**1**) (153 mg, 0.26 mmol), $Fe(acac)_3$ (94 mg, 0.26 mmol), and KI (44 mg) resulted in **1b** (120 mg, 0.19 mmol, 72%): IR (KBr, cm^{-1}) 3140 υ(N−H), 3000−2750 υ(C−H), 1620 υ(C═N), 1555 δ(N−H), 1605, 1480, 1440 υ(C═C); κ (Ω^{-1} mol^{-1} cm^2) 149; MS (FAB) for $[C_{24}H_{32}N_4O_4Fe]^+$ m/z = 496.2. Anal. ($C_{24}H_{32}N_4O_4FeI$) C, H, N.

[(4,6-DiOMe-ENBPI)Fe]$^+$I$^-$ (2b). Using method B involving H_3DMabi ligand (**2**) (110 mg, 0.17 mmol), $Fe(acac)_3$ (60 mg, 0.17 mmol), and KI (28 mg) yielded **2b** (90 mg, 0.13 mmol, 77%): IR (KBr, cm^{-1}) 3420 υ(O−H), 3110 υ(N−H), 3000−2850 υ(C−H), 1610 υ(C═N), 1550 δ(N−H), 1595, 1460, 1430 υ(C═C); κ (Ω^{-1} mol^{-1} cm^2) 127; MS (FAB) for $[C_{26}H_{36}N_4O_6Fe]^+$ m/z = 556.2. Anal. ($C_{26}H_{36}N_4O_6FeI$·0.5 H_2O) C, H, N.

[(3-OMe-ENBPI)Ga]$^+$I$^-$ (1c). Using method B involving H_3Mabi ligand (**1**) (140 mg, 0.24 mmol), $Ga(acac)_3$ (89 mg, 0.24 mmol), and KI (40.3 mg) yielded **1c** (125 mg, 0.19 mmol, 80%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.62 (m, 2H), 1.95 (m, 2H), 2.72 (m, 2H), 3.02 (m, 4H), 3.10−3.65 (m, 6H), 3.81 (s, 6H, OCH_3), 5.18 (bs, 2H, NH), 6.62 (t, 2H, Ar-H), 6.85 (d, 2H, Ar-H), 7.05 (d, 2H, Ar-H), 8.28 (s, 2H, CH═N); IR (KBr, cm^{-1}) 1635 υ(C═N); κ (Ω^{-1} mol^{-1} cm^2) 137; MS (FAB) for $[C_{24}H_{32}N_4O_4Ga]^+$ m/z = 509.3. Anal. ($C_{24}H_{32}N_4O_4GaI$·$2H_2O$) C, N, H.

[(4,6-DiOMe-ENBPI)Ga]$^+$I$^-$ (2c). Using method B involving H_3DMabi ligand (**2**) (130 mg, 0.19 mmol), $Ga(acac)_3$ (72 mg, 0.19 mmol), and KI (32 mg) yielded **2c** (110 mg, 0.16 mmol, 81%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.55 (m, 2H), 1.94 (m, 2H), 2.65 (m, 2H), 2.95 (m, 4H), 3.35 (m, 4H), 3.67 (t, 2H), 3.75 (s, 6H), 3.78 (s, 6H), 4.96 (bs, 2H, NH), 5.87 (2H, Ar-H), 5.96 (2H, Ar-H), 8.28 (s, 2H, CH═N); IR (KBr, cm^{-1}) 1610 υ(C═N); κ (Ω^{-1} mol^{-1} cm^2) 133; MS (FAB) for $[C_{26}H_{36}N_4O_6Ga]^+$ m/z = 569.1. Anal. ($C_{26}H_{36}N_4O_6GaI$·$2H_2O$) C, H, N.

[(3-OMe-ENBPI)In]$^+$NO$_3^-$ (1d). Using method A involving H_3Mabi ligand (**1**) (132 mg, 0.23 mmol), potassium hydroxide (3M, 230 μL), and $In(NO_3)$·$5H_2O$ (89 mg, 0.23 mmol) on final washings with ether containing 15% methanol yielded **1d** (100 mg, 0.16 mmol, 71%; spectroscopically identical compound was obtained by an *in situ* method[29] as an iodide): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.75 (m, 2H), 2.12 (m, 2H), 2.65 (m, 2H), 3.10 (m, 4H), 3.46 (m, 2H), 3.67 (m, 2H), 3.75 (s, 6H, OCH_3), 4.17 (t, 2H), 5.22 (bs, 2H, NH), 6.54 (t, 2H, Ar-H), 6.79 (d, 2H, Ar-H), 6.97 (d, 2H, Ar-H), 8.34 (s, 2H, CH═N); IR (KBr, cm^{-1}) 1626 υ(C═N), 1280−1244b υ(NO_3^-); κ (Ω^{-1} mol^{-1} cm^2) 110; MS (FAB) for $[C_{24}H_{32}N_4O_4In]^+$ m/z = 555.0. Anal. ($C_{24}H_{32}N_4O_4InI$·KCl) C, N; H: calcd, 4.26; found, 4.86.

[(4,6-DiOMe-ENBPI)In]$^+$I$^-$ (2d). Using method B involving H_3DMabi ligand (**2**) (118 mg, 0.17 mmol), $In(acac)_3$ (73 mg, 0.17 mmol), and KI (30 mg) resulted in **2d** (100 mg, 0.13 mmol, 76%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.70 (m, 2H), 2.10 (m, 2H), 2.55 (m, 2H), 3.05 (m, 4H), 3.40 (m, 4H), 3.72 (s, 6H, OCH_3), 3.76 (s, 6H, OCH_3), 3.94 (t, 2H), 5.14 (bs, 2H, NH), 5.83 (2H, Ar-H), 5.94 (2H, Ar-H), 8.34 (s, 2H, CH═N); IR (KBr, cm^{-1}) 1608 υ(C═N); κ (Ω^{-1} mol^{-1} cm^2) 174; MS (FAB) for $[C_{26}H_{36}N_4O_6In]^+$ m/z = 615.1. Anal. ($C_{26}H_{36}N_4O_6InI$·$2H_2O$) C, H, N.

Cell Culture and Cytotoxicity Assays. Monolayers of parental KB-3-1 and multidrug resistant KB-8-5 cell lines were routinely grown in DMEM (GIBCO, Grand Island, NY) supplemented with L-glutamine (2 mM), penicillin/streptomycin (0.1%), and heat-inactivated fetal bovine serum (10%) in the absence and presence of 10 ng/mL colchicine (Sigma Chemical Co.), respectively. Multidrug resistant cells were cultured in drug-free media for 96 h prior to cytotoxicity assays. Cytotoxicity potencies of R-ENBPI metal complexes, metal salts, or colchicine were determined in 96-well microtiter plates as described.[16] Cells (5000/well) were plated in media containing fetal bovine serum (5%) and allowed to recover for 4 h. The indicated concentrations of R-ENBPI complex, metal(III) nitrate with matched vehicle or a cytotoxic concentration of colchicine (10 μg/mL, 25 μM) were added in triplicate wells for each cell line. Drug solubility and vehicle concentration limited the highest test concentration to 100 μM. Cells were then incubated for 72 h under normal growth conditions (37 °C, 5% CO_2 atmosphere). Cell survival was assayed using sulforhodamine B (SRB) as previously described with slight modification.[48] Briefly, cells were fixed in 10% trichloroacetic acid for 60 min at 4 °C, washed five times with tap water, and allowed to air-dry overnight (25 °C). Protein precipitates were then stained with 0.4% SRB in 1% acetic acid for 20 min at room temperature. Excess SRB was removed with four 1% acetic acid washes, plates were air-dried (25 °C), and then the stain was redissolved in 10 mM unbuffered Tris base. Quantitation was performed on an ELISA plate reader using an absorption difference technique (490−450 nm).

Survival is expressed as the percentage of surviving cells relative to growth in media containing drug vehicle alone (ligand **1**, 1% ethanol; ligand **2** and metal(III) nitrates, 0.85% ethanol/0.15% DMSO). LC_{50} determinations were obtained from the cell survival curves by computer fitting with a sigmoid equation: $S = \{(S_{max} - S_{min})/[1 + (C/LC_{50})^{\gamma}]\} + S_{min}$, where S is cell protein, S_{max} is cell protein in control buffer, S_{min} is residual cell protein at highest drug toxicity, γ is the slope, C is cytotoxic agent concentration, and LC_{50} represents the half-maximal cytotoxic concentration. For analysis, S_{min} was constrained to zero at high drug concentrations. S_{max} ranged from

79% to 94% of control, with no significant differences between groups by one-way analysis of variance (*F*-ratio, 1.52[49]). Paired data were compared by the Student's *t*-test.[49] Dependencies of fitted curves were generally < 0.5 indicating that the curves were not overparameterized. Most analogs were tested in at least two separate culture experiments with essentially identical results.

Acknowledgment. This work was supported by a grant from the Department of Energy (ER61885). D.P.W. is an Established Investigator of the American Heart Association.

Supporting Information Available: Proton-decoupled ^{13}C-NMR spectrum (Figure 1) (2 pages). Ordering information is found on any current masthead page.

References

(1) Lichtshtein, D.; Kaback, H. R.; Blume, A. J. Use of a lipophilic cation for determination of membrane potential in neuroblastoma-glioma hybrid cell suspensions. *Proc. Natl. Acad. Sci. U.S.A.*, **1979**, *76*, 650−654.
(2) Johnson, L.; Walsh, M.; Chen, L. Localization of mitochondria in living cells with rhodamine 123. *Proc. Natl. Acad. Sci. U.S.A.*, **1980**, *77*, 990−994.
(3) Davis, S.; Weiss, M.; Wong, J.; Lampidis, T.; Chen, L. Mitochondrial and plasma-membrane potentials cause unusual accumulation and retention of rhodamine-123 by human breast adenocarcinoma-derived MCF-7 cells. *J. Biol. Chem.* **1985**, *260*, 13844−13850.
(4) Langston, J. W.; Ballard, P.; Tetrud, J. W.; Irwin, I. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* **1983**, *219*, 979−980.
(5) Ramsay, R. R.; Salach, J. I.; Singer, T. P. Uptake of the neurotoxin 1-methyl-4-phenylpyridine (MPP^+) by mitochondria and its relation to the inhibition of the mitochondrial oxidation of NAD^+-linked substrates by MPP^+. *Biochem. Biophys. Res. Commun.* **1986**, *134*, 743−748.
(6) Piwnica-Worms, D.; Kronauge, J. F.; Chiu, M. L. Uptake and retention of hexakis (2-methoxy isobutyl isonitrile) technetium-(I) in cultured chick myocardial cells: mitochondrial and plasma membrane potential dependence. *Circulation* **1990**, *82*, 1826−1838.
(7) Backus, M.; Piwnica-Worms, D.; Hockett, D.; Kronauge, J.; Lieberman, M.; Ingram, P.; LeFurgey, A. Microprobe analysis of Tc-MIBI in heart cells: calculation of mitochondrial potential. *Am. J. Physiol: Cell* **1993**, *265*, C178−C187.
(8) Crane, P.; Laliberte, R.; Heminway, S.; Thoolen, M.; Orlandi, C. Effect of mitochondrial viability and metabolism on technetium-99m-sestamibi myocardial retention. *Eur. J. Nucl. Med.* **1993**, *20*, 20−25.
(9) Piwnica-Worms, D.; Kronuage, J. F.; LeFurgey, A.; Backus, M.; Hockett, D.; Ingram, P.; Lieberman, M.; Holman, B. L.; Jones, A. G.; Davison, A. Mitochondrial localization and characterization of 99-Tc-SESTAMIBI in heart cells by electron probe X-ray microanalysis and 99-Tc-NMR spectroscopy. *Magn. Reson. Imaging* **1994**, *12*, 641−652.
(10) Ritchie, R. J. A critical assessment of the use of lipophilic cations as membrane potential probes. *Prog. Biophys. Mol. Biol.* **1984**, *43*, 1−32.
(11) Chernoff, D. M.; Strichartz, G. R.; Piwnica-Worms, D. Membrane potential determination in large unilamellar vesicles with hexakis(2-methoxyisobutyl isonitrile)technetium(I). *Biochim. Biophys. Acta* **1993**, *1147*, 262−266.
(12) Gluck, M.; Krueger, M.; Ramsay, R.; Sablin, S.; Singer, T.; Nicklas, W. Characterization of the inhibitory mechanism of 1-methyl-4-phenylpyridinium and 4-phenylpyridine analogs in inner membrane preparations. *J. Biol. Chem.* **1994**, *269*, 3167−3174.
(13) Wackers, F. J.; Berman, D.; Maddahi, J.; Beller, G.; Straus, H.; Boucher, C.; Picard, M.; Holman, B.; Fridrich, R. Tc-99m-hexakis 2-methoxy isobutlisonitrile:human biodistribution, dosimetry, safety and preliminary comparison to thallium-201 for myocardial perfusion imaging. *J. Nucl. Med.* **1989**, *30*, 301−309.
(14) Chen, L. B. Mitochondrial membrane potential in living cells. *Annu. Rev. Cell Biol.* **1988**, *4*, 155−181.
(15) Koya, K.; Li, Y.; Wang, H.; Ukai, T.; Tatsuta, N.; Kawakami, M.; Shishido, T.; Chen, L. MKT-077, a novel rhodacyanine dye in clinical trials, exhibits anticarcinoma activity in preclinical studies based on selective mitochondrial accumulation. *Cancer Res.* **1996**, *56*, 538−543.
(16) Piwnica-Worms, D.; Chiu, M. L.; Budding, M.; Kronauge, J. F.; Kramer, R. A.; Croop, J. M. Functional imaging of multidrug-resistant P-glycoprotein with an organotechnetium complex. *Cancer Res.* **1993**, *53*, 1−8.
(17) Bryce-Smith, D. Environmental chemical influences on behaviour and mentation. *Chem. Soc. Rev.* **1986**, *15*, 93−123.
(18) May, P. M.; Bulman, R. A. The present status of chelating agents in medicine. *Prog. Med. Chem.* **1983**, *20*, 225−336.
(19) Deutsch, E.; Libson, K.; Jurisson, K.; Lindoy, L. F. Technetium chemistry and technetium radiopharmaceuticals. *Prog. Inorg. Chem.* **1983**, *30*, 75−139.
(20) Lauffer, R. Paramagnetic metal complexes as water proton relaxation agents for NMR imaging: theory and design. *Chem. Rev.* **1987**, *87*, 901−927.
(21) Kumar, K.; Tweedle, M. F. Macrocyclic polyaminocarboxylate complexes of lanthanides as magnetic resonance imaging contrast agents. *Pure Appl. Chem.* **1993**, *65*, 515.
(22) Xu, J.; Franklin, S. J.; Whisenhunt, D. W.; Raymond, K. N. Gadolinium complex of tris[(3-hydroxy-1-methyl-2-oxo-1,2-didehydropyridine-4-carboxamido)ethyl]-amine: a new class of gadolinium magnetic resonance relaxation agents. *J. Am. Chem. Soc.* **1995**, *117*, 7245−7246.
(23) Abrams, M.; Murrer, B. Metal compounds in therapy and diagnosis. *Science* **1993**, *261*, 725−730.
(24) Palucki, M.; McCormick, G. J.; Jacobsen, E. N. Low temperature assymmetric epoxidation of unfunctionalzed olefins catalyzed by (salen)Mn(III) complexes. *Tetrahedron Lett.* **1995**, *36*, 5457−5460.
(25) Isobe, T.; Kida, S.; Misumi, S. Preparation of schiff base complexes of lanthanides(III) ions. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 1862−1863.
(26) Bailey, N. A.; Mckenzie, E. D.; Worthington, J. M. Molecular structure (x-ray analysis) of a dinuclear iron(III) compound formed with the $[N_4O_3]$ ligand 'sal$_3$trien'. *Inorg. Chim. Acta* **1977**, *25*, L137−138.
(27) Evans, D. F.; Jakubovic, D. A. Water soluble hexadentate schiff-base ligands as sequestrating agents for iron(III) and gallium-(III). *J. Chem. Soc. Dalton Trans.* **1988**, 2927−2933.
(28) Sarma, B. D.; Bailar, J. C. The stereochemistry of metal complexes with polydentate ligands. Part I. *J . Am. Chem. Soc.* **1955**, *77*, 5476−5480.
(29) Sarma, B. D.; Ray, K. R.; Sievers, R. E.; Bailar, J. C. The stereochemistry of metal chelates with multidentate ligands. Part II. *J. Am. Chem. Soc.* **1964**, *86*, 14−16.
(30) Tweedle, M. F.; Wilson, L. J. Variable spin iron(III) chelates with hexadentate ligands derived from triethylenetetramine and various salicyaldehydes. *J. Am. Chem. Soc.* **1976**, *98*, 4824−4834.
(31) Wong, E.; Liu, S.; Lugger, T.; Hahn, F. E.; Orvig, C. Hexadentate N_4O_2 amine phenol complexes of gallium and indium. *Inorg. Chem.* **1995**, *34*, 93−101.
(32) Yang, L. W.; Liu, S.; Wong, E.; Rettig, S. J.; Orvig, C. Complexes of trivalent metal ions with potentially heptadentate N_4O_3 Schiff base and amine phenol ligands of variable rigidity. *Inorg. Chem.* **1995**, *34*, 2164−2178.
(33) Yang, L. W.; Liu, S.; Rettig, S. J.; Orvig, C. Complexation of potentially heptadentate ligand H_3dha$_3$tren with lanthanides: Architecture of $[Nd(NO_3)_3(H_3dha_3tren)]_n$ polymer with profuse modes of nitrate coordination. *Inorg. Chem.* **1995**, *34*, 4921−4925.
(34) Green, M. A.; Welch, M. J.; Huffman, J. C. Synthesis and crystallographic characterization of a gallium salicylaldimine complex of radiopharmaceutical interest. *J. Am. Chem. Soc.* **1984**, *106*, 3689−3691.
(35) Tsang, B. W.; Mathias, C. J.; Green, M. A. A gallium-68 radiopharmaceutical that is retained in myocardium: ^{68}Ga-[(4,6-MeO)$_2$sal)$_2$BAPEN]$^+$. *J. Nucl. Med.* **1993**, *34*, 1127−1131.
(36) Tsang, B. W.; Mathias, C. J.; Fanwick, P. E.; Green, M. A. Structure-distribution relationship for metal-labelled myocardial imaging agents: Comparison of a series of cationic gallium(III) complexes with hexadentate bis(salicylaldimine) ligands. *J. Med. Chem.* **1994**, *37*, 4400−4406.
(37) Shannon, R. D. Revised effective ionic radii and systematic studies of interatomic distances in halides and chalcogenides. *Acta Crystallogr.* **1976**, *A32*, 751−767.
(38) Geary, W. J. The use of conductivity measurements in organic solvents for the characterization of coordination compounds. *Coord. Chem. Rev.* **1971**, *7*, 81−122.
(39) Chandra, S. K.; Chakravorty, A. Two Manganese(IV) complexes with isomeric MnN_4O_2 spheres incorporating hexadentate amide-amine-phenolate coordination. *Inorg. Chem.* **1992**, *31*, 760−765.
(40) Ito, T.; Sugimoto, M.; Ito, H.; Toriumi, K.; Nakayama, H.; Mori, W.; Sekizaki, M. A chelate ring size effect on spin states of iron-(III) complexes with hexadentate ligands derived from salicylaldehyde and 4,8-diazaundecane-1,11-diamine (3,3,3-tet) or 4,7-diazadecane-1,10-diamine (3,2,3-tet) and their X-ray structures. *Chem. Lett.* **1983**, 121−124.
(41) Sharma, V.; Reichert, D.; Crankshaw, C.; Welch, M. J.; Piwnica-Worms, D. Effects of the central coordination metal on recognition of ethylenediamine-N,N′-bis(propyl(R-benzylimino)]metal-(III) cations by multidrug resistance (MDR1) p-glycoprotein. *J. Nucl. Med.* **1996**, *37*, 71P.

(42) Gros, P.; Ben Neriah, Y.; Croop, J. M.; Housman, D. E. Isolation and expression of a complementary DNA that confers multidrug resistance. *Nature* **1986**, *323*, 728−731.
(43) Shen, D. W.; Fojo, A.; Chin, J. E.; Roninson, I. B.; Richert, N.; Pastan, I.; Gottesman, M. M. Human multidrug-resistant cell lines: increased mdr1 expression can precede gene amplification. *Science* **1986**, *232*, 643−645.
(44) Gottesman, M. M.; Pastan, I. Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu. Rev. Biochem*. **1993**, *62*, 385−427.
(45) Ford, J. M.; Hait, W. N. Pharmacology of drugs that alter multidrug resistance in cancer. *Pharmacol. Rev*. **1990**, *42*, 155−199.
(46) Akiyama, S. I.; Fojo, A.; Hanover, J. A.; Pastan, I.; Gottesman, M. M. Isolation and genetic characterization of human KB cell lines resistant to multiple drugs. *Somatic Cell. Mol. Genet*. **1985**, *11*, 117−126.
(47) Dolci, E. D.; Abramson, R.; Xuan, Y.; Siegfried, J.; Yuenger, K. A.; Yassa, D. S.; Tritton, T. R. Anomalous expression of P-glycoprotein in highly drug-resistant human KB cells. *Int. J. Cancer* **1993**, *54*, 302−308.
(48) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst*. **1990**, *82*, 1107−1112.
(49) Glantz, S. A. *Primer of Biostatistics*, 2nd ed.; Mcgraw-Hill, Inc.: New York, 1987; p 379.

JM950823C