Synthesis, Molecular Structure, and Properties of a Neutral Schiff Base Phenolic Complex of Magnesium

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Received November 19, 1997

Introduction

Multidrug resistance (MDR) in cancer mediated by the *MDR1* P-glycoprotein (Pgp), a 140−180 kDa plasma membrane protein,[1,2] renders chemotherapeutic treatment ineffective by pumping a variety of natural product cytotoxic agents and xenobiotic compounds out of cancer cells.[2,3] Pgp has been a major target for synthesis and development of both therapeutic antagonists that block its transport function[4−6] and diagnostic radiopharmaceuticals that are transported by the protein for use in functional imaging of Pgp transport activity in tumors in vivo.[7−14] Most, but not all, compounds that interact with Pgp are hydrophobic and cationic at physiological pH.[4] Cationic amine phenol[15,16] and Schiff base phenolic Ga(III) complexes[17,18] have been shown to be Pgp-transported cationic radiopharmaceuticals for possible use in positron emission tomographic imaging.[19] While analogous Fe(III) complexes are potential cytotoxic agents,[20,21] tumor cell expression of *MDR1* Pgp reduces the potency of some of these complexes, implying Pgp-mediated transport of the agents.[20] To further understand the Pgp targeting properties of these compounds, we sought to directly evaluate the effect of charge of the complex on Pgp interactions. This could be done by comparing the cytotoxicity profile of a neutral complex to that of an identical, but positively charged, complex in both drug-sensitive and multidrug-resistant cancer cells. Thus, a neutral analogue of the Ga(III) and Fe(III) complexes was desired. Herein we describe the synthesis and structure of a novel neutral Schiff base Mg complex (**1**) and evaluate its cytotoxic potency in human drug-sensitive KB-3-1 and multidrug-resistant KB-8-5 tumor cells.

Experimental Section

Chemistry. The Mg complex of *N*,*N*′-bis{3-[(2-hydroxy-3-methoxybenzyl)imino]propyl}ethylenediamine (**1**) was prepared by a dropwise addition of *o*-vanillin (2.28 g; 0.015 mol) in 60 mL of CH_2Cl_2 to a mixture of *N*,*N*′-bis(3-aminopropyl)ethylenediamine (3.49 g; 0.02 mol) and $MgSO_4$ (6.0 g) in 300 mL of CH_2Cl_2. After the addition, the mixture was stirred for 24 h at room temperature. The solution was separated from solid material and solvent removed under low pressure (yield based on *o*-vanillin: 3.4 g; 97%). Solid residue was then crystallized from nitromethane. ^{1}H NMR ($CDCl_3$): δ 7.81 (s, 2H), 6.71 (d, 2H), 6.61 (d, 2H), 6.31 (t, 2H), 4.15 (m, 2H), 3.89 (m, 2H), 3.79 (s, 6H), 2.92 (m, 8H), 2.00 (d, 2H), 1.56 (m, 2H), 1.39 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 165.1 (C(10), C(20)); 160.3 (C(12), C(22)); 152.6 (C(13), C(23)); 125.3 (C(11), C(21)); 121.9 (C(24), C(14)); 112.5 (C(26), C(16)); 110.6 (C(15), C(25)); 60.5 (C(17), C(27)); 55.5 (C(1), C(8)); 49.7 (C(4), C(5)); 49.0 (C(3), C(6)); 33.9 (C(2), C(7)). IR: 3229 (ν_{NH}), 3049 ($\nu_{CH(arom)}$), 2913 and 2858 ($\nu_{CH(aliph)}$), and 1630 ($\nu_{C=N}$) cm^{-1}. LRMS (M + 1): 465 *m*/*z*. Anal. Calcd for $C_{24}H_{32}N_4O_4Mg$: C, 62.01; H, 6.94; N, 12.05; Mg, 5.23; Found: C, 61.25; H, 6.84; N, 12.12; Mg, 5.39. Further description of the synthesis, NMR, IR, and mass spectral experiments as well as the X-ray data collection are given in the Supporting Information.

Cytotoxicity Assay. Cytotoxic potency of **1** was determined in a cell survival assay using parental human epidermal carcinoma KB-3-1 cells and colchicine-derived multidrug-resistant KB-8-5 cells which express *MDR1* Pgp.[13] Following a 72 h incubation in growth media in the absence or presence of increasing concentrations of **1**, cell mass was determined using a colorimetric protein assay with sulforhodamine B as described in the Supporting Information.[22] The cytotoxic potency of **1** was determined by computer fitting cell survival curves to estimate the half-maximal lethal concentration (LC_{50}).[20]

Results and Discussion

Group IIA elements offer several attractive choices as the central metallic core for neutral Schiff base phenolic complexes. Among them, Mg has several advantages, including a similar six-coordinate ionic radius compared with Ga (0.72 Å for Mg^{2+} and 0.62 Å for Ga^{3+})[23] and an ability to adopt octahedral

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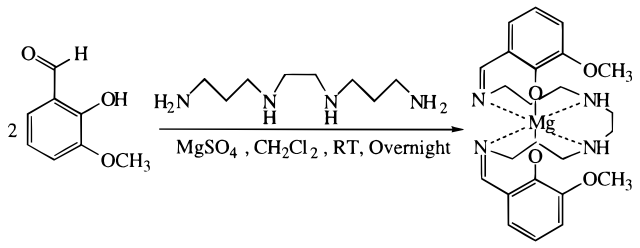
S0020-1669(97)01466-3 CCC: $15.00
Published on Web 08/14/1998

Scheme 1



coordination with a marked preference for oxygen donors.[24] In addition, Mg is of bioinorganic interest because of its ability to bind biological macromolecules such as proteins, nucleic acids, and membrane lipids.[25,26] Schiff base phenoxo complexes of Mg involving the participation of two phenolic oxygens and four nitrogens are unknown, and furthermore, only a few structurally characterized monomeric complexes with phenols are reported.[27−29]

As expected, attempts to isolate a Schiff base phenoxo complex of Mg using a known tris(salicylaldimine) precursor ligand[20] stirred in methylene chloride at room temperature in the presence of excess $MgSO_4$ failed to generate the desired product, putatively due to the lack of water required for cleavage of the five-membered imidazolidine ring. Conversely, aqueous conditions also failed, presumably a result of hydration of Mg^{2+} ions.

Application of direct synthesis[30] resulted in the desired product (**1**) in one step (Scheme 1) and with quantitative yield. Through activation of tetraamines by absorption onto $MgSO_4$ particles, the condensation reaction with aldehydes likely occurred on the surface of the particles. Direct synthesis shows especially favorable results when one reactant is insoluble (vs an insoluble product wherein the reaction is driven to completion by classic equilibrium interactions), thereby presenting the opportunity for the other two reactants to meet on its surface. This decreases the reaction order from 3 to 2 or 1. In this case, while the reaction mechanism is unknown, the net effect was that phenolic oxygens bound the Mg atom and liberated protons. Excess tetraamine in solution may have contributed to the success of the reaction by deprotonating phenolic oxygens. Furthermore, formation of **1** as a result of this reaction suggests that $MgSO_4$ may not be an ideal desiccant for use in condensation reactions involving salicylaldehydes and linear tetraamines.

Highly soluble in methylene chloride, **1** is slightly soluble in methanol and sparingly soluble in water. The ^{1}H NMR spectrum of **1** recorded in $CDCl_3$ at room temperature demonstrates a characteristic series of complex multiplets in the aliphatic region (δ 1.39, 1.56, 2.00, 2.92, 3.89), in addition to the presence of a single set of aromatic signals (δ 6.31, 6.61, 6.71) and the anticipated single methoxy resonance at δ 3.79. The complexity of the multiplets observed in the aliphatic region, due to the chirality of the coordinated secondary amines, provides evidence for rigidity of **1**. In addition, resonance

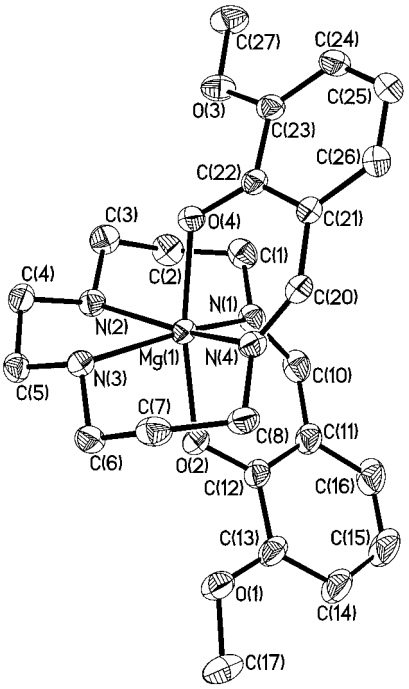


Figure 1. Fragment of the molecular structure of **1** (20% probability level). H atoms have been omitted for clarity.

signals of the coordinated secondary amino hydrogens, which are typically observed around δ 4.9−5.2 for the analogous cationic Ga(III) complexes,[20] are shifted upfield in the neutral complex and likely masked by overlapping aliphatic resonance signals. The signal from the aldimino proton was observed at δ 7.81 for the Mg complex versus δ 8.28 for the previously reported Ga(III) complex,[20] indicating a shift of electron density from the imino carbon atom toward nitrogen for the Ga(III) complex. This implies stronger N−Ga interaction and better stabilization of the partial negative charge on the nitrogen by the Ga^{3+} ion vs Mg^{2+}. Proton decoupled ^{13}C NMR of **1** in $CDCl_3$ at room temperature demonstrates 12 carbon resonance signals suggesting a symmetrical structure in solution analogous to that of Ga(III) complexes of the same[20] or similar[18] ligands.

The molecular structure of **1** is shown in Figure 1. Details of the crystal data and refinement parameters for **1** are given in Table 1, and principal bond lengths and angles are listed in Table 2. Each unit cell holds two pairs of independent stereoisomeric molecules, resulting in a racemic crystal. The principal bond lengths and angles are similar for both molecules in the asymmetric unit and are listed separately in the Supporting Information. The nearest structural analogue to the Mg compound is a positively charged Ga(III) complex[18] with a similar bis(salicylaldimine) that had been crystallized as the iodine salt. As with the Ga(III) complex, the Mg atom exhibits an octahedral geometry, surrounded by four nitrogen atoms in an equatorial plane and two axial oxygen atoms. Metal−nitrogen bond lengths in the case of Mg are up to 0.2 Å longer than for Ga. Given similar crystal atomic radii for Mg and Ga,[23] these data confirm the NMR observations indicating that Mg forms weaker bonds to nitrogen atoms than Ga. However, the metal−oxygen bond lengths are similar (only 0.06 Å longer for the Mg complex) confirming the known preference of Mg for oxygen donors.

It was essential before biological investigations to determine if **1** would remain stable in extracellular buffers for sufficient time to target cells (72 h at 37 °C). Demetalation of **1** produced characteristic ^{1}H NMR signals of an aldimino proton at δ 8.10

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Table 1. Crystal and Structure Refinement Details[a]

compound	$C_{24}H_{32}MgN_4O_4$
fw	464.85
temp, (K)	294(2)
wavelength (Å)	1.54178
color	light yellow
cryst syst	triclinic
space group (no.)	$P\bar{1}$ (2)
θ range for cell determination	4.32−27.89
a, b, c (Å)	11.316(1), 11.698(1), 21.553(2)
α, β, γ (deg)	75.22(1), 75.29(1), 61.86(1)
vol (Å^3)	2402.5(4)
Z	4
calcd density (mg/mm^3)	1.285
abs coeff (mm^{-1})	0.948
F(000)	992
cryst size (mm)	0.6 × 0.25 × 0.2
θ range	2.15−69.20
index ranges	$-13 \le h \le 13$, $-13 \le k \le 13$, $-23 \le l \le 26$
reflns collected	16400
indep reflecns	8618 [R(int) = 0.065]
obsd data/restrictions/parameters	8600/0/852
goodness-of-fit (S)	1.026
final R1, wR2 [$I > 2\sigma(I)$]	0.0656, 0.1762
R1, wR2 (all data)	0.0860, 0.2177
(Fourier-map) max, min (e Å^{-3})	0.282, −0.358

[a] $R_{int} = \sum|F_o^2 - F_o^2(\text{mean})|/\sum|F_o^2|$; R1 = $\sum||F_o| - |F_c||/\sum|F_o|$; wR2 = $[\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)^2]]^{1/2}$; $S = [\sum[w(F_o^2 - F_c^2)^2]/(n-p)]^{1/2}$; where F_o = observed structure factors; F_c = calculated structure factors; w = weighting scheme (see instructions to SHELXL-93); n = number of elections; and p = total number of parameters refined. Corrections for extinction used the expression $F_c^{new} = kF_c[1 + 0.001eF_c^2 \times \lambda^3/\sin(2\theta)]^{-0.25}$.

Table 2. Selected Bond Lengths (Å) and Angles (deg) for **1**

Mg(1)−O(2)	1.998(2)	Mg(1)−N(3)	2.215(3)
Mg(1)−O(4)	2.001(2)	Mg(1)−N(4)	2.201(3)
Mg(1)−N(1)	2.183(3)	N(4)−C(20)	1.280(4)
Mg(1)−N(2)	2.219(3)		
O(4)−Mg(1)−O(2)	169.96(9)	N(1)−Mg(1)−N(2)	83.6(1)
O(4)−Mg(1)−N(1)	91.9(1)	N(3)−Mg(1)−N(2)	80.8(1)
O(2)−Mg(1)−N(1)	83.0(1)	N(4)−Mg(1)−N(3)	83.7(1)
O(4)−Mg(1)−N(2)	87.5(1)	N(1)−Mg(1)−N(4)	113.9(1)
O(2)−Mg(1)−N(2)	100.5(1)	N(1)−Mg(1)−N(3)	160.2(1)
O(4)−Mg(1)−N(3)	99.43(9)	N(4)−Mg(1)−N(2)	160.2(1)
O(2)−Mg(1)−N(3)	87.99(9)	C(20)−N(4)−C(8)	117.5(3)
O(4)−Mg(1)−N(4)	82.89(9)	C(20)−N(4)−Mg(1)	124.3(2)
O(2)−Mg(1)−N(4)	91.27(9)		

and variation in the aliphatic region of the decomposition product. The relative abundance of **1** was analyzed through evaluation of integration ratios of both aldimino and selected aliphatic signals compared with its decomposition product. Greater than 85% of **1** remained intact after 72 h of incubation in solution containing an equimolar mixture of Mg^{2+} and $HPO_4^{2-}/H_2PO_4^-$ ions (pH 7.4). Note that the $Mg^{2+}/HPO_4^{2-}/H_2PO_4^-$ solution emulated the physiological buffer used for cytotoxicity assays. However, rates of hydrolysis of **1** significantly increased in acidic solution (pH 6.0) or in the presence of 5 mM $HPO_4^{2-}/H_2PO_4^-$ alone.

The relative cytotoxic potency of **1** was examined under conditions identical to those used previously for cationic analogues.[20] In contrast to the cationic 3-methoxy Fe(III) and Ga(III) complexes wherein the multidrug-resistant KB-8-5 cells showed 20-fold and 3-fold resistance, respectively, to the cytotoxic action of the complexes compared to drug-sensitive KB-3-1 cells,[20] the neutral Mg complex resulted in equal cytotoxic profiles in both cell lines (Figure 2).[31] These data would indicate that Pgp does not transport **1** out of the multidrug-resistant cells. Overall, when combined with the structural similarity of the Mg and Ga complexes, the results suggest that Pgp interactions for a given member of this class of compounds may be directly dependent on the charge of the molecule.

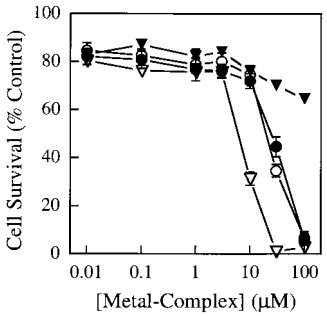

Figure 2. Cell survival studies and LC_{50} determination. Survival of drug-sensitive KB-3-1 (○, ▽) and Pgp-expressing KB-8-5 (●, ▼) cells grown in the presence of increasing concentrations of **1** (○, ●) or the cationic Fe(III) analogue (▽, ▼). Cells grown in the presence of vehicle (0.85% ethanol/0.15% DMSO) alone served as control preparations; data for cell survival in the presence of complexes are plotted as a percent of vehicle control. LC_{50} (μM), mean ± SEM: **1**, KB-3-1, 26 ± 1.6; KB-8-5, 34 ± 2.5; Fe(III) complex, KB-3-1, 9.0 ± 0.5; KB-8-5, >200. Data for the Fe(III) complex are replotted for comparison from ref 20. Each point represents the mean of triplicate determinations; bars represent ± SEM when larger than symbol.

Summary

A new method for synthesis of a stable Schiff base neutral phenoxo Mg complex is described. The novel complex is structurally similar to analogous cationic Ga(III) complexes. However, the Mg complex showed cytotoxic potencies that are not modified by expression of *MDR1* Pgp in the target human cancer cells, emphasizing the importance of charge for promoting *MDR1* Pgp interactions with these complexes. In addition, since metal complexes may form, these results highlight caution in the indiscriminate use of $MgSO_4$ as a drying agent in condensation reactions involving linear tetraamines and salicylaldehydes.

Acknowledgment. This work was supported by grants from the U.S. Department of Energy (DE-FG02-94ER61885) and National Institutes of Health (RO1 CA65735). We thank the Washington University Resource for Biomedical and Bioorganic Mass Spectrometry for their technical assistance.

Supporting Information Available: A description of general techniques and of details of the synthesis of the Mg complex, X-ray crystallography, and cell culture and cytotoxicity assays and tables of crystal data and refinement, atomic coordinates, bond lengths and angles, and anisotropic displacement parameters (19 pages). Ordering information also is given on any current masthead page.

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(31) Stability data would indicate that a significant quantity of the intact Mg complex was present in the extracellular buffer (pH 7.4) throughout the time course of the cytotoxicity assay. However, unidentified intracellular hydrolysis within lysosomal compartments cannot be ruled out.