Synthesis of Two 3,5-Disubstituted Sulfonamide Catechol Ligands and Evaluation of Their Iron(III) Complexes for Use as MRI Contrast Agents

Daniel D. Schwert,*,† Nicholas Richardson,‡ Gongjun Ji,† Bernd Radüchel,§ Wolfgang Ebert,§ Peter E. Heffner,† Rick Keck,⊥ and Julian A. Davies†

Department of Chemistry, University of Toledo, 2801 W. Bancroft Street, Toledo, Ohio 43606, Department of Chemistry and Physics, Wagner College, Staten Island, New York 10301, Research Laboratories of Schering AG, 13342 Berlin, Germany, and Department of Urology, Medical College of Ohio, Toledo, Ohio 43614

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Two 3,5-disubstituted sulfonamide catechol ligands were synthesized. Tris(ligand) iron(III) complexes were prepared and investigated as MRI contrast agents. Longitudinal relaxivity (r1) values were determined for the complexes. The r1 values in water were substantially higher than those of typical six-coordinate iron(III) complexes. The r1 values in plasma under the same conditions increased. The iron(III) complexes were administered to rats, and the kidney and liver signal intensities were measured by T_1-weighted MR imaging experiments.

Introduction

Magnetic resonance imaging (MRI) has received much attention over the past two decades as a technique for the diagnosis of disease states and to test organ function in the body.[1] The signal intensity (SI) from different tissues varies with the density of protons in the tissues and both the longitudinal (T_1) and transverse (T_2) relaxation times of those protons. While the inherent image contrast of different body tissues is sufficient in some cases, it may be necessary to use a contrast agent to improve the quality of the image and aid in diagnosis. Contrast agents act by changing the relaxation times and/or the proton density within a tissue so that the objective tissue may be differentiated from adjacent tissues.

Paramagnetic metal ions have been investigated as MRI contrast agents because they change the T_1 and T_2 relaxation times of water protons.[2] Due to concerns about toxicity of metal aquo ions, chelating ligands are often employed in contrast agent development. In addition to decreasing the toxicity of the metal ions, ligands may be functionalized in order to provide tissue specificity. Unfortunately, as the ligands occupy coordination sites on the metal ion the number of water molecules directly bound to the metal center decreases and therefore the overall effectiveness of the contrast agent decreases.

Paramagnetic gadolinium(III),[3] manganese(II),[4] and iron(III) have received the most attention in MRI contrast agent research. When used in T_1-weighted MRI, these agents act by increasing the SI of the target organs and tissues (positive contrast).[2] Other agents, such as superparamagnetic iron oxides (SPIO) and perfluorocarbon solutions, act by decreasing the SI (negative contrast). Currently one manganese(II)- and four gadolinium(III)-based T_1 (positive) contrast agents are available in the United States for clinical imaging. No iron(III)-based positive (but Feridex I.V. as a T_2 agent) contrast agents are available.[5]

In part the lack of iron(III)-based contrast agents can be attributed to the accessibility of the iron(II)/iron(III) redox couple to physiological systems, leading to the need for coordinatively saturated complexes. This eliminates the possibility of inner-sphere coordination of water molecules, potentially limiting the effectiveness of the contrast agents.

Iron(III)-tris(tironate) was investigated as a contrast agent for MRI.[6] This compound was intended to maximize second-sphere interactions with water molecules through hydrogen bonding to the oxygen atoms of the Fe−O−R linkages while maintaining the coordinative saturation of the iron center. The longitudinal (T_1) relaxivity (r1) value of the iron(III)-tris(tironate) far exceeded the values reported for typical six-coordinate, coordinatively saturated iron(III) complexes, suggesting that second-sphere coordination of water may indeed be important with this contrast agent. However, the complex was found to be highly toxic, possibly due to the inherent incompatibility of the nine-minus charge carried by the contrast agent with biological systems. This toxicity was not believed to be due to the release of iron(III) from the complex as the intact metal−ligand complex was detected in the urine of the animals injected. In addition, this charge necessitates nine counterions per contrast agent anion, increasing the osmolality of the injection solution.

In an attempt to remedy this situation, two 3,5-disubstituted catecholate derivatives have been prepared. The iron(III)-tris(catecholate) complexes of these ligands each carry a three-minus charge, reducing the osmotic stress caused by the injection solution. The r1 values of these complexes have been determined in water and plasma at 0.47 T (20 MHz) and 37 °C (Bruker Minispec pc 20), and the kidney and liver SI enhancements obtained by T_1-weighted MR imaging experiments (GE Signa 1.5 T) on rats injected with the agents

* To whom correspondence should be addressed. Currently at Division of Chemistry, Indiana University Southeast, 4201 Grant Line Rd., New Albany, IN 47150-6405. Phone: 812-941-2462. E-mail: dschwert@ius.edu.
† University of Toledo.
‡ Wagner College.
§ Schering AG.
⊥ Medical College of Ohio.

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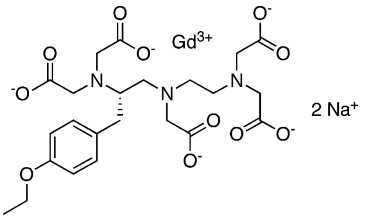

Figure 1. Structure of Gd−EOB−DTPA.

Scheme 1. Synthesis of Disubstituted Sulfonamide Catechols

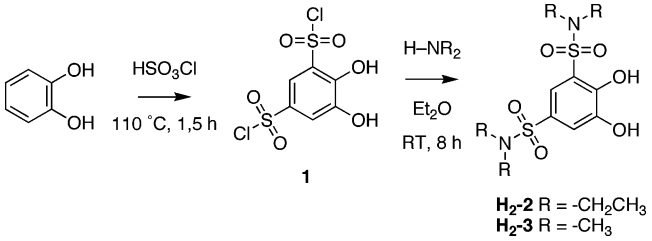

Table 1. Relaxivity Data for Iron(III)-Based Contrast Agent Complexes and Gd−EOB−DTPA

compound	r1 (H_2O) ($mmol^{-1}$ s^{-1})	r1 (plasma) ($mmol^{-1}$ s^{-1})
Fe(**2**)$_3$	1.9	4.0
Fe(**3**)$_3$	2.0	3.0
Gd−EOB−DTPA[a]	5.3	8.7

[a] From ref 7.

were investigated. These results were compared with those obtained in MR imaging experiments with gadolinium-(4*S*)-4-(4-ethoxybenzyl)-3,6,9-tris(carboxylatomethyl)-3,6,9-triazaundecanoic acid, disodium salt (Gd−EOB−DTPA, Primovist, Figure 1), a liver-specific contrast agent in the late phase of clinical development.[7,8]

Results and Discussion

Synthesis. The synthesis of compound **1** was adapted from a previous published procedure.[9] Two different disubstituted sulfonamide catechol ligands (H_2**2** and H_2**3**, Scheme 1) were synthesized for use as chelating agents for iron(III) as potential MRI contrast agents. Alkyl chain length was varied to determine the effect of such variation on the in vitro relaxivity values and on the in vivo SI enhancements of the iron(III) complexes (Na_3Fe(**2**)$_3$ and Na_3Fe(**3**)$_3$) of these ligands. Two synthetic routes were used to generate the metal complexes. For relaxivity experiments, the trisodium salts of the tris(ligand) complexes of iron(III) were synthesized using Fe(acac)$_3$ as the source of iron(III), and the complexes were isolated. For the animal experiments, the metal complexes were generated in situ by the addition of 1 mol equiv of $FeCl_3$ to 3 mol equiv of ligand in aqueous solution, and the pH of the solution was adjusted to 7.2 with sodium hydroxide.

Relaxivity Experiments. The results of the relaxivity measurements are shown in Table 1. The measured values of r1 for the iron(III) complexes are higher than those reported for typical six-coordinate, coordinatively saturated iron(III) complexes.[5] As expected, the iron(III) catecholate complexes have lower r1 values than Gd−EOB−DTPA due to the higher number of unpaired electrons on gadolinium(III) and the one water molecule bound directly to the metal center. In plasma, Gd−EOB−DTPA also had a higher r1 value than the iron(III) complexes but the percentage increases in r1 values was greater for one of the iron(III)-based contrast agents. The r1 increase for Gd−EOB−DTPA was 64% while the relaxivities of Fe(**2**)$_3$ and Fe(**3**)$_3$ increased by 110% and 50%, respectively. Part of the increase in r1 is due to the higher viscosity of plasma. The rest of the relaxivity increase is likely due to some binding of the contrast agents to plasma proteins.

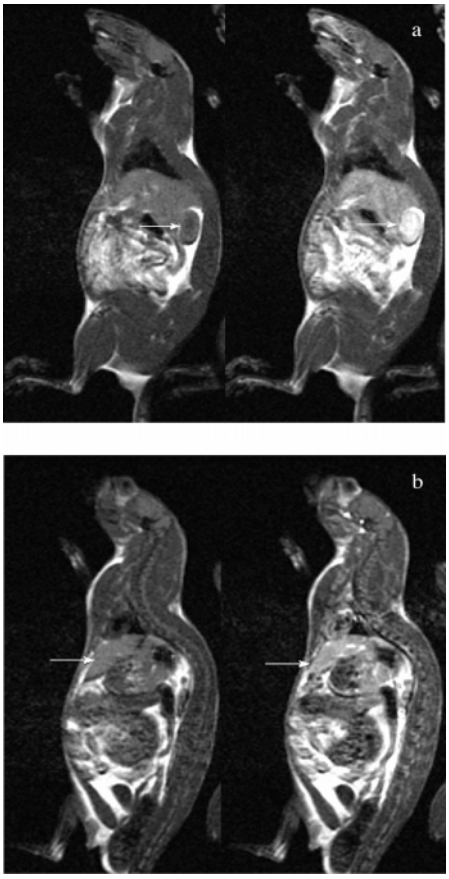

Figure 2. MRI scans of rats pre and postinjection of Fe(**2**)$_3$. (a) Pre- (left) and 4 min postinjection (right) images showing pronounced enhancement (168%) of the kidney (arrow). (b) Pre- (left) and 4 min postinjection (right) images showing pronounced (53.0%) enhancement of the liver (arrow). Images are adjacent 3 mm slices separated by a 1.5 mm intersection gap.

Animal Experiments. Female Fischer 344 rats were injected intravenously with solutions of contrast agent and the liver and kidney SI postinjection were measured and compared with the preinjection values. An example of a typical animal scan for the iron(III) complexes is shown in Figure 2. A similar scan for an animal injected with Gd−EOB−DTPA has been previously published.[10] The injected solutions had a concentration of 45.0 mM and the applied dosage was 0.1 mmol Fe per kg body weight (bw). Slight variations in the weights of the rats resulted in marginally different final injection concentrations.

The results of the experiments with 0.1 mmol/kg bw contrast agent solution are shown in Figure 3 and Figure 4. Note that the error bars represent the standard deviation values of the voxel intensities and are not a representation of experimental error but rather a measure of the interindividual differences

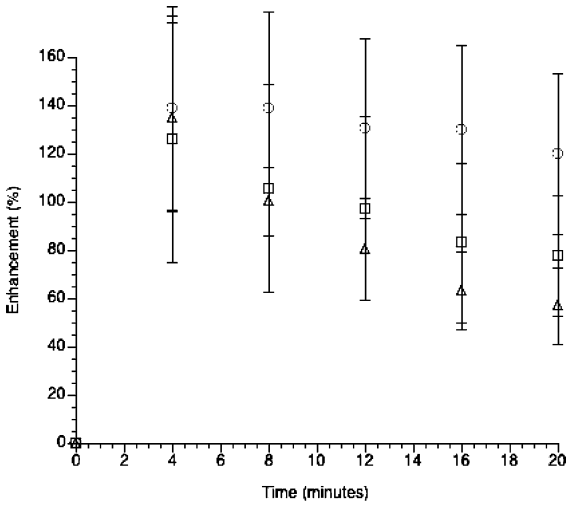

Figure 3. SI enhancements of the kidneys after injection of Fe(**2**)$_3$ (circle, eight rats, 0.101 ± 0.006 mmol/kg), Fe(**3**)$_3$ (square, four rats, 0.100 ± 0.008 mmol/kg), and Gd−EOB−DTPA (triangle, five rats, 0.10 ± 0.01 mmol/kg). Error bars represent the standard deviation of the data.

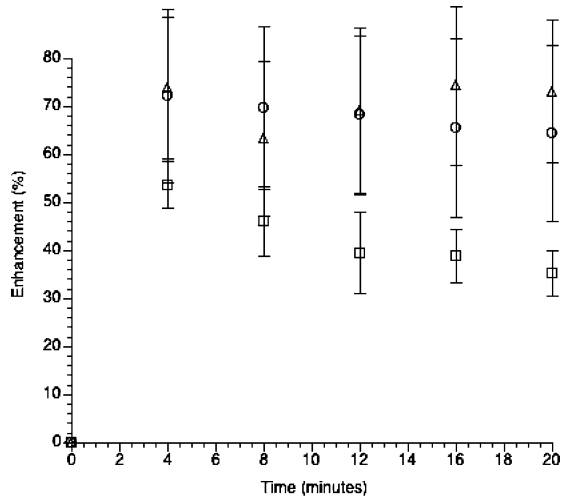

Figure 4. SI enhancements of the liver after injection of Fe(**2**)$_3$ (circle, eight rats, 0.101 ± 0.006 mmol/kg), Fe(**3**)$_3$ (square, four rats, 0.100 ± 0.008 mmol/kg), and Gd−EOB−DTPA (triangle, five rats, 0.10 ± 0.01 mmol/kg). Error bars represent the standard deviation of the data.

between animals. Of the two iron(III) complexes, it was found that Fe(**2**)$_3$ provided significantly ($P < 0.05$) higher liver SI enhancement and greater kidney SI enhancement over the course of the experiments. The levels of SI enhancements for both the liver and the kidney after injection of Fe(**2**)$_3$ remained relatively constant over the time course of the experiments. Removal of one methylene group on the alkyl side chains (resulting in Fe(**3**)$_3$) resulted in lower kidney and liver image SI enhancements and a faster decrease in the levels of SI. This is possibly due to the rapid clearance of the contrast agent from the bloodstream thus an early peak in SI enhancement may be missed.

In both cases, the iron(III)-based contrast agents provided evidence of greater kidney SI enhancements as compared to Gd−EOB−DTPA at 0.1 mmol/kg bw over the course of the MRI scans (for Fe(**2**)$_3$ and Gd−EOB−DTPA, $P < 0.001$; for Fe(**3**)$_3$ and Gd−EOB−DTPA, $P < 0.10$). The liver SI enhancements with Gd−EOB−DTPA were found to be comparable to the enhancements by Fe(**2**)$_3$ ($P > 0.7$). This result is impressive considering the large differences in in vitro r1 values between Fe(**2**)$_3$ and Gd−EOB−DTPA. The other iron(III)-based contrast agent, Fe(**3**)$_3$, provided significantly ($P < 0.001$) lower liver SI enhancement than Gd−EOB−DTPA.

Conclusions

Two new catechol-based ligands were synthesized, complexed with iron(III), and investigated as MRI contrast agents. The r1 values of the complexes in water were found to be greater than those of typical six-coordinate, coordinatively saturated iron(III) complexes. The iron(III) complexes showed increases in r1 value of at least 50% when measured in plasma. Investigations are currently underway to determine the protein binding of the complexes in order to determine the cause of the increase in r1 values. The iron(III) complexes injected into animals at 0.1 mmol/kg bw showed greater kidney SI enhancements than Gd−EOB−DTPA, a promising liver-specific contrast agent. One of the iron(III) complexes, Fe(**2**)$_3$, provided liver SI enhancements comparable to Gd−EOB−DTPA when injected at 0.1 mmol/kg bw.

Experimental Section

All reagents (each above 97% purity except for dimethylamine [40% in water]) were purchased from Aldrich Chemical Co. (Milwaukee, WI) and were used without further purification. All ^{1}H and ^{13}C{^{1}H} NMR data were recorded on a Varian VXR-400 spectrometer. Tetramethylsilane and 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid were used as internal standards in organic and aqueous samples, respectively. Elemental analysis was performed by the Instrumentation Center at the University of Toledo.

3,5-Bis(dichlorosulfonyl)catechol (1). The synthesis of compound **1** was adapted from a previously published procedure. A 1000-mL, three-necked, round-bottomed flask was equipped with a nitrogen inlet, a thermometer, and a magnetic stir bar. Chlorosulfonic acid (100 mL, 1.50 mol) was added to the flask and heated to 110 °C under nitrogen. Ten grams of catechol (0.0908 mol) was slowly added to the flask. The resulting solution was heated for 90 min and then cooled to room temperature. The nitrogen inlet was removed, and a water condenser was attached. The flask was placed in a dry ice/acetone/water bath and cooled to below −10 °C. Concentrated hydrochloric acid (100 mL) was slowly added, keeping the temperature below 0 °C at all times. After all of the acid was added, 300 mL of diethyl ether was added. The organic layer was collected, and the aqueous layer was washed with diethyl ether (10 × 100 mL). The ether solution was dried over sodium sulfate and filtered, and then the solvent was removed by rotary evaporation. An orange/brown oil was recovered (10.4, 0.0339 mol, 37% yield). ^{1}H NMR in acetone-d_6 (ppm): 7.8 (d, 1H, ArH), 8.0 (d, 1H, ArH). ^{13}C{^{1}H} NMR in acetone-d_6 (ppm): 118.4, 120.1, 130.5, 134.6, 148.7, 152.7 (C_6H_2).

3,5-Bis(diethylsulfonamide)catechol ($H_2$2). A 1000-mL round-bottomed flask was equipped with a Claisen adapter, reflux condenser, and an addition funnel. Diethylamine (42.4 mL, 0.410 mol) was dissolved in 100 mL of diethyl ether and cooled to 0 °C in an ice bath. Compound **1** (10.0 g, 0.0326 mol), dissolved in 100 mL of diethyl ether, was added slowly. The reaction was allowed to come to room temperature and stirred overnight. The ether was removed by rotary evaporation. Concentrated HCl (80 mL) was added to the resulting solid. The aqueous layer was washed with CH_2Cl_2 (6 × 150 mL). The CH_2Cl_2 solution was dried over sodium sulfate and filtered, and the solvent was removed by rotary evaporation leaving a black solid. The black solid was extracted with hot

hexanes (10 × 1000 mL). The combined hexanes extracts were cooled in the freezer. The product precipitated as white crystals (3.3 g, 0.0087 mol, 26% yield). ^{1}H NMR in $CDCl_3$ (ppm): 1.10 (t, 6H, CH_3), 1.13 (t, 6H, CH_3), 3.21 (q, 4H, CH_2), 3.38 (q, 4H, CH_2), 7.4 (d, 1H, ArH), 7.6 (d, 1H, ArH). ^{13}C{^{1}H} NMR in $CDCl_3$ (ppm): 14.0 (CH_3), 14.3 (CH_3), 42.3 (CH_2), 42.4 (CH_2), 116.9, 118.1, 123.7, 133.0, 145.7, 146.5 (C_6H_2). Anal. ($C_{14}H_{24}N_2O_6S_2$) C, H, N.

3,5-Bis(dimethylsulfonamide)catechol ($H_2$3). A 1000-mL round-bottomed flask was equipped with a Claisen adapter, reflux condenser, and an addition funnel. Dimethylamine (52 mL, 40% in water, 0.410 mol) was dissolved in 100 mL of diethyl ether and cooled to 0 °C in an ice bath. Compound **1** (10.0 g, 0.0326 mol) dissolved in 100 mL of diethyl ether was added slowly. The reaction was allowed to come to room temperature and stirred overnight. The ether was removed by rotary evaporation. Concentrated HCl (80 mL) was added to the resulting solid. The aqueous layer was washed with CH_2Cl_2 (6 × 150 mL). The CH_2Cl_2 solution was dried over sodium sulfate and filtered, and the solvent was removed by rotary evaporation. The product was recrystallized using CH_2Cl_2/hexanes (1.1 g, 0.0034 mol, 10% yield). ^{1}H NMR in $CDCl_3$ (ppm): 2.7 (s, 6H, CH_3), 2.8 (s, 6H, CH_3), 7.4 (d, 1H, ArH), 7.5 (d, 1H, ArH). ^{13}C{^{1}H} NMR in $CDCl_3$ (ppm): 37.7 (CH_3), 37.9 (CH_3), 118.0, 119.1, 119.6, 127.7, 146.6, 146.7 (C_6H_2). Anal. ($C_{10}H_{16}N_2O_6S_2$) C, H, N.

Preparation of Iron(III) Complexes for Relaxivity Experiments. For relaxivity experiments, Fe(**2**)$_3$ and Fe(**3**)$_2$ were isolated as trisodium salts. The appropriate ligand (3 mmol) was dissolved in water. Sodium hydroxide (0.0800 g, 2 mmol) and Fe(acac)$_3$ (0.3532 g, 1 mmol) were added, and the solution was refluxed for 24 h. The solution was allowed to cool and the pH adjusted with NaOH to pH 7 and extracted with diethyl ether (6 × 150 mL). The solution was filtered, and the solvent was removed by lyophilization. Anal. ($C_{52}H_{80}N_6FeNa_3O_{25}S_6$ for **2**, $C_{40}H_{66}N_6FeNa_3O_{25}S_6$ for **3**) C, H, N.

Preparation of MRI Injection Solutions. For a 45.0-mM injection solution, compound H_2**2** (0.257 g, 0.675 mol) or compound H_2**3** (0.219 g, 0.675 mmol) and NaOH (0.0540 g, 1.35 mmol) were dissolved in 3 mL of water. Ferric chloride hexahydrate (0.0608 g, 0.225 mol) was added with stirring. The pH was adjusted to 7.2 using NaOH, and the volume was adjusted to 5 mL. Injection solutions of Gd−EOB−DTPA at a concentration of 45.0 mM were made by the dissolution of the disodium salt of Gd−EOB−DTPA (Schering, AG, Berlin, Germany) in water.

Relaxivity Experiments. The prepared lyophilized samples, Fe(**2**)$_3$ and Fe(**3**)$_3$ were dissolved in either water or plasma to give samples at three concentrations (0.25, 0.5, and 1.0 mmol Fe/L). The T_1 relaxation rate of each sample was measured by use of a Minispec P20 (Bruker, Rheinstetten, Germany) at 20 MHz (0.47 T) and 37 °C. Linear regression analysis of relaxation rates $R_1 = 1/T_1$ versus concentration gave the relaxivity values (r1) of each complex in water and plasma.

Animal Experiments. Animal experiments were performed on a Signa 1.5-T superconducting MR scanner (GE Medical Systems, Milwaukee, WI). Two Fisher 344 rats were anesthetized with 65 mg/kg bodyweight of sodium pentobarbital and placed in a send-and-receive head coil. A localizer image was obtained followed by a sagittal preinjection T_1-weighted spin−echo (SE) pulse sequence with $T_R = 300$ ms and $T_E = 20$ ms. The matrix was 256 × 256 over a 20-cm field of view. Three signals were acquired of 3-mm slices with a 1.5-mm intersection gap. The total scan time was 3 min and 56 s. The contrast agent (0.5 mL per animal) was injected through a 27-gauge butterfly needle in the tail vein over 2 min and 15 s followed by a 0.75−1.00 mL saline flush over 45 s. Five postinjection scans were then acquired using the same scan parameters as the pre-injection scans. The postinjection scans were separated by an approximately five-second delay between scans.

SI measurements were made in operator-defined regions of interested (ROI) on the pre- and postcontrast images. For each animal imaged, three or four liver image slices were analyzed and one image slice was analyzed for each kidney. The ROI data were then converted to percent enhancement values (eq 1). The percent enhancement values for the liver and kidney image slices were then averaged and standard deviation values were determined. The significance of the differences in the data was determined using Student's t test (95% confidence interval). The data were plotted against time, with the completion of the injection being time = 0 min. The time of completion of the scans (approximately 4, 8, 12, 16, and 20 min) was used as the time of the image, even though the image is actually an average of the data acquired over the time of the scan.

$$\text{percent enhancement} = \frac{\text{ROI}_{\text{postinj}} - \text{ROI}_{\text{preinj}}}{\text{ROI}_{\text{preinj}}} \times 100\% \quad (1)$$

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Supporting Information Available: A table listing the elemental analysis for the target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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