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A heteronuclear ZnGd complex as a potential contrast agent for magnetic resonance imaging

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Abstract

A new ligand H_3L, with internal compartments for allocating 3*d* metal ions and external donors to bind 4*f* ions, was synthesized and completely characterized. Reaction of H_3L with zinc(II) and gadolinium(III) salts allows isolating the heteronuclear complex $\{[ZnGd(HL)(NO_3)(OAc)(CH_3OH)](NO_3)\}\cdot 6H_2O$ (**1**·$6H_2O$). The ability of **1**·$6H_2O$ to act as a magnetic resonance imaging (MRI) contrast agent was evaluated and this study shows that both the transversal and longitudinal relaxivities are quite high but the T_1/T_2 ratio of 7.9 indicates that it could have even greater potential as a T_2 contrast agent.

Keywords

Gadolinium, zinc, MRI contrast agent, Schiff base, imidazolidine

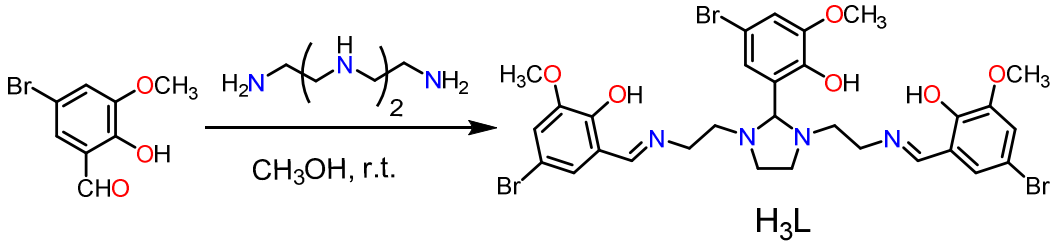
Introduction

The application of gadolinium(III) complexes as contrast agents (CAs) in magnetic resonance imaging requires stable complexation of the metal ion under physiological conditions to avoid the release of the toxic free Gd(III).[1] Several pathways leading to the potential dissociation of Gd^{3+} complexes in vivo have been identified: 1) acid-catalyzed dissociation; 2) dissociation catalyzed by endogenous metal ions such as Zn^{2+} and Cu^{2+} and 3) dissociation assisted by endogenous ligands like citrate, phosphate or bicarbonate. Accordingly, the continuous search for new CAs with increasing stability and relaxivities respect to the commercial ones is still a challenge. In this sense, it should be noted that several gadolinium CAs have shown not only to be stables in the presence of Zn^{II} but to improve their relaxation time.[2,3]

With these considerations in mind, in this work we prepare the new ligand H_3L (Scheme 1), with predesigned compartments to allocate 3*d* and 4*f* ions, with the aim of isolating a ZnGd heretonuclear complex and evaluate its ability to act as CA for MRI.

Results and discussion

H_3L was obtained according to Scheme 1



Scheme 1

Analytical and spectroscopic data corroborate the isolation of the desired compound, with high purity, and unequivocally validate the formation of the imine groups and the

imidazolidine ring. In this way, the H4 and H17 protons (Fig. 1) appear as singlets in the ^{1}H NMR spectrum at 3.83 and 8.16 ppm, respectively, and the IR spectrum shows an intense band at 1633 cm^{-1}, which can be assigned to ν(C=N).

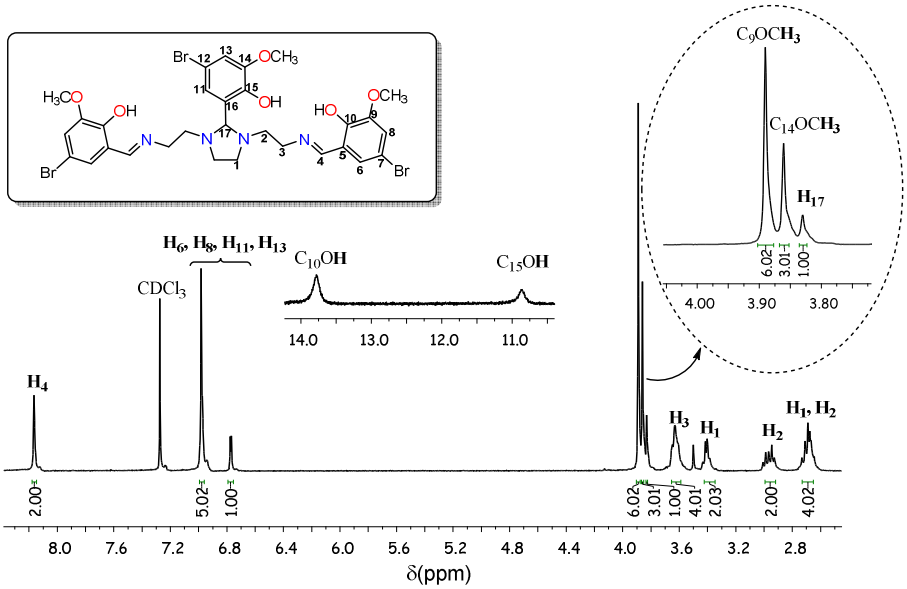
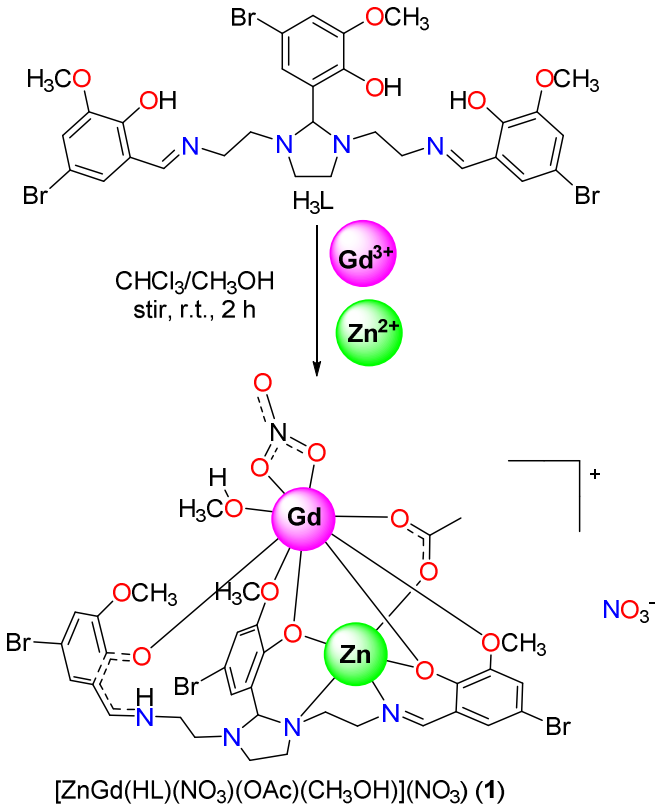


Fig. 1. ^{1}H NMR spectrum of H_3L in $CDCl_3$

H_3L reacts with $Zn(OAc)_2\cdot 2H_2O$ and $Gd(NO_3)_3\cdot 6H_2O$ in 1:1:1 molar ratio to yield the heteronuclear complex {[ZnGd(HL)(NO_3)(OAc)(CH_3OH)](NO_3)} (**1**), as shown in Scheme 2, which precipitates as the hexahydrate **1**·$6H_2O$.



Scheme 2. Reaction scheme for isolating **1**.

The analytical, spectroscopic and X-ray diffraction characterization of **1**·$6H_2O$ agrees with the structure proposed in Scheme 2. Thus, the comparison of the IR spectrum of the complex with that of the free ligand shows that the band assigned to ν(C=N) splits into two

new bands at 1636 and 1648 cm^{-1}, due to the loss of symmetry of the ligand in the complex. In addition, new bands are observed at 1556 cm^{-1}, assigned to $\nu(COO^-)$, and at 1300 and 1285 cm^{-1}, assigned to NO_3^- vibrations, in agreement with the presence of the acetate and nitrate donors in the metal complex.[4] Finally, it is possible to observe a broad band centred at 3264 cm^{-1}, which indicates the presence of water and methanol in the compound.

Several attempts to grow single crystals of **1**·$6H_2O$ were unsuccessful and only allowed to obtain small single crystals. Thus, X-ray diffraction studies were not of enough quality to solve the structure but they let to know the unit cell parameters, which are the following ones: crystal system: monoclinic; a = 10.82(3), b = 16.29(5), c = 26.67(7) Å; β = 97.37(6) º; V=4659(37) $Å^3$. These parameters are very similar to those found for a ZnDy complex with the same ligand[5] and, therefore, the structure should be very akin and should agree with the proposed one in Scheme 2.

The potentiality of **1**·$6H_2O$ as a MRI contrast agent was evaluated. Thus, longitudinal R_1 and transversal R_2 relaxivities were measured. The R_1 value of 4.90 $mM^{-1}s^{-1}$ is comparable with those found for other commercial agents,[6] and, therefore, it could be said that **1**·$6H_2O$ should be a good T_1 contrast agent. Nevertheless, the R_2 value is 38.63 $mM^{-1}s^{-1}$, and, therefore, the T_1/T_2 (R_2/R_1) ratio is 7.9, value that strongly differs from 1. As a consequence, this precludes the use of **1**·$6H_2O$ as a positive MRI contrast.[7] However, the R_2 value is also quite large and the T_1/T_2 ratio is higher than 6, which is a condition for a species being used as a T_2 contrast agent. Accordingly, it seems that this heteronuclear ZnGd complex could be a candidate as a negative MRI contrast, in spite of usual T_2 agents are based, for example, on magnetic iron oxide nanoparticles.[8]

The potential of the GdZn system to serve as contrast agent was also demonstrated using *in vitro* MRI on tube phantoms. The MRI experiments were conducted using a reference without complex (at 12 o'clock in phantoms, see Fig. 2) and six different increasing concentrations (clockwise) of the synthesized complex (0.05, 0.2, 0.5, 1, 1.2 and 1.5 mM).

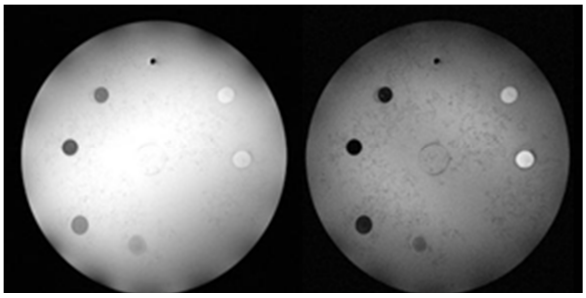


Fig. 2. T_1-weighted (left) and T_2-weighted (right) MR images for **1**·$6H_2O$

The T_1-weighted phantom shows that a bright image can be gradually obtained by increasing the complex concentration up to 0.5 mM; in contrast, at higher concentrations the image becomes darker. On the other hand, the T_1-weighted phantom darkens as the concentration increases. Accordingly, this seems to indicate that, as previously anticipated on the basis of the T_1/T_2 ratio, **1**·$6H_2O$ has a better performance as a T_2 contrast agent.

Conclusions

The new H_3L ligand, with differentiated compartments for 3*d* and 4*f* metal ions, has been satisfactorily isolated and characterized. The ligand yields the heteronuclear ZnGd complex {[ZnGd(HL)(NO_3)(OAc)(CH_3OH)](NO_3)}·$6H_2O$. This complex shows longitudinal relaxivities comparable to those of commercial T_1 CAs but the T_1/T_2 ratio considerable higher than 1 indicates that it is not a good candidate for being used as a positive MRI contrast agent. Nevertheless, the relatively high transversal relaxivity R_2 and the T_1/T_2 ratio higher than 6 suggest a better performance as magnetic negative imaging contrast agent. These results are validated by *in vitro* MRI phamtons.

Experimental

Synthesis of H_3L

H_3L was isolated as follows: to a solution of 5-bromo-2-hydroxybenzaldehyde (1.386 g, 6 mmol) in methanol (40 mL), triethylentetramine (0.292 g, 2 mmol). The solution was stirred in air for 4 h. and a yellow solid precipitated, being subsequently filtered off and dried in air 1.2 g (76%). MW: 785.32 $gmol^{-1}$. M.P.: 200-202 ºC. Elemental analysis: experimental: C 45.89, N 6.96, H 4.25%; calcd. for $C_{30}H_{33}N_4O_6Br_3$: C 45.88, N 7.13, H 4.23. IR spectrum (ATR, $\tilde{\nu}/cm^{-1}$): 1633 (C=N). ^1H NMR (250 MHz, $CDCl_3$, δ ppm): 2.65-2.74 (m, 4H, 2H1+2H2); 2.93-3.01 (m, 2H, 2H2); 3.43-3.38 (m, 2H, 2H1); 3.61 (t, 4H, 4H3); 3.83 (s, 1H, H17); 3.86 (s, 3H, CH_3); 3.89 (s, 6H, $2CH_3$); 6.77 (s, 1H, H11); 6.95-7.01 (m, 5H, 2H6 + 2H8 + H13); 8.16 (s, 2H, 2H4); 10.86 (s, 1H, OH); 13.78 (s, 2H, 2OH). RMN-^1H (250 MHz, DMSO-d_6, δ ppm): 2.57-1.69, 2.71-2.80 (m, 6H, 2H1 + 4H2); 3.27-3.34 (m, 2H, 2H1); 3.53-3.65 (m, 4H, 4H3); 3.71 (s, 3H, CH_3); 3.77 (s, 6H, $2CH_3$); 4.09 (s, 1H, H17); 6.94 (s, 1H), 6.97 (s, 1H) (H11 + H13); 7.06 (s, 2H), 7.18 (s, 2H) (2H6 + 2H8); 8.34 (s, 2H, 2H4).

Synthesis of **{[ZnGa(HL)(NO_3)(OAc)(CH_3OH)](NO_3)}·$6H_2O$ (1·$6H_2O$)**

To a $CHCl_3$ (5 mL) solution of H_3L (0.074 g, 0.094 mmol), $Zn(OAc)_2·2H_2O$ (0.021 g, 0.094 mmol) was added. Then, to the resultant yellow solution $Gd(NO_3)_3·6H_2O$ (0.042, 0.094 mmol) and 5 mL of methanol were added. The mixture was stirred at room temperature for 2 h and a finely divided yellow powder is obtained. The solid is separated by centrifugation and dried in air. 072 g (58%). MW: 1329.12 $gmol^{-1}$. Elemental analysis: experimental: C 29.35, N 6.54, H 3.24%; calcd. For $ZnGdC_{33}H_{50}N_6O_{21}Br_3$: C 29.82, N 6.32, H 3.79%. IR spectrum (ATR, $\tilde{\nu}/cm^{-1}$): 1636, 1648 (C=N); 1557 (COO^-); 1285, 1300 (NO_3^-); 3264 (OH).

MRI measurements

MRI studies at high magnetic field were conducted on a 9.4 T MR system (Bruker Biospin, Ettlingen, Germany) with 440 mT/m gradients. A quadrature radio-frequency transmit-receive resonator was used for data acquisition. T_2-weighted images were acquired using a multi slice multi echo sequence of 11.32 ms echo time, 3000 ms repetition time, 16 echoes, 14 slices, 1 average, FOV of 7.5 cm X 7.5 cm and matrix size of 300 X 300. T_1-weighted images were acquired using a RARE-VTR sequence of 10.81 ms echo time, rare factor 4, repetition times of 3000 ms, 3235 ms, 3491 ms, 3770 ms, 4078 ms, 4422 ms, 4810 ms, 5256 ms, 5779 ms, 6414 ms, 7221 ms, 8327 ms, 10098 ms and 15000 ms. 14 slices, 1 average, FOV of 7.5 cm X 7.5 cm and a matrix size of 300 X 300. Post-processing was performed using ImageJ software (Rasband, W. NIH).

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