Design and Synthesis of Manganese-ligand Based Magnetic Resonance Imaging Contrast Agents

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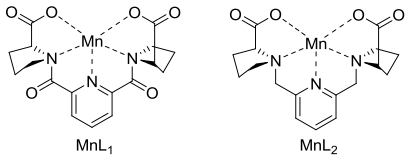
Introduction: A contrast agent (CA) used in magnetic resonance imaging (MRI) clinical diagnosis can enhance the image quality. Obtain highly efficient and low mammalian toxicity contrast agent is the ultimate target in research. Optimization of molecular structure of CA can increase relaxivity and stability. We designed two new manganese complexes (MnL_1 and MnL_2), which are potential candidates as MRI contrast agents. The molecular structure of manganese complexes are as following.



MnL_1 MnL_2

Methods:

Pyridine-2, 6-dicarboxylic acid (99%,aladdin) and L-Proline (AstaTech) were used, (S)-2-(methoxycarbonyl) pyrrolidinium chloride obtained by esterifing L-Proline in methanol.

Synthesis of ligand1 (L_1):

Pyridine-2, 6-dicarboxylic acid (0.02mol) and thionyl chloride (50ml) were mixed in an ice bath. 0.4ml DMF was added in. Then, the solution was refluxed for 2 h. The excess thionyl chloride was evaporated. The residue was dissolved in 50ml chloroform. (S)-2-(methoxycarbonyl) pyrrolidinium chloride (0.04mol) and triethylamine (0.2mol) were dissolved in 100ml chloroform. The two solutions were mixed and stirred for 8h at room temperature. The solution was washed with water, product was obtained by recrystallization. The product was hydrolyzed in alkaline condition to obtained corresponding diacid (L_1).

Synthesis of ligand2 (L_2):

2, 6-bis(chloromethyl) pyridine was synthesized from pyridine-2, 6-dicarboxylic acid through three steps (Esterification in ethanol, reduction by sodium borohydride[1], chlorination in thionyl chloride[2]). 2, 6-bis (chloromethyl) pyridine (8 mmol), (S)-2-(methoxycarbonyl) pyrrolidinium chloride (16mmol) and K_2CO_3 (80 mmol) were mixed in 30ml acetonitrile, and stirred for 1h. Then, the solution was heated to reflux for 20 h.Filtered K_2CO_3 from solution, acetonitrile was evaporated off. The product was purified by silica gel column chromatography. The product was hydrolyzed in alkaline condition to obtained corresponding diacid (L_2).

Synthesis of manganese complexes (MnL_1 /MnL_2):

1mmol Diacid (L_1 or L_2) was dissolved in 10ml water,4mmol NaOH was added in and the mixture was degas under N_2. Then, 2 mmol $MnCl_2 \cdot 4H_2O$ was added in and stirred 12 h in room temperature. The water was removed by rotary evaporation and the resulting solid was suspended in 10 ml chloroform. The insoluble salts were removed by filtration and the filtrate was evaporated.

T_1 relaxivity studies:

T_1 relaxivities were measured at 1.5 T on a clinical MR scanner (Siemens Sonata) at room temperature [3]. The T_1-weighted images were acquired with a conventional spin-echo acquisition (TE= 5.3 ms) with TR values ranging from 20 to 1000ms. Relaxivity values of r_1 were calculated through the curve fitting of $1/T_1$ relaxation time (s^{-1}) from the versus concentration for 0.1, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45 and 0.5 mM Mn samples. All solutions were assayed for manganese concentration by atomic absorption spectroscopy (AAS).

Results:

The manganese complexes MnL_1 and MnL_2 were synthesized, and characterized by ESI-MS. The T_1 relaxivitiyies of two manganese complexes, $MnCl_2$ and a commercial contrast agent (Gd-DTPA, magnevist) were measured, indicated in Figure 1.

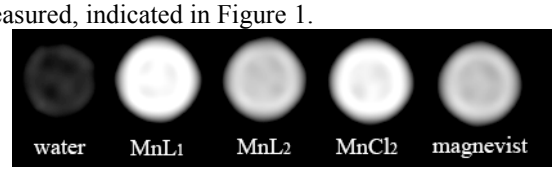


Figure 1. T_1 weighted spin-echo MR phantom images of MnL_1, MnL_2, $MnCl_2$ and magnevist in water at equal molar concentration (0.4mM) of each contrast agent. Images were acquired at 1.5 T at room temperature (TR/TE=90/5.3ms)

The r_1 values are listed in table 1. Relaxivity of manganese complexes are all better than magnevist, and MnL_1 has a highest r_1 value (6.4 $mM^{-1}S^{-1}$). The results are expectant. The MnL_1complex has higher rigidity in molecular conformation, which may have some effects on T_1 relaxivity.

Table 1. Relaxivities of MRI Contrast Agents at 1.5T, room temperature

Contrast Agents	$r_1(mM^{-1}S^{-1})$
MnL_1	6.4
MnL_2	3.4
$MnCl_2$	6.0
Magnevist	3.0

Conclusions:

We successfully designed and synthesized two new manganese contrast agents, which have better contrast over traditional commercial contrast agent. Biocompatibility and in vivo imaging tests are undergoing to examine their potentials as effective MRI probes.

References:

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