# Synthesis and Evaluation of Novel MRI Contrast Agents of Chemically Modified Gd-DTPA Complexes with Sugars

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## Abstract:

*MRI is one of medical diagnostic imaging technologies that can draw the cross section in the body. To obtain a clearer image, Gd complexes are often used as MRI contrast agents. Gd-DTPA (Gd-Diethylenetriaminepentaacetate, Magnevist®) is used in particular as the MRI contrast agents. We prepared and evaluated novel MRI contrast agents that were chemically modified Gd-DTPA with sugars (represented as Gd-DTPA-Sugar) via hydrolysis route for providing specificity to target organs and tissues. Gd-DTPA-Sugar complex showed an excellent potential for the MRI contrast agent ($r_1$=31.2 $s^{-1}mM^{-1}$). Gd-DTPA-Sugar complexes alternatively prepared by shorter synthetic route without protection/ deprotection (hydrolysis) method showed inferior results ($r_1$=6.3 and 8.1 $s^{-1}mM^{-1}$) to the hydlized product.*

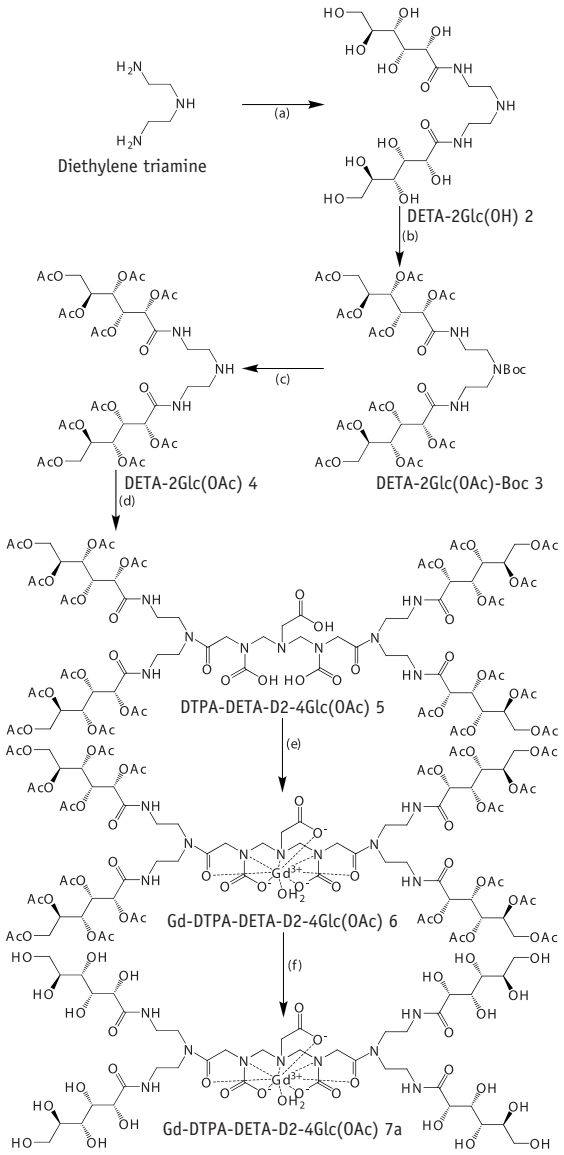
***Keywords:*** *MRI contrast agent, Gd(III)-DTPA, tumor imaging.*

## 1. Introduction

MRI is one of a medical diagnostic imaging technology, and it can obtain the cross section of all angles in the body. MR imaging is obtained from the difference of nuclear relaxation time of protons, which are resonated water and fat protons in the body by irradiation in high magnetic field. Therefore, even if the contrast agents need not to be used for MRI, the imaging is possible. But to obtain a clearer image, Gd complexes are used as MRI contrast agents. Gd complexes enhance contrast by shortening $T_1$ relaxation times of water protons. Because $T_1$ relaxation time depends on Gadolinium concentration of MRI contrast agent, $r_1$ relaxivity that divided $T_1$ relaxation time by Gadolinium concentration is used as a guide to contrast intensification of MRI contrast agent. Now, Gd-DTPA is used extensively as MRI contrast agents [1]. However, Gd-DTPA has problems such as that it's not so high $r_1$ relaxivity, low retention in blood vessels and no specificity in the body. Our laboratory designed novel Gd-DTPA complexes for that chemistry modified sugar [2]. To give organ and tissue specificity, we focused an attention on function of sugars as organ and tissue specificity, and then chemically modified Gd-DTPA complexes with sugars became the candidates for resolving the problems of the Gd-DTPA. We prepared some Gd-DTPA-Sugar complexes and evaluated *in vivo* and *in vitro*. Gd-DTPA-Sugar complex was showed great result. So, Gd-DTPA-Sugar complex was prepared by short route for large quantity synthesis. Then, because $r_1$ relaxivity is improved when molecular size big, Gd-DTPA-Sugar complexes having extended carbon chains were prepared with shorter synthtic route.

## 2. Results and discussion

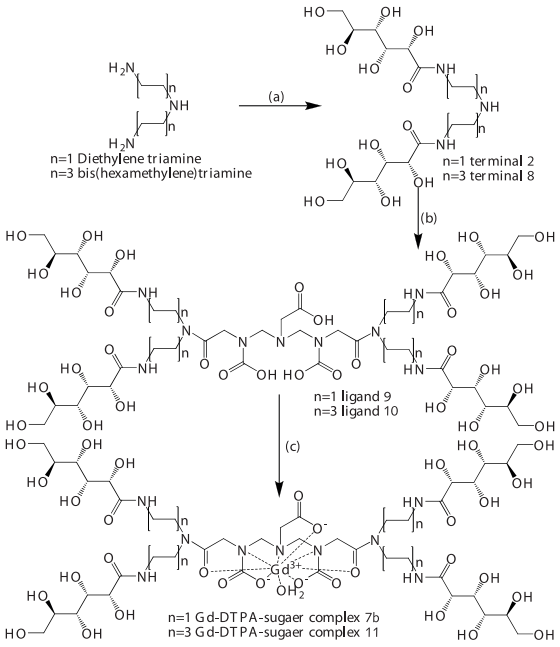
### 2.1. Synthesis of Gd-DTPA-Sugar complex




*Scheme 1. Synthesis of Gd-DTPA-Sugar complex. Reagents and conditions: (a) D-(+)-Glucono-1,5-lactone, DMF, r.t., 24 h; (b) $(Boc)_2O$, DMF, r.t., 24 h, $Ac_2O$, $Et_3N$, r.t., 48 h; (c) TFA, $CH_2Cl_2$, r.t., 4 h; (d) DTPA dianhydride, DMF, Pyridine, r.t., 4 h; (e) $GdCl_3 6H_2O$, 95 °C, 1 h; (f) NaOH aq(1N), $H_2O$, r.t., 24 h.*

A pathway of synthesis of Gd-DTPA-Sugar complex is shown in Scheme 1. Synthesis of Gd-DTPA-sugar complexes, that consist of dendrimer structure, used a convergent method. DTPA dianhydride **1** of dendrimer core was prepared by dehydration-condensation of DTPA. DETA-2Glc(OH) **2** was prepared by reaction of D-(+)-Glucono-1,5-lactone and primary amine groups of diethylenetriamine. DETA-2Glc(OAc)-Boc **3** was prepared by t-Boc protection of secondary amine group by $(Boc)_2O$ and acetylation of hydroxyl groups by $Ac_2O$. DETA-2Glc(OAc) **4** of dendrimer terminal was prepared by deprotection reaction of t-Boc group. DTPA-DETA-D2-4Glc(OAc) **5** of ligand was prepared by reaction of DTPA dianhydride 1 of dendrimer core and DETA-2Glc(OAc) **4.** Gd-DTPA-DETA-D2-4Glc(OAc) **6** was prepared by chelation reaction of DTPA-DETA-D2-4Glc(OAc) **5** and Gadolinium(III) ion. Gd-DTPA-DETA-D2-4Glc(OH) **7a** of Gd-DTPA-sugar complex was prepared by hydrolysis of acetyl groups of Gd-DTPA-DETA-D2-4Glc(OAc) **6** by NaOH aq(1N).

### 2.2. Short route syntheses of Gd-DTPA-Sugar complexes




*Scheme 2. Short route syntheses of Gd-DTPA-Sugar complexes **7b** and **11**. Reagents and conditions: (a) $Ac_2O$, pyridine, 65 °C, 24 h; (b) D-(+)-Glucono-1,5-lactone,DMF, **2** r.t., 24 h, **8** 80 °C, 12 h; (c) pyridine, DMSO, 60 °C, 24 h; (d) $GdCl_3 6H_2O$, pyridine, 40 °C, 12 h.*

Shorter routes of syntheses of Gd-DTPA-Sugar complexes were shown in Scheme 2. DETA-2Glc(OH) **2** and HMTA-2Glc(OH) **8** of Dendrimer terminals were prepared by reaction of D-(+)-Glucono-1,5-lactone and primary amine groups of diethylene triamine and bis(hexamethylene)triamine. DTPA-DETA-D2-4Glc(OH) **9** and DTPA-HMTA-D2-4Glc(OH) **10** of ligands were prepared by reaction of DTPA dianhydride **1** of dendrimer core with DETA-2Glc(OH) **2** and HMTA-2Glc(OH) **8** of dendrimer terminals. Gd-DTPA-DETA-D2-4Glc(OH) **7b** and Gd-DTPA-HMTA-D2-4Glc(OH) **11** of Gd-DTPA-sugar complexes were prepared by chelation reaction of DETA-D2-4Glc(OH) **9** and DTPA-HMTA-D2-4Glc(OH) **10** and Gadolinium(III) ion.

### 2.3. *in vitro* evaluation

The value of $r_1$ relaxivity, that is caluculated by being divided $T_1$ relaxation time by gadolinium concentration, is used as a guide to contrast intensification of MRI contrast agent, because the relaxation time depends on the gadolinium concentration of MRI contrast agent. Because gadolinium complex formation constants depend on the pH value of the aqueous media and the free gadolinium ion concentration that did not form the complexes have influence on measurements of relaxation time, the media for gadolinium complex preparation were adjusted to pH 7.0 in water, and to the media was added Chelex®100 Resin, stirred for six hours, and thus removal of the free gadolinium ion was performed. The removal of free gadolinium ion was confirmed by the color test by using Xylenol Orange. Gadolinium concentration was measured by an ICP-AES instrument because relaxation time depended on gadolinium concentration of contrast agents. $T_1$ was measured by TD-NMR of 0.47 T at 37 °C. $T_1$ was measured not only in water but also in serum albumin which is the mostly existing protein in blood.

*Table 1. Comparison of $r_1$ relaxivity.*

| Gd complexes | $r_1$ [$s^{-1}mM^{-1}$] | |
|---|---|---|
| | in $H_2O$ | in albumin |
| Gd-DTPA | 3.5 | 3.5 |
| **7a** | 31.2 | - |
| **7b** | 6.3 | 6.8 |
| **11** | 8.1 | 7.7 |

### 2.4. *in vivo* evaluation

The relaxivity constant $r_1$ was calculated by the following expression.

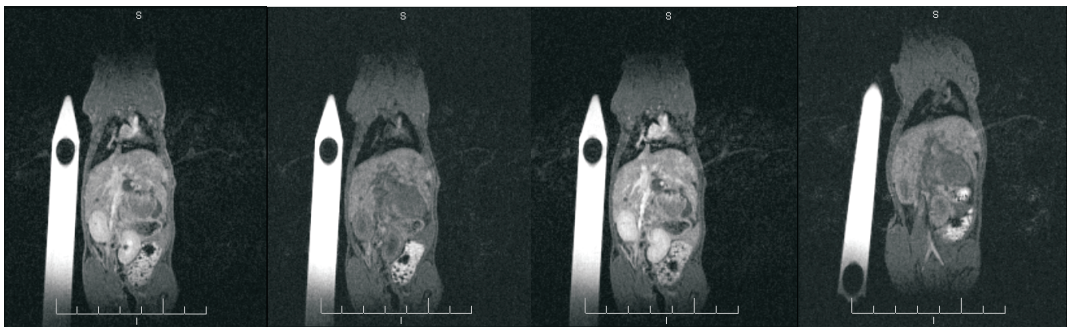


*Fig. 1. Rat's MRI when Gd-DTPA (0.1 mmol/kg) was administered.*

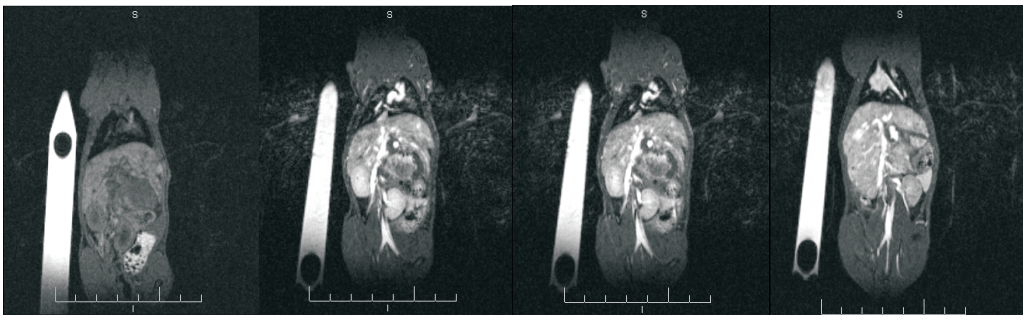


*Fig. 2. Rat's MRI when Gd-DTPA-DETA-D2-4Glc(OH) **7a** (0.05 mmol/kg) was administered.*

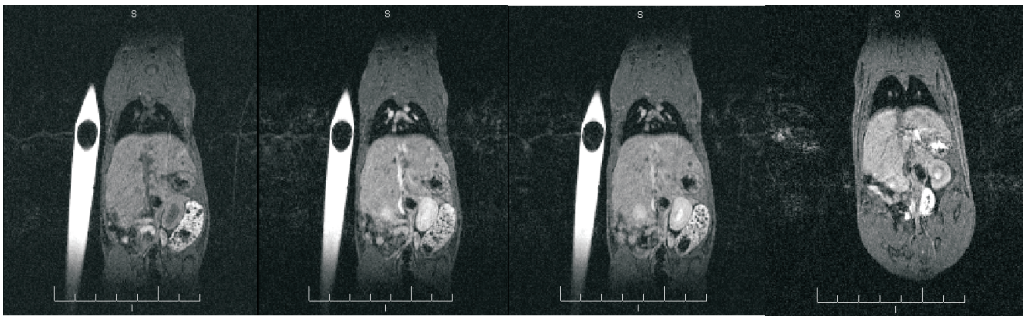


*Fig. 3. Rat's MRI when Gd-DTPA-DETA-D2-4Glc(OH) **7b** (0.05 mmol/kg) was administered.*

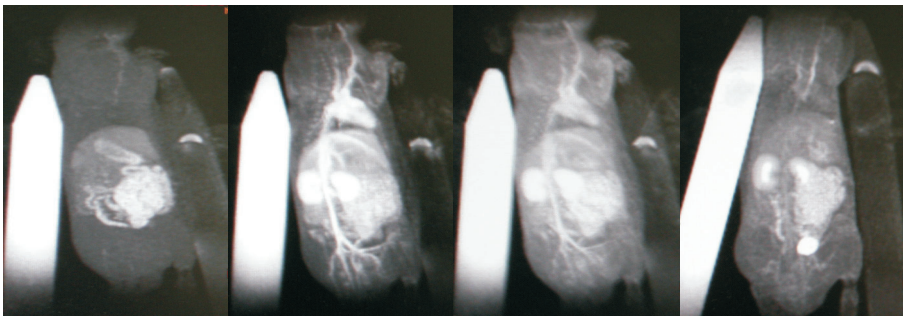


*Fig. 4. Rat's MRI when Gd-DTPA-HMTA-D2-4Glc(OH) **11** (0.05 mmol/kg) was administered.*

$$r_1 = \frac{\frac{1}{T_1} \times 1000 - r_1^{H_2O}}{[Gd^{3+}]}$$

$r_1$ ; relaxivity [$s^{-1}mM^{-1}$]
$T_1$ ; relaxation time [ms]
$r_1^{H_2O}$ ; water of relaxivity [$s^{-1}mM^{-1}$]
$[Gd^{3+}]$ ; Gadolinium concentration [mM]

The MR images of the rats were drawn by MRI machine at 3.0 T. Concentration of MRI contrast agent were adjusted by normal saline solution, Gd-DTPA solution used was 0.1 mmol/kg, solutions used for Gd-DTPA-DETA-D2-4Glc(OH) **7a** (Fig. 2), Gd-DETA-D2-4Glc(OH) **7b** (Fig. 3) and Gd-DTPA-HMTA-D2-4Glc(OH) **11** (Fig. 4) were 0.05 mmol/kg. The each MR image of Fig. 1-4 shows before administration, and then about 1, 5, and 20 minutes after the administration, respectively, from left to right.

## 3. Conclusion

Gd-DETA-D2-4Glc(OH) **7(a)** showed great result to give quite higher $r_1$ relaxivity by *in vitro*, clearer contrast effect, higher retention in blood vessels, and specificity in the liver by *in vivo*. But Gd-DETA-D2-4Glc(OH) **7(b)** being prepared by shorter route showed not so great result.

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