Tetrahedron Letters 51 (2010) 2431–2433

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Synthesis and in vitro studies of Gd–DTPA derivatives as new potential MRI contrast agents

Arigala Uma Ravi Sankar [a,b], Mitsuji Yamasitha [a,*], Kambam Srinivasulu [a,b], Nobuhisa Ozaki [a], Takashi Aoki [a], Michio Fujie [a,c], Keisuke Ogawa [a], Shingo Okada [a], Manubu Yamada [a], Yasutaka Tanaka [d], Motohiko Kimura [d], Mitsuo Toda [d]

[a] Graduate School of Science and Technology, Shizuoka University, Naka-Ku, Hamamatsu 432-8561, Japan
[b] Japan Association for the Advancement of Medical Equipment (JAAME) Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
[c] Faculty of Medicine, Hamamatsu University School of Medicine, Kita-Ku, Hamamatsu 431-3192, Japan
[d] Department of Materials Science and Chemical Engineering, Faculty of Engineering, Shizuoka University, Hamamatsu 432-8561, Japan

ARTICLE INFO

Article history:
Received 23 December 2009
Revised 20 February 2010
Accepted 23 February 2010
Available online 26 February 2010

Keywords:
Dendritic ligands
Gd-complexes
MRI contrast agents
T_1 and r_1 values

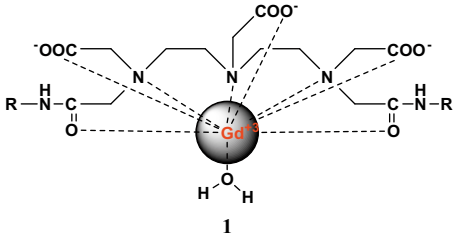
ABSTRACT

A new type of dendritic molecules, Gd–DTPA derivatives, which work as a functionalized ligand coordinating gadolinium(III) ion at the center of their frameworks with different terminal moieties on the molecular surfaces, was readily synthesized with high yield. The structures were established by ^{1}H, ^{13}C NMR, and mass spectral studies. In vitro studies showed them to have enhanced r_1 value in albumin medium and good potentiality as MRI contrast agent.

Over the past two decades Magnetic Resonance Image (MRI) has become a very powerful tool of diagnostic medicine.[1,2] Paramagnetic materials have been investigated as MRI contrast agents (CAs). These materials enhance the contrast of the image indirectly by remarkably shortening the magnetic relaxation time of water protons coordinated by comparison with protons of the surrounding tissues.[3] The most frequently used CAs are stable gadolinium(III) complexes with hydrophilic poly(aminocarboxylate) ligands resulting in rapid extracellular distribution and renal elimination. Gd(III) is preferred because of its favorable magnetic properties. Gd-complexes with amphiphilic properties have previously been prepared and evaluated as blood-pool and liver-imaging agents. Long chain amides and esters of Gd–DTPA are the most common.[4]

In continuation of our work on MRI contrast agents[5] we designed a more water-soluble molecules **1a–e** (Fig. 1). The terminal groups of diethylenetriaminepentaacetic acid (DTPA) have a specific target and combine only with asialoglycoprotein receptor (ASGPR) on the surface of hepatocyte, and also improve the water solubility of contrast agent. So DTPA was used as a core, and free amine was used as a biofunctional group to prepare a series of dendritic Gd-complexes for use as novel MRI contrast agents that improve blood-pool property and enhance T_1 and r_1 values in albumin medium.

The condensation of terminal **2a–e** and DTPA dianhydride **3**[6] results in the polyamine ligand **4**[7] which on further reaction with $GdCl_3{\cdot}6H_2O$ forms compound (**1a–e**)[8] (Fig. 1 and Scheme 1).



1

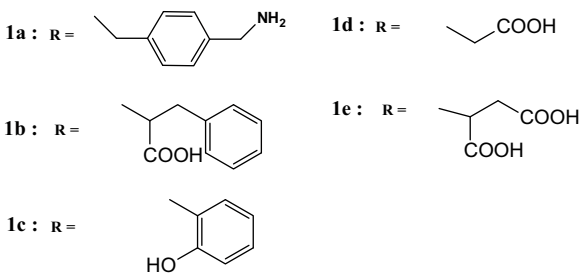
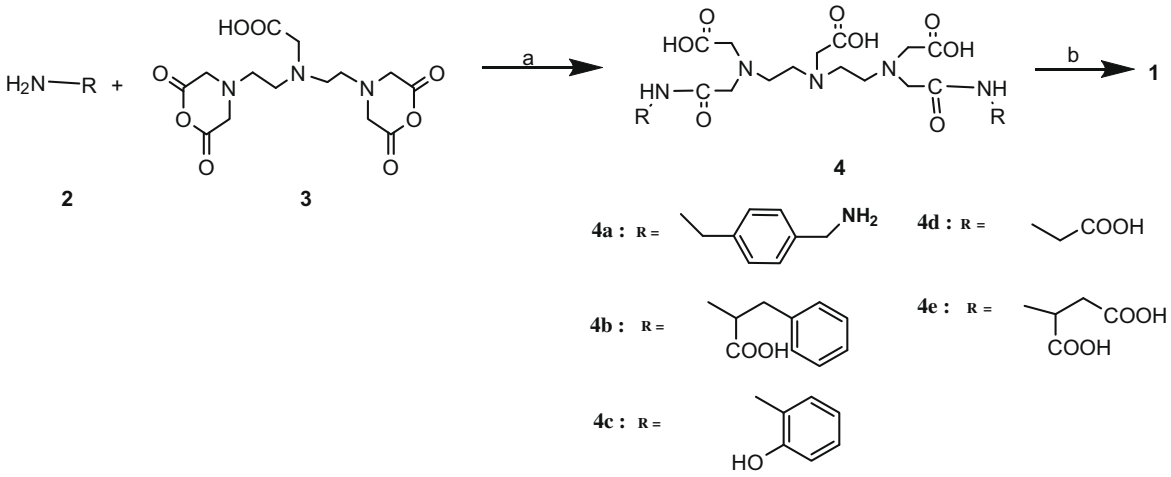


Figure 1. The structure of Gd–DTPA-complexes **1a–e**.

* Corresponding author. Tel./fax: +81 53 478 1144.
E-mail address: tcmyama@ipc.shizuoka.ac.jp (M. Yamasitha).

0040-4039/$ - see front matter © 2010 Published by Elsevier Ltd.
doi:10.1016/j.tetlet.2010.02.138



Scheme 1. Reagents and conditions: (a) DMF, 60 °C, 24 h; (b) $GdCl_3{\cdot}6H_2O$, pyridine, H_2O, 60 °C, 24 h.

Table 1
Comparison of r_1 relaxivity of Gd–DTPA and Gd-complexes **1a–e**

Gd-complexes	r_1 (s^{-1} mM^{-1})	
	In H_2O	In albumin
Gd–DTPA	3.5	3.5
1a	8.0	10.25
1b	5.8	10.00
1c	4.8	6.2
1d	5.5	6.4
1e	5.4	9.5

These ligands are composed of DTPA[6] and terminal units **2a–e**, which may immobilize gadolinium ion at the focal points by eight coordination sites, allowing one water molecule to chelate and encapsulates the metal ions inside the glycoside clusters. Like the previous reports on Gd(III)–DTPA complexes[9,10] our new Gd-complex has showed good solubility in aqueous solutions, although the acetylated free amines might reinforce their own hydrophobic features. The Gd–DTPA-complexes **1a–e** with different terminal functionalities were designed to have a rigid frame work in the molecule and to facilitate the protein recognition with the amines as well. Hence Gd–DTPA-derived complexes **1a–e** are expected to possess better r_1 values in water and enhanced r_1 values in albumin.

The r_1 values obtained for **1a–e** in water and albumin are shown in Table 1. As expected, the r_1 value in albumin medium was enhanced moderately but not remarkably. Along with the 'glycoside cluster effect'[11] the carbohydrate aggregation may offer a potential advantage for site-specific delivery of the contrast agents at a molecular level since carbohydrates play significant roles in recognition processes on cell surface.[11–13] Therefore, the combined contribution of amines and carbohydrates to the enhancement of r_1 value of Gd-complexes by hybridization of the terminal of free amine will be preferable.[14]

We developed the synthesis of new dendritic molecules having functionalized ligands useful for the preparation of Gd-complexes. This synthetic methodology can be scaled up for multigram for practical use as radiopharmaceuticals. The chelates with higher-molecular weight groups are indispensable for the prevention of their diffusion from the intravascular space during MRI examinations.[15] Accordingly, these gadolinium(III) chelates after creation and structural modifications may fulfill many criteria for superior contrast agents. Following the intensive investigations on a wide variety of carbohydrate-modified ligands, the feasibility studies on their metal complexes as new potential candidates for MRI contrast media are now in progress.[14]

In vitro evaluation: Relaxivity r_1 that divided relaxation time T_1 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 ion that did not form complexes has influence on measurements of relaxation time, free gadolinium ion were removed by adjusting pH 7.0 in water, adding Chelex®100 Resin, and stirring at room temperature for six hours. The removal of free gadolinium ion was confirmed with color test by xylenol orange. Gadolinium concentration was measured with ICP-AES 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, the most existing protein in blood. The r_1 values were calculated by the following expression, the values are shown in Table 1.

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

where $r_1^{H_2O}$ is the relaxivity (s^{-1} mM^{-1}); T_1 is the relaxation time (ms); r_1 is the water of relaxivity (s^{-1} mM^{-1}); and [Gd^{3+}] is the gadolinium concentration (mM).

We have succeeded in the synthesis of a new gadolinium complex, Gd–DTPA derivatives as an MRI CAs. Their higher accumulation in the blood vessel and higher tumor-selectivity indicate that they have potential as MRI angiography agents and for early diagnosing medical treatments of tumors.

Acknowledgments

We would like to thank the Ministry of Health, Labor, and Welfare, Japan, and JAMME, Japan Association for the Advancement of Medical Equipment Japan; the Headquarters of Japan Science and Technology Agency (JST); Shizuoka Prefecture Industry Creating Agency; and Grants-in-Aid for Scientific Research by Japan Society for the promotion of Science (JSPS) for providing financial support.

References and notes

1. Lauffer, R. B. *Chem. Rev.* **1987**, *87*, 901–927.
2. Tweedle, M. F. In *Lanthanide Probes in Life, Chemical, and Earth Sciences*; Bunzli, J. C. G., Choppin, G. R., Eds.; Elsevier: Amsterdam, 1989. Chapter 5.
3. Caravan, P.; McMurry, J. J.; Lauffer, R. B. *Chem. Rev.* **1999**, *99*, 2293.
4. Kabalka, G. W.; Davis, M. A.; Moss, T. H.; Buonocore, E.; Hubner, K.; Holmberg, E.; Maruyama, K.; Haung, L. *Magn. Reson. Med.* **1991**, *19*, 406.

5. Takahashi, M.; Hara, Y.; Aoshima, K.; Kurihara, H.; Oshikawa, T.; Yamasitha, M. *Tetrahedron Lett.* **2000**, *41*, 8485.
6. Blish, S. W. A.; Chowdhury, A. H. M. S.; Mcpartlin, M.; Scowen, T. J.; Bulman, R. A. *Polyhedron* **1995**, *14*, 567.
7. The synthesis of dendritic ligand (**4**) employed a convergent method to couple core and free amine branch. **Compound 4a**: To the solution of terminal bis *p*-xylylenediamine (**2**, 0.76 g, 5.6 mmol) in DMF (25 mL) was added DTPA anhydride (**3**, 1.0 g, 2.8 mmol) and stirred for 24 h at 55–60 °C. After completion of the reaction the solvent was removed under reduced pressure, and dried to obtain pure compound **4a** with 95% Yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ: 8.06–8.68, 7.10–7.11, 4.23, 3.26–3.31, 2.27. ^{13}C NMR (75 MHz, $CDCl_3$): δ: 43.4, 45.6, 52.6, 54.8, 59.3, 59.8, 136.2, 141.6, 171.2, 173.4, 177.6. MALDI-TOF MS: *m*/*z* (%) = 629 (100) M^+. The title compounds (**4b**–**e**) were prepared by the above-mentioned procedure. Compound **4b**: ^{1}H NMR (300 MHz, $CDCl_3$): δ: 8.18, 7.24–7.20, 4.45, 3.79–3.06, 2.93–2.49, 1.07–1.02. MALDI-TOF MS: *m*/*z* (%) = 687 (100) $[M-H]^-$. Compound **4c**: ^{1}H NMR (300 MHz, $CDCl_3$): δ: 9.58, 7.95–7.92, 6.96–6.72, 3.48–2.49, 1.08–1.03. MALDI-TOF MS: *m*/*z* (%) = 574 (100) $[M-H]^-$. Compound **4d**: ^{1}H NMR (300 MHz, $CDCl_3$): δ: 8.33, 7.93, 4.25–4.05, 3.75–3.03, 2.87–2.07, 1.17–1.01. ^{13}C NMR (75 MHz, $CDCl_3$): δ: 43.0, 52.6, 54.8, 59.3, 59.8, 60.7, 171.2, 173.4, 174.8, 177.6. MALDI-TOF MS: *m*/*z* (%) = 508 (100) $[M-H]^-$. Compound **4e**: ^{1}H NMR (300 MHz, $CDCl_3$): δ: 8.43–8.15, 7.95, 4.54–4.01, 3.72–3.10, 2.93–2.08, 1.98–1.15. MALDI-TOF MS: *m*/*z* (%) = 622 (100) $[M-H]^-$.
8. To a solution of ligand (**4**, 2.0 g, 3.2 mmol) in water, pyridine (2.6 mL, 32 mmol) was added and the reaction mixture was stirred for 10 min at rt. To this $GdCl_3{\cdot}6H_2O$ (1.2 g, 3.2 mmol) was added slowly and the reaction was kept at 60 °C and stirred for 24 h. After completion of the reaction water was removed under vacuum and the crude product was dissolved in water and the excess of Gd(III) was removed by using Chelex® resign and checked by use of xylenol orange indicator. After removal of excess Gd(III) the resin was filtered off and under reduced pressure the solvent was removed by rotary-evaporator, dried to obtain pure compound **1a** with 90% Yield. MALDI-TOF MS: *m*/*z* (%) = 802 (100).
9. Kawa, M.; Frechet, J. M. *J. Chem. Mater.* **1998**, *10*, 286.
10. Newkome, G. R.; Guther, R.; Moorefield, C. N.; Cardullo, F.; Echegoyen, L.; Perez-Cordero, E.; Luftmann, H. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2023.
11. Konings, M. S.; Dow, W. C.; Love, D. W.; Raymond, K. N.; Quay, S. C.; Rocklage, S. M. *Inorg. Chem.* **1990**, *29*, 1488.
12. Lee, Y. C.; Lee, R. T. *Acc. Chem. Res.* **1995**, *28*, 321.
13. Zanini, D.; Roy, R. *J. Am. Chem. Soc.* **1997**, *119*, 2088.
14. Ozaki, N.; Sankar, A. U. R.; Yamasitha, M.; Aoki, T.; Tanaka, Y.; Kimura, M.; Fujie, M.; Takehara, Y.; Takahara, H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 932.
15. Formation of 1:1 Gd(III) complex was deduced on the basis of titration experiment, where 0.5, 1.0, or 10.0 equiv of Gd(III) was added relative to the ligands upon complexation.