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Utilization of dendritic framework as a multivalent ligand: a functionalized gadolinium(III) carrier with glycoside cluster periphery

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Abstract

A new type of designed and functionalized ligand for a lanthanide ion based on dendritic architecture was synthesized. The dendrimers, which contain four and twelve glucose moieties on the molecular surfaces, were readily synthesized with good yields in every step. The NMR and GPC analyses precisely demonstrated successful constructions of the dendritic structures, while the formations of gadolinium chelates were deduced on the basis of HPLC study. © 2000 Elsevier Science Ltd. All rights reserved.

A wide variety of hyperbranched polymers have been described in the last decade and their expanded applications have considerably enhanced their own significance.[1,2] Among lots of elaboration, the use of spherically dendritic macromolecules as drug carriers has attracted a great deal of interest because of the feasibility for new drug delivery systems.[3] In this regard, some groups have described intriguing examples of new contrast media for the preclinical studies of magnetic resonance imaging (MRI), where dendritic frameworks were applied as a paramagnetic metal support.[4,5] Lanthanide metals such as Gd(III) shorten the relaxation time and presuppose a possibility of achieving higher sensitivities for the MRI examinations.[6] To date, much effort has been devoted to create new candidates in this field, and most of them immobilize the metals in the exterior surfaces under chelation.[7–9] On designing more instructive contrast agents, we explored an alternative approach to a tunable candidate for the metal carrier by utilization of the dendritic framework as a ligand for lanthanide metals (Fig. 1). These ligands are composed of diethylenetriaminepentaacetic acid (DTPA)[10] and glycodendrimers, which may immobilize

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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. 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 surfaces.[12–14] In this publication, we disclose a new type of dendritic macromolecule, which works as a functionalized ligand coordinating gadolinium(III) ion at the centers of their frameworks under complexation.[15,16]

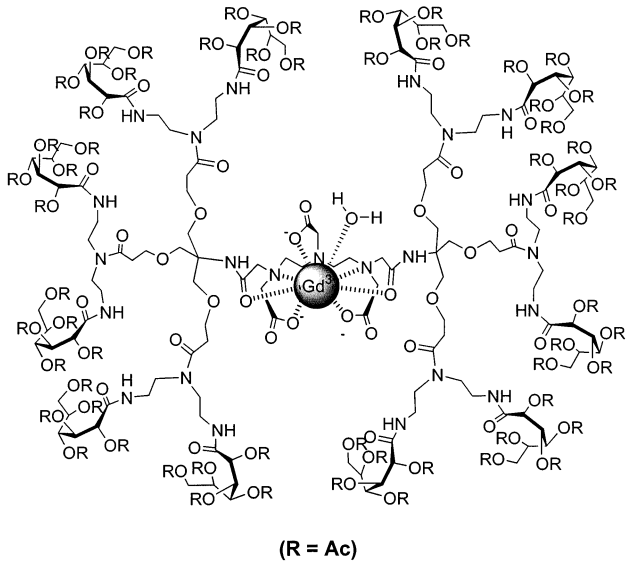
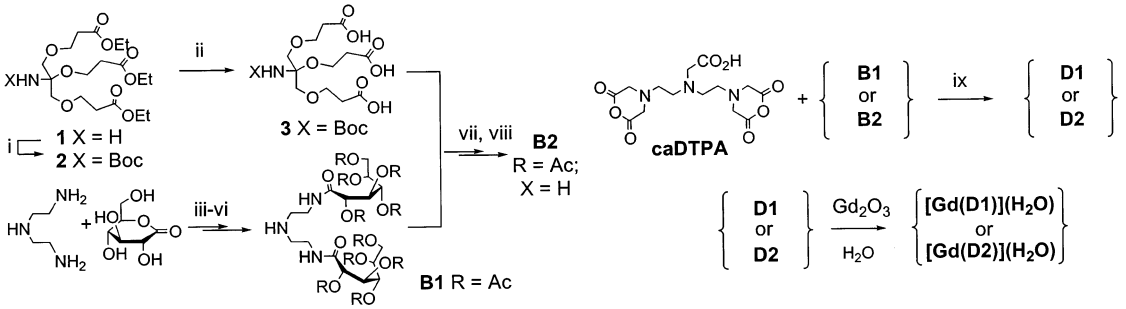


Figure 1. Structural drawing of gadolinium(III)–dendrimer chelate [Gd(**D2**)](H_2O)

The syntheses of target dendrimers **D1** and **D2** employed a convergent method to couple diethylenetriaminepentaacetic acid bis(cyclic anhydride) (caDTPA) and glycoside branches (**B1** and **B2**), respectively (Scheme 1). The higher ordered branch **B2**, responsible for the dendrimer **D2**, was synthesized by a coupling between **B1** and tricarboxylic acid **3**. The building block **1** was readily accessible by Newkome's protocol,[17] and its amino group was then protected as the *tert*-butyl carbamate (Boc group) to give **2**. Subsequent hydration of three ester groups in basic conditions led to the linker unit **3** in 80% total yield from **1**.



Scheme 1. Reagents and conditions: (i) $(Boc)_2O$, Et_3N, THF, rt, 81%; (ii) NaOH, CH_3OH–H_2O (2:1), rt, >99%; (iii) DMF, rt, 99%; (iv) $(Boc)_2O$, DMF, rt, 96%; (v) Ac_2O, pyridine, rt, 98%; (vi) TFA, CH_2Cl_2, rt, 95%; (vii) DCC–HOBt, DMF, rt, 62%; (viii) TFA, CH_2Cl_2, rt, 90%; (ix) DMF, rt, >95%

In regard to the carbohydrate domain, D-glucose amide derivatives were chosen as representative compounds for our first model. The synthesis of double-glycoside branch **B1** was achieved by direct amidation of diethylenetriamine with δ-gluconolactone. After Boc protection of the amino group, all glycoside hydroxyls were acetylated by treatment with excess acetic anhydride in the presence of pyridine. At this stage, *N*-Boc protected **B1** was obtained in 93% overall yield from diethylenetriamine and cleavage of the Boc group by a brief exposure to trifluoroacetic acid (TFA) gave the amine **B1** in 95% yield. On the other hand, a coupling reaction between **3** and **B1** led to another branch unit **B2** bearing six glycoside portions. Condensation of these compounds in the presence of DCC and HOBt gave *N*-protected **B2** (62% yield), and subsequent removal of the Boc group by TFA cleavage led to another amine **B2** in 90% yield. With these glycoside branches **B1** and **B2** in hand, the dendrimers **D1** and **D2** were quantitatively produced by their nucleophilic additions to caDTPA, respectively.

The formations of dendritic structures were well demonstrated by the spectroscopic data.[18] In the ^{1}H NMR spectra, the intense acetyl and glycoside peaks appeared in the regions of δ 2.0–2.3, δ 4.1–4.4, and δ 5.0–5.7 ppm, while the characteristic protons of the metal chelating portions showed broad absorption around δ 3.5 ppm as observed for other DTPA analogs. Additionally, these structures were further established by GPC analyses evaluating their relative molecular sizes. Fig. 2 shows GPC traces for the dendrimers, where intense peaks appearing at the retention time of 14.9 and 13.5 min corresponded to **D1** and **D2** with molecular weights of up to 2117 and 6203 Da, respectively.

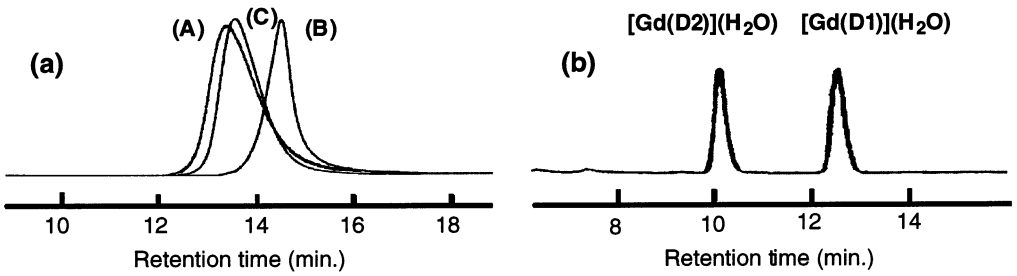


Figure 2. (a) Overlay of GPC traces for (A) polystyrene standard ($Mw/Mn = 1.06$, $Mn = 3957$), (B) **D1** and (C) **D2**; (b) Reversed-phase HPLC analyses of gadolinium(III) chelates [Gd(**D1**)](H_2O) and [Gd(**D2**)](H_2O)

Like previous works on Gd(III)–DTPA complexes,[10] our dendritic ligands should leave the three carboxylate arms free to coordinate to Gd(III) ion. As demonstrated by HPLC analyses (Fig. 2), the formations of well-stabilized complexes [Gd(**D1**)](H_2O) and [Gd(**D2**)](H_2O) were readily realized by heating mixtures of the ligands and Gd_2O_3 in aqueous media at 100°C.[19,20] Remarkably, these complexes showed good solubility in aqueous solutions, although the acetylated glycosides might reinforce their own hydrophobic features. Besides, these acetyl groups could be cleaved by additional sodium methoxide with good yields (74% for **D1** and 60% for **D2**), respectively.

Finally, we presented syntheses of new dendritic macromolecules and their utilization as functionalized ligands. Along with our synthetic strategy, a multigram synthesis responsible for practical use as radiopharmaceuticals can be administered. The chelates with higher-molecular weight compounds are indispensable for prevention of their diffusion from the intravascular space during MRI examinations.[21] Accordingly, these dendrimer–gadolinium(III) chelates may fulfill many criteria for superior contrast agents after certain structural modifications. Following

intensive investigations on a wide variety of carbohydrate-modified dendrimers, the feasibility of their metal complexes as new potential candidates for MRI contrast media are now in progress.

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18. IR and NMR data for dendrimers: **D1**: ν_{max}(NaCl)/cm^{-1} 1651, 1670, 1682, 1747, and 3400; δ_H (300 MHz, $CDCl_3$) 2.03 (s, 12H, Ac*4), 2.06 (s, 24H, Ac*8), 2.09 (s, 12H, Ac*4), 2.18 (s, 6H, Ac*2), 2.19 (s, 6H, Ac*2), 3.2–4.0 (m, 34H, NCH_2), 4.1–4.4 (m, 8H, CH_2OAc), 5.1 (br s, 4H, CH(OAc)), 5.23 (br s, 4H, CH(OAc)), 5.45 (br s, 4H, CH(OAc)), 5.6 (br s, 4H, CH(OAc)), 7.65 (br s, 4H, NH), 7.96 (br s, 2H, NH); δ_C(75 MHz, $CDCl_3$) 20.4, 20.6, 20.7, 37.7, 37.8, 61.6, 68.8, 68.9, 69.1, 69.5, 69.6, 71.7, 71.8, 71.9, 77.2, 167.0, 167.2, 169.5, 169.6, 169.7, 169.8, 170.0, 170.1, 170.2, 170.3, 170.6, and 170.7. **D2**: ν_{max}(NaCl)/cm^{-1} 1635, 1653, 1684, 1697, 1749, and 3300; δ_H (300 MHz, $CDCl_3$) 2.04 (s, 48H, Ac*16), 2.07 (s, 72H, Ac*24), 2.10 (s, 36H, Ac*12), 2.18 (s, 12H, Ac*4), 2.23 (s, 12H, Ac*4), 2.62 (br s, 12H, OCH_2CH_2CON), 3.4–3.8 (m, 90H, NCH_2 and OCH_2CH_2CON), 4.1–4.4 (m, 24H, CH_2OAc), 5.08 (br s, 12H, CH(OAc)), 5.25 (br s, 12H, CH(OAc)), 5.45 (br s, 12H, CH(OAc)), 5.62 (br s, 12H, CH(OAc)), 7.58 (br s, 12H, NH), and 8.01 (br s, 2H, NH); δ_C(75 MHz, $CDCl_3$) 20.4, 20.6, 20.7, 32.8, 38.1, 38.8, 45.6, 47.4, 61.5, 61.6, 68.1, 68.7, 68.8, 69.0, 69.1, 69.6, 69.8, 71.7, 77.2, 77.6, 167.1, 167.3, 169.5, 169.7, 169.8, 169.9, 170.1, 170.6, and 173.3.
19. Formations of 1:1 Gd(III)–dendrimer chelates were deduced on the basis of titration experiment, where 0.5, 1.0, or 10.0 equiv. of Gd(III) was added relative to the dendrimers upon complexation. Each sample gave no significant difference in chromatographic retention time during HPLC analysis, indicating 1:1 stoichiometry.
20. The stability constants ($\log K = 20.9$ for [Gd(**D1**)]$^{3+}$ and 21.3 for [Gd(**D2**)]$^{3+}$ at pH 7.0) were determined by reacting the complexes with a known Gd(DTPA) chelate, see: Deal, K. A.; Motekaitis, R. J.; Martell, A. E.; Welch, M. J. *J. Med. Chem.* **1996**, *39*, 3096–3106.
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