STUDIES ON PREPARATION AND CHARACTERIZATION OF NOVEL MRI CONTRAST AGENTS FOR TARGETING ORGANS AND BLOOD VESSELS

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Abstract: To improve the poor properties of currently clinically used MRI contrast agent of Gd-DTPA (Gadolinium-Diethylenetriamine-N,N,N',N",N"-pentaacetic acid) complex, sugar dendritic derivatives which contains Gd-DTPA as the core part and four or twelve sugars as the terminal part, dendrimer **8(Gd-DTPA-D1Glc(OAc))** or dendrimer **10(Gd-DTPA-D2Glc(OAc))**, respectively, were prepared. From the result of relaxivity profile of these Gd complexes depending on temperature and magnitude of magnetic field, the smaller size MRI contrast agent of Gd-DTPA, **8(Gd-DTPA-D1Glc(OAc))**, showed similar behavior to that of Gd-DTPA and the dendrimer constructs DDS of Gd-DTPA and was proven to have the improved properties for MR imaging of blood vessel (MRA) as well as for targeting specific organs.

Introduction

The MRI (Magnetic Resonance Imaging) is a clinically diagnostic modality, which prompted the need for a new class of drugs (1). Among the MRI contrast agents being developed, Gd(III)-DTPA (Diethylenetriamine-N,N,N',N",N"-pentaacetic acid) has been shown to have highly effective properties in contrasting the MR imaging. The most practical and popular MRI contrast agents, Gd(III)-DTPA, Gd(III)-DOTA (DOTA=1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate), and Gd(III)-DTPA-BMA (BMA=bis(methylamide)), were simple chelates of gadolinium, whose molecular sizes are small and have no molecule recognition site in the contrast agent molecule for living body, therefore, these MRI contrast agents leak from blood vessel and have poor function for MRA (MR angiography) and for organ specificity.

Recent attempts to improve the poor functionality of these MRI contrast agents are reported to provide the targeting property by formation of particular binding *in vivo* (2). The clinical utility of a contrast agent depends not only on absolute contrast enhancing properties but also on concentration of the contrast agent in target tissues and in blood vessels.

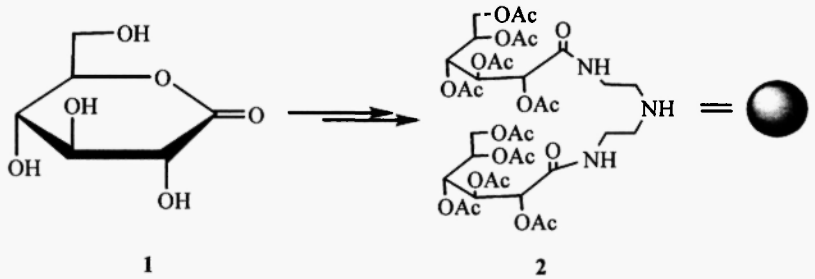
The liver has asialoglycoprotein receptor which especially recognizes lactose (3). We have attempted to synthesize Gd-DTPA derived ligands having several kinds of sugar terminal to recognize sites of the liver and to enlarge the molecular size. Dendrimers have a sphere structure whose character enables to control and to enlarge the molecular size and the weight. By using the character of dendrimers and sugars provided on Gd-DTPA, increasing the blood vessel storability and enhancing the recognition property of molecules of organs were attempted to improve blood vessel MR imaging for realizing efficient MRA and also to specify organs by constructing efficient drag delivery system (DDS) to organs. In this paper we will deal with the synthesis and characterization of the physical property of novel potential MRI contrast agents containing sugars, four

sugar dendrimer **8(Gd-DTPA-D1Glc(OAc))** and/or twelve sugar dendrimer **10(Gd-DTPA-D2Glc(OAc))**, the former of which was proved *in vivo* to recognize organs and blood vessels by the terminal sugar molecules linked on the Gd-DTPA core of the dendrimers.

Results and Discussion

Synthesis

Convergent method was applied for the preparation of dendrimer structure. The terminal, branch, and core parts were separately prepared first, and then the parts were combined to construct the dendrimers for Gd(III)-DTPA derived ligands. In the beginning of the Gd(III) ligand preparation, D-(+)-glucono-1,5-lactone (**1**) was used for providing the recognition site of organs such as liver and kidney in the dendrimer. To prepare terminal, gluconolactone **1(Glc)** was converted into **2(Glc)** by protection of the hydroxyl groups of the sugar lactone by acetate formation (Scheme-1). The ^{1}H NMR spectrum of **2(Glc)** is shown in Figure-1.



1 **2**

Scheme-1 : The preparation route to terminal **2(Glc)** from D-(+)-glucono-1,5-lactone (**1**).

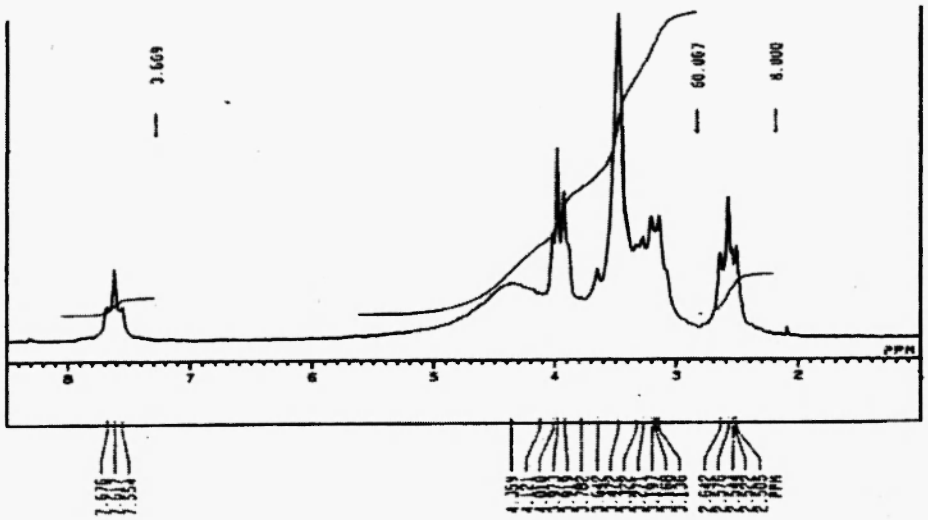
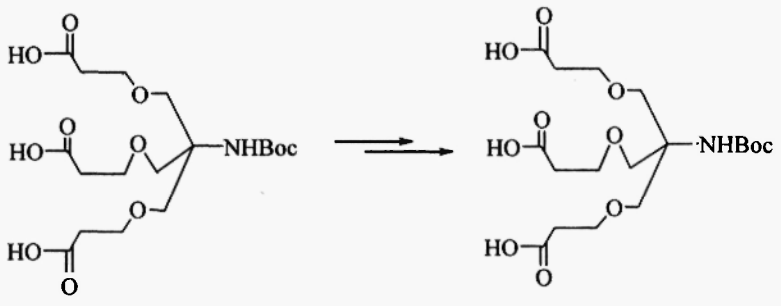


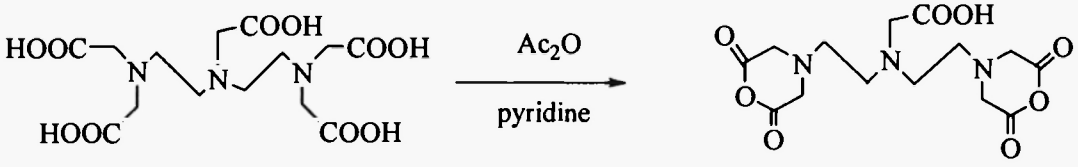
Figure-1 : ^{1}H NMR spectrum of **2(Glc)**.

Acetylation of D-(-)-galactono-1,4-lactone (**1(Gal)**) by the usual reaction conditions gave **2(Gal)**. In accordance with the synthetic strategy, compound **4** was synthesized from tris(hydroxymethyl)aminomethane (**3**) (Scheme-2) as the branch [4,5], and DTPA anhydride, compound **6**, was synthesized from DTPA (**5**) (Scheme 3) as the core [**6**].



3 **4**

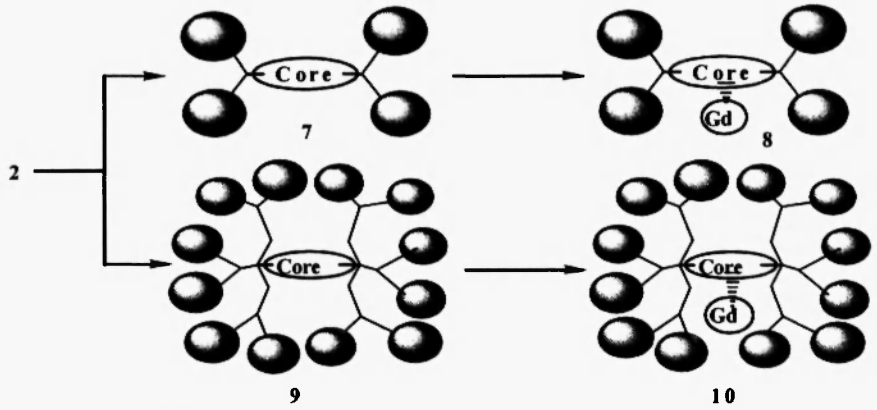
Scheme-2 : The preparation of branch 4.



5 **6**

Scheme-3 : The preparation of DTPA anhydride from Diethylenetriamine-N,N,N',N",N"-pentaacetic acid.

Reaction of sugar derivative **2** with **6** afforded compound **7(DTPA-D1Glc(OAc))** (Figure-4). The ^{1}H NMR spectrum of **7(DTPA-D1Glc(OAc)** is shown in Figure-2. Gd-DTPA complex **8(Gd-DTPA-D1Glc(OAc))** (Scheme-4) was obtained from **7(DTPA-D1Glc(OAc))** with equivalent amount of Gd_2O_3 or $GdCl_3$. Formation of **8(Gd-DTPA-D1Glc(OAc))** was confirmed by the ^{1}H NMR spectrum of **7(DTPA-D1Glc(OAc))** and GPC analysis of **8(Gd-DTPA-D1Glc(OAc))** (Figure-5). Dendrimer **9(DTPA-D2Glc(OAc))** was prepared similarly via dendrimer wedge which was synthesized by the reaction of **2** with **4** followed by deprotection of Boc group and successive reaction of the dendrimer wedge with **6**. The ^{1}H NMR spectrum of **9(DTPA-D2Glc(OAc))** is shown in Figure-3. Then, Gd-DTPA complex **10(Gd-DTPA-D2Glc(OAc))** was prepared by the same synthetic method for compound **8(Gd-DTPA-D1Glc(OAc))** (Scheme-4).



9 **10**

A circle ● represents a sugar residue which linked with the core.

Scheme-4 : The preparation route to **8(Gd-DTPA-D1Glc(OAc))** and **10(Gd-DTPA-D2Glc(OAc))**.

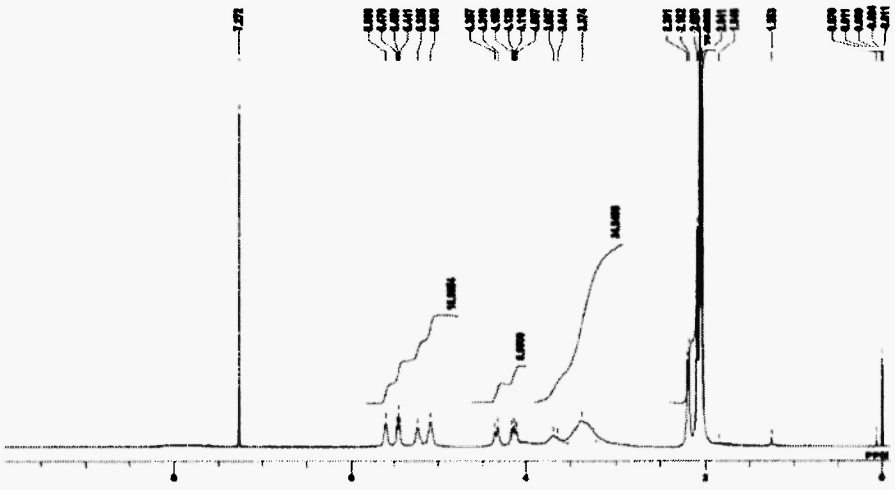


Figure 2. ^{1}H NMR spectrum of **7(DTPA-D1Glc(OAc))**.

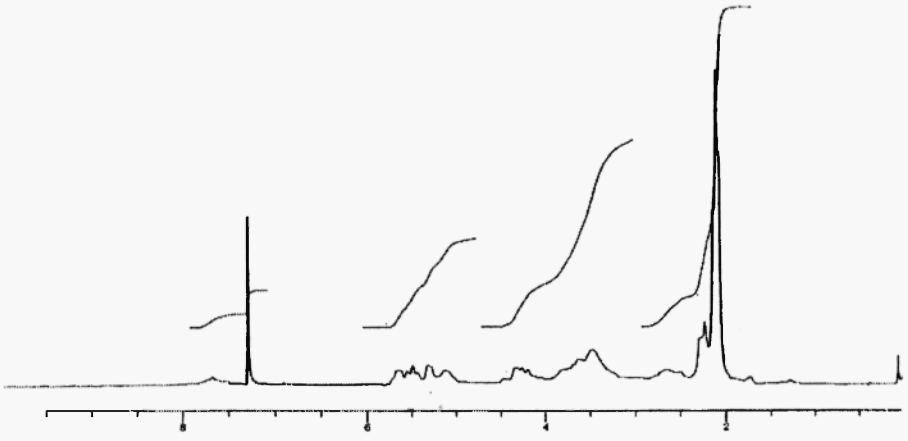


Figure 3. ^{1}H NMR spectrum of **9(DTPA-D2Glc(OAc))**.

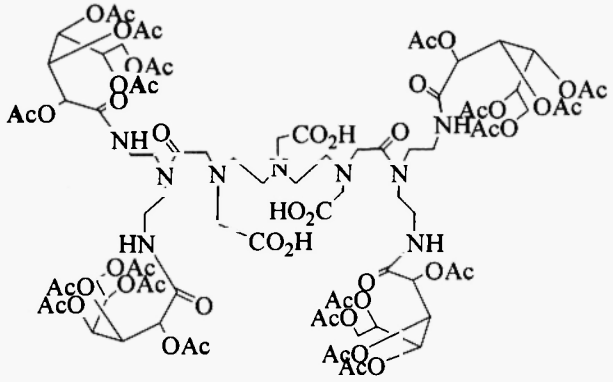


Figure-4 : Structure of dendrimer **7(DTPA-D1Glc(OAc))**.

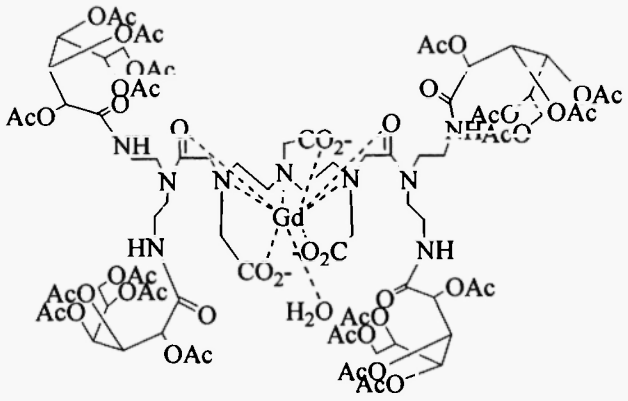


Figure-5 : Structure of Gd(III) complex **8(Gd-DTPA-D1Glc(OAc))**.

Physical Property

The MR image intensity in ^{1}H NMR signal of water protons linked with Gd(III) is dependent on nuclear relaxation times [7]. They have a good correlation to the relaxation rate of the protons. The parameters of relaxation times for compounds **8(Gd-DTPA-D1Glc(OAc))** and **10(Gd-DTPA-D2Glc(OAc))** were examined. The relaxivity vs. temperature profile is shown in Figure-4. Figure-4 shows that **8(Gd-DTPA-D1Glc(OAc))** has larger relaxivity than that of **10(Gd-DTPA-D2Glc(OAc))** at 37 °C, and then **8(Gd-DTPA-D1Glc(OAc))** is superior to **10(Gd-DTPA-D2Glc(OAc))** as a MRI contrast agent.

The parameters of relaxation rates for compounds **8(Gd-DTPA-D1Glc(OAc))** and **10(Gd-DTPA-D2Glc(OAc))** vs. Larmor frequency were examined and the profile observed is shown in Figure 5. By the comparison of Larmor frequency profile, the curves of Gd-DTPA and **8(Gd-DTPA-D1Glc(OAc))** resemble each other, and the observation implies that these two complex have same Gd chelate structure.

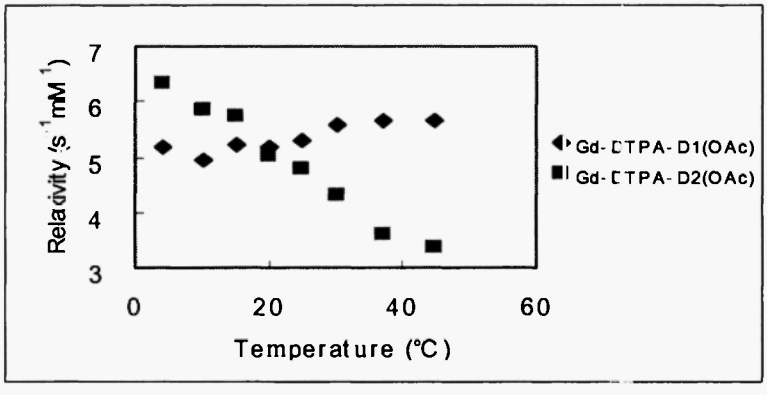


Figure-4 : Relaxivity of the protons of **8(Gd-DTPA-D1Glc(OAc))** and **10(Gd-DTPA-D2Glc(OAc))** vs. temperature profile.

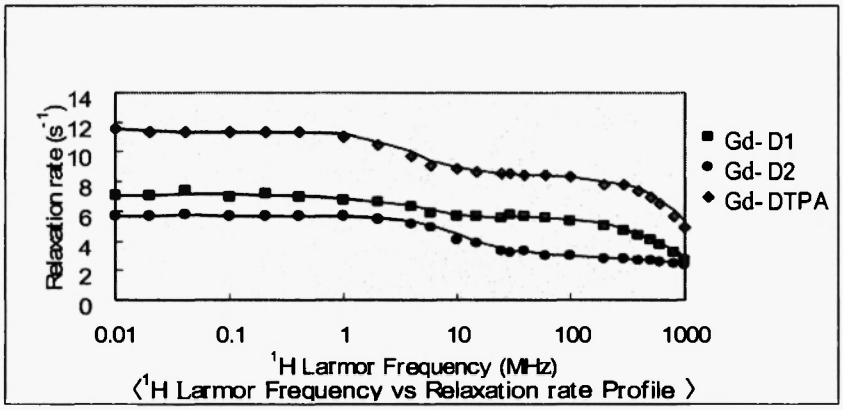


Figure-5 : Relaxation rate of the protons of **8(Gd-DTPA-D1Glc(OAc))** and **10(Gd-DTPA-D2Glc(OAc))** vs. ^{1}H Larmor frequency profile.

These results show that relaxivity of **10(Gd-DTPA-D2Glc(OAc))** (=Gd-DTPA-D2(OAc) or Gd-D2) decreased as temperature risen, while the change of relaxivity of **8(Gd-DTPA-D1Glc(OAc))** (=Gd-DTPA-D1(OAc) or Gd-D1) vs. temperature was very little, or rather increasing. Further, relaxation rate profile vs. ^{1}H Larmor frequency of Gd-DTPA in Figure 5 is similar to that of **8(Gd-DTPA-D1Glc(OAc))**, but is not similar to that of **10(Gd-DTPA-D2Glc(OAc))**. From this fact observed, it may be concluded that Gd(III) ion coordinates with **8(Gd-DTPA-D1Glc(OAc))** securely in the same pattern as Gd-DTPA complex, but may not do for **10(Gd-DTPA-D2Glc(OAc))**. The Gd(III) ion may not be complexed with the core DTPA part of ligand **9(DTPA-D2Glc(OAc))**, but the Gd(III) ion may be entangled by amide linking with the sugar residue and/or the amide linker part. Therefore, in order to make Gd(III) ion complex with the core part of the dendrimer, DTPA derivatives as the ligand may not exert steric hindrance for the chelation with Gd(III) for **7(DTPA-D1Glc(OAc))**, and then **8(Gd-DTPA-D1Glc(OAc))** may be expected to deliver Gd-DTPA as a MRI contrast agent in the DDS. Then alkaline hydrolysis of **8(Gd-DTPA-D1Glc(OAc))** was performed to prepare **8(Gd-DTPA-D1Glc(OH))**, where acetate of the hydroxyl groups of sugars are deprotected to free hydroxyl groups and thus, providing an increased hydrophilicity.

The improved MR imaging of blood vessels as well as specificity of organs by using **8(Gd-DTPA-D1Glc(OH))** as the contrast agent was observed *in vivo* experiments. The excellent results for MRA as well as targeting and specificity for organs will be published soon separately.

Conclusion

Gd(III)-DTPA derivatives **8(Gd-DTPA-D1Glc(OAc))** and **10(Gd-DTPA-D2Glc(OAc))**, which link sugar molecules by the number of 4 and 12, respectively, were synthesized and characterized. From the result of physical property measurement, ^{1}H Larmor frequency vs. relaxation rate profile of **8(Gd-DTPA-D1Glc(OAc))** is similar to that of Gd-DTPA and **8(Gd-DTPA-D1Glc(OAc))** has larger relaxivity than that of **10(Gd-DTPA-D2Glc(OAc))** at 37 °C. Therefore, Gd(III) complex **8(Gd-DTPA-D1Glc(OAc))** is expected to be a good DDS of Gd-DTPA to enhance MRI signal and to improve the poor function for MRA by Gd-DTPA complex. The alkaline hydrolyzed product of the Gd(III) complex, **8(Gd-DTPA-D1Glc(OH))**, thus is expected to show an excellent imaging of blood vessels as well as specific organs, then **8(Gd-DTPA-D1Glc(OH))** will innovate on MRI and MRA technology for clinical treatment.

Experimental

General Procedures and Methods

NMR spectra were collected on HITACHI R90H (90 MHz) and JEOL EX300 (300MHz). Preparative recycle GPC for isolation of the products were performed by using Japan Analytical Industry Co. Ltd. (JAI), LC918, for purification of ligands.

Sugar Derivative 2(Glc)

The solution of D-(+)-glucono-1,5-lactone (7.05 g) in 45 mL of DMF was added into diethylenetriamine (2.04 g) and the mixture was stirred for 5 h at room temperature, and then the mixture was left in the refrigerator for an overnight. To the solution of the mixture (4.23 g) was added $(Boc)_2O$ (2.01 g) at 0 °C then stirred for an overnight at room temperature. The mixture was added into Et_3N (10.0 g) and Ac_2O (9.40 g) at 0 °C then stirred for 2 days at room temperature. After the completion of the reaction, extraction of the product with 30 mL ethyl acetate followed by neutralization with aqueous sodium hydrogencarbonate (15 mL × 3 times), drying over anhydrous Na_2SO_4, and evaporation of the solvent gave a brown crystal. To the solution of the crystal (8.42 g) in CH_2Cl_2 (5 mL) was added trifluoroacetic acid (6.40 g) and the mixture was allowed to react for 2 h at room temperature. After the completion of the reaction, extraction of the product with 30 mL ethyl acetate followed by neutralization with aqueous sodium hydrogencarbonate (15 mL × 3 times), drying over anhydrous Na_2SO_4, removal of the solvent by evaporator, and the product isolation by recycle GPC gave **2(Glc)** in 74% yield (6.00 g).

DTPA Derivative with Four Sugars 7(DTPA-D1Glc(OAc))

To the solution of **2(Glc)** (2.17 g) in 20 mL of CH_3CN was added **6** (0.441 g) and the mixture was refluxed for 2 h. After the completion of the reaction and the evaporation of the solvent gave 7(**DTPA-D1Glc(OAc)**) in 97% yield (2.50 g).

Contrast Agent 8(Gd-DTPA-D1Glc(OAc))

DTPA derivative 7(**DTPA-D1Glc(OAc)**) (0.150 g) was dissolved in 3.5 mL of Gd_2O_3 (0.0123 g) solution of H_2O/MeOH (2/1). The mixture was refluxed for 45 min. And then, cation exchange resin was added to the mixture to remove the free gadolinium(III) (Gd(III)) ion. After the resin was filtered off, the filtrate was adjusted to neutrality with 1 N NaOH, and then the solvent was evaporated to give **8(Gd-DTPA-D1Glc(OAc))** (0.150 g) in 95% yield.

DTPA Derivative with Twelve Sugars 9(DTPA-D2Glc(OAc))

The solution of **2(Glc)** (6.04 g) and **4** (0.500 g) in CH_2Cl_2 (5 mL) was added into THF (15 mL) solution of HOBt (1.12 g) and DCC (2.93 g) at 0 °C, then the mixture was stirred for 24 h at room temperature. After the completion of the reaction, the solvent was removed off by evaporator. Extraction of the product from the residue with 30 mL ethyl acetate followed by neutralization of the extract with aqueous sodium hydrogencarbonate (15 mL × 3 times), removal of the solvent, and column chromatography of the residue on silica gel (eluent $CHCl_3$) gave dendrimer wedge protected by Boc group. To the solution of this product in 5 mL of CH_2Cl_2, TFA (10.5 mL) was added, and then the mixture was stirred for 2 h at room temperature. After the completion of the reaction, extraction of the product with 30 mL ethyl acetate followed by neutralization of the extract with aqueous sodium hydrogencarbonate (15 mL × 3 times) and evaporation of the solvent gave the deprotected wedge as a residue. To the solution of the residue in 20 mL of CH_3CN was added 6 (0.153 g) and the mixture was refluxed for 2 h. After the completion of the reaction, evaporation of the solvent gave 9(**DTAP-D2Glc(OAc)**) (2.60 g) in 75% yield.

Contrast Agent 10(Gd-DTPA-D2Glc(OAc))

In a mixed solvent of 12 mL of H_2O/MeOH (2/1) was dissolved DTPA derivative 9(**DTPA-D2Glc(OAc)**) (2.64 g), and then Gd_2O_3 (0.0771 g) was added and the solution was refluxed for 45 min. Cation exchange resin was added to the mixture in

order to remove the free gadolinium(III) ion. After the resin was filtered off, the filtrate was adjusted to neutrality with 1 N NaOH. Evaporation of the solvent gave **10(Gd-DTPA-D2Glc(OAc))** (2.60 g) in 96% yield.

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Refrences and Notes

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