High Relaxivity MRI Contrast Agents based on a closo-borane platform

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Introduction: Macromolecular CAs (dendrimers, polymers, proteins) carrying multiple copies of Gd^{3+} chelates show great promise towards sensitivity and diagnostic imaging time. However, such CAs often have limited relaxivity (r_1) at clinically relevant fields and are difficult to characterize due to their inherent polydispersity. Recently, intermediate size molecules have become favored over macromolecules due to enhanced relaxivity over a broad range of imaging field strengths. The higher r_1 values of such multimeric MRI CAs is attributed to their rigid framework and the presence of multiple Gd^{3+}-chelates in a small molecular space. Herein, we present a new class of polyfunctional MRI CAs built on the three dimensional icosahedral [*closo*-$B_{12}H_{12}$]$^{2-}$ anion (**I**, **Figure 1**). This unique design provides a multi-center core that can be used to anchor up to twelve radial arms with desired pendant functionalities that originate at close proximity to each other, resulting in a highly symmetrical and compact architecture. This novel configuration is ideally suited for the construction of a nanomolecular assembly having twelve Gd^{3+}-chelates in a sterically constrained fashion that can restrict rotational motion of individual Gd^{3+}-chelates resulting in high relaxivity values and an enhanced MRI contrast image. The *closo*-B_{12}^{2-} moiety can therefore be used as a platform for the targeted and high payload delivery of drug molecules and imaging agents. The 6-amino-6-methylperhyro-1,4-diazepine-1,4-*N*^{6}, *N*^{6}-tetraacetic acid ring system, abbreviated as *AAZTA*, was introduced in 2004[1] and consists of a seven membered ring that wraps around a Gd^{3+} ion giving rise to a system with two inner sphere water molecules (q = 2) in fast exchange with bulk water. Gd-AAZTA exhibits high relaxivity (r_1 = 7.1 $s^{-1}mM^{-1}$ at 20 MHz and 298 K) and is resistant to transmetallation and ligand exchange with various endogenous ions. Based on this general concept, an icosahedral *closo*-B_{12} scaffold supporting twelve copies of a Gd^{3+}-AAZTA chelate was envisioned.

Materials and Methods: The hydroxylation of all of the B-H vertices of **I** using 30% H_2O_2 provides [*closo*-$B_{12}(OH)_{12}$]$^{2-}$ (**II**, **Figure 1**) in 95% yield.[2] The B-OH vertices resemble alcohols in their reactivity and consequently twelve-fold carboxylate ester, ether and carbamate derivatives, described by us as "*closomers*", are now available.[3-5] This chemistry was recently extended to generate twelve fold azido ester analogues (**III**, **Figure 1**), that were used to carry out Cu(I) catalyzed Huisgen 1,3-dipolar click chemistry with terminal alkynes to obtain twelve equivalent 1,2,3-triazole rings tethered to the closomer scaffold.[6] Consequently, a modified AAZTA ligand with a terminal alkyne group (**IV**, **Figure 1**) was synthesized. Using the well-established Click Chemistry strategy, **IV** was reacted with the *closo*-borane motif **III** to generate the twelve fold AAZTA bound closomer **V** (**Figure 1**) in 76% yield. Next steps involved the de-protection of pendant *tert*-butyl ester groups in **V**, followed by twelve fold Gd-complexation to generate the closomer contrast agent **VI**. The final closomer contrast agent **VI** was purified via exhaustive dialysis in ultrapure water and characterized using IR, ICP-OES and HPLC.This unique closomer motif **VI** was tested for r_1 relaxivity and compared to the clinically used CA Omniscan®.

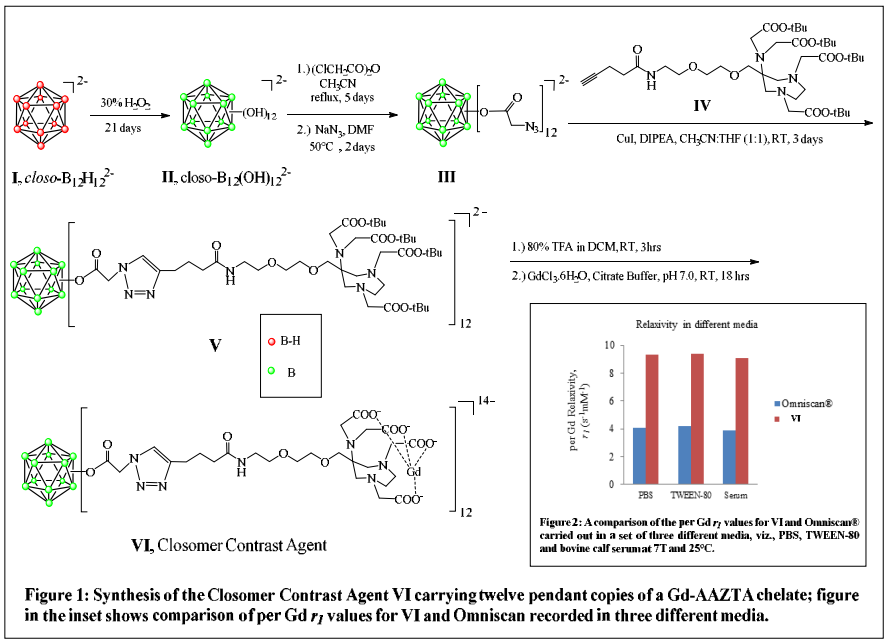

Figure 1: Synthesis of the Closomer Contrast Agent VI carrying twelve pendant copies of a Gd-AAZTA chelate; figure in the inset shows comparison of per Gd r_1 values for VI and Omniscan recorded in three different media.

Figure 2: A comparison of the per Gd r_1 values for VI and Omniscan® carried out in a set of three different media, viz., PBS, TWEEN-80 and bovine calf serum at 7T and 25°C.

Results and Discussions: The IR spectrum of **VI** exhibited the characteristic shift of the carbonyl stretch from 1736 cm^{-1} to 1595 cm^{-1}, which demonstrates the complexation of Gd^{3+} with AAZTA ligand. The Gd^{3+} loading was also determined by ICP-OES, which showed the formation of essentially fully loaded chelates with average of 10.8 Gd^{3+} ions per closomer. **VI** exhibited very high r_1 relaxivity value of 9.3 $s^{-1}mM^{-1}s$ per Gd (100.4 $s^{-1}mM^{-1}$ per closomer) at 298 K and 7 T in water and phosphate buffered saline (PBS) solution. This almost 250% increase in the r_1 value for **VI** over Omniscan® (r_1 = 4.05 $s^{-1}mM^{-1}$ at 7 T) can be attributed to the confinement of the twelve Gd^{3+} ions in a sterically constrained space on the icosahedral *closo*-borane scaffold and the two inner-sphere water molecules (q = 2) of the Gd^{3+}-chelate. The dynamic light scattering (DLS) analysis of **VI** in water and PBS solution gave the average particle size of 900 nm, indicating possible aggregation of the **VI**. To negate the possibility of a high r_1 value due to aggregation of **VI** in water or PBS solutions, a formulation of **VI** in polysorbate-80, a nonionic surfactant, also termed as TWEEN-80, was prepared, which lowered its average particle size to 20 nm range. The relaxivity measurements of this formulation at 7 T gave an r_1 value of 9.4 $s^{-1}mM^{-1}$, almost identical to that obtained in water and PBS solution. **Figure 2** in the inset shows a graphical comparison of the higher r_1 relaxivity values for **VI** and Omniscan® in PBS, TWEEN-80 and bovine calf serum at 7T and 25°C.

Conclusions: In summary, we hereby report a novel monodisperse, nano-sized, multimeric, high-performance MRI CA based on icosahedral *closo*-borane architecture. Closomer **VI** is water-soluble, carries up to twelve Gd^{3+}-chelates tightly in a sterically confined space. This unique configuration exhibits a high relaxivity value (per Gd relaxivity, r_1 = 9.3 $s^{-1}mM^{-1}$, per molecule r_1 = 100.4 $s^{-1}mM^{-1}$ at 7T in water or PBS). The closomer synthesis methodology presented here is very versatile and can easily be adapted to other ligand types such as DTPA, DOTA and DTTA ligands coupled with biological receptor-specific targeting moieties. The development of **VI** also led us to the design of novel AAZTA chelates (e.g. **IV**) that involves an easy synthetic route towards the incorporation of a PEG linker arm with varying end-groups at the 1,4-diazepine ring. Further, the chemistry of the *closo*-$B_{12}H_{12}^{2-}$ allows for heterogeneous vertex substitution and this opens up the possibility of a targeted or dual-mode imaging platform based on the *closo*-borane core.

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