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Demonstration of a sucrose-derived contrast agent for magnetic resonance imaging of the GI tract

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ABSTRACT

A scaffold bearing eight terminal alkyne groups was synthesized from sucrose, and copies of an azide-terminated Gd–DOTA complex were attached via copper(I)-catalyzed azide-alkyne cycloaddition. The resulting contrast agent (CA) was administered by gavage to C3H mice. Passage of the CA through the gastrointestinal (GI) tract was followed by T_1-weighted magnetic resonance imaging (MRI) over a period of 47 h, by which time the CA had exited the GI tract. No evidence for leakage of the CA from the GI tract was observed. Thus, a new, orally administered CA for MRI of the GI tract has been developed and successfully demonstrated.

Gastrointestinal (GI) radiography using barium contrast media has been widely used as a first-choice diagnostic imaging modality for detection of GI pathologies.[1] However, this approach is limited by radiation dosage[2] and the lack of lesion specificity. Colonoscopy is the standard of care for colorectal cancer (CRC) screening. However, colonoscopy is an invasive procedure that requires intravenous sedation and suffers from patient noncompliance.[3,4] CT colonography (CT-C) has high sensitivity to identify large polyps, but sensitivity decreases with a decrease in polyp size,[5] and radiation dosage is a concern. MRI colonography (MR-C) uses non-ionizing radiation and provides CRC lesion detection with high specificity, but modest sensitivity.[6,7] MR-C has been indicated in cases of incomplete colonoscopy due to obstruction, and is increasingly being applied as a non-invasive screening tool without the need for sedation.[5] MR-C has been performed with bright lumen,[8] and more recently in rodent models using dark lumen distended intestines in combination with intravenous contrast administration, typically Gd–DTPA or Gd–DOTA.[9–11] Dark lumen contrast has shown somewhat better performance, yet it is beneficial to the patient to undergo screening procedures that do not require enema or bowel distension.

Contrast agents (CAs) that have multiple Gd–chelates per molecule exhibit increased molar relaxivities (r_1),[12–15] making them detectable at lower concentrations than MR-C agents such as Gd–DTPA and Gd–DOTA. Targeted contrast agents[16] bind with high specificity and affinity, and for extended periods of time, to cell-surface markers that are expressed by aberrant cells, reducing the amount of CA needed and lengthening the time available for observation. If orally administered targeted CAs with multiple Gd–chelates per molecule were available for MR-C detection of lesions and pathologies of the GI tract, the drawbacks detailed in the previous paragraph would be overcome. This would result in a significantly lower limit of detectability for tumors in T_1-weighted images, without the need to use bright or dark lumen methods.

To be useful for construction of a CA for use in the GI tract, a molecular scaffold must possess multiple attachment points for

Abbreviations: CA, contrast agent; CRC, colorectal cancer; CT-C, X-ray computed tomography colonography; CuAAC, copper(I)-catalyzed azide-alkyne cycloaddition; DTPA, diethylenetriaminepentaacetic acid; DOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; FOV, field of view; FSEMS, fast spin echo multi-slice; GE3D, 3D spoiled gradient echo images; GI, gastrointestinal; MR-C, magnetic resonance imaging colonography; PS, progressive saturation; SEMS, spin echo multi-slice; TE, echo time; TR, repetition time.

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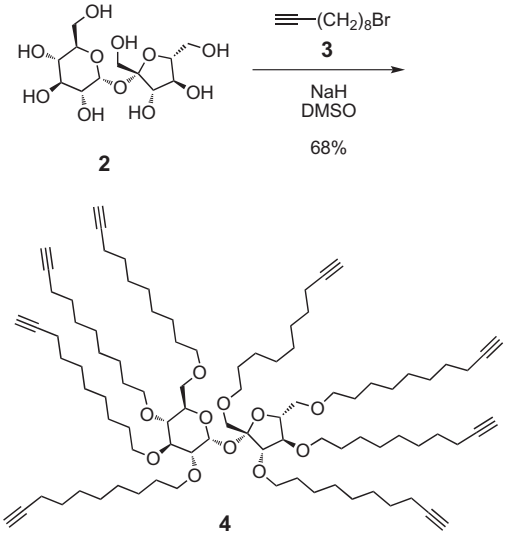
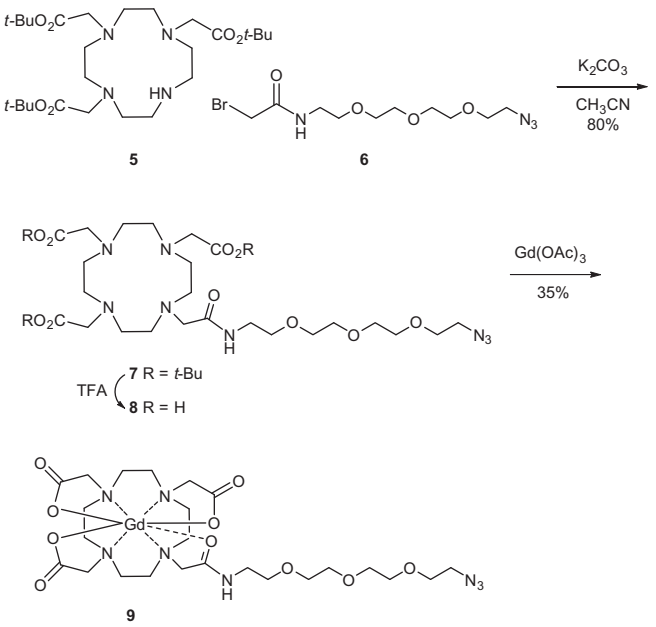
Gd–chelates and targeting ligands. Unbound CA must be non-toxic and pass through the GI tract intact, without being degraded by stomach acid or digestive enzymes or absorbed by the intestines. With these considerations in mind, our attention was drawn by olestra (**1**), a non-digestible fat substitute derived from sucrose and mixtures of fatty acids.[17,18] We reasoned that this molecule might serve as a useful scaffold if appropriate reporter groups and targeting ligands could be attached to the termini of the fatty acid chains. Olestra contains acid- or base-labile ester links and is a complex mixture of compounds. To make our construct more stable and less complex, we elected to employ ether links to sucrose involving chains of the same or similar constitution. We recently demonstrated that small peptide ligands[19] and prodrugs[20] could be attached to a sucrose-derived scaffold by means of the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC). As the next step toward development of targeted CAs, we report herein the synthesis of an untargeted, sucrose-derived CA and its use in magnetic resonance imaging of the GI tract.

Reaction of sucrose (**2**) with 24 equiv of sodium hydride in DMSO and 16 equiv of 10-bromo-1-decyne (**3**)[21] afforded octaalkyne **4** in 68% yield after purification by silica gel column chromatography (Scheme 1). Reaction of ester **5**[22] with bromide **6**[23] in the presence of potassium carbonate in acetonitrile gave DOTA derivative **7** in 80% yield (Scheme 2). Following cleavage of the *t*-butyl esters with TFA, reaction of **8** with gadolinium(III) acetate produced azide **9** in 35% yield over the two steps. CuAAC reaction[24] of octaalkyne **4** with 16 equiv of azide **9** using $CuSO_4$ and sodium ascorbate in 9/1 THF/water at rt for 4 days produced, after reversed phase column chromatography, a mixture of triazole-containing compounds that differed in the number of unreacted alkynes remaining in the molecule. The principal component of this mixture, hereafter referred to as the Gd–DOTA-sucrose CA, determined by MALDI-TOF MS, was the octatriazole **10**. The average number of Gd–DOTA chelates attached per sucrose was 7.1 (Fig. 1).

Initial MRI characterization of the Gd–DOTA-sucrose CA was accomplished in a phantom. An initial high concentration stock solution was prepared, and two separate phantoms were created by serial dilution. These phantoms were studied using progressive saturation experiments (PS), with 11 TR values exponentially spaced from 30 s to 60 ms. Nonlinear least squares regression allowed for the determination of the relaxation time constant (T_1) leading to the relaxation rate constant ($R_1 = 1/T_1$). The two highest concentration mixtures exhibited T_1 values that were shorter than



Scheme 1. Synthesis of octaalkyne **4**.



Scheme 2. Synthesis of azide **9**.

the minimum TR used in the relaxation series, which led to large variances in the fitted R_1 values. Figure 2 shows the relationship between R_1 and [Gd–DOTA-sucrose CA], where weighted linear regression was used to determine the r_1 value, 29.5 ± 0.1 mM s^{-1}, for the average multimer (r_1 per Gd = 4.1 mM s^{-1}). The weights used in the fit were inversely proportional to the variance in R_1 for each concentration, $1/\sigma^2_{R_1,i}$.

In vivo MRI studies were performed in C3H mice. Animals were anesthetized and prepared for MR imaging by induction with isoflurane. They were placed in a mouse-specific holder within an Agilent 72 mm birdcage coil (Agilent Technologies Inc., Santa Clara, CA) and inserted into an Agilent ASR 310 7T MRI system for scanning, which typically required 1 h.

Imaging parameters and conditions were identical throughout the course of the experiment. The VnmrJ 3.1 pulse-sequences included scout (FLASH) scans with three-plane geometry, axial planning scans with T_2-weighted Fast Spin Echo (FSEMS) with TR/TE = 2800/48 ms, and 25 slices. Coronal T_2-weighted FSEMS (TR/TE = 1600/48 ms) images were acquired for anatomical reference. Coronal planes were oriented using the kidneys as a frame of reference, while placing the read dimension along the *x*-axis to minimize breathing artifacts. 3D spoiled gradient echo images (GE3D) were acquired (TR/TE of 25/2.4 ms) with a field of view (FOV) of $40 \times 90 \times 30\ mm^3$, an in-plane resolution of $156 \times 352\ \mu m^2$, a 0.94 mm slice thickness, and a flip angle of 90°. A total of four averages were acquired with a scan time of 7 min. To protect from acidity in the stomach, the Gd–DOTA-sucrose CA was suspended in 0.1 M phosphate buffer, pH 7.4, and administered orally via gavage for contrast enhanced MRI experiments. Post-contrast experiments were initiated immediately following gavage to include the time-points 0.5, 2.5, 3.3, 6.5, 7.7, 8.5, 16.7, 24.5, 27.5, and 47 h. Spin-lattice relaxation experiments were performed using PS, with multiple TR values of 5, 1.87, 0.7, 0.26, and 0.098 s. A 2D spin echo experiment was used (SEMS) with a FOV of $45 \times 90\ mm^2$, an in-plane resolution of $176 \times 352\ \mu m^2$, and a slice thickness of 1.2 mm.

The levels of detection are quite promising, in that the r_1 is relatively large compared to Gd–DOTA and in keeping with other reported multimeric Gd contrast agents.[12–15] After orally introducing 0.5 mL of a 2.5 mM solution of the Gd–DOTA-sucrose CA mixture by gavage, high contrast in vivo [~11 relative enhancement (Enh − Bckgrnd)/Bckgrnd] was observed, and the untargeted agent