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Bioorganic & Medicinal Chemistry Letters 18 (2008) 2505–2508
Bioorganic & Medicinal Chemistry Letters

# A novel cholic acid-based contrast enhancement agent for targeted MRI

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Received 12 November 2007; revised 9 January 2008; accepted 14 January 2008
Available online 18 January 2008

**Abstract**—The novel Gd(III) complexes of heptadentate ligands NE3TA and NE3TA-Bn were prepared, and their relaxivities were measured and favorably compared to the commercially available MRI contrast enhancement agent Gd(DOTA). NE3TA was conjugated with cholic acid (CA) to produce CA-NE3TA. TEM images of Gd(CA-NE3TA) indicate that the complex self-assembles forming nano-sized micelles and displays an over threefold increased relaxivity compared to Gd(DOTA). The new cholic acid-conjugated nanoparticle MR contrast enhancement agent, Gd(CA-NE3TA) possesses great promise for use in targeted MRI.


Magnetic resonance imaging (MRI) is a non-invasive and high resolution imaging technique that has become a powerful diagnostic tool in the clinic. The images due to the MR signal of water protons provide a sharp contrast between tissues with different proton relaxation times ($T_1$ or $T_2$). Signal intensity, i.e., relaxivity ($1/T_1$ or $1/T_2$) results from proton exchange between a slowly exchanging gadolinium-bound water molecule and bulk water.[1] In order to enhance contrast between tissues, paramagnetic metal complexes have been introduced in vivo. The lanthanide Gd(III) is known to be an optimal paramagnetic metal for MRI due to its high electronic spin (7/2) and slow electronic relaxation rate.[2] A number of Gd(III) complexes such as Gd(DOTA) and Gd(DTPA) are clinically approved for use in MRI.[1] However, most contrast agents have non-specific extracellular distribution and the disadvantages of low relaxivity, low tissue specificity, and rapid clearance.[3] Considerable research efforts have been directed towards developing safe Gd(III)-based MR contrast agents with high tissue specificity and sensitivity. MRI is proven to be more sensitive and specific than other medical tests for detecting liver malignancies and for distinguishing them from benign lesions.[4] The gadolinium complexes of two DTPA analogues, benzyloxypropionictetraacetate (BOPTA) and ethoxybenzyl-diethylenetriaminepentaacetic acid (EOB-DTPA) are the clinically approved hepatobiliary agents.[5] The Gd(III) complexes provide low detection and characterization of metastatic lesions, although the agents are useful in detection and characterization of hepatocelluar lesions.[6]

In our continued effort to develop liver-specific MRI contrast agents,[7,8] we planned to use bile acid as a liver or intestine targeting moiety. The property of amphifacial bile acid to undergo enterohepatic circulation and form helical aggregates makes it a useful shuttle system to deliver various drugs to the liver and intestine with favorable intestine absorption and pharmacokinetic profile.[9,10] Bile acids are efficiently taken up into the cells by two types of carriers: apical sodium-dependent bile salt transporters (ASBT) and $Na^+$-independent carriers.[11] Experimental studies demonstrate that bile acids enter liver and colon cancer cells which over express bile acid transporters and carriers.[11,12]

We have recently reported a new series of bimodal chelators, NETA, NPTA, NE3TA, NE3TA-Bn containing both macrocyclic and acyclic components that have various biomedical applications.[8,13,14] The Gd(III) com-

*Keywords*: Chelating agents; Macrocyclic ligands; MRI contrast agents; Gd(III) complexes; Cholic acid; CA-NE3TA; NE3TA; NE3TA-Bn.
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0960-894X/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2008.01.044

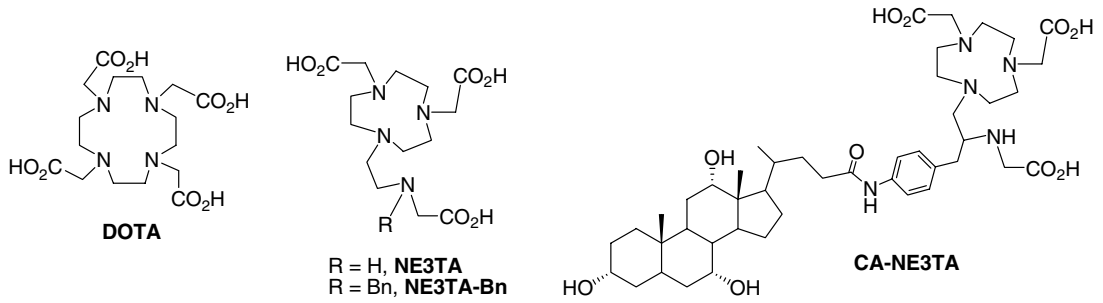



**Figure 1.** MRI contrast enhancement agents in preclinical and clinical use.

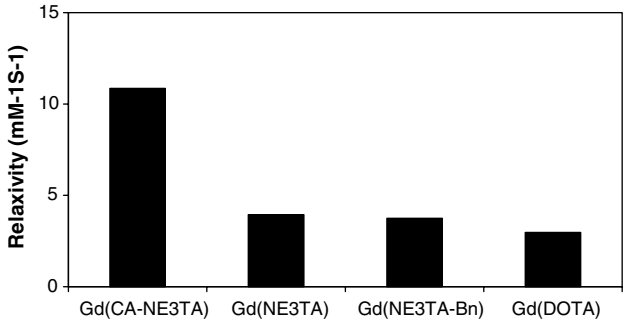
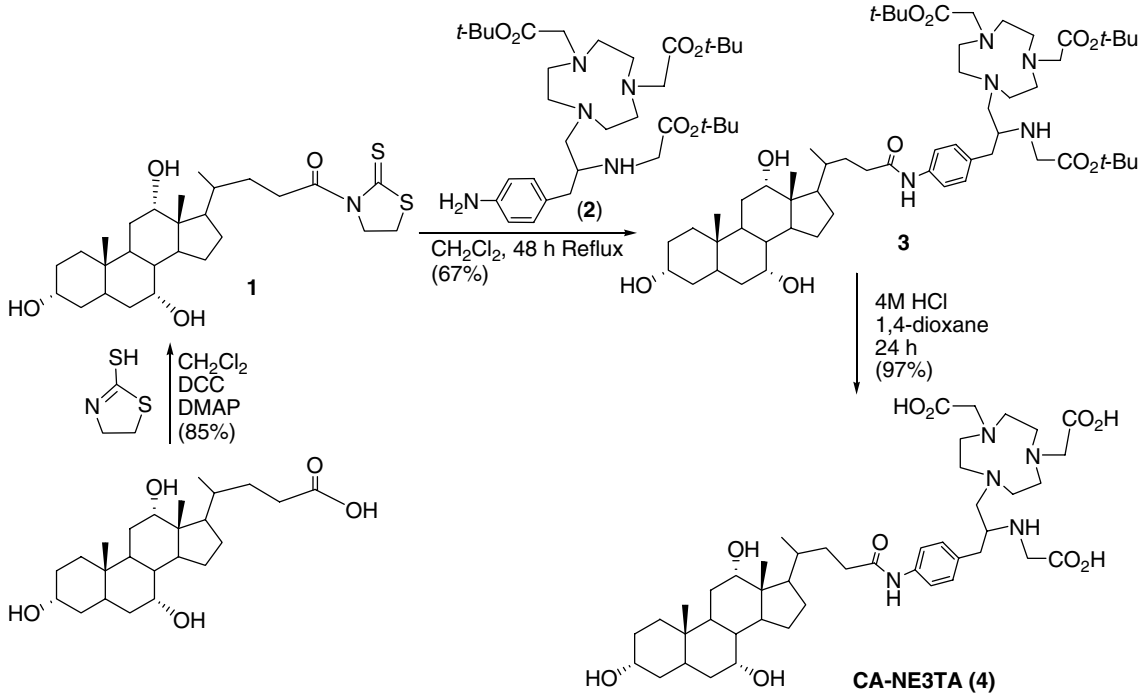



**Figure 2.** Relaxivity of the new Gd(III) complexes.

plexes of octadentate NETA and NPTA are stable in both serum and mice and possess enhanced relaxivity compared to those of DOTA.[8] In the present study, the Gd(III) complexes of heptadentate NE3TA and NE3TA-Bn were prepared as potential MRI contrast agents. Heptadentate NE3TA contains four amines and three carboxylates as potential donor groups. NE3TA–Bn is a heptadentate ligand with a benzyl group which can be further modified for conjugation to a targeting moiety. Both NE3TA and NE3TA-Bn can produce neutral Gd(III) complexes that have an advantage of less protein interaction and a potentially more favorable in vivo tissue distribution, and the corresponding Gd(III) complexes may provide enhanced relaxivity due to increase in hydration number ($q$) when compared to that of DOTA (Fig. 1) and DTPA.

Herein, we report the synthesis and characterization of the new Gd(III) complexes, Gd(NE3TA), Gd(NE3TA-Bn), and Gd(CA-NE3TA). Transmission electron microscopy (TEM) images of cholic acid analogues, CA-NE3TA and Gd(CA-NE3TA) were obtained.

Gd(NE3TA) and Gd(NE3TA-Bn) were synthesized by reacting $GdCl_3$ with NE3TA and NE3TA-Bn, respectively. NE3TA or NE3TA-Bn were mixed with $GdCl_3$ in a molar ratio of 1–0.9, and the resulting mixture was adjusted to pH 7 and heated to 90 °C and stirred until no free Gd(III) ions were detected using Arsen-




**Scheme 1.** Synthesis of CA-NE3TA.

azoIII (AAIII) assay. Relaxivity of the aqueous Gd(III) complexes (pH 7) was measured on a Bruker MQ60 NMR analyzer, and the concentration of the Gd(III) complexes was measured by ICP-MS. The relaxivity data (Fig. 2) indicate that the Gd(III) complexes of NE3TA (3.94 $mM^{-1} s^{-1}$) and NE3TA-Bn (3.74 $mM^{-1}s^{-1}$) provided higher relaxivity as compared to Gd(DOTA) (2.97 $mM^{-1}s^{-1}$), probably due to increase in $q$. NE3TA was successfully conjugated with the most common bile acid, cholic acid (Scheme 1). A number of reports have shown that conjugation of cholic acid to various drugs via the amide bond little affects interaction of the conjugates with the human bile acid transporters.[9,12] Amphifacial cholic acid is a good targeting moiety, and its conjugation to MR contrast agent is expected to form helical globular aggregates[15] with a favorable pharmacokinetic and relaxivity profile.[1] Cholic acid was preactivated with 2-mercaptothiazoline to afford **1**, which was further reacted with a functionalized NE3TA (**2**)[16] containing an amino group to provide **3**. The *t*-butyl group of **3** was removed by treatment with 4 M HCl in 1,4-dioxane to provide CA-NE3TA (**4**). TEM image of CA-NE3TA (10 μM aqueous solution) indicates that the bile acid conjugated NE3TA forms discrete spherical micells in nanometer size (~10–50 nm, Fig. 3) due to the presence of hydrophobic cholic acid moiety surrounded by hydrophilic NE3TA ligand. The Gd(III) complex of CA-NE3TA was prepared as described above. Gd (CA-NE3TA) self-assembles into nano-sized micells as evidenced by TEM measurement (~10 nm, Fig. 4). Interestingly, when the complex Gd(CA-NE3TA) was taken in low concentration (10 μM aqueous solution), it was shown to rapidly agglomerate into stacked spherical micells (Fig. 5). Aggregation of Gd(CA-NE3TA) as evidenced by TEM images is proposed to result from heating during the complexation reaction and/or gadolinium induced cross linking.[17] $T_1$ relaxivity of Gd(CA-NE3TA) in aqueous solution (0.8 mM) was measured to 10.85 $mM^{-1} s^{-1}$. The relaxivity data indicate that incorporation of cholic acid with NE3TA produced more than threefold increase in $T_1$ relaxivity. This increase in relaxivity may be a result of slower molecular rotation due to aggregation of the complex as evidenced by TEM image.

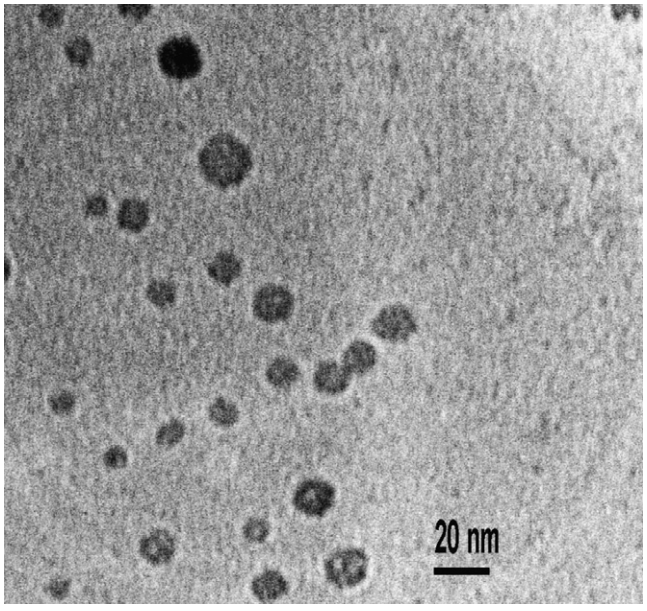



**Figure 3.** TEM image unstained of CA-NE3TA (10 μM aqueous solution).

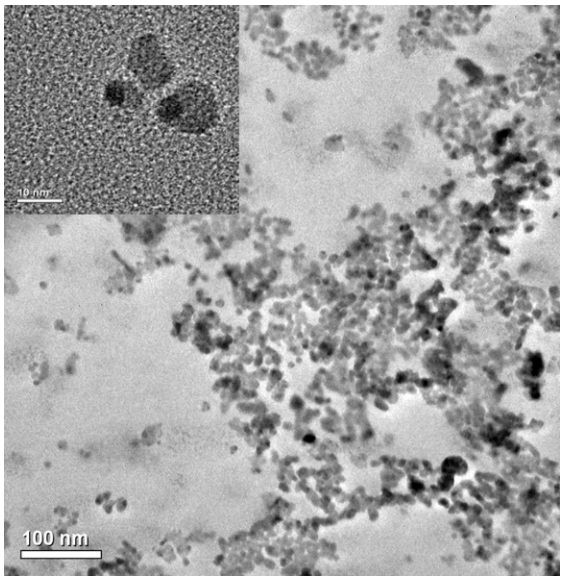



**Figure 4.** TEM image unstained of Gd(CA-NE3TA) (100 μM aqueous solution); (inset, scale bar in 10 nm).

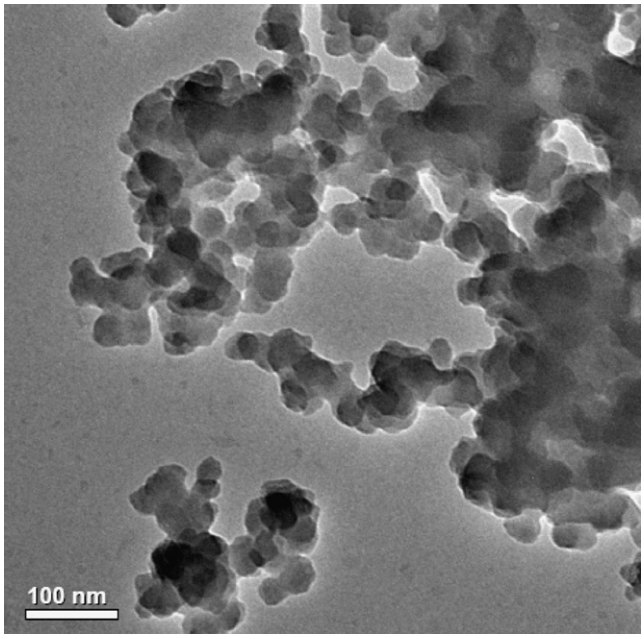



**Figure 5.** TEM image unstained of Gd(CA-NE3TA) (10 μM aqueous solution).

In summary, we have prepared a series of novel Gd(III) complexes, Gd(NE3TA), Gd(NE3TA-Bn), and Gd(CA-NE3TA). Relaxivity of Gd(NE3TA) and Gd(NE3TA-Bn) is high when compared to that of the commercially available MR contrast agent Gd(DOTA). Both the cholic acid-based NE3TA, CA-NE3TA and the corresponding Gd(III) complex, Gd(CA-NE3TA) self assemble forming nano-sized micells as shown by TEM images.

Gd(CA-NE3TA) displayed much increased relaxivity as compared to Gd(DOTA). The new cholic acid-conjugated Gd(III) complex, Gd(CA-NE3TA) possesses promise for use in targeted MRI. Studies of serum stability and cellular uptake and in vivo biodistribution, MRI of Gd(CA-NE3TA) in mice are underway.

## Acknowledgment

This research was supported by the National Institutes of Health (K22CA102637 to C.H.S. and 1R01-EB00-5866 to T.M.).

## Supplementary data

Full Experimental details. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.01.044.

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