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# Fast and easy access to efficient bifunctional chelators for MRI applications

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ABSTRACT

Novel bifunctional agents based on the AAZTA (6-amino-6-methylperhydro-1,4-diazepinetetraacetic acid) structure with hydroxyl, carboxyl and chloro functional groups were prepared. The $Gd^{III}$ complexes show optimal magnetic properties making them very good candidates for conjugation to biomolecules for molecular imaging applications. An example of conjugation to a bile acid derivative is also reported.



The superb anatomical resolution of Magnetic Resonance Imaging (MRI) and its outstanding capacity of differentiating soft tissues made this procedure the technique of choice in modern diagnostic investigations. Currently, about one-third of MRI clinical scans are carried out in the presence of Gd-contrast agents (CA) that cause dramatic acceleration of the water proton relaxation rates, thus providing physiological information beyond the impressive anatomical resolution commonly obtained in the uncontrasted images. The efficacy of a CA is measured by its *relaxivity* ($r_1$), a parameter defined as the increase in the $^1H$ relaxation rate of water protons in the presence of a 1 mM concentration of the paramagnetic complex. Among the main factors influencing the relaxivity are the number, $q$, of the coordinated water molecule(s) and their mean residence lifetime ($\tau_M$).[1]

The recently reported Gd-complex based on the polyaminocarboxylate ligand AAZTA is endowed with optimal physico-chemical properties for its use as MRI CA. It is characterized by high thermodynamic stability and kinetic inertness towards transmetallation reactions with endogenous cations[2] and shows favourable magnetic properties associated with the presence of two inner-sphere water molecules ($q = 2$) characterized by a relatively fast water exchange rate ($\tau_M$=90 ns) and a remarkable relaxivity value of 7.1 $mM^{-1}\ s^{-1}$ at 20 MHz and 298 K.[3]

However, in order to be of practical use in biomedical applications, a $Gd^{III}$ chelate characterized by an optimized relaxation efficiency needs also to contain an appropriate functional group for conjugation to specific biological vectors. At the same time, it is crucial that such derivative maintain the favourable properties of the parent complex in terms of hydration number and rate of water exchange. Since many of the bifunctional chelating agents used for MRI applications are based on $q = 1$ Gd-complexes[4] and only a few examples of $q = 2$ systems conjugated to biological carriers have been reported,[5] the synthesis of AAZTA derivatives containing various functional groups useful for bio-conjugation is highly relevant for the future development of efficient molecular imaging probes.

Two derivatives containing a differently spaced hydroxyl group for further functionalization have been recently published: one of them is conjugated to a lipophilic organic chain,[6] while the other is characterized as crystal structure of the corresponding $Gd^{III}$ complex.[7] The preparation of the former derivative requires seven steps, while no synthetic details were reported for the latter. AAZTA derivatives with one or two glutarate moieties replacing the cyclic acetic pendant arms have very recently been published.[8] Unfortunately, in this latter case the derivatization resulted in a significant decrease of the residence lifetime of the coordinated water molecules for the corresponding Gd-complexes.

We describe herein a general synthetic procedure towards a series of AAZTA bifunctional prochelators (Scheme 1) for coupling both to $NH_2$-containing biomolecules and to other reactive functional groups relevant for biomedical applications. The AAZTA-derived chelator should provide four intact carboxylic acid functions in addition to the cyclic diazepane ring for a stable and efficient binding of metal ions and a function for coupling to biomolecules. A preliminary $^1H$ and $^{17}O$ NMR relaxometric study of the $Gd^{III}$ complex with **L1** and **L2** was carried out in order to compare their magnetic properties with those of the parent $[GdAAZTA]^-$ complex.

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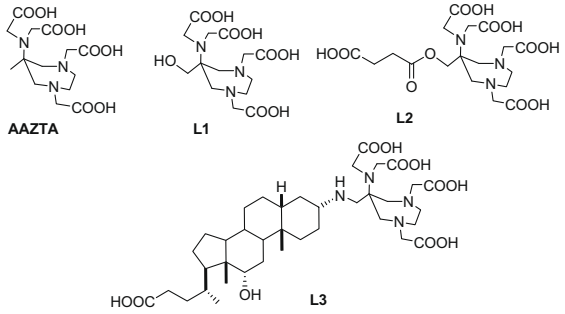
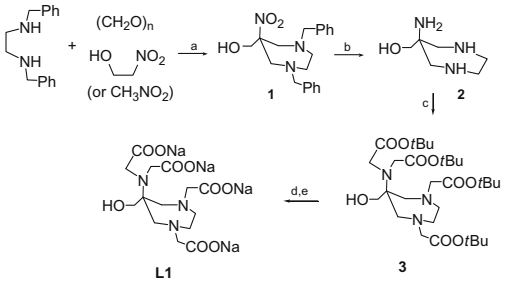


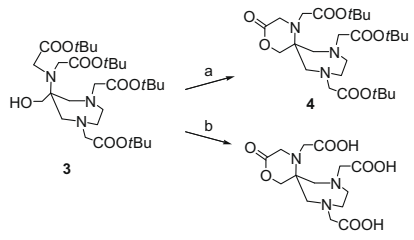

**Scheme 1.** Ligands discussed in this Letter.




**Scheme 2.** Synthesis of **L1**. Reagents and conditions: (a) Toluene/EtOH (1/1 *v*/*v*), reflux, 6 h, purification on silica gel (99.5% from nitroethanol, 62% from nitromethane); (b) Pd/C, $HCOONH_4$, HCOOH, MeOH, 50 °C, 1 h; ion exchange resin (97.6%); (c) Bromo-*tert*-butylacetate, $K_2CO_3$, MeCN, rt, 18 h, purification on silica gel (53%); (d) TFA/$CH_2Cl_2$, rt, 16 h; (e) NaOH 1 M, reflux,1 h.

**L1** was synthesised as shown in Scheme 2 modifying the procedure reported by Aime et al.[3] The nitrodiazepine **1** was obtained by double nitro-Mannich reaction between *N*,*N*'-dibenzyl ethylenediamine, paraformaldehyde and 2-nitroethanol in nearly quantitative yield. Alternatively **1** may be prepared by using nitromethane and excess paraformaldehyde to form nitroethanol in situ through the Henry reaction; in these conditions, the yield is reduced to about 60%.

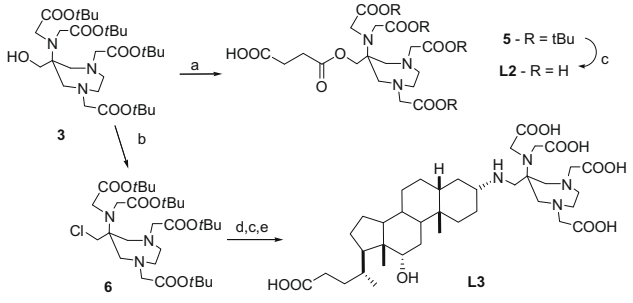
Reduction of the nitro and the *N*-benzyl groups is achieved by catalytic hydrogenation on Pd/C catalyst using ammonium formate and formic acid as source of hydrogen at T ⩽ 50 °C. The acidic environment and the temperature control were necessary to prevent the formation of the alkoxide of **1** and subsequent retro-Henry reaction.[9] After removal of the formate anions by passing the ammonium salt **2** through an anion exchange resin column, the strongly basic amine was reacted with *t*-butyl bromoacetate in the presence of anhydrous $K_2CO_3$ to generate the protected chelate **3**. This alkylation was carried out in $CH_3CN$ and at room temperature in order to avoid the formation of a variable amount of a 2-oxomorpholine by-product which was formed by intramolecular reaction between the hydroxyl group and one of the two equivalent *t*-butyl esters on the exo-cyclic amine (Scheme 3). This lactonization is favoured by the basic environment and by the correct orientation of the hydroxyl and carboxy groups. The protected chelate **3** has to be stored refrigerated as it is unstable towards lactonization at room temperature. The hydrolysis of the *t*-butyl esters by reaction with a 50% solution of trifluoroacetic acid in $CH_2Cl_2$ resulted in the formation of a mixture of **L1** and of an 2-oxomorpholinic product by acid catalysed intramolecular Fisher transesterification. Using less harsh conditions, for example phosphoric acid[10] or sulphuric acid,[11] the same mixture of products was obtained although with different percentages as revealed after HPLC-MS analysis. After treatment of the mixture with a 1 M solution of NaOH the lactone was converted quantitatively to **L1** and stored at basic pH and at T ⩽ 5 °C. Remarkably, there are only a few reports on intramolecular lactonization with *t*-butyl esters and to the best of our knowledge only in our case the cyclization occurs spontaneously.[12]




**Scheme 3.** Intramolecular cyclization to the 2-oxomorpholine derivative: (a) spontaneous at rt or using a base such as $K_2CO_3$ or DIPEA; (b) TFA/$CH_2Cl_2$, rt, 16 h.

In order to form a derivative that could be easily conjugated to biomolecules the Williamson etherification reaction was tested on the protected ligand **3** following various reaction conditions reported in the literature.[13] However, the basic conditions that had to be used resulted in the complete conversion of **3** into the 2-oxomorpholinic derivative **4**. Hence we decided to exploit the formation of an ester bond with the hydroxyl group of **3** synthesising the derivative (**5**) obtained by the opening of succinic anhydride (Scheme 4). **5** has the great advantage that, having one free carboxyl group and the other protected as *t*-butyl esters can be used for conjugation in organic solvents and in the classical solid phase peptide synthesis approach, too. To validate this potential application, the stability of the ester bond of **5** was tested in the acidic conditions used for deprotection of the *t*-butyl esters (i.e., TFA/$CH_2Cl_2$ 1:1) thus obtaining the ligand **L2**. In addition, the protected ligand **3** was reacted with thionyl chloride to convert the hydroxymethyl group into a chloromethyl derivative (**6**). In order to demonstrate its versatility, we reacted **6** with 3-amino-3,7-dideoxycholic methyl ester[14] (Scheme 4). Deprotection of both *t*-butyl and methyl esters yielded the final ligand **L3**. On complexation with $Gd^{III}$ ligand **L3** gives rise to a complex which will be studied as MRI contrast agent in comparison with other bile acid conjugates. In fact, several liver-specific Gd-based CAs in which a bile acid conjugated to a Gd-DOTA or DTPA derivative have been reported demonstrating their ability to target the hepatocytes and to bind Human Serum Albumin or lipoproteins.[15]

In order to check the effect of the chemical derivatization on the relaxometric properties, the $Gd^{III}$ complexes of **L1** and **L2** were prepared by mixing stoichiometric amounts of the ligands and $GdCl_3$ solution. Unchelated $Gd^{3+}$ ions were eliminated by precipitation of the hydroxide at basic pH by adding aliquots of a concentrated NaOH solution. Interestingly, [Gd**L1**]$^-$ and [Gd**L2**]$^-$ are bifunctional




**Scheme 4.** Synthesis of functional derivatives **5**, **6** and of the deprotected ligands **L2** and **L3**. Reagents and conditions: (a) succinic anhydride, $CH_2Cl_2$, rt, 6 h (67%); (b) thionyl chloride, $CH_2Cl_2$, rt, 2 h; (c) TFA/$CH_2Cl_2$ 1:1, rt, 16 h; (d) aminocholic methyl ester, $NEt_3$, $CH_3CN$, rt, 24 h; (e) NaOH 1 M, reflux 1 h; HPLC purification (26.2% from **3**).

Gd-complexes which could exploit the free hydroxyl or carboxyl functions for conjugation. In fact, they can be anchored in aqueous media onto sensitive systems such as proteins which do not tolerate the acidic conditions necessary for the hydrolysis of the *t*-butyl esters.

The $^{1}$H NMR relaxometric behaviour of [Gd(**L1**)]$^{-}$ and [Gd(**L2**)]$^{-}$, studied at 20 MHz and 298 K, does not differ markedly from the data obtained for the parent [Gd(AAZTA)]$^{-}$ complex as the relaxivities are 7.6 and 9.1 $mM^{-1}s^{-1}$, respectively, and constant over a large pH range (2–11). Also the exchange lifetimes of the two water molecules, determined for both complexes by Nuclear Magnetic Resonance Dispersion (NMRD) and $^{1}$H VT NMR profiles and confirmed by preliminary $^{17}$O NMR data, were about 90 ± 20 ns, in line with the value found for [Gd(AAZTA)]$^{-}$.

In conclusion, the procedure for the synthesis of functionalized AAZTA-ligands described in this Letter represents a significant advance with regard to the following:

(1) The number of synthetic steps is limited and analogous to those of the parent AAZTA ligand.
(2) A large variety of functional groups can be introduced starting from the protected compound **3**.
(3) The conjugation to the vector molecules can be achieved either in organic solvent (using **3**, **5** and **6**) or in aqueous media (using [Gd(**L1**)]$^{-}$ or [Gd(**L2**)]$^{-}$).
(4) The corresponding $Gd^{III}$ complexes maintain the favorable relaxation properties of [Gd(AAZTA)]$^{-}$.

Then, these new compounds may have widespread utility for the synthesis of Magnetic Resonance-Molecular Imaging probes, not excluding the possibility to use **L1** or **L2** with other diagnostic ($^{111}$In, $^{67/68}$Ga) or therapeutic ($^{90}$Y, $^{177}$Lu) radiometals for nuclear medicine applications.

## Acknowledgments

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## Supplementary data

Supplementary data (detailed synthetic procedures and spectroscopic data for final products and intermediates) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.05.024.

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