ORGANIC LETTERS

2004
Vol. 6, No. 8
1201−1204

Mannich Reaction as a New Route to Pyridine-Based Polyaminocarboxylic Ligands

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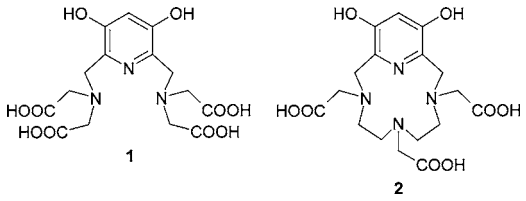
Received December 26, 2003

ABSTRACT



The Mannich reaction was successfully employed to obtain two pyridine-based ligands 1 and 2. The latter represents the first example of a Mannich reaction leading to a 12-membered pyridine-containing macrocycle.

Since its inception in the early 1970s, magnetic resonance imaging (MRI) has come to play a pivotal role in medical diagnosis.[1] In this context, much attention has been devoted to the development of paramagnetic metal complexes able to act as contrast agents. In a magnetic resonance image, the contrast relies essentially on differences of proton relaxation times. Contrast agents are able to markedly enhance the relaxation rates of tissular water. Among paramagnetic metal ions, Gd(III) is the candidate of choice, having seven unpaired electrons and a relatively long electronic relaxation time. Because uncomplexed Gd(III) aqua ion (or its hydroxo species) are highly toxic (LD_{50} 0.1−0.2 mmol/kg), the lanthanide ion has to be administered in the form of a stable chelate. To this end,[2] numerous acyclic and macrocyclic ligands have been considered with particular attention to the class of poly(aminocarboxylate) ligands.

Notably, some Gd chelates (e.g., MAGNEVIST, DOTAREM, PRO-HANCE),[2a] based on diethylene-triaminepentaacetic acid (DTPA), 12-membered tetrazamacrocyclic ligand DOTA, and its derivatives HP-DO3A, respectively (Figure 1), currently enjoy widespread clinical use.

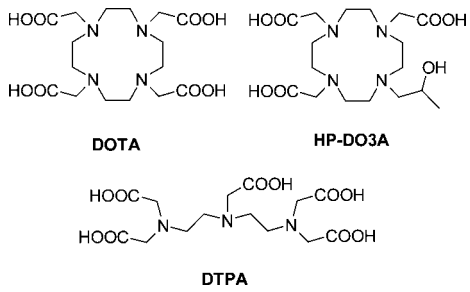


Figure 1.

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10.1021/ol036510q CCC: $27.50
Published on Web 03/13/2004

The key requirements for a potential Gd-containing contrast agents are (i) high thermodynamic (and possibly kinetic) stability, (ii) good water solubility, (iii) low osmolality, and (iv) high proton relaxivity. The latter property directly refers to the efficiency of a paramagnetic substance to enhance the relaxation rate of water protons and is largely dependent upon the number of the inner-sphere water molecule(s).

Therefore, the search for Gd-based contrast agents is aimed at designing novel multidentate ligands able to yield stable Gd(III) complexes with an enhanced hydration of the metal ion and, possibly, safer toxicity profile.

Pyridine-based macrocyclic ligands[3] were shown to bind efficiently lanthanide(III) ions, the affinity being strongly affected by the ring size. Twelve-membered rings with acetate or phosphonate sidearms yield very stable Gd(III) complexes.[4] Moreover, these ligands as well as their acyclic congeners were used to prepare Tb(III) and Eu(III) chelates with high luminescence yields.[5] The corresponding conjugates with biomolecules such as proteins or antibodies are of interest as very sensitive bioaffinity probes.[6]

The synthesis of pyridine-based ligands has been carried out by direct alkylation of bis(halomethyl)pyridine or bis(imine) reduction-alkylation protocols. These methods, although often effective, suffer from a number of drawbacks. The direct alkylation is based on the availability of bis(halomethyl)pyridines, which except for commercial 2,6-bis(chloromethyl)pyridine must be prepared with tedious procedures and are prone to polymerization that could adversely affect reactions involving these intermediates.

Although quick and easy, the bis(imine) reduction-alkylation protocol worked poorly when used to make polyazaarenophanes with less than 14-membered rings. A further limitation is the difficulty attending the preparation of suitably functionalized dialdehydes.[7]

Therefore, improvements in this field are highly desirable, especially if they lead to a streamlined approach under milder conditions.

The aminomethylation reaction, in the form of the Mannich reaction and its several modifications, is one of the most useful protocols for the introduction of nitrogenated functionalities.[8] The synthesis of polyaminopolycarboxylic acids via aminomethylation of electron-rich aromatics is well documented.[9] In fact, the reaction of iminodiacetic acid/paraformaldehyde with substituted phenols is the route of choice to various ligands for mono- and polynuclear complexes.[10]

However, to the best of our knowledge, the use of the Mannich reaction to operate either a cyclization involving a pyridine ring or a macrocyclization to a 12-membered ring has never been applied. In this paper we report on the syntheses of pyridine-based polyaminopolycarboxylic ligands **1** and **2**, via aminomethylation. Pyridines are usually reluctant to undergo aromatic electrophilic substitution, especially by weak electrophiles such as the iminium ions involved in the Mannich reaction. Nevertheless, the aminomethylation of the pyridine ring was successfully reported on 3-hydroxypyridine **3** (with *N*-methylpiperazine/formaldehyde),[11] where the electron-releasing OH group overrides the electron deficiency of the azine ring and activates positions 2 and 6, paving the way to the synthesis of double-armed pyridine-containing ligands.

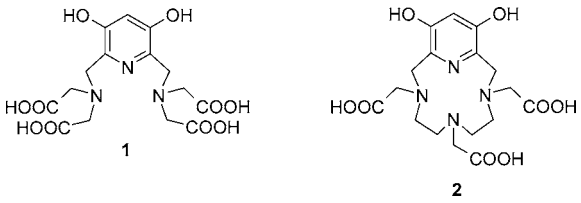

Figure 2. Ligands synthesized.

By analogy with the work of Stempel and Buzzi,[4] we obtained the 2-substituted product **4** as the only product (35%) when reacting **3** with diethyl iminodiacetate/paraformaldehyde in refluxing ethanol. Although promising, these experiments were thwarted by our inability to achieve even modest conversion to 2,6-disubstituted products, despite using a variety of stoichiometries, higher temperatures, and longer reaction times (Scheme 1). Even if the issue of reluctance of **3** toward a 2-fold Mannich did not change, we reasoned that the entropic advantage inherent to the use of 1,4,7-triazaheptane-1,4,7-triacetic acid tri-*tert*-butyl ester **5**[12] and paraformaldehyde could be favorable. However, these tactics proved unsuccessful and **6** was isolated (29−36% yield) as the only products from numerous attempts.

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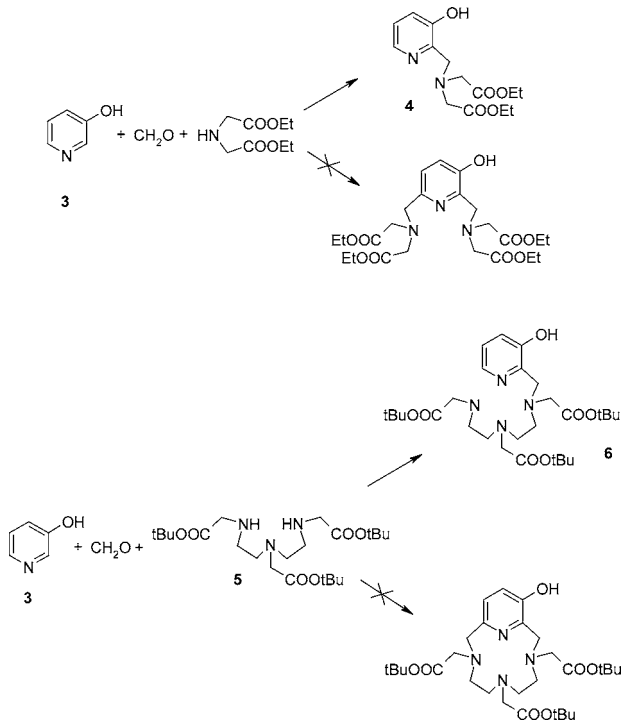
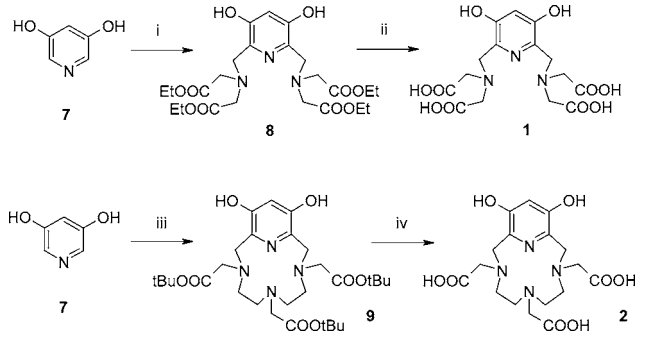
Scheme 1



Assessing the difficulty for double aminomethylation reaction as residing in the insufficient nucleophilicity of **3**, we switched to 3,5-dihydroxypyridine **7**.[13] This substrate behaved very well under typical conditions for Mannich reactions. Pleasingly, on treatment of **7** with diethyl iminodiacetate/paraformaldehyde (refluxing EtOH, 18 h) or **5**/paraformaldehyde in the presence of an organic acid (refluxing MeOH, 6 h), clean bis-Mannich reactions ensued at the 2- and 6-positions to produce the required adducts **8** (58%) and **9** (62%), respectively (Scheme 2). Interestingly,

Scheme 2[a]



[a] (i) $(CH_2O)_n$, $NH(CH_2COOEt)_2$, EtOH, reflix; (ii) 6 M HCl, reflux; (iii) $(CH_2O)_n$, **5**, tartaric acid, MeOH, reflux; (iv) TFA, $PhOCH_3$.[14]

omission of tartaric acid (cat.) as an organic acid resulted in the isolation of a mere 38% of the 12-membered macrocycle **9**. Finally, full deprotection of ethyl esters in **8** to reach the target **1** (90%) was cleanly achieved with 6 N HCl (reflux, 8 h), whereas unmasking of carboxylic groups in **9** took place in neat TFA−anisole (room temperature, 24 h), yielding **2** in 94% yield.

These two new ligands embody interesting features linked to the presence of the two hydroxy groups. They increase the electron density of the azine ring, providing a stronger donation ability to the nitrogen atom. Even more important, they can act as handles for further functionalization (e.g., derivatization and conjugation to biomolecules) either as reacting sites or as activating groups toward aromatic electrophilic substitution at the 4-position.

The Gd(III) complexes $[Gd(\mathbf{1})]^-$ and $[Gd(\mathbf{2})]$ were prepared by mixing stoichiometric amounts of $GdCl_3 \cdot 6H_2O$ with an aqueous solution of the ligands **1** and **2**, respectively, while maintaining the pH at 6 by the addition of 1 N NaOH.

The proton relaxivities (r_{1p}) (at 0.47 T, 25 °C, pH 7) for [Gd-**1**]$^-$ and [Gd-**2**] were 9.1 and 8.5 $mM^{-1}\ s^{-1}$, respectively, and are significantly higher than those of contrast agents currently used in clinical practice (i.e., 4.3 $mM^{-1}\ s^{-1}$ for $[Gd(\mathbf{DTPA})(H_2O)]^{2-}$ and 4.2 $mM^{-1}\ s^{-1}$ for $[Gd(\mathbf{DOTA})(H_2O)]^-$).[15] When previously reported pyridine-containing Gd(III) complexes were taken into account,[4] their proton relaxivities were indeed somewhat lower, a rather surprising result if one considers the close structural similarity (i.e., denticity and nature of the donor atom set). Likely, an additional contribution to the observed relaxivity may arise from an increase due to the polar hydroxy groups. Although the reasons for this remain unclear at this time, it does appear to be related to the increase of the second coordination sphere due to the presence of two (extra) pendant OH groups.

[Gd-**1**]$^-$ and [Gd-**2**] showed a remarkable kinetic stability in aqueous solution over a wide pH range. As shown in Figure 3, the water proton relaxivity for [Gd-**1**]$^-$ was

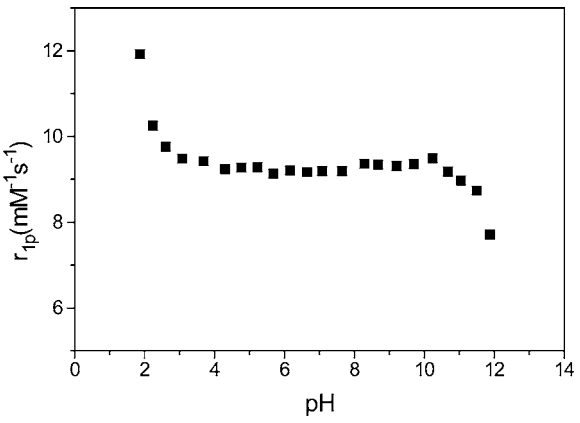


Figure 3. Proton longitudinal relaxivity (r_{1p}) vs pH for [Gd-**1**]$^-$ (20 MHz, 298 K).

relatively constant in the pH range between 3 and 10. This implies the absence of any dissociation process in this interval, extending well outside the domain of physiological values. The decrease in relaxivity observed at pH > 10 was

correlated to the replacement of water by hydroxyl ion in the inner coordination sphere. Competitive titration of [Gd-**1**]$^{-}$ and [Gd-**2**] with DTPA showed no evidence of ligand exchange even after 10 days (pH 7, room temperature), implying either a high degree of kinetic inertness or a higher thermodynamic stability.

A further noticeable property of [Gd-**1**]$^{-}$ is represented by its failure to react with chelating bidentate anions such as carbonate and lactate. These endogenously available anions, i.e., present in variable concentrations in blood plasma, can compete with water molecules for binding and decrease the effective relaxivity of the complex, thus facilitating its dissociation. This inertness is a promising indication for the in vivo use of [Gd-**1**]$^{-}$ as a contrast agent for MRI.

In summary, entry was gained to new pyridine-based ligands (e.g., **1** and **2**) by double Mannich reaction. This smooth approach circumvents the need for protection-deprotection steps and works well in cyclization reactions, such as the 12-membered ring closure to **2**. The ease of this strategy allows rapid access to a novel class of molecules, whose utility has been exemplified by their use as heptadentate ligands for lanthanide(III) ions. Finally, the Gd(III)-complexes of these new ligands exhibit interesting relaxometric properties that make them potentially useful contrast agents for MRI.

Acknowledgment. Financial support from MIUR and Bracco Imaging SpA. (Milan, Italy) is gratefully acknowledged. Two of the authors (C.C. and M.S.) are thankful to the Dipartimento di Chimica Organica e Industriale, Università degli Studi, Milan, Italy, for kind hospitality in their laboratories.

Supporting Information Available: Experimental procedures and full characterization for compounds **1**, **2**, **8**, and **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL036510Q

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