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COMMUNICATION

One-pot synthesis of a piperidine-based rigidified DTPA analogue and its bifunctional chelating agent†

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The core structure of *cis*-3,5-diaminopiperidine was *N*-alkylated with excess *t*-butylbromoacetate in order to exploit the successive *N*-quaternarization and Stevens rearrangement to access the pentaalkylated product and the bifunctional chelating agent containing a *N*-butanedioic acid pendant arm at the same time. The relaxometric properties of the Gd^{III} complexes with these ligands were studied also in terms of pH and serum stabilities.

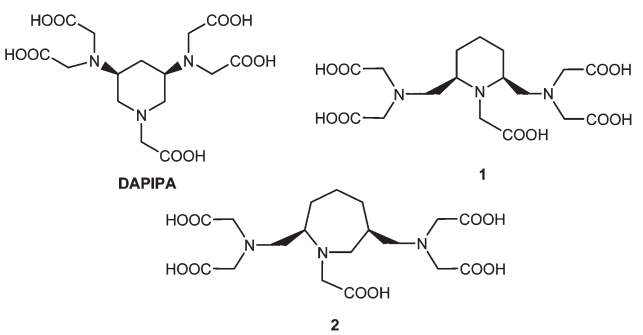
The development of Gd^{III}-based Magnetic Resonance Imaging (MRI) contrast agents (CAs) has flourished in the last twenty years focusing mainly on the improvement of the efficiency of the clinically approved ones, even though such substances are being widely and successfully used in clinical applications.[1,2] The two main properties that an optimal CA should present are high relaxivity, *i.e.* the increase of the water proton relaxation rate observed in a 1 mM solution of the paramagnetic system, and high thermodynamic and kinetic stabilities in order to avoid any toxic Gd^{III} release *in vivo*.[1,2] More recently, an important challenge in the development of novel chelates, which has gained increasing attention, is the improvement of CAs capability to target certain organs and tissues, in order to meet the needs of the growing area of Molecular Imaging and the achievement of precise delivery to specific cellular targets.[3] The most general strategy consists in the synthesis of a chelating unit bearing a remote functional group suitable for the covalent linkage to a specific biological carrier, leading to the so-called bifunctional chelating agents (BFCAs).[4]

It is well reported in the literature that the relatively rigid cavity of a macrocyclic ligand allows enhanced complex stability when there is a match between the size of the cavity and that of the metal ion being complexed.[5] In general, conformational constraint of chelating agents such as the inclusion of a rigid structure to control the geometry of the metal binding donor groups may have a remarkable effect especially on kinetic stability of the formed metal complexes.[6] In a rigid structure, the dissociation of the metal complex is disfavoured by the tight packing of the coordination cage that prevents the protonation of the nitrogen atoms. It has also been reported that the complexation/decomplexation kinetics of a macrocyclic complex in which a rigid cyclohexyl ring is directly grafted onto the tetraaza cycle become even slower.[7] Moreover, an increased rigidity in multidentate acyclic ligands such as EDTA, DTPA or EGTA (ethylenediamine *N*,*N*,*N*′,*N*′-tetraacetic acid, diethylenetriamine *N*,*N*,*N*′,*N*″,*N*″-pentaacetic acid and ethyleneglycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid, respectively) prevents their metal complexes from undergoing a process of simultaneous unwrapping and transfer of their donor groups from one metal ion to another.[8–10]

Brechbiel and coworkers reported two different stereoisomeric forms of a phenylisothiocyanate DTPA-based BFCA with a backbone rigidified by a cyclohexyl ring which showed considerable improvement of *in vivo* stability.[9] More interestingly, the same group synthesized and studied two DTPA analogues based on piperidine and azepane rings (**1** and **2**, respectively, Scheme 1). Serum stability studies showed that the Gd^{III} complexes with these rigidified compounds are more stable than GdDTPA under the same conditions.[10]

Results and discussion

This being the state of the art, we approached the synthesis of the DTPA-like ligand DAPIPA (*cis*-3,5-diaminopiperidine *N*,*N*′,*N*′,*N*″,*N*″-pentaacetic acid, Scheme 1) in which the rigid structure is a piperidine ring with two iminodiacetic groups in 3,5



Scheme 1 DAPIPA ligand and other DTPA analogues based on piperidine and azepane rings.

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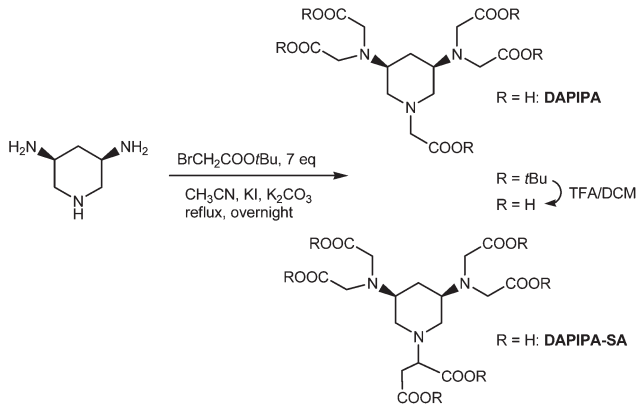

Scheme 2 Synthetic scheme of the one-pot *N*-alkylation and Stevens rearrangement on *cis*-3,5-diaminopiperidine.

and *cis* positions. The polyamine *cis*-3,5-diaminopiperidine is well known and its coordination chemistry towards transition metal ions such as Co^{II}, Ni^{II}, Cu^{II}, Zn^{II} or Cd^{II} has been studied in detail.[11] It was recognized that the rigid structure, the restriction to exclusively facial coordination and the structure of the ligand backbone provided a specific degree of preorientation of the donor groups in the process of complex formation. Different synthetic procedures for the preparation of *cis*-3,5-diaminopiperidine have been reported in the literature starting from either 2-chloro-3,5-dinitropyridine or 3,5-dibromopyridine.[11,12] We followed the procedure reported by Hegetschweiler and coworkers which converted 3,5-dibromopyridine to the 3,5-diamino and then hydrogenated the pyridine in aqueous HBr with 5% Rh/C catalyst and H_2 at 5 bar.[11]

Alkylation with *t*-butyl bromoacetate could yield the desired protected ligand DAPIPA (Scheme 1). In order to access further functionalisation, aiming at a BFCA built upon the DAPIPA core structure, we decided to apply the chemistry reported on a recent communication detailing a strategy to access structural modification of acyclic polyamino polycarboxylic ligands such as EGTA and DTPA by the one-pot *N*-alkylation–Stevens rearrangement approach.[13] The authors reported the use of allyl bromide and benzyl bromide for the *N*-alkylation of the *t*-butyl protected ligands. The quaternary ammonium salt formed was followed by a base (K_2CO_3) induced migration of the alkyl group on the *t*-Bu ester activated α-position. Although the mechanism of the Stevens reaction is not yet entirely clarified, a radical pathway seems to be responsible for the alkyl migration step.[14] Interestingly, in case of DTPA, only the α-position of the central arm was alkylated;[13] thus, this reaction was found to be highly regioselective mainly due to the greater basicity and nucleophilicity of the central nitrogen atom. We then thought about the possibility to react the *cis*-3,5-diaminopiperidine with excess *t*-butyl bromoacetate in order to form in the same reaction the pentaalkylated compound and the product derived by the successive *N*-alkylation of the endocyclic nitrogen atom followed by Stevens rearrangement. These successive reactions were successfully attained by refluxing *cis*-3,5-diaminopiperidine with excess *t*-butyl bromoacetate in acetonitrile in the presence of KI and K_2CO_3 (Scheme 2). Only the two expected products were obtained and, after chromatographic purification, both could be isolated in similar amounts. Full NMR (^1H, ^{13}C, COSY, HSQC), ESI MS and IR characterisation (see ESI† for details) confirmed the formation of the pentaalkylated derivative DAPIPA(*t*Bu)$_5$ and the ligand DAPIPA-SA(*t*Bu)$_6$ (*cis*-3,5-diaminopiperidine *N*-butanedioic-*N*′,*N*′,*N*″,*N*″-tetraacetic acid hexa *t*-butyl ester), in which the acetate group on the endocyclic nitrogen atom was α-substituted by a *t*-butylacetate moiety. The protected DAPIPA and DAPIPA-SA were obtained in approximately 42% and 35% yield, respectively. Both compounds were then deprotected by trifluoroacetic acid hydrolysis of *t*-butyl esters to obtain the two final products in quantitative yield.

It is worth noting that, although the superior homologue glutaric acid pendant arm has been used in a wide range of bifunctional chelating ligands,[4,15–18] a butanedioic acid pendant arm has only been reported for the DOTA-like BFCA called DOTASA,[15] which could be obtained only in very low yields (2% overall yield) as the reagent α-*t*-butyl γ-benzyl 2-bromobutanedioate, employed in the monoalkylation of cyclen, undergoes unproductive elimination side-reaction to give the fumaric acid diester as the main product. The strategy herein reported delineates a potentially general method to access functionalisation with butanedioic residues by *N*-alkylation–Stevens multiple reaction. This approach gives access to both the chelating ligand and its bifunctional chelating agent containing a butanedioic acid pendant arm at the same time and in good yields starting from *cis*-3,5-diaminopiperidine. To the best of our knowledge it is the first report of a simultaneous synthesis of a chelating ligand and of its BFCA. Furthermore, it must be noted that the DAPIPA-SA ligand can be used as BFCA after metal ion complexation as the protecting groups on the carboxylic acids are not orthogonal, but the reactivity of the carboxylic acid is well differentiated after metal ion coordination. This procedure has in fact been employed in several conjugation reactions reported in the literature ranging from the linkage of chelating agents to silica nanoparticles[17] to the functionalisation with lipophilic pendant arms leading to amphiphilic metal complexes.[18]

The Gd^{III} complexes of DAPIPA and DAPIPA-SA were prepared by adding small volumes of a stock solution of $Gd(NO_3)_3$ to a solution of the ligand, maintaining the pH at 6.5 with diluted NaOH. The complexation process was monitored by measuring the change in the longitudinal water proton relaxation rate (R_1) at 20 MHz and 25 °C (pH = 6.0) as a function of the concentration of Gd^{III}. The r_1 values of $GdDAPIPA^{2-}$ and $GdDAPIPA\text{-}SA^{3-}$, measured under the same conditions, were found to be 5.2 ± 0.1 mM^{-1} s^{-1} and 6.0 ± 0.1 mM^{-1} s^{-1}. These relaxivity values are slightly higher than those of currently used MRI contrast agents such as $[Gd(DTPA)(H_2O)]^-$ and $[Gd(DOTA)(H_2O)]^-$ ($r_1 \sim 4.7$ mM^{-1} s^{-1} under identical experimental conditions) as a consequence of the increased molecular dimension of the complexes.[1] These r_1 values also suggest the presence of one water molecule coordinated to the paramagnetic centre (*inner-sphere* water molecule, $q = 1$). For $GdDAPIPA^{2-}$, the magnetic field dependence of the relaxivity, the so-called nuclear magnetic relaxation dispersion (NMRD) profile, has been measured at 25 and 37 °C in the proton Larmor frequency range 0.01–70 MHz, corresponding to magnetic field strengths varying between 2.34×10^{-4} T and 1.64 T (Fig. 1). The profiles have the typical shape of low molecular weight complexes, with a plateau at low fields, a dispersion around 3–4 MHz and another plateau in the high fields region (>20 MHz). The

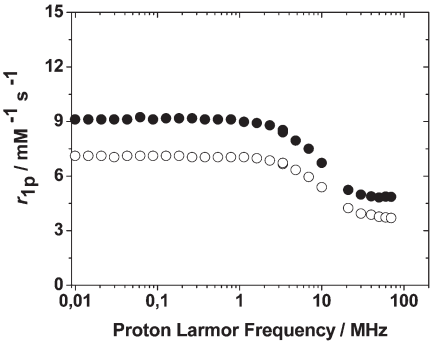

Fig. 1 $1/T_1$ ^{1}H NMRD profiles for GdDAPIPA^{2-} at 25 °C (●) and 37 °C (◯) (pH = 7.5).

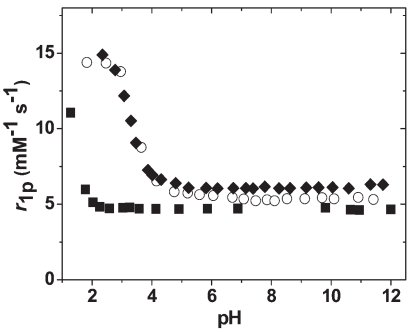

Fig. 2 Plot of ^{1}H relaxivity r_1 *vs.* pH for GdDAPIPA^{2-} (◯) and GdDAPIPA-SA^{3-} (◆) compared with GdDTPA^{2-} (■) (20 MHz; 25 °C).

temperature dependence over the entire range of proton Larmor frequencies indicates that r_1 is not limited by the water exchange rate but rather by the fast rotational motion of the complex.[1,2]

The pH dependencies of r_1 of both Gd^{III} complexes (Fig. 2) show a constant r_1 value from pH 4.5 to basic pH and an increase of r_1 at pH lower than 4.5 indicating a pH mediated decomplexation occurring at acidic pH. The comparison with the pH dependence of GdDTPA reported in Fig. 2 demonstrates that the two novel complexes are less stable at acidic pHs than GdDTPA. Thus, the protonation of the carboxylic acids and subsequent reduction of the number of donor atoms is probably the cause of the dissociation of Gd^{3+} from the chelating ligands. In order to explain this behaviour, it is worth noting that the conformational study carried out on the transition metal complexes with *cis*-3,5-diaminopiperidine shows a significant distortion of the chair conformation of the piperidine ring towards an envelope conformation. This may be due to a rearrangement of the donor atoms that brings them closer to the metal centre.[11] We assume that the exocyclic N atoms are also in the pseudo-axial positions required for the cooperative binding to the Gd^{3+} ion in our case. The high energy of this conformation and the tight steric requirements of the Gd^{3+} ion in GdDAPIPA^{2-} and GdDAPIPA-SA^{3-} complexes probably are the cause of the proton assisted decomplexation occurring at pH lower than 4.5.

The serum stability of both Gd^{III} complexes was also assessed by measuring the time dependence of the r_1 values (20 MHz and 25 °C) of *ca.* 1 mM solutions of GdDAPIPA^{2-} and GdDAPIPA-SA^{3-}. Although serum stability is not necessarily an absolute indicator of *in vivo* stability, the constant value of r_1 over 48 h (see ESI†) is an indication that Gd^{3+} in these conditions does not dissociate from the chelates.

Conclusions

In summary, this communication reports the one pot synthesis of a novel polyaminocarboxylic ligand and of its BFCA bearing a butanedioic moiety through a *t*-butyl ester *N*-alkylation followed by Stevens rearrangement. The high yields and the ease of synthesis open the way to the exploitment of this approach for a series of potential BFCAs without the use of long and low yielding procedures involving protecting groups.

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