

Novel heteroditopic chelate for self-assembled gadolinium(III) complex with high relaxivity†

Robert Ruloff,^a Gerard van Koten^b and André E. Merbach^{*a}

^a Ecole Polytechnique Fédérale de Lausanne, Laboratoire de Chimie Inorganique et Bioinorganique, BCH Lausanne, Switzerland. E-mail: andre.merbach@epfl.ch; Fax: +41 21 693 9875; Tel: +41 21 693 9871

^b Utrecht University, Debye Institute, Department of Metal-Mediated Synthesis, Padualaan 8, Utrecht, The Netherlands. E-mail: g.vankoten@chem.uu.nl; Fax: +31 30 252 3615; Tel: +31 30 253 3120

Received (in Cambridge, UK) 8th January 2004, Accepted 10th February 2004

First published as an Advance Article on the web 26th February 2004

[Fe(tpy-DTTA)₂Gd₂] is a self-assembled trinuclear complex based on a novel ligand in which a terpyridine and a poly(amino carboxylate) moiety are connected;‡ it has a well-defined topology with favourable features to attain high relaxivities, *i.e.* a rigid Fe^{II}(tpy)₂ core, reduced flexibility at the periphery thanks to a short linker, and efficient separation of the two Gd^{III} centres.

The Nobel Assembly recently awarded the prize in medicine for discoveries concerning magnetic resonance imaging (MRI) and described this technique as a breakthrough in medical diagnostic and research.¹ Nowadays, around 30% of all MRI examinations use contrast agents, mostly Gd^{III} complexes, which enhance the intrinsic contrast, thus the anatomical resolution of the magnetic resonance images. Contrast agents of much higher efficacy (relaxivity)§ than those available on the market are required for novel applications, such as molecular imaging, where the amount of the agent delivered to a given target is strongly limited by biological constraints. The Solomon–Bloembergen–Morgan theory that relates the observed paramagnetic relaxation rate enhancement to microscopic properties predicts that a 20-fold relaxivity increase is possible for Gd^{III} complexes as compared to commercial agents. Such improvement requires the simultaneous optimization of the three most important factors: water exchange, rotation and electron spin relaxation.² The tuning of the water exchange rate, k_{ex} , from 10⁶ s⁻¹ for commercial agents to the optimal value (10⁷ to 10⁸ s⁻¹) has been recently reported.³ Optimization of the rotation involves using slowly tumbling, usually macromolecular agents. The Gd^{III} chelate is fixed by covalent or non-covalent binding to the macromolecule *via* a short and rigid linking unit to reduce local flexibility. Here we present a model compound that possesses favourable water exchange and rigidity in view of developing high relaxivity MRI contrast agents. The novel heteroditopic ligand synthesized exhibits distinct and specific binding sites for Fe^{II} and Gd^{III} and is capable of self-assembling in aqueous solution in the presence of these metals to give a highly rigid structure.

The ligand design is based on the combination of two different complexing moieties, each with structural characteristics for a preferential coordination mode (Scheme 1). The terpyridine unit is known to form stable and inert complexes of well-defined topology

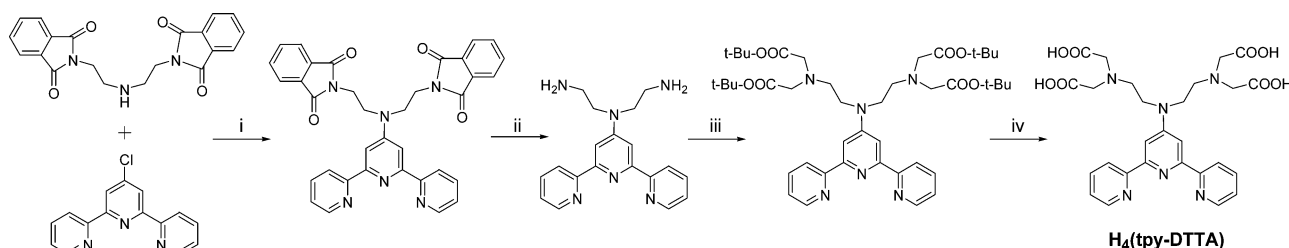
with divalent transition metal ions. On the other hand, the poly(amino carboxylate) DTTA⁴⁻ with a N₃O₄ donor atom set is well-suited for coordinating trivalent metal ions such as lanthanides. The two units are directly connected *via* a covalent C–N bond between the central nitrogen of the poly(amino carboxylate) and the 4' carbon of tpy to have the shortest and most rigid linker.

The first step of the ligand synthesis is the nucleophilic substitution of 4'-chloro-2,2':6',2''-terpyridine (Cl-tpy) with the terminal protected diethylenetriamine under solvent-free conditions (Scheme 1). This turned out to be the key reaction of the synthesis. Initially we attempted Fe^{II} complexation to activate Cl-tpy for nucleophilic attack as reported for similar reactions.⁴ The subsequent substitution with the protected triamine was tried in a variety of solvents (alcohols, acetonitrile, DMF); however, the yields never exceeded 4%. In contrast, high yields were achieved in this first step (i) by optimizing a method previously reported for the direct attachment of aza-crowns to Br-tpy in a high-temperature melt under inert atmosphere.⁵ Our optimization yielded more than 85% even with the poorer electrophile, but commercially available Cl-tpy. All further steps, deprotection, carboxymethylation, ester hydrolysis and final purification by ion exchange chromatography, were straightforward. The ligand tpy-DTTA⁴⁻, which is highly water soluble at pH > 5, was characterized by ¹H NMR, ESI-MS and elemental analysis.

Trinuclear complexes with tpy-DTTA⁴⁻ have been prepared in aqueous solution either stepwise or by self-assembly. In the stepwise procedure, first the bis complex [Fe(tpy-DTTA)₂]⁶⁻ was prepared by adding a Fe^{II} salt to the ligand in 1 : 2 ratio. The deep violet complex was characterized by ¹H NMR and ESI-MS. In a second step we added 2 equivalents of a Eu^{III} salt and adjusted the pH to 7. Concentration of the solution yielded a deep violet precipitate that is the hydrated heteronuclear complex [Fe(tpy-DTTA)₂Eu₂] as proved by elemental analysis. The same Fe^{II}Eu^{III} type complex forms directly by self-assembly when Fe^{II} and Eu^{III} salts are simultaneously added to the ligand followed by adjusting the pH to 7. Likewise, the stepwise procedure and self-assembly were applied to prepare the Fe^{II}Gd^{III} complex solutions (Fig. 1) used for ¹H relaxivity measurements.

Relaxivities of [Fe(tpy-DTTA)₂Gd₂] were determined at variable temperature and at two magnetic fields (0.94 and 1.41 T corresponding to 40 and 60 MHz; Table 1). For comparison, we intended to measure the relaxivity of the non-assembled complex [Gd(tpy-DTTA)]⁻ but failed due to its poor solubility. On mixing

† Electronic Supplementary Information (ESI) available: synthesis and analytical data for tpy-DTTA⁴⁻ and for the complexes. See <http://www.rsc.org/suppdata/cc/b4/b400169a/>



Scheme 1 Reagents and conditions: (i) solvent-free, 180 °C, (ii) 1. 6 M HCl, 2. extraction (iii) *tert*-butyl bromoacetate, K₂CO₃, DMF (iv) 1. 6 M HCl, 2. ion-exchange chromatography (for details, see ESI†).

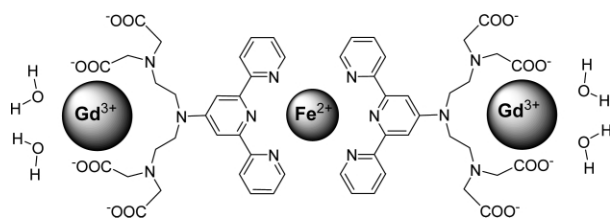


Fig. 1 [Fe(tpy-DTTA)₂Gd₂].

a Gd^{III} or Eu^{III} salt with the ligand, a white precipitate forms immediately. Elemental analysis of the precipitate for the Eu^{III} complex gives the composition [EuH(tpy-DTTA)]·1.5H₂O. Since the coordination moiety of the ligand tpy-DTTA⁴⁻ is identical to that of TTAHA⁶⁻, we use [GdH(TTAHA)]²⁻ as a reference compound (Fig. 2).⁶ [GdH(TTAHA)]²⁻ is known to have two inner sphere water molecules and a water exchange which is faster than that of commercial, Gd^{III} based contrast agents ($k_{\text{ex}}^{298} = 8.6 \times 10^6 \text{ s}^{-1}$). By analogy, we assume that [Fe(tpy-DTTA)₂Gd₂] also has two water molecules per Gd^{III} in the inner sphere. The water exchange rate is likely similar to that on [GdH(TTAHA)]²⁻; to determine its exact value ¹⁷O NMR measurements are planned and will be reported in due course.

The relaxivity significantly increases from the low molecular weight [GdH(TTAHA)]²⁻ to the trinuclear [Fe(tpy-DTTA)₂Gd₂] ($r_1 = 7.3 \text{ mM}^{-1}\text{s}^{-1}$ at 20 MHz, 40 °C vs. $r_1 = 17.4 \text{ mM}^{-1}\text{s}^{-1}$ at 40 MHz, 37 °C). This increase is particularly remarkable when compared to other complexes with two Gd^{III} centres for which relaxivities similar to that of the corresponding mononuclear Gd^{III} compounds have been reported (3.5–5.5 mM⁻¹s⁻¹).⁷ These previously investigated Gd₂^{III} complexes all had a flexible linker between the two chelating units which reduced the relaxivity gain due to fast internal rotation. In addition, the proximity of the Gd^{III} ions can contribute to faster electronic relaxation *via* an intramolecular dipole–dipole mechanism, which again will be disfavorable for attaining high relaxivities. In contrast, in our novel self-assembled [Fe(tpy-DTTA)₂Gd₂] the low-spin Fe^{II}(tpy)₂ unit represents an entirely rigid core. This core also functions as an efficient spacer between the two Gd^{III} ions to avoid dipolar interactions that could accelerate electronic relaxation. Conse-

Table 1 Relaxivities, r_1 (mM⁻¹s⁻¹) for [Fe(tpy-DTTA)₂Gd₂] (mean of two samples: $c_{\text{Gd}} = 4.74 \text{ mM/pH} = 5.8$ and $c_{\text{Gd}} = 4.94 \text{ mM/pH} = 6.1$)^a

$T/^\circ\text{C}$	r_1 (40 MHz)	r_1 (60 MHz)
5.0	27.3	27.4
25.0	22.0	22.9
37.0	17.4	17.1
50.0	12.7	12.6

^a Measured with Bruker Minispec mq40 and mq60.

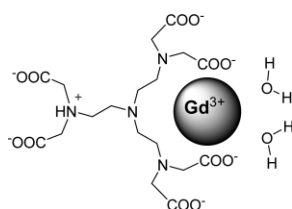


Fig. 2 [GdH(TTAHA)]²⁻.

quently, the high relaxivity can be attributed both to the rigidity of the whole complex ensured by the rationally designed core and to the relatively long Gd^{III}–Gd^{III} distance which excludes dipole–dipole interactions between the paramagnetic centers. The relaxivities increase with decreasing temperature which shows that—as expected—water exchange is fast enough not to limit relaxivity.

Desreux *et al.* published a preliminary report on a phenanthroline derivative of Gd^{III}DO3A that self-assembles around Fe^{II} in aqueous solution to give a tetranuclear Fe^{II}(L₃Gd₃^{III}) structure.⁸ They measured a relaxivity gain from 3.7 to 12.2 mM⁻¹s⁻¹ (20 MHz, 37 °C) *in vitro* due to the self-assembly of the three Gd^{III} units around one Fe^{II}. This system was proposed as a contrast agent responsive to Fe^{II} concentration; however, so far no further results have been published in this respect.

In conclusion, we prepared a self-assembled Fe^{II}Gd₂^{III} complex with high relaxivity. It is based on a novel ligand in which a terpyridine and a poly(amino carboxylate) moiety are connected for the first time. The [Fe(tpy-DTTA)₂Gd₂] complex has a well-defined topology with favourable features to attain high relaxivities. It has a rigid Fe^{II}(tpy)₂ core, a reduced flexibility at the periphery thanks to a short linker, and an efficient separation of the two Gd^{III} centres. The tpy-DTTA⁴⁻ is a potential terminal ligand for the construction of high relaxivity macromolecular MRI contrast agents with numerous Gd^{III}. In addition, it opens new perspectives for luminescent probes with a Ru^{II}(tpy)₂ core, or as a building block for crystal engineering.

We acknowledge Dr Éva Tóth for helpful discussions and reviewing the draft, Laurent Quebatte for assistance with initial test reactions, and the Swiss National Science Foundation for financial support. This work was performed within the frame of EU COST Action D18.

Notes and references

‡ tpy = 2,2':6',2''-terpyridine; DTTA⁴⁻ = diethylenetriamine-*N,N,N'',N''*-tetraacetate.

§ Proton relaxivity is defined as the paramagnetic enhancement of the longitudinal water proton relaxation rate, referred to 1 millimolar concentration of the paramagnetic agent.

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