Bull Soc Chim Fr (1996) 133, 267-272

Synthesis, characterization and relaxivity of functionalized aromatic amide DTPA-lanthanide complexes†

Catherine Gèze[1], Corinne Mouro[1], François Hindré[1], Maryvonne Le Plouzennec[1], Claude Moinet[2], Raymond Rolland[3], Lucia Alderighi[4], Alberto Vacca[4], Gérard Simonneaux[1]*

[1] *Laboratoire de chimie organométallique et biologique, URA CNRS 415, Université de Rennes-1;*
[2] *Laboratoire d'électrochimie, URA CNRS 439, Université de Rennes-1;*
[3] *Institut de recherche mathématique, URA CNRS 305, Université de Rennes-1, 35042 Rennes, France;*
[4] *Dipartimento di Chimica, Universita Degli Studi di Firenze, 50144, Florence, Italy*

(Received 31 October 1995; accepted 22 February 1996)

Summary — Aromatic DTPA-amide derivatives have been synthesized and their protonation constants determined by potentiometry. The stability constants of the gadolinium(III) complexes have been also measured using a spectrophotometric method. These values are compared with corresponding values reported in the literature for mono and other bis-amide DTPA derivatives. The bis-aromatic amide conjugates of DTPA show similar protonation constants but slightly lower stability constants. The measurements of the proton spin-lattice (T_1) relaxation time of these new chelates suggest that they contain only one water molecule in the internal coordination sphere, as was previously found for the parent DTPA complex.

diethylenetriaminepentaacetic acid / aromatic amide / gadolinium / contrast agent / relaxivity

Résumé — Synthèse, caractérisation et relaxivité de complexes DTPA-lanthanide possédant des liaisons amide aromatique. *Des dérivés du DTPA possédant des liaisons amide aromatique ont été préparés et leurs constantes de protonation ont été déterminées par potentiométrie. Les constantes de stabilité des complexes de gadolinium (III) ont été mesurées à l'aide d'une méthode spectrophotométrique. Les valeurs obtenues sont comparées avec les valeurs correspondantes reportées dans la littérature pour des dérivés du DTPA mono et bis amides. Les dérivés DTPA bis amide aromatique révèlent des constantes de protonation similaires à celles obtenues pour le DTPA, les constantes de stabilité sont cependant légèrement inférieures. Les mesures des temps de relaxation spin-réseau (T_1) réalisées avec ces nouveaux chélates suggèrent la présence d'une seule molécule d'eau dans la sphère de coordination interne, comme ceci a déja été démontré avec le DTPA.*

acide diéthylènetriaminepentaacétique / amide aromatique / gadolinium / agent de contraste / relaxivité

Introduction

Paramagnetic gadolinium chelates are currently used for improving the contrast enhancement in magnetic resonance imaging (MRI) [1]. The first agent to be widely used was $[Gd(DTPA)(H_2O)]^{2-}$ (DTPA is diethylenetriaminepentaacetate). However the presence of a negative charge results in high osmolarity under clinical formulations. In order to solve this problem, efforts have recently focused on modifying DTPA derivatives to prepare neutral lanthanide complexes. Therefore, several bis-amide derivatives of DTPA have been prepared as alternatives [2]. In this area, the bis(methylamide) derivative of DTPA, Gd(DTPA-BMA), is the first non ionic MRI contrast agent developed for clinical application [3].

However the sensitivity of this modern technique is still inadequate for the recognition of metastatic deposits, particularly focal hepatic lesions [4, 5]. The efficacy for tumor detection of the hepatocyte-specific contrast agent gadobenate dimeglumine was recently demonstrated [4]. A new hepatobiliary contrast agent, gadolinium ethoxybenzyldiethylenetriaminepentaacetate was also found clinically advantageous for MRI detection of focal hepatic masses [5]. The observed hepatocellular uptake was related to the presence of some active transporters and the lipophilic benzyl moiety covalently bound to the DTPA chelator [6]. Thus current efforts are mainly devoted to the search for a higher specificity towards organs and tissues, in particular tumoral tissues. Our approach to this problem has been to use Gd(DTPA) coupling with tetraphenylporphyrin derivatives [7]. Magnetic resonance images showed an enhancement of contrast between tumor and the adjacent tissues after injection of this agent. In order to extend this coupling method to different porphyrin derivatives, and thus to

† Dedicated to Professor René Dabard on the occasion of his 64th birthday.

* Correspondence and reprints

design new generations of MRI contrast reagents, we need a suitable functionalized derivative of the DTPA-gadolinium complex. In this paper, we report our initial attempts to reach this goal by preparing DTPA chelates with aromatic amine functionality [8]. The presence of two lipophilic aromatic groups covalently bound to the chelate may also favor a hepatic uptake. The protonation constants of these ligands together with the stability constants and the relaxivity of the lanthanide complexes are also presented.

Experimental section

General procedures

The DTPA-bis-(aromatic amide) chelates were synthesized by the condensation of the appropriate aromatic amines with DTPA-bis-(anhydride) according to the literature [9] with minor modification. The procedure for covalent coupling only one aromatic amine to the parent DTPA chelate via amide linkage was realized using the method reported by Krejcarek and Tucker [10]. Controlled potential electrolysis was carried out in a batch cell at a mercury pool cathode [11]. The products were characterized by ^{1}H and ^{13}C NMR spectroscopy, FAB mass spectra and elemental analysis.

- *DTPA-bis-(anilide)* **1**

Aniline (2.6 g, 2.8×10^{-2} mol) was added to the dianhydride of DTPA (1 g, 2.8 mmol) dissolved in 5 mL DMSO using Schlenk tubes and stirred under argon at room temperature for 8 h. The solvent was removed under vacuum at 60 °C. After addition of 20 mL THF and stirring the resulting mixture for 6 h, the precipitate was filtered and recrystallized twice (methanol/ether, 1:1) to yield 1.5 g (95%).

^{1}H NMR (D_2O, pD 7) δ (ppm): 7.39 (m, 8H), 7.20 (t, 2H, J = 7.1 Hz), 3.81 (s, 4H), 3.47 (s, 2H), 3.39 (t, 4H, J = 5.8 Hz), 3.28 (s, 4H), 3.11 (t, 4H, J = 5.8 Hz).

^{1}H NMR (DMSO d_6) δ (ppm): 10.08 (s, 2H, NH), 7.66 (d, 4H, J = 7.9 Hz), 7.26 (t, 4H, J = 7.9 Hz), 7.02 (t, 2H, J = 7.4 Hz), 3.63 (s, 2H), 3.47 (s, 8H), 3.12 (s, 4H), 2.98 (s, 4H).

^{13}C NMR (D_2O, pD 7) δ (ppm): 181.68, 175.05, 173.14, 139.01, 131.86, 128.34, 124.58, 62.17, 61.42, 57.40, 55.60, 53.07.

^{13}C NMR (DMSO d_6) δ (ppm): 173, 172.50, 138.62, 128.51, 123.19, 119.27, 58.14, 55.28, 55.18, 52.17, 50.64.

Anal calc for $C_{26}H_{33}N_5O_8$: C, 57.46; H, 6.08; N, 12.89. Found: C, 55.43; H, 6.21; N, 12.85.

- *DTPA-bis-(p-NO_2-anilide)* **2**

4-Nitroaniline (2.7 g, 1.97×10^{-2} mol) was added to the dianhydride of DTPA (1 g, 2.8 mmol) dissolved in 5 mL DMSO using Schlenk tubes and stirred under argon at room temperature for 8 h. The solvent was removed under vacuum at 60 °C. After addition of 20 mL THF and stirring the resulting mixture for 6 h, the precipitate was filtered and recrystallized twice (methanol/ether, 1:1) to yield 1.15 g (65%).

^{1}H NMR (D_2O, pD 7) δ (ppm): 7.88 (d, 4H, J = 9.1 Hz), 7,49 (d, 4H, J = 9.1 Hz), 3.86 (s, 2H), 3.57 (s, 4H), 3.43 (t, 4H, J = 5.4 Hz), 3.33 (s, 4H), 3.16 (t, 4H, J = 5.4 Hz).

^{1}H NMR (DMSO d_6) δ (ppm): 10.69 (s, 2H, NH), 8.11 (d, 4H, J = 8 Hz), 7.88 (d, 4H, J = 8 Hz), 3.59 (s, 2H), 3.54 (s, 4H), 3.47 (s, 4H), 3.08 (s, 4H), 2.98 (s, 4H).

^{13}C NMR (D_2O, pD 7) δ (ppm): 181.62, 175.27, 173.28, 145.96, 145.56, 127.43 , 122.62, 62.08, 60.96, 57.87, 55.84, 52.91.

FAB-MS (m/e) for $C_{26}H_{28}N_7O_{12}Na_3$: 700 (M + 1), 678, 656, 632, 610.

- *DTPA-bis-(p-CF_3-anilide)* **3**

4-(trifluoromethyl)aniline (4.52 g, 2.81×10^{-2} mol) was added to the dianhydride of DTPA (1 g, 2.8×10^{-3} mol) dissolved in 5 mL DMSO using Schlenk tubes and stirred under argon at room temperature for 72 h. The solvent was removed under vacuum at 60 °C. After addition of 20 mL THF and stirring the resulting mixture for 48 h, the precipitate was filtered and recrystallized twice (methanol/ether, 1:1) to yield 1.77 g (93%).

^{1}H NMR (D_2O, pD 7) δ (ppm): 7.50 (s, 8H), 3.83 (s, 2H), 3.54 (s, 4H), 3.42 (t, 4H, J = 5.6 Hz), 3.31 (s, 4H), 3.16 (t, 4H, J = 5.6 Hz).

^{13}C NMR (D_2O, pD 7) δ (ppm): 181.47, 175.02, 173.21, 142.53, 128.68, 123.39, 62.06, 61.00, 57.45, 55.85, 53,02.

Anal calc for $C_{28}H_{31}N_5F_6O_8$: C, 49.48; H, 4.56; N, 10.31; F, 16.79. Found: C, 49.57; H, 4.71; N, 10.35; F, 16.89.

- *DTPA-bis-(p-NH_2-anilide)* **4**

For the electrosynthesis of the amino derivative, direct reduction of **2** (1.32 g, 2.08 mmol), dissolved in a solution of 70 cm^3 methanol and 50 cm^3 H_2SO_4 N, was performed at -0.9 V_{sce} for 5 h. At the end of the electrolysis, the solution was neutralized with NaOH 2N. After precipitation and filtration, the solid residue was washed three times with methanol (**4**: 85% yield).

^{1}H NMR (D_2O, pD 7) δ (ppm): 7.17 (d, 4H, J = 8.4 Hz), 6.77 (d, 4H, J = 8.4 Hz), 3.79 (s, 2H), 3.45 (s, 4H), 3.37 (t, 4H, J = 6 Hz), 3.25 (s, 4H), 3.08 (t, 4H, J = 6 Hz).

^{13}C NMR (D_2O, pD 7.6) δ (ppm): 181.67, 174.85, 173.14, 146.54, 130.86, 126.45, 61.95, 61.35, 57.34, 55.54, 52.98.

^{1}H NMR (DMSO d_6) δ (ppm): 10,73 (s, 2H, NH-CO).

- *DTPA-mono-(p-NO_2-anilide)* **5**

Pentakis-triethylammonium DTPA was first prepared from DTPA (1 g, 2.54 mmol) and 5 equiv of triethylamine (12.7 mmol) in 50 mL acetonitrile. The solution was stirred for 1 h at 40 °C. The solution was then cooled to 0 °C and 3.05 mmol isobutyl chloroformate (1.2 equiv) was added dropwise, followed by immediate addition of a solution of the 4-nitroaniline (17.75 mmol) in 20 mL dry acetonitrile. The resulting mixture was stirred for 30 min, then the solvent was distilled off under vacuum. The residue was dissolved in water (pH 8.0), and the unreacted 4-nitroaniline was extracted with ether. The solution was loaded onto a 5 × 80 cm sephadex G 25 column, and eluted with water (pH 8.0). Two fractions were collected corresponding to the mono-amide (840 mg, 1.4 mmol, 55% yield) and to the bis-amide (275 mg, 0.38 mmol, 15% yield).

^{1}H NMR (D_2O, pD 10) δ (ppm): 8.23 (d, 2H, J = 9.1 Hz), 7.74 (d, 2H, J = 9.1 Hz), 3.48 (s, 4H), 3.28 (s, 6H), 3.05 (s, 4H), 2.93 (4H).

FAB-MS (m/e) for $C_{20}H_{23}N_5O_{11}Na_4$: 602 (M + 1).

Protonation

Deionized water was purified with a MilliQ-Reagent system to produce water with a specific resistance > 15 Mohm cm. Sodium perchlorate (C Erba ACS grade) was purified as previously described [12]. $HClO_4$ and NaOH solutions were prepared under nitrogen and were standardized by conventional methods. The ionic strength was adjusted to 0.15 mol dm^{-3} with $NaClO_4$.

Potentiometric titrations were carried out using a Crison Micro pH 2002 potentiometer fitted with a Radiometer GK2401 combined pH glass electrode in conjunction with a Hamilton Microlab M motor-driven syringe under the

control of an appropriate program running on a IBM PS/2 mod 20 computer [13]. The solutions to be titrated were kept at 298.2 + 0.1 K and magnetically stirred in a water vessel with a jacket. The vessel was closed to the atmosphere, apart from a vent to allow egress of a nitrogen steam which was passed over the solution to exclude contamination by atmospheric carbon dioxide. The instrumentation was calibrated by titration of 0.100 mol dm^{-3} NaOH (about 1 cm^3 from the syringe) against 4 mmol dm^{-3} $HClO_4$ (20 cm^3).

Two main procedures were used in performing the potentiometric experiments: (a) addition of a 0.100 mol dm^{-3} NaOH solution to a solution ($\approx$ 20 cm^3) of modified acidic DTPA ($\approx$ 0.5 mmol dm^{-3}) and excess $HClO_4$ (0.5 mmol dm^{-3}); (b) addition of a 0.200 mol dm^{-3} $HClO_4$ solution to a solution ($\approx$ 20 cm^3) of modified DTPA (0.5 mmol dm^{-3}) previously neutralized, if necessary, by reaction with NaOH, in slight excess with respect to the stoichiometric amount. The data were processed using Hyperquad [14].

Stability

The gadolinium-chelate stability constants were determined using a spectrophotometric method, previously reported by Sherry [2a, 15] and Kumar and Tweedle [16]. All spectrophotometric measurements were made with an Uvikon 941 spectrophotometer, using arsenazo III as indicator. Solutions of mixtures of Gd(III), arsenazo III and variable amounts of the ligand were prepared. The absorbance variation with ligand concentration was used to calculate the equilibrium constant. The method was verified i) by checking the extinction coefficients of the 1:1 and 1:2 gadolinium complexes of arsenazo III, of 35 000 and 50 000 L $mol^{-1}cm^{-1}$ respectively, and ii) by the determination of the thermodynamic stability constant of the parent DTPA and comparison with literature values. The solutions were buffered at pH 4 with 0.01 mol dm^{-3} acetate and the ionic strength was adjusted to 0.1 M with 0.1 mol dm^{-3} NaCl. All the measurements were made at 25 °C. Stability constants were calculated using a computer program described previously [2a, 18].

Relaxivity

The relaxivities of the gadolinium complexes were measured at 25 °C in deionized aqueous solutions for relaxation enhancement evaluation. A Bruker PC 20 spectrometer was used with the following pulse sequences: T_1 were measured from an inversion–recovery pulse sequence with 8τ values ranging from 0.04–5 T_1 (typically 20 ms to 2.5 s for a T_1 of 500 ms); T_2 were obtained from a Meiboom–Gill–Carr–Purcell sequence with 20 measurement points ranging from 0.1–2.3 T_2 (typically 11–230 ms for a T_2 of 100 ms).

NMR

The ^1H and ^{13}C NMR measurements were recorded on a Bruker AC 300P spectrometer at 300 MHz (^1H) and 75 MHz (^{13}C) in D_2O (pH 7) or DMSO (d_6) using Me_4Si as a reference standard. NMR pH titrations in D_2O were carried out at ambient temperature of 25 °C, from pH 4 to 12 at intervals of approximately 0.3 pH units. Stock solutions of the free ligands were prepared by dissolving appropriate amounts of the ligand in D_2O (pH = 4). The pH of the stock solutions were adjusted by the addition of 0.1 mol dm^{-3} NaOD in D_2O. Apparent pD (pD_{app}) was measured with a glass combination electrode standardized with H_2O on the basis of pH buffers. Observed values for pD_{app} were taken without correction.

Results and discussion

Ligand synthesis

We have been interested in preparing lanthanide complexes of polyaminocarboxylate chelates as MRI contrast agents, achieving high local concentrations at lower dosages. An example of tumor-specific contrast agents is tetra-*p*-aminophenylporphyrin conjugated with Gd(DTPA) [7]. Since the reaction employed to conjugate DTPA to the porphyrin involves formation of aromatic amide linkages with the carboxyl groups, the first goal of this study was to synthesize model compounds in order to better understand the role of the aromatic group on the chelation effect and on the relaxivity of the gadolinium complexes. Recent reports have employed a similar synthesis using aromatic amines and chelates in order to prepare heterobimetallic complexes [17], reagents for the determination of glycated proteins [18] and a label for fluorescent immunoassays [19].

Thus, new DTPA-bis-(aromatic amide) ligands have been prepared through the coupling of DTPA-bis-(anhydride) and *para*-substituted aniline-producing ligands with functional groups in the *para* position. The yield of the coupling was good but longer reaction times were necessary due to the lower basicity of the aniline derivatives in comparison to that of the alkylamine. The ^1H NMR spectra confirm the expected structures. The structure of ligand **1** is illustrated in figure 1. The aliphatic proton magnetic resonance spectrum of the ligand **1** (pH 6.4), shown in figure 2, displays three singlets at 3.81 (2H), 3.47 (4H) and 3.28 (4H) ppm and two triplets at 3.39 and 3.11 ppm with an intensity ratio 4H:4H. The assignments of the peaks follow those previously reported for DTPA-BMA (BMA: bismethylamide) in which the distinction between the two terminal CH_2 groups was based on the fact that amide groups have a larger inductive effect compared to an α-carboxylate group [3b]. The two triplets are assigned to the protons of the two methylene groups between the nitrogen atoms, using ^1H-detected heteronuclear multiple-bond connectivity spectroscopy (HMBC) [20]. Similar results were found with the other aromatic chelates. The low-field part of the spectrum displays a triplet at 7.20 ppm (4H, *o*-phenyl) and a multiplet centered at 7.36 ppm [6H, (*m* + *p*)-phenyl].

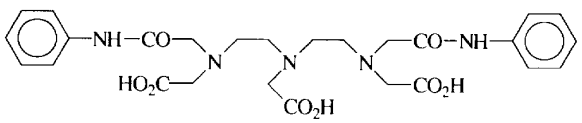

Fig 1. DTPA-bis-(anilide) **1**.

Protonation constants

At least two different acid–base titrations for each ligand (DTPA, **1**, **2**, **3**, **4** and **5**) have been analyzed using the program Hyperquad [14] to obtain the stepwise basicity constants, which are summarized in table I. These constants are first compared with the corresponding values for DTPA [21]. As previously ob-

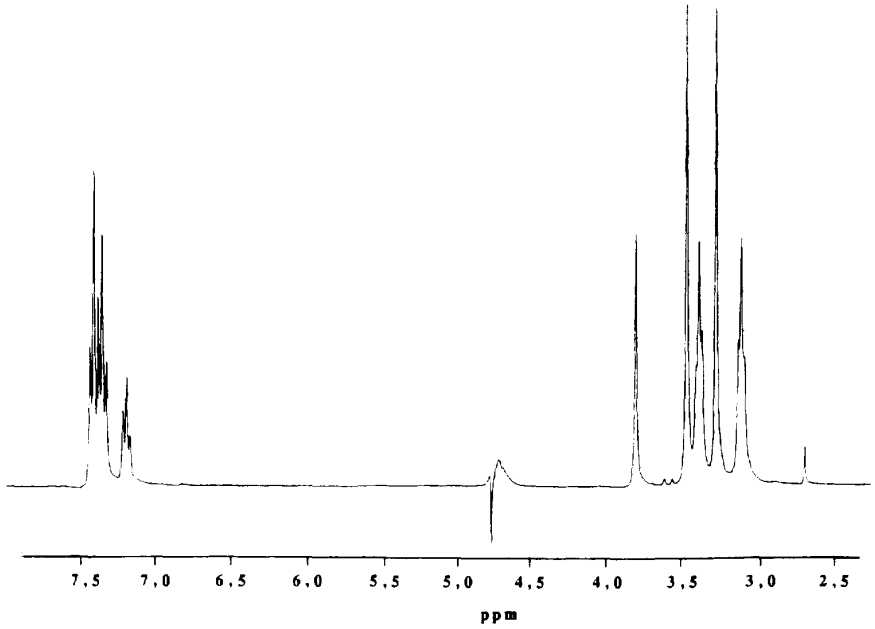

Fig 2. ^{1}H NMR spectrum of DTPA-bis-(anilide) at 25 °C.

Table I. Logarithms of the stepwise protonation constants for DTPA and DTPA derivatives[a].

ligand	*log* K_1	*log* K_2	*log* K_3	*log* K_4	*log* K_5	*ref*
DTPA	9.76(1)	8.33(1)	4.19(1)	2.68(2)	2.01(1)	b
DTPA-mono-(*p*-NO_2-anilide)	9.60(9)	6.20(1)	3.98(8)	2.63(8)	1.60(4)	b
DTPA-bis-(anilide)	9.10(6)	4.39(6)	3.41(6)	2.10(1)		b
DTPA-bis-(*p*-NO_2-anilide)	9.10(1)	4.30(1)	3.24(9)	2.10(2)		b
DTPA-bis-(*p*-NH_2-anilide)	9.65(9)	5.00(1)	3.70(1)	2.10(2)		b
DTPA-bis-(*p*-CF_3-anilide)	9.02(9)	4.40(9)	3.35(9)	2.10(2)		b
DTPA	9.86	8.32	4.12	2.85		c
DTPA	10.2	8.6	4.2	2.9	2.4	d
DTPA-mono propylamide	9.9	6.4	3.8	1.8		d
DTPA-BMA	9.4	4.5	3.4			d

[a] The values in parentheses are 3 σ on the last significant figure. [b] This work: T = 298; ionic strength 0.15 M $NaClO_4$. [c] Ref [21c]: T = 298; ionic strength 0.50 M KNO_3. [d] Ref [2a]: T = 298; ionic strength 0.1 M NaCl.

served, the greater electron-withdrawing ability of an amide group compared to that of a carboxyl group increases the acidity of all the nitrogen atoms (see also NMR studies). In table I, the potentiometrically-determined constants of DTPA-bis-aromatic amide are also compared with other bis-alkylamide derivatives reported by Sherry and Cacheris [2a]. The presence of an aromatic amide linkage instead of an alkyl amide group increases the acidity of the first protonated nitrogen, the pKa of the two other amino groups not being strongly affected by the aromatic moiety. The carboxylate protonations are typical of the amine carboxylate chelates occurring in the pH range 1–2.7.

NMR studies

Two protonation constants for all DTPA-bis-(anilide) chelates were observed in the pH range 4–12, corresponding to the stepwise protonation of the amino groups. First, figure 3 clearly shows three main inflections at pH 9–10, corresponding to protonation of the central nitrogen. The (a), (d) and (e) protons first move downfield due to the nitrogen protonation. The second protonation (pH 4–5) seems to occur at the terminal nitrogen atoms, whereas the central nitrogen loses its proton, thus the two protons reside on the two outer nitrogens. Accordingly the (e), (b) and (c) protons move downfield and an upfield shift is observed for the (a) and (d) protons. This behavior of the chemical shifts of the chelate **1** as a function of pH is very similar to that of the parent compound DTPA which has been discussed by Letkemann and Martell [21]. It is interesting to note, however, that a different protonation sequence has been reported for DTPA-bis-(methoxyethylamide) [22].

Stability constants

The equilibrium constant from the reaction of the mixture of Gd and arsenazo–Gd complexes with the new

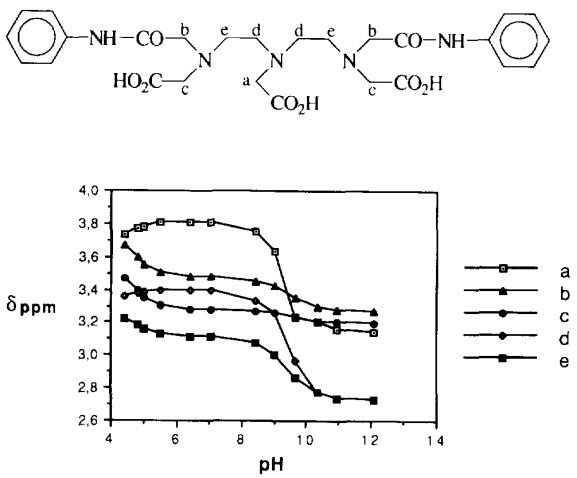

Fig 3. Chemical shift of DTPA-bis-(anilide) vs pH.

ligands at pH 4 was determined by the spectrophotometric method previously reported by Sherry et al [2a]. Thus the conditional stability constants of DTPA complexes could be obtained at pH 4. The thermodynamic stability constants for the complexes may then be calculated from the equilibrium constants measured at pH 4 by:

$$K_{\text{therm}} = K_{\text{cond}}[1 + K_1[H^+] + K_1K_2[H^+]^2 + K_1K_2K_3[H^+]^3 + \ldots]$$

where K_1, K_2 and K_3 are the stepwise protonation constants for the DTPA conjugate.

The thermodynamic stability constants for the Gd(DTPA)-bis-(aromatic amide) complexes are reported in table II. Also shown are the Gd(DTPA)-bis-(alkylamide) complexes and the parent Gd(DTPA) complex for comparison. It is thought that the participation of the amide carbonyl oxygen is maintained in the metal coordination (vide infra). The lower stability of the amide complexes, as compared to the parent DTPA complex, reflects the lower basicity of the amide carbonyl oxygen relative to the carboxylate oxygen. The stability constants of Gd(DTPA)-bis-(aromatic amide) complexes, with the exception of Gd(DTPA)-bis-(p-NH_2-anilide) ($\log k_{\text{therm}} = 16.13$), did not change significantly throughout the series and are about one order of magnitude smaller than the corresponding values for the Gd(DTPA)–BMA complex: $\log k_{\text{therm}} = 16.86$ [23]. However the value we found for the DTPA complex is also slightly lower than that found by Sherry [2a]; this difference, which is about a factor of 10, may reflect slight differences in experimental conditions. Actually, the stability of alkyl and phenyl amide derivatives seem very similar.

Relaxivity

A plot of the water proton longitudinal relaxation rate of a solution of DTPA-bis-(anilide) as a function of Gd concentration at 25 °C (pH 7) is shown in figure 4. The [Gd(DTPA)-bis-(anilide)] complex has a relaxivity of 4.1 mmol dm^{-3} s^{-1} at 25 °C and 20 MHz.

Table II. Thermodynamic stability constants measured at 25 °C.

ligand	*log* K_{therm}	*reference*
DTPA	21.04	a
DTPA-mono-(p-NO_2-anilide)	17.63	a
DTPA-bis-(p-NH_2-anilide)	16.13	a
DTPA-bis-(p-CF_3-anilide)	15.33	a
DTPA-bis-(anilide)	15.16	a
DTPA-bis-(p-NO_2-anilide)	14.90	a
DTPA	20.73	b
DTPA	22.26	c
DTPA-mono-(propylamide)	19.68	c
DTPA-bis-(propylamide)	16.23	c
DTPA-bis-(methylamide)	16.85	d

a This work: $T = 298$; ionic strength 0.15 M $NaClO_4$. b Ref [21b]: $T = 298$; ionic strength 0.50 M KNO_3. c Ref [2a]: $T = 298$; ionic strength 0.10 M NaCl. d Ref [1b]: $T = 298$; ionic strength 0.10 M KCl.

Similar measurements of the proton relaxation times following stepwise addition of Gd allow the determination of the relaxivity of the other ligands (table III). These results are only sligthly lower than the value (4.8 mmol dm^{-3} s^{-1}) found for the parent Gd(DTPA) complex. This finding leads us to suggest that the number of coordination sites available for inner-sphere water binding is similar in both complexes: one site is occupied by a water molecule and the other eight sites by the ligand, ie, three nitrogens, three carboxylate oxygens and two carboxamido groups. Such a situation has been previously suggested for other Gd(DTPA)-bis-(amides) [2a, 24] and is confirmed by the X-ray structures of Gd(DTPA)-bis-(ethylamide) [9] and Gd(DTPA)-bis-(methyl-amide) [25] . In the classical theory underlying hard and soft behavior of acids and bases [26], oxygen donors such as carboxylate groups are hard bases and will form strong bonds with hard acids, such as Gd. Aromatic amides will be softer but still strong enough to complete the coordination sphere. Thus the contribution of the amide functional group to the stability of gadolinium(III) complexes has been recently reported [27]. Moreover the amide functional groups seem to contribute to the relative selectivity of the bis-(amide) ligands as gadolinium complexing agents in comparison with calcium complexation [27].

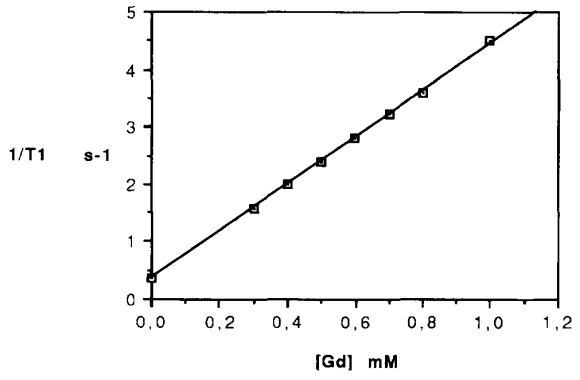

Fig 4. Plot of the water proton longitudinal relaxation rate (R_1) of a 1 mM solution of DTPA-bis-(anilide) as a function of Gd concentration, measured at 20 MHz, 25 °C and pH 7.

Table III. Relaxivities of the gadolinium complexes measured at 20 MHz, 25 °C in H_2O.

Ligand	r_1 ($mmol^{-1}$ $dm^3 s^{-1}$)
Gd-DTPA-mono-(*p*-NO_2-anilide)	5.08
Gd-DTPA	4.82
Gd-DTPA-bis-(anilide)	4.66
Gd-DTPA-bis-(*p*-NH_2-anilide)	4.12
Gd-DTPA-bis-(*p*-NO_2-anilide)	3.78
Gd-DTPA-bis-(*p*-CF_3-anilide)	3.71

a This work: $T = 298$; ionic strength 0.15 M $NaClO_4$. b Ref [21b]: $T = 298$; ionic strength 0.50 M KNO_3. c Ref [2a]: $T = 298$; ionic strength 0.10 M NaCl. d Ref [1b]: $T = 298$; ionic strength 0.10 M KCl.

Conclusion

The physical properties, stability constants and relaxivity of Gd(DTPA)-bis-(aromatic amide) complexes are approximately the same as for those formed with DTPA–BMA, which is currently in clinical use. These lipophilic chelates, which remain hydrosoluble, may offer new alternatives for use as tissue-specific contrast agents, in particular for liver imaging. In vivo trials will be necessary to confirm this hypothesis.

Acknowledgments

The authors would like to thank A Bondon for recording HMBC spectra and helpful discussions. We are also grateful to J de Certaines for the very kind use of his relaxometry facility.

References

1 a) Lauffer RB, *Chem Rev* (1987) 87, 901;
b) Rocklage SM, Watson AD, *J Magn Reson Imaging* (1993) 3, 167

2 a) Sherry AD, Cacheris WP, Kuan KT, *Magn Reson Med* (1988) 8, 180;
b) Rizkalla EN, Choppin GR, Cacheris W, *Inorg Chem* (1993) 32, 582;
c) Geraldes CFGC, Urbano AM, Alpoim MC, Sherry AD, Kuan KT, Rajagopalan R, Maton F, Muller RN, *Magn Reson Imaging* (1995) 13, 3, 401

3 Quay SC, *Diamine-DTPA Paramagnetic Contrast Agents For MR Imaging US Patent No 4* (687) 1987

4 Kreft BP, Tanimoto A, Stark DD, Baba Y, Zhao L, Chen JT, Compton CC, Finn JP, Cavagna FM, *J Magn Reson Imaging* (1993) 3, 41

5 a) Weinmann HJ, Schuhmann-Giamperi G, Schmitt-Willich H, Vogler H, Frenzel T, Gries H, *Magn Reson Med* (1991) 22, 233;
b) Clement O, Mühler A, Vexler VS, Kuwatsuru R, Berthezène Y, Roseneau W, Brasch R, *J Magn Reson Imaging* (1993) 3, 71

6 de Haën C, Gozzini L, *J Magn Reson Imaging* (1993) 3, 179

7 Hindré F, Le Plouzennec M, de Certaines J, Foultier MT, Patrice T, Simonneaux G, *J Magn Reson Imaging* (1993) 3, 59

8 During this work was in preparation, one of this system was also investigated. It is shown in this paper that the smaller relaxivity of the DTPA-bis-(anilide) complex is due to a long exchange lifetime of the coordinated water molecule. See Aime S, Fasano M, Paoletti S, Terreno E, *Gazz Chim Ital* (1995) 125, 125

9 a) Hnatowich DJ, Layne WW, Childs RL, *Int J Appl Radiat Isotop* (1982) 33, 327;
b) Konings MS, Dow WC, Love DB, Raymond KN, Quay SC, Rocklage SM, *Inorg Chem* (1990) 29, 1488

10 Krejcarek GE, Tucker KL, *Biochem Biophys Res Commun* (1977) 77, 581

11 Moinet C, Peltier D, *Bull Soc Chim Fr* (1969) 690

12 Micheloni M, May P, Williams DR, *J Inorg Nucl Chem* (1978) 40, 1209

13 Fontanelli M, Micheloni M, *Proceedings of the 1st Spanish–Italian Congress on Thermodynamics of Complexes*, Peniscola, 3–6 June (1990)

14 a) Gans P, Sabatini A, Vacca A, *J Chem Soc, Dalton Trans* (1985) 1195;
b) Sabatini A, Vacca A, Gans P, *Coord Chem Rev* (1992) 120, 389

15 Cacheris WP, Nickle SK, Sherry AD, *Inorg Chem* (1987) 26, 958

16 Kumar K, Jin T, Wang X, Desreux JF, Tweedle MF, *Inorg Chem* (1994) 33, 3823

17 Aime S, Botta M, Fasano M, Terreno E, *Spectrochim Acta* (1993) 49A, 1315

18 Aime S, Botta M, Dastrau W, Fasano M, Panero M, Arnelli A, *Inorg Chem* (1993) 32, 2068

19 Bailey MP, Rocks BF, Riley C, *Analyst* (1984) 109, 1449

20 Bax A, Summers MF, *J Am Chem Soc* (1986) 108, 2093

21 a) Sudmeier JL, Reilley CN, *Anal Chem* (1964) 36, 1698;
b) Gritmon TF, Goedken MP, Choppin GR, *J Inorg Nucl Chem* (1977) 39, 2021;
c) Choppin GR, Goedken MP, Gritmon TF, *J Inorg Nucl Chem* (1977) 39, 2025;
d) Letkeman P, Martell AE, *Inorg Chem* (1979) 18, 1284

22 White DH, deLearie LA, Dunn TJ, Rizkalla EN, Imura H, Choppin GR, *Invest Radiol* (1991) 26, S229

23 Cacheris WP, Quay SC, Rocklage SM, *Magn Reson Imaging* (1990) 177, 101

24 Geraldes FGC, Urbano AM, Alpoim MC, Hoefnagel MA, Peters JA, *J Chem Soc, Chem Commun* (1991) 656

25 Ehnbom L, Pedersen B, *Acta Chem Scand* (1992) 46, 126

26 Pearson RG, *Science* (1966) 151, 172

27 Paul-Roth C, Raymond KN, *Inorg Chem* (1995) 34, 1408