DOTASA Revisited: ^{1}H NMR and Potentiometric Studies of a Highly Asymmetrical Ligand and its Lanthanide(III) Complexes

João P. André [a], Ernö Brücher [b], Robert Kiraly [b], Rui A. Carvalho [c], Helmut Mäcke [d], Carlos F. G. C. Geraldes [c]

[a] Centro de Química, Campus de Gualtar, Universidade do Minho, 4710-057 Braga, Portugal; [b] Department of Inorganic and Analytical Chemistry, University of Debrecen, 4010, Hungary; [c] Department of Biochemistry; NMR Cer ter of Neurosciences and Cell Biology, University of Coimbra, 3001-401 Coimbra, Portugal; [d] Division of Radiological Chemistry, Institute of Nuclear Medicine, University Hospital, Petersgraben 4, 4031 Basel, Switzerland

chelating agents in regard of the thermodynamic and kinetic stability of their Gd(III) chelates. DOTASA (= 1, 4, 7, 10 – tetraazacyclodo-decane -1-(*R*,*S*)-succinic acid - 4, 7, 10 -triacetic acid) [1] is a DOTA-like macrocyclic ligand (DOTA = 1, 4, 7, 10 - tetraazacyclododecane -1, 4, 7, 10 -tetraacetic acid) showing a carboxymethyl -CH_2COOH substituent moiety at a C_α carbon of one of the four acetate pendant arms, present as a racemic mixture of *R* and *S* configurations.

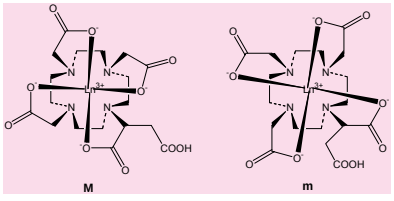

Fig. 1 - Schematic representation of the two diastereoisomers of **[Gd(DOTASA)(H$_2$O)]$^{2-}$** complexes, endowed with the same square [3333] conformation of the tetraaza ring, but with a different layout of the acetate groups: **M** isomer (square antiprismatic) and **m** isomer (twisted antiprismatic). The H_2O molecule (in the apical position above the plane of the four oxygen atoms has been omitted for clarity).

	DOTASA		DOTA		
log K_i	0.1 M Me_4NCl	0.5 M Me_4NNO_3	0.1 M Me_4NCl [15]	0.1 M Me_4NCl [14]	0.1 M Me_4NNO_3 [13]
logK_1	10.99 (0.02)	11.17	11.74	11.73	12.09
logK_2	9.18 (0.02)	9.38	9.76	9.40	9.68
logK_3	5.35 (0.02)	5.25	4.68	4.50	4.55
logK_4	4.40 (0.02)	4.21	4.11	4.19	4.13
logK_5	3.75 (0.02)	3.28	2.37	-	-
logK_6	2.93 (0.02)	-	-	-	-
logK_7	1.8 (0.04)	-	-	-	-
logK_8	1.2 (0.06)	-	-	-	-

Table 1 - Protonation constants (log *Ki*) of DOTASA and DOTA at 25 ºC obtained by potentiometry.

Results and discussion

A) Protonation constants of the ligand

i) Potentiometry gave values close to DOTA except for the extra pK_3 value of 5.35 assigned to protonation of the extra carboxylate group in the succinyl arm (**Table 1**) .

ii) ^{1}H NMR spectra of DOTASA at different pH values are too complex to allow the full determination of its microscopic protonation scheme, due the the presence of multiple isomeric structures in solution. The first two protonations are distributed among the three N-atoms bound to the acetate arms thus excludingthe N-atom bound to the succinate arm. Only at pH values lower than 8,95 the carboxylate groups, excluding the C_α substituint, are protonated.

B) Thermodynamic stability constant of [Gd(DOTASA)(H$_2$O)]$^{2-}$

Potentiometry gave log K_{ML} = 27.2 (0.2), which is higher than that of [Gd(DOTA)(H$_2$O)]$^-$.

C) NMR studies of the [Ln(DOTASA)(H$_2$O)]$^{2-}$ complexes

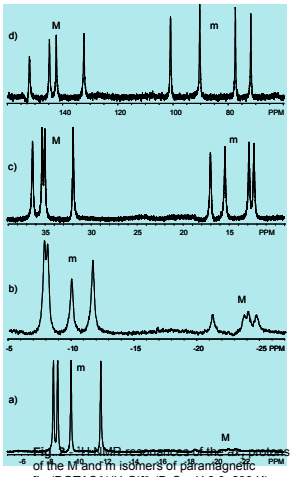

Fig. 2 - ^{1}H NMR resonances of the 2D protons of the M and m isomers of paramagnetic [Ln(DOTASA)(H$_2$O)]$^{2-}$ (D$_2$O, pH 8.0, 298 K): a) Ln = Ce; b) Ln = Eu; c) Ln = Yb.

Ln	m isomer	M isomer	M / m
Ce	-8.31, -8.62, -9.58, -11.73	-18.52, -20.48, -20.94, -21.43	1:11.5
Pr	-23.53, -23.99, -27.65, -30.39	-40.35, -46.05, -46.17, -46.30	1:6.7
Nd	-7.80, -8.05, -9.97, -11.67	-21.18, -23.65, -23.99, -24.63	1:3.3
Sm	not assigned	-2.47, -2.96, -2.96, -3.60	-
Eu	17.93, 16.36, 13.76, 13.22	37.28, 36.23, 35.94, 32.85	1:0.9
Yb	101.01, 90.44, 77.56, 71.92	152.22, 145.05, 142.52, 132.49	1:1.2

Table 2 - NMR shifts (δ – ppm) of the ax_i protons of the paramagnetic [Ln(DOTASA)(H$_2$O)]$^{2-}$ chelates in the M and m isomeric forms.

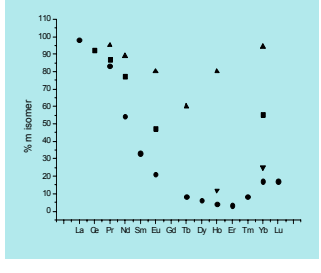

Fig. 3 - Molar fractions of the isomer m for Ln-tetraazamacrocycles in aqueous solution, pH 7, 298 K, as a function of the complexes metal ion obtained from ^{1}H NMR:
(■) [Ln(DOTASA)]$^{2-}$(this work);
(●) [Ln(DOTA)(H$_2$O)]$^-$[11,25];
(▲) [Ln(*RRRR*)-(TCE-DOTA)(H$_2$O)]$^{2-}$ [31];
(▼) [Ln(DOTA-pNP)(H$_2$O)]$^-$[32].

Conclusions

The present studies illustrate the multiple effects that derivatisation of the DOTA ligand has on the properties of the corresponding Gd(III) chelate, with consequences on its potential application as an MRI contrast agent. While the protonation constants of DOTASA are not much changed relative to DOTA except for an extra p*K* value due to protonation of the extra carboxylate group in the succinyl arm, the stability constant of its Gd(III) chelate is significantly increased relative to [Gd(DOTA)(H$_2$O)]$^-$.

The number of isomers in solution of the Ln(III) chelates of DOTASA also doubles, combining the M and S structures of the framework of the complexes with the *R* and *S* configurations of the substituted pendant arm C_α atom. More importantly, the m isomer population of the Gd(III) complex with DOTASA is 3-4 times increased relative to Gd(III)-DOTA. This is in contrast with the C_α effect of the more sterically bulky para-nitrophenyl group, like in the Gd(III)-DOTA-pNP chelate [32], where the % m increase is much smaller. The effect of the flexible carboxymethyl group in Gd(III)-DOTASA on the % m increase is almost as high as that resulting from *RRRR* tetrasubstitution of DOTA [31], and can thus be considered a good strategy of connecting a Gd(III)-tetrazamacrocycle to a carrier while increasing its water exchange rate, and thus its relaxivity.