Eur J Med Chem (1995) 30, 539–546

Molecular modelling of tetrahydroxymethyl-substituted DOTA derivatives and their Gd(III) ion complexes

R Fossheim[1,2], H Dugstad[2], SG Dahl[1]

[1]*Institute of Medical Biology, Department of Pharmacology, University of Tromsø, MH-Breivika, 9037 Tromsø;*
[2]*Nycomed AS, PO Box 4220 Torshov, N-0401 Oslo, Norway*

(Received 20 January 1995; accepted 27 February 1995)

Summary — Molecular mechanics calculations and molecular dynamics simulations were used to examine the molecular structures and stabilities of the four isomers (*RRRR*, *RRRS*, *RRSS* and *RSRS*) of DOTA-THM (1,4,7,10-tetraaza-2,5,8,11-tetrahydroxymethylcyclododecane-*N*,*N'*,*N''*,*N'''*-tetraacetic acid) and their Gd(III) ion complexes. The stabilities of the *RRRR* and *RRRS* isomers were similar to the parent compound DOTA, whereas the *RRSS* and *RSRS* isomers were the least stable. The calculations suggest that the positioning of the substituents on the [3333] square ring is important for stability. A correlation between experimental activation energies for complex formation and our calculated ligand constraint energies for GdNOTA, GdDOTA and GdDO3A, which has been demonstrated by others, suggests that the *RRRR* and *RRRS* isomers have higher activation energies than the *RSRS* and *RRSS* isomers, which, in turn, are higher than for GdDOTA. Molecular lipophilicity potentials for the complexes demonstrated that a large fraction of the water-accessible surfaces are equi-hydrophilic, as opposed to the GdDOTA and GdDTPA complexes.

Gd chelate / MRI / molecular modelling / molecular lipophilicity potential (MLP)

Introduction

DOTA (1,4,7,10-tetraazacyclododecane-*N*,*N'*,*N''*,*N'''*-tetraacetic acid), DTPA (1,4,7-triazaheptane-1,1,4,-7,7-pentaacetic acid) and other aminopolycarboxylates form highly stable Gd(III) ion complexes that are used as chelating agents in magnetic resonance imaging (MRI) techniques [1, 2]. Both the kinetic and thermodynamic stabilities of such complexes are important for their diagnostic use, since lanthanide ions are highly toxic in man.

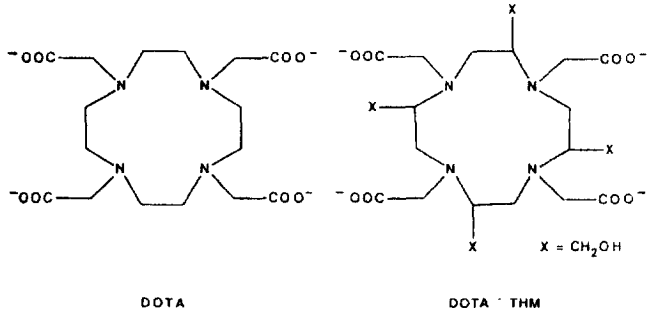
Molecular mechanics calculations and molecular dynamics simulations have previously been performed for several polyaminocarboxylate ligands and their Gd(III) complexes, in order to study structure–stability relationships [3, 4]. As a continuation of this work we have performed molecular mechanics calculations and molecular dynamics simulations of the DOTA derivative, DOTA-THM (1,4,7,10-tetraaza-2,5,8,11-tetrahydroxymethylcyclododecane-*N*,*N'*,*N''*,*N'''*-tetraacetic acid) (scheme 1) and of Gd(III) complexes of this chelate.

*Correspondence and reprints: R Fossheim, Nycomed AS, PO Box 4220 Torshov, N-0401 Oslo, Norway

DOTA-THM contains four hydroxymethyl groups and would be expected to be more hydrophilic than the parent compound DOTA. This might reduce toxicity, provided the thermodynamic and kinetic stability is not significantly altered. In order to investigate the hydrophilic properties of DOTA-THM more fully, molecular lipophilicity potentials (MLPS) [5] have been calculated and compared with those of GdDOTA and GdDTPA.

Methods

Molecular mechanics calculations and molecular dynamics simulations were performed with the all-atom force field of the Amber software package [6] on a Vax 8600/VMS system. Molecular graphics were performed with the Midas programs [7] on an Evans and Sutherland PS390 graphics terminal using a MicroVax II/Ultrix system as the host computer. Energy minimizations were done by 10 steps of steepest descent, followed by full conjugate gradient minimization until convergence. The molecular dynamics simulations were performed for 10 ps at 300 K, after a 1 ps equilibration period starting from 0.1 K. The step-length was 1 fs, and coordinates were stored every 1000 steps. The *in vacuo* calculations were performed with a distance-dependent dielectric function $\varepsilon = r_{ij}$. A constant dielectric function, $\varepsilon = 1.0$, was used for the calculations performed with solvent water molecules. A non-bonded cutoff of 8.0 Å was used in these calculations.



Scheme 1.

Atomic point charges

Atomic charges used in the molecular mechanics calculations and the molecular dynamics simulations were obtained from *ab initio* calculations with the Quest 1.0 program [8], using a STO-3G basis set. Quest calculates electrostatic potentials in several layers around the molecule. Atomic charges are obtained by least squares fitting to the electrostatic potentials. The quantum mechanical calculations were performed on a Cray X-MP computer. Charges for DOTA-THM were obtained from a previous charge calculation on DOTA [3]. Common atoms in DOTA-THM and DOTA were assigned the same charges. The charges for the four hydroxymethyl groups were obtained by a charge calculation on methanol. The charges of atoms in overlapping areas were adjusted in order to give the correct total charge.

Ligands

There are four chiral centres in DOTA-THM. However, due to the four-fold rotation symmetry there are only four isomers (not counting mirror images) having the configurations *RRRR*, *RRRS*, *RRSS* and *RSRS*. Starting structures for the four isomers were obtained with the molecular modelling program Alchemy [9] on a MS-DOS personal computer, and the ligands were then energy minimized *in vacuo*. The energy-minimized structures were then subjected to molecular dynamics simulations. The three structures with lowest potential energy were selected from the coordinate sets and then energy minimized.

In order to examine hydration effects, each of the ligands with lowest conformational energy were hydrated out to 8.0 Å and the energy of the solute–solvent systems were minimized.

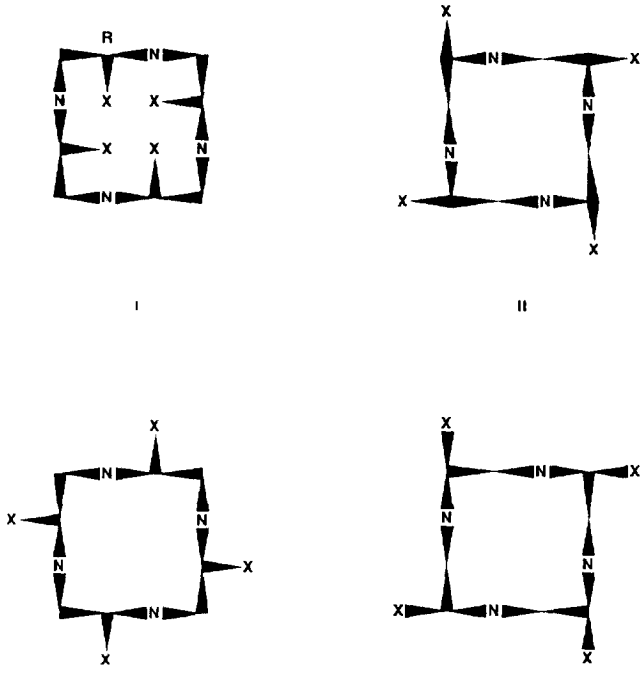
Complexes

Starting models for the complexes were constructed with the graphics terminal and the molecular graphics program Midas [7]. Assuming a [3333] conformation of the 12-membered ring, which is defined by four sequences of *gauche, gauche, anti* torsion angles, the ligand conformations were adjusted to give a coordination geometry as close as possible to a square antiprism, as has been found in the crystal structure of EuDOTA [10] and in calculated structures containing this macrocyclic ring [3, 4]. The crystal structure of GdDOTA, which has been reported since [11], was almost identical to that of EuDOTA.

There are four different substituent positions when the ring adopts a [3333] conformation, corresponding to the four conformers illustrated in scheme 2 for the *RRRR* isomer. The conformers differ in the sense that **I** and **III** have the substituents on the edges of the [3333] square, whereas **II** and **IV** have the substituents on the corners. The pairs **I/II** and **III/IV** are interconvertible through ethylene inversion, *ie* by changing the N-C-C-N torsion angles from *+gauche* to *–gauche*. The pairs **I/III** and **II/IV** are not true conformers, in the sense that they are only interconvertible by processes involving rearrangement of cation coordination. They can be envisaged interconverted by complexation from opposite sides of the macrocyclic ring plane; the process involves inversion of the N-atoms and exchange of the 'up' and 'down' positions for all edge atoms of the 12-membered ring. Which of the 'edge in', 'edge out', 'corner down' or 'corner up' positions the four $-CH_2OH$ groups will occupy, is thus dependent on the configuration and preferred conformation of the complex (scheme 2).

Starting models of the conformers **I–IV** were made for each of the four unique isomers. The pairs **I/III** and **II/IV** are mirror images for the *RSRS* and *RRSS* isomers. The starting models were energy minimized with a water molecule docked into the vacant coordination site on the 4-fold symmetry axis of the antiprismatic coordination polyhedron.

In addition to the different ring conformations described above, two orientations of the four carboxylate groups are compatible with an antiprismatic coordination geometry. The carboxylate groups may reside over the edges of the [3333] square as in the crystal structure of EuDOTA [10] (designated **A**) or they may reside over the corners (**B**), corresponding to a



Scheme 2. Possible [3333] ring conformations for the *RRRR* isomer of DOTA-THM. The *x* position for the $-CH_2OH$ group in the four conformers is labelled 'edge in' (**I**), 'corner down' (**II**), 'edge out' and 'corner up' (**IV**). The chirality of one position (*R*) is shown in **I**.

90° rotation of the square formed by the four coordinating O (carboxylate) atoms. For each of the different ring conformations, starting structures corresponding to **A** and **B** were constructed and energy minimized.

Coordination with the hydroxyl groups is in principle possible in GdDOTA-THM. However, it is only possible if the ring adopts conformations other than the [3333] conformation having the N-atoms on the edges as shown in scheme 2. Such conformations are likely to be high in energy due to the strong preference for the [3333] conformation in such complexes [10], and they have thus not been considered.

In order to examine hydration effects, the four conformers which had lowest energy were hydrated out to 8 Å and energy minimizations were performed on the solute–solvent systems.

Complex stability

The results from energy minimizations of ligands and complexes were used to calculate complex stability. The *in vacuo* stability was calculated as:

$$E_{R,g} = E_{ML} - E_L \quad [1]$$

E_{ML} is the energy of the complex (ligand and cation) and E_L is the energy of the free ligand in its lowest energy conformation found. E_{ML} is derived from the total energy of the ML•H_2O complex (E_T) by subtracting the interaction energy with the coordinated water molecule (E_{II}). Complex stability can also be expressed as:

$$E_{R,g} = E_I + E_{D.I.} \quad [2]$$

E_I is the interaction energy between ligand and cation and $E_{D.I.}$ is the constraint energy of the ligand, taken as the difference in energy between the ligand in the complex conformation and the energy of the free ligand.

The contribution of hydration effects to complex stability [3] was calculated as:

$$E_{R,aq} = E_{R,g} + E_{H1} + E_{H2} \quad [3]$$

E_{H1} is the difference in solvation energy for the carboxylate groups in free ligand and complex. E_{H2} is the solvation energy resulting from cation coordination of water molecules in the complex.

Molecular lipophilicity potential (MLP)

MLP profiles were calculated for the energy-minimized GdDOTA and GdDTPA complexes, and the four GdDOTA-THM isomers. Atomic hydrophobicity constants (f_i-values) for the ligand atoms were taken from Ghose and Crippen [12]. The f_i-value for Gd was derived from experimental log*P* values for GdDOTA (–2.87), GdDTPA (–3.63) and GdDO3A (–2.15) [13] by subtracting the calculated log*P* values of the ligands from the experimental log*P* values. Log*P* is defined as $\log([X]_b/[X]_w)$, where $[X]_b$ is the concentration of the complex in butanol and $[X]_w$ is the concentration of the complex in water. This gives f_{Gd} equal to 0.95, 0.72 and 1.21 for GdDOTA, GdDTPA and GdD03A. The mean value (f_{Gd} = 0.96) was used in the calculations. The water-accessible surface was calculated for the complexes and the values of MLP at a point x this surface were calculated using the esp-program of the Midas package [7] with a constant dielectric function ε = 1.0. MLP is thus calculated according to the equation:

$$\mathrm{MLP}(x) = \Sigma_i f_i/d_i \quad [4]$$

where d_i is the distance from x to atom i. In order to test whether this function gives relative MLP values in agreement with those obtained using the slightly different functional form of MLP suggested by Furet *et al* [5], calculations were also performed for the model compounds acetamide and acetone.

The fact that f(Gd) is derived from the water/butanol system, whereas the f_i-values for the ligand atoms are derived from the water/octanol system, may introduce a systematic error in the calculations. However, this eventual error is expected to be relatively constant, since the surfaces are fairly spherical with the cation at the centre of the sphere.

Results

Ligands and complexes

A common conformational feature for all the free ligands is the presence of intramolecular hydrogen bonds. In all compounds the four hydroxyl groups are hydrogen bond donors to the carboxylate groups, making four intra-molecular hydrogen bonds in each ligand. The molecular mechanical energies of the four ligands were also fairly equal: 79.8, 102.4, 87.8 and 92.4 kJ/mol for the *RRRR*, *RRRS*, *RRSS* and *RSRS* isomers with the lowest energy, respectively. None of the preferred conformations of the free ligands correspond to the [3333] complex conformation. The fairly equal ligand energies suggest that eventual differences in complex stabilities will be determined by the structure and energies of the corresponding complexes.

The energies of the complexes (E_T) and the parent compound DOTA are given in table I, together with the substituent positions occupied in each complex conformation. Stereoscopic drawings of the four complexes having lowest energy are shown in figure 1. The corresponding coordination polyhedra are shown in figure 2. For all the four DOTA-THM isomers, the [3333] conformation **II** is more stable than **I** (table I). Energy minimizations of the isomers *RRRR* and *RRRS* starting from **I** resulted in conformers with unsymmetric ring conformations with high complex energy. This suggests that steric strain would exclude more than two bulky substituents occupying the 'edge in' (ei) positions. From table I it is evident that the calculated complex energy can be related to the positions occupied by the hydroxymethyl groups in the general case. Considering the *RRRR* and *RRRS* isomers, the stability sequence is **II** > **III** > **IV** >> **I**, corresponding to the preference order 'corner down' > 'edge out' > 'corner up' > 'edge in'. The fact that the *RRRR* conformers **II** and **III** have lower energies than the corresponding *RRRS* conformers, whereas the opposite is true for conformer **IV**, also confirms this finding. Furthermore, the smaller energy difference between **I** and **II** for the *RSRS* isomer than the *RRSS* isomer suggests that when two substituents occupy 'edge in' positions, it is energetically more favourable

Table I. Complex energies (E_T) [GdDOTA-THM·H_2O], hydration energies (E_H), ligand–cation interaction energies (E_I) and ligand constraint energies ($E_{D,L}$) for GdDOTA, and GdDOTA-THM conformers.

Compound	*Conformation*	*Substituent position*[a]	*Complex energies (kJ/mol)*			
			E_T	E_H	E_I	$E_{D,L}$
DOTA[b]			−1795.7	−79.8	−2260.5	383.3
DOTA-THM						
RRRR	**II, B**	4 cd	−1728.0	−77.3	−2235.9	504.5
	III, A	4 eo	−1710.0	−78.2	−2209.1	496.2
	IV, B	4 cu	−1628.9	−73.6	−2240.5	604.8
RRRS	**II, B**	1 cu; 3 cd	−1713.0	−76.9	−2221.7	482.8
	III, B	3 eo; 1 ei	−1686.2	−78.2	−2202.0	991.2
	IV, B	3 cu; 1 cd	−1639.4	−75.2	−2245.0	578.5
RRSS	**I, B**	2 eo; 2 ei	−1623.9	−76.5	−2211.2	575.6
	II, B	2 cu; 2 cd	−1668.7	−74.8	−2261.0	578.9
RSRS	**I, B**	2 ei; 2 eo	−1646.9	−78.6	−2192.4	530.9
	II, B	2 cu; 2 cd	−1674.1	−75.2	−2246.3	554.7

[a]Substituent positions occupied by the four hydroxymethyl groups: cu ('corner up'), cd ('corner down'), eo ('edge out') and ei ('edge in'); [b]results from reference [3].

to have the substituents on opposite edges than on neighbouring edges since they are further apart in the former case.

The energy differences between the two different orientations of the carboxylate groups (**A** and **B**), were generally small, and in favour of (**B**). Comparison of *RRRR* (**II**), *RRRS* (**III**), *RRSS* (**I**) and *RSRS* (**I**) shows energy differences of 5.4, 7.9, 7.1 and 0.8 kJ/mol, respectively. Only for *RRRR* (**III**) is **A** more stable (1.3 kJ/mol). It is only when 'corner up' positions are occupied that the energy differences become substantial between the two orientations of the carboxylate groups (range 14.2–31.8 kJ/mol), and always in favour of **B**.

The coordination polyhedra of the four complexes (fig 2) corresponding to carboxylate conformation **B**, may be described as distorted monocapped antiprisms. The oxygen atoms are located slightly closer to one of the nitrogens of the square base.

Complex stability

Calculated complex stabilities according to equations [1]–[3] are given in table II for the DOTA-THM isomers and DOTA. For the four DOTA-THM isomers both the $E_{R,g}$ and $E_{R,aq}$ values suggest the following stability sequence, *RRRR* = *RRRS* > *RRSS* = *RSRS*, within the uncertainties of the calculations. Comparison of the energies needed to bring the free ligands into their respective complex conformations ($E_{D,L}$) show that this parameter is significantly smaller for the *RRRR* and *RRRS* isomers than the *RSRS* and *RRSS* isomers, in accordance with the calculated energies of the complexes described in the preceding section.

Table I shows that the ligand constraint energy ($E_{D,L}$) is significantly higher for all DOTA-THM isomers than for DOTA. On the other hand, the calculated desolvation energies of the carboxylate groups resulting from complex formation (E_{HL}), are all smaller for DOTA-THM than for DOTA. Both these effects can be ascribed to the presence of intramolecular hydrogen bonds in the DOTA-THM free ligands [4]. Thus, when comparing the $E_{R,aq}$ values, which have previously been related to experimental log*K* values [4], the *RRRR* and *RRRS* isomers are calculated to be equi-stable with GdDOTA.

It has recently been shown by others [14] that our calculated ligand constraint energies are correlated with the experimental activation energy ($\Delta G^{\#}$) for reorganization of the intermediate Gd(·HL) for the macrocyclic ligands NOTA, DO3A and DOTA. This relationship suggests that the DOTA-THM isomers have different kinetics of complex formation. The activation energies that can be inferred from the regression line are 37.2, 36.8, 39.3 and 38.9 kJ/mol for the *RRRR*, *RRRS*, *RRSS* and *RSRS* isomers, respectively. Thus, the two isomers that are calculated to be least stable are those which we predict to have the highest activation energies. The activation energies for all four isomers are higher than for GdDOTA (34.2 kJ/mol), but lower than for GdDO3MA (45.0 kJ/mol).

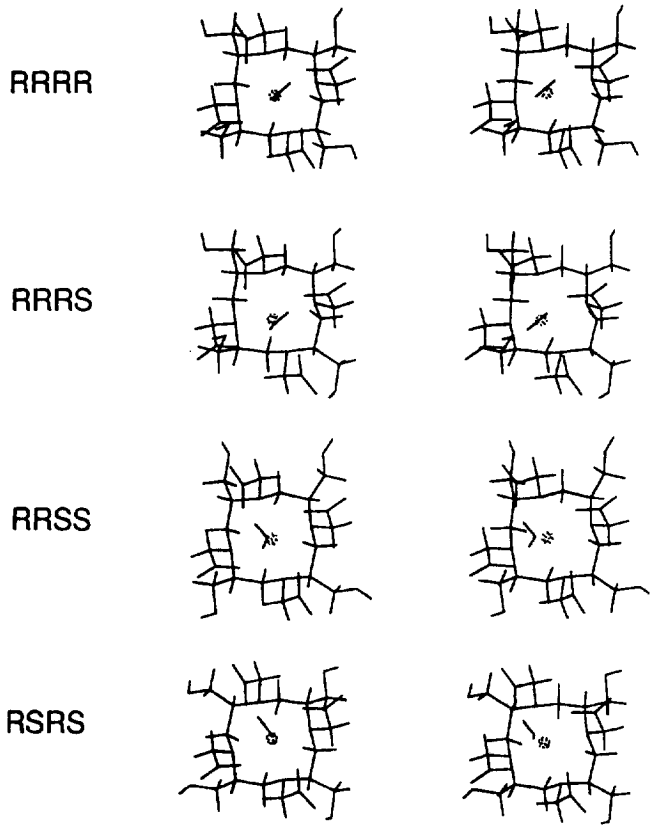


Fig 1. Stereoscopic drawings of the *RRRR*, *RRRS*, *RRSS* and *RSRS* configurated GdDOTA-THM·H_2O complexes.

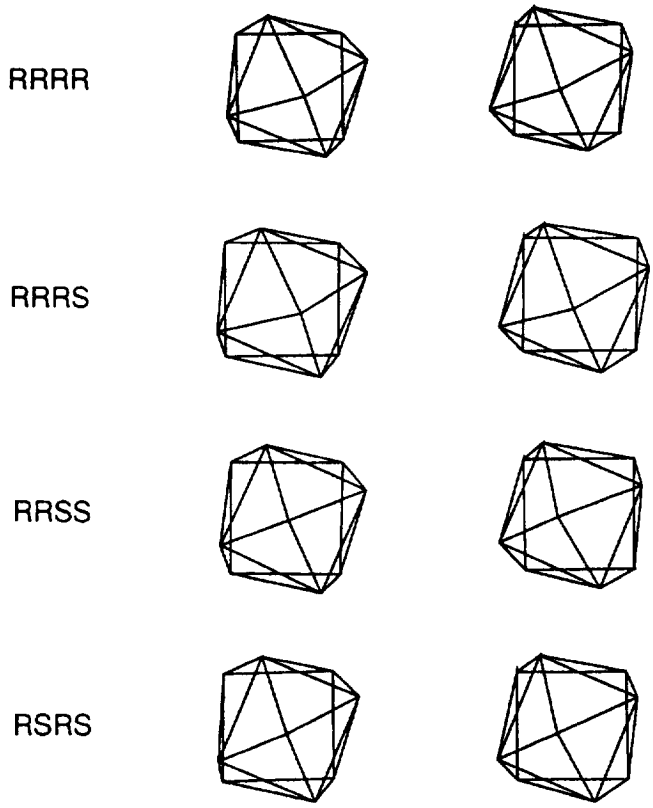


Fig 2. Stereoscopic drawings of the coordination polyhedra for the *RRRR*, *RRRS*, *RRSS* and *RSRS* configurated GdDOTA-THM·H_2O complexes. The coordination polyhedra have been tilted 45° with respect to the view given in figure 1.

Molecular lipophilicity potentials

The calculations performed for acetamide and acetone suggest that equation [3] gives MLP values in relative agreement with those found using a slightly different functional form of MLP [5]. Frequency distributions for the MLP-values calculated on the water-accessible surfaces of GdDTPA, GdDOTA and the four DOTA-THM isomers are shown in figure 3. Only negative MLP values appear on the water accessible surfaces of the six complexes, reflecting their hydrophilic character. However, the range and distribution of MLP values differs among the compounds. GdDTPA has a larger fraction of strongly negative MLP values than GdDOTA, corresponding to a more hydrophilic complex. Comparison of the GdDOTA-THM isomers with those of GdDOTA and GdDTPA shows that the MLP range is shifted towards more negative values. Furthermore, the distribution functions of these four complexes are clearly closer to Gaussian type functions than those of GdDOTA and GdDTPA. The histograms in figure 3 do not show any distinct differences between the four GdDOTA-THM complexes.

Discussion

The present molecular mechanics calculations and molecular dynamics simulations on the four isomers of DOTA-THM (1,4,7,10-tetraaza-2,5,8,11-tetrahydroxymethylcyclododecane-*N*,*N*',*N*'',*N*'''-tetra-acetic acid) and Gd(III) complexes of these, suggest a certain preference order for the four unique substituent positions. The calculated energies of the possible [3333] conformations of the four isomers suggest that the position designated 'corner down' is energetically favoured, followed by 'edge out', 'corner up' and 'edge in' which is the least favoured

Table II. Reaction energy *in vacuo* ($E_{R,g}$), solvation energy difference of carboxylate groups in the free ligand and the complex (E_{H1}), solvation energy of Gd(III) in the complex (E_{H2}) and reaction energy in an aqueous environment ($E_{R,aq}$) for DOTA and DOTA-THM (kcal/mol).

Complex	$E_{R,g}$	E_{H1}	E_{H2}	$E_{R,aq}$
GdDOTA[a]	−1877.2	400.0	−123.7	−1600.9
GdDOTA-THM (*RRRR*)	−1731.4	275.9	−120.8	−1576.3
GdDOTA-THM (*RRRS*)	−1738.9	262.9	−120.4	−1596.8
GdDOTA-THM (*RRSS*)	−1682.0	302.2	−118.3	−1498.1
GdDOTA-THM (*RSRS*)	−1691.2	280.5	−118.7	−1529.5

[a]Results from reference [3].

position. This finding is in agreement with both the stability order of the possible [3333] conformations for each isomer, and the calculated stabilities of the lowest energy conformers of the four isomers. When the *RRSS* and *RSRS* isomers adopt their most stable [3333] conformation, two hydroxymethyl groups are forced to occupy 'corner up' positions as opposed to one and none for the *RRRS* and *RRRR* isomers, respectively.

The two orientations of the four carboxylate groups that are compatible with a monocapped antiprismatic coordination geometry differed only slightly in energy (less than 7.9 kJ/mol), provided the hydroxymethyl groups do not occupy 'corner up' positions. The conformation having the carboxylate groups over the corners of the [3333] square (**B**) were preferred in most cases (table I). When 'corner up' positions are occupied, steric interactions between the carboxylate groups and the hydroxymethyl groups clearly favours this orientation (**B**). As a result of this, the corresponding coordination polyhedra show slight distortions of the monocapped square antiprismatic coordination geometry (C_{4v} symmetry) towards that of a monocapped cube (fig 2). It may be noted that fairly large distortions of lanthanide ion coordination polyhedra towards D_4 symmetry have been found in crystal structures, most notably in the crystal structure of the octakis-pyridine-*N*-oxide-La(III) ion [15].

These calculations do not consider entropy effects. However, we believe entropy differences to be small when comparing the GdDOTA-THM isomers and GdDOTA. The ligands are all cyclic with the same number of ring atoms, and all the complexes coordinate one water molecule. Large differences in reaction entropies might be expected when comparing cyclic *versus* non-cyclic ligands and when comparing complexes that coordinate different numbers of water molecules.

The calculated MLP of the four GdDOTA-THM isomers, GdDOTA and GdDTPA, suggest that the four hydroxymethyl groups make the complexes more hydrophilic than GdDTPA and GdDOTA, as may have been expected. Equally important, the four $-CH_2OH$ groups make large parts of the water-accessible surface equi-hydrophilic, as suggested by the near Gaussian frequency distribution function of the MLP values found for the GdDOTA-THM isomers, but not for GdDOTA and GdDTPA. Judged from the histograms shown in figure 3, the four isomers are difficult to distinguish with respect to MLP. However, it may be noted that intramolecular hydrogen bonding and the effect this may have on water solubility are poorly accounted for by MLP values. Intramolecular hydrogen bonding between the carboxylate groups is only possible in complexes having the hydroxymethyl groups in 'corner up' positions. Figure 1 shows that intramolecular hydrogen bonding may be achieved for the most stable conformers of isomer *RRSS*, *RSRS* and *RRRS*, but not for *RRRR*.

Considering the four isomers as potential MRI agents, the present calculations suggest that the *RRRR* and *RRRS* isomers are the best candidates when judging complex stability. The four hydroxymethyl groups give the four isomers of the DOTA-THM complexes a molecular surface that is highly and uniformly hydrophilic. High hydrophilicity of GdDOTA derivatives has been shown to reduce toxicity [16], thus suggesting that GdDOTA-THM may have reduced toxicity compared with the parent compound GdDOTA.

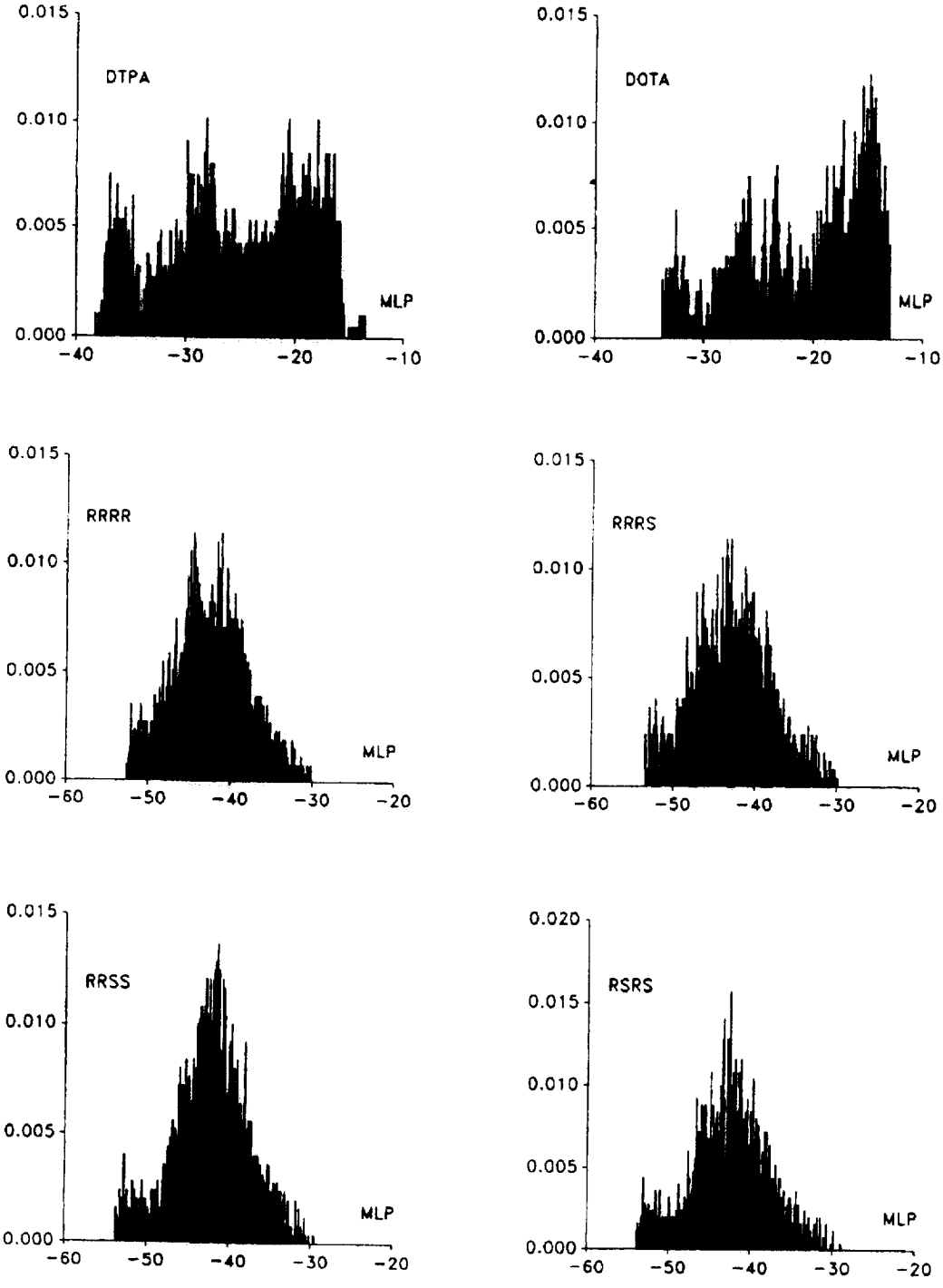


Fig 3. Histograms showing the frequency distribution of MLPs, calculated on the water-accessible surface of GdDTPA, GdDOTA and the four GdDOTA-THM isomers.

References

1 Desreux JF, Barthelemy PP (1988) *Nucl Med Biol* 15, 9–15
2 Lauffer RB (1987) *Chem Rev* 87, 901–927
3 Fossheim R, Dahl SG (1990) *Acta Chem Scand* 44, 698–706
4 Fossheim R, Dugstad H, Dahl SG (1991) *J Med Chem* 34, 819–826
5 Furet P, Sele A, Cohen NC (1988) *J Mol Graphics* 6, 182–189
6 Singh UC, Weiner PK, Caldwell JW, Kollman PA (1986) Amber (UCSF Version 3.0), Department of Pharmaceutical Chemistry, University of California, San Francisco, CA, USA
7 Ferrin TE, Huang CC, Jarvis LE, Langridge R (1988) *J Mol Graphics* 6, 13–37
8 Singh UC, Kollman PA (1984) *J Comp Chem* 5, 129–135
9 Alchemy, Tripos Associates Inc, 1699, South Hanley Road, Suite 303, Saint Louis, MO 63144, USA
10 Spirlet MR, Rebizant J, Desreux JF, Loncin MF (1984) *Inorg Chem* 23, 359–363
11 Dubost JP, Leger JM, Langlois MH, Meyer D, Schaefer M (1991) *CR Acad Sci Paris Ser II* 312, 349–354
12 Ghose AK, Crippen GM (1986) *J Comp Chem* 7, 565–577
13 Wedeking P, Dischino D, Emswiler J, Chang CA, Tweedle MF, Eckelman WC (1989) Society of Magnetic Resonance in Medicine, Eighth Annual Meeting, Amsterdam, The Netherlands
14 Tweedle MF, Kumar K (1993) *Inorg Chem* 32, 4193–4199
15 Al-Karaghouli AR, Wood JS (1972) *Chem Comm* 516–518
16 Gries H, Frenzel T, Niedball U *et al* (1991) Society of Magnetic Resonance in Medicine, Tenth Annual Meeting, San Francisco, CA, USA