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New designs for MRI contrast agents

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Summary

New designs for Magnetic Resonance Imaging contrast agents are presented. Essentially, they all are host–guest inclusion complexes between γ-cyclodextrins and polyazamacrocycles of gadolinium (III) ion. Substitutions have been made to the host to optimise the host–guest association. Molecular mechanics calculations have been performed, using the UFF force field for metals, to decide on the suitability of the substitutions, and to evaluate the host–guest energies of association. Interesting general conclusions have been obtained, concerning the improvement of Magnetic Resonance Imaging contrast agents; namely, a set of rational methodologies have been deduced to improve the association between the gadolinium (III) chelates and the cyclodextrins, and their efficiency is demonstrated with a large set of substituted complexes, opening new doors to increase the diagnostic capabilities of Magnetic Resonance Imaging.

Introduction

Nuclear Magnetic Resonance (NMR) is a widely used technique with exciting biomedical applications such as Magnetic Resonance Imaging (MRI) *in vivo*. In MRI, a powerful diagnostic tool in clinical practice, the use of contrast agents (CA) is essential to increase the contrast of the images obtained by this technique, allowing a clear distinction between healthy and diseased tissues [1].

Lanthanide chelates are extremely effective as CAs, increasing the T_1 relaxation rate of the water protons. They allow the obtention of very high resolution images. These compounds have been used with much success, and recent research has focussed on the design of new polyazamacrocyclic ligands for some trivalent lanthanide cations (in particular for Gd), namely DOTA- and DOTP-like derivatives[1] [1, 2], resulting in the Gd-DOTA and Gd-DPTA chelates.

This study is devoted to the optimisation of the contrast agent $[GdDOTA]^-$ (trade name: Dotarem®). $[GdDOTA]^-$ is currently being used with success in the diagnosis of cerebral and medullar pathologies, as well as other vascular diseases, being one of the most important contrast agents for Magnetic Resonance Angiography (MRA) [3, 4].

The paramagnetic gadolinium (III) ion acts predominantly on the T_1 relaxation of water protons, which results in signal enhancement and 'positive' contrast. Translational diffusion of solvent molecules and the chelate as well as specific chemical interactions, that bring the solvent molecules near the metal ion, seem to be determinant to the efficiency with which the T_1 relaxivity of water is enhanced [1–2, 5].

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[1] (DOTA≡1, 4, 7, 10-tetraazacyclododecane-1, 4, 7, 10-tetraacetate; DOTP≡1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonate).

Proton T_1 relaxivities may be further enhanced through the formation of host–guest non-covalent complexes between the CA and slowly tumbling macromolecules such as oligosaccharides or some proteins like albumin [6]. The decreased rate of molecular tumbling enhances the electron-nuclear interaction between the Gd(III) ion and water, leading to very short relaxation times and consequently very bright blood vessels on an MRA image [1, 7]. Additionally, it seems fair to assume that the biodistribution of the agent could be altered *in vivo* with such inclusion complexes. In this way, the specific biodistribution of the CAs, as well as its blood half life and excretion rate can be optimised according to the diagnostic needs. The use of cyclodextrins (CD) as CAs hosts may address the above mentioned aspects. Cyclodextrins are one of the most promising and widely used oligosaccharide hosts in host–guest drug complexation. It is known that cyclodextrin encapsulated drugs usually have increased bioavailability and half-life in the organism, as well as good excretion properties and absence of toxicity [8, 9]. Additionally, it is thought that the increase of the total mass of the complex, as well as the distortion of the shape of the CA, from the quasi-spherical shape of the $[GdDOTA]^-$ complex to the more irregular shape of the CD:$[GdDOTA]^-$ complex would result in a slower dynamics of rotation in water [10], enhancing contrast. In spite of the enormous potential of CD:$[GdDOTA]^-$ complexes as contrast agents, previous studies [11] led us to conclude that the association between the CDs and lanthanide (III) chelates with polyazamacrocyclic ligands of the type described above is very weak. The main problem is that the largest of the CDs that is able to make inclusion complexes in physiological media (the γ-CD) has a cross section too small to fully include the CA. Instead, the guest penetrates only partially in the CD, making the inclusion inefficient. A possible solution would be the use of substituted γ-CDs that associate more efficiently with the CA. For that purpose we have investigated the results of several substitutions carried out in host–guest complexes of the γ-CD:lanthanide (III) chelate type. All substitutions have been performed in the host, i.e., the γ-CD. The results have been surprisingly good and of general interest to those involved in the field.

It could also be thought that changes in the ligand $DOTA^{4-}$ could lead to a better association with the CD. Successful techniques for *de novo* ligand design have been developed by Hay and co-workers, among others [12–15]. However, we did not attempt to re-design the ligand because $DOTA^{4-}$ is already an excellent ligand for Gd(III) [1, 2]. Moreover, it might be dangerous tampering with the lanthanide (III) chelate for danger of weakening the complexation with the lanthanide ion, the toxicity of which is well known to be fatal to the human body. Nevertheless, the main concepts derived for host metal design can indeed be applied here for the γ-CD:$[GdDOTA]^-$ complex.

There are three main lines drawn in the work of Hay [12–15] to design a good host: (1) existence of multiple binding sites; (2) the ability to adopt a conformation where all binding sites are oriented to complement the guest; (3) a limited degree of conformational freedom. All these points were explored here. The frequent use of multiple mutations in a single CD is an example of the first rule. Point 2 was the most difficult to optimize. The γ-CD was not large enough to fully accommodate the guest, and bigger CDs have several disadvantages, as discussed in the text. Several attempts to increase the γ-CD height were made. The most used approach was to use 2–3 atom chains with the binding sites at the end of the chain. Finally, point 3 is fully adopted by the host γ-CD, which has a rather limited conformational freedom.

Methodology

Geometries

Crystallographic structures for some lanthanide (III) chelates of $DOTA^{4-}$ or closely related ligands are available [16–20]; the same is true for DOTP-like chelates [21]. Reports of NMR studies on the solution dynamics of lanthanide (III) polyazamacrocycles [17, 22], and on their interactions with possible host molecules are also known [23–25].

We have used a crystallographic structure for $[GdDOTA]^-$. Figure 1 shows a representation of the structure of $[GdDOTA]^-$, alone and included in the γ-CD. The crystal structure of the γ-cyclodextrin-12-crown-4 1:1 inclusion complex [26] was used as a starting template to model the host–guest complex structures. $[GdDOTA]^-$ was placed inside the empty cavity of the γ-CD molecule, and an appropriate conformational analysis was carried out for the complex [11]. In fact, to find out the best fit of the guest inside the host, we first carried out systematic searches to minimize the energy of the different scanned conformations. These were obtained by gradually varying distance d of the host to the guest, and angle θ defined

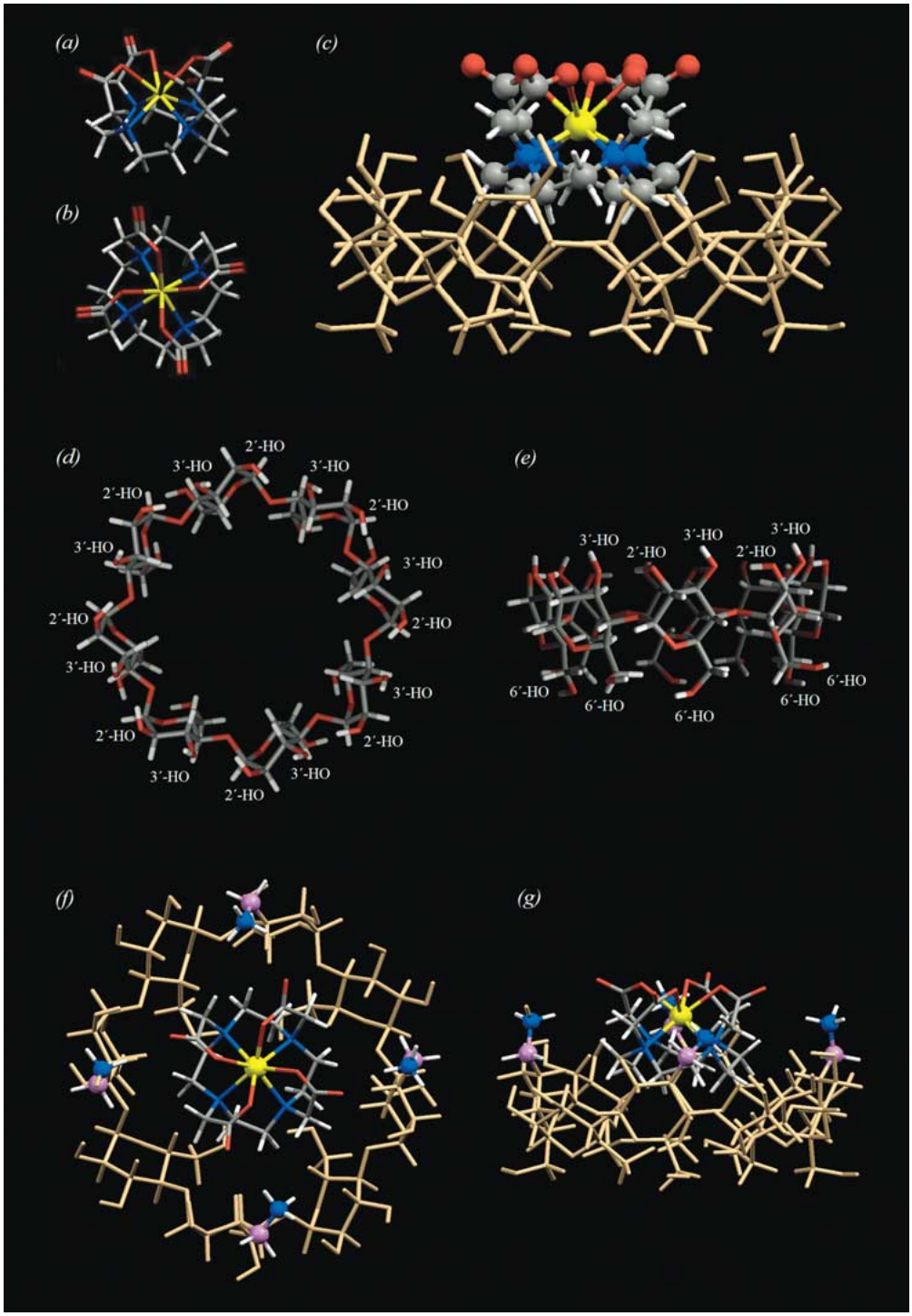


Figure 1. *(a, b)* Structure of $[GdDOTA]^{-}$ complex (side and top views); *(c)* structure of host–guest complex γ-CD:$[GdDOTA]^{-}$; *(d, e)* structure of γ-cyclodextrin and numbering for the hydroxyl groups used throughout the text (top and side views); *(f, g)* top view and side view of the optimised geometry of the γ-CD:$[GdDOTA]^{-}$ complex with substitution 1.10.

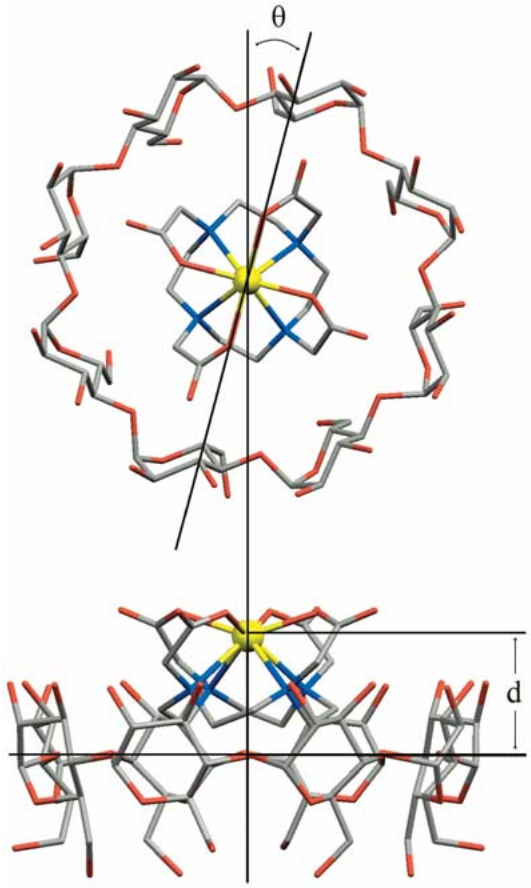


Figure 2. Parameters used on the conformational analysis performed for the complex γ-CD:[GdDOTA]$^{-}$.

by the position of the chelate relative to the γ-CD (see Figure 2) [11].

Molecular mechanics calculations

All calculations were performed *in vacuo*, and with the software package Gaussian98 [27]. The UFF force field for metals [28] was used, within Gaussian98, to run molecular mechanics geometry optimisation calculations. Here, we chose to use classical mechanics to perform our geometry optimisations due to the size of the system. Both Gd and the γ-CD have been parameterised for the UFF force field. In fact, this is the only force field which has been parameterised for all elements of the Periodic Table. Several UFF benchmarks have been published. The average errors have been determined for molecules where reliable experimental data was available for comparison. Typical deviations from experiment found in several organic and inorganic molecules are just a few (1–8) kJ/mol, and at most, a few dozens (less than 40) kJ/mol [29, 30]. For the purpose of this work, this error amplitude is perfectly acceptable since, as will be shown, the binding energies obtained with the substitutions were in the several hundred kJ/mol scale.

The geometries are also well reproduced by this force field. In organic molecules, average deviations from experiment are 0.021 Å for C-C bond length and 0.024 Å for C-N, and 5° to 10° in angle bend [31]. With metallic complexes, typical errors are generally less than 0.05 Å for bond lengths, and angles usually do not show errors larger than 5° to 10° [30].

The visualization of all geometries as well as all substitutions in both the host and guests were carried out with the help of the programs Gaussview [32] and Molden [33].

UFF uses partial charges calculated by the method of charge equilibration [34]. The advantage of this method is that the point charges are geometry-dependent. However, it is not efficient to calculate the point charges at each optimisation step, as it would make the calculations too slow. Hence, we calculated the point charges only at the beginning and at the end of the geometry optimisation. After each optimisation calculation was complete the charges were recalculated for the optimised geometry, and the convergence criteria were checked again. If the geometry was considered as not optimised with the final charges, the optimisation was repeated in a self-consistent procedure until the geometry fulfilled the convergence criteria with both the initial and final charges. This usually was achieved after 3–6 optimisation runs. The viability of this approximation was tested by running the same calculation both determining the point charges at every optimization step, and by using the self consistent method calculating the point charges only at the beginning and at the end of each optimization. The final result was exactly the same, proving that the minimum found in both calculations is the same.

To calculate the energy of association we used the following procedure. We began by introducing the substitutions on the γ-CD:[GdDOTA]$^{-}$ complex and optimising the geometry. Starting from that geometry the substituted γ-CD was obtained by deleting the central [GdDOTA]$^{-}$ complex and re-optimising the geometry. The energy of association was calculated as (with obvious notation):

$$\Delta E^{subs}_{assoc} = \Delta E^{original}_{assoc} + \left\{ (E^{subs}_{CD:(Gd(DOTA))^-} - E^{subs}_{CD}) - (E^{original}_{CD:(Gd(DOTA))^-} - E^{original}_{CD}) \right\} \quad (1)$$

Assuming that the entropic contribution for ΔG_{assoc} is similar for the original and substituted complexes, the free energy variation induced by the substitution can be approximated by the corresponding energy difference:

$$\Delta\Delta G_{assoc} = \Delta G^{subs}_{assoc} - \Delta G^{original}_{assoc} \approx \Delta E^{subs}_{assoc} - \Delta E^{original}_{assoc} \quad (2)$$

Results and discussion

Due to the massive number of substitutions here explored we divided the results into four tables, each dealing with a different substituent. Unless otherwise specified, the substitutions in the top part of the γ-CD were made on the 2′-HO group and the substitutions in the bottom part of the γ-CD were made on the 6′-HO group. Figures 1d and 1e show the numbering of the groups in which substitutions were carried out.

Before beginning the discussion, some points need to be clarified, namely:
– all values presented in Tables 1 to 4 are absolute energy values calculated from expression (1);
– when discussing the quality of a substitution, a favourable one refers to a substitution with a negative association energy lower than the original complex's association energy of -105 kJ mol^{-1};
– where stereochemical (steric) strain or tension is invoked, it refers to local repulsions, resulting from atomic distances too short.

Ammonium substituents

We began by exploiting substitutions in the top region of the γ-CD. This region is hydrophilic, with the four negative oxygens of the ligand DOTA^{4-} pointing to the interior of the γ-CD. The most intuitive substitution would be to replace 2′-HO groups by positively-charged groups, to form ion-dipole interactions between the host and the guest. We have used two positively-charged groups, the ammonium and the trimethylammonium groups. Initially we believed that the former would be more appropriate, due to its reduced size. However, it has the disadvantage of changing its protonation state (and hence the charge) with the pH. Although that can be easily overcome by performing additional substitutions in the host that assure a protonated state at physiological pH, the use of substituted ammonium groups (as the trimethylammonium group) have been confirmed to be preferential in energetic terms. Another important point is that all substitutions were made symmetrically, i.e., when a given hydroxyl group was substituted, the same substitution was performed in the opposite glucose ring. That assured that the ligand did not move from the centre of the γ-CD, maximizing all interaction between the host and the guest. Table 1 shows the obtained results with the ammonium substituent and its derivatives. Figures 1f and 1g show the result of substitution 1.10, where the aliphatic chains have been coloured pink.

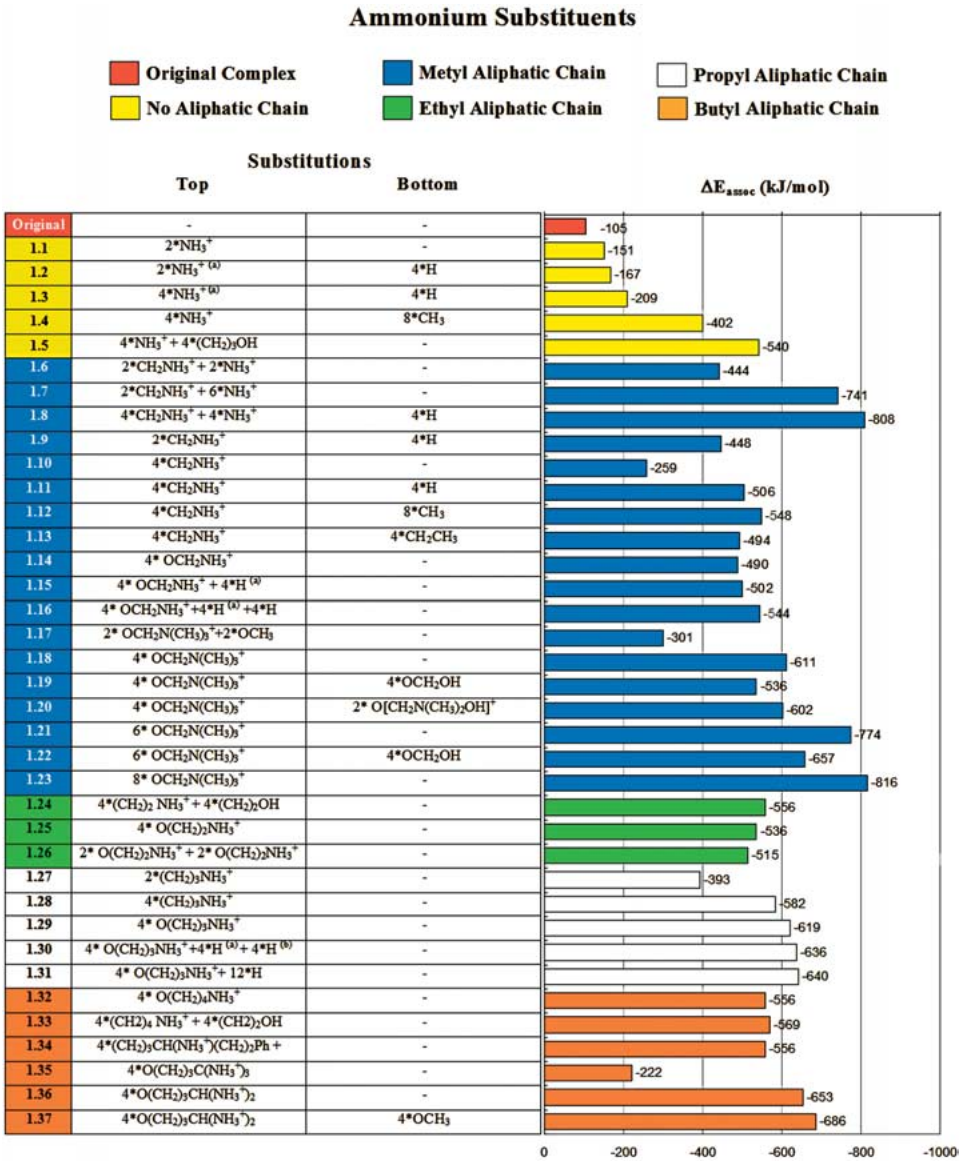
The first substitutions (1.1–1.5) consisted in the introduction of ammonium groups in the top region, and directly connected to the γ-CD. Substitutions were also performed in the bottom part of the γ-CD. Substitution of the 6′-HO group by hydrogens has the advantage of decreasing steric tension, and allowing the ligand to penetrate deeper into the γ-CD, especially when large groups are introduced in the top. Aliphatic chains in the bottom of the γ-CD do not allow such steric relaxation, but increase the van der Waals interactions with the hydrophobic bottom region of the ligand. All these substitutions have proved to be very favourable, greatly enhancing binding, mostly due to the interaction of the ammonium groups with the DOTA^{4-} oxygens.

Upon increasing the number of ammonium substituents (from two to four) binding was further improved. However, the optimised geometries showed that the ammonium groups were not in an optimal position, being too distant from the DOTA^{4-} oxygens (3.4 Å). Therefore, the next substitutions did include an aliphatic chain to approach them both.

In substitutions 1.6–1.23 a methyl group was inserted between the γ-CD and the ammonium group. In general, the results were better than without the aliphatic chain. The larger the number of substituents, the larger the stabilization in terms of energy.

Substitutions in the bottom part of the γ-CD also proved favourable. With substitutions 1.10–1.13 it can be seen that the best compromise between steric tension and increased van der Waals interactions is achieved with all 6′-HO groups replaced by methyl groups. Substitutions 1.14–1.16 clarify that the elimination of the remaining hydroxyl groups in the top of the γ-CD also stabilized further the complex. Those groups were indeed competing for establishing hydrogen bonds with the ammonium groups, and this did not contribute to a better host–guest complexation.

Table 1. Substitutions (1.1–1.37) carried out in the γ-CD of the γ-CD:[GdDOTA]$^{-}$ complexes. By default, top substitutions replaced 2′-hydroxyl groups and bottom substitutions replaced 6′-hydroxyl groups. All energies in kJ/mol. (a) Substitutions in 3′-hydroxyl groups; (b) Substitutions on the adjacent glucose unit.



In substitutions 1.17–1.23 the trimethylammonium substituent was tested. The results have shown that this substituent is better than ammonium, in the sense that this substitution resulted in stronger binding. It was possible to successively improve binding by increasing the number of substituents, up to a maximum of eight (one in each ring).

In the bottom region all substitutions proved unfavourable. This is due to the large volume of the top substitutions, which preclude DOTA^{4-} to be displaced upwards by the bottom substituents, causing steric tension. The successive increase of the aliphatic chain, from a methyl chain to a propyl chain revealed to be favourable (compare 1.14 with 1.25 (ethyl chain)

and with 1.29 (propyl chain)). Increasing the aliphatic chain even more does not stabilize further the complex, due to the large volume of the chains (1.32). The elimination of further hydroxyl groups also revealed to be favourable (1.30 and 1.31), for the same reasons given above. The trimethylammonium groups attached to a butyl chain become too bulky and steric repulsion between them destabilized the complex. The removal of one of the methyl groups (dimethylammonium substituents) resulted in a considerable stabilization of the complex (1.35–1.37).

Hydroxyl substituents

Having explored the effect of the ammonium substituents we proceeded by using a different approach to optimise the host–guest association. The γ-CD itself already has very good terminal groups to form strong intermolecular hydrogen bonds. Those groups are the hydroxyl groups at the top (2′-HO and 3′-HO). The problem is that the $DOTA^{4-}$ oxygens are too distant from them (3.9 Å–4.1 Å), and the interaction is weak. One can optimise those hydrogen bonds just by giving mobility to the hydroxyl groups. To that purpose we replaced them by aliphatic chains terminated by a hydroxyl group. The results are described in Table 2.

The association energies presented in Table 2 are not as low as those with ammonium substituents (Table 1), as expected. However, when compared with the unsubstituted complex, the host–guest association improved markedly if an ethyl or propyl chain was used. Considering that the association energy of the unsubstituted complex is −105 kJ/mol, we can see that a simple methyl chain is not enough to improve the interaction (2.1–2.2). Instead it drives the hydroxyl groups further away from the $DOTA^{4-}$ oxygens, forming hydrogen bonds with the other γ-CD hydroxyl groups, and increasing the association energy. The removal of the remaining 2′-HO improved the association by eliminating intramolecular hydrogen bonds with the substituents.

The ethyl substituent (2.4) gave much better results, with the tetra-substituted γ-CD exhibiting very low association energy. The increase of the number of substituents improved the association (2.3–2.4). The same was noted using longer propyl chains (2.5–2.6), where we obtained a good stabilizing effect with only two substitutions. However, when we increased the number of substitutions to four, the binding did not improve as much as expected and resulted in an energy equivalent to the one obtained with ethyl chains. The main reason is that the steric crowding in the top of the γ-CD began to be important. An evidence of that is obtained analysing the results of the substitutions using butyl chains (2.7–2.8), where the double-substituted γ-CD associated better to the guest than the one with four substitutions.

Non-polar substituents

To further explore the capacities of the γ-CD as a host to the contrast agent we decided to study another conceptual approach to optimise the host–guest complex. As can be noted in Figures 1c, 1f and 1g the cross section of the γ-CD is not large enough to accommodate the ligand. To improve that we tried to insert large aliphatic groups in the top region to increase the γ-CD depth. That would allow the ligand to become fully inserted in the host. Therefore, the next substitutions are not directed to interact with the $DOTA^{4-}$ oxygens, but instead to interact with the ligand as a whole, whose central and bottom parts are largely hydrophobic.

Another simple approach to improve the inclusion would be not to increase the γ-CD height, but instead to increase its cross section, by removing specific groups. The results obtained for both methodologies are shown in Table 3.

The first two substitutions (3.1 and 3.2) resorted to the concept of increasing the internal volume of the γ-CD. In the first (3.1) four 2′ hydroxyl groups were removed. As they are pointing to the interior of the host, the substitution increases the internal free volume of the γ-CD. The result is positive, in the sense that binding is enhanced when compared with the original system. To further increase this effect we removed all the hydroxyl groups from the top and simultaneously from the bottom of the γ-CD (3.2). The substitutions in the bottom part allow the guest to penetrate more deeply into the host. The result was extremely valuable, resulting in much stronger binding, comparable with the best substitutions using ammonium substituents. Moreover, this last modified γ-CD has not the problem of changing its complexation properties with its protonation state. It has the further advantage of using a philosophy that is directly transferable to the physiological medium, whereas in the preceding cases (Tables 1 and 2) the water molecules can eventually compete with the $DOTA^{4-}$ for the establishment of hydrogen bonds with the polar substituents. The next substitutions (3.3–3.9) have been devised to improve the γ-CD ‘cage’. They are all very similar and all of them improved complexa-

Table 2. Substitutions (2.1–2.8) carried out in the γ-CD of the γ-CD:[GdDOTA]$^-$ complexes. By default, top substitutions replaced 2′-hydroxyl groups and bottom substitutions replaced 6′-hydroxyl groups. All energies in kJ/mol.

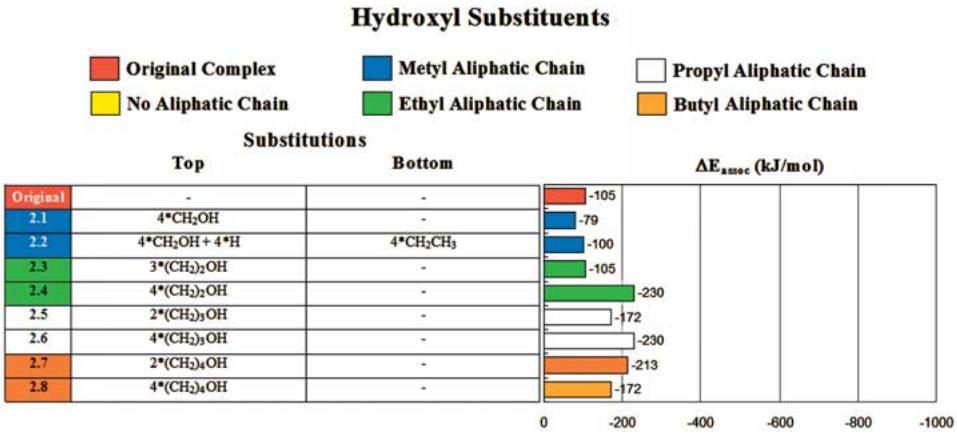
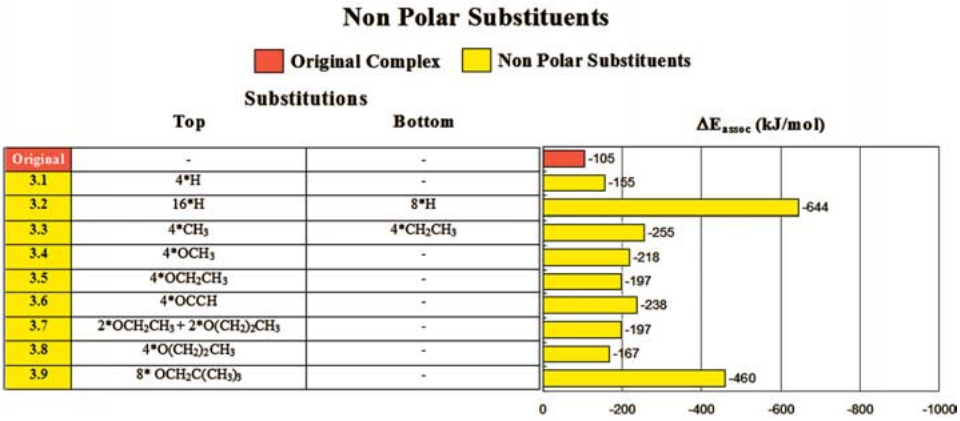


Table 3. Substitutions (3.1–3.9) carried out in the γ-CD of the γ-CD:[GdDOTA]$^-$ complexes. By default, top substitutions replaced 2′-hydroxyl groups and bottom substitutions replaced 6′-hydroxyl groups. All energies in kJ/mol.



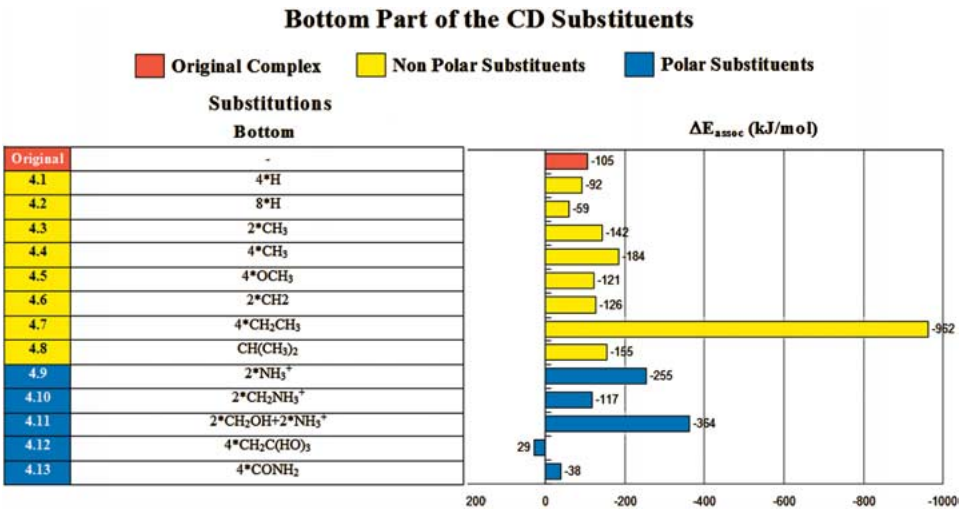
tion. The resulting binding is, however, not as strong as previously obtained by increasing the host internal volume. The best result (3.9) is obtained upon substituting all the 2′-HO groups by ethyl chains terminated with 2,2-dimethylbutyl groups. The overall results from this philosophy can be considered quite pleasing.

Bottom part of the CD substituents

Finally, a last approach was undertaken in this work, which consisted in making substitutions only in the bottom part of the γ-CD. Such a method is based in the assumption that substitutions in the bottom region will displace the guest molecule upwards. In that sense, the inexistence of top substitutions would allow the guest to move upwards without the steric tension that would be imposed by bulky substitutions on the top. The results are shown in Table 4.

Substitution 4.1 indicates that elimination of the bottom hydroxyl groups is unfavourable for complexation, and substitution 4.2 reinforces this finding. It looks as if to increase only the bottom free volume of the host is not a good strategy. Therefore, we turned to

Table 4. Substitutions (4.1–4.13) carried out in the γ-CD of the γ-CD:[GdDOTA]$^{-}$ complexes. By default, top substitutions replaced 2′-hydroxyl groups and bottom substitutions replaced 6′-hydroxyl groups. All energies in kJ/mol.



increasing the interactions between the γ-CD bottom part and the guest, expecting that those interactions would overcome additional steric strain. Substitutions 4.3–4.6 reveal that insertion of a hydrophobic methyl chain favours complexation, by enhancing binding. In this case, the newly formed van der Waals interactions overcome additional steric strain. As the results were not excellent, we tried to increase the length of the aliphatic substituent to an ethyl chain (4.7). The stabilization of the complex was then much more significant, resulting in an excellent host–guest association energy. Following that line of thought we increased even further the aliphatic substituent, to increase more the van der Waals interactions. Starting from the previous substitution we inserted an additional methyl group in the hydrophobic chain, to generate an i-propyl substituent. As the bottom cross section of the host is shorter than the top one, we could not use n-propyl as a substituent because the four propyl chains would overlap. Binding was reduced, showing that the balance between the new van der Waals interactions and the increased steric strain was biased towards the second (4.8). Thus, the bigger bottom part of the CD substituent that optimises complexation is the ethyl substituent. Larger ones destabilize the complex due to their large volume.

Although the base of the ligand DOTA^{4-} is not hydrophilic, we also tested the effect of using polar and charged residues in the host–guest association. Analysing the obtained results (Table 4, substitutions 4.9–4.13) we can see that polar groups in the bottom region also favour association. However, in this region, the substituents must be as small as possible, and the aliphatic chains must be shorter, to avoid stereochemical tension. In the case of the ammonium substituent, no chain should be used (4.9–4.10) to avoid an unfavourable approach between the charged substituents. Bulky substituents also proved unfavourable (4.12). The best result is obtained with alternate hydroxyl and ammonium groups, the first attached to a methyl chain and the last without any chain at all (4.13).

Conclusions

This work is devoted to the design of new contrast agents for MRI. At this initial stage there is no need for high-level quantitative results, but instead it is sufficient to derive qualitative/semi-quantitative data. Hence, the results presented here are not intended to reproduce very high accuracy association Gibbs energies, but instead to point to general trends to increase the association constant between the γ-CD and the ligand. Only the most promising substitutions should be selected for further (more rigorous) studies, which should then include the physiological solvent.

A list of the substitutions executed in this work can be found in Tables 1 to 4. All substitutions were carried out in the γ-CD; also given are the energies of complexation (kJ/mol) for the corresponding host–guest complexes *substituted γ-CD:[GdDOTA]*$^{-}$. To resume this work we will derive a set of conclusions that summarize the observed changes, and help to interpret and predict the effect of future substitutions.

The basic problem in the association is that the cross-section of the γ-CD is too small to accommodate the ligand. In Figures 1c, 1f and 1g we can see that the ligand cannot penetrate fully into the γ-CD, half of it remaining outside the ligand. The use of larger CDs (e.g. δ-CDs) has several disadvantages, namely their expensiveness and their tendency to distort into an 'eight' shape in aqueous solution, precluding association. So, the major strategy developed was to introduce substitutions in the γ-CD to increase the interactions with the ligand. Usually substitutions have two opposite effects: on one hand the increased interactions between the pair favour association, and on the other the larger volume of the substituents (compared to the replaced groups) causes stereochemical strain, making association more difficult. Successful substitutions usually result from a good balance between both factors.

Generally speaking, we can say that there are two main regions of the γ-CD that can be successfully substituted: the top 2′-HO groups and the bottom 6′-HO groups. The effects of the substitutions are not always additive. Substitutions at the bottom of the γ-CD tend to increase the stereochemical strain in that region, moving the ligand up, and substitutions at the top of the γ-CD tend to increase the stereochemical strain in that region, moving the ligand down. If bulky substituents are simultaneously introduced on the top and bottom parts, the stereochemical strain will increase significantly, and overcome the increased interaction energy. The result can be worse than replacing groups only in one of the two regions.

In the top region, the ligand is strongly hydrophilic, having four anionic carboxylate groups disposed symmetrically around the lanthanide ion. Each glucose ring has two hydroxyl groups. The 2′-HO groups point inwards and the 3′-HO groups point outwards. Substitutions in the 2′-HO groups cause the substituents to become orientated towards the ligand, and therefore they result in a larger stabilization of the complex.

In the bottom region, the ligand is more hydrophobic. Each glucose ring has one 6′-HO group pointed inwards. Substitutions in that region increase the interactions between the γ-CD and the ligand, especially if the inserted groups are hydrophobic (e.g. aliphatic chains); however, if they are very bulky they also tend to move the ligand up, making the inclusion more incomplete.

It was noticed that replacing the 2′-HO groups by polar or anionic groups has only a limited stabilizing effect, as the new groups remain too distant from the carboxylate oxygens to strongly interact with them. The inclusion of an aliphatic chain connecting the substituent to the γ-CD allows a closer approximation between the two. The optimal size of the aliphatic chain was found to include 2–3 carbon atoms. If longer chains are used, the stereochemical strain between those bulky substituents and the ligand results in decreased binding. The top substituents that were found to give the best associations were the ammonium/trimethyl ammonium groups; the ammonium group alone also gave very good results. The inclusion of an aliphatic chain terminated by the same hydroxyl group also stabilized the complex, although not as much as the ionic substituents.

Another problem detected is that such polar groups can also make hydrogen bonds to the other top hydroxyl groups of the γ-CD. Those intramolecular hydrogen bonds do not stabilize the complex. To avoid competition between hydrogen bonding to the γ-CD hydroxyl groups and to the ligand carboxylates, the top hydroxyl groups can be replaced by hydrogens. This procedure proved to be a very successful way to improve binding.

The number of substitutions is also important. As a general rule, the larger the substituents, the lower the number of replacements that can be done favourably. If an ionic substituent is used, the coulomb repulsion between the substituted groups can also limit the number of simultaneous substitutions. Using aliphatic chains with 2–3 carbon atoms, the best associated energies are obtained with eight substitutions, one in each ring.

In the bottom region of the γ-CD the aliphatic chains must be much smaller. If non-polar substituents are considered the optimal substituent would be an ethyl group. Such a substituent resulted in the larger stabilization obtained in this work, and we can say that it is the most promising substituted γ-CD, due to its simplicity, stability in solution and easyness to synthesise. Moreover, it is reasonable to assume that the physiological medium will not affect the complexation because water molecules do not interact strongly with the ethyl chains.

If polar groups are used the maximum chain length would be a single methyl group. This region has a smaller cross section that makes the substituents overlap more easily.

Last but not least, a different philosophy can be employed to increase association, which is to reduce the steric strain of the association by increasing the internal volume of the γ-CD. Eliminating all the hydroxyl groups from the γ-CD makes its cavity somewhat larger, leading to a substantial stabilization of the complex.

In summary, we have derived a set of substituted γ-CDs with a higher affinity to complex polyazamacrocycles of lanthanide (III) chelates. The results are very promising, and future work will be directed to select only the best ones, and to study them more deeply in a physiological environment.

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