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Effect of the second coordination sphere on new contrast agents based on cyclodextrin scaffolds for MRI signals†

Hussein Idriss,[a] François Estour,[a] Ibrahim Zgani,[a] Cécile Barbot,[a] Anais Biscotti,[a] Samuel Petit,[b] Chantal Galaup,[c] Marie Hubert-Roux,[a] Lionel Nicol,[d] Paul Mulder[d] and Géraldine Gouhier*[a]

Two new imaging tools using polydentate cyclodextrins were obtained using an innovative synthetic strategy. For the first time the influence of hydrogen bonding interactions of the cyclodextrin free rim was studied by MRI. The positive second coordination sphere effect was then quantified.

One of the current aims of research in the field of magnetic resonance imaging (MRI) is the design of new contrast agents (CAs) in order to significantly improve the sensitivity of this technique. The most important class of CAs for MRI is currently represented by Gadolinium(III) (Gd) chelates including cyclic tetraazacyclododecanetetraacetic acid (DOTA) or linear diethylenetriaminepentacetic acid (DTPA) ligands.[1] These clinically used complexes are nine coordinated with eight polyaminopolycarboxylate donor ligands and a water molecule occupying the ninth coordination site. Despite their wide use, DOTA or DTPA could lead to serious allergies after several injections.[2] By increasing the sensitivity of this medical diagnostic tool, it would then be possible to inject a weaker amount of CA and to prevent toxicological risks for the patients.

Functionalized cyclodextrins (CDs) have already been described as interesting scaffolds to access to efficient CAs.[3] Our challenge was to design new CAs based on β-CD, with high relaxivities, in order to increase the detection threshold. β-CD is a natural macrocyclic molecule composed of seven glucose units β-(1–4) in the shape of a conical cylinder delimiting an internal hydrophobic cavity.[4] The secondary face is more crowded than the primary side due to the presence of a double number of hydroxyl groups and constitutes the larger rim. Due to the higher molecular mass of this cyclic oligosaccharide, Gd(III) complexes of β-CD decrease the rotation rate in water, increase the water proton relaxation rates (r_1) and then enhance the contrast of images.

Nevertheless, efficient results have only been obtained with CAs bearing more than one metal atom per CD, increasing the risk of releasing free toxic gadolinium in the blood circulation.[2]

To reach our objectives we have synthesised heptacoordinated Gd(III) CAs based on 2,3-dimethylated β-CD **1a** and native β-CD **2a** thus containing only one metal ion (Fig. 1). The metal complexation occurs thanks to seven acetate ligands grafted on the β-CD primary face *via* stable ether functions under physiological conditions. A molecular modeling study showed that all the carboxylate functions could be localized at a distance of 2.4–2.5 Å from the Gd^{3+} ion.[5] From this design, two water molecules fill the inner spheres of the metal ion center *via* the oxygen atoms.

The aim of this study was also to compare MRI results obtained from these two new similar structures **1a** and **2a** and to evaluate for the first time the influence of the hydroxyl groups fixed to the crown of the CD on the MRI signal (Fig. 1).

Four other metal ion complexes based on Europium Eu(III) **1b** and **2b** and Lanthane La(III) **1c** and **2c**, respectively, have also been isolated to complete the structural and analytical studies by luminescence and NMR techniques (Fig. 1).

Results and discussion

The 2,3-dimethylated β-CD was successfully synthesized in three steps from native β-CD **3** as already described.[6] Seven ligands were then grafted on the lower face with 55% yield by reaction with

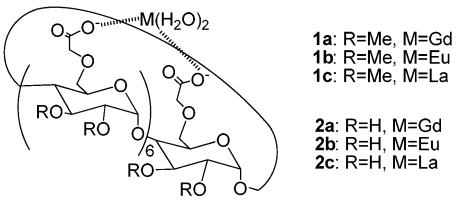

Fig. 1 Metal complexes based on CD scaffolds.

[a]*Normandie Univ, COBRA, UMR 6014, FR 3038, INSA Rouen, CNRS, IRIB, IRCOF 1 rue Tesnière 76821 Mont-Saint-Aignan, France. E-mail: geraldine.gouhier@univ-rouen.fr; Fax: 33-235522959; Tel: 33-235522909*

[b]*EA 3233, SMS, 76821 Université of Rouen, France*

[c]*SPCMIB, UMR CNRS 5068 Université Paul Sabatier, 118, route de Narbonne 31062 Toulouse Cedex, France*

[d]*INSERM U1096, Institute for Research and Innovation in Biomedicine, UFR de Médecine et de Pharmacie, 76821 Université de Rouen, France*

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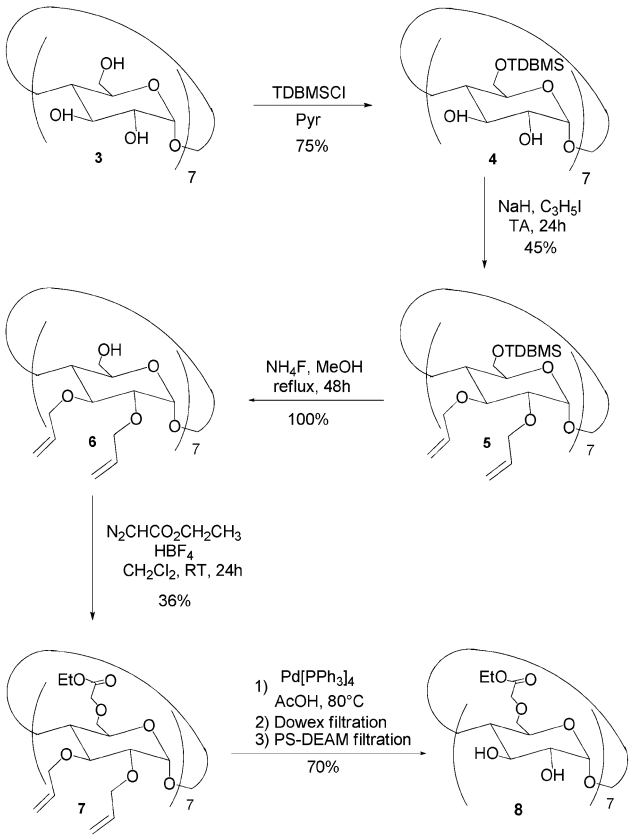
ethyl diazoacetate in the presence of tetrafluoroboric acid in anhydrous dichloromethane at room temperature according to Zavada's procedure.[7]

However the application of this methodology to native β-CD **3** brought about difficulties to obtain selective per-*O*-6-functionalized compounds due to the insolubility of **3** in dichloromethane. The use of other solvents such as DMF, DMSO or pyridine did not improve the reaction. The functionalization of the primary face of **3** was then tested by addition of sodium iodoacetate in the presence of pyridine in DMF but without success.[8] In order to enhance the solubility of the macrocycle in dichloromethane, the secondary face was temporarily protected by silyl[9] or acetyl groups[10]. However the complete substitution by seven carboxylate arms was not observed even after optimization tests such as the variation of the number of reagent equivalents (1-5 per β-CD) and/or of the reaction time (24–72h). In all cases mixtures of substituted CDs (3 to 7 ethyl acetate groups) inseparable by column chromatography were observed by mass spectrometry analysis.

Looking for an alternative strategy we have selected the allylic group as a convenient synthon. To our knowledge allylic functionalization had never been used as a temporary protective group for CDs synthesis. Only mono, perallylated, or selectively alkylated β-CDs in various positions have already been reported in successive reaction schemes such as oxidation, reduction and addition leading to many multifunctionalized CDs.[11] The suitable compound **5** was obtained with 45% yield from 6-*O*-persilylated β-CD **4** (Scheme 1).[12] Quantitative deprotection of the primary face in the presence of ammonium fluoride led to new compound **6** being soluble in dichloromethane. The introduction of seven acetate functions on the primary face of **6** using Zavada's procedure led to derivative **7** with 36% yield.[7] A mixture of compounds bearing four to six functions was also isolated, but an oversubstitution of these derivatives was tested without success.

In order to access to the desired compound **8**, complete deprotection of **7** had to be adjusted. Deallylation conditions have been optimized using $Pd(PPh_3)_4$ in acetic acid. After purification by chromatography, compound **8** was isolated in the presence of catalyst. The use of supported $Pd(PPh_3)_4$ on insoluble polymer did not facilitate the purification step.[13] The elimination of residual palladium required two successive filtrations on Dowex® 50WX4 and diethanolamine polystyrene (PS-DEAM) resins.[14] Finally the compound **8** was isolated with 70% yield.

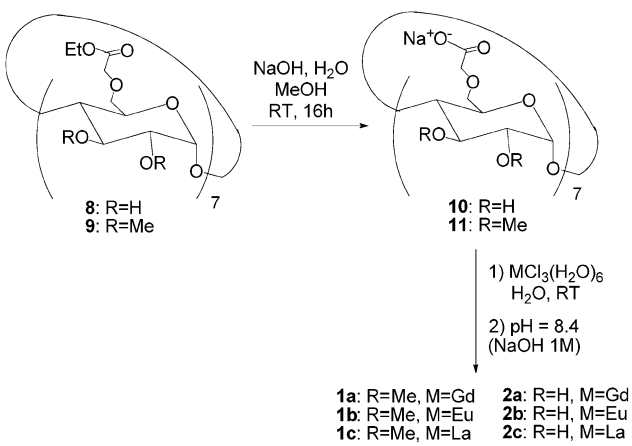
Through this original synthetic strategy, we developed an alternative methodology that could be applied in order to selectively prepare various CDs derivatives bearing groups only on the primary rim. The new important key intermediate **6** extends the panel of known selective protective groups to be easily removable such as acetyl, benzyl or silyl functions usually used in the synthesis of modified CDs. Moreover the high solubility in organic media of this allylic precursor is an alternative to the permanent methylation functionalization commonly used to avoid the CD solubility problems. Thus, this new methodology could be useful in order to reach new CD structures with other flexibilities, solubilities and new binding properties.[4]



Scheme 1 Synthesis of per-6-ethylacetate CD **8**.

For access to metal complexes **1a–c** and **2a–c**, a saponification reaction of the ester functions of **8** and **9** was quantitatively carried out (Scheme 2). Gd(III), Eu(III) or La(III) chloride hexahydrate salts were then complexed by **10** and **11** in water under controlled pH.

Mass spectrometry analyses and luminescence studies proved a stoichiometry 1/1 M/CD for complexes **1** and **2** respectively (ESI†). Due to the paramagnetic property of Gd(III), NMR analyses have been carried out on **1c** and **2c** complexes. The ^{13}C NMR spectrum



Scheme 2 Synthesis of complexes **1** and **2**.

showed significant chemical shifts of the carbon atoms around the carboxylate functions proving the complexation of the lanthanide with the seven ligands. Finally hydration numbers have been calculated for luminescent Eu^{3+} complexes **1b** and **2b** by using the well-established isotope effect and the Horrocks equation (ESI†). Indeed the comparison of the luminescence lifetimes of these europium complexes obtained in H_2O and D_2O allows an assessment of the hydration state. Hydration numbers of 2.48 and 2.18 were obtained for **1b** and **2b**, respectively, and confirm the presence of heptacoordinated active sites.

MRI tests were carried out at 0.5 Tesla on minispec mq 20 in TRIS buffer under physiological pH and at 37 °C. The relaxivities (r_1) of **1a** and **2a** CAs were 4.56 and 6.53 mM^{-1} s^{-1} respectively. These results correspond to an increase of 30% and 87% of the signal imaging compared to the reference value of DOTA (Chart 1). This variation observed between DOTA and CAs **1a–2a** could be explained by a higher molecular size of the CD scaffold increasing the reorientational correlation times (τ_r) and consequently the relaxivities.[3] Nevertheless the high signal variation of 43% between CAs **1a** and **2a** is mainly due to the second coordination sphere effect because the variation of mass between the adducts here is negligible.[15] Indeed **2a** may yield stronger interactions with water molecules on the upper face of the macrocycle, and lengthens their lifetime rate in the proximity of the paramagnetic centre.

Such hydrogen-bonding networks involving the coordinated water(s) are then reinforced and perturb the relaxivity.[16] In order to reduce the water molecules around the complexes and present in the cavity of the macrocycle, dioxane was selected due to its high miscibility with water and its slow self-diffusion.[17] A similar drop of the relaxivities for **1a** and **2a** at 2.2 $mM^{-1}s^{-1}$ and 2.5 $mM^{-1}s^{-1}$, respectively, was observed proving the key role of the outersphere for these two complexes.

Water molecules around **1a** and **2a** complexes may present different rotational mobilities due to the distortion of the shape of the CD slowing down the dynamics of rotation and enhancing the contrast imaging.

As the presence of free Gd^{3+} in CAs may have no negligible impact on the contrast in the MR image and could provide erroneous information on the contrast effect induced, a titration has been carried out following the protocol described by Barge.[18]

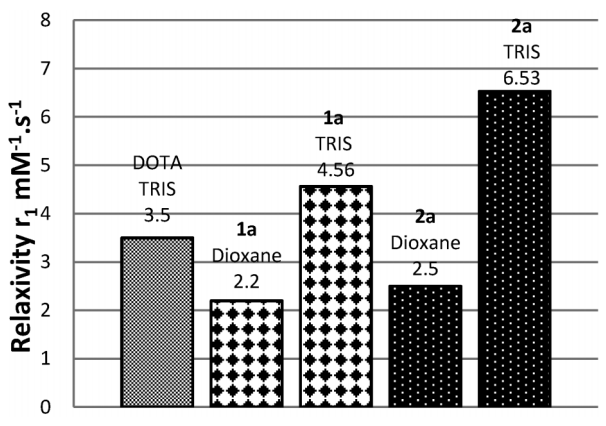


Chart 1 Comparison of MRI results between DOTA, **1a** and **2a**.

After complexometric titration using EDTA in the presence of xylenol orange as an indicator, weak percentages of 0.6% of free Gd(III) for **1a** and of 0.4% of free Eu(III) for **2b** were measured validating the MRI results observed.

Conclusions

In summary, we developed a new synthetic method using allylic functions to access to C6-functionalized CDs that enlarge the panel of known selective protections and can be a solution to solve the common problem of weak solubility in organic solvents of these scaffolds.

The MRI results obtained with two new CAs confirmed the potential of this strategy for relaxation enhancement using polydentate hydrophilic CDs as new imaging tools. A comparative study underlined the individual contributions of the rim of CD on the MRI signal and allowed the first quantification of the positive influence of the second coordination shell of the CD. The relaxivity gain could be attributed to a high number of water molecules being involved in a network of hydrogen bonding interactions.

A nuclear magnetic relaxation dispersion profile (NMRD) measurement will pinpoint the respective roles of the three hydration spheres contributions (inner, second and outer coordination spheres).

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