Ga(III) chelates of amphiphilic DOTA-based ligands : synthetic route and *in vitro* and *in vivo* studies

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Abstract

In this work we report a synthetic strategy of amphiphilic DOTA-based chelators bearing a variable size α-alkyl chain at one of the pendant acetate arms (from six to fourteen carbon atoms), compatible with their covalent coupling to amine-bearing biomolecules. The amphiphilic behavior of the micelles-forming Ga(III) chelates (critical micelle concentration), their stability in blood serum and their lipophilicity (logP) were investigated. Biodistribution studies with the ^{67}Ga-labeled chelates were performed in Wistar rats showing a predominant liver uptake with almost no traces of the radiochelates in the body after 24 hours.

1. Introduction

Gallium offers three useful radioisotopes for medical imaging, ^{67}Ga ($t_{1/2}$ = 3.25 days), ^{66}Ga ($t_{1/2}$ = 9.5 h) and ^{68}Ga ($t_{1/2}$ = 68 min). The first one, a γ emitter, can be used for γ-scintigraphy, while the others, being β^+ emitters, are adequate for positron emission tomography (PET). ^{68}Ga is a very attractive radionuclide for PET as it can be produced *in loco* from a ^{68}Ge generator allowing easy routine manufacture in the hospital facilities, similar to what happens with the ^{99}Mo/^{99m}Tc-generator. Gallium-based radiopharmaceuticals preparation is easy and fast, contrarily to the preparation of ^{18}F or ^{11}C covalently labeled PET agents, leading to a minimum loss of activity [1, 2]. The most important requirements for a Ga(III)-based radiopharmaceutical agent are the thermodynamic stability and the kinetic inertness during the period of clinical use in order to avoid ligand exchange with the blood serum proteins, such as transferrin (Tf) [3].

Ga(III) is a hard acidic cation that forms thermodynamically stable chelates with ligands displaying multiple anionic oxygen donor sites. Several ligands have been used to

sequester Ga(III) up to its maximum octa-coordination forming pseudo-octahedral structures [4]. The macrocyclic ligands NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid) and DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) are commonly used for Ga(III) [5, 6] as both provide highly stable chelates. Despite the relatively lower thermodynamic stability of the DOTA complex (approximately 10 orders of magnitude) than that of Ga(III)-NOTA [6], DOTA presents the advantages of being commercially better available and of having a less arduous synthesis. DOTA is also advantageous considering that it can more than saturate gallium´s common six-coordination sphere [7], offering two free carboxymethyl arms for conjugation to targeting molecules [8, 9, 10]. Additionally DOTA is known to form stable complexes with many other radiometals such as In(III), Cu(II), Y(III), lanthanide(III) ions or Ac(III) [8, 11, 12].

In a previous publication we reported the synthesis of a small library of four amphiphilic NOTA-based chelators for Ga(III) presenting a α-alkyl chain of variable size bound at one of the pendant arms [13]. These ligands showed to form micelles in solution. Micelles are known to constitute efficient drug carriers that can improve its pharmacokinetic properties or its bioavailability; likewise they can increase the target-to-background ratio of the drug or to deliver hydrophobic molecules [14-17]. These colloidal particles tend to accumulate in macrophage-rich tissues, undergoing endocytosis/phagocytosis [14, 18]. ^{125}I, ^{111}In or ^{153}Sm labeled micelles have been used for the scintigraphic visualization of macrophage-rich tissues such as lymph nodes, liver and spleen [19-21].

In this paper we describe the synthesis and characterization of three amphiphilic DOTA-derivative chelators, bearing in one of the acetate arms a α-alkyl chain with a variable number of carbon atoms (from six to fourteen atoms, see scheme 1). The critical micelle

concentration (cmc) values of their Ga(III) chelates have been determined by a fluorescent method based on the use of ANS (8-anilino-1-napthalene sulfonic acid) [22]. The stability of the ^{67}Ga(III) labeled chelates in blood serum has been investigated. Biodistribution studies of the ^{67}Ga(III) labeled chelates have been performed in Wistar rats.

2. Experimental

2.1. Materials and methods

Analytical grade reagents were purchased from Sigma-Aldrich, Fluka, Acros Organics and Chematech. [^{67}Ga](citrate) was purchased from [^{67}Ga](citrate) was purchased from CIS-BIO (Gif-sur- Yvette, France). The reactions were monitored by thin layer chromatography (TLC) on aluminum plates coated with silica gel 60 F_{254} (Macherey Nagel). Chromatographic separations were performed on silica gel Whatman 230-240 Mesh. The NMR spectra were recorded on a Varian Unity Plus 300 spectrometer or on a Bruker Avance III 400 spectrometer. The ^{1}H chemical shifts are reported in ppm, relative to TMS and the following abbreviations are used: s = singlet; s_b = broad singlet; d = doublet; dd = double doublet; t = triplet; t_b = broad triplet; m = multiplet; m_b = broad multiplet. pH measurements were performed on a pH meter Crison micro TT 2050 with an electrode Mettler Toledo InLab 422. Mass spectra (ESI^+) were performed on a VG Autospec M spectrometer or on a Finnigan LXQ MS Detector.

The ITLC-SC stripes (instant thin layer chromatograpy) used for the control of the radiochemical purity of the [^{67}Ga]L solutions were from Gelman Sciences Inc.

2.2. Synthetic procedures

2.2.1. Diphenyldiazomethane (DDM)

DDM was prepared according to the method of Miller [23]. To 10.03 g of benzophenonehydrazone (51.1 mmol), 11.51 g of anhydrous sodium sulfate, 26.91 g of yellow mercuric oxide (II) (124.2 mmol) and 3.8 mL of ethanol saturated with potassium oxide, 160 mL of ethyl ether were added and the suspension was stirred in a 1 L round flask at room temperature during 75 minutes. The reaction mixture was filtered by gravity and the solvent removed from the filtrate under reduced pressure. The dark red oil thus obtained was dissolved in petroleum ether 40-60 ºC and filtered again by gravity. The solvent was evaporated under reduced pressure giving a dark red oil which was left to freeze. When heated to room temperature it afforded 9.90 g of dark red crystals (99.7%).

2.2.2.1 – 2-Bromooctanoic acid benzhydryl ester (4)

A solution of 2.56 g of DDM (13.19 mmol) in 90 mL of acetone was added over a period of 2 hours to a solution of 2.5 mL of 2-bromooctanoic acid **(1)** (14.51 mmol) in 110 mL of acetone. This mixture was kept with stirring in an ice bath during 12 hours and then left at room temperature for an additional 12 hours until the red color of DDM had vanished. The acetone was evaporated under reduced pressure and a yellow oil was obtained. This oil was purified by column chromatography over silica gel 60 (cyclohexane/ethyl acetate 4:1). 5.12 g of a yellow oil (99.8%) were obtained. ^{1}H NMR (300 MHz, $CDCl_3$, $SiMe_4$, δ): 0.89 (t, 3H, J=6.6 Hz, CH_3), 1.24-1.30 (m, 8H, CH_2), 1.96-2.18 (m, 2H, CHBr-C*H*$_2$), 4.34 (t, 1H, J=7.2 Hz, C*H*Br), 6.92 (s, 1H, C*H*Ph_2), 7.36 (m, 10H, Ph_2).

2.2.3.1 – 2-Bromodecanoic acid benzhydryl ester (5)

Using a similar procedure with 2-bromodecanoic acid **(2)**, 6.78 g (99.0%) of a yellow oil were obtained. ^{1}H NMR (300 MHz, $CDCl_3$, $SiMe_4$, δ): 0,95 (t, 3H, J=6.6 Hz, CH_3), 1.25-1.44 (m, 12H, CH_2), 2.00-2.22 (m, 2H, CHBr-C*H*$_2$), 4.38 (t, 1H, J=8.3 Hz, C*H*Br), 6.97 (s, 1H, C*H*Ph_2), 7.31-7.46 (m, 10H, Ph_2).

2.2.4.1 – 2-Bromohexadecanoic acid benzhydryl ester (6)

Using a similar procedure with 2-bromohexadecanoic acid **(3)**, 6.04 g (94.0%) of a yellow oil were obtained. ^{1}H NMR (300 MHz, $CDCl_3$, $SiMe_4$, δ): 0.93 (t, 3H, J=6.8 Hz, CH_3), 1.17-1.42 (m, 24H, CH_2), 1.98-2.20 (m, 2H, CHBr-C*H*$_2$), 4.35 (t, 1H, J=7.4 Hz, C*H*Br), 6.94 (s, 1H, C*H*Ph_2), 7.30-7.43 (m, 10H, Ph_2).

2.2.2.2 – 1,4,7,10-tetraazacyclododecane-1-(2-carboxyheptyl) benzhydryl ester (7)

199.3 mg of 1,4,7,10-tetraazacyclododecane (1.16 mmol) were dissolved in 10 mL of dichloromethane and to this solution a second solution of 347.3 mg of 2-bromooctanoic acid benzhydryl ester **(4)** (0.89 mmol) in 10 mL of dichloromethane was added over a period of 1.5 hours. The mixture was stirred overnight at room temperature, filtered under vacuum and concentrated under reduced pressure, affording a brown oil. This oil was purified by column chromatography over silica gel 60 (dichloromethane/ethanol 7:3, later dichloromethane/ethanol/ammonia 7:3:0.5). At the end 211.1 mg of a yellow oil (49.9%) were obtained. ^{1}H NMR (300 MHz, $CDCl_3$, $SiMe_4$, δ): 0.83 (t, 3H, J=6.8 Hz, CH_3), 1.12-1.36 (m, 8H, CH_2), 1.74-1.89 (m, 2H, CHN-C*H*$_2$), 2.42-3.40 (m, 16H, NC*H*2), 6.87 (s, 1H, C*H*Ph_2), 7.19-7.36 (m, 10H, Ph_2).

2.2.3.2 – 1,4,7,10-tetraazacyclododecane-1-(2-carboxynonyl) benzhydryl ester (8)

Using a similar procedure with the convenient ester **(5)**, 1.5 g (65.7%) of a yellow oil were obtained. ^{1}H NMR (300 MHz, $CDCl_3$, $SiMe_4$, δ): 0.88 (t, 3H, J=6.2 Hz,

CH_3), 1.16-1.41 (m, 12H, CH_2), 1.81-1.97 (m, 2H, CHN-C*H*$_2$), 2.22-3.63 (m, 16H, NC*H*2), 4.19-4.35 (m, 1H, C*H*N), 6.92 (s, 1H, C*H*Ph_2), 7.22-7.41 (m, 10H, Ph_2).

2.2.4.2 – 1,4,7,10-tetraazacyclododecane-1-(2-carboxypentadecyl) benzhydryl ester (9)

Using a similar procedure with the convenient ester **(6)**, 279.4 mg (42,5%) of a yellow oil were obtained. ^{1}H NMR (300 MHz, $CDCl_3$, $SiMe_4$, δ): 0.87 (t, 3H, J=7.2 Hz, CH_3), 1.15-1.42 (m, 24H, CH_2), 1.74-3.61 (m, 18H, CHN-C*H*$_2$, NC*H*$_2$), 4.28-4.37 (m, 1H, C*H*N), 6.91 (s, 1H, C*H*Ph_2), 7.24-7.42 (m, 10H, Ph_2).

2.2.2.3 – 1,4,7,10-tetraazacyclododecane-1-(2-carboxyheptyl) benzhydryl ester-4,7,10-triacetic acid *tert*-butyl ester (10)

211.3 mg of 1,4,7,10-tetraazacyclododecane-1-(2-carboxyheptyl) benzhydryl ester **(7)** (0.44 mmol) were dissolved in 10 mL of acetonitrile and to this solution, 364.8 mg of potassium carbonate (2.63 mmol) and 208 µl of *tert*-butyl bromoacetate (1.41 mmol) were added. The mixture was stirred overnight at room temperature, filtered under vacuum and concentrated under reduced pressure to afford a brown oil. This oil was purified by column chromatography over silica gel 60 (dichloromethane/ethanol 7:3). At the end it was obtained 253.3 mg of a brown oil (70,1%). ^{1}H NMR (300 MHz, $CDCl_3$, $SiMe_4$, δ): 0.77 (t, 3H, J=7.3 Hz, CH_3), 1.08-1.30 (m, 8H, CH_2), 1.39 (s, 27H, $C(CH_3)_3$), 1.55-1.71 (m, 2H, CHN-C*H*$_2$), 1.90-3.50 (m, 22H, NC*H*$_2$,CH_2-COO*t*Bu), 6.78 (s, 1H, C*H*Ph_2), 7.15-7.29 (m, 10H, Ph_2).

2.2.3.3 – 1,4,7,10-tetraazacyclododecane-1-(2-carboxynonyl) benzhydryl ester-4,7,10-triacetic acid *tert*-butyl ester (11)

Using a similar procedure with the convenient compound **(8)**, 985.0 mg (70.8 %) of a brown oil were obtained. ^{1}H NMR (300 MHz, $CDCl_3$, $SiMe_4$, δ): 0.89 (t, 3H, J=7.0

Hz, CH_3), 1.16-1.37 (m, 12H, CH_2), 1.49 (s, 27H, $C(CH_3)_3$), 1.83-3.81 (m, 24H, NC*H*$_2$, CHN-C*H*$_2$,CH_2-COO*t*Bu), 6.89 (s, 1H, C*H*Ph_2), 7.26-7.40 (m, 10H, Ph_2).

2.2.4.3 – 1,4,7,10-tetraazacyclododecane-1-(2-carboxypentadecyl) benzhydryl ester-4,7,10-triacetic acid *tert*-butyl ester (12)

Using a similar procedure with the convenient compound **(9)**, 142.9 mg (32.4%) of a brown oil were obtained. ^{1}H NMR (300 MHz, $CDCl_3$, $SiMe_4$, δ): 0.86 (t, 3H, J=6..8 Hz, CH_3), 1.15-1.37 (m, 24H, CH_2), 1.43-1.47 (m, 27H, $C(CH_3)_3$), 1.63-1.80 (m, 2H, CHN-C*H*$_2$), 1.96-3.59 (m, 22H, NC*H*$_2$, CH_2-COO*t*Bu,), 6.86 (s, 1H, C*H*Ph_2), 7.24-7.38 (m, 10H, Ph_2).

2.2.2.4 – 1,4,7,10-tetraazacyclododecane-1-(2-carboxyheptyl)-4,7,10-triacetic acid (DOTAC8 (13))

253.3 mg of 1,4,7,10-tetraazacyclododecane-1-(2-carboxyheptyl) acid benzhydryl ester-4,7,10-triacetic acid *tert*-butyl ester **(10)** (0.31 mmol) were dissolved in 5 mL of trifluoroacetic acid (98%). The mixture was stirred overnight at room temperature and concentrated under reduced pressure affording a brown oil which was washed with 3x70 mL of water. The aqueous solution was filtered with a cellulose membrane and concentrated under reduced pressure, affording 183.0 mg of a crystalline brown solid (62.9%). MS (ESI^+): m/z (relative intensity): 511.58 (MNa^+, 100).

2.2.3.4 – 1,4,7,10-tetraazacyclododecane-1-(2-carboxynonyl)-4,7,10-triacetic acid (DOTAC10 (14))

The same procedure with the convenient compound **(11)** afforded 455.0 mg (67.4%) of a crystalline brown solid. MS (ESI^+): m/z (relative intensity): 517.58 (MH^+, 100).

2.2.4.4 – 1,4,7,10-tetraazacyclododecane-1-(2-carboxypentadecyl)-4,7,10-triacetic acid (DOTAC16 (15))

The same procedure with the convenient compound **(12)** afforded 119.2 mg (65.3%) of a crystalline brown solid. MS (ESI^+): m/z (relative intensity): 601.42 (MH^+, 100).

2.3. Determination of the critical micellar concentration

The cmc value can be estimated by a fluorescence method using the ANS as fluorescent probe [22]. The emission intensities of ANS were obtained at 25 °C in microplates optimized for fluorescence where the excitation wavelength used was 350 nm, while the wavelength of recorded emission was 480 nm. Measurements were made in 1 M phosphate buffer (pH 7.4), where every microplate well contained a known amount of chelate and $1x10^{-5}$ M ANS. The fluorescence measurements were recorded on a Bio-Tek® Synergy™ HT spectrofluorimeter using the software KC4™.

The chelates were prepared by adding an equivalent quantity of Ga(III) nitrate to a weighted quantity of ligand, dissolving in water at pH 4 and heating at 75°C over 1 hour. The water was evaporated and the solid was redissolved in phosphate buffer pH 7.4. In order to know the exact number of ligand equivalents existing in a weighted amount of ligand, an excess of a standard Al(III) solution was added to a weighted quantity of ligand, leaving the complexation to occur at 75°C. To this solution an excess of a standard EDTA solution was added and this was back-titrated with a standard Ca(II) solution using eriochrome black T as indicator [24].

2.4. Radiochemistry

[^{67}Ga] chelates for *in vivo* and *in vitro* experiments were prepared by adding 1 mCi of [^{67}Ga](citrate) to a solution of 1 mg of the chelator in HEPES (0.150 mL, 0.1 M,

pH 5) and heated at 80 ºC for ca 1 h. The radiochemical purity of the [^{67}Ga]L solutions was determined either by TLC eluting with methanol or using ITLC-SC stripes eluting with a saline/acetic acid (9:1) mixture. In the first case the ^{67}Ga(III) ion remains in the origin and [^{67}Ga]L migrates (rf=0.6) while in the second case [^{67}Ga]L remains in the origin and the ^{67}Ga(III) ion migrates (rf=0.8). The percentage of bound metal averaged 96%.

2.4.1. Determination of logP and stability in blood serum

The octanol/water partition coefficients (logP) of [^{67}Ga](DOTAC8)$^{-}$, [^{67}Ga](DOTAC10)$^{-}$ and [^{67}Ga](DOTAC16)$^{-}$ were determined using the *shake-flask* method. The partition coefficient was determined by adding 25 μL of the chelate solution to a tube containing 1 mL of saline solution and 1 mL of 1-octanol. The resulting mixture was shaken at room temperature for 1 h and then centrifuged at 3000 rpm during 3 min. After the centrifugation, 100 μL of each phase were collected and the activity was measured. The partition coefficient was calculated as a ratio of the counts in the octanol fraction to the counts in the water fraction being this the result of the average of 5 determinations (S.D. < 0.01).

For the blood serum stability studies, 5 μCi of the standard solution of [^{67}Ga](DOTAC10)$^{-}$ were added to 5 mL of fresh human serum, previously equilibrated in 5% CO_2 (95% air) environment at 37 ºC. The mixture was stored in the same environment conditions, and aliquots of 100 mL (in triplicate) were taken at appropriate periods of time (0 min, 30 min, 1 h and 3 h). The aliquots were treated with 200 μL of ethanol, cooled (4 ºC), and centrifuged during 15 min at 4000 rpm, at 4 ºC, in order to precipitate the serum proteins. A 100 μL aliquot of supernatant was collected for activity counting in a γ well-counter. The sediment was washed twice with 1 mL of ethanol and its activity was counted. The activity of the supernatant was compared to

that of the sediment in order to determine the percentage of the chelate associated to the proteins. The activity of the supernatant at 3 h was evaluated by TLC in order to check whether the chelate remained intact.

2.4.2. Biodistribution studies

Groups of four animals (Wistar rat males weighting ca 200 g) were anaesthetized with Ketamine (50.0 mg/mL)/chloropromazine (2.5%) (10:3) and injected in the tail vein with ca 100 μCi of the tracer and sacrificed 30 min and 24 h later. The major organs were removed, weighted and counted in a γ well-counter.

3. Results and discussion

3.1. Synthesis

In the preparation of the ligands described in this work, an orthogonal protection strategy was followed using protecting groups for the carboxylic moieties that may be cleaved, if wished, at two different moments. Such groups were the *tert*-butyl ester (cleaved in acid) and the benzhydryl ester (cleaved through Pd catalyzed hydrogenolysis or in acid). This synthetic strategy is compatible with the preparation of bifunctional ligands that, after cleavage of the single benzhydryl ester, can be covalently coupled to targeting biomolecules with a free amine group. Preliminary coupling experiments with linear RGD peptides have shown this strategy to be very effective [results not published]. The targeting of the prochelators with suitable biomolecules is of chief importance for receptor mediated medical imaging or radiotherapeutic applications [8, 9]. In this work we treated the pro-chelators **10-12** at once with TFA, affording the unprotected chelators DOTAC8, DOTAC10 and DOTAC16 (**13-15**).

3.2 Determination of the critical micellar concentration

Micelles formation by amphiphilic compounds occurs above the critical micellar concentration (cmc), and this value can be determined by a fluorescence method using ANS as fluorescent probe. This aromatic molecule has fluorescent properties that depend on the polarity of the medium. Therefore, in polar media like water ANS is not fluorescent, whereas in nonpolar media such as the interior of the micelles it is fairly luminescent [22]. The cmc values of [Ga(DOTAC8)$^-$], [Ga(DOTAC10)$^-$] and [Ga(DOTAC16)$^-$] chelates were estimated by linear least-square fitting of the fluorescence emission at 480 nm versus the concentration of the chelates (Fig. 1). The calculated cmc values were 3.98 mM for [Ga(DOTAC8)]$^-$, 3.13 mM for [Ga(DOTAC10)]$^-$ and 0.87 mM for [Ga(DOTAC16)]$^-$, showing, as expected, a decrease of the cmc value with the increase of the alkyl chain, reflecting a superior capacity to self-aggregate by the longer alkyl chain chelates. Although a correlation between the alkyl chain length and the cmc value can be observed, the aqueous behavior of the three systems is quite different. For the [Ga(DOTAC8)]$^-$ and [Ga(DOTAC16)]$^-$ systems, there is a substantial increase in the fluorescence intensity with the increase of the chelate concentration until the cmc value is reached, and a smaller increase in the fluorescence intensity after de cmc. Such behavior is not shown by the [Ga(DOTAC10)]$^-$ system, suggesting that [Ga(DOTAC8)]$^-$ and [Ga(DOTAC16)]$^-$ might form pre-micellar aggregates. The formation of pre-micellar aggregates has been previously reported by Torres et al for the chelate [Gd(EPTPAC$_{16}$)]$^{2-}$, which formed particles with dimensions superior to 100 nm below the cmc, as detected by DLS [25]. The cmc value obtained for [Ga(DOTAC16)]$^-$ is comparable to the value found for the amphiphilic Gd(III) chelate with the same alkyl chain length [26].

3.3 Determination of logP

The calculated values of the octanol/water partition coefficients (logP) were -2.83 for $[^{67}Ga](DOTAC8)^-$, -1.50 for $[^{67}Ga](DOTAC10)^-$ and 0.27 for $[^{67}Ga](DOTAC16)^-$. These values show that the chelates are highly hydrophilic, and, as expected, the hydrophylicity decreases with the increasing size of the α-alkyl chain.

3.4 Stability in blood serum

Incubation studies of $[^{67}Ga](DOTAC10)^-$ in fresh human serum, followed by precipitation of its protein content, showed that the percentage of the activity in the protein pellet steadily increased with the incubation time (6.25% at 0 min, 9.07% at 60 min), reaching a value of 12.57% at 3 h. The increasing activity associated to the blood proteins can be explained by the hydrophobic interactions involving the α-alkyl substituent and the hydrophobic regions of the proteins, and it is expected that this interaction can increase with the increasing size of the α-alkyl side chain.

The stability of the chelate was verified through TLC analysis, where the radioactivity present in the fraction 3 h after incubation was measured and analyzed. It was found that after this time the radiochelate was intact, reflecting the high stability of $[^{67}Ga](DOTAC10)^-$ towards transchelation.

3.5 Biodistribution studies

The biodistribution data for $[^{67}Ga](DOTAC10)^-$ and $[^{67}Ga](DOTAC16)^-$ are expressed as the percentage of injected dose per gram of tissue (%ID/g) in Fig. 2. At 30 minutes after injection both chelates mainly localize at the kidneys and the liver which correlates with a rapid body excretion. Nevertheless we have to consider that, in both cases, the somewhat high activity in the blood at this time results in a lack of biospecificity as every organ with good blood irrigation has an increased chelate concentration. However, there are some differences in the way the two chelates are

excreted. It can be seen that [^{67}Ga](DOTAC16)$^{-}$ is much more hepatospecific (approximately 1.7% of the activity is present in hepatobiliary transit at 30 min) than [^{67}Ga](DOTAC10)$^{-}$ (just approximately 0.4% of the activity is present in hepatobiliary transit at the same time). These effects can be explained considering the higher lipophilicity of [^{67}Ga](DOTAC16)$^{-}$ (logP = 0.27) in comparison to [^{67}Ga](DOTAC10)$^{-}$ (logP = -1.50), which results in a higher kidney uptake (0.9% of the activity) for the last chelate. It would be expected that the lung uptake of [^{67}Ga](DOTAC16)$^{-}$ would be much higher than that observed, due to the formation of pre-micellar aggregates, which could be trapped in lung capillaries, as it has been observed for the long chain tracer [^{153}Sm](EPTPAC$_{16}$)]$^{2-}$ [25]. From the biodistribution data it is noticeable that most of the radioactivity was cleared off from the tissues and organs within 24 h with virtually no deposition in the bones which is a clear indication of the high chelates stability.

According to the specifications of the [^{67}Ga](citrate) supplier, less than 4.5 ng of Ga(III) (1 mCi) should be present in the final solution of [^{67}Ga](DOTAC10)$^{-}$ or [^{67}Ga](DOTAC16)$^{-}$. Thus, in both cases, the concentrations were well below the cmc determined for the complexes, and no micelles were supposed to be present in solution.

4. Conclusions

We have developed an easy and efficient synthetic route that leads to DOTA-based amphiphilic chelators, suitable for gallium(III) complexation. This synthetic approach makes use of an orthogonal protection strategy that is well-suited for the conjugation to biomolecules bearing a free amine function.

The in vitro evaluation of [^{67}Ga](DOTAC10)$^{-}$ in serum demonstrated its high stability toward transchelation. The biodistribution studies in Wistar rats with the ^{67}Ga-labeled chelates showed that their uptake occurs mainly in the liver and, as expected, is

proportional to the length of the pendant α–alkyl chain and consequently to the lipophilicity. Their clearance from the body after 24 hours is also noticeable indicating that the chelates are very stable and no toxicity effects are foreseen.

As previously found by us for the analogous NOTA-based Ga(III) chelates [13], the injection of the radiochelates at concentrations below the determined cmc precluded the formation of micelles in vivo, with lower hepatic uptake than expected. Despite the similar in vivo results obtained for the triaza- and tetraaza-based complexes, the tetraaza chelators, besides being somewhat more hydrophilic, offer the possibility of covalent coupling to biomolecules via a non-coordinating carboxylic moiety, without compromising the chelate stability. Preliminary work of conjugation of these amphiphilic chelators to model linear RDG peptides stresses the utility of this family of Ga(III) chelators, whose lipophilicity can be tuned in accordance to the pendant alkyl chain length used.

5. Abbreviations

%ID/g = percentage of injected dose per gram of tissue

ANS = 8-anilino-1-naphtalene sulfonic acid

cmc = critical micellar concentration

DDM = diphenyldiazomethane

DLS = dynamic light scattering

$EPTPAC_{16}$ = ethylenepropylenetriaminepentaacetic acid

HEPES = *N*-(2-Hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid)

ITLC (instant thin layer chromatograpy)

L = ligand

MRI = magnetic resonance imaging

PET = positron emission tomography

RGD = Arginine-Glycine-Aspartic Acid

TFA = trifluoracetic acid

TLC = thin layer chromatography

TMS = tetramethylsilane

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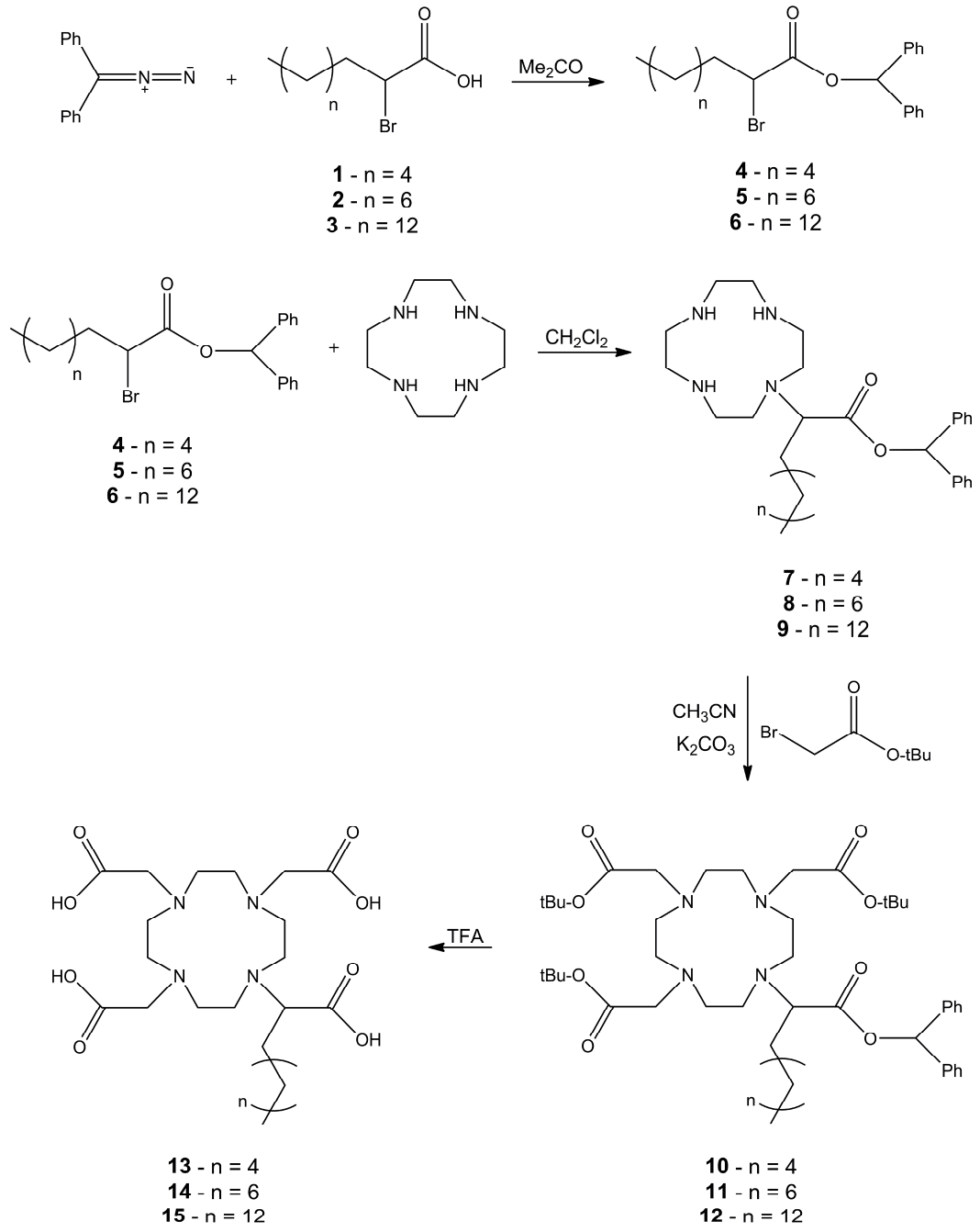

Scheme 1 – Schematic synthesis of DOTAC8, DOTAC10 and DOTAC16.

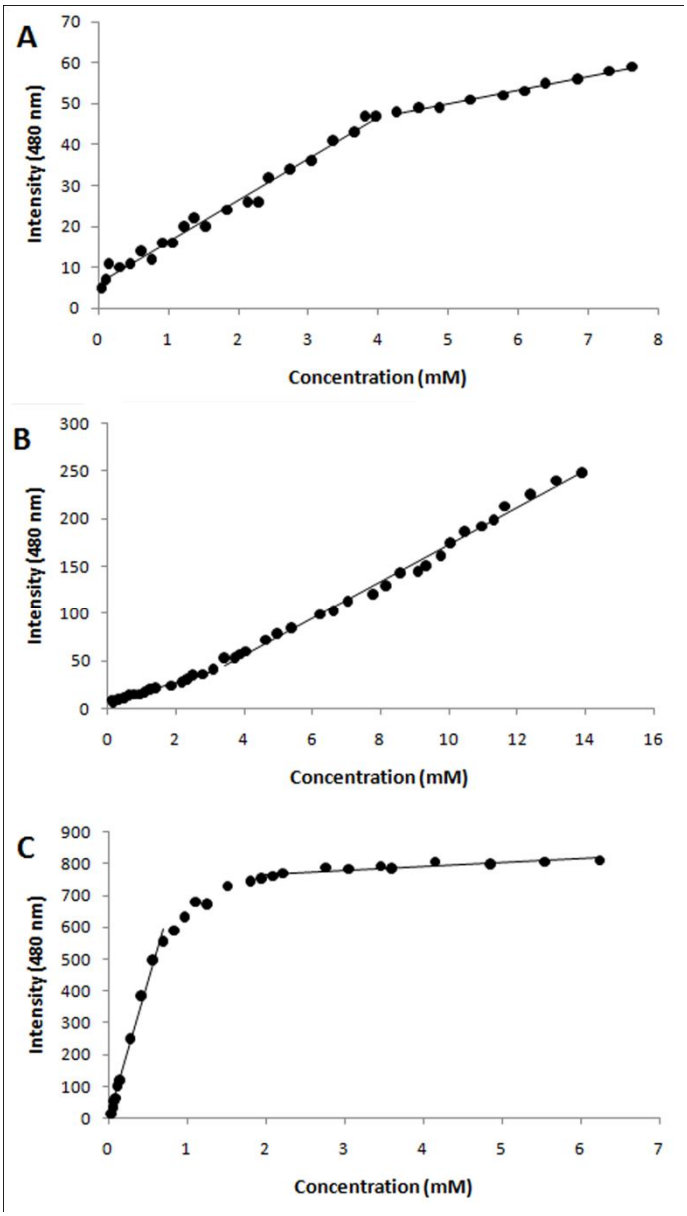

Figure 1 – Fluorescence intensity at 480 nm versus: A – [Ga(DOTAC8)$^-$] concentration; B – [Ga(DOTAC10)$^-$] concentration; C – [Ga(DOTAC16)$^-$].

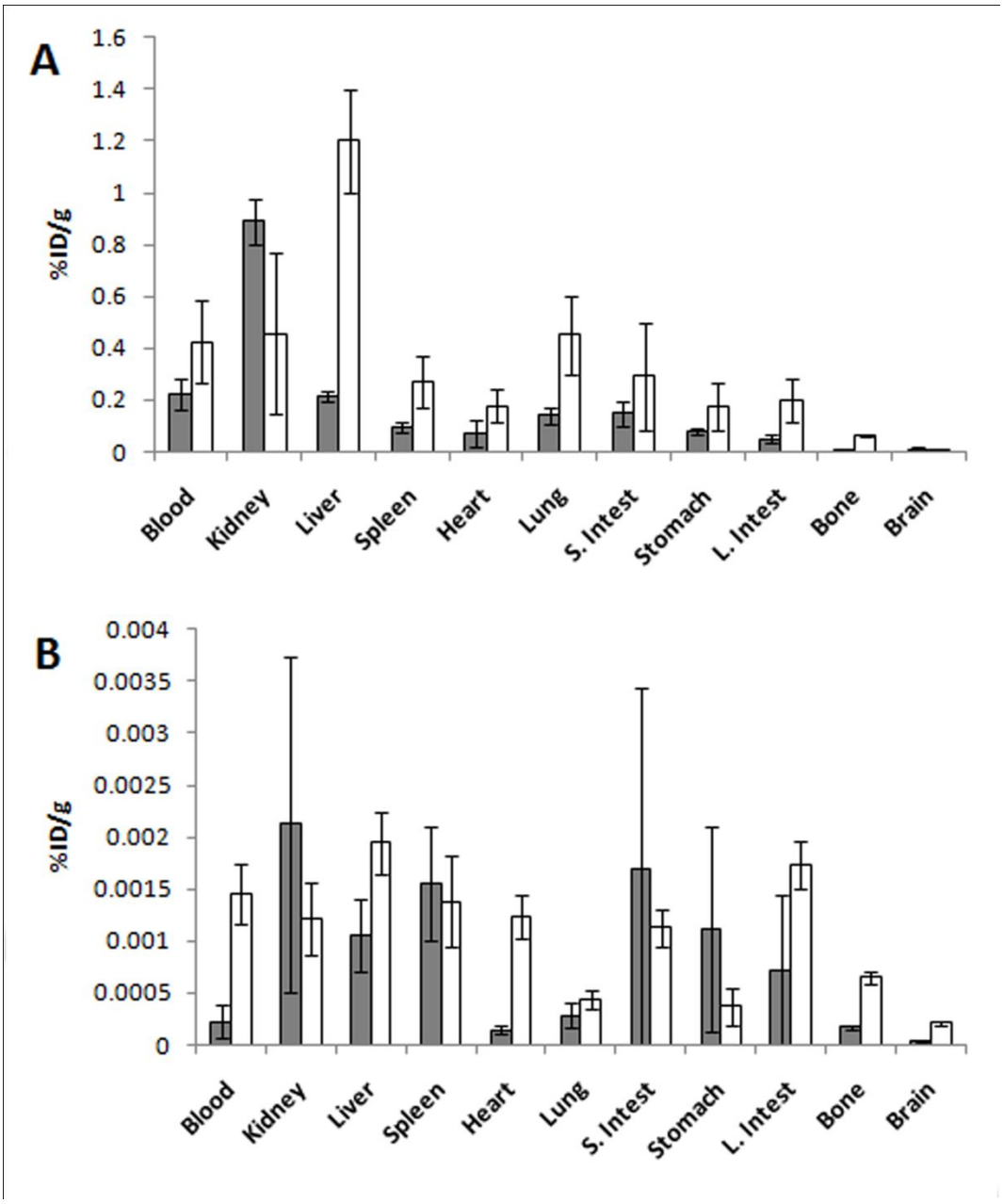

Figure 2 – Biodistribution profile for Wistar rats (percentage of the injected dose/g of organ) of [^{67}Ga](DOTAC10)$^{-}$ (grey bars) and [^{67}Ga](DOTAC16)$^{-}$ (white bars) at 30 min (A) and 24 h (B) after injection.