Studies towards the development of lipophilic bifunctional N₃S₃ chelators for ⁶⁸Ga

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**Gallium-68 / Trithiolate chelators / Radiopharmaceutical chemistry / Lipophilic complexes / Bifunctional chelators**

**Summary.** The present study is concerned with a concept of charge-neutral, lipophilic, macrocyclic bifunctional chelators, suitable for the introduction of a gallium-68 label into small molecules. The synthesis of a novel bifunctional N₃S₃-type chelator, derived from 1,4,7-triazacyclononane, initial ⁶⁸Ga-radiolabelling and the determination of stability and calculated lipophilicity of the compound are described. The ⁶⁸Ga-labelled chelate was obtained in a maximum radiochemical yield of 93±5% after a reaction time of 2 min. It remained intact over 3 h in a DTPA-challenge and a transferrin challenge experiment, indicating sufficient stability for PET studies.

**1. Introduction**

In recent years, the positron emitter ⁶⁸Ga is undergoing a renaissance as a generator-derived PET-nuclide for clinical routine. This is due to recent improvements in the performance of commercially available ⁶⁷Ge/⁶⁸Ga generator systems and post-processing of generator eluents [1–3]. The latter includes purification of the cationic [⁶⁸Ga]Ga³⁺ by separation of metal contaminants as well as eluate concentration for labelling purpose [1]. Apart from one exception, bifunctional chelates suitable for complexation of [⁶⁷,⁶⁸Ga]Ga³⁺ are very hydrophilic compounds which lead to highly polar, sometimes charged complexes. Due to beneficial permeative properties and the potential crossing of the blood-brain-barrier, more lipophilic bifunctional chelators present a milestone in ⁶⁸Ga-PET. For this reason, several groups elucidated the synthesis [4–12], stability [10, 11] and biological properties [12] of less hydrophilic chelators [4–12].

Luyt and Katzenellenbogen have described a bifunctional chelator based on a tripodal NS₁₃-chelator (Fig. 1) [5]. Despite the value of the ligand for the intended use as labelling agent for peptides and proteins, the high molecular weight and the particular requirements for conjugation utilising the included aniline-NH₂ donor function limit the value of this compound for labelling of small molecules [5, 24].

Less weighty aliphatic chelators were derived from the macrocyclic polyamine 1,4,7-triazacyclononane (TACN) using mercaptoethyl pendant arms. Moore and coworkers have introduced a corresponding compound 2a [7, 8] (Table 1). The more lipophilic analogue 2b was studied in rats with regard to a potential application as radio-gallium labelled tracer for hepatobiliary imaging by John et al. [12]. Those authors generally claimed “lipophilicity” and the available biological properties support this claim. For example, the ⁶⁷Ga-chelate of 2b is mainly excreted via the liver, which is a general hint on lipophilicity. Nevertheless, the compound did not show any uptake into the brain [7, 8, 12]. Being aware that high liver uptake of [⁶⁷,⁶⁸Ga]Ga³⁺ labelled compounds might also originate from

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**Table 1.** Hexadentate N₃S₃-type chelators based on 1,4,7-triazacyclononane.

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<tr>
<td>2a</td>
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<td>2b</td>
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\[^{[67,68]}\text{Ga}\]\text{Ga}^{3+}, bound to transferrin, we decided to investigate the stability of a bifunctional derivative of 2a. In addition, no bifunctional derivatives of 2a have been described in the literature so far, although these are highly promising candidates for the synthesis of small molecule \[^{68}\text{Ga}\] brain imaging agents.

2. Results and discussion

The present work elucidates a charge-neutral, macrocyclic bifunctional chelator derived from compound 2a. The corresponding gallium chelate should possess a reasonably low molecular weight, a low tendency to form intermolecular hydrogen bonds, kinetic inertness and sufficient thermodynamic stability to facilitate PET-imaging. The kinetic inertness of the complex containing a chain branch in one pendant arm is verified. Furthermore, the claim of lipophilicity of the complex containing a chain branch in one pendant arm was branched with an adjacent hydroxymethyl substituent. Thereby, various donor and acceptor functions can be incorporated by interconversion of the versatile hydroxyl-group.

To get an estimate on the feasibility of the synthesis and an estimate on the properties of a potential bio-molecule conjugated 1,4,7-trismercaptatoethyl-1,4,7-triazacyclononane (TACN-TM, 2a), model compound 9 was synthesised. 1,4,7-triazacyclononane (3) was synthesized via the common route described by Richman et al. [13, 14]. Deprotection and work up was slightly modified to result in a reproducible yield of 83 ± 5%. The reaction time for deprotection in concentrated sulphuric acid was reduced to 120 min at 110 °C. The conversion of the protected polyamino macrocycle was monitored using the method of Raßhofer and Voegtle [23].

The sulphuric acid was removed by two subsequent precipitations in EtO and MeOH. The obtained colourless slurry was taken up in water and made alkaline to pH 12, prior to the extraction with n-butanol to obtain compound 3 as colourless crystals of sufficient purity for all further reactions.

The synthesis of 9 was performed as shown in Scheme 1. Allyl-benzyl ether (4) was epoxidised as described previously to obtain 5 in 95% yield [15, 17]. Subsequent ring-opening with lithium triphenylmethyl mercaptate afforded compound 6 in 88% yield [16]. Mesylation of alcohol 6 under standard conditions gave sulphide 7 in 97% yield [17]. Compound 8 was obtained in 33% yield by mono-alkylation of 3 in dichloromethane [22].

The final chelator 9 was obtained from 8 in 55% yield via alkylation of the remaining secondary amines with ethylenethiol followed by immediate deprotection of the monothiol-protected intermediate [12, 18], resulting in an overall yield of 15%.

All initial labelling and stability experiments were carried out with model compound 9 bearing a benzyl ether at the intended coupling moiety.

Compound 9 was labelled with \[^{68}\text{Ga}\] in anhydrous chloroform as described previously, isolated by solid phase extraction and analysed by HPLC and TLC [21]. Fig. 2 gives a potential structure of the final complex 10.

The time dependence of the radiochemical yield is shown in Fig. 3. Precursor solution 9 (10 μl, 13 nmol) was added to the reaction mixture and the reaction was conducted under conventional heating (Fig. 3). Alternatively, using microwave irradiation (CEM discover focussed microwave, 300 W, 2 min), the product 10 was obtained in 93 ± 5% yield. The chloroform was evaporated and the product was taken up in purified water and passed through a strong cation exchanger (Merck Lichrolut® SCX, 200 mg)\(^1\).

The product was formulated in DPBS, filtered through a Millex\(^1\) sterile filter and analysed for radiochemical purity and specific radioactivity by radio-HPLC (Merck LiChroSorb\(^1\) RP-18, 7 μ, 150 × 4.6 mm, 25% MeOH in PBS at pH 7.4) and radio-TLC (silica-gel 60, 0.1 M citrate solution pH 4 or 30% EtOH in 5% NaCl). The radiochemical purity of the product exceeded 98% and the specific activity of the product was ≥ 7 GBq/μmol.

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\[^1\] Notably, the trapped non-reacted gallium can be eluted from the cartridge with 15% HCL in Acetone solution and the non-reacted precursor can be recovered by alkaline elution to some extent.
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Fig. 2. Potential structure of the gallium complex 10. Minimised energy conformation from molecular mechanics calculation.

Fig. 3. $^{68}$Ga labelling of chelator 9 at two different temperatures. Diamonds: 10 µg 9 at 90 °C; squares: 10 µg 9 at 40 °C. Values are mean ±1 SD from three independent determinations.

The stability of product $[^{68}\text{Ga}]10$ was determined via apo-transferrin transchelation and DTPA-challenge experiments [22]. Therefore, aliquots of the radioactive product were added to 1 nM, 10 nM, 100 nM and 1 µM concentrations of DTPA and 10 mg apo-transferrin in PBS (5 ml) at pH 7.4 and incubated at 37 °C for 180 min. Samples were withdrawn from the solutions after 5 min, 15 min, 30 min, 60 min, 120 min and 180 min. The percentage of intact complex $[^{68}\text{Ga}]10$ was determined by TLC.

It was found that practically no transchelation occurred between $[^{68}\text{Ga}]10$ and DTPA or apo-transferrin, indicating that the known high stability for the non-modified 1,4,7-trismercaptoethyl-1,4,7-triazacyclo-nonane was retained, despite the introduction of the pendant arm branch (Figs. 4 and 5).

The octanol-water partition coefficient of Ga10 was calculated using Cambridgesoft® ChemBioDraw/ChemBio3D 2007. A log $P$ value of 5.36 was obtained for the charge-free octahedral complex shown in Fig. 2. Most of the bioactive molecules suitable for conjugation with an appropriate radiolabel are less lipophilic than the simple aromatic hydrocarbon used as model. Therefore, representative values obtained for the model conjugate are expected to be higher than those obtained with real targeting vectors. However, the high theoretical value for the model compound supports the utility of 10 as a moiety suitable for the synthesis of lipophilic small molecules. Furthermore, the complex $[^{68}\text{Ga}]10$ shows reasonable kinetic inertness towards transchelation, which is not negatively affected by the pendant arm branch.

3. Conclusion

In conclusion, $[^{68}\text{Ga}]10$ is a highly promising candidate bifunctional chelator for the synthesis of lipophilic $^{68}$Ga imaging agents. Its synthesis is straightforward concerning the labelling precursor 9, and $^{68}$Ga labelling reactions proceed fast and efficient. The compound remains stable in vitro over the duration of a typical PET examination. It provides a lipophilic $c$ log $P$ which indicates suitability for the synthesis of lipophilic imaging agents.

4. Experimental

NMR-spectra were recorded with a Bruker AC 200 FT-NMR-spectrometer, $J$ values are given in Hertz, chemical shifts are reported downfield from TMS ($\delta = 0$ ppm) referred to the solvent residual signal $^1$H NMR (300 MHz,
CHCl₃ 7.224 ppm). Field desorption (FD) mass spectra were recorded on a Finnigan MAT90 FD spectrometer. All chemicals were obtained in commercial quality from Acros Organics, Sigma Aldrich, VWR, TCI or STREM and used without further purification unless otherwise stated. TLC was conducted on self-cut Merck silica gel 60 covered aluminium plates. Detection and staining was performed either using iodine on silica gel, potassium permanganate solution, UV fluorescence or vanillin–sulfuric acid. Column chromatography was performed on Acros silica gel 60, 0.063–0.200 mesh, p.a. solvents for chromatography were washed with CH₂Cl₂ (15 ml) and cooled to 0 °C. 3.48 (dd, \( J = 7.37 \) Hz, 2 H, \( CH₂O \)), 3.51 (“p”, \( J = 11 \) Hz, 1 H, \( CH₂O \)), 3.68 (dd, \( J = 6.6 \) Hz, 1 H, \( CH₂O \)). MS (ESI) \( m / z = 518.2 \) (100), \( C_{30}H_{30}O_{4}S_{2} \) requires 518.16. 2.20 (brd, \( J = 6.6 \) Hz, 1 H, \( CH₂O \)), 2.51 (brd, \( J = 6.6 \) Hz, 1 H, \( CH₂O \)). MS (FD) \( m / z = 551.3 \) (100) [M⁺]+, \( C_{30}H_{30}O_{4}S_{2} \) requires 551.30.

Preparation of [56Ga]Ga(acac)₃, as reported recently [21]: Briefly, the hydrochloric acid (0.1 M) containing [56Ga]GaIII was passed through the Biorad AG 50 W8 cation exchange resin to trap the [56Ga]³⁺. The resin was subsequently eluted with HCl (0.1 M) in acetonitrile (1 ml) by air to remove the non-gallium metal contamination. The n.c.a. radionuclide was eluted in a mixture of 2% acetylcetone and 5 mg of gentisic acid in acetonitrile, directly into a round-bottom, pressure-tight glass vial. Evaporation of the volatiles following by reconditioning in dry chloroform (5 ml) afforded n.c.a. [56Ga]Ga(acac)₃.

Radiolabelling under conventional heating in chloroform: Chelator 9 was dissolved in acetonitrile (1 mg/ml) and used for radio-labelling without further purification. Labelling precursor stock solution (10 µl) was added to [56Ga]Ga(acac)₃ in CHCl₃ (20 MBq, 5 ml) and the mixture was heated to 40 or 90 °C. To monitor reaction progress, aliquots were removed from the reactions mixture after 1, 3, 7 or 10 min, analysed by radio-HPLC (Merck LiChroSorb® RP-18, 7 µm, 150 × 4.6 mm, 25% MeOH in PBS at pH 7.4) and radio-TLC (silica-gel 60, 0.1 M citrate solution pH 4 or 30% EtOH in 5% NaCl).

Radiolabelling under microwave irradiation in chloroform: Labelling precursor stock solution (10 µl) was added
to $[^{68}\text{Ga}]{\text{Ga\text{acac}}}_3$ in CHCl$_3$ (100 MBq, 5 ml) in a pressure tight microwave reaction vial and irradiated in a CEM discover® focussed microwave reactor for 1, 2 or 5 min at 300 W. The solvent was removed with heating in a stream of nitrogen and the residue was taken up in purified water. The slightly acidic solution was passed through a strong cation exchanger (Merck Lichrolut® SCX, 200 mg) and formulated in PBS solution (1 ml).

Stability determination via DTPA and apo-transferrin challenge: Aliquots of the radioactive product in PBS (100 µl) were added to 1 nM, 10 nM, 100 nM and 1 µM concentrations of DTPA or 10 mg apo-transferrin in PBS (5 ml) at pH 7.4 and incubated for 180 min. Samples were withdrawn from the solutions after 5 min, 15 min, 30 min, 60 min, 120 min and 180 min. The percentage of intact complex $[^{68}\text{Ga}]{\text{Ga\text{acac}}}_3$ was determined by TLC.

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References