1,4,7-Triazacyclononane-1-succinic acid-4,7-diacetic acid (NODASA): a new bifunctional chelator for radio gallium-labelling of biomolecules

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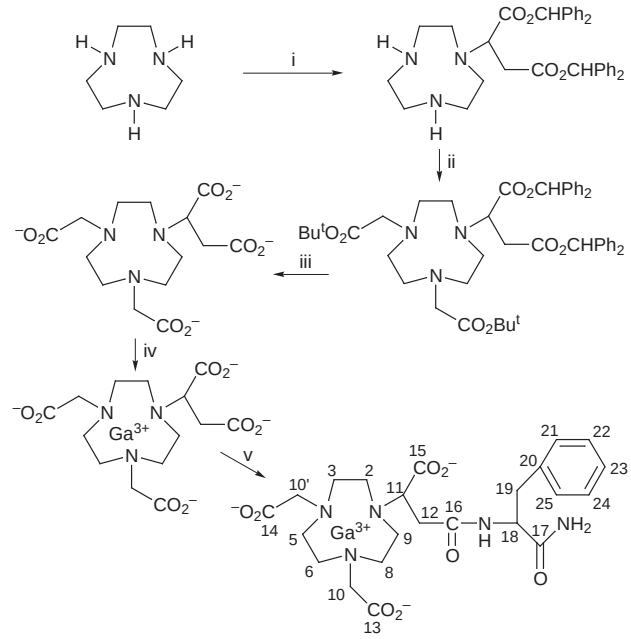
A new bifunctional chelator NODASA (1,4,7-triazacyclononane-1-succinic acid-4,7-diacetic acid) has been synthesised, its kinetically inert gallium(III) complex was crystallographically characterized and conjugated to a model aminoacidamide showing the feasibility of a prelabelling approach with ^{68,67}Ga.

Over the past ten years a constant interest in the chemistry of bifunctional chelators useful in biomedical applications has been evident.[1,2] Many were designed for coupling to monoclonal antibodies[3,4] and other biomolecules.[5] Recently bioactive peptides were successfully introduced into the clinic for *in vivo* visualization of tumours.[6,7] As these peptides show very fast blood clearance and diffusion into tissues the use of short lived metallic positron emitters becomes possible. In this context ^{68}Ga ($t_{1/2}$ = 68 min) is of special interest because its half-life is compatible with the rate of localization of small targeting molecules. For our purpose a bifunctional chelator is needed which is comprised of a gallium immobilizing moiety and a short carboxylate arm for coupling to the N-terminal end of bioactive peptides. We aim at using this bifunctional chelator in a preconjugation ^{67,68}Ga-labelling approach of somatostatin analogues. This preconjugation allows the covalent coupling of a well defined radiometal complex of high specific activity; this will be very important with regard to most radiopeptides because of their potential pharmacological effects.

Therefore we synthesised a new N-functionalised 1,4,7-triazamacrocycle in three steps by alkylation of 1,4,7-triazacyclononane (Scheme 1): (i) with 1 equiv. of bis(diphenylmethyl) D,L-bromosuccinate in chloroform; (ii) with 2 equiv. of *tert*-butyl bromoacetate in acetonitrile in the presence of K_2CO_3 followed by deprotection with 6 M HCl (iii). The overall yield of the synthesis of 1,4,7-triazacyclononane-1-succinic acid-4,7-diacetic acid (NODASA) was slightly > 50%.‡

Crystals of Ga(NODASA) were obtained from an aqueous solution of the ligand and $Ga(NO_3)_3$ in equimolar amounts (pH 3, 70 °C, 1 h; Scheme 1 (iv), followed by slow evaporation. Characterization by X-ray diffraction§ showed that the complex is electrically neutral and the Ga^{III} ion is fully chelated in a slightly distorted octahedral environment (Fig. 1). The β-carboxylate remains protonated and does not participate in the complexation. The three nitrogen atoms of the triazacyclononane define a plane in a facial arrangement and three of the pendant carboxylate oxygens constitute another one. These planes are almost coplanar with a dihedral angle of 1.75°. The *trans* N–Ga–O bond angles average 165.4°. This leads to a relative twist of the N_3 and O_3 planes by 14.6° away from a symmetrically staggered conformation, similar to the parent complex Ga(NOTA)[8] and different previously synthesised nickel(II) and chromium(III) NOTA complexes.[9] The variation in the individual Ga–O and Ga–N bond lengths is very small showing overall high similarity to the parent complex Ga(NOTA).[10] Ga(NODASA) was also characterized by its ^{1}H, ^{13}C and ^{69}Ga NMR spectra.¶ A multiplet centered at δ 3.75 (Fig. 2) due to the magnetically non equivalent acetate hydrogens is the most dramatic effect in the ^{1}H NMR spectrum of the chelate in relation to the free ligand, which exhibits a singlet at δ 3.50. The sharp multiplets of the ethylenic protons might show the existence of slow intramolecular processes of interconversion



Scheme 1 Synthesis of the chelate Ga(NODASA) and coupling to D-phenylalanineamide

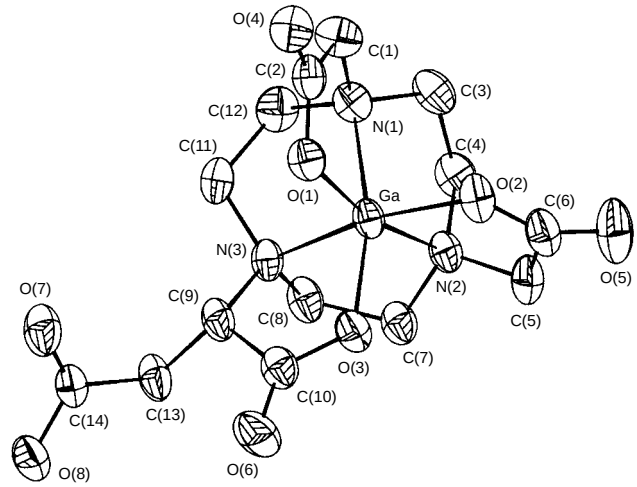


Fig. 1 ORTEP drawing of the Ga(NODASA) complex. Ga–N(1) 2.101(3), Ga–N(2) 2.098(3), Ga–O(1) 1.937(3), Ga–O(3) 1.942(3) Å; O(1)–Ga–O(3) 94.7(1), O(2)–Ga–N(3) 165.6(1), O(2)–Ga–N(2) 82.5(1), N(2)–Ga–N(3) 84.1(1)°.

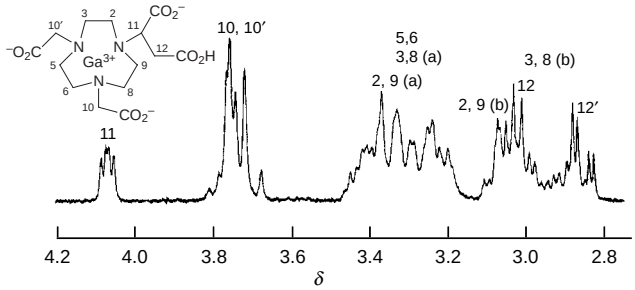


Fig. 2 ^{1}H NMR (400 MHz) spectrum of Ga(NODASA) in D_2O, 7 mM, pD = 3.6 and T = 22 °C

between the (λλλ) and (δδδ) conformations[11] of the ring backbone arising from the high rigidity of the system.

Although the structural results give some important indications about the binding of Ga^{III} to the macrocycle, the stability of this chelate is very important for successful applications *in vivo*. With a complexation competition method using ^{67}Ga as a radiotracer and NOTA‖ as an auxiliary competing ligand it has been possible to estimate the conditional stability constant for the complex at different pH values. The equilibration has been followed for nine days. The determination of the ligand protonation constants allows the calculation of the thermodynamic stability constant of Ga(NODASA) to $\log K_{Ga(NODASA)}$ = 30.9(0.2) compared to 30.98 of Ga(NOTA).[12]

An even more important indicator for *in vivo* applications is the measurement of the rate of exchange of Ga^{III} in blood serum under the physiological conditions.[14] For this experiment $^{67}Ga^{III}$ is first incubated with about 50 times excess of NODASA at pH 6.2 in 0.5 M ammonium acetate buffer (25 min, 90 °C) in order to incorporate the metal ion. Then the complex is mixed with blood serum and the exchange kinetics with transferrin are measured at 37 °C. This was done by taking aliquots of the serum, separating them by gel filtration, which allows the separation of ^{67}Ga(NODASA) from gallium(III)–transferrin ($\log K$ = 23.7),[13] and measuring the activity in both fractions with use of radiometric detection. The results clearly show that ^{67}Ga–NODASA virtually does not transfer any ^{67}Ga to transferrin over the observed period of 5 days, fulfilling the criterion of high kinetic stability.

The kinetic stability of Ga(NODASA) with respect to the acid-catalysed dissociation has been demonstrated with the aid of the ^{67}Ga complex, kept in 0.1 M glycine–HCl buffer, pH 2, at 37 °C. Aliquots of this solution were analysed by HPLC. After 5 days the complex was still 100% intact.

The fact that the β-carboxylate remains free while the other three carboxylates are involved in five-membered chelate rings, upon coordination to the metal ion, offers a very interesting possibility to couple the chelate to a biomolecule. As a model peptide we coupled D-phenylalanineamide to Ga(NODASA) [Scheme 1 (v), in DMSO–DMF (2 : 1)] using HATU** [15] as the coupling reagent with almost quantitative yield.†† HATU allows coupling of carboxylate functions to primary amines within minutes rendering even the coupling of ^{68}Ga(NODASA) to peptides feasible.

In summary, ^{67,68}Ga(NODASA) can be used in a pre-labelling approach followed by conjugation to a biomolecule. This approach is currently being followed using somatostatin analogues.

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Notes and References

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‡ The ligand had satisfactory elemental analysis, ^{1}H, ^{13}C NMR and mass spectra. The pK_a values were determined by pH potentiometry: p$K_{HL^{3-}}$ = 11.71, p$K_{H_2L^{2-}}$ = 5.94, p$K_{H_3L^-}$ = 4.27, pK_{H_4L} = 3.22 and p$K_{H_5L^+}$ = 1.95 (0.50 M KNO_3).

§ *Crystal data*: $C_{14}H_{20}GaN_3O_8 \cdot 3H_2O$, monoclinic, space group $P2_1/n$ [a = 7.6077(6), b = 20.573(3), c = 12.186(2) Å, β = 97.726(9)°], Z = 4, F(000) = 1000, μ = 2.56 mm^{-1}, Cu-Kα = 1.54180 Å, T = 293 K, θ_{max} = 77.50°, ω–2θ scan technique, 3561 independent reflections, 3011 used in refinement, 284 parameters refined, final R = 5.08, final R_w = 0.0626, Chebychev polynomial weighting. CCDC 182/850.

¶ ^{13}C NMR 100 MHz (D_2O), δ 31.3 (C12), 44.9 (C2,9, 52.8–53.5 (C3,5,6,8), 61.9 and 62.0 (C10,10′), 65.8 (C11), 174.8 and 175.0 (C13,14,15,16). ^{69}Ga NMR 72.05 MHz (D_2O) shows a single resonance at δ + 165 ($w_{1/2}$ = 1000 Hz).

‖ 1,4,7-Triazacyclononane-1,4,7-triacetic acid.

** O-(7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate.

†† R_f[SiO_2, isopropyl alcohol–NH_3(aq) (7 : 3)] = 0.40; *m/z* (ESI^+) : 574.4(MH^+, 15), 594.1 (MNa^+, 100); ^{13}C NMR 100 MHz (D_2O), δ 32.0 (C12), 38.3 (C19), 44.0 (C2,9), 52.4–53.0 (C3,5,6,8) 54.4 and 54.8 (C18), 61.5 (C10,10′), 64.5 and 65.0 (C11), 128.4–128.9 (C21–25), 136.8 (C20), 169.4 (C17), 171.5 (C13,14), 172.4 (C15), 174.0 (C16).

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