Structure and stability of hexadentate complexes of ligands based on AAZTA for efficient PET labelling with gallium-68

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Pre-organised tricarboxylate ligands based on 6-amino-perhydro-1,4-diazepine bind 68Ga rapidly and selectively in acetate buffer at pH 4 to 7, forming kinetically stable complexes suitable for use in PET imaging.

We present a series of hexadentate tribasic ligands based on perhydro-1,4-diazepine that bind 68Ga rapidly in the pH range 4–7, forming radiolabelled complexes suitable for imaging studies using positron emission tomography.1

There are many examples of aza-carboxylate (e.g. NOTA)2 and related aza-phosphinate ligands based on 1,4,7-triazacyclononane,3,4 that form stable 1 : 1 complexes with small metal ions. An octahedral coordination geometry is favoured, for example with Ga3+, Fe3+, Mn2+ and Zn2+. This has drawn the attention of such related aza-phosphinate ligands with lanthanide (log K = 0.1 KCl). The relative steric demand of the methyl and N-substituents at the 6-position means that AAZTA, as a di-N-protonated ligand, is not pre-organised for metal binding (Scheme 1). The methyl or related primary alkyl groups (A value ca. 7 kJ mol−1) have a lower steric demand than the protonated nitrogen substituent, (A value ca. 10 kJ mol−1). Hence, it prefers to take up the pseudo-axial site (Scheme 1) so that the ligand presents isolated EDDA (ethylenediamine diacetate) and iminodiacetate binding units in the major conformer, that could lead to kinetically trapped complexes, under conditions that do not allow formation of the most stable complex by cooperative donor ligation. Such conditions occur during radiolabelling of ligands with the positron emitting isotope 68Ga, (t1/2 = 67.7 min) as the eluate from a 68Ge generator is acidic (pH 0.2 to 1.3).7

Moreover, gallium aqua species tend to hydrolyse above pH 6.

With this background in mind, we have compared AAZTA with ligands L1−4, examining the efficiency of radiolabelling as a function of pH, as well as assessing the relative stability of 68Ga complexes. The selection of the new ligands L1 and L2 was made in order to introduce a bulkier phenyl substituent (A value 11.7 kJ mol−1) at the 6-position, favouring population of the desired metal-binding conformation (Fig. 1). Ligands L1 and L2 were made following established methods.5,6

In modifying the synthesis for L3 and L4, ak e yi s s u e w a st h en e e need accessible by N-functionalisation. Indeed, the archetypal ligand AAZTA, 2, (pKa 11.23, 6.52)3c forms stable complexes in water with lanthanide (log K ca. 20) and calcium ions (log K 12.8, 298 K, I = 0.1 KCl). The relative steric demand of the methyl and N-substituents at the 6-position means that AAZTA, as a di-N-protonated ligand, is not pre-organised for metal binding (Scheme 1). The methyl or related primary alkyl groups (A value ca. 7 kJ mol−1) have a lower steric demand than the protonated nitrogen substituent, (A value ca. 10 kJ mol−1). Hence, it prefers to take up the pseudo-axial site (Scheme 1) so that the ligand presents isolated EDDA (ethylenediamine diacetate) and iminodiacetate binding units in the major conformer, that could lead to kinetically trapped complexes, under conditions that do not allow formation of the most stable complex by cooperative donor ligation. Such conditions occur during radiolabelling of ligands with the positron emitting isotope 68Ga, (t1/2 = 67.7 min) as the eluate from a 68Ge generator is acidic (pH 0.2 to 1.3).7 Moreover, gallium aqua species tend to hydrolyse above pH 6.

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In modifying the synthesis for L3 and L4, a key issue was the need to remove the N-benzyl groups and reduce the nitro group in the cyclic intermediate, 3, without ring opening, e.g. by concomitant hydrogenolysis at the quaternary centre (Scheme 2). By using a...
2,4-dimethoxybenzyl group in place of a simple benzyl moiety, debenzylation with TFA was undertaken by RANEY® nickel hydrogenation, averting solvolysis at the benzylic site, with loss of the nitro group. The more bulky phenyl ring in \( \text{L}^1 \) suppresses the lactamisation reaction that occurs readily in acidic aqueous media between an endocyclic \( \text{N}^- \text{acetate} \) group and the secondary amine site. This has been observed to occur readily, even at pH 3 for the analogue of \( \text{L}^3 \) (Me at the quaternary centre), and is apparent in aged aqueous solution samples of \( \text{L}^2 \).

The gallium(m) complexes of \( \text{L}^1\text{--L}^3 \) crystallised readily from aqueous solution at pH 4. Their molecular structures were determined by X-ray crystallography at 120 K. Complexes [GaL\(^1\)] and [GaL\(^2\)] crystallized as hemi- and monohydrates respectively; crystals of complex [GaL\(^3\)] contain two almost identical crystallographically independent molecules. Each gallium ion is coordinated by the \( \text{N}_{2}	ext{O}_{4} \) donors forming charge neutral complexes, and the geometry around the Ga(m) ion is a slightly distorted octahedron (Table 1 and Fig. 2).

The radiolabelling performance of ligands L\(^1\text{--L}^4 \) was assessed in comparison to AAZTA at pH 4.0, 5.3 (acetate buffer, 0.2 M) and 6.8 (1 M HEPES), using 100 MBq \( ^{68}\text{Ga} \) (0.66 nM) and a ligand concentration of 10 \( \mu \text{M} \), (Table 2). With AAZTA, three radio-labelled species were observed, whose relative ratio varied with pH; in contrast, with \( \text{L}^2\text{--L}^4 \), only one species was formed at each pH examined.

The relative stability of the radiolabelled complexes was assessed by diluting the labelled complexes in fresh PBS buffer (1 M, pH 7.4, 310 K, [Ga] = 0.16 nM), comparing behaviour as a function of time by radio-TLC. Similar protocols were used in the presence of either excess DTPA, Fe(III) (each 5000 eqs), apotransferrin (130 eqs) or in foetal calf serum. In each case, the complexes of ligands \( \text{L}^2\text{--L}^4 \) were much less stable and did not resist the DTPA and serum challenge. This behaviour was even more pronounced for preparations made at pH 2.3 or 3.3. Comparing sets of complexes labelled at pH 2.3, the phenyl-substituted pair of ligands, \( \text{L}^3\text{--L}^4 \) showed better stability profiles than their methyl analogues, \( \text{L}^1\text{--L}^2 \).
by factors of 2 to 6, with 50% of the observed species unchanged. The formation of multiple species with AA'TA (and to a lesser extent with L1) of differing relative stability is consistent with the formation of 'kinetically trapped', constitutionally isomeric complexes (Scheme 1). These less stable complexes may involve weaker binding to the EDDA moiety. For AA'TA, the adoption of N2O4 as well as the favourable N2O4 coordination type may occur competitively. Importantly, with L2,45 the formation of a single, major stable gallium bound species is most probably assisted by adoption of a favourable, 'pre-organised' conformation of the di-N-protonated ligand, in which the exocyclic N-substituent adopts an axial site.

These gallium-labelled characteristics compare favourably with the behaviour reported for related acyclic and macrocyclic hexadentate ligands.3,4,13–15 Indeed, in separate challenge experiments with L1 and NOTA (20 μM of each ligand, 0.66 nM [68Ga], 298 K, pH 4.0, 0.2 M acetate), the acyclic ligands were each found in preference within 1 minute and the order of preferential binding was L3 > L4 > L2 > L1. The ratio of the gallium labelled L1/NOTA complexes was 3 : 1 under these conditions and did not vary thereafter with time, reflecting the relative rates of formation of these kinetically stable complexes.

Finally, preparations of [68GaL1] and [68GaL4] were injected into Sprague-Dawley rats to assess preliminary biodistribution behaviour. The compounds appear to be biologically inert. The only organs detectable by positron emission tomography were the liver, kidneys and bladder, with no evidence for complex retention in any other organ. Twenty-five minutes after administration of [68GaL1] via the tail vein, the signal was only detectable by positron emission tomography in the kidney and bladder, with no evidence for retention in any other organ (ESI†). Such behaviour is consistent with the high kinetic stability profile observed in vitro, and suggests that these complexes offer scope as efficient and effective imaging probes, and may be simply adapted structurally to allow the labelling of biomolecules. Current work is exploring this behaviour.

In conclusion, a new series of hexadentate ligands has been created, suitable for radiolabelling with 68Ga over the pH range 4 to 7. Using a ligand with an aryl substituent at the quaternary site, a preferred binding conformation is adopted that suppresses the formation of less stable, kinetically trapped complexes.

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Notes and references
† The X-ray single crystal data were collected at 120 K on an Agilent Gemini S-Ultra diffractometer (graphite monochromator, MoKα, λ = 0.70073 Å) equipped with Cryostream (Oxford Cryosystems) open-flow nitrogen cryostat. The structures were solved by direct methods and refined by full-matrix least squares on F2 for all data using Olex212 and SHELXTL.12 software. [GaL1]: C13H27GaN3O6, M = 393.05, orthorhombic, space group Fdd2, a = 28.8536(7), b = 27.2438(7), c = 7.57981(19) Å, V = 5938.2(2) Å3, Z = 16, μ(Mo Kα) = 1.888 mm−1, Dcalc = 1.753 g mm−3, 18,337 reflections measured (5.64 ≤ 2θ ≤ 59.98), 4,319 unique (Rint = 0.0417) were used in all calculations. Final R1 = 0.0307 (>2σ(f)); wR2 = 0.0674 (all data). [GaL2]: C16H34GaN3O6, M = 430.11, monoclinic, space group P21, a = 7.85267(20), b = 12.6657(3), c = 9.2182(2) Å, β = 100.735(3), V = 689.85(4) Å3, Z = 2, μ(Mo Kα) = 1.627 mm−1, Dcalc = 1.642 g mm−3, 12,473 reflections measured (5.46 ≤ 2θ ≤ 57.98), 4501 unique (Rint = 0.0443) used in all calculations. Final R1 = 0.0322 (>2σ(f)); wR2 = 0.0702 (all data). [GaL3]: C16H29Ga2N4O6, M = 432.08, triclinic, space group P1, a = 10.6961(8), b = 13.0778(12), c = 13.8414(12) Å, α = 116.076(9), β = 105.208(7), γ = 92.002(7), V = 1652.8(2) Å3, Z = 4, μ(Mo Kα) = 1.709 mm−1, Dcalc = 1.736 g mm−3, 16,836 reflections measured (5.04 ≤ 2θ ≤ 55), 7571 were unique (Rint = 0.0881) and used in all calculations. Final R1 = 0.0699 (>2σ(f)); wR2 = 0.1864 (all data).


6 During the course of this work, managanese(II) complexes of L1 and L4 were described and evaluated as contrast agents in MRI: L. Tei, G. Gugliotta, M. Fekete, F. K. Kalman and M. Bott, Dalton Trans., 2011, 40, 2025.


8 Such substituted, heterocyclic seven membered rings adopt several low energy conformers, of which the twist-chair is often the lowest in energy: F. Freeman, J. H. Hwang, E. H. Junge, P. D. Parmar, Z. Renz and J. Trinh, Int. J. Quantum Chem., 2008, 108, 339. Full details of the conformational analysis of the gallium ligands will be reported elsewhere.