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

Cite this: *Chem. Commun.*, 2013, **49**, 579

Received 16th October 2012,
Accepted 13th November 2012

DOI: 10.1039/c2cc37544c

www.rsc.org/chemcomm

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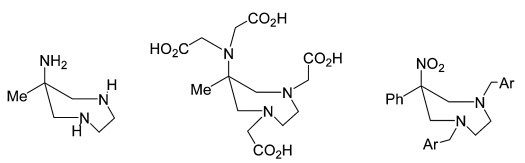
Pre-organised tricarboxylate ligands based on 6-amino-perhydro-1,4-diazepine bind ^{68}Ga rapidly and selectively in acetate buffer at pH 4 to 7, forming kinetically stable complexes suitable for use in PET imaging.

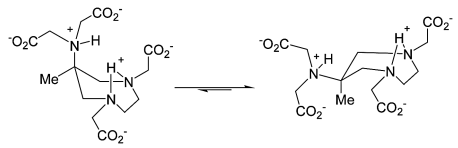
We present a series of hexadentate tribasic ligands based on perhydro-1,4-diazepine that bind ^{68}Ga rapidly in the pH range 4–7, forming radiolabelled complexes suitable for imaging studies using positron emission tomography.[1]

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 Ga^{3+}, Fe^{3+}, Mn^{2+} and Zn^{2+}. This has drawn the attention of such ligands for radiolabelling studies, *e.g.* with $^{67/68}$Ga, and ^{111}In. Recently, the coordination chemistry of various ligands derived from 6-amino-6-methyl-perhydro-1,4-diazepine, **1**, has also been studied.[5,6]



1 2 3

The parent triamine **1** is constitutionally isomeric with 1,4,7-triazacyclononane and presents three N atoms that serve as donors in a facial array. The presence of the primary amine group means that heptadentate ligands are particularly accessible by N-functionalisation. Indeed, the archetypal ligand AAZTA, **2**, (pK_a 11.23, 6.52)[5c] 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 imino-diacetate 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 ^{68}Ga, ($t_{1/2}$ = 67.7 min) as the eluate from a ^{68}Ge generator is acidic (pH 0.2 to 1.3).[7] Moreover, gallium aqua species tend to hydrolyse above pH 6.



Scheme 1 Schematic diagram[8] of solution conformers for $[H_2AAZTA]^{2-}$ (equilibrium shifts right to reduce repulsion).

With this background in mind, we have compared AAZTA with ligands L^{1-4}, examining the efficiency of radiolabelling as a function of pH, as well as assessing the relative stability of ^{68}Ga complexes. The selection of the new ligands L^3 and L^4 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 L^1 and L^2 were made following established methods.[5,6] In modifying the synthesis for L^3 and L^4, 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

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† Electronic supplementary information (ESI) available: Ligand and complex synthesis and characterisation; pH/^{1}H NMR titration data; radiochemical labelling details; X-ray structures of the 3 gallium complexes as well as two intermediates in the synthesis of L^1 and L^3. CCDC 906036–906040. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2cc37544c

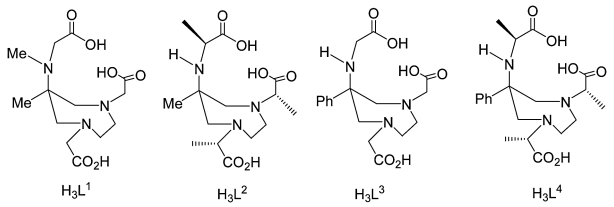
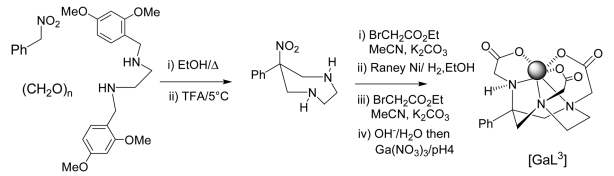


Fig. 1 Hexadentate ligands based on perhydro-1,4-diazepine, shown in their likely metal ion binding conformation.



Scheme 2 Synthetic scheme for [GaL^3].

2,4-dimethoxybenzyl group in place of a simple benzyl moiety, debenzylation with TFA was achieved at room temperature; subsequent reduction of the nitro group could be undertaken by RANEY® nickel hydrogenation, averting solvolysis at the benzylic site, with loss of the nitro group. The more bulky phenyl ring in L^3 suppresses the lactamisation reaction that occurs readily in acidic aqueous media between an endocyclic *N*-acetate group and the secondary amine site. This has been observed to occur readily, even at pH 3 for the analogue of L^3 (Me at the quaternary centre), and is apparent in aged aqueous solution samples of L^2.

The gallium(III) complexes of L^1–L^3 crystallised readily from aqueous solution at pH 4. Their molecular structures were determined by X-ray crystallography at 120 K. Complexes [Ga·L^1] and [Ga·L^2] crystallized as hemi- and monohydrates respectively; crystals of complex [Ga·L^3] contain two almost identical crystallographically independent molecules.‡ Each gallium ion is coordinated by the N_3O_3 donors forming charge neutral complexes, and the geometry around the Ga(III) ion is a slightly distorted octahedron (Table 1 and Fig. 2).

The radiolabelling performance of ligands L$^{1–4}$ 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}Ga (0.66 nM) and a ligand concentration of 10 μM, (Table 2). With AAZTA, three radiolabelled species were observed, whose relative ratio varied with pH; in contrast, with L$^{2–4}$, only one species was formed at each pH examined.

Table 1 Selected bond distances (Å) and geometric parameters for [Ga·L$^{1–3}$] (120 K;[11,12] for numbering system, see Fig. 2) (ESI)

	[Ga·L^1]	[Ga·L^2]	[Ga·L^3]
Ga–O(1)	1.939(2)	1.967(1)	1.945(4)
Ga–O(3)	1.904(2)	1.895(2)	1.912(4)
Ga–O(5)	1.933(2)	1.934(2)	1.918(4)
Ga–N(1)	2.140(2)	2.158(2)	2.128(4)
Ga–N(2)	2.138(2)	2.150(2)	2.144(5)
Ga–N(3)	2.111(2)	2.075(2)	2.091(4)
Σ^a	9.27	8.72	10.3
Θ^b	10.7	16.9	13.8

[a] Octahedral distortion parameter $\Sigma = \Sigma(|90 - \varphi i|)/12$ [$\Sigma = 0^\circ$ for an ideal octahedron; φi represents the 12 smallest L–M–L angles].[9] [b] Average trigonal distortion angle $\Theta = \Sigma(|60 - i|)/24$ [$\Theta = 0^\circ$ for an ideal octahedron; i represents the trigonal angles of the eight faces of the octahedron].[10]

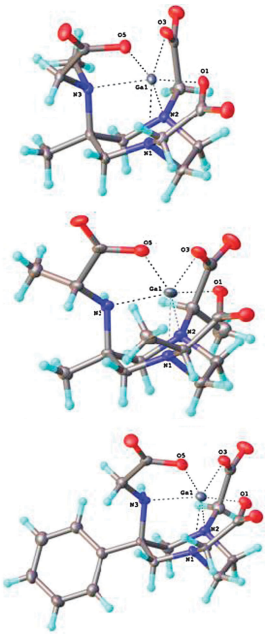


Fig. 2 Molecular structures of gallium(III) complexes (120 K): *upper*: [Ga·L^1]; *centre*: (*SSS*) [Ga·L^2]; *lower*: [Ga·L^3].

Table 2 Radiolabelling yields (%, s.d. ±1)) and times (min, in parenthesis) for labelling of AAZTA and L$^{1–4}$ (295 K, [^{68}Ga = 0.66 nM (t = 0); [L] = 10 μM; 1.4 mL) (ESI)

pH	AAZTA	L^1	L^2	L^3	L^4
4.0[b,c]	97[a] (1)	98[a] (1)	99 (3)	97 (3)	99 (3)
5.3	96[a] (1)	97[a] (1)	98 (3)	98 (3)	99 (3)
6.8	96[a] (3)	97[a] (1)	98 (3)	97 (3)	97 (3)

[a] Formation of more than one species occurred, *e.g.* for AAZTA at each pH, three species were observed and ratio was typically 9 : 9 : 1, and for L^1, 18 : 1. [b] At pH 3.3 ([^{68}Ga] = 0.2 nM, [L] = 10 mM] > 98% labelling was achieved in every case within 10 min at 323 K, but multiple species were observed notably with AAZTA (2 : 2 : 1) and L^1 (9 : 1), of differing relative stability. [c] Ligands L$^{1–4}$ also exhibited quantitative labelling at pH 4 with less ligand (298 K, 5 μM [L], 0.66 nM [^{68}Ga].

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, [GaL] = 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 L$^{2–4}$ prepared at pH 4 and above resisted these challenges completely, over a 2 h period.

However, with labelled AAZTA, the minor species formed 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, L$^{3/4}$ showed better stability profiles than their methyl analogues, L$^{1/2}$,

by factors of 2 to 6, with 50% of the observed species unchanged. The formation of multiple species with AAZTA (and to a lesser extent with L^1) 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 AAZTA, the adoption of N_2O_4 as well as the favourable N_3O_3 coordination type may occur competitively. Importantly, with L^{2-4}, the formation of a single, major stable gallium bound species is most probably assisted by adoption of a favourable, 'pre-organised' conformer of the di-*N*-protonated ligand, in which the exocyclic N-substituent adopts an axial site.

These gallium-labelling characteristics compare favourably with the behaviour reported for related acyclic and macrocyclic hexadentate ligands.[3,4,13–15] Indeed, in separate challenge experiments with L^{1-4} and NOTA (20 μM of each ligand, 0.66 nM [^{68}Ga], 298 K, pH 4.0, 0.2 M acetate), the acyclic ligands were each bound in preference within 1 minute and the order of preferential binding was $L^3 > L^1 > L^2 > L^4$. The ratio of the gallium labelled L^3/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 [^{68}GaL^1] and [^{68}GaL^4] 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 [^{68}GaL^1] *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 ^{68}Ga 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.

We thank the Association of Commonwealth Universities for a scholarship (BPW) and ESF COST Action D38 for support.

Notes and references

‡ The X-ray single crystal data were collected at 120 K on an Agilent Gemini S-Ultra diffractometer (graphite monochromator, λMo Kα, 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 F^2 for all data using Olex2[11] and SHELXTL[12] software. [Ga·L^1]: $C_{13}H_{21}GaN_3O_{6.5}$, M = 393.05, orthorhombic, space group *Fdd*2, a = 28.8536(7), b = 27.2438(7), c = 7.57981(19) Å, V = 5958.3(2) Å^3, Z = 16, μ(Mo Kα) = 1.888 mm^{-1}, D_{calc} = 1.753 g mm^{-3}, 18 337 reflections measured ($5.64 \leq 2\Theta \leq 59.98$), 4319 unique ($R_{int}$ = 0.0417) were used in all calculations. Final R_1 0.0307 (>2σ(I)); wR_2 0.0674 (all data). [Ga·L^2]: $C_{15}H_{26}GaN_3O_7$, M = 430.11, monoclinic, space group $P2_1$, a = 7.58267(20), b = 12.6657(3), c = 9.2185(2) Å, β = 100.735(3)°, V = 869.85(4) Å^3, Z = 2, μ(Mo Kα) = 1.627 mm^{-1}, D_{calc} = 1.642 g mm^{-3}, 12 473 reflections measured ($5.46 \leq 2\Theta \leq 57.98$), 4501 unique ($R_{int}$ = 0.0433) used in all calculations. Final R_1 0.0322 (>2σ(I)); wR_2 0.0702 (all data). [Ga·L^3]: $C_{17}H_{20}GaN_3O_6$, M = 432.08, triclinic, space group $P\bar{1}$, 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) Å^3, Z = 4, μ(Mo Kα) = 1.709 mm^{-1}, D_{calc} = 1.736 g mm^{-3}, 16 836 reflections measured ($5.04 \leq 2\Theta \leq 55$), 7571 were unique ($R_{int}$ = 0.0881) and used in all calculations. Final R_1 0.0699 (>2σ(I)); wR_2 0.1864 (all data).

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