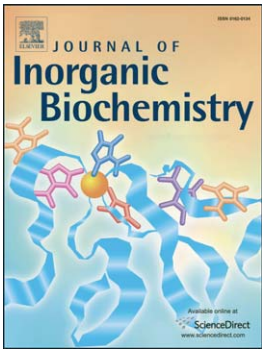


A gallium(III) Schiff Base-curcumin complex that binds to amyloid-β plaques

Jaclyn L. Lange, David J. Hayne, Peter Roselt, Catriona A. McLean, Jonathan M. White, Paul S. Donnelly

PII: S0162-0134(16)30056-3
DOI: doi: 10.1016/j.jinorgbio.2016.02.029
Reference: JIB 9943

To appear in: *Journal of Inorganic Biochemistry*

Received date: 12 November 2015
Revised date: 18 February 2016
Accepted date: 25 February 2016

Please cite this article as: Jaclyn L. Lange, David J. Hayne, Peter Roselt, Catriona A. McLean, Jonathan M. White, Paul S. Donnelly, A gallium(III) Schiff Base-curcumin complex that binds to amyloid-β plaques, *Journal of Inorganic Biochemistry* (2016), doi: 10.1016/j.jinorgbio.2016.02.029

A Gallium(III) Schiff Base-Curcumin Complex That Binds to Amyloid-β Plaques

Jaclyn L. Lange[1], David J. Hayne[1], Peter Roselt[2], Catriona A. McLean[3], Jonathan M. White[1] and Paul S. Donnelly[*1]

[1] School of Chemistry and Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Melbourne, 3010, Victoria, Australia.

[2] Research Division, Peter MacCallum Cancer Centre, St Andrews Place, East Melbourne, Victoria, Australia.

[3]Department of Anatomical Pathology, The Alfred Hospital, Victoria 3181, Australia

Highlights

- A neutral gallium complex with curcumin acting as a bidentate ligand has been prepared.
- The complex binds to amyloid-beta plaques present in human Alzheimer's disease brains.

Keywords

Gallium(III) complexes; Gallium radiopharmaceuticals; Alzheimer's disease; Diagnostic imaging; Positron-emission tomography; Amyloid-beta

Abstract

Gallium-68 is a positron-emitting isotope that can be used in positron-emission tomography imaging agents. Alzheimer's disease is associated with the formation of plaques in the brain primarily comprised of aggregates of a 42 amino acid protein called amyloid-β. With the goal of synthesizing charge neutral, low molecular weight, lipophilic gallium complexes with the potential to cross the blood-brain barrier and bind to Aβ plaques we have used an ancillary tetradentate N_2O_2 Schiff base ligand and the β-diketone curcumin as a bidentate ligand to give a six-coordinate Ga^{3+}

complex. The tetradentate Schiff base ligand adopts the *cis*-β configuration with deprotonated curcumin acting as a bidentate ligand. The complex binds to amyloid-β plaques in human brain tissue and it is possible that extension of this chemistry to positron-emitting gallium-68 could provide useful imaging agents for Alzheimer's disease.

Introduction

Alzheimer's disease (AD), the most common form of neurodegenerative dementia, is characterised by the presence of extracellular amyloid plaques and intracellular neurofibrillary tangles in the brain.[1] A major constituent of the extracellular amyloid plaques is an aggregated peptide called amyloid-β (Aβ), a 39-43 amino acid peptide derived from the amyloid precursor protein (APP).[2-5] These amyloid plaques do not consistently correlate with cognitive impairment and some argue that smaller soluble oligomeric species are the toxic species responsible for neuronal death.[6] However, oligomers and plaques are thought to be in equilibrium. The Neurofibrillary Tangles (NFT) consist of a hyper-phosphorylated form of a microtubule-associated protein called tau.[7]

Definitive diagnosis of AD has traditionally relied on post-mortem histological analysis but in the last decade diagnostic imaging of Aβ plaque burden in patients has become possible using positron emission tomography (PET) and radiolabeled tracers that bind to Aβ plaques. These new imaging agents have provided valuable insights into the role of Aβ plaques to cognitive impairment and are assisting in re-defining the requirements for clinical diagnosis.[8, 9] One of the first tracers used for Aβ imaging in humans was a benzothiazole derivative radiolabelled with positron-emitting carbon-11, ^{11}C-PIB (Pittsburgh compound B) (Fig. 1).[10-12] Selected benzothiazole derivatives are known to bind to Aβ fibrils and plaques and this interaction is mediated by a combination of hydrophobic and hydrogen bonding interactions. Stilbene and styrylpyridine derivatives are structurally related to benzothiazoles and also exhibit selective binding to Aβ plaques.[13] A group of stilbene and styrylpyridine derivatives have been identified as having potential to be of use in the

differential diagnosis of AD from other conditions. Efforts focusing on preparing stilbene or styrylpyridine derivatives radiolabelled with positron emitting fluorine-18 have culminated with the recent FDA approval of ^{18}F-AV45 (florbetapir).[14-16] The synthesis of compounds radiolabelled with ^{18}F often relies on covalent modification of an organic molecule with the radionuclide that is produced by a cyclotron. In principle the simple and rapid incorporation of a positron-emitting metallic isotope into a ligand designed to bind to Aβ plaques is an attractive alternative to covalent bond formation with ^{18}F. Positron-emitting radionuclides of potential interest in this context include ^{61}Cu, ^{62}Cu, ^{64}Cu and ^{68}Ga.[17-22] Relatively recent developments have led to availability of positron-emitting ^{68}Ga from a ^{68}Ge/^{68}Ga generator. The parent nuclide, ^{68}Ge, possesses a half-life of 271 days and the generators can provide sufficient quantities of ^{68}Ga for up to one year resulting in a relatively inexpensive and reliable source of a positron-emitting radionuclide. The favorable decay properties of ^{68}Ga and a half-life of 68 minutes mean that the radionuclide is attractive for imaging applications based on peptide-targeted systems and this has been pursued with considerable success.[23-33] To date there has been less focus on developing ^{68}Ga labeled lipophilic small molecules suitable for brain imaging.[34, 35]

Curcumin (curcH = 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is a component of the Indian spice turmeric that has a long history of use in traditional medicine. Sustained interest in the broad pharmacological activity of curcumin has led to several clinical trials investigating the compounds potential as an anticancer agent and as an anti-inflammatory. Curcumin is also known to bind to Aβ plaques presumably through similar interactions to the structurally similar Congo Red and substituted stilbene derivatives.[36, 37] As a natural β-diketone curcumin is a good ligand for metal ions and a large number of metal complexes of curcumin have been prepared including complexes with the [^{99m}Tc(CO)$_3$] core that have potential application in single photon emission tomography (SPECT) imaging.[38-40] The [Ga(curc)] complex has been prepared and this lead us to investigate the potential of radiolabeled ^{68}Ga^{3+} curcumin complexes as the basis of new Aβ plaque imaging agents.[41] During the preparation of this manuscript the

synthesis of complexes proposed to be $[^{68}Ga(curc)_2]^+$ was reported and these complexes demonstrated some affinity for $A\beta_{1-40}$ fibrils.[42] Herein we described the synthesis of a charge neutral lipophilic Ga^{3+} complex that binds to Aβ plaques in human brains. Our approach employs an ancillary tetradentate N_2O_2 Schiff base ligand and the β-diketone curcumin as a bidentate ligand to give a six-coordinate Ga^{3+} complex.

Experimental

All reagents and solvents were obtained from standard commercial sources and unless otherwise stated were used as received. Commercial curcumin was purified by chromatography.[41] Elemental analysis for C, H and N were carried out by Chemical & Microanalytical Services (CMAS) Pty. Ltd. Belmont, Vic and Microanalytical Unit, Australian National University, ACT. ^{1}H-NMR spectra were recorded with a Varian FT-NMR 500 spectrometer (Varian, California USA) at 500 MHz. ^{13}C-NMR spectra were recorded with a Varian FT-NMR 400 spectrometer (Varian, California USA) and acquired at 101 MHz. All NMR spectra were recorded at 298˚K. The reported chemical shifts (in parts per million) are referenced relative to residual solvent signal. Mass spectra were recorded on an Agilent 6510 ESI-TOF LC/MS Mass Spectrometer (Agilent, California USA). Absorbance spectra were obtained on a Shimadzu UV-1650 PC spectrophotometer (Shimadzu, Sydney NSW) and emission spectra were obtained on a Varian CAREY Eclipse fluorescence spectrophotometer (Varian, California USA).

Syntheses

N,N-bis(2-ethoxysalicylidene)-1,3-diaminopropane (H_2L): A mixture of 3-ethoxysalicylaldehyde (0.60 g, 3.6 mmol) and 1,3-diaminopropane (0.13 g, 1. 80 mmol) in methanol (10 mL) was heated at reflux for 30 minutes. A yellow precipitate formed which was collected by filtration, washed with cold methanol (2 x 5 mL) and diethyl ether (2 x 5 mL) and dried *in vacuo* to give yellow crystals of H_2L (0.50 g, 1.35 mmol, 75%); HR-MS: [m/z $C_{21}H_{26}N_2O_4$ + H^+]$^+$ m/z 371.196 (experimental),

371.190 (calculated). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.68 (s, 2H, -O*H*), 8.56 (s, 2H, N=C*H*), 7.00 (d, *J* = 7.9 Hz, 4H, Ar*H*), 6.77 (m, 2H, Ar*H*), 4.02 (q, *J* = 7.0 Hz, 4H, -C*H*$_2$-CH$_3$), 3.68 (t, *J* = 6.7 Hz, 4H, -C*H*$_2$-CH$_2$-C*H*$_2$-), 2.01 (quintet, *J* = 6.8 Hz, 2H, -CH$_2$-C*H*$_2$-CH$_2$-), 1.32 (t, *J* = 7.0 Hz, 6H, -CH$_2$-C*H*$_3$). {^{1}H}^{13}C NMR (101 MHz; DMSO-d_6) 166.4, 151.9, 147.1, 123.3, 118.5, 117.7, 116.1, 64.0, 55.4, 31.5, 14.8.

[GaL(OH$_2$)(OHCH$_3$)]NO$_3$: A mixture of H_2L (1.19 g, 3.2 mmol) and Ga(NO$_3$)$_3$.*x*H$_2$O (1.09 g, 3.0 mmol, assuming *x* = 6) in methanol (20 mL) was heated at reflux for 1.5 hours. A yellow precipitate was formed which was collected by filtration, washed with cold methanol (10 mL) and diethyl ether (20 mL) and dried *in vacuo* to give [GaL(solvent)$_2$]NO$_3$ (1.14 g, approx. 2.1 mmol, ~ 71%, assuming solvent = H_2O), λ_{max}(DMF)/nm 369 (ε/dm^3 cm^{-1} mol^{-1} 51,330), λ_{em}(DMF)/nm 498 (λ_{ex} 372); HR-MS: [m/z $C_{21}H_{24}GaN_2O_4]^+$ m/z 437.098 (experimental), 437.099 (calculated); ^{1}H NMR (400 MHz; DMSO-d_6) δ 8.42 (s, 2H, N=C*H*), 6.99 (m, 4H, Ar*H*), 6.61 (m, 2H, Ar*H*), 4.15 (q, *J* = 7.0 Hz, 4H, C*H*$_2$-CH$_3$), 3.93-3.90 (m, 4H, N-C*H*$_2$, 1.99-1.98 (m, 2H, CH$_2$-C*H*$_2$-CH$_2$), 1.30 (t, *J* = 7.0 Hz, 6H, CH$_2$-C*H*$_3$); {^{1}H}^{13}C NMR (101 MHz; DMSO-d_6) δ 170.3, 158.9, 150.0, 126.8, 120.4, 118.5, 115.1, 64.8, 60.5, 36.3, 28.1, 25.2, 15.3. Crystals of sufficient quality for structure determination were grown by exchange of vapours between diethyl ether and a solution of the complex in ethanol and were revealed by X-ray crystallography to be [GaL(OH$_2$)(OHCH$_3$)]NO$_3$.

[*Ga(curc)$_3$*]: Minor modifications to published procedures.[41] A mixture of curcumin (0.88 g, 2.39 mmol), Ga(NO$_3$)$_3$.*x*H$_2$O (0.34 g, 0.93 mmol, assuming *x* = 6) and triethylamine (0.16 mL) in methanol (25 mL) was heated at reflux for 1 hour. The mixture was allowed to cool to ambient temperature. A red-orange precipitate was isolated using a centrifuge and the supernatant discarded. The red-orange solid was washed with cold methanol (10 mL) and diethyl ether (2 x 10 mL) and dried *in vacuo* to give [Ga(curc)$_3$] (0.44 g, 0.38 mmol, 41%); λ_{max}(DMF)/nm 305, 432, 455.5 (ε/dm^3 cm^{-1} mol^{-1} 17,710, 128,870, 107,350), λ_{em}(DMF)/nm 520 ($\lambda_{ex.}$458); ^{1}H NMR (500 MHz; DMSO-

d_6): δ 9.54 (s, 6H, O*H*), 7.42 (m, 6H, Ar*H*), 7.23 (m, 6H, Ar*H*), 7.04 (m, 6H, C*H*=C*H*), 6.75 (m, 12H, Ar*H*), 5.97 (s, 3H, C*H*), 3.78 (s, 18H, O-CH_3). $\{^1H\}^{13}C$ NMR (101 MHz; DMSO-d_6): δ 183.2, 148.9, 148.0, 139.8, 126.6, 124.8, 122.7, 115.7, 111.0, 55.6, 55.5

[GaL(curc)]: A mixture of H_2L (0.10 g, 0.27 mmol) and [Ga(curc)$_3$] (0.16 g, 0.14 mmol) in methanol (5 mL) was heated at reflux for 3 hours. The mixture was allowed to cool to ambient temperature. An orange precipitate formed was collected by filtration, washed with cold methanol (2 x 5 mL) and dried *in vacuo* to give [GaL(curc)] (0.05 g, 0.06 mmol, 43%). λ_{max}(DMF)/nm 433.5, 457 (ε/dm^3 cm^{-1} mol^{-1} 54,480, 51,710), λ_{em}(DMF)/nm 515 ($\lambda_{ex.}$424). A sample for microanalysis was recrystalized from a concentrated mixture of the complex dissolved in dimethylsulfoxide (left for several days), calc for $C_{42}H_{43}GaN_2O_{10}$: C, 62.62; H, 5.38; N 3.48%. Found: C, 62.4; H, 5.7; N, 3.4%; ^1H NMR (500 MHz; DMSO-d_6): δ 9.59-9.50 (br s, 2H, O*H*), 8.47 (s, 1H, N-C*H*), 8.23 (s, 1H, N-C*H*), 7.47 (d, *J* = 15.5 Hz, 1H, Ar*H*), 7.29 (d, *J* = 1.8 Hz, 1H, Ar*H*), 7.11 (m, 1H, Ar*H*), 6.99-6.94 (m, 2H, Ar*H*), 6.90 (m, 1H, Ar*H*), 6.83-6.79 (m, 3H, Ar*H* (2H) C*H*=C*H* (1H)), 6.76-6.65 (m, 3H, Ar*H*), 6.54-6.39 (m, Ar*H*), 6.29 (m, 1H, C*H*=C*H*), 5.69-5.66 (m, 1H, C-C*H*-C), 4.19 (m, 1H, CH_2-C*H*H-CH_2), 4.09-3.95 (m, 4H, 2 x CH_2-CH_3), 3.83 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.51 (m, 1H, CH_2-C*H*H-CH_2), 2.27-2.25 (m, 2H, CH_2-CH_2-CH_2), 1.86 (m, 2H, CH_2-CH_2-CH_2), 1.21-1.16 (m, 3H, CH_2-CH_3), 1.07-1.03 (m, 3H, CH_2-CH_3). $\{^1H\}^{13}C$ NMR (101 MHz; DMSO-d_6): δ 183.3, 181.9, 168.9, 168.1, 161.7, 160.2, 150.4, 149.7, 148.97, 148.78, 148.0, 147.7, 140.7, 139.6, 126.84, 126.65, 126.53, 126.2, 124.6, 123.4, 122.8, 122.5, 122.2, 121.4, 119.0, 118.0, 117.6, 115.6, 113.6, 111.24, 111.19, 102.9, 65.1, 64.0, 59.7, 57.8, 55.70, 55.52, 30.6, 15.17, 15.08. Crystals suitable for analysis by x-ray crystallography were grown from a solution of the complex dissolved in dimethyl sulfoxide.

Crystallography

Intensity data for [GaL(OH_2)($OHCH_2CH_3$)]NO_3 and [GaL(curc)] were acquired using either Mo-K□ or Cu-K□ radiation, the temperature during data collection was maintained at 130.0(1) using an

Oxford Cryosystems cooling device. The structures were solved by direct methods and difference Fourier synthesis.[43] Thermal ellipsoid plots were generated using the program ORTEP-3 integrated within the WINGX suite of programs.[44]

Crystal data for [GaL(OH$_2$)(OHCH$_2$CH$_3$)]NO$_3$: $C_{23}H_{32}N_3O_9Ga$ = 564.24, *T* = 130.0 K, λ = 1.7107, triclinic, space group P -1 , *a* = 12.4587(8), *b* = 13.6613(11), *c* = 14.9608(12) *Å,* α = 86.004(7)° β = 82.486(6)° γ = 76.117(6) *V* = 2448.8(3) *Å*^{3}, Z = 4, Z- = 2, D_c = 1.530 Mg M^{-3} μ(Mo-Kα) 1.182 mm^{-1}, F(000) = 1176, crystal size 0.45 x 0.23 x 0.09 mm^3, 17916 reflections measured θ_{max} = 30.02° , 12285 independent reflections [R(int) = 0.0559], the final R was 0.1017 [I > 2σ(I) 6451 data] and *w*R(F^2) was 0.2857 (all data). CCDC 1436486.

Crystal data for [GaL(curc)]: ($C_{42}H_{42}N_2O_{10}Ga$).(DMSO).(3(H_2O), *M* = 936.67, *T* = 130.0 K, λ = 1.54184, triclinic, space group P-1, *a* = 11.6419(5)), *b* = 12.2119(5), *c* = 16.0212(7)) *Å,* α = 81.251(4)° β = 83.506(4)° γ = 77.765(3)° *V* = 2192.37(17) *Å*^{3}, Z = 2, D_c = 1.419 Mg M^{-3} μ(Cu-Kα) 1.893 mm^{-1}, F(000) = 982, crystal size 0.30 x 0.21 x 0.02 mm^3, 13663 reflections measured θ_{max} = 76.94° , 8762 independent reflections [R(int) = 0.0345], the final R was 0.0600 [I > 2σ(I) 7349 data] and *w*R(F^2) was 0.1804 (all data). CCDC 1436487.

Assessment of plaque binding in human tissue - Paraffin preserved brain tissue blocks were provided by the Victoria Brain Bank Network. Brain tissue was collected at autopsy. The National Neural Tissue Resource Centre performed sourcing and preparation of human brain tissue. AD pathologic diagnosis was made according to standard National Institute on Aging-Reagan Institute criteria. Determination of age matched human control (HC) cases was subject to the above criteria. The AD and HC brain tissues sections (5 μM) were first recovered from paraffin (xylene, 4 × 2 min) followed by rehydration (soaking in a series of 100%, 90%, 70%, and 0% v/v ethanol/water baths). The hydrated tissue sections were washed in phosphate buffer saline (PBS, 5 min). Autofluorescence of the tissue was quenched using potassium permanganate (0.25% in PBS, 20 min) and washing with PBS (2 × 2 min). The now brown coloured sections were washed with potassium metabisulfite and oxalic acid (1% in PBS) until the brown colour was removed followed by washing with PBS (3 × 2 min). The sections were blocked with bovine serum albumin (2% BSA in PBS, pH 7.0, 10 min) and then treated with [GaL(curc)] (50 μM in 15% v/v DMSO/PBS, 1 hour).

The sections were treated with BSA again to remove any [GaL(curc)] non-specifically bound to the tissue. Finally, the sections were washed with PBS (3 × 2 min), deionised water, and mounted with non-fluorescent mounting media (Dako). Fluorescence images were visualized using a Leica (Bannockburn, IL) DM1RB microscope (λ_{ex} = 359 nm, λ_{em} = 461 nm). The Aβ plaques in every second section of brain tissue (5 μM serial sections) were identified by treatment with primary antibody to Aβ (1E8) and visualization with standard a peroxidase-based visualization kit (DAKO).[20]

Electronic spectroscopy

The UV/visible spectra of [Ga(L)(curc)] and $[Ga(L)(OH_2)(OHCH_2CH_3)]^+$ (50 □M) were measured in 1% bovine serum albumin (w/w) in 15:85 DMSO:phosphate buffered saline pH 7.4. Absorbance spectra were taken immediately after mixing then the periodically up to 2 hours using a Shimadzu UV-1650PC UV/visible spectrophotometer and capped quartz cuvettes.

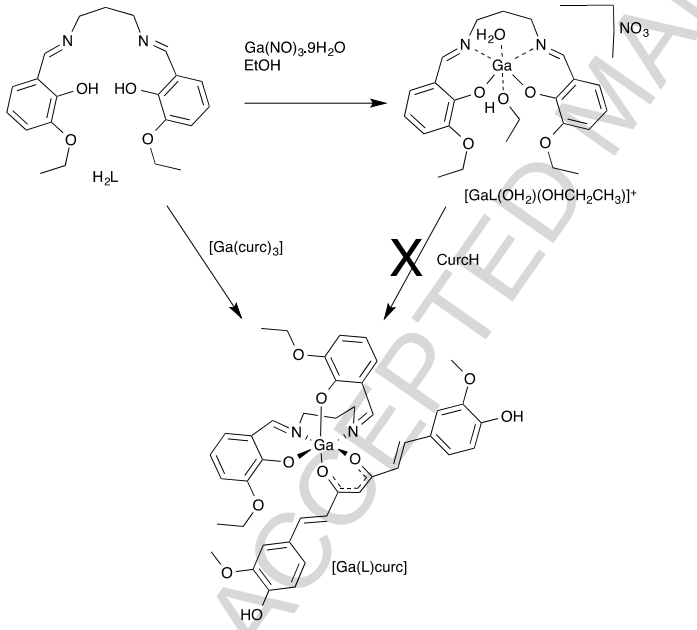
Results and Discussion

Neutral gallium complexes of tripodal hexadentate Schiff base ligands such as 1,1,1-tris((5-methoxysalicylaldimino)methyl)ethane have been investigated as myocardial perfusion imaging agents but in general do not cross the blood-brain barrier to allow assessment of cerebral perfusion.[45-47] Lipophilic monocationic gallium(III) complexes formed with hexadentate N_4O_2 Schiff base ligands have been investigated as myocardial perfusion imaging agents and as substrates of P-glycoprotein for imaging multi-drug resistance.[48-50] [51-53]

In this manuscript we report using a tetradentate N_2O_2 Schiff base ligand, N,N-bis (2-ethoxysalicylidene)-1,2-diaminopropane (H_2L), derived from the condensation of 1,3-diaminopropane and 3-ethoxysalicylaldehyde as supporting ancillary ligands in the formation of neutral octahedral gallium(III) complexes. The ethoxy substituents *ortho*- to the phenol group were incorporated to increase lipophilicity and hopefully increase blood-brain barrier permeability as our previous work demonstrated that the presence of the ethoxy substituent in cobalt(III) Schiff base

complexes increased cell membrane permeability in neuronal-like SH-SY5Y cells.[54] When tetradentate N_2O_2 Schiff base ligands are used to form 6-coordinate metal complexes it is possible for the ligand to adopt three different configurations, trans(2), *cis*-β, and *cis*-α.[55, 56] If the remaining two coordination sites of a six-coordinate metal ion are provided by monodentate ligands the trans configuration is often encountered but the *cis*-β configuration is required if the remaining two coordination sites are provided by a bidentate ligand.

Reaction of H_2L with gallium nitrate in ethanol allowed isolation of $[GaL(OH_2)(OHCH_2CH_3)]^+$ as yellow crystals of the nitrate salt with two monodentate solvent ligands situated *trans* to each other (Scheme 1).



Scheme 1. The synthesis of $[GaL(OH_2)(OHCH_2CH_3)]^+$ and [GaL(curc)].

Analysis of the crystals by X-ray crystallography revealed the Ga^{3+} is in a distorted octahedral environment with the Ga-N and Ga-O(phenolate) bonds similar to previous reported Ga^{3+} structures with Schiff base ligands (Figure 1).[57] The Ga(1)-O(1) and Ga(1)-O(3) bond lengths to the oxygen of the Schiff base L^2 are 1.898(5) Å and 1.917(5) Å respectively. The Ga-N_{imine} bonds are 2.067(6) Å and 2.050(7) Å.

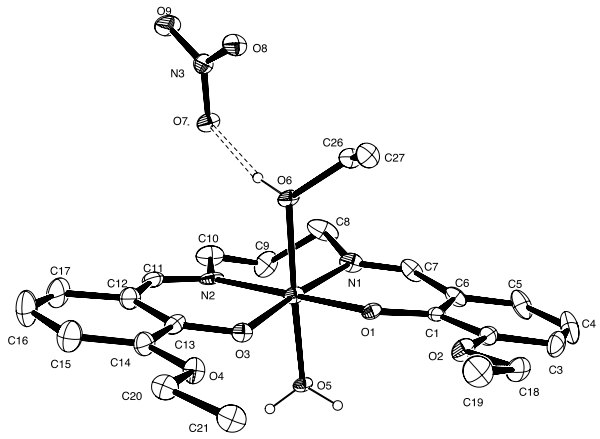


Figure 1. ORTEP representation of $[GaL(OH_2)(OHCH_2CH_3)]^+$ (ellipsoids are at 30% probability level). Hydrogen atoms attached to carbon omitted for clarity.

Reactions of $[GaL(OH_2)(OHCH_2CH_3)]^+$ with curcumin in an attempt to form a Ga^{3+} complex with the Schiff base in the *cis*-β configuration to accommodate the bidentate curcumin ligand were unsuccessful. This contrasts with our experience with $[Co(salen)]^+$ complexes (salen = N,N-bis(salicylidene)-1,2-diaminothane), with the ligand in the *trans* configuration, where the ligand could be forced to adopt a *cis*-β configuration when the complexes were reacted with acetylacetonate (acac).[54] The reaction of three equivalents of curcH with gallium nitrate allows the isolation of neutral tris-bidentate complex [Ga(curc)].[41] The reaction of $[Ga(curc)_3]$ with H_2L in ethanol allowed isolation of neutral [GaL(curc)] in good yields. The ^1H NMR spectrum of [GaL(curc)] reveals that the tetradentate Schiff-base ligand has adopted the *cis*-β configuration with two separate resonances due to the two imine hydrogen atoms at δ 8.23 ppm and 8.47 ppm when compared to the single resonance at 8.61 ppm in the C_2 symmetric complex $[GaL(HOCH_2CH_3)(OH_2)]^+$. Two singlets for each methoxy functional groups of the coordinated and deprotonated curcumin are at δ 3.83 ppm and 3.80 ppm.

Crystals of [GaL(curc)] suitable for X-ray crystallography were grown from a solution of the complex dissolved in dimethyl sulfoxide (Figure 2). The gallium(III) ion is an distorted octahedral

environment with the tetradentate ligand in the *cis*-β conformation combining with the curcumin anion to provide an N_2O_4 donor environment in a racemic mixture Δ and Λ enantiomers. The bond angle of the N(1)-Ga(1)-N(2) six-membered chelate ring is 86 ˚ and the gallium to phenolic oxygen atoms bond angle, O(1)-Ga-O(3), is 93 ˚. In [Ga(L)curc] the metal ion-phenolate bond lengths, Ga-O(1) and Ga(1)-O(3) are 1.932(2) Å and 1.924(2) Å respectively and are longer than comparable bonds when the ligand adopts the trans(2) configuration in $[GaL(OH_2)OHCH_2CH_3]^+$. The Ga-O(curcumin) bond lengths are 1.975(2) and 1.991(2). The bond lengths and angles are comparable to those found in of [Ga(salpn)(acac)] (salpn = N,N-bis(salicylidene)-1,2-diaminopropane) that was reported recently.[58]

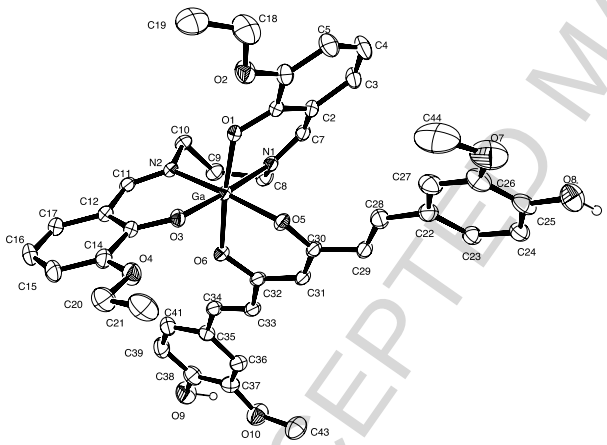


Figure 2. ORTEP plot of [GaL(curc)] (ellipsoids are at the 30% probability level). Solvent (dimethyl sulfoxide and water), the minor component atoms of the disordered methoxyphenol ring (C22-C27, O7, O8, and C44) and protons attached to carbon have been omitted for clarity.

In the solid state the molecules of [GaL(curc)] form centrosymmetric dimers of enantiomers through π-πinteractions of the aromatic curcumin ligand. The angle between the planes defined by the terminal aromatic rings of the curcumin ligand is 13.2(4)° while the distances between the atoms of one plane to the second aromatic ring range from 3.27(3) – 3.90(3) Å (Figure 3).

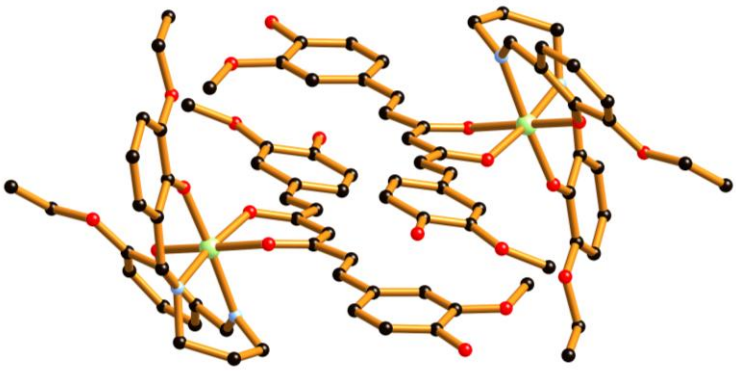


Figure 3. Representation of the centrosymmetric dimers found in the X-ray crystal structure of [GaL(curc)] formed through π-π interactions of the curcumin ligand.

The stability of [Ga(L)(curc)] in bovine serum albumin was assessed by electronic spectroscopy. The UV/visible spectrum of [Ga(L)(curc)] (50 mM) in an aqueous solution (15:85 DMSO:phosphate buffered saline, pH 7.4) with 1% serum (w/w) featured the expected broad absorption at λ = 430 nm characteristic of curcumin but also an absorption at λ = 295 nm. There was a small reduction (16 %) in the intensity of this band over a two hour incubation period (chosen to be approximately twice the half-life of gallium-68) suggesting the complex was sufficiently stable in serum to warrant further more detailed investigations. The electronic spectrum of $[Ga(L)(OH_2)(OHCH_2CH_3)]^+$ had a similar absorption at λ = 295 nm but underwent significant changes within the first five minutes of being incubated with bovine serum albumin possibly due to rapid exchange of the monodentate solvent ligands with accessible donor atoms on the protein.

Curcumin is fluorescent (λ_{max} = 522 nm, λ_{ex} = 453 nm) and [GaL(curc)] is also fluorescent with a small hypsochromic shift in the emission wavelength (λ_{max} = 508 (λ_{ex} = 424 nm) and this fluorescence can be used to characterise the binding of the complex to Aβ plaques. The ability of [GaL(curc)] to bind to Aβ plaques was assessed in post-mortem human frontal cortex tissue from a subject diagnosed with clinical Alzheimer's disease. The brain tissue (5 μm serial sections) was pre-treated with bovine serum albumin to minimise non-selective binding and the tissues were treated with a solution of [Ga(L)(curc)] (50 μM). The localization of [GaL(curc)] on the treated brain tissue

was measured by epi-fluorescent microscopy (λ_{ex} = 359 nm, λ_{em} = 461 nm) and compared to the contiguous section of tissue stained with an Aβ antibody (1E8) to identify plaques revealing significant co-localisation of the complex with Aβ plaques (Figure 4). The Aβ plaques are typically 40−60 μm in diameter so 5 μm serial sections should largely include features of the same Aβ plaque.[59] The complex was not retained in samples of human brain tissue from age-matched control subjects suggesting some degree of specificity for Aβ plaques.

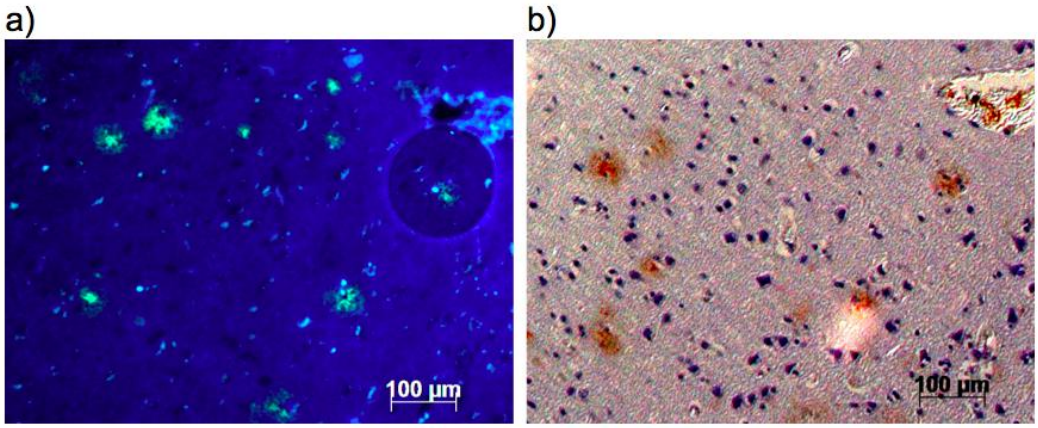


Figure 4. a) Sections of frontal cortex brain tissue from Alzheimer's disease subjects treated with [Ga(L)(curc)] and imaged with epi-fluorescent microscopy (λ_{ex} = 359 nm, λ_{em} = 461 nm). b) Image of contiguous section of brain tissue stained with Aβ selective antibody (1E8).

Concluding Remarks

In aiming to develop charge neutral, low molecular weight, lipophilic complexes with the potential to cross the blood-brain barrier and bind to Aβ plaques we have employed an ancillary tetradentate N_2O_2 Schiff base ligand and the β-diketone curcumin as a bidentate ligand to give a six-coordinate Ga^{3+} complex. The tetradentate Schiff base ligand adopts the *cis*-β configuration with the deprotonated curcumin ligand acting as a bidentate ligand. Promisingly the complex binds to Aβ plaques in human brain tissue. Given that the synthesis of hexadentate Schiff-base gallium-68 complexes often use [^{68}Ga(acac)$_3$] as a convenient starting material and the recent report of the preparation of [Ga(curc)$_2$]$^+$ complexes it appears feasible that the approach presented here could be

translated to $^{68}Ga^{3+}$.[42, 49] Further studies are required to develop radiolabeling procedures, assess the stability of the complexes *in vivo* and their ability to cross the blood-brain barrier.

Dedication

In memory of Professor Graeme Hanson and his contributions to Bioinorganic Chemistry and Electron Paramagnetic Resonance

Acknowledgements

The Australian Research Council for funding this research. The Victorian Brain Bank for provision of human brain tissue. PSD is and ARC Future Fellow.

References

[1] D.J. Selkoe, Nat. Med., 17 (2011) 1060-1065.

[2] C.L. Masters, G. Simms, N.A. Weinman, G. Multhaup, B.L. McDonald, K. Beyreuther, Proc. Nat. Acad. Sci. (USA), 82 (1985) 4245-4249.

[3] G.G. Glenner, C.W. Wong, V. Quaranta, E.D. Eanes, Appl Pathol, 2 (1984) 357-369.

[4] G.G. Glenner, C.W. Wong, Biochem Biophys Res Commun, 120 (1984) 885-890.

[5] G.G. Glenner, C.W. Wong, Biochem Biophys Res Commun, 122 (1984) 1131-1135.

[6] D.M. Walsh, D.J. Selkoe, J. Neurochem., 101 (2007) 1172-1184.

[7] M. Goedert, Science, 349 (2015) 601.

[8] V.L. Villemagne, S. Burnham, P. Bourgeat, B. Brown, K.A. Ellis, O. Salvado, C. Szoeke, S.L. Macaulay, R. Martins, P. Maruff, D. Ames, C.C. Rowe, C.L. Masters, Lancet Neurol., 12 (2013) 357-367.

[9] C.C. Rowe, P. Bourgeat, K.A. Ellis, B. Brown, Y.Y. Lim, R. Mulligan, G. Jones, P. Maruff, M. Woodward, R. Price, P. Robins, H. Tochon-Danguy, G. O'Keefe, K.E. Pike, P. Yates, C. Szoeke, O.

Salvado, S.L. Macaulay, T. O'Meara, R. Head, L. Cobiac, G. Savage, R. Martins, C.L. Masters, D. Ames, V.L. Villemagne, Ann. Neurol., 74 (2013) 905-913.

[10] C.A. Mathis, B.J. Bacskai, S.T. Kajdasz, M.E. McLellan, M.P. Frosch, B.T. Hyman, D.P. Holt, Y. Wang, G.-F. Huang, M.L. Debnath, W.E. Klunk, Bioorg. Med. Chem. Lett., 12 (2002) 295-298.

[11] C.A. Mathis, Y. Wang, D.P. Holt, G.-F. Huang, M.L. Debnath, W.E. Klunk, J. Med. Chem., 46 (2003) 2740-2754.

[12] C.A. Mathis, B.J. Lopresti, W.E. Klunk, Nucl. Med. Biol, 34 (2007) 809-822.

[13] H.F. Kung, S.R. Choi, W. Qu, W. Zhang, D. Skovronsky, J. Med. Chem., 53 (2010) 933-941.

[14] S.R. Choi, G. Golding, Z. Zhuang, W. Zhang, N. Lim, F. Hefti, T.E. Benedum, M.R. Kilbourn, D. Skovronsky, H.F. Kung, J. Nucl. Med., 50 (2009) 1887-1894.

[15] L. Yang, D. Rieves, C. Ganley, New Engl. J. Med., 367 (2012) 885-887.

[16] C.M. Clark, M.J. Pontecorvo, T.G. Beach, B.J. Bedell, R.E. Coleman, P.M. Doraiswamy, A.S. Fleisher, E.M. Reiman, M.N. Sabbagh, C.H. Sadowsky, J.A. Schneider, A. Arora, A.P. Carpenter, M.L. Flitter, A.D. Joshi, M.J. Krautkramer, M. Lu, M.A. Mintun, D.M. Skovronsky, Lancet Neurol., 11 (2012) 669-678.

[17] P.S. Donnelly, Dalton Trans., 40 (2011) 999-1010.

[18] J.L. Hickey, S.C. Lim, D.J. Hayne, B.M. Paterson, J.M. White, V.L. Villemagne, P. Roselt, D. Binns, C. Cullinane, C.M. Jeffery, R.I. Price, K.J. Barnham, P.S. Donnelly, J. Am. Chem. Soc., 135 (2013) 16120-16132.

[19] S. Lim, B.M. Paterson, M.T. Fodero-Tavoletti, G.J. O'Keefe, R. Cappai, K.J. Barnham, V.L. Villemagne, P.S. Donnelly, Chem. Commun., 46 (2010) 5437-5439.

[20] M.T. Fodero-Tavoletti, V.L. Villemagne, B.M. Paterson, A.R. White, Q.-X. Li, J. Camakaris, G. O'Keefe, R. Cappai, K.J. Barnham, P.S. Donnelly, J. Alzheimer's Dis., 20 (2010) 49-55.

[21] D.J. Hayne, S.C. Lim, P.S. Donnelly, Chem. Soc. Rev., 43 (2014) 6701-6715.

[22] J.L. Hickey, P.S. Donnelly, Coord. Chem. Rev., 256 (2012) 2367-2380.

[23] I. Velikyan, Theranostics, 4 (2014) 47-80.

[24] J. Notni, K. Pohle, H.-J. Wester, Nucl. Med. Biol., 40 (2013) 33-41.

[25] J. Simecek, M. Schulz, J. Notni, J. Plutnar, V. Kubicek, J. Havlickova, P. Hermann, Inorg. Chem., 51 (2012) 577-590.

[26] I. Velikyan, J. Labelled Compd. Radiopharm., 58 (2015) 99-121.

[27] M.T. Ma, O.C. Neels, D. Denoyer, P. Roselt, J.A. Karas, D.B. Scanlon, J.M. White, R.J. Hicks, P.S. Donnelly, Bioconjugate Chem., 22 (2011) 2093-2103.

[28] R.A. Dumont, F. Deininger, R. Haubner, H.R. Maecke, W.A. Weber, M. Fani, J. Nucl. Med., 52 (2011) 1276-1284.

[29] M. Fani, L. Del Pozzo, K. Abiraj, R. Mansi, M.L. Tamma, R. Cescato, B. Waser, W.A. Weber, J.C. Reubi, H.R. Maecke, J. Nucl. Med., 52 (2011) 1110-1118.

[30] M. Henze, A. Dimitrakopoulou-Strauss, S. Milker-Zabel, J. Schuhmacher, L.G. Strauss, J. Doll, H.R. Maecke, M. Eisenhut, J. Debus, U. Haberkorn, J. Nucl. Med., 46 (2005) 763-769.

[31] E.C.S.d.C. Etchebeherd, A.d.O. Santos, B. Gumz, A. Vicente, P.G. Hoff, G. Corradi, W.A. Ichiki, J. Geraldo de Almeida Filho, S. Cantoni, E.E. Camargo, F.P. Costa, J. Nucl. Med., 55 (2014) 1598-1604.

[32] M. Eder, M. Schaefer, U. Bauder-Wuest, W.-E. Hull, C. Waengler, W. Mier, U. Haberkorn, M. Eisenhut, Bioconjugate Chem., 23 (2012) 688-697.

[33] M.T. Freitag, J.P. Radtke, B.A. Hadaschik, A. Kopp-Schneider, M. Eder, K. Kopka, U. Haberkorn, M. Roethke, H.-P. Schlemmer, A. Afshar-Oromieh, Eur. J. Nucl. Med. Mol. Imaging, (2015) Ahead of Print.

[34] K. Chauhan, A. Datta, A. Adhikari, K. Chuttani, A. Kumar Singh, A.K. Mishra, Org. Biomol. Chem., 12 (2014) 7328-7337.

[35] H. Watanabe, M. Ono, S. Iikuni, M. Yoshimura, K. Matsumura, H. Kimura, H. Saji, Bioorg. Med. Chem. Lett., 24 (2014) 4834-4837.

[36] F. Yang, G.P. Lim, A.N. Begum, O.J. Ubeda, M.R. Simmons, S.S. Ambegaokar, P.P. Chen, R. Kayed, C.G. Glabe, S.A. Frautschy, G.M. Cole, J. Biol. Chem., 280 (2005) 5892-5901.

[37] X. Zhang, Y. Tian, C. Zhang, X. Tian, A.W. Ross, R.D. Moir, H. Sun, R.E. Tanzi, A. Moore, C. Ran, Proc. Natl. Acad. Sci. U. S. A., 112 (2015) 9734-9739.

[38] S. Wanninger, V. Lorenz, A. Subhan, F.T. Edelmann, Chem. Soc. Rev., 44 (2015) 4986-5002.

[39] M. Sagnou, D. Benaki, C. Triantis, T. Tsotakos, V. Psycharis, C.P. Raptopoulou, I. Pirmettis, M. Papadopoulos, M. Pelecanou, Inorg. Chem., 50 (2011) 1295-1303.

[40] C. Triantis, T. Tsotakos, C. Tsoukalas, M. Sagnou, C. Raptopoulou, A. Terzis, V. Psycharis, M. Pelecanou, I. Pirmettis, M. Papadopoulos, Inorg. Chem., 52 (2013) 12995-13003.

[41] K. Mohammadi, K.H. Thompson, B.O. Patrick, T. Storr, C. Martins, E. Polishchuk, V.G. Yuen, J.H. McNeill, C. Orvig, J. Inorg. Biochem., 99 (2005) 2217-2225.

[42] M. Asti, E. Ferrari, S. Croci, G. Atti, S. Rubagotti, M. Iori, P.C. Capponi, A. Zerbini, M. Saladini, A. Versari, Inorg. Chem., 53 (2014) 4922-4933.

[43] G.M. Sheldrick, Acta Crystallogr., Sect. A Found. Crystallogr., 64 (2008) 112-122.

[44] L.J. Farrugia, J. Appl. Crystallogr., 45 (2012) 849-854.

[45] M.A. Green, M.J. Welch, J.C. Huffman, J. Am. Chem. Soc., 106 (1984) 3689-3691.

[46] M.A. Green, M.J. Welch, C.J. Mathias, K.A.A. Fox, R.M. Knabb, J.C. Huffman, J. Nucl. Med., 26 (1985) 170-180.

[47] M.A. Green, J. Labelled Compd. Radiopharm., 23 (1986) 1227.

[48] B.W. Tsang, C.J. Mathias, M.A. Green, J. Nucl. Med., 34 (1993) 1127-1131.

[49] B.W. Tsang, C.J. Mathias, P.E. Fanwick, M.A. Green, J. Med. Chem., 37 (1994) 4400-4406.

[50] Y.-M. Hsiao, C.J. Mathias, S.-P. Wey, P.E. Fanwick, M.A. Green, Nucl. Med. Biol., 36 (2009) 39-45.

[51] V. Sharma, J.L. Prior, M.G. Belinsky, G.D. Kruh, D. Piwnica-Worms, J. Nucl. Med., 46 (2005) 354-364.

[52] J. Sivapackiam, S.E. Harpstrite, J.L. Prior, H. Gu, N.P. Rath, V. Sharma, Dalton Trans., 39 (2010) 5842-5850.

[53] V. Sharma, J. Sivapackiam, S.E. Harpstrite, J.L. Prior, H. Gu, N.P. Rath, D. Piwnica-Worms, PLoS One, 9 (2014) e109361/109361-e109361/109310, 109310 pp.

[54] C. Schieber, J. Howitt, U. Putz, J.M. White, C.L. Parish, P.S. Donnelly, S.-S. Tan, J. Biol. Chem., 286 (2011) 8555-8564.

[55] T. Katsuki, Chem. Soc. Rev., 33 (2004) 437-444.

[56] K. Matsumoto, B. Saito, T. Katsuki, Chem. Commun. (Cambridge, U. K.), (2007) 3619-3627.

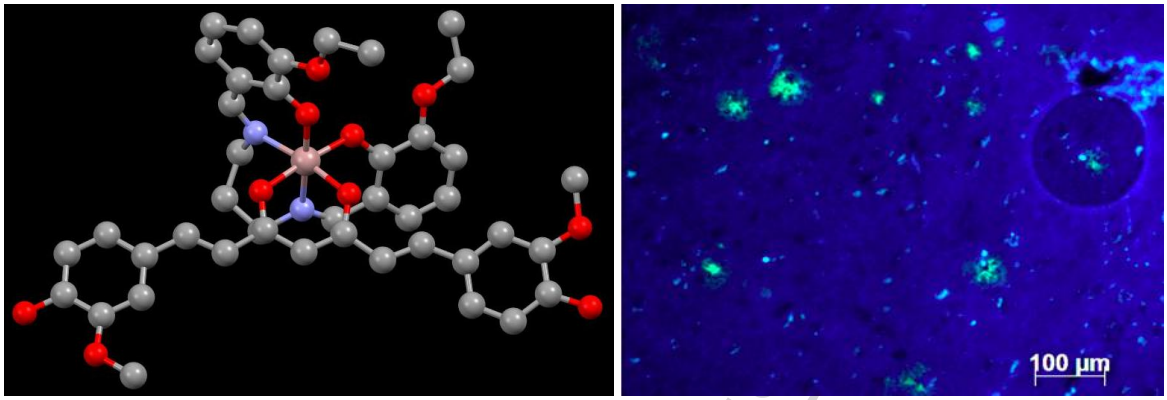
[57] M.S. Hill, D.A. Atwood, Eur. J. Inorg. Chem., (1998) 67-72.

[58] S. Tella, V. Bekiari, V.G. Kessler, G.S. Papaefstathiou, Polyhedron, 64 (2013) 77-83.

[59] M.T. Fodero-Tavoletti, D.P. Smith, C.A. McLean, P.A. Adlard, K.J. Barnham, L.E. Foster, L. Leone, K. Perez, M. Cortes, J.G. Culvenor, Q.-X. Li, K.M. Laughton, C.C. Rowe, C.L. Masters, R. Cappai, V.L. Villemagne, J. Neurosci., 27 (2007) 10365-10371.

Graphical abstract

TOC Synopsis graphic and text:



A gallium(III) complexes that binds to amyloid-β plaques associated with Alzheimer's disease has been prepared. The complex features a tetradentate N_2O_2 Schiff base as an ancillary ligand and the β-diketone curcumin as a bidentate ligand to give a six-coordinate Ga^{3+} complex.

Highlights

- A neutral gallium complex with curcumin acting as a bidentate ligand has been prepared.
- The complex binds to amyloid-beta plaques present in human Alzheimer's disease brains.