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Author/s:

Koay, Hui Jing

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Exploiting the Gallium-Fluoride Bond in the Design of New Radiopharmaceuticals

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Exploiting the Gallium-Fluoride Bond in the Design of New Radiopharmaceuticals

Hui Jing Koay

[Orcid.org/0000-0002-5475-8242](https://orcid.org/0000-0002-5475-8242)

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Abstract

Positron emission tomography (PET) is a molecular imaging technique that produces three-dimensional images in high resolution and is used to assist in the diagnosis and monitoring of disease. The technique relies on molecules radiolabelled with a positron-emitting radionuclide called ‘tracers’ injected into patient at ‘trace’ concentrations. The most used radionuclide for PET is fluorine-18. Conventional approaches to fluorine-18 radiolabelling rely on the formation of a covalent C-¹⁸F bond and such synthetic chemistry can be challenging. It has been suggested that coordinate bond formation between Al³⁺/Ga³⁺ and F⁻ may lead to complexes of sufficient stability due to the high metal-fluoride bond energy. The work presented in this thesis investigates the potential of the formation of Ga³⁺-F⁻ complexes as alternative method for rapid incorporation of fluorine-18 into molecules.

A series of pentadentate acyclic ligands based on the dipicolinic acid scaffold (H₂L³⁻⁵) that coordinate to Ga³⁺ were prepared. Characterisation of [GaL³(OH)], [GaL³Cl] and [GaL³F] by X-ray crystallography revealed that in each case the ligand acted as tetradentate donor with a weak interaction with a sixth nitrogen donor. Treatment of [GaL³(OH)] / [GaL³Cl] with [¹⁸F]/H₂O at room temperature readily formed [GaL³F]. Although the synthesis of [GaL³F] was straightforward, it was only possible to form [¹⁸F][GaL³F] in very low radiochemical yields.

In a second approach a family of macrocyclic ligands was adopted to develop a single chelate system that has the potential to be radiolabelled with three radionuclides; fluorine-18, copper-64 and gallium-68. A stilbene derivative attached to the NODA chelator, H₂L¹² was designed to bind to amyloid-β (Aβ) plaques associated with Alzheimer’s disease (AD). The ability to identify the burden of insoluble aggregates in the brain in a non-invasive manner may assist our understanding of AD. The new ligand, H₂L¹² binds to extracellular Aβ plaques present in human brain tissue, but there was no evidence that [GaL¹²F] and [CuL¹²] bound to

A β plaques. The radiolabelled [^{18}F][GaL ^{12}F], [^{68}Ga][GaL ^{12}F] and [^{64}Cu][CuL ^{12}] formed in high radiochemical purity at pH 4 - 5.5. The biodistribution and PET imaging potential of [^{18}F][GaL ^{12}F] and [^{64}Cu][CuL ^{12}] was evaluated in mice and this revealed the initial uptake in brain of [^{18}F][GaL ^{12}F] and [^{64}Cu][CuL ^{12}] were $0.85 \pm 0.13\%$ IA/g and $0.71 \pm 0.03\%$ IA/g respectively. An increase in uptake in bone suggested that the [^{18}F]Ga $^{3+}$ -F $^{-}$ system is unstable *in vivo*.

A tetradentate azamacrocyclic ligand with N $_4$ donor set, tetramethyltetraaza[14]annulene (tmtaa) was investigated to replace the carboxylate donor groups in [GaL ^3F] and [GaL ^{12}F]. A five coordinate Ga $^{3+}$ -F $^{-}$ complex of tmtaa was prepared. [Ga(tmtaa)F] was characterised by X-ray crystallography revealing the complex has a saddle-shaped geometry. The radioactive complex, [^{18}F][Ga(tmtaa)F] can be prepared by a halogen exchange reaction starting with [Ga(tmtaa)Cl]. Functionalisation at the methine carbon atoms of H $_2$ tmtaa could see future development to include biomolecules.

Translation of the non-radioactive Ga $^{3+}$ -F $^{-}$ chemistry to systems containing fluorine-18 remains challenging. The work presented here demonstrated that the [^{18}F]Ga $^{3+}$ -F $^{-}$ complexes are less stable than the Al $^{3+}$ analogues. It would be of great interest to investigate the potential of the [^{18}F]Al $^{3+}$ -F $^{-}$ complexes of these families of ligands as PET imaging agents.

Declaration

This is to certify that

- i. This thesis comprised only my original work except where indicated in the text,
- ii. Due acknowledgement has been made in the text to all other material used,
- iii. This thesis is fewer than 100 000 words in length, exclusive of tables, maps, bibliographies and appendices.

Hui Jing Koay

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Abbreviation

[¹¹ C]SB-13	<i>N</i> -[¹¹ C]methylamino-4'-hydroxystilbene
[¹⁸ F]DOPA	6-[¹⁸ F]-fluoro- <i>L</i> -dihydroxyphenylalanine
[¹⁸ F]EF5	2-(2-nitro-1 <i>H</i> -imidazol-1-yl)- <i>N</i> -(2,2,3,3,3-[¹⁸ F]pentafluoropropyl)-acetamide
[¹⁸ F]FACBC	anti-1-amino-3-[¹⁸ F]fluorocyclobutane-1-carboxylic acid
[¹⁸ F]FAZA	[¹⁸ F]fluoroazomycin-arabinofuranoside
[¹⁸ F]FBA	4-[¹⁸ F]fluorobenzaldehyde
[¹⁸ F]FBEM	<i>N</i> -[2-(4-[¹⁸ F]fluorobenzamido)ethyl]maleimide
[¹⁸ F]FDDNP	2-(1,1-dicyanopropen-2-yl)-6-(2-[¹⁸ F]-fluoroethyl)-methylamino-naphthalene
[¹⁸ F]FDEA	5'-[¹⁸ F]fluorodeoxy-2-ethynyladenosine
[¹⁸ F]FDG	2-deoxy-2-[¹⁸ F]fluoro- <i>D</i> -glucose
[¹⁸ F]FES	[¹⁸ F]fluoroestradiol
[¹⁸ F]FET	<i>O</i> -(2-[¹⁸ F]fluoroethyl)- <i>L</i> -tyrosine
[¹⁸ F]FETA	<i>N</i> -(2-([¹⁸ F]fluoroethyl)-2(2-nitroimidazol-1-yl)-acetamide
[¹⁸ F]FLT	3'-deoxy-3'-[¹⁸ F]fluorothymidine
[¹⁸ F]FMAU	2'-Deoxy-2'-[¹⁸ F]Fluoro-5-Methyl- 1-β-d-arabinofuranosyluracil
[¹⁸ F]FMISO	[¹⁸ F]fluoromisonidazole
[¹⁸ F]FMT	6-[¹⁸ F]Fluoro- <i>L</i> - <i>m</i> -tyrosine
[¹⁸ F]FPPDB	(<i>E</i>)-4-4((6-(2-(2-(2-fluoroethoxy)ethoxy)benzo[d]thiazol-2-yl)-diazenyl)- <i>N,N</i> -dimethylaniline
[¹⁸ F]NFSI	<i>N</i> -[¹⁸ F]fluorobenzenesulfonimide
[¹⁸ F]SFB	<i>N</i> -succinimidyl-4-[¹⁸ F]fluorobenzoate
[2.2.2]Cryptand	4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo-(8.8.8)-hexacosane
[Ni(tmtaa)]	5,7,12,14-tetramethyldibenzo[<i>b,i</i>][1,4,8,11]tetraazacyclotetradecaheptaenatonickel (II)
[NMe ₄]F	Tetramethylammonium fluoride
5'-[¹⁸ F]FDA	5'-[¹⁸ F]fluoro-5'-deoxyadenosine
5'-[¹⁸ F]FDI	5'-[¹⁸ F]fluoro-5'-deoxyionosine
5'-[¹⁸ F]FDR	5'-[¹⁸ F]fluoro-5'-deoxy- <i>D</i> -ribose
5'-[¹⁸ F]FDU	5'-[¹⁸ F]fluoro-5'-deoxyuridine
5'-FDA	5'-fluoro-5'-deoxyadenosine
5'-FDAS	5'-fluoro-5'-deoxyadenosine synthase
AD	Alzheimer's Disease
APP	Amyloid precursor protein
AZD2184	2-(6-(methylamino)pyridine-3-yl)benzothiazole-6-ol
AZD4694	2-(2-fluoro-6-(methylamino)pyridine-3-yl)benzofuran-5-ol
Aβ	amyloid-β
BACE-1	β APP cleavage enzyme
bipy	2,2'-bipyridyl
BTA	Benzothiazole-aniline
CAA	Cerebral amyloid angiopathy
CDCl ₃	[² H]chloroform
CIDEA	5'-chlorodeoxy-2-ethynyladenosine
C-NETA	{4-[2-(bis-carboxy-methylamino)-5-(4-nitrophenyl)pentyl]-7-carboxymethyl-[1,4,7]triazanonan-1-yl} acetic acid
CT	Computed Tomography

d ₆ -DMSO	[² H] ₆ Dimethylsulfoxide
DCE	Dichloroethane
DCM	Dichloromethane
DIPEA	<i>N,N</i> -Diisopropylethylamine
DMF	<i>N,N</i> -Dimethylformamide
DMSO	Dimethylsulfoxide
DMTr	dimethoxytrityl
DOTA	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid
EC	Electron capture
EDTA	Ethylenediaminetetraacetic acid
ESI-MS	Electrospray ionisation-mass spectrometry
FDA	Food and Drug Administration
FDEA	5'-fluorodeoxy-2-ethynyladenosine
Fmoc	Fluorenylmethyloxycarbonyl
gCOSY	gradient homonuclear correlation spectroscopy
gHSQC	gradient heteronuclear single-quantum correlation spectroscopy
GRPR	Gastrin-releasing peptide receptor
H ₂ tmtaa	1,8-dihydro-5,7,12,14-tetramethyldibenzo[<i>b,i</i>][1,4,8,11] tetraazacyclotetradeca-4,6,11,13-tetraene
HF	Hydrofluoric acid
HFIP	1,1,1,3,3,3-hexafluoro-2-propanol
IA/g	Injected activity per gram of tissue
K ₂₂₂	Kryptofix-222
K _i	Inhibition constant
MPAA	Methyl phenylacetic acid
NFT	Neurofibrillary tangles
NK1R	Neurokinin 1 receptor
NMR	Nuclear magnetic resonance
NODA	1,4,7-triazacyclononane-1,4-diacetic acid
NODA-GA	1,4,7-triazacyclononane-1-glutaric acid-4,7-acetic acid
NOTA	1,4,7-triazacyclononane-1,4,7-triacetic acid
PBS	Phosphate-buffered saline
PEG	Polyethyleneglycol
PET	Positron emission tomography
phen	1,10-phenanthroline
PiB	Pittsburgh Compound B (2-(4-methylaminophenyl)-6-hydroxybenzothiazole)
PSMA	Prostate-specific membrane antigen
RGD	arginylglycylaspartic acid
RP-HPLC	Reversed phase high performance liquid chromatography
R _t	Retention time
SAAC	Single amino acid chelate
SAM	(<i>S</i>)-adenosyl- <i>L</i> -methionine
SiFA	Silicon-based fluoride acceptor
SPPS	Solid-phase peptide synthesis
SsNMR	Solid-state nuclear magnetic resonance
t _{1/2}	half-life
taa	Tetraaza[14]annulene
tacn	triazacyclononane
tau	tubulin associating unit

TBAF	[¹⁸ F]tetraethylammonium fluoride (NBu ₄ F)
TEG	tetraethyl glycol
terpy	2,2',6'2''-terpyridyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
ThT	thioflavin T (2-(4-methylaminophenyl)-6-hydroxybenzothiazole)
TIPS	triisopropylsilane
TK1	Human thymidine kinase
β ⁺	Positron

Chapter 1 Introduction

1.1 Positron emission tomography imaging

Positron emission tomography (PET) imaging is a non-invasive molecular imaging technique that is used to assist in diagnosing and monitoring the progression of diseases.¹⁻

² The basic principle underlying PET imaging is that positron-emitting radionuclides are attached to biologically active molecules to form a tracer that is injected into the human body. These radiotracers travel *via* blood circulation, and accumulate around the target tissue (Figure 1.1).³ These unstable radionuclides decay by the emission of a positron (β^+), which travels a short distance before colliding with an electron. The annihilation results in the production of two coincident gamma photons, each with an energy of 511 keV.⁴ These pairs of gamma (γ) photons travel in the opposite direction at about a 180° angle to each other and are detected by the PET detector in the form of a closed ring, producing clear three-dimensional images of the biodistribution of a radiotracer at the molecular level as a function of time. The distribution of the labelled probe hence provides quantitative information regarding the physiological and biochemical processes in living organisms. One of the advantages of PET is its sensitivity which allows the use of radionuclides in small concentrations, often quoted in pico- to nanomolar, thus minimising potential pharmacological side effects.⁵⁻⁶

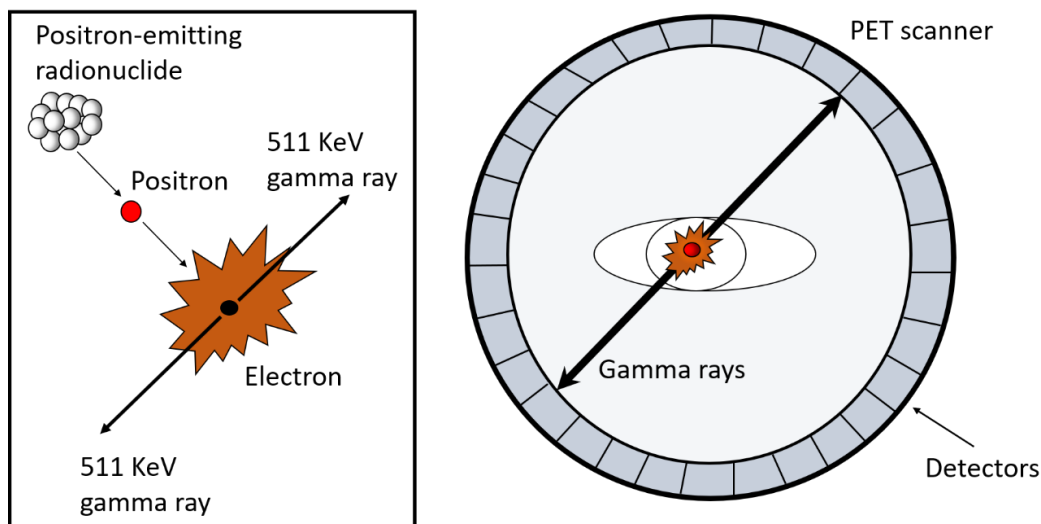


Figure 1.1. The illustration of the fundamental principle underlying PET imaging.

Another molecular imaging technique widely used clinically is Computed Tomography (CT). CT relies on different levels of X-ray attenuation by tissues and computer processing to map and create three-dimensional images of the body. It is often combined with PET as a multimodality tool.³ The PET/CT scanner provides insights into the pathophysiological processes alongside the molecular events with correlation to the anatomical reference frame.⁷

The physiological and molecular mechanisms of diseases can be studied by utilising tracers incorporated with suitable positron-emitting radioisotopes. A variety of positron-emitting radioisotopes that are used in developing PET radiopharmaceuticals are listed in Table 1.1.⁸ Carbon-11 is a radionuclide with a short half-life ($t_{1/2} = 20.4$ min), ideal for labelling small molecules. However, its widespread use is limited as its half-life can be too short for routine synthesis of tracers and subsequent imaging. The longer half-life of gallium-68 ($t_{1/2} = 68$ min), fluorine-18 ($t_{1/2} = 109.8$ min) and copper-64 ($t_{1/2} = 12.7$ h) radionuclides allow sufficient time for multistep radiochemical synthesis and transportation of the labelled tracers from the production sites to imaging centres for same-day imaging.

Table 1.1. The positron-emitting radioisotopes and their properties.

Radioisotopes	Half-life ($t_{1/2}$)	Decay mode
Carbon-11	20.39 min	β^+ (961 keV), EC
Fluorine-18	109.8 min	β^+ (97%, 635 keV), EC
Copper-64	12.7 h	β^+ (18%, 0.28 MeV), EC
Gallium-68	68 min	β^+ (90% 2.92 MeV), EC

1.2 The properties of fluorine

The fluoride ion is highly electronegative and small in size with very low polarizability.⁹ When strategically positioned, a fluorine substituent can enhance the overall pharmacokinetic properties of a molecule or imaging agent. The fluorine atom is often used to replace a hydrogen atom attached to a carbon atom partly due to their similar sizes, but the substituent can have an electronic effect that changes a molecule's behaviour. Substituting a hydroxy group leads to minimal variation with respect to the comparable electronegativity and hydrogen bond ability as well as the similar van der Waals radii of fluorine and oxygen atoms.¹⁰

Table 1.2. The Van der Waals radii and electronegativity on the Pauling scale of fluorine, oxygen and hydrogen.

Properties	F	O	H
Van der Waals radius (Å)	1.47	1.52	1.20
Electronegativity (Pauling)	3.98	3.44	2.20

The incorporation of fluorine atom onto medicinal compounds is thought to be advantageous due to: (1) Increased metabolic stability of the compounds by blocking metabolically labile sites; (2) Improved pharmacokinetics especially lipophilicity and basicity and (3) Enhanced binding affinity to the target protein by changing the

conformation of a molecule.¹¹ The unique properties of fluorine make it an attractive element exploited in both drug discovery and application in PET.

Fluorine-18 ($[^{18}\text{F}]\text{F}^-$) is a positron-emitting isotope that is widely used in PET to radiolabel small molecules, peptides and proteins. It is an excellent candidate for PET in both clinical and preclinical settings due to its nuclear and pharmacochemical properties. Fluorine-18 has high positron decay ratio (97%), low positron energy (635 keV) and lack of side emissions as it only travels a short distance before annihilation occurs.¹² The short diffusion range (< 2.4 mm) provides images with high resolution while exposing patients to low overall radiation.¹³ These favourable properties provide a strong spur to develop a greater variety of fluorine-18 labelled PET imaging agents through different methods.

1.3 Fluorine-18 labelled PET imaging agents

1.3.1 $[^{18}\text{F}]\text{FDG}$: The workhorse of PET imaging

The majority of clinical PET scans use 2-deoxy-2- $[^{18}\text{F}]$ fluoro-D-glucose ($[^{18}\text{F}]\text{FDG}$) as a tracer (Figure 1.2). $[^{18}\text{F}]\text{FDG}$ is a glucose analogue in which a fluorine atom replaces the hydroxyl group at position 2' and is used routinely as a PET agent for cancer imaging.¹⁴ In 2000, the Food and Drug Administration (FDA) approved $[^{18}\text{F}]\text{FDG}$ for the evaluation of malignancies in patients with suspected abnormalities or patients clinically diagnosed with cancer. Like glucose, $[^{18}\text{F}]\text{FDG}$ is taken up by living cells and phosphorylated by hexokinases to FDG-6-phosphate; however, further metabolism is blocked as the hydroxyl on the second carbon has been replaced by fluorine.¹⁵ Although it remains controversial, the Warburg Effect describes that elevated glucose metabolism is one of the characteristics of cancer cells.¹⁶⁻¹⁸ Cancer cells use aerobic glycolysis to generate an abnormally high amount of energy compared to healthy cells, followed by the metabolism of pyruvate into lactate.¹⁹ $[^{18}\text{F}]\text{FDG}$ accumulates and is retained by these highly

glycolytic tissues as the metabolism of cancer cells is different from most normal cells. The ability to measure glucose metabolism based on the uptake of [^{18}F]FDG reflected by the distribution of the radiotracer can provide information to assist in the analysis and characterisation of diseases related to alterations in glucose metabolism.

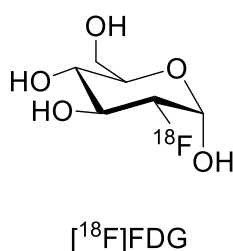


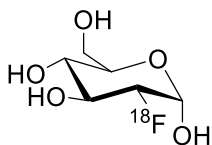
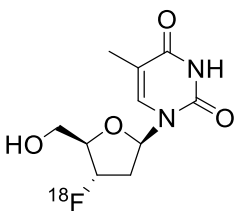
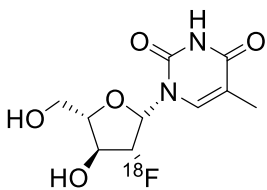
Figure 1.2. The fluorine-18 labelled PET imaging agents, [^{18}F]FDG.

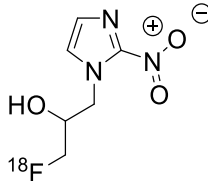
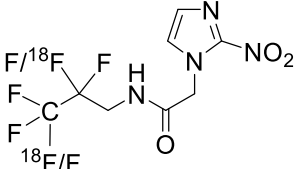
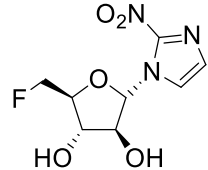
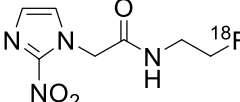
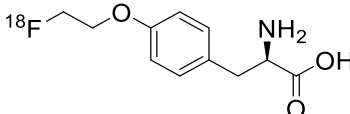
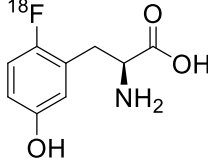
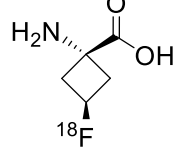
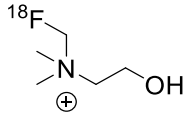
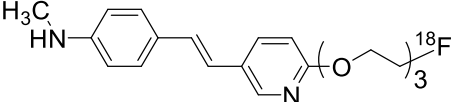
Besides having the capability to measure glucose metabolism, [^{18}F]FDG is a useful PET imaging agent due in part to its rapid excretion through urine and the resulting presence of few radiolabelled metabolites in the blood and thus has gained widespread use for the diagnosis and monitoring of cancers such as Hodgkin disease, non-Hodgkin's lymphoma, breast cancer, melanoma and lung cancer.²⁰⁻²³ Early detection of malignant tumours using PET scans with [^{18}F]FDG allows a clinician to follow up with an appropriate anticancer treatment.

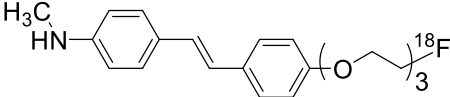
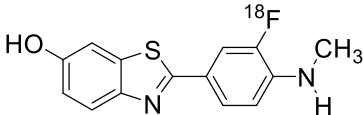
Despite the high sensitivity of PET imaging with [^{18}F]FDG, a major drawback is that [^{18}F]FDG is not selective as glucose is highly taken up by cells. It cannot selectively target cancer cells, which can result in false-positive and false-negative findings, making the delineation of tumour extent difficult. For example, uptake and accumulation of [^{18}F]FDG in metabolically active inflammatory tissues or certain benign tumours is a common issue of unspecific tracer retention.^{21, 24-25} False-negative findings are typically associated with androgen-dependent prostate cancer as the tumour cells have a low metabolic rate, thus minimises the uptake of [^{18}F]FDG.²⁶⁻²⁷ As the glucose uptake in the normal frontal cortex

is high, the low metabolic contrast between healthy and tumour cells in the brain results in low tumour-to-background resolution when imaged with [^{18}F]FDG, obscuring the difference between healthy tissue and malignant tumour in the brain.²⁸ Nevertheless, [^{18}F]FDG remains a powerful imaging agent to assist in the characterisation of disease for patients with an established cancer diagnosis during and after treatment and for evaluation of the ongoing therapeutic regimen. Despite the success of [^{18}F]FDG, few other tracers have entered routine clinical use despite gaining FDA approval due to challenges in developing scalable synthesis to enable widespread clinical use. Several fluorine-18 labelled PET imaging agents that are FDA approved or currently in clinical trials for the diagnosis of diseases are listed in Table 1.3. The reaction synthesis, as well as their applications of selected agents, are discussed below.

Table 1.3. Selected fluorine-18 labelled PET imaging agents that are FDA approved or are currently in clinical trials.

Class/ Imaging agent	Chemical structure	Target	Clinical Status
Metabolism			
[^{18}F]FDG		Hexokinase	FDA approved
[^{18}F]FLT		Thymidine kinase	Phase III
[^{18}F]FMAU		Thymidine kinase	Phase I

Hypoxia		
¹⁸ F]FMISO		Low oxygen
¹⁸ F]EF5		Low oxygen
¹⁸ F]FAZA		Low oxygen
¹⁸ F]FETA		Low oxygen
Protein synthesis		
¹⁸ F]FET		Amino acid transporters
¹⁸ F]FMT		Amino acid transporters
¹⁸ F]FACBC		Amino acid transporters
Membrane metabolism		
¹⁸ F]Fluorocholine		Choline kinase
Osteoporosis		
¹⁸ F]sodium fluoride		Bone
Alzheimer's disease		
¹⁸ F]florbetapir		Amyloid-β plaques

^[18F] florbetaben 	Amyloid-β plaques	FDA approved
^[18F] flutemetamol 	Amyloid-β plaques	FDA approved

1.3.2 [¹⁸F]FLT for cell proliferation

A fluorine-18 imaging tracer currently in phase III clinical trial is 3'deoxy-3' [¹⁸F]fluorothymidine ([¹⁸F]FLT) which is a radiolabelled analogue of the DNA base thymidine, used to target human thymidine kinase (TK1).²⁹ Thymidine is used by proliferating cells for DNA synthesis during the S phase of the cell cycle but is not incorporated into RNA.³⁰ As the elevated replication of tumour cells is a hallmark of cancer, the ability to visualise the rate of cell proliferation could assist the clinician in monitoring tumour growth. Typically, the reduction of tumour size is an indication of reduced cell proliferation as a result of chemotherapy.³¹⁻³² For this purpose, analogues of thymidine have been synthesised and studied in the development of radiotracers. Although the carbon-11 labelled [¹¹C]thymidine proved to be a promising imaging agent, the short half-life of carbon-11 and it being rapidly metabolised limited its use in the clinical setting.³³⁻³⁵ As an alternative, [¹⁸F]FLT was developed, and its clinical application has been studied extensively.³⁶⁻⁴² [¹⁸F]FLT is taken up by nucleoside transporters in the same way as thymidine, and the phosphorylated form of thymidine is incorporated into DNA. Due to the absence of the hydroxyl group at the 3' position, [¹⁸F]FLT is trapped within cells after being phosphorylated by the enzyme TK1. The intracellular accumulation of [¹⁸F]FLT provides an indicator of the activity of TK1, as a measure of cell proliferation before and after anticancer treatment, where a decrease in [¹⁸F]FLT

uptake reflects the effectiveness of therapy.⁴³ Advantages of [^{18}F]FLT over [^{18}F]FDG include the higher specificity to tumour proliferation and the inability to accumulate in inflammatory lesions, reducing the false-negative findings.⁴⁴ Unfortunately, there is lower uptake of [^{18}F]FLT in low-grade brain tumour compared to [^{18}F]FDG which reduces its sensitivity to detect tumours in the brain at early stages, limiting its use for brain imaging.⁴⁵⁻⁴⁸ In contrast, high uptake of [^{18}F]FLT due to the proliferation of lymphocytes in reactive lymph nodes and high cellular activity in the bone marrow and liver results in low specificity of the tracer and thus can produce false-positive findings.⁴⁹⁻

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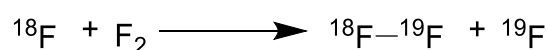
Both [^{18}F]FDG and [^{18}F]FLT have demonstrated their roles as glucose metabolism and cell proliferating marker, respectively. These imaging agents are less useful at the initial diagnosis stages of some cancers. Still, they serve as good predictors of cancer therapeutic effectiveness and remain as useful diagnostic imaging tools in clinical settings.

1.4 Formation of C- ^{18}F bonds via electrophilic substitution

Conventional approaches to fluorine-18 radiolabelling rely on the formation of a covalent C- ^{18}F bond. The C-F bond is intrinsically strong and highly polarised, attributed to the electrostatic attraction between $\text{C}^{\delta+}$ and $\text{F}^{\delta-}$ rather than the electron sharing within the covalent bond.⁵¹ The short and unreactive C-F bond that is not prone to cleavage is an advantage in the design of radiotracers for PET imaging.

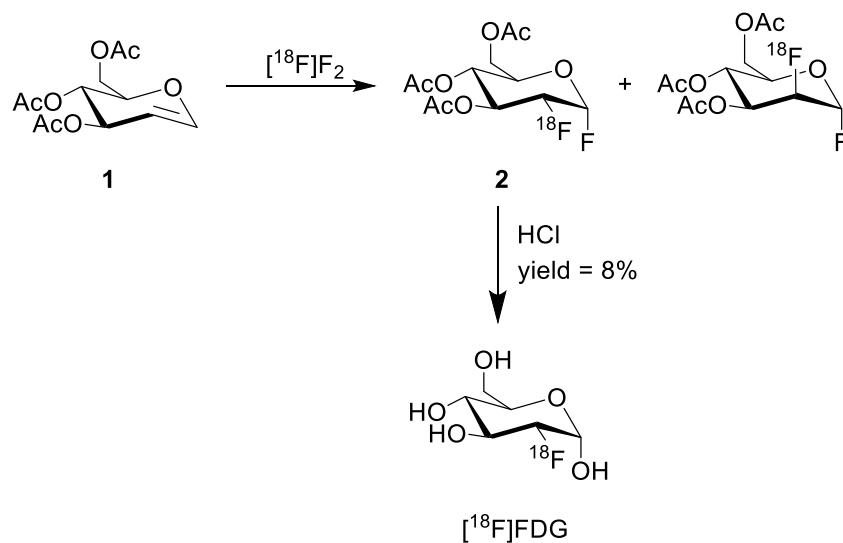
Electrophilic fluorine gas, [^{18}F]F₂, prepared from either the deuteron-irradiation of Ne, ($^{20}\text{Ne}(\text{d},\alpha)^{18}\text{F}$) or proton bombardment of water ($^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$) can either be used as a reagent for electrophilic substitution or converted to the less reactive acetylhypofluorite ([^{18}F]CH₃CO₂F). These two reagents are the most common sources of fluorinating agents for electrophilic fluorination. Specific activity is the activity of a radioisotope per unit

mass. The $^{18}\text{F}]\text{F}_2$ gas liberated from cyclotron has low specific activity (0.04 – 0.4 GBq/ μmol) as it also contains $^{19}\text{F}]\text{F}_2$, the non-radioactive isotope of fluorine with a 1:1 $^{18}\text{F}/^{19}\text{F}$ ratio (Scheme 1.1). An improved method to produce $^{18}\text{F}]\text{F}_2$ gas with a specific activity of up to 55 GBq/ μmol was reported in 1997 using fluorine-18 labelled methyl fluoride ($^{18}\text{F}]\text{CH}_3\text{F}$) and F_2 gas (0.8%).⁵² Radiolabelling with the electrophilic fluorination reactions is seldom used due to low radiochemical yield and low specific activity. Nevertheless, it has played a crucial role in the early development of some valuable fluorine-18 labelled imaging agents for PET.



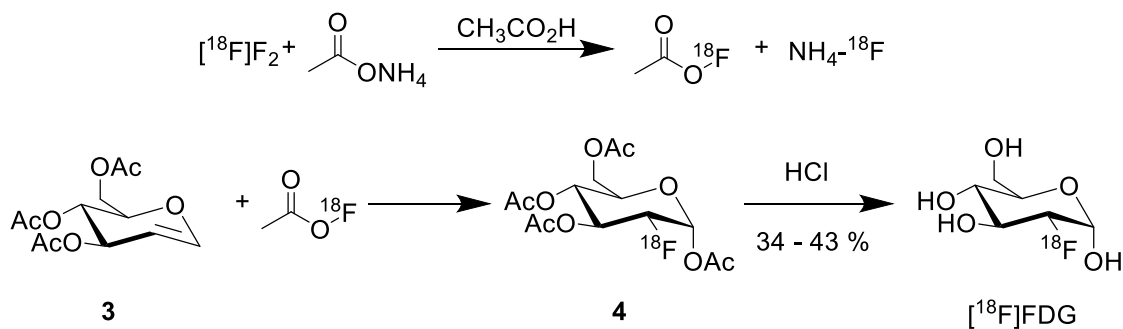
Scheme 1.1. Method for the preparation of electrophilic $^{18}\text{F}]\text{F}_2$.

The compound $^{18}\text{F}]\text{FDG}$ was first synthesised in 1976 by electrophilic fluorination.⁵³⁻⁵⁴ The glucal precursor, 3,4,6-tri-*O*-acetyl-D-glucal (**1**) was treated with $^{18}\text{F}]\text{F}_2$, producing a mixture of 3:1 fluorine-18 labelled difluoro-glucose and difluoro-mannose derivatives (Scheme 1.2). The mixture was separated using gas chromatography. The difluoro-glucose (**2**) was then hydrolysed with hydrochloric acid to form 2-fluoro-2-deoxyglucose. The total time of synthesis was about 2 hours, with an overall radiochemical yield of 8%. The first FDG trial in humans was carried out in 1978 to map glucose metabolism in the human brain.⁵⁵



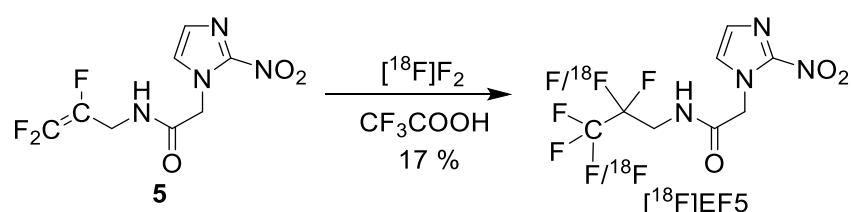
Scheme 1.2. Synthesis of $[^{18}\text{F}]\text{FDG}$ using electrophilic substitution reaction.

Several improvements have enabled increasing the radiochemical yield (34 - 43%) and decreasing the production time, such as replacing $[^{18}\text{F}]\text{F}_2$ with acetylhydropofluorite ($[^{18}\text{F}]\text{CH}_3\text{CO}_2\text{F}$) (Scheme 1.3). Treatment of $[^{18}\text{F}]\text{F}_2$ with aqueous ammonia in glacial acetic acid allows isolation of $[^{18}\text{F}]\text{CH}_3\text{CO}_2\text{F}$.



Scheme 1.3. Synthesis of $[^{18}\text{F}]\text{FDG}$ using electrophilic substitution reaction utilising $[^{18}\text{F}]\text{CH}_3\text{CO}_2\text{F}$.

The hypoxia-selective biomarker 2-(2-nitro-1*H*-imidazol-1-yl)-*N*-(2,2,3,3,3- ^{18}F)pentafluoropropyl-acetamide (^{18}F]EF5) was prepared by the introduction of ^{18}F]F₂ to the alkene double bond on the corresponding allyl precursor (**5**) (Scheme 1.4).⁵⁶⁻⁵⁷ The reaction required a low concentration of ^{18}F]F₂ (0.1%) to achieve a radiochemical yield of 17%. Trifluoroacetic acid (CF₃COOH) was employed as a solvent to ensure protonation of the nitrogen atom in the 3' position to reduce the reactivity of the nitroimidazole ring and favour selective reaction at the double bond.

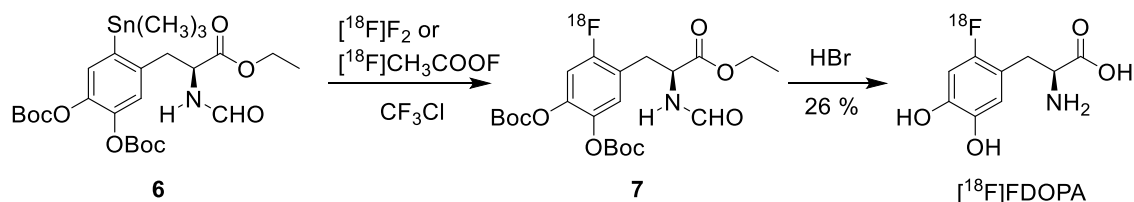


Scheme 1.4. Synthesis of ^{18}F]EF5 via electrophilic substitution reaction.

Notably, the radiochemical yield obtained from electrophilic fluorination of enol ethers or alkenes using ^{18}F]F₂ gas is limited to 50% as for every ^{18}F]F⁻ atom there is an ^{19}F]F⁻. The use of the less reactive ^{18}F]CH₃CO₂F as the source of fluoride can overcome this issue.

A fluoride analogue of L-DOPA, 6- ^{18}F -fluoro-L-dihydroxyphenylalanine (^{18}F]FDOPA), is used to image cerebral dopamine metabolism. The fluorine-18 labelled compound was first synthesised *via* electrophilic aromatic substitution of 3,4-dihydroxyphenyl-L-alanine with ^{18}F]F₂ gas.⁵⁸⁻⁵⁹ This method produced a mixture of isomers which was separated by purification to obtain the 6-fluoro isomer in low radiochemical yield (1.3 - 3%). To overcome the non-regiospecificity issue, a protected trialkyl tin derivative of DOPA (**6**) is treated with either ^{18}F]F₂ or ^{18}F]CH₃CO₂F (Scheme 1.5).⁶⁰⁻⁶¹ Regioselective radiofluorodestannylation of the organometallic precursor followed by

deprotection of the hydroxyl and carboxyl group using hydrogen bromide generated [^{18}F]FDOPA with a radiochemical yield of 26%.



Scheme 1.5. Synthesis of [^{18}F]FDOPA using electrophilic substitution reaction.

Apart from [^{18}F]CH₃CO₂F, a range of electrophilic fluorination reagents can be prepared from [^{18}F]F₂ include [^{18}F]XeF₂, [^{18}F]CF₃OF, [^{18}F]FCIO₃, *N*-[^{18}F]fluoropyridinium triflate,⁶² 1-[^{18}F]fluoro-2-pyridone, [^{18}F]Selectfluor⁶³ and *N*-[^{18}F]fluorobenzenesulfonimide ([^{18}F]NFSI) (Figure 1.3). An extensive review on the topic of fluorine-18 radiolabelling in aqueous media using [^{18}F]selectfluor as an electrophilic fluorinating reagent is available.⁶⁴

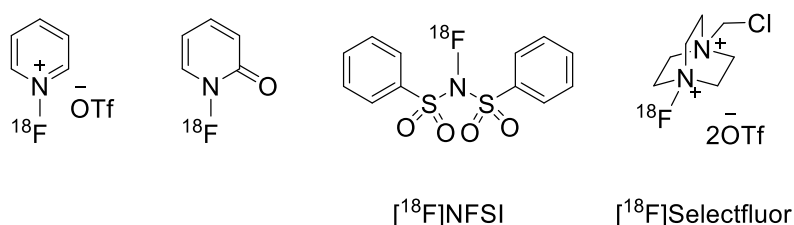
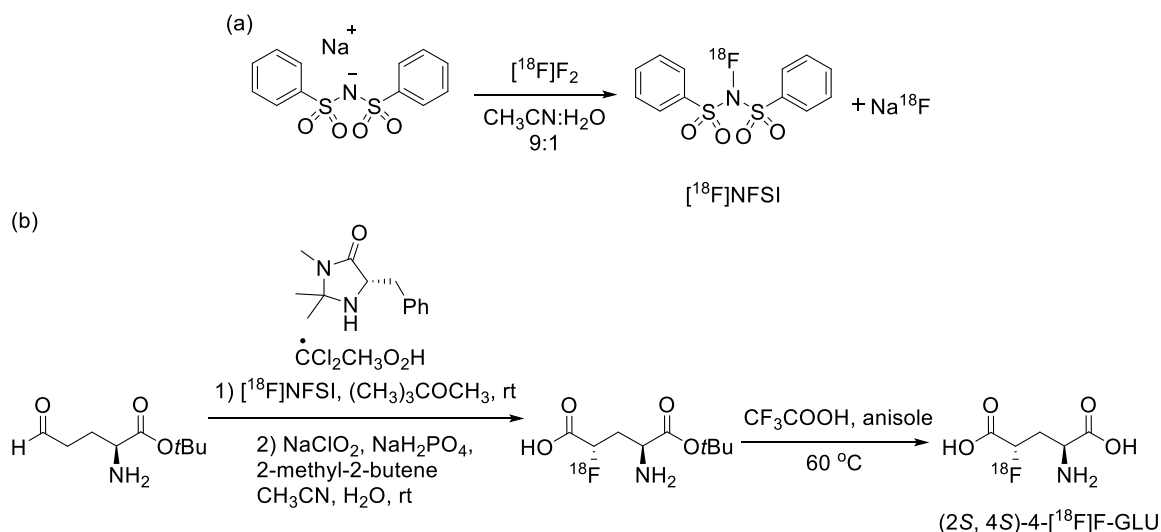


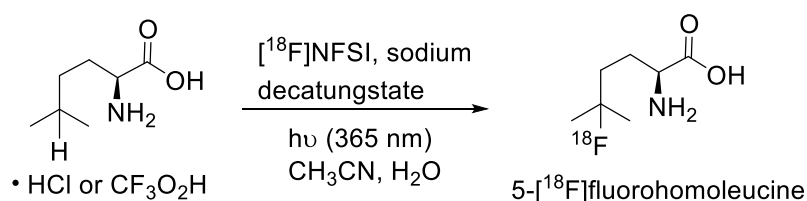
Figure 1.3. Chemical structures of some electrophilic fluorinating reagents.

The fluorinating agent [^{18}F]NFSI is prepared by bubbling [^{18}F]F₂ through a solution of 0.01 M NaN(SO₂Ph)₂ in acetonitrile and water (9:1) (Scheme 1.6 a). This reagent can be used to convert silyl-end ether and alkylsilane to fluorine-18 labelled ketone and allylic fluoride respectively.⁶⁵ Another example of an electrophilic fluorination using [^{18}F]NFSI is the synthesis of fluorine-18 labelled amino acid, (2*S*,4*S*)-4-[^{18}F]fluoroglutamic acid ((2*S*,4*S*)-4-[^{18}F]F-GLU) with high enantioselectivity (>99% *ee*) and high diastereoselectivity (19:1 d.r) (Scheme 1.6 b).⁶⁶



Scheme 1.6. (a) Production of [¹⁸F]NFSI and (b) synthesis of (2S,4S)-4-[¹⁸F]fluoroglutamic acid ((2S,4S)-4-[¹⁸F]F-GLU).

Recently, a selective photocatalytic fluorine-18 radiolabelling was achieved with [¹⁸F]NFSI to synthesise 5-[¹⁸F]fluorohomoleucine with a radiochemical yield of 28% ± 3% and a specific activity of 0.006 GBq/μmol (Scheme 1.7).⁶⁷ In this reaction, sodium decatungstate catalyses the hydrogen atom abstraction, followed by the transfer of [¹⁸F]F⁻ atom from [¹⁸F]NFSI to the resulting carbon radical. High tumour accumulations of the fluorine-18 labelled leucine analogue were observed in mice bearing human glioma (5.9 ± 0.7% ID/g (n = 4)) and prostate cancer (5.6 ± 3.2% ID/g (n = 5)) at 60 min post-injection.

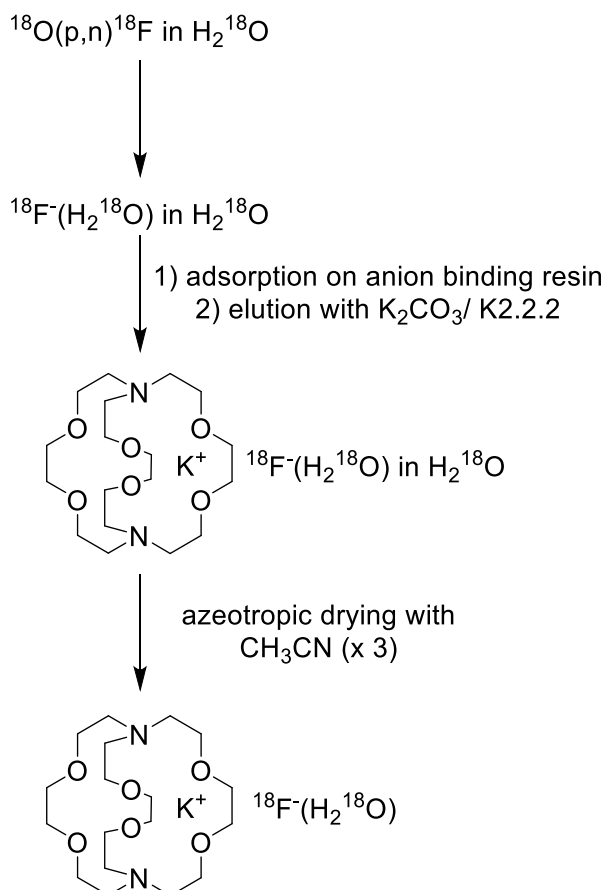


Scheme 1.7. Synthesis of 5-[¹⁸F]fluorohomoleucine using [¹⁸F]NFSI and sodium decatungstate with UV radiation at 365 nm.

Ultimately, the low radiochemical yield of the labelled product and low specific activity of the carrier-added method of production limited the use of the electrophilic substitution approach to fluorine-18 labelling.

1.5 Formation of C-¹⁸F bonds via nucleophilic substitution

Nucleophilic [¹⁸F]F⁻ is produced through ¹⁸O(p,n)¹⁸F nuclear reaction by proton bombardment of isotopically enriched [¹⁸O]H₂O in a cyclotron. The presence of the [¹⁸O]H₂O reduces reactivity of the [¹⁸F]F⁻ ion due to solvation and hydrogen bonding. Evaporation of water in a dipolar aprotic organic solvent with a phase transfer catalyst allows isolation of anhydrous [¹⁸F]F⁻. The dehydrating step is crucial to minimise the formation of hydrofluoric acid (HF), which can interfere with fluorination reactions. The [¹⁸F]F⁻ ion is adsorbed onto an anion exchange resin and is eluted with potassium carbonate and [4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo-(8.8.8)-hexacosane] ([2.2.2]Cryptand). The molecule [2.2.2]Cryptand is a bicyclic crown ether that binds to the potassium ion to form an ion pair soluble in organic solvents (Scheme 1.8). The mixture is dried with heat under inert gas and then dissolved in acetonitrile before drying azeotropically to remove the remaining water in a few successive cycles.



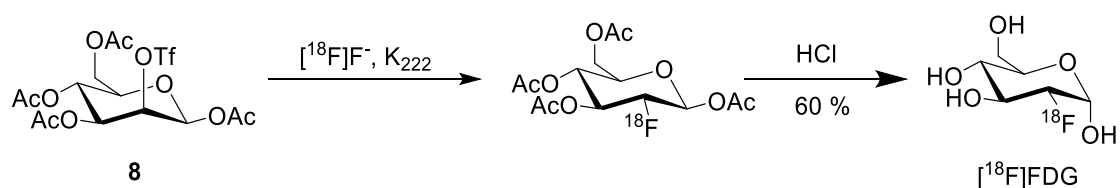
Scheme 1.8. Preparation of $[^{18}\text{F}]\text{F}^-$ from $[^{18}\text{O}]\text{H}_2\text{O}$ in a cyclotron showing the chemical structure of [2.2.2]cryptand.

The $[^{18}\text{F}]\text{F}^-$ ion is a strong nucleophile in non-aqueous media and well-suited for labelling through nucleophilic aliphatic substitution ($\text{S}_{\text{N}}2$) or nucleophilic aromatic ($\text{S}_{\text{N}}\text{Ar}$) substitution. These reactions are generally more favourable compared to electrophilic substitution as the source does not contain $[^{19}\text{F}]\text{F}^-$ and the fluorine-18 products are generally obtained in higher specific activity.

1.5.1 Aliphatic Nucleophilic Substitution

Aliphatic nucleophilic substitution proceeds via the reaction of $[^{18}\text{F}]\text{F}^-$ with an aliphatic precursor that contains a leaving group such as the following, in the order of leaving ability: $\text{Cl} < \text{Br} < \text{I} < 4\text{-methyl benzenesulfonate (tosylate)} \sim \text{methanesulfonate (mesylate)} < 4\text{-nitrobenzenesulfonate (nosylate)} < \text{trifluoromethanesulfonate (triflate)}$.⁶⁸⁻⁶⁹

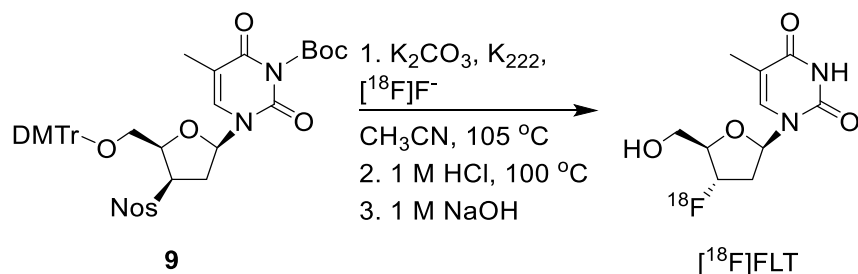
A significant improvement in the synthesis of [^{18}F]FDG was reported in 1986 where [^{18}F]F $^-$ was used to displace the triflate group of the mannose triflate precursor, methyl 4,6-*O*-benzylidene-3-*O*-methyl-2-*O*-trifluoromethanesulfonyl- β -D-mannopyranoside (**8**) in the presence of [2.2.2]Cryptand in acetonitrile (Scheme 1.9). The overall radiochemical yield of the reaction is usually higher than 60%.⁷⁰



Scheme 1.9. Synthesis of [^{18}F]FDG using nucleophilic substitution reaction.

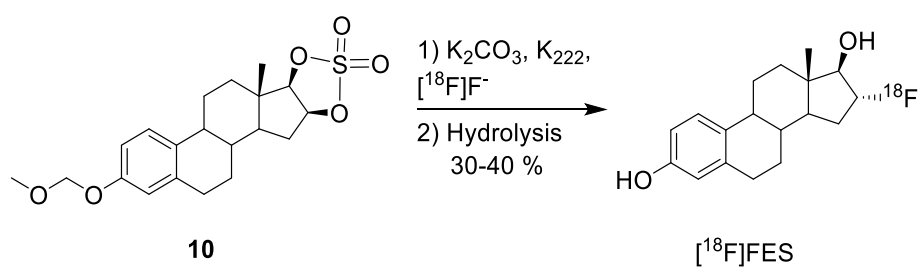
The synthesis of [^{18}F]FLT was first reported in 1991 through nucleophilic aliphatic substitution as a carrier-added synthetic method.⁷¹ The reaction involved converting thymidine to 3'-mesyl-5'-trityl- β -D-lyxofuranosyl thymidine followed by treatment with KF/[^{18}F]KF/crown ether to generate [$^{18/19}\text{F}$]FLT with low specific activity. High specific activity is desired to ensure a low amount of non-radioactive substance is present in the doses. In order to improve the radiochemical yield and specific activity, non-carrier-added methods were developed using alternative precursors. The most favourable and widely used is the nosylate precursor with a dimethoxytrityl (DMTr) group at the 5' position.²⁹ The nosylate precursor with an *N*-2,4-dimethoxybenzyl protected pyrimidine-NH required a deprotection step using ceric ammonium nitrate which led to the formation of unwanted side products. The 3-*N*-Boc protection of the precursor (**9**) simplified the deprotection step by avoiding the side reaction (Scheme 1.10).⁷² The subsequent investigation into the different reaction temperatures, precursor concentration and precursor-to-base ratio further improved radiochemical yield and

specific activity.⁷³⁻⁷⁴ The production of [^{18}F]FLT using a fully automated synthesis was later developed with a reported radiochemical yield of ~50%.⁷⁵



Scheme 1.10. Synthesis of [^{18}F]FLT using nucleophilic aliphatic substitution reaction.

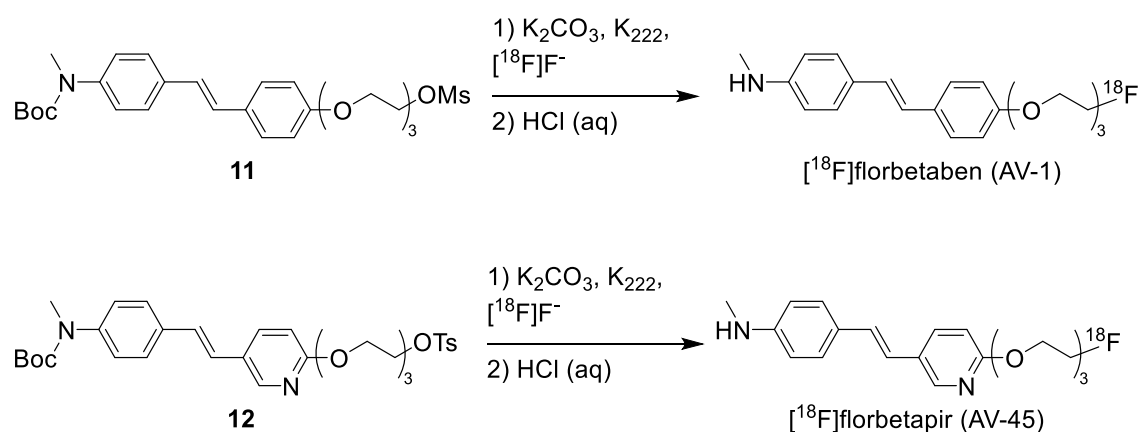
An example of nucleophilic aliphatic substitution with a cyclic sulphate precursor is the synthesis of [^{18}F]fluoroestradiol ([^{18}F]FES), a PET imaging agent used to assist in the diagnosis of breast cancer (Scheme 1.11).⁷⁶⁻⁷⁷ The precursor 3-methoxymethyl-16 β ,17 β -epiestriol-O-cyclic sulfone (**10**) treated with [^{18}F]F $^-$ undergoes a ring-opening reaction followed by hydrolysis of the methoxymethyl protecting group provided [^{18}F]FES in 30 - 40% radiochemical yield.



Scheme 1.11. Synthesis of [^{18}F]FES using nucleophilic aliphatic substitution reaction.

Several fluorine-18 labelled PET imaging agents designed to assist in the diagnosis of Alzheimer's disease can be synthesised using nucleophilic substitution reactions. Alzheimer's disease is an irreversible neurodegenerative disorder clinically characterised by memory loss and decline in cognitive function. The intracellular neurofibrillary tangles and extracellular amyloid plaques in the brain are pathological hallmarks of the disease

that serve as biomarkers. Amyloid plaque-specific PET imaging agents [^{18}F]florbetaben (AV-1) and [^{18}F]florbetapir (also known as Amyvid or AV-45) were developed using the aliphatic substitution approach. Substitution of the mesylate group on precursor (**11**) with [^{18}F]F $^-$ yields the fluorine-18 labelled polyethyleneglycol (PEG)-stilbene [^{18}F]florbetaben while substitution of the tosylate group on precursor (**12**) with [^{18}F]F $^-$ yields the styrylpyridine derivative of [^{18}F]florbetaben, [^{18}F]florbetapir (Scheme 1.12).⁷⁸⁻⁷⁹

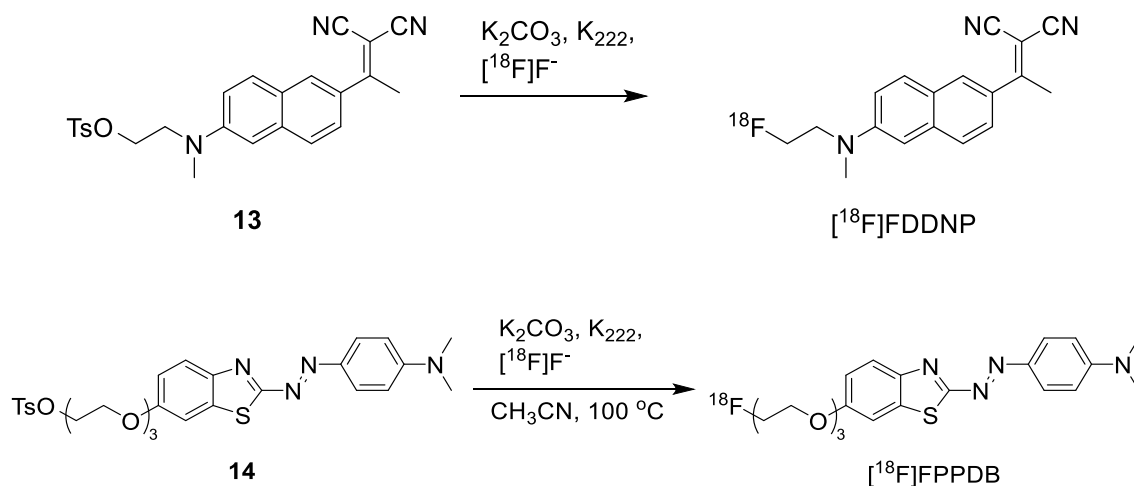


Scheme 1.12. Synthesis of [^{18}F]florbetaben (AV-1) and [^{18}F]florbetapir (AV-45) via nucleophilic substitution reactions.

Both the FDA approved fluorine-18 labelled radiotracers [^{18}F]florbetaben and [^{18}F]florbetapir integrate oligoethylene linkers that separate the positron-emitting radioisotopes from the targeting moiety, enabling modification of the lipophilicity without significantly altering the binding affinity to amyloid plaques.

Tosylate precursors are often employed to synthesise fluoro-pegylated (FPEG) imaging agents for the visualization of fibrillary amyloid depositions in the brain such as 2-(1,1-dicyanopropen-2-yl)-6-(2-[^{18}F]-fluoroethyl)-methylamino-naphthalene ([^{18}F]FDDNP) and (E)-4-4((6-(2-(2-(2-fluoroethoxy)ethoxy)benzo[d]thiazol-2-yl)-diazenyl)-N,N-dimethylaniline ([^{18}F]FPPDB). The compound [^{18}F]FPPDB is a fluorine-18 labelled phenyldiazenyl benzothiazole for the imaging of neurofibrillary tangles in Alzheimer's

disease.⁸⁰ The fluorine-18 labelling was performed on a tosyl precursor (**13**) undergoing a nucleophilic displacement reaction with [¹⁸F]F⁻ (Scheme 1.13).



Scheme 1.13. Synthesis of [¹⁸F]FDDNP and [¹⁸F]FPPDB via nucleophilic substitution reaction.

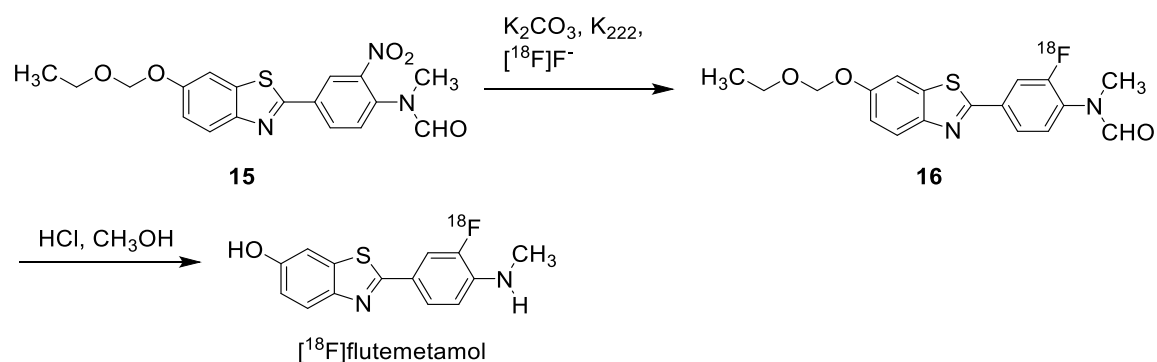
Fluorine-18 labelling via the formation of C-¹⁸F bond using nucleophilic aliphatic substitution remains a commonly used method as the reaction conditions are mild compared to aromatic nucleophilic substitution and electrophilic substitution, and the products are often obtained in desirable yields. A drawback of this method is the inherent necessity to protect any other potential sites of nucleophilic attack in the precursor, which prolongs the preparation time to include a deprotection step at the end of the synthesis.

1.5.2 Aromatic Nucleophilic Substitution

Aromatic nucleophilic substitution (S_NAr) requires an electron-withdrawing substituent to sufficiently activate an aromatic precursor on the *ortho* or *para* position to a leaving group. The affinity of F⁻ towards the electron-deficient aromatic molecule is dependent on the electron-withdrawing groups, with their respective approximate ability increasing in the order of 3-NO₂ < 4-Ac < 4-CHO < 4-CN ~ 4-CF₃ < 4-NO₂. The reaction proceeds via the displacement of a leaving group with the approximate leaving ability increases in

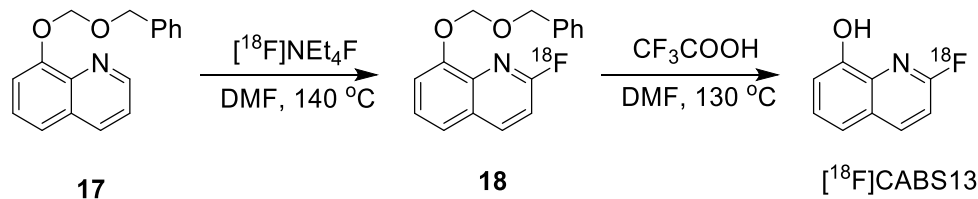
the order of $I < Br < Cl < F < NO_2 \sim N^+CH_3$.⁸¹ These aromatic nucleophilic substitution reactions often proceed under high temperature (120 - 180 °C) in a polar aprotic solvent (DMSO) in the presence of K_{222} and a base.

An example of a fluorine-18 labelled PET imaging agent synthesised using nucleophilic aromatic substitution is [^{18}F]flutemetamol, an FDA approved diagnostic probe for Alzheimer's disease.⁸²⁻⁸⁴ The benzothiazole derivative precursor (**15**) has an N^+CH_3 group *ortho* to the leaving group (NO_2). The displacement of the nitro group with [^{18}F]F⁻ followed by deprotection with acid yields [^{18}F]flutemetamol (Scheme 1.14).



Scheme 1.14. Synthesis of [^{18}F]flutemetamol using nucleophilic aromatic substitution reaction.

The prerequisite of having both electron-withdrawing and leaving groups on an aromatic ring limits the versatility of aromatic substitution. The use of nitrogenous heteroarenes as precursors which tend to be electron-deficient in place of the *ortho*-activating groups can overcome this problem. Hence, the compound [^{18}F]CABS13 was synthesised by the addition of [^{18}F]tetraethylammonium fluoride ([^{18}F]NEt₄F) onto the precursor containing a pyridine group (**17**) followed by deprotection using CF_3CO_2H (Scheme 1.15).⁸⁵



Scheme 1.15. Nucleophilic aromatic substitution of heteroarene (17) to form [¹⁸F]CABS13.

Other examples of fluorine-18 molecules synthesised using nitrogenous heteroarenes precursors include the pyridinyl benzofuran derivative 2-(2-fluoro-6-(methylamino)pyridine-3-yl)benzofuran-5-ol ([¹⁸F]AZD4694), an A β plaque imaging agent and [¹⁸F]T807, a potential tau imaging agent (Figure 1.4).⁸⁶⁻⁸⁷ [¹⁸F]AZD4694 currently in phase III clinical trials showed promising results with lower white matter binding compared to its carbon-11 labelled derivative, [¹³C]PiB.

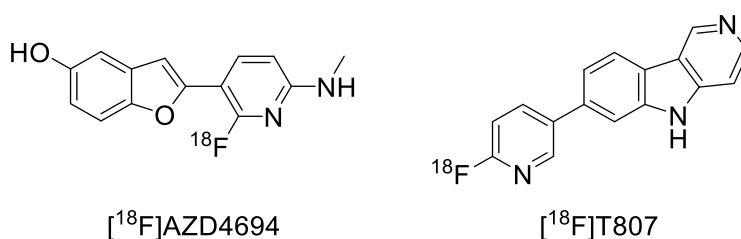
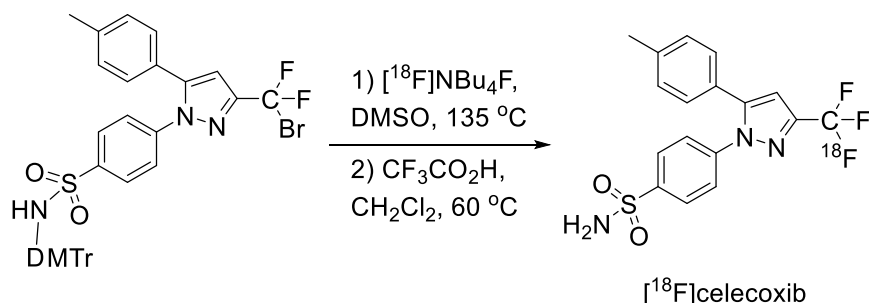


Figure 1.4. Structures of [¹⁸F]AZD4694 and [¹⁸F]T807.

1.5.3 Fluorine-18 labelled Multifluoromethyl Motifs

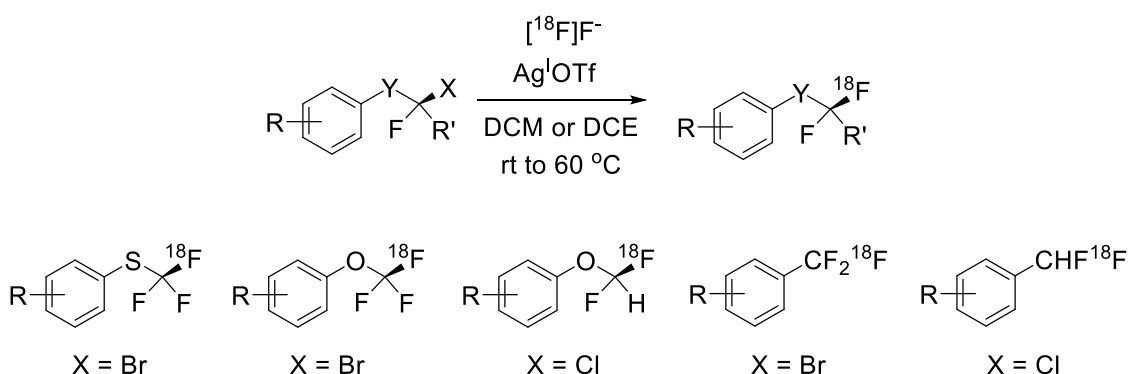
Radiopharmaceuticals containing multifluoromethyl motifs (-CF₃ and -CF₂H) can be radiolabelled with fluorine-18 via halogen X/¹⁸F (X = Cl, Br) exchange or isotopic ¹⁹F/¹⁸F exchange. For instance, the compound [¹⁸F]4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide ([¹⁸F]celecoxib) was obtained from a halogen exchange reaction between the DMTr protected aryl-CF₂Br derivative and [¹⁸F]tetrabutylammonium fluoride ([¹⁸F]NBu₄F) in DMSO at 135 °C with a radiochemical yield of 10% $\pm$ 2% and a molar activity of 4.44 $\pm$ 1.48 GBq/ μ mol (Scheme 1.16).⁸⁸ This

reaction requires elevated temperature due to the presence of two α -fluorine atoms which impedes the displacement of Br^- with $[^{18}\text{F}]\text{F}^-$.



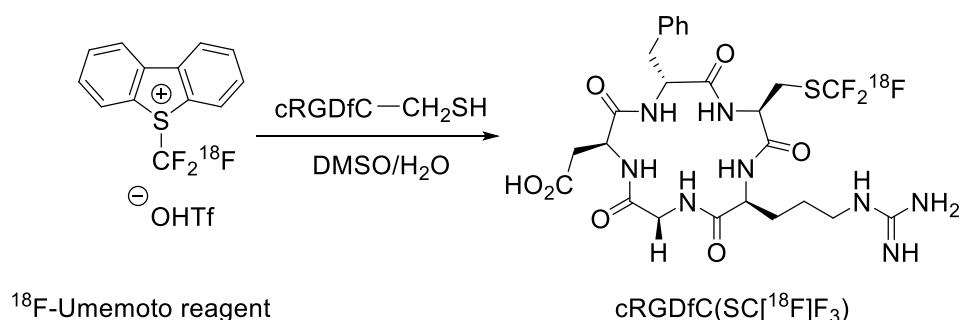
Scheme 1.16. Synthesis of $[^{18}\text{F}]\text{celecoxib}$ via halogen $\text{Br}/^{18}\text{F}$ exchange reaction.

To overcome the harsh conditions required, Gouverneur and co-workers discovered that $\text{Ag}(\text{I})$ catalyst can facilitate the fluorine-18 radiolabelling of a range of multifluoromethyl motifs. The $\text{Ag}(\text{I})$ salt, silver trifluoromethanesulfonate (AgOTf) was used to convert molecules with aryl- SCF_2Br , aryl- OCF_2Br , aryl- OCF_2H , aryl- CF_3 and aryl- CHFCl to aryl- $\text{SC}[^{18}\text{F}]\text{F}_3$, aryl- $\text{OC}[^{18}\text{F}]\text{F}_3$, aryl- $\text{OC}[^{18}\text{F}]\text{F}_2\text{H}$, aryl- $\text{C}[^{18}\text{F}]\text{F}_3$ and aryl- $\text{CH}[^{18}\text{F}]\text{FCl}$ at room temperature or 60°C in dichloromethane (DCM) or dichloroethane (DCE) (Scheme 1.17).⁸⁹⁻⁹⁰



Scheme 1.17. The general method of the $\text{Ag}(\text{I})$ -mediated halogen exchange reaction for a range of multifluoromethyl motifs.

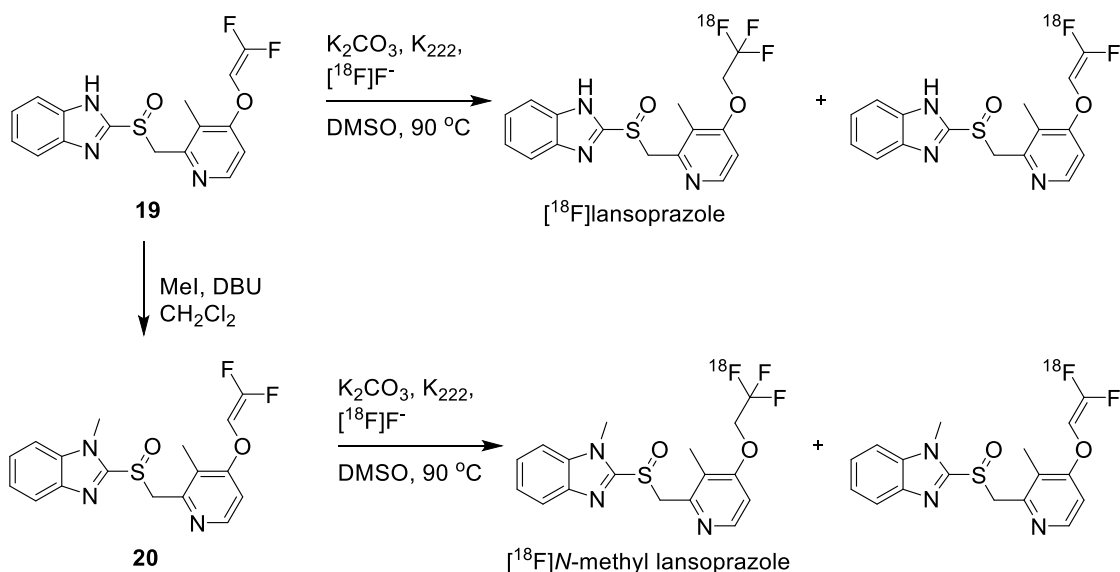
This work was further extended to radiolabel biomolecules attached to aryl-SCF₂Br motifs with fluorine-18. For instance, fluorine-18 labelled 5-(trifluoromethyl)dibenzothiophenium trifluoromethanesulfonate, known as the Umemoto reagent, is used as a fluorinating reagent to label a cRGDfC peptide with fluorine-18 (Scheme 1.18).⁹¹



Scheme 1.18. Fluorine-18 radiolabelling of a cRGDfC peptide attached to an aryl-SCF₃ motif using the ¹⁸F-Umemoto reagent.

A proton-pump inhibitor, Lansoprazole (PrevacidTM) was first radiolabelled with carbon-11. It was later discovered that Lansoprazole and its *N*-methyl variant have high affinities for aggregated tau neurofibrillary tangle *in vitro*. The fluorine-18 labelled [¹⁸F]lansoprazole and [¹⁸F]*N*-methyl lansoprazole were synthesised using a one-step method *via* nucleophilic reaction of aliphatic trifluoroalkyl CF₃ groups (Scheme 1.19).⁹²⁻

⁹⁴ The enol ether precursors (**19**) and (**20**) with benzoimidazole moiety were treated with [¹⁸F]F⁻ to produce a mixture of fluorine-18 labelled trifluoro alkyl and enol ether in a 1:3 ratio.⁹⁵ Although the isotopic exchange reaction can be achieved under mild conditions, the mixed products with low radiochemical yield (12 - 28%) is not ideal.



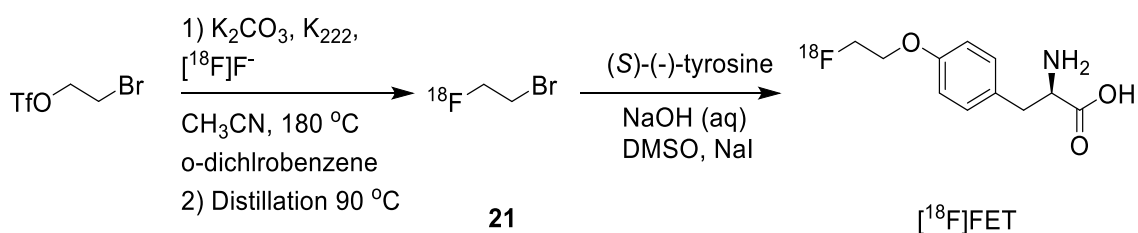
Scheme 1.19. Synthesis of $[\text{}^{18}\text{F}]$ lansoprazole and $[\text{}^{18}\text{F}]$ N-methyl lansoprazole.

Recently, fluorine-18 labelled aryl- CF_3 motifs were also prepared from Cu(I)-mediated fluorination of aryl iodides or boronic acid with $\text{Cu}-[\text{}^{18}\text{F}]\text{CF}_3$ as well as oxidative fluorination of benzylic difluoromethyl groups catalysed by $[\text{Mn}(\text{salen})\text{Cl}]$.⁹⁶⁻¹⁰⁰ Transition metal mediated cross-coupling reactions using organopalladium-based and organometallic nickel-based fluorination reagents have also been explored to label aromatic molecules with fluorine-18.¹⁰¹⁻¹⁰² These methods have further extended the range of fluorine-18 labelled compounds but are less suitable for labelling biomolecules as elevated temperature and the use of polar organic solvents during synthesis are not always compatible with peptides and proteins. Furthermore, the absence of activated aromatic moieties on biomolecules requires an alternate method to introduce $[\text{}^{18}\text{F}]\text{F}^-$. Therefore, incorporation of fluorine-18 into biomolecules using prosthetic groups represents a better approach.

1.5.4 Utilising prosthetic groups for the incorporation of fluorine-18

Indirect fluorine-18 radiolabelling of biomolecules can be achieved using prosthetic groups with orthogonally reactive functional groups available for conjugation to biomolecules. These prosthetic groups bearing fluorine-18 are more suitable to be conjugated to peptides and proteins of large molecular weights under more tolerable conditions to avoid denaturing of heat-sensitive biologically active molecules. Besides being able to react rapidly under mild conditions, it is important that these small prosthetic groups do not alter the biological properties of the targeting molecules unfavourably.

As an example below, the tyrosine analogue [^{18}F]FET was synthesised using a prosthetic group with an [^{18}F]F $^-$ and a bromine leaving group (**21**) (Scheme 1.20).



Scheme 1.20. Synthesis of [^{18}F]FET, an example of fluorine-18 labelling using a prosthetic group.

A series of fluorine-18 labelled prosthetic groups has been developed for coupling to peptides and proteins mostly *via* acylation, alkylation, amidation or oxime formations (Figure 1.5). Each of these prosthetic groups has different functional groups that can target the amino or carboxylic acid functional groups within peptides and proteins. The most commonly used prosthetic group is the active ester *N*-succinimidyl-4- ^{18}F fluorobenzoate ([^{18}F]SFB), which can be conjugated to peptides at either the primary amino groups at the *N*-terminus or lysine residues through an acylation reaction after activation of the *N*-succinimide functional group. The 4- ^{18}F fluorobenzaldehyde

(^{18}F]FBA) forms an oxime group with peptides containing an aminooxyl functional group whereas the *N*-[2-(4- ^{18}F]fluorobenzamido)ethyl]maleimide (^{18}F]FBEM) conjugates to peptides with a free thiol group by forming a thioether bond.

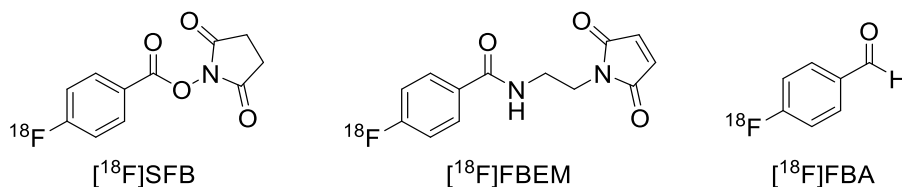


Figure 1.5. The chemical structures of ^{18}F]SFB, ^{18}F]FBEM and ^{18}F]FBA.

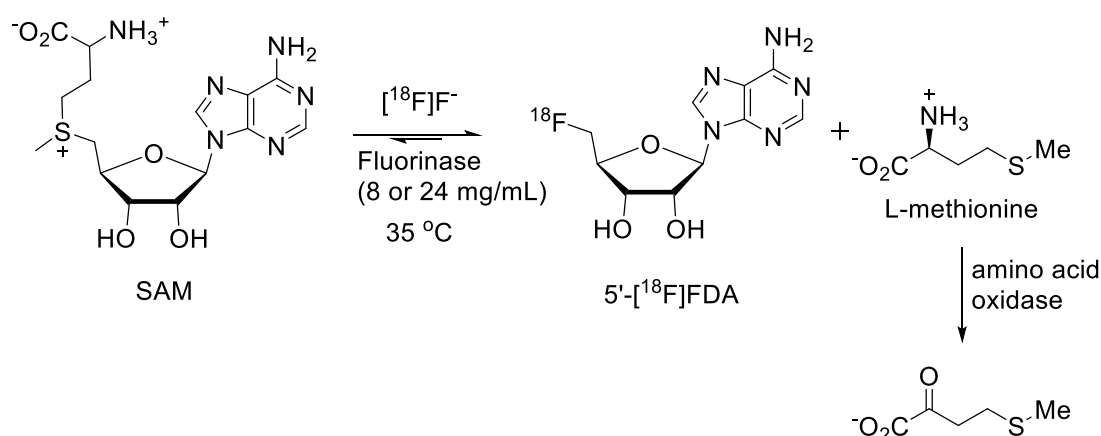
The incorporation of fluorine-18 *via* a prosthetic group allows the labelling of molecules of biological interest under mild conditions. However, the multi-step procedure is often time-consuming and inefficient as the fluorine-18 is introduced onto the compound in the early stages of synthesis, resulting in low radiochemical yield.

1.5.5 Fluorinase mediated C- ^{18}F bond formation

An interesting method was developed by O'Hagan and co-workers by which a fluorinase enzyme was utilised to catalyse the nucleophilic C- ^{18}F bond formation. A fluorinase enzyme (5'-fluoro-5'-deoxyadenosine synthase, 5'-FDAS, E.C. 2.5.1.63) isolated from the bacterium *Streptomyces cattleya* is capable to convert (S)-adenosyl-L-methionine (SAM) to 5'-fluoro-5'-deoxyadenosine (5'-FDA) and L-methionine.¹⁰³⁻¹⁰⁵ Fluorine-18 radiolabelling was achieved in the presence of $^{18}\text{F}/\text{H}_2^{18}\text{O}$ at 35 °C to give 5'- ^{18}F]fluoro-5'-deoxyadenosine (5'- ^{18}F]FDA) with a radiochemical yield of 1% (Scheme 1.21).¹⁰⁶ The low radiochemical yield was attributed to the reversible process that did not favour the forward reaction. An *in situ* oxidation of L-methionine with an L-amino acid oxidase with various fluorinase enzymes shifted the chemical equilibrium to give the fluorine-18 labelled product with a radiochemical yield of 95% within 2 h.¹⁰⁷ Other ^{18}F]-5'-fluorodeoxy nucleosides such as 5'- ^{18}F]fluoro-5'-deoxyionosine (5'- ^{18}F]FDI) (adenyl

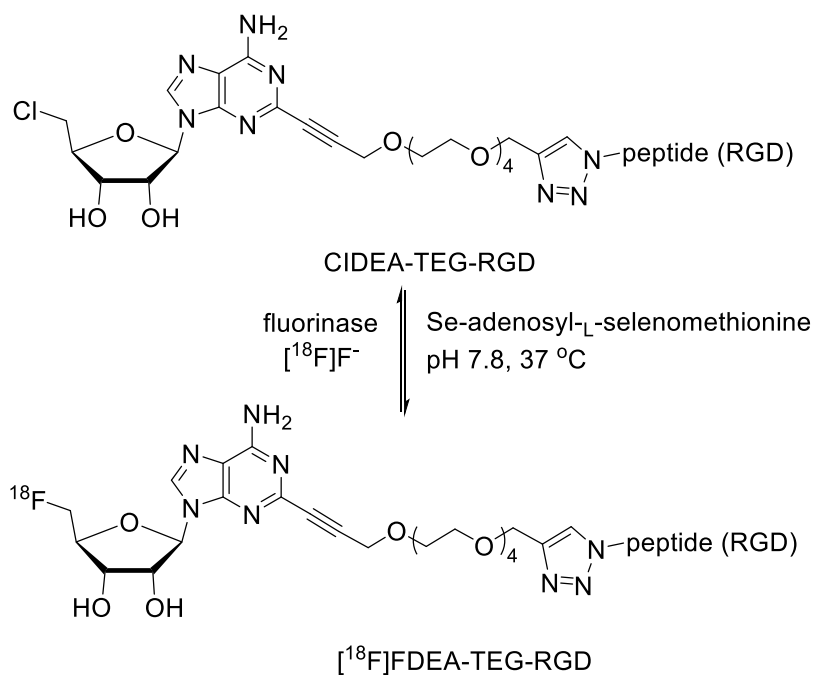
acid deaminase, 4 h, yield 75%), 5'-[¹⁸F]fluoro-5'-deoxy-D-ribose (5'-[¹⁸F]FDR) (purine nucleotide phosphorylase-phytase, 4 h, yield 45%) and 5'-[¹⁸F]fluoro-5'-deoxyuridine (5'-[¹⁸F]FDU) (purine nucleotide phosphorylase and thymidine phosphorylases, 4 h, yield 33%) were synthesised by base-swapping reactions with combinations of fluorinase and purine/pyridine nucleotide phosphorylase enzymes under similar reaction conditions.¹⁰⁷⁻

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Scheme 1.21. Synthesis of 5'-[¹⁸F]FDA in the presence of fluorinase enzyme.

The advantages of using fluorinase are the selectivity and solubility in water which does not require the use of anhydrous fluoride. Recently, the enzymatic-catalysed reaction was employed to form a 5'-[¹⁸F]fluorodeoxy-2-ethynyladenosine ([¹⁸F]FDEA) substrate conjugated to an arginyglycylaspartic acid (RGD) peptide with a tetraethyl glycol (TEG) linker at pH 7.8 at 37 °C using the chloride analogue, ClDEA-TEG-RGD as the precursor (Scheme 1.22).¹⁰⁹



Scheme 1.22. The enzymatic-catalysed fluorine-18 labelling of an RGD peptide derivative.

The late-stage fluorine-18 radiolabelling of peptide at neutral pH proved that the biocatalytically controlled enzymatic fluorination reaction is an attractive method for the formation of C- ^{18}F bond in aqueous media. Nonetheless, this approach needs to be further optimised as the long reaction time (2-4 h) required to achieve high radiochemical yields can limit its use for the production of fluorine-18 radiopharmaceuticals. Despite some success using the traditional approach of fluorine-18 labelling based on the formation of the covalent C- ^{18}F bond, optimization of synthetic methods and development of automation are ongoing to improve efficiency and to reduce preparation time.

1.6 Bond formation between non-carbon elements and $[^{18}\text{F}]\text{F}^-$

To address the limitations of the conventional methods of fluorine-18 labelling, there is growing interest in developing strategies utilising inorganic elements to incorporate $[^{18}\text{F}]\text{F}^-$ onto biomolecules. The principle behind the approach of fluorine-18 labelling using non-carbon elements is based on the strength of the bonds between F^- and cations

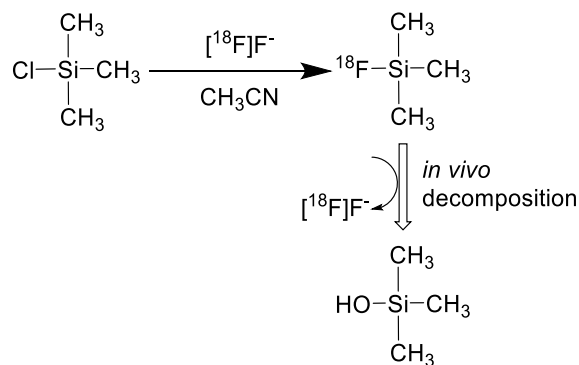
that are known to be higher than the C-F bond. This is reflected by their bond dissociation energies, a measure of the energy required to break the bonds (Table 1.4).¹¹⁰⁻¹¹¹. The strength of these bonds provides thermodynamic and kinetic driving forces that can be exploited for fluorine-18 labelling.

Table 1.4. Comparison of bond dissociation energies (kJ/mol).

Bond	Bond dissociation energies (kJ/mol)
B-F	766 (13)
C-F	536 (21)
Si-F	540 (13)
Ga-F	577 (15)
Al-F	664 (6)

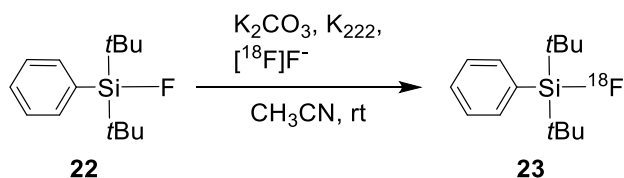
1.6.1 The formation of the Silicon-¹⁸F bond

The formation of a Si-¹⁸F bond dates back to 1959 to a preparation of [¹⁸F]silicon tetrafluoride with high specific activity but fast hydrolysis of the compound.¹¹² In 1985, an *in vivo* evaluation of [¹⁸F]fluorotrimethylsilane synthesised from chlorotrimethylsilane and [¹⁸F]tetramethylammonium fluoride in aqueous acetonitrile *via* halogen exchange reaction (60% radiochemical yield) revealed high uptake in bone (5.53 ± 0.53 ID/g) and low uptake in brain (0.06 ± 0.02 ID/g) (Scheme 1.23). These observations in rats exposed to [¹⁸F]fluorotrimethylsilane suggested *in vivo* hydrolysis to the corresponding silanol and release of fluoride, rendering it unsuitable for imaging applications.¹¹³



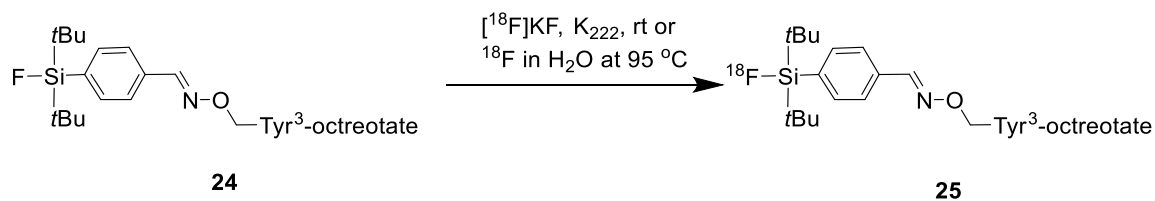
Scheme 1.23. Synthesis of [^{18}F]fluorotrimethylsilane and the hydrolysed silanol *in vivo*.

To improve stability *in vivo*, the rate of hydrolysis can be lowered by employing a more sterically hindered molecule to shield the Si- ^{18}F bond. The silicon-based fluoride acceptor (SiFA) was developed to address this poor hydrolytic profile. It features a silicon core, two bulky *tert*-butyl substituents at the silicon atom for a stabilising effect and a phenyl system available for functionalisation (Scheme 1.24). As chlorosilane compounds are unstable and prone to hydrolysis, the ^{19}F - ^{18}F isotopic exchange method was adopted for the formation of Si- ^{18}F bonds using fluorine-19 compounds as precursors.



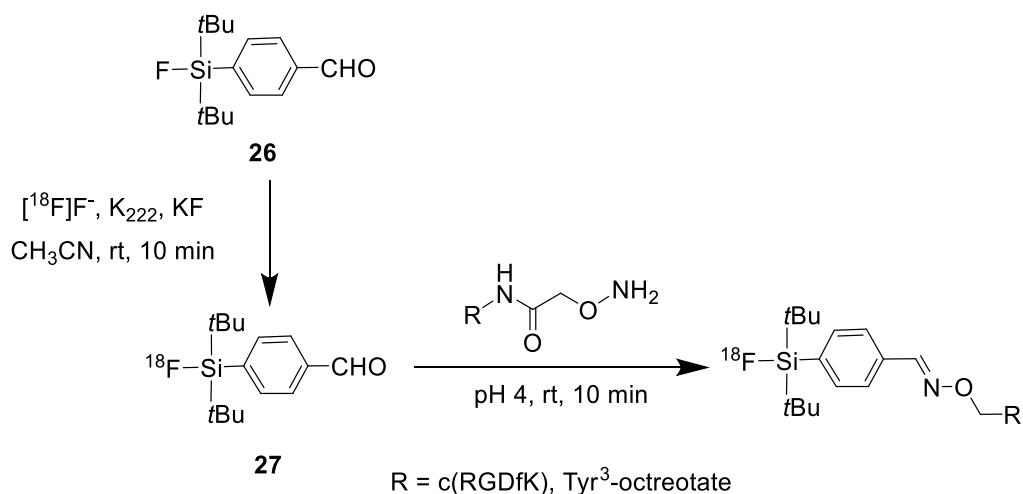
Scheme 1.24. The silicon-based fluoride acceptor (SiFA).

The SiFA approach was adopted to directly label peptide by $^{19}\text{F}/^{18}\text{F}$ isotopic exchange using the precursor (**24**) derivatized with a Tyr³-octreotate (Scheme 1.25).¹¹⁴⁻¹¹⁵



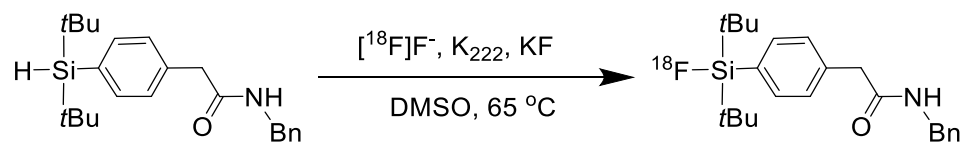
Scheme 1.25. Fluorine-18 labelling of Tyr³-octreotate by isotopic $^{19}\text{F}/^{18}\text{F}$ exchange using a fluoroorganosilane.

Although the isotopic exchange method of the above synthesis afforded relatively high radiochemical yields (95 - 97% at room temperature and 70 - 90% at 95°C), the large amount of unlabelled precursor required can compromise the specific activity. This is because the non-radioactive ^{19}F -compound also binds to the biological target site but does not get detected by the PET detector, resulting in low quality PET images and is not suitable for imaging application. The elevated temperature and aprotic solvent required also restrict the applicability to label other heat-sensitive molecules. To overcome these limitations, a two-step procedure was introduced where the Si- ^{18}F bond was formed using the aldehyde (**26**) before purification and conjugation to peptides (Scheme 1.26).¹¹⁶ Such method allows the use of an aqueous solvent at the later stage, which is more compatible with peptides.



*Scheme 1.26. Synthesis of $[^{18}\text{F}]\text{-SiFA-p-CHO}$ (**27**) for the conjugation of peptides.*

A series of silicon building blocks was also designed, which incorporates different leaving groups to achieve higher specific activity using the leaving group approach compared to the isotopic exchange approach (Scheme 1.27).



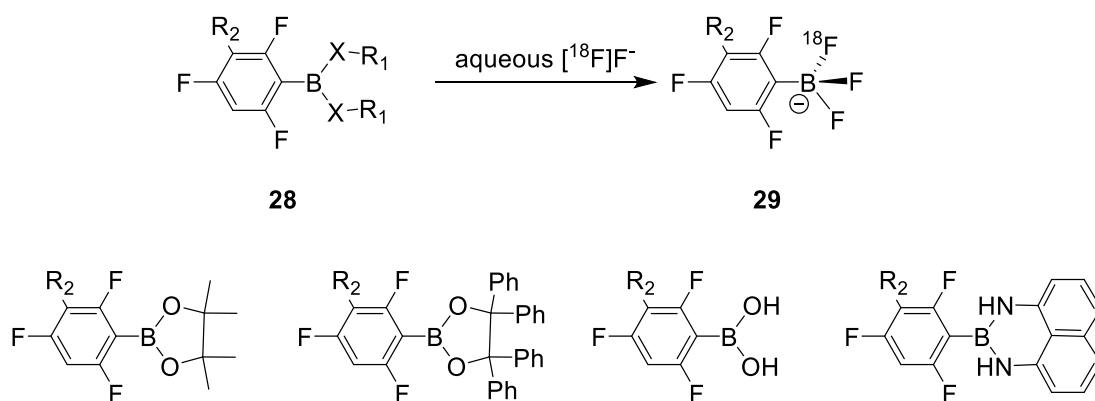
Scheme 1.27. The leaving group approach to the formation of Si-¹⁸F bond.

One of the drawbacks of the SiFA moiety is the high lipophilicity of the overall compound observed when a somatostatin analogue bearing a SiFA motif labelled with fluorine-18 presents non-specific binding and hepatobiliary excretion. This requires the inclusion of lipophilicity-reducing auxiliaries such as a PEG functional group.¹¹⁷

The isotopic exchange approach to fluorine-18 radiolabelling has long been perceived to produce low to moderate specific activity, especially for compounds based on the formation of C-¹⁸F bonds. The formation of Si-¹⁸F bonds using this method has produced labelled compounds with a specific activity of 0.8-1.7 Ci μmol⁻¹ using a small amount of precursors (< 25 nmol), which is sufficient for imaging.¹¹⁷⁻¹¹⁸ The fluorination of silicon-based compounds generally proceeds in an aprotic solvent with anhydrous [¹⁸F]F⁻, although in one instance the reaction was achieved in aqueous media at 95 °C which might be a useful labelling method.¹¹⁴ Much effort has been put into the structural modification of the SiFA motif as its high lipophilicity promotes high uptake in liver due to hepatobiliary metabolism and reduced uptake into target sites. Owing to this requirement, the imaging application of SiFA is limited as these modifications would be more applicable to large molecules. Small molecules such as ligands for target receptor are less likely to tolerate such extensive changes.

1.6.2 The formation of the Boron-¹⁸F bond

The extremely strong bond between boron and fluoride facilitates stable boron trifluorides, and B-F bonds can be formed in aqueous media. A biotinylate boronic ester can be converted into a fluorine-18 labelled trifluoroborate molecule using [¹⁸F]F⁻ in water.¹¹⁹ A subsequent study of the hydrolytic stability of the aryltrifluoroborate compounds showed that the introduction of electron-withdrawing substituents on the aryl ring decreased the rate of hydrolysis by stabilising the trifluoroborate moiety in aqueous solution, thus reducing the possibility of fluoride ion release (Scheme 1.28).¹²⁰⁻¹²¹

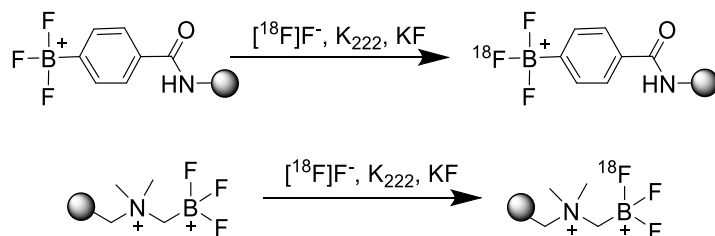


Scheme 1.28. Arylboronate precursors that can be labelled with fluorine-18 to form the fluorine-18 labelled aryltrifluoroborates (29).

A fluorine-18 labelled biotinylated aryltrifluoroborate prepared in aqueous solution (pH 4.5) was the first fluorine-18 labelled trifluoroborate studied *in vivo*.¹²²⁻¹²³ The purified fluorine-18 labelled molecule accumulated and cleared *via* the bladder with no detected hydrolysis and decomposition.

An RGD-trifluoroborate conjugate was labelled with fluorine-18 using a kit-like isotopic exchange ¹⁹F-¹⁸F approach, yielding the product with a specific activity of 14 Ci μmol⁻¹ (Scheme 1.29).¹²⁴ This proves the feasibility of fluoroborates in the design of new fluorine-18 PET imaging agents. A wide variety of biologically active molecules was

attached to this trifluoroborate moiety as fluoride captors undergo ^{19}F - ^{18}F isotopic exchange reaction to produce the fluorine-18 labelled compounds.¹²⁵



Scheme 1.29. Isotopic exchange reaction with trifluoroborates. Black circle indicates biomolecules.

The success of ^{19}F - ^{18}F isotopic exchange reaction with the advantage of simplified labelling steps and no necessity for purification may have broken the paradigm with the radiochemical yield and specific activity higher than predicted. Despite the efficiency of this methodology, the acidification using an acidic buffer at pH 2 to form the three B-F bonds may not be amenable for a broad range of biomolecules. The relatively high lipophilicity of the Si- ^{18}F and B- ^{18}F building blocks that accelerates the rate of uptake in kidney and hepatobiliary clearance limits their use for PET imaging. An alternative approach to Si- ^{18}F and B- ^{18}F based radiotracers is using other group 13 (Al^{3+} and Ga^{3+}) metal complexes to develop Al- ^{18}F and Ga- ^{18}F radiotracers. The properties of Al^{3+} and Ga^{3+} as well as their aqueous chemistry is described below.

1.6.3 Al^{3+} and Ga^{3+} Coordination Chemistry

Aluminium is the most abundant metal in the earth's crust (8.1%), and the third most abundant element after oxygen and silicon. Contrarily, gallium is less abundant and only makes up about 0.0019% of the earth's crust. Due to their low redox potentials, aluminium and gallium exist in oxidation states of +1 and +2 but are most stable and exist predominantly as trivalent cations in aqueous solution under physiological conditions.¹²⁶

Al^{3+} has an ionic radius of 50 pm and is highly electropositive. The similar ionic radii of Ga^{3+} (62 pm) and Fe^{3+} (65 pm) leads to similarities in their coordination chemistry, specifically how Ga^{3+} mirrors Fe^{3+} in *in vivo* settings.¹²⁷ For example, Ga^{3+} binds to the iron-transport protein, transferrin, with high affinity.

Both Al^{3+} and Ga^{3+} are hard Lewis acids, that readily form complexes with ligands with hard donor atoms (F, N and O atoms) and often form six-coordinate complexes.¹²⁸ An important characteristic of the aqueous chemistry of aluminium and gallium is the formation of the hydrated species ($[\text{M}(\text{H}_2\text{O})_6]^{3+}$) and the hydroxides ($[\text{M}(\text{OH})_3]$ and $[\text{M}(\text{OH}_4)]^-$) where $\text{M} = \text{Al}$ and Ga .¹²⁹⁻¹³⁰ The insoluble hydroxides $[\text{Al}(\text{OH})_3]$ and $[\text{Ga}(\text{OH})_3]$, dominate from pH 3–7 but in pH higher than 7–9 the soluble species $[\text{Al}(\text{OH}_4)]^-$ and $[\text{Ga}(\text{OH}_4)]^-$ predominate. To prevent the precipitation of the hydroxide species in aqueous media, weakly coordinating anions such as citrate and acetate are often used as stabilising ligands in the buffer solutions. Metal complexation can be achieved between pH 3 - 7 in these mildly acidic buffers.

An understanding of the coordination chemistry of group 13 metal fluoride complexes is necessary to assess its potential for use with fluorine-18 towards PET imaging applications. The group 13 metal fluorides (MF_3 , $\text{M} = \text{Al}, \text{Ga}$) are inert polymeric solids and often crystallised as the hydrates. Aluminium trifluoride exists mainly in two forms, the α -form, $[\text{AlF}_3(\text{OH}_2)_3]$ and the β -form $[\text{AlF}_2(\text{OH}_2)_2(\mu\text{-F})_n] \cdot n\text{H}_2\text{O}$.¹³¹ Gallium trifluoride in its trihydrate form, $\text{GaF}_3 \cdot 3\text{H}_2\text{O}$ is highly volatile and hygroscopic. Pure GaF_3 can be synthesised by reacting HF with metallic gallium at 550 °C. The crystals of GaF_3 have a densely packed polymorphous octahedral structure. The trifluoride hydrates are generally insoluble in aqueous and organic solvents, which limits their use as

precursors for the formation of metal fluoride complexes as the reactions usually require extremely high temperatures.¹³²

Metal trifluoride complexes can be synthesised by hydrothermal reaction with $\text{MF}_3 \cdot x\text{H}_2\text{O}$ as a precursor at 180 °C for ~15 h or a Cl/F halide exchange reaction with pre-formed metal trichloride analogues which can be achieved at a lower temperature using MCl_3 . The incorporation of fluoride can be achieved using tetrabutylammonium fluoride (TBAF or NBu_4F) or tetramethylammonium fluoride (NMe_4F) in acetonitrile or KF in aqueous acetonitrile. The metal trifluoride (MF_3 , $\text{M} = \text{Al, Ga}$) complexes containing a variety of nitrogen donor ligands (2,2',6'2''-terpyridyl (terpy), 1,10-phenanthroline (phen) and 2,2'-bipyridyl (bipy)) can be synthesised using both methods.¹³² Interestingly, the Al^{3+} complex failed to form while using NBu_4F or NMe_4F in acetonitrile, which was potentially due to the smaller ionic radius of Al^{3+} compared to Ga^{3+} , suggesting that the Al^{3+} complex does not undergo an associative mechanism under these conditions. The halide exchange reaction was achieved using a solvent-assisted substitution mechanism using aqueous acetonitrile.¹³³ The metal trifluoride hydrates have distorted octahedral geometries at the metal with *mer*-trifluoride coordination (Figure 1.6). These trifluoride complexes have relatively high stability in aqueous solutions with the Cl/F exchange reaction proceeding at a higher rate than the competing hydrolysis reactions.

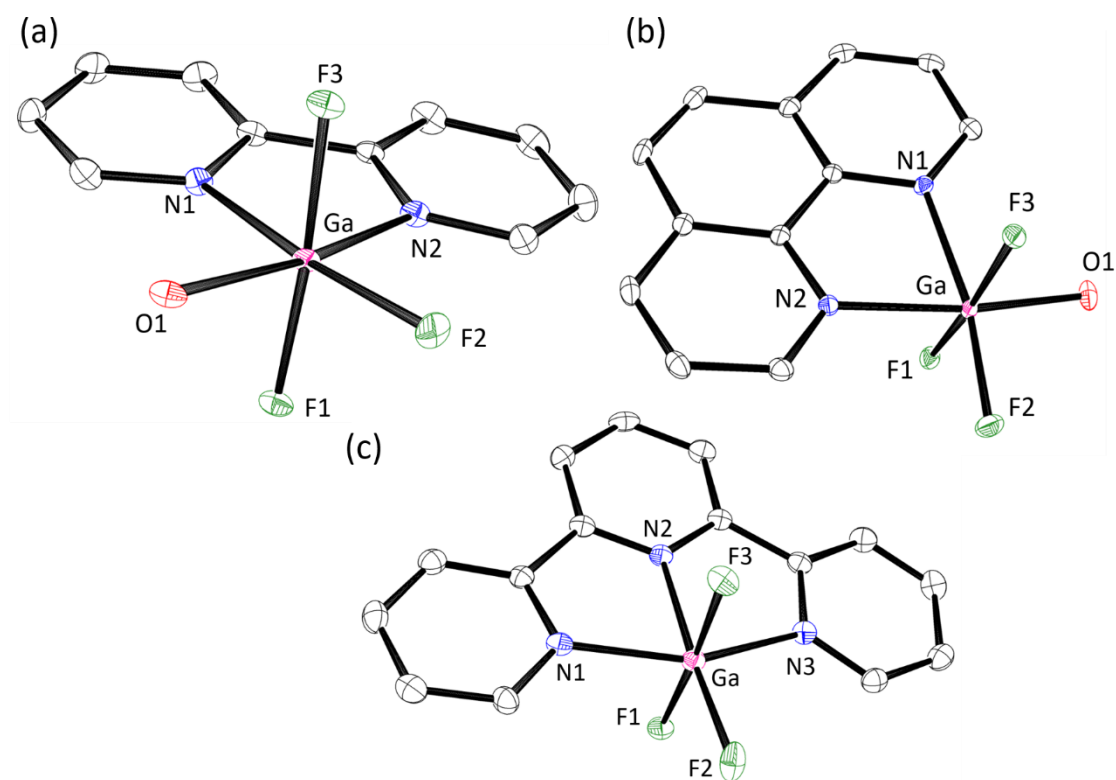


Figure 1.6. ORTEP representations of the X-ray crystal structures (30% ellipsoids) of $[Ga(bipy)(OH_2)F_3]$ (a), $[Ga(phen)(OH_2)F_3]$ (b) and $[Ga(terpy)F_3]$ (c).¹³²

The coordination chemistry of other multidentate ligands with high affinities towards Al^{3+} and Ga^{3+} has been investigated. Penta- and hexadentate ligands with nitrogen and oxygen donor atoms are commonly used to react with $AlCl_3$ and $GaCl_3$ to form six-coordinate Al^{3+} and Ga^{3+} complexes followed by Cl/F exchange reaction yielding the metal fluoride complexes. The Al^{3+} and Ga^{3+} monofluoride complexes have distorted octahedral geometries at the metal centre with bond distances of (a) Al-F 1.709(14) Å; and (b) Ga-F 1.821(2) Å (Figure 1.7).¹³⁴⁻¹³⁵ In some instances, the monochloride complex is prone to hydrolysis, leading to the formation of the hydroxide complex. Nonetheless, both monochloride and hydroxide complexes are suitable precursors that react with sodium fluoride or potassium fluoride to form the monofluoride complexes.

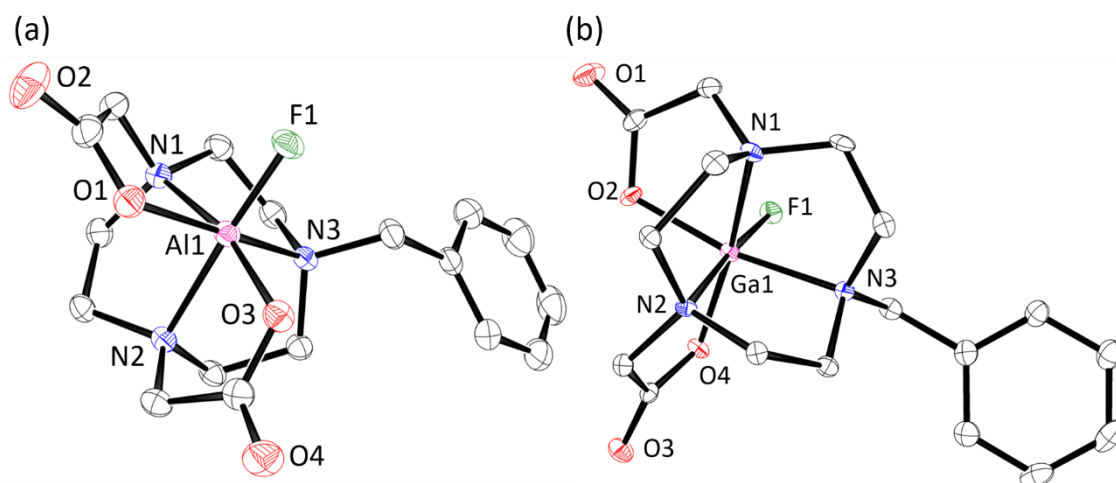
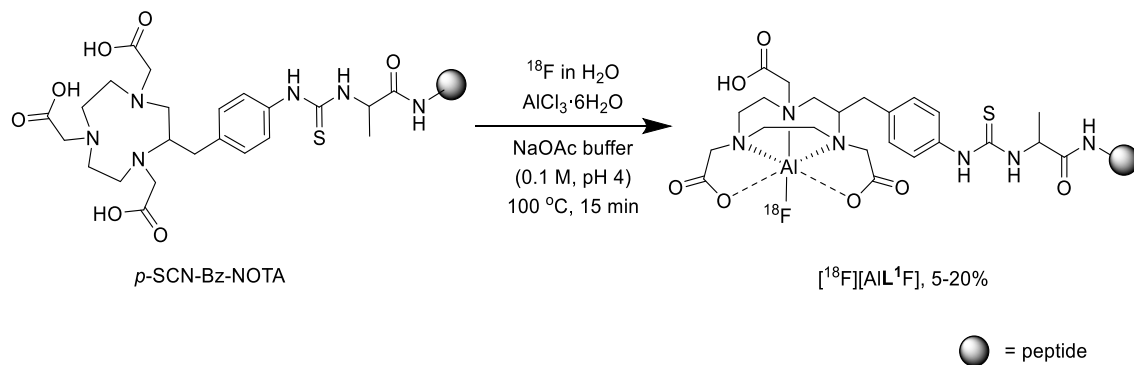


Figure 1.7. ORTEP representations of the X-ray crystal structures (30% ellipsoids) of [Al(MeBz-NODA)F] (a) and [Ga(MeBz-NODA)F] (b) complexes with distorted octahedral geometries.¹³⁴⁻¹³⁵

1.6.4 The formation of the Aluminium-¹⁸F and Gallium-¹⁸F bonds

The use of Al³⁺ and Ga³⁺ complexes to incorporate fluorine-18 has led to the development of new strategies for the synthesis of PET diagnostic imaging agents. The first fluorine-18 labelled peptide using Al³⁺ complex was reported in 2009.¹³⁶ Reaction of [¹⁸F]F⁻ with AlCl₃·6H₂O resulted in [¹⁸F]{AlF}²⁺, which then formed metal complex with the macrocyclic ligand, 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) that was conjugated to a peptide *via* a benzyl-isothiocyanate linker at the carbon backbone (Scheme 1.30). The overall yield of the radiolabelled peptide was 5-20% with an estimated specific activity of 500 - 1300 Ci mmol⁻¹. This approach created much interest due to its potential to revolutionise fluorine-18 labelling as it does not require [2.2.2]Cryptand and azeotropic drying of water, allowing fluorine-18 to be incorporated in the final step with a shorter reaction time and a single purification step at the end.



Scheme 1.30. The method for the formation of $[^{18}\text{F}][\text{AlL}^1\text{F}]$ complex. Black circle indicates peptide.

The strength of the chelated $\text{Al-}^{18}\text{F}$ bond is reflected in the lack of uptake in bone in nude mice bearing LS174T human colonic tumours. The uptake in bone at 1.5 h post-injection of $[^{18}\text{F}][\text{AlL}^1\text{F}]$ ($0.13 \pm 0.14\%$ ID/g) was low compared to the non-chelated $[^{18}\text{F}]\text{F}^-$ ($19.88 \pm 2.12\%$ ID/g) and $[^{18}\text{F}]\{\text{AlF}\}^{2+}$ species ($19.03 \pm 2.7\%$ ID/g) that accumulated in bone, showing minimal dissociation of the $\text{Al-}^{18}\text{F}$ bond from the chelator.

A range of NOTA derivatives were prepared to study the influence of ligand structures on the stability, specific activity and radiochemical yield of the $[^{18}\text{F}][\text{AlL}^1\text{F}]$ complex.¹³⁷ These chelators are known to form thermodynamically stable and kinetically inert metal complexes. The chelators NOT2A, 1,4,7-triazacyclononane,1-gluteric acid-4,7-acetic acid (NODA-GA) and {4-[2-(bis-carboxy-methylamino)-5-(4-nitrophenyl)pentyl]-7-carboxymethyl-[1,4,7]triazanonan-1-yl} acetic acid (C-NETA) conjugated to a peptide were chelated to $[^{18}\text{F}]\{\text{AlF}\}^{2+}$ under similar reaction conditions as $[^{18}\text{F}][\text{AlL}^1\text{F}]$, shown in the general methodology below (Figure 1.8). The reaction involves the addition of a mixture of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ and aqueous $[^{18}\text{F}]\text{F}^-$ to a conjugate with a biologically active molecule at pH 4 - 5 using acetate buffer at 100 °C. The chemical structures and the radiochemical yields labelled with peptide (500 nmol) are listed in Table 1.5.

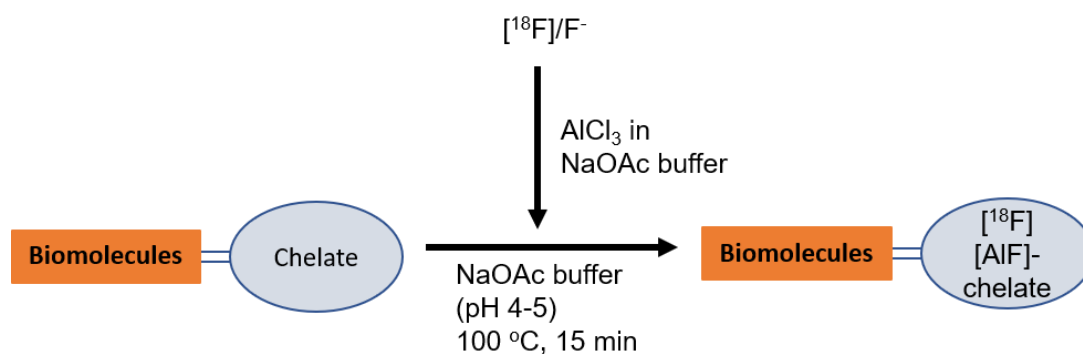
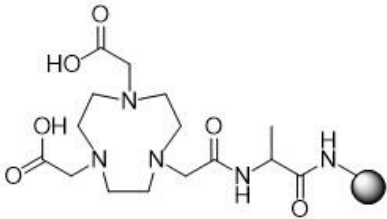
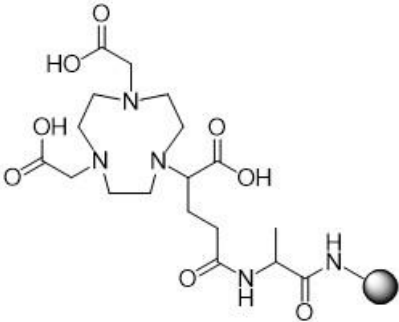
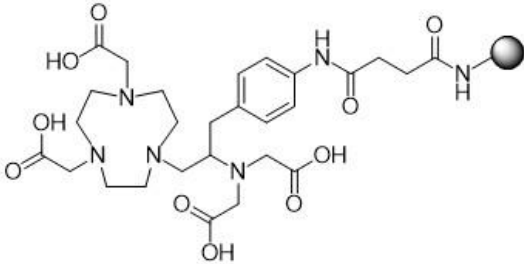


Figure 1.8. The general methodology for the synthesis of fluorine-18 PET imaging agents.

Table 1.5. Chemical structures of peptide-bearing NOT2A, NODA-GA and C-NETA and the radiochemical yields after labelling with $[^{18}\text{F}]\{\text{AlF}\}^{2+}$. Black circle indicates peptide.

Chelate-peptide	Chemical structure	Radiochemical yield (%)
NOT2A-peptide		6
NODA-GA-peptide		31
C-NETA-peptide		87

The orientation of the third carboxylate group on the chelators may play a role in the coordinate bond formation with fluoride as the carboxylate group has a high affinity for Al^{3+} due to the partially covalent nature of the Al-O bond, which can restrict the complexation of the pre-formed $[\text{}^{18}\text{F}]\{\text{AlF}\}^{2+}$ to the chelator if it is in the vicinity to interact with Al^{3+} . The poor radiochemical yield for $[\text{}^{18}\text{F}]\text{AlF-NODA-GA}$ (6%) can be attributed to the close proximity of three carboxymethyl functional groups to the metal binding site. The 3rd and 4th carboxylate functional groups on C-NETA are further from the metal binding site, which lead to minimal interaction between Al^{3+} and O^{2-} . Notably, all of the $[\text{}^{18}\text{F}]\text{AlF-NOTA}$ complexes were stable in human serum and also *in vivo*, but formed diastereomeric products which can be unfavourable due to the possibility of side effects.

In the last ten years, fluorine-18 labelling using Al^{3+} complexes has been explored extensively, mainly focusing on the use of 1,4,7-triazacyclononane-1,4-diacetic acid (NODA) and NOTA analogues (Figure 1.9).^{134, 138} The versatility of this fluorine-18 labelling method has been tested on different biological targets such as RGD, a peptide for integrin receptor,¹³⁹ $[\text{}^{18}\text{F}]\text{-AlF-PSMA-11}$ (PSMA = prostate-specific membrane antigen) for prostate cancer imaging,¹⁴⁰⁻¹⁴² folate receptor¹⁴³⁻¹⁴⁵ and octreotide for hypoxia¹⁴⁶.

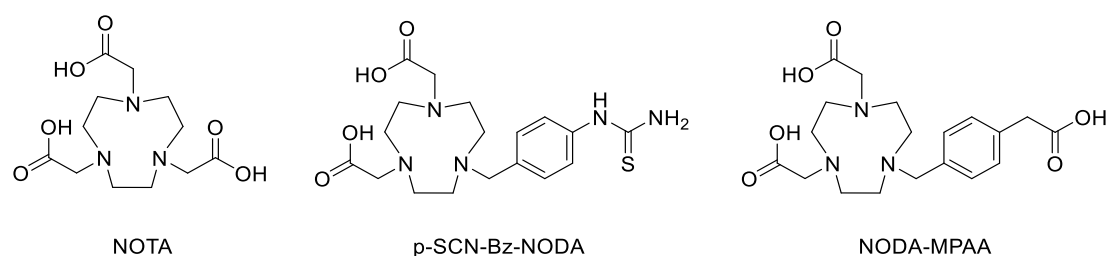


Figure 1.9. A variety of chelators used to label small molecules and peptides with fluorine-18 via the formation of $\text{Al}^{3+}\text{-}^{18}\text{F}^-$ bond.

A lyophilised kit formulation for fluorine-18 labelling was later developed.¹³⁸ The prototype kit is made up of a NODA-MPAA (methyl phenylacetic acid)-peptide chelator (2 mM in H₂O), potassium biphthalate (0.06 M, pH 4.0) as buffer, AlCl₃·6H₂O (2 mM in buffer solution), α,α -trehalose dihydrate (5%) as a bulking agent and ascorbic acid (5 mg/mL) as radioprotectant. The simple, one-step labelling procedure with [¹⁸F]F⁻ in saline followed by purification by Alumina-N or a reversed phase solid phase extraction cartridge demonstrated its potential to be translated to clinical settings.

Accordingly, careful control of the pH is crucial as high pH leads to the formation of insoluble hydroxides. In contrast, low pH promotes competitive protonation of the [¹⁸F]F⁻ at the same time impedes the deprotonation of oxygen atoms, preventing the coordination of the [¹⁸F]{AlF}²⁺ to the chelator. The high temperature, typically above 100 °C required to drive the formation of the [¹⁸F]Al³⁺-F⁻ complexes remains a limitation of the Al³⁺-F⁻ framework as this methodology has only been applied for peptides that are stable at high temperature. Recent work demonstrated a two-step method of first incorporating [¹⁸F]{AlF}²⁺ onto the ligand at 100 °C, followed by conjugation to a biomolecule at room temperature, which prolongs the preparation time and compromises the labelling efficiency.¹⁴⁷ New chelators were developed by Cleeren and co-workers that can form [¹⁸F][AlF] complexes at 20-40 °C, allowing the fluorine-18 radiolabelling of heat-sensitive biomolecules in a two-step protocol. The acyclic polydentate chelators, Glu-NH-CO-NH-Lys(Ahx)L3 and (±)-H₃RESCA, have less rigid frameworks compared to macrocyclic NOTA ligands and thus required lower activation energies for the coordination of [¹⁸F]{AlF}²⁺. A chelate conjugated to the urea-based PSMA inhibitor, Glu-NH-CO-NH-Lys(Ahx)L3, was radiolabelled with [¹⁸F]Al³⁺-F⁻ at pH 4.5 using acetate buffer at 40 °C with a radiochemical yield of 25% and molar activity of 27 GBq/μmol (Figure 1.10).¹⁴⁸ A new restraining complexing agent, (±)-H₃RESCA with

either a tetrafluorophenyl ester or maleimide was conjugated with three different proteins via the lysine residues: human serum albumin (HSA), anti-Kupffer cells nanobody (NbV4m119) and anti HER2 affibody ($Z_{\text{HER2:2891}}$) (Figure 1.10). These constructs were radiolabelled with $[^{18}\text{F}]\text{Al}^{3+}\text{-F}^-$ at pH 4.5 using acetate buffer at room temperature to produce $(\pm)\text{-}[^{18}\text{F}]\text{AlF(RESCA)-HSA}$, $(\pm)\text{-}[^{18}\text{F}]\text{AlF(RESCA)-NbV4m119}$ and $(\pm)\text{-}[^{18}\text{F}]\text{AlF(RESCA)-}Z_{\text{HER2:2891}}$ with radiochemical yields of 52-63%, 35-53% and $20\%\pm 7\%$ respectively.¹⁴⁹

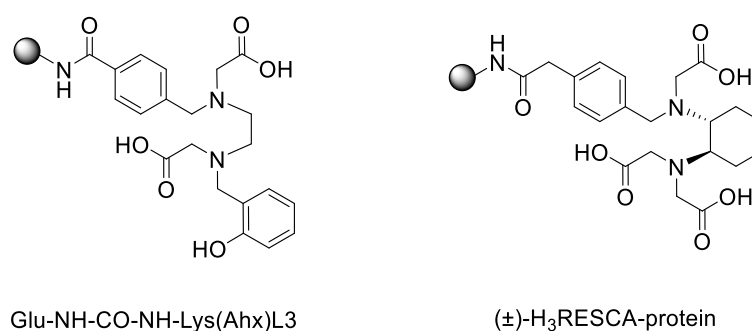
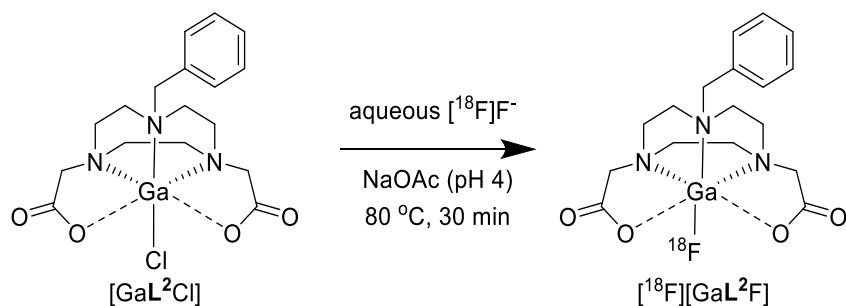


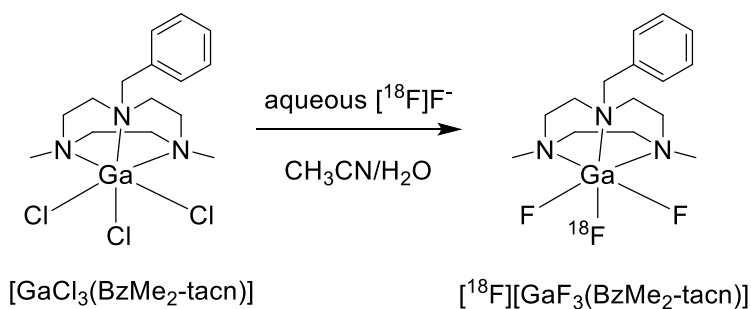
Figure 1.10. Chemical structures of Glu-NH-CO-NH-Lys(Ahx)L3 and $(\pm)\text{-H}_3\text{RESCA}$ -protein used to label heat-sensitive biomolecules with fluorine-18 via the formation of $\text{Al}^{3+}\text{-}[^{18}\text{F}]\text{F}^-$ bond at 20-40 °C.

An alternative approach of using Ga^{3+} complex has been suggested. The ease of the incorporation of fluorine-18 into Ga^{3+} complexes in mildly acidic aqueous solution at a temperature below 100 °C provides an advantage over the formation of $\text{Al}^{3+}\text{-F}^-$ bond. A chelating strategy was developed using a pre-formed Ga^{3+} complex via halide $\text{Cl}/^{18}\text{F}$ exchange reaction. The addition of carrier-free aqueous $[^{18}\text{F}]\text{F}^-$ to a gallium chloride complex $[\text{GaL}^2\text{Cl}]$ on a 210 nmol scale led to the incorporation of $[^{18}\text{F}]\text{F}^-$ with a radiochemical yield of 60 - 70% (Scheme 1.31).¹³⁵



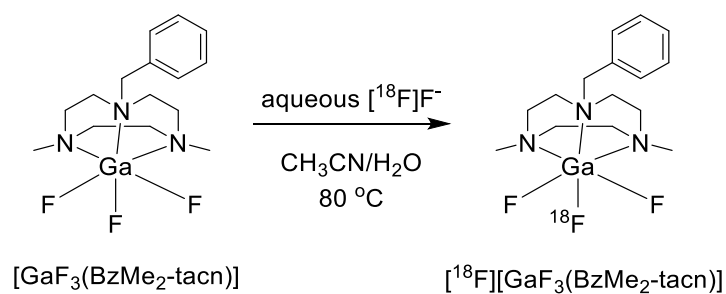
Scheme 1.31. Fluorine-18 labelling of a pre-formed gallium chloride complex to form $[^{18}\text{F}][\text{GaL}^2\text{F}]$.

The fluorine-18 labelling of $[\text{GaCl}_3(\text{BzMe}_2\text{-tacn})]$ (tacn = triazacyclononane) can be achieved in aqueous acetonitrile at room temperature at pH 4 by halogen $\text{Cl}/^{18}\text{F}$ exchange reaction to yield $[^{18}\text{F}][\text{GaF}_3(\text{BzMe}_2\text{-tacn})]$ (Scheme 1.32).¹⁵⁰ The fluorine-18 labelled product showed high radiochemical stability in phosphate-buffered saline solution at pH 7.4.



Scheme 1.32. Halide $\text{Cl}/^{18}\text{F}$ exchange reaction for the incorporation of fluorine-18 into $[\text{GaCl}_3(\text{BzMe}_2\text{-tacn})]$.

An isotopic $^{19}\text{F}/^{18}\text{F}$ exchange reaction using $[\text{GaF}_3(\text{BzMe}_2\text{-tacn})]$, which is more resistant to hydrolysis than $[\text{GaCl}_3(\text{BzMe}_2\text{-tacn})]$, was achieved, leading to high incorporation of $[^{18}\text{F}]\text{F}^-$ (radiochemical yield between 65 - 73%) (Scheme 1.33).¹⁵¹



Scheme 1.33. Isotopic $^{19}\text{F}/^{18}\text{F}$ exchange reaction for the incorporation of fluorine-18 into $[\text{GaF}_3(\text{BzMe}_2\text{-tacn})]$.

The incorporation of fluorine-18 into Ga^{3+} complexes proved to be achievable at room temperature or 80 °C. This is in contrast to the temperature higher than 100 °C required for the formation of $[\text{}^{18}\text{F}]\text{Al}^{3+}\text{-F}^-$ complexes. Preliminary studies highlighted the potential advantages of $[\text{}^{18}\text{F}]\text{Ga}^{3+}\text{-F}^-$ complexes over $[\text{}^{18}\text{F}]\text{Al}^{3+}\text{-F}^-$ complexes, such as the ability to incorporate heat-sensitive biomolecules onto Ga^{3+} complexes before performing fluorine-18 labelling. This approach can avoid the two-step synthesis in which $[\text{}^{18}\text{F}]\{\text{AlF}\}^{2+}$ is prepared before conjugating to biomolecule as the introduction of fluorine-18 in the early stages of synthesis can compromise the specific activity. Research into the versatility of this approach is limited, namely its use on a wider variety of ligand systems containing biologically active molecules as well as the *in vivo* stability and its use for suitable nuclear imaging applications.

1.7 Aim

Fluorine-18 remains the most widely used positron-emitting radionuclide in PET with [^{18}F]FDG being the most commonly available imaging agent but not without its limitations. In the search for better fluorine-18 PET radiotracers, an evaluation on the Ga- ^{18}F bond may have some advantages over the C- ^{18}F and Al- ^{18}F system. Ideally, the Ga $^{3+}$ -F $^{-}$ bond is thermodynamically stable and kinetically inert for imaging applications.

The work presented herein focuses on the strategy to prepare chelators for the formation of neutral Ga $^{3+}$ -F $^{-}$ complexes. The Ga $^{3+}$ complexes are designed to have a free site for the incorporation of a fluoride ion. The overall goal is to expand the knowledge of the non-radioactive chemistry and allowing further development into radiopharmaceuticals based on the non-carbon element with fluorine-18.

The picolinic acid family of ligands with different denticities have found several uses in the radiopharmaceutical applications with various radioisotopes. The work presented in Chapter 2 will investigate the acyclic ligand based on the picolinic acid scaffold, consisting of two picolinic acid groups connected through a methylene bridge to a central nitrogen atom, forming a pentadentate ligand system with an N $_3$ O $_2$ binding cavity to coordinate to Ga $^{3+}$ -F $^{-}$ as the Ga ^{18}F -chelate moiety. The tertiary nitrogen atom provides a platform for modification on the backbone of the chelator to incorporate biologically relevant molecules. Much of the work in this chapter involves the preparation, characterisation and evaluation of the properties displayed by the ligands and complexes.

Encouraged by early reports, the research in Chapter 3 sets about to investigate the versatility of a single chelate system that is capable of labelling a variety of radionuclides using the azamacrocyclic ligand as the chelator, incorporating a targeting vector for cerebral A β plaques to assist in the diagnosis of Alzheimer's disease as a proof-of-concept

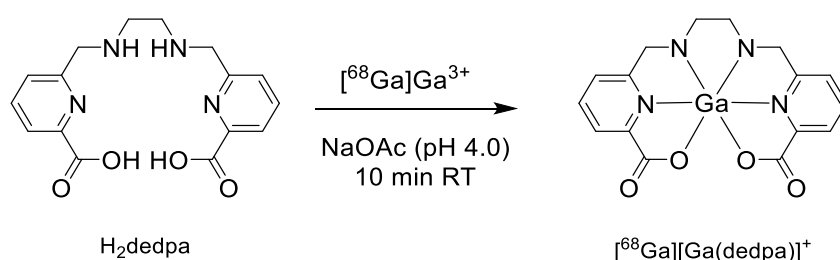
application. In addition to the synthesis, characterisations and binding properties of the compounds to extracellular A β plaques in human tissue, methodologies for the radiolabelling of the [^{64}Cu]Cu, [^{18}F]F $^-$ -Ga $^{3+}$ and [^{68}Ga]Ga complexes along with the biodistribution of the radiolabelled compounds in mice will be explored.

Chapter 4 aims to explore the N $_4$ donor system with high affinity for Ga $^{3+}$. Of interest is the Ga $^{3+}$ -F $^-$ complex that has the potential to cross the blood-brain barrier. The focus will be on preparing Ga $^{3+}$ -F $^-$ complex with tetraaza[14]annulene as chelator and assessing the suitability to be translated into fluorine-18 chemistry.

Chapter 2 Acyclic Chelators for Gallium-Fluoride Complexes

2.1 Acyclic picolinate-containing ligands and their applications

The application of the family of acyclic chelator ‘dedpa’ based on the picolinic acid scaffold in nuclear medicine in particular with gallium-68 was pioneered by Orvig and co-workers.¹⁵²⁻¹⁵⁶ The ‘dedpa’ family is made up of two picolinic acids joined by a backbone and consists of amine, pyridine and carboxylic acid functionalities. The H₂dedpa chelator coordinates to [⁶⁸Ga]Ga³⁺ at room temperature within minutes to form stable [⁶⁸Ga][Ga(dedpa)]⁺ (Scheme 2.1).



Scheme 2.1. Formation of [⁶⁸Ga][Ga(dedpa)]⁺.

The use of the ‘dedpa’ system is attractive as the room temperature labelling conditions are compatible with heat-sensitive biologically active molecules. Since the first report of [⁶⁸Ga][Ga(dedpa)]⁺ in 2010, the functionalisation at the secondary amines on the backbone of ‘dedpa’ led to the development of a series of its derivatized forms. The modification can alter the chemical properties of the [⁶⁸Ga]Ga³⁺ complex and thus must be taken into consideration to ensure high *in vivo* stability. For example, the high lipophilicity and cationic characteristic of [⁶⁸Ga][Ga(dedpa)]⁺ rendered it a poor myocardial perfusion imaging agent, but the derivative with an RGD conjugate has a considerable tumour uptake.¹⁵³⁻¹⁵⁴

In 2008, the ligands H₂dedpa and H₂CHXdedpa, originally named H₂bcpe and H₂bcpc respectively, were proposed to form stable Zn²⁺, Cd²⁺ and Pb²⁺ complexes.¹⁵⁷ The derivatives of the ‘dedpa’ with higher denticities ranging from 6 to 10 have also been developed to coordinate to metal ions of larger size such as In³⁺ and Y³⁺. These derivatives are H₄octapa, H₄CHXoctapa, H₅decapa and H₄tpaen (Figure 2.1).^{155, 158-159}

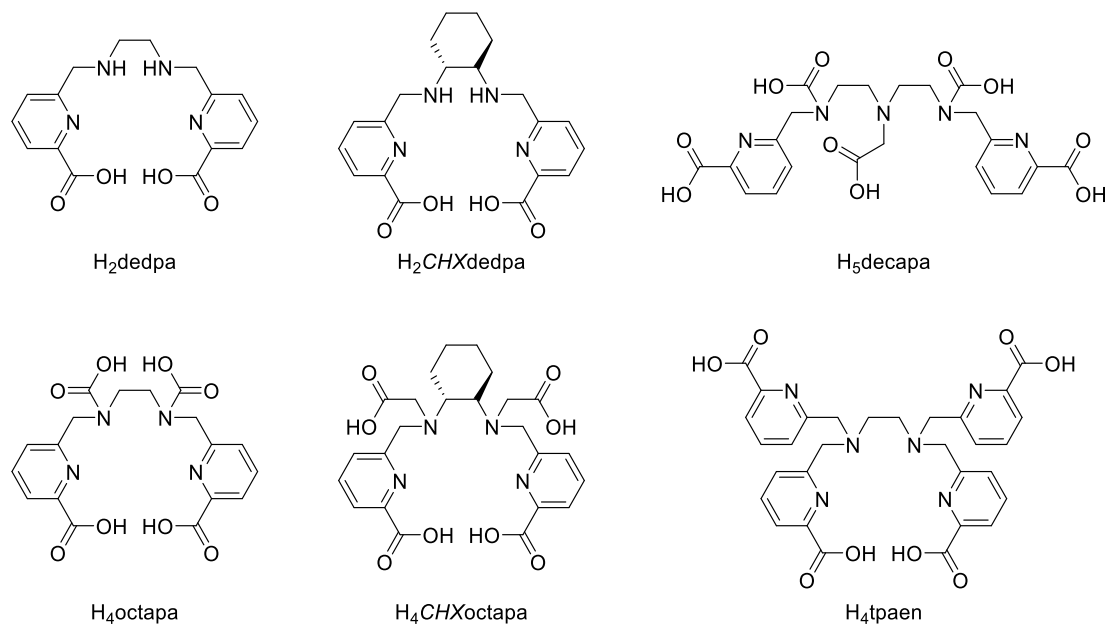


Figure 2.1. Acyclic ligands that form stable Ga³⁺ complexes.¹⁵²⁻¹⁵⁶

The ‘dpa’ variant is formed by connecting two picolinic acids at the 6’ position via methylene bridges to a nitrogen atom. The ‘dpa’ ligand with an N₃O₂ donor set has a lower denticity than the ‘dedpa’ ligands. Derivatives of ‘dpa’ such as H₂dpaea and H₂dpaMea chelators can coordinate to Mn²⁺ and Gd³⁺ and are potential MRI contrast agents shown to have substantial high-field relaxivities (Figure 2.2).¹⁶⁰⁻¹⁶¹

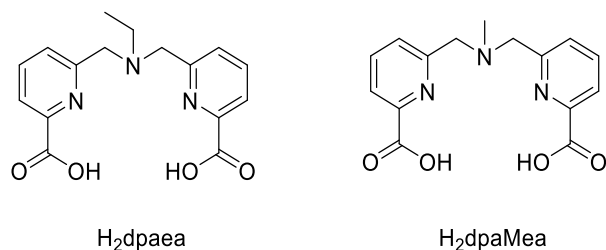


Figure 2.2. Chemical structures of H_2dpaea and H_2dpaMea that form stable complexes with Mn^{2+} and Gd^{3+} .

A ‘dpa’ derivatised with benzyl-nitro functional group (H_2dipin) coordinates to Cu^{2+} with a square pyramidal geometry (Figure 2.3).¹⁶² The chelator coupled to biotin also forms complexes with Co^{2+} as well as the $[\text{Re}(\text{CO})_3]^+$ -core.

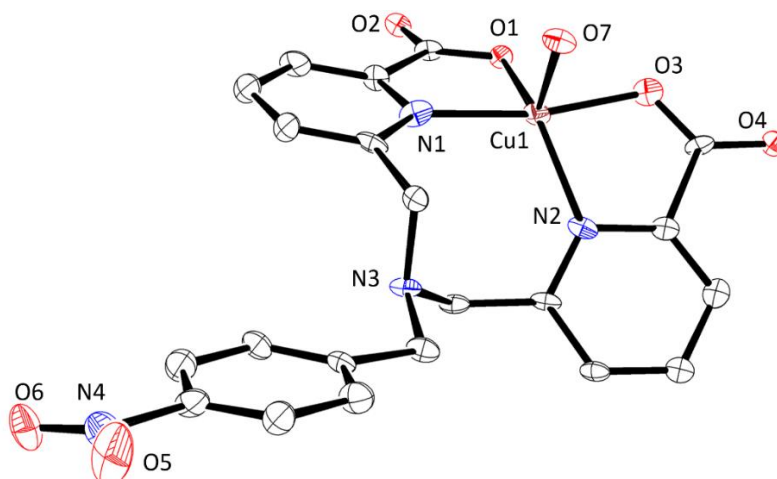


Figure 2.3. ORTEP representation of the X-ray crystal structure (30% ellipsoids) of $[\text{Cu}(\text{dipin})]$.¹⁶²

During the course of this PhD program, a carboxylate analogue of ‘dpa’, H_3dpaa , that can be radiolabelled with gallium-67 at ambient temperature at pH 6.5 was reported in September 2016.¹⁶³ The structure as revealed by X-ray crystallography showed that the $[\text{Ga}(\text{dpaa})(\text{H}_2\text{O})]$ complex has a six-coordinate structure with the Ga^{3+} in a distorted octahedral geometry, with the 6th site occupied by a water molecule (Figure 2.4). The displacement of one of the ligand donor atoms by water alleviates the strain induced by

coordinating to all six donor atoms in the ligand, but the coordinating water molecule might lead to a decrease in the *in vivo* stability as endogenous ligands can compete to coordinate to Ga^{3+} , which is reflected by the decrease in radiochemical yield (58%) after 2 hours of incubation with human serum.

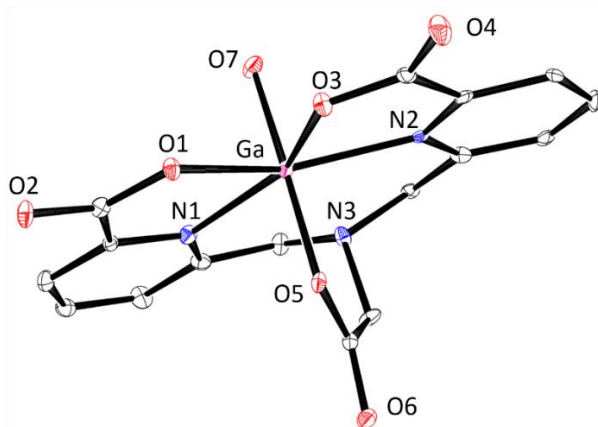
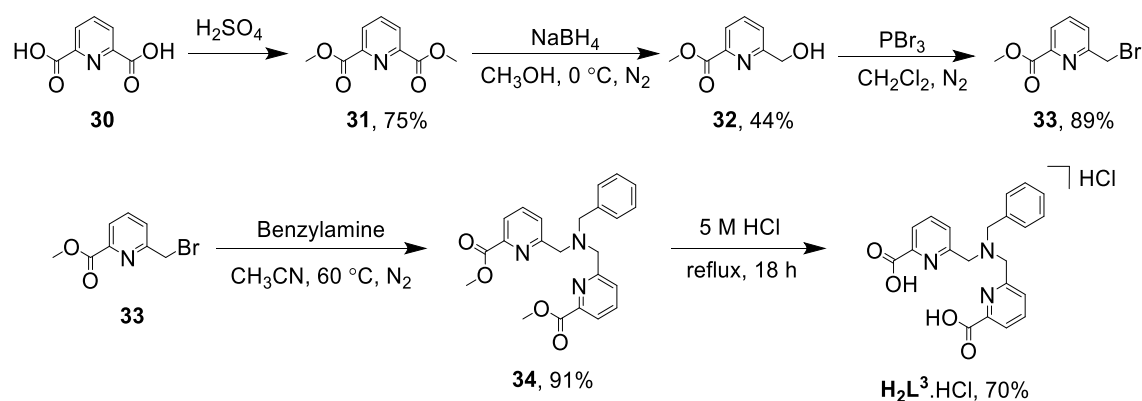


Figure 2.4. ORTEP representation of the X-ray crystal structure (30% ellipsoids) of $[\text{Ga}(\text{dpaa})(\text{H}_2\text{O})]$ showing a water molecule occupying the 6th site.¹⁶³

The stability and ease of labelling at ambient temperature of the ‘dedpa’ and ‘dpa’ chelators and their derivatives gave the inspiration in the design of the ligand framework for our purpose of fluorine-18 radiolabelling with Ga^{3+} complex. By utilising the ‘dpa’ framework with lower denticity, the chelator can coordinate to Ga^{3+} with a free site for the incorporation of a fluoride ion. It is of our interest to utilise this ligand scaffold to incorporate functional groups that allow for conjugation to target molecules and coordinate to Ga^{3+} and subsequently introduce a fluoride ion *via* the formation of $\text{Ga}^{3+}\text{-F}^-$ bond.

2.2 Synthesis of H_2L^{3-5} and their Ga^{3+} complexes, structural determination by nuclear magnetic resonance spectroscopy and characterisation by X-Ray Crystallography

A ‘dpa’ chelator with an appended methylbenzyl at the central nitrogen atom was desired. The ligand H_2L^3 was synthesised in a few steps by adapting a reported procedure.¹⁶⁴ Esterification of the carboxylic acid groups of commercially available 2,6-pyridinedicarboxylic acid (**30**) followed by a selective reduction of one methyl ester with sodium borohydride in methanol allowed isolation of methyl 6-(hydroxymethyl)picolinate (**32**) (Scheme 2.2). Treatment of (**32**) with phosphorus tribromide in anhydrous dichloromethane gave methyl 6-(bromomethyl)picolinate (**33**) in a high yield. A doubly reductive amination reaction between benzylamine and two equivalents of methyl 6-(bromomethyl)picolinate (**33**) in anhydrous acetonitrile yielded the product (**30**). Hydrolysis of the methyl ester functional group in compound (**34**) with 5 M HCl yielded H_2L^3 as the hydrochloride salt. The compounds were characterised by ^1H and $^{13}\text{C}\{^1\text{H}\}$ nuclear magnetic resonance (NMR) spectroscopy and electrospray ionisation-mass spectrometry (ESI-MS).



Scheme 2.2. Synthesis of $H_2L^3 \cdot HCl$.

Crystals suitable for single X-ray crystallography of $H_2L^3 \cdot HCl$ were grown by slow evaporation of an aqueous solution of the compound at ambient temperature (Figure 2.5).

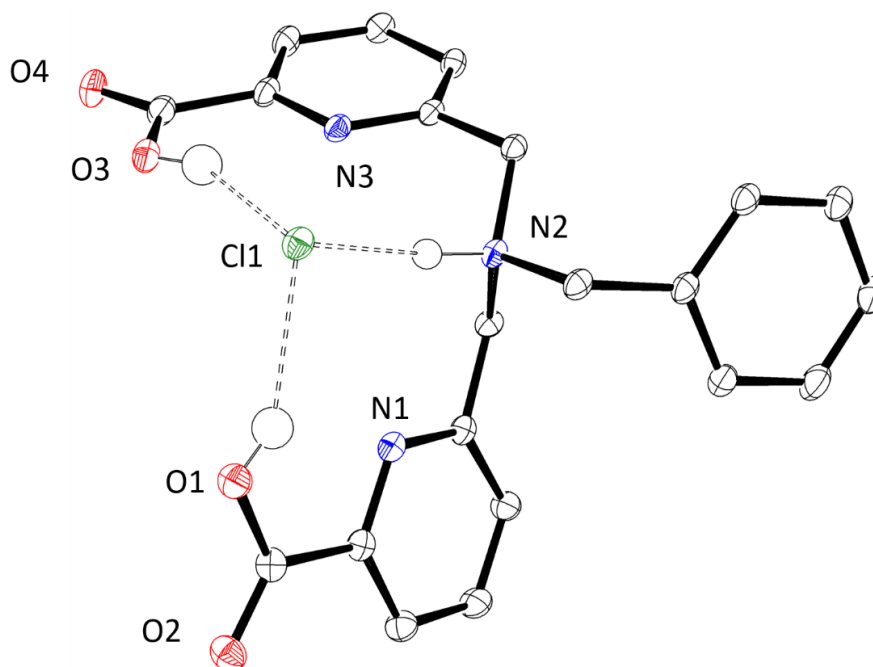


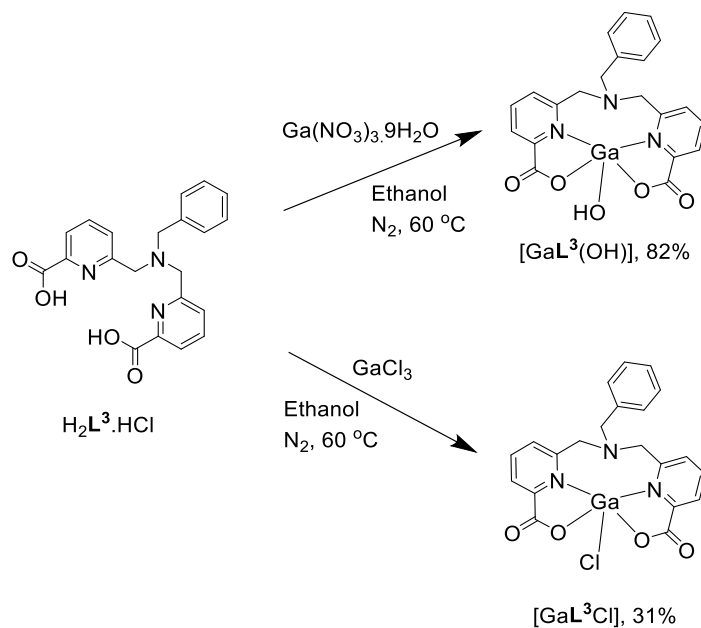
Figure 2.5. ORTEP representation of the X-ray crystal structure (30% ellipsoids) of $H_2L^3 \cdot HCl$ showing the hydrogen bonding. Hydrogen atoms attached to carbon atoms have been omitted for clarity.

The structure as revealed by X-ray crystallography crystallised with one molecule of hydrochloric acid. The ORTEP representation of $H_2L^3 \cdot HCl$ shows the hydrogen bonding between Cl^- and three hydrogen atoms attached to two oxygen atoms (O(1) and O(3)) and the central nitrogen atom (N(2)).

Table 2.1. Summary of the crystallography data of $H_2L^3 \cdot HCl$.

	$H_2L^3 \cdot HCl$
Empirical formula	$C_{21}H_{20}ClN_3O_4$
MW	413.85
Space group	P1
T/K	100(10)
Crystal size/mm ³	0.551 x 0.205 x 0.123
Crystal system	Triclinic
$\lambda/\text{\AA}$	1.54184
$a/\text{\AA}$	8.1655(6)
$b/\text{\AA}$	10.5274(4)
$c/\text{\AA}$	12.3713(6)
$\alpha/^\circ$	75.104 (4)
$\beta/^\circ$	79.386(5)
$\gamma/^\circ$	89.774(4)
$V/\text{\AA}^3$	1009.08
Z	2
$D_{\text{calc}}/\text{Mg/m}^3$	1.362
μ/mm^{-1}	1.957
$F(000)$	432
Reflections measured	11600
Independent reflections	4130 [$R_{\text{int}} = 0.0685$]
final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0599$, $wR^2 = 0.1720$
final R indices (all data)	$R_1 = 0.0639$, $wR^2 = 0.1861$
Goodness-of-fit on F^2	1.081

The reaction of $\text{H}_2\text{L}^3\cdot\text{HCl}$ with $\text{Ga}(\text{NO}_3)_3\cdot 9\text{H}_2\text{O}$ in ethanol at 60 °C under an atmosphere of nitrogen allowed isolation of $[\text{GaL}^3(\text{OH})]$ (Scheme 2.3). Analysis of the solid by ESI-MS gave signals at m/z 444.05 $[\text{GaL}^3]^+$ and 462.06 $[\text{GaL}^3 + (\text{H}_2\text{O})]^+$ with the expected isotope patterns. By replacing $\text{Ga}(\text{NO}_3)_3\cdot 9\text{H}_2\text{O}$ with GaCl_3 , $[\text{GaL}^3\text{Cl}]$ was formed.



Scheme 2.3. Synthesis of $[\text{GaL}^3(\text{OH})]$ and $[\text{GaL}^3\text{Cl}]$.

Comparison of the ^1H NMR spectra of $\text{H}_2\text{L}^3\cdot\text{HCl}$ and $[\text{GaL}^3(\text{OH})]$ in d_6 -DMSO revealed an upfield shift of the signals attributed to the methylene protons from $\delta_{\text{H}} = 4.67$ and 4.61 ppm to $\delta_{\text{H}} = 4.18$ and 3.99 ppm (Figure 2.6).

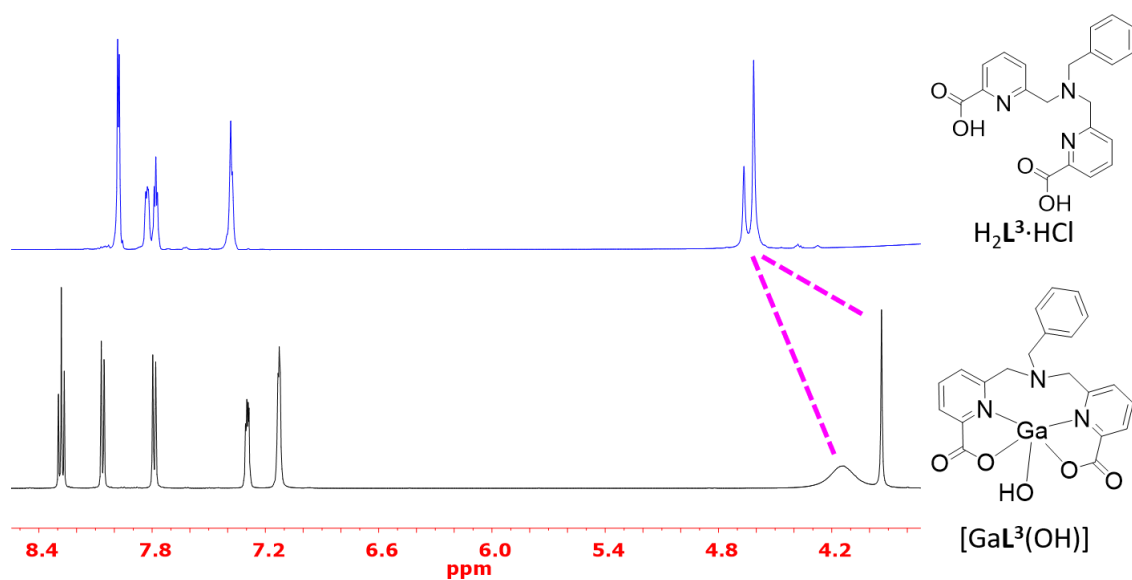


Figure 2.6. Region of ^1H NMR spectra of $\text{H}_2\text{L}^3\cdot\text{HCl}$ (blue) and $[\text{GaL}^3(\text{OH})]$ (black) in d_6 -DMSO.

The gradient homonuclear correlation spectroscopy (gCOSY) spectrum allowed the assignment of the resonances attributed to the pyridyl hydrogen and benzyl hydrogen atoms (Figure 2.7). For $H_2L^3 \cdot HCl$, the resonances at $\delta_H = 7.97$ and 7.77 ppm were assigned to the benzyl hydrogen atoms (Figure 2.7 green box). The resonances at $\delta_H = 7.82$ and 7.38 ppm integrated to four and two protons respectively were assigned to the pyridyl hydrogen atoms (Figure 2.7 red box).

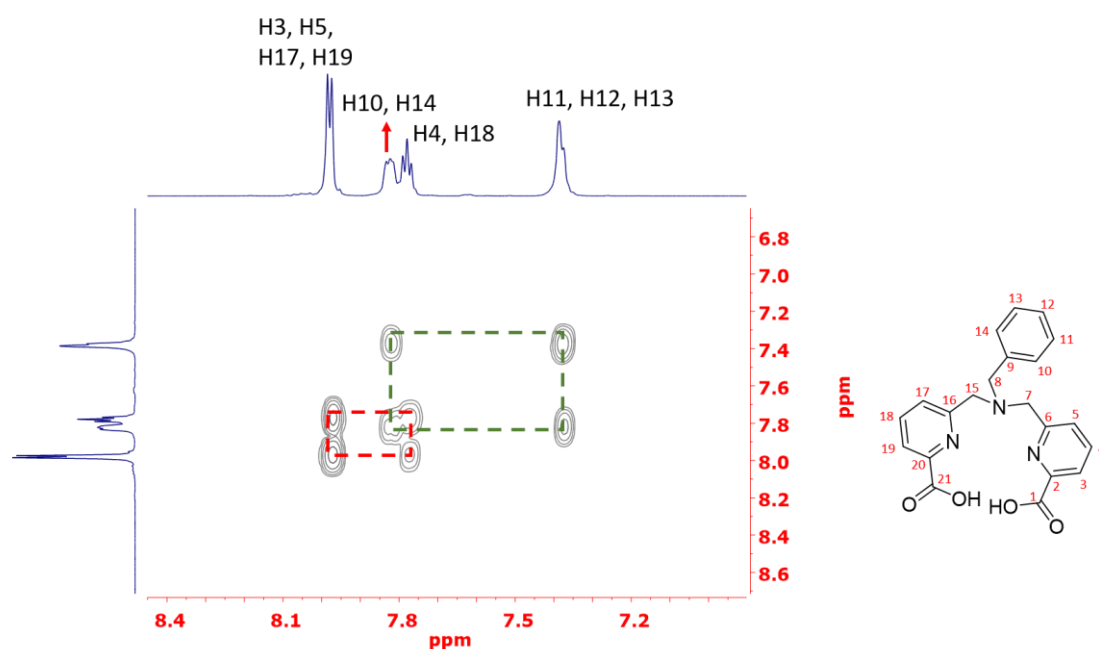


Figure 2.7. A section of the gCOSY NMR spectrum of $H_2L^3 \cdot HCl$ in d_6 -DMSO.

For $[\text{GaL}^3(\text{OH})]$, the resonances at $\delta_{\text{H}} = 7.31$ and 7.08 ppm were assigned to the benzyl hydrogen atoms (Figure 2.8 red box). The resonance at $\delta_{\text{H}} = 8.27$ ppm is involved in two spin systems and was assigned to the pyridyl hydrogen atoms (H4 and H18) furthest away from the pyridyl nitrogen atom (Figure 2.8 green and orange box).

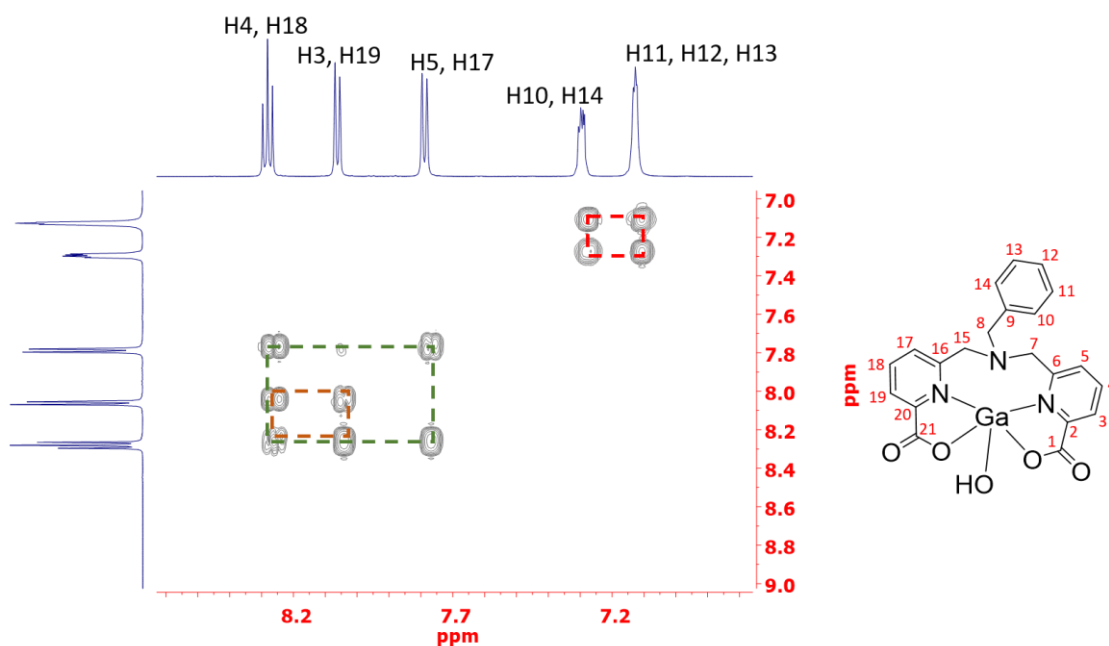
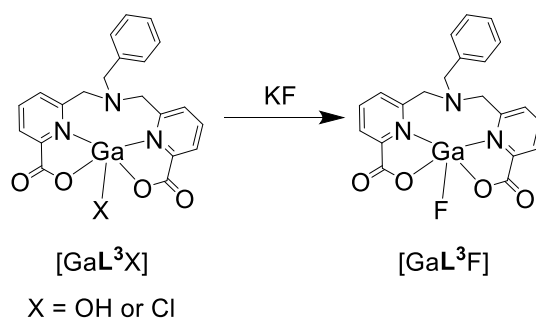


Figure 2.8. A section of the gCOSY NMR spectrum of $[\text{GaL}^3(\text{OH})]$ in d_6 -DMSO.

Methodology for the formation of the $\text{Ga}^{3+}\text{-F}^-$ bond was developed, either by displacement of OH^- with F^- using $[\text{GaL}^3(\text{OH})]$ or through halide Cl/F exchange reaction with $[\text{GaL}^3\text{Cl}]$ as the precursor (Scheme 2.4).



Scheme 2.4. Synthesis of gallium (III) fluoride complex, $[\text{GaL}^3\text{F}]$.

The incorporation of a fluoride ion into the Ga^{3+} complex was first attempted using a phase transfer catalyst in an aprotic solvent. In this process, coordination of K^+ ion with 18-crown-6 increases the solubility of the fluoride salt and subsequently bound to Ga^{3+} . The ^{19}F NMR spectrum shows a single resonance at $\delta_{\text{F}} = -141$ ppm, confirming the formation of the $\text{Ga}^{3+}\text{-F}^-$ bond. To investigate the likelihood of the reaction proceeding in aqueous media, $[\text{GaL}^3(\text{OH})]$ was dissolved in a mixture of $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ (1:1) before dropwise addition of one molar equivalent of aqueous KF into the solution. The product precipitated after the concentration of the solution and was collected by filtration. A single peak at the same resonance ($\delta_{\text{F}} = -141$ ppm) in the ^{19}F NMR spectrum confirmed the formation of the $\text{Ga}^{3+}\text{-F}^-$ complex (Figure 2.9). Given the success of the synthesis in aqueous solution, the halide Cl/F exchange reaction was carried out under the same conditions. This method is preferred as it excludes the use of phase transfer catalyst and that further purification step is not necessary.

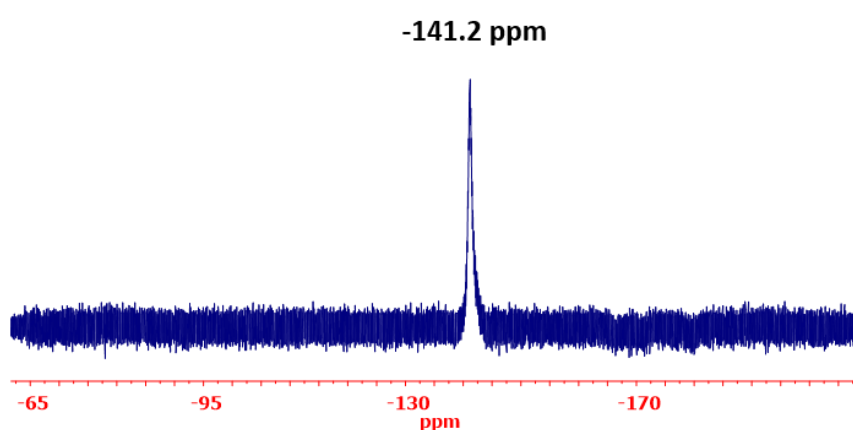


Figure 2.9. A portion of ^{19}F NMR spectrum of $[\text{GaL}^3\text{F}]$ in $d_6\text{-DMSO}$.

Crystals suitable for single X-ray crystallography of $[\text{GaL}^3(\text{OH})]$, $[\text{GaL}^3\text{Cl}]$ and $[\text{GaL}^3\text{F}]$ were grown by evaporation of aqueous solutions of the complexes (Figure 2.10). The complexes were all monoclinic, whereas the ligand $\text{H}_2\text{L}^3\cdot\text{HCl}$ was triclinic. Besides, the $[\text{GaL}^3(\text{OH})]$ and $[\text{GaL}^3\text{F}]$ were isomorphous while $[\text{GaL}^3\text{Cl}]$ has a different space group.

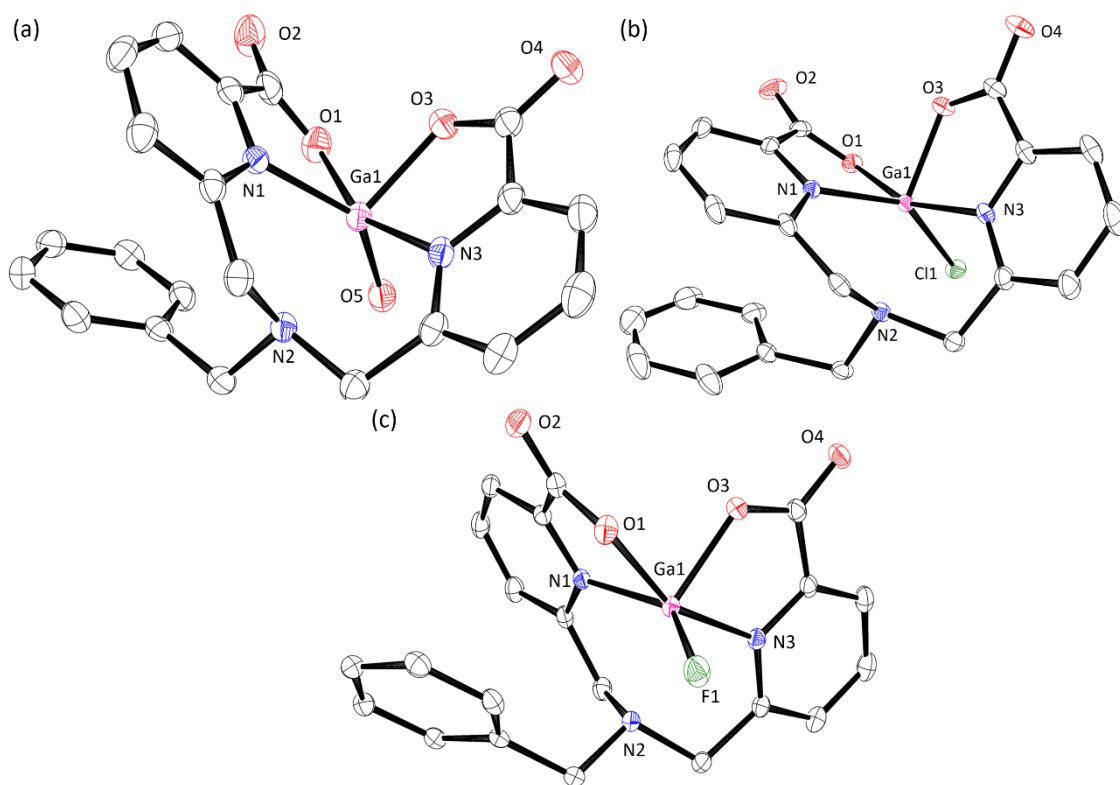


Figure 2.10. ORTEP representations of the X-ray crystal structures (30% ellipsoids) of $[\text{GaL}^3(\text{OH})]$ (a), $[\text{GaL}^3\text{Cl}]$ (b) and $[\text{GaL}^3\text{F}]$ (c). Solvent and hydrogen atoms have been omitted for clarity.

In each complex, the ligand acts as tetradentate N_2O_2 donor to Ga^{3+} . The distances between Ga^{3+} and central tertiary nitrogen (Ga(1)-N(2)) (2.440 (2) – 2.471 (2) Å) are greater than the sum of the effective ionic radii for gallium and nitrogen (Van der Waals radii of Ga-N ~ 2.08 Å). Hence, in each case, the central nitrogen atom N(2) is not bonded to Ga^{3+} though it is slightly pointed toward the metal centre, which is an indicative of a weak interaction. The Ga(1)-N(1) and Ga(1)-N(3) bond lengths range from 2.001 Å to

2.075 Å and the Ga(1)-O(1) and Ga(1)-O(3) bond lengths range from 1.981 Å to 2.056 Å as expected (Table 2.2).

Table 2.2. Selected bond lengths (Å) for the Ga³⁺ complexes.

Bond length (Å)	[GaL ³ (OH)]	[GaL ³ Cl]	[GaL ³ F]
Ga(1)-N(1)	2.029(2)	2.001(2)	2.063(2)
Ga(1)-N(2)	2.471(2)	2.442(1)	2.440(2)
Ga(1)-N(3)	2.075(2)	2.072(2)	2.012(2)
Ga(1)-O(1)	2.056(2)	2.002(2)	1.996(2)
Ga(1)-O(3)	2.006(2)	1.981(1)	2.024(2)
Ga(1)-O(5)	1.831(2)		
Ga(1)-Cl(1)		2.2194(6)	
Ga(1)-F(1)			1.797(2)

As Ga³⁺ only binds to four donor atoms of the ligand and that it generally prefers five or six-coordinate, one of the sites is occupied by an anion (OH⁻, Cl⁻ or F⁻). There is a distortion of bond angles about Ga³⁺, where the N-Ga-N and N-Ga-O angles are less than 180°. To identify the coordination geometry of the five-coordinated complexes, the tau parameters were calculated by taking the two largest angles involving two pairs of donor atom at the metal centre and subtracting the second largest from the largest and dividing the value by 60°. The tau parameters obtained were 0.192, 0.271 and 0.193 for [GaL³(OH)], [GaL³Cl] and [GaL³F] respectively. As the tau parameters approach 0, the coordination geometry of the Ga³⁺ complexes are best described as distorted square pyramidal.

The Ga(1)-F(1) bond length of [GaL³F] was found to be 1.797(2) Å, which is relatively short compared to the average Ga-F bond length published in literature. The crystal structure shows extensive hydrogen bonding (F...H-OH) interaction between the fluorine

atom and the lattice water molecules (Figure 2.11a), which is not observed in the crystal structure of $[\text{GaL}^3\text{Cl}]$. In addition, there is π -stacking of aromatic rings between molecules in the crystal structure of $[\text{GaL}^3\text{F}]$ (Figure 2.11b).

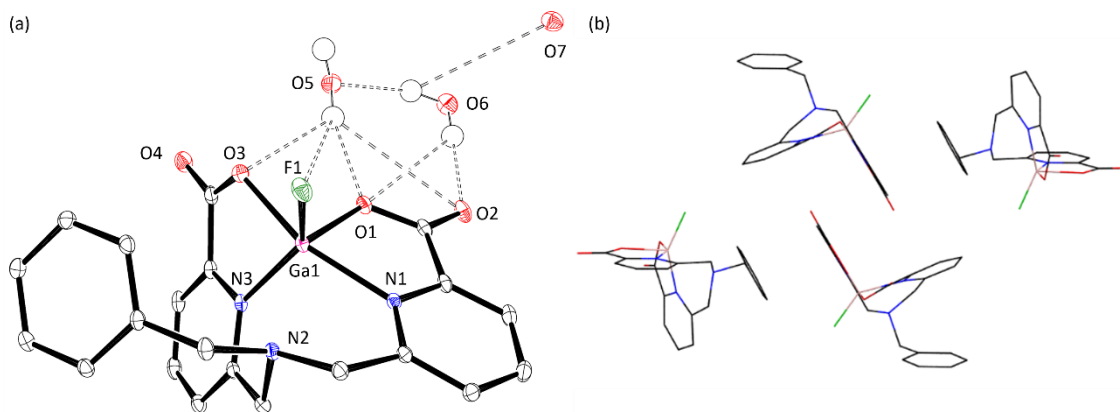


Figure 2.11. ORTEP representation of the X-ray crystal structure (30% ellipsoids) of the H-bonding network of $[\text{GaL}^3\text{F}]$ (a) and MERCURY representation of the π -stacking of aromatic rings between $[\text{GaL}^3\text{F}]$ (b). Selected hydrogen atoms and solvent atoms have been omitted for clarity.

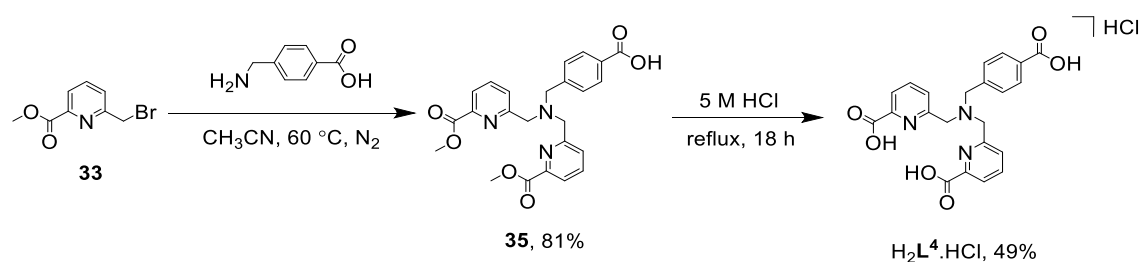
The weak interaction between the central tertiary nitrogen atom N(2) in the backbone of the chelator and Ga^{3+} may hinder incoming nucleophilic ligands and reduce the propensity to form six-coordinate Ga^{3+} complexes. This may also prevent the formation of transition states with higher coordination number and increase stability of the Ga^{3+} complexes.

Table 2.3. Summary of the crystallographic data for $[\text{GaL}^3(\text{OH})]$, $[\text{GaL}^3\text{Cl}]$ and $[\text{GaL}^3\text{F}]$.

	$[\text{GaL}^3(\text{OH})]\cdot 5\text{H}_2\text{O}$	$[\text{GaL}^3\text{Cl}]$	$[\text{GaL}^3\text{F}]\cdot 5\text{H}_2\text{O}$
Empirical formula	$\text{C}_{21}\text{H}_{18}\text{GaN}_3\text{O}_{10}$	$\text{C}_{21}\text{H}_{17}\text{ClGaN}_3\text{O}_4$	$\text{C}_{21}\text{H}_{17}\text{FGaN}_3\text{O}_9$
MW	552.18	480.55	554.17
Space group	P 2 ₁ /n	I 2/a	P 2 ₁ /n
T/K	130.0(1)	130.00(10)	130.0(1)
Crystal size/mm ³	0.544 x 0.323 x 0.313	0.361 x 0.267 x 0.135	0.377 x 0.320 x 0.203
Crystal system	Monoclinic	Monoclinic	Monoclinic
$\lambda/\text{\AA}$	0.71073	1.54184	0.71073
$a/\text{\AA}$	11.6817(2)	14.4262(2)	11.7761(5)
$b/\text{\AA}$	16.2098(3)	9.08430(10)	16.0638(6)
$c/\text{\AA}$	12.2210(3)	31.6400(5)	12.1853(4)
$\alpha/^\circ$	90	90	90
$\beta/^\circ$	91.268(2)	99.306(2)	91.841(3)
$\gamma/^\circ$	90	90	90
$V/\text{\AA}^3$	2313.58(8)	4091.91(10)	2303.89(15)
Z	4	8	4
$D_{\text{calc}}/\text{Mg/m}^3$	1.585	1.560	1.598
μ/mm^{-1}	1.252	3.336	1.261
$F(000)$	1144	1952	1144
Reflections measured	14031	14985	16907
Independent reflections	4060 [$R_{\text{int}} =$ 0.0202]	4289 [$R_{\text{int}} =$ 0.0205]	6130 [$R_{\text{int}} =$ 0.0223]
final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0328$, $wR^2 =$ 0.0853	$R_1 = 0.0323$, $wR^2 =$ 0.0852	$R_1 = 0.0356$, $wR^2 =$ 0.0904
final R indices (all data)	$R_1 = 0.0352$, $wR^2 =$ 0.0866	$R_1 = 0.0330$, $wR^2 =$ 0.0858	$R_1 = 0.0427$, $wR^2 =$ 0.0952

Goodness-of-fit on F ²	1.052	1.059	1.063
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Functionalisation of the chelator at the tertiary nitrogen atom in the backbone can be achieved by replacing benzylamine with aminobenzoic acid, which allowed the isolation of (**35**). Hydrolysis under acidic conditions afforded H₂L⁴ as the hydrochloride salt (Scheme 2.5).



Scheme 2.5. Synthesis of H₂L⁴.HCl.

For compounds H₂L⁴.HCl and [GaL⁴Cl], there was a small shift and a splitting of the signal attributed to the methylene hydrogen atoms ($\delta_{\text{H}} = 4.24$ ppm to $\delta_{\text{H}} = 4.19$ and 4.04 ppm) (Figure 2.12). Coordination of Ga³⁺ to H₂L⁴.HCl results in a significant change in resonance attributed to the aromatic protons.

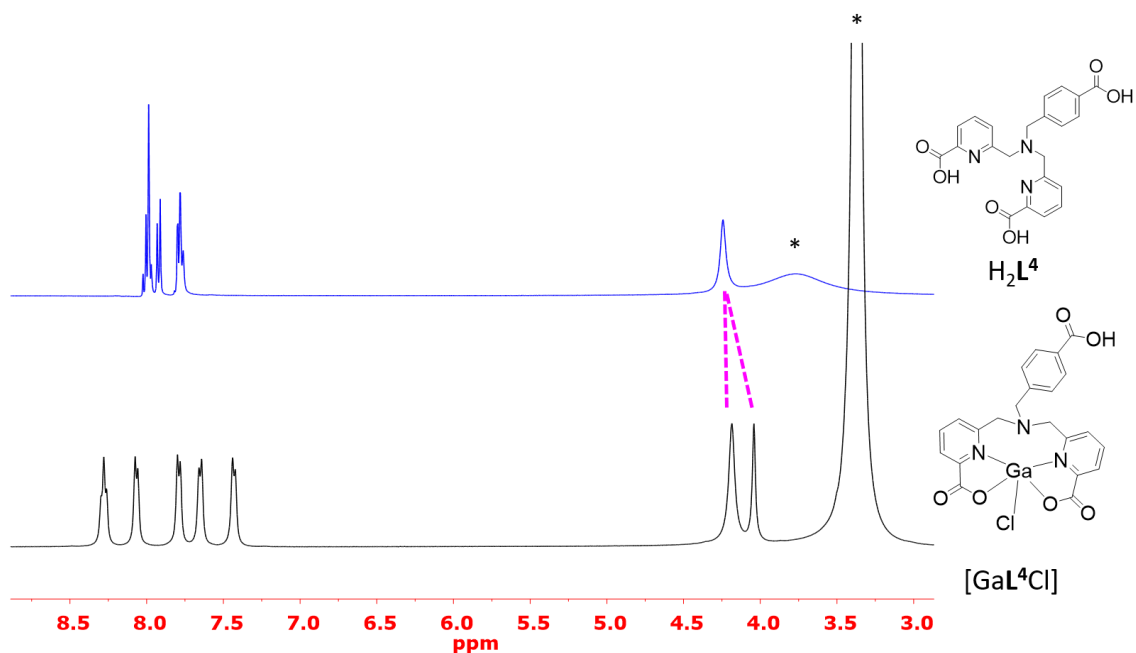


Figure 2.12. ^1H NMR of $H_2L^4 \cdot HCl$ (blue) and $[GaL^4Cl]$ (black) in d_6 -DMSO. The asterisks indicate solvent signal for H_2O ($\delta_H = 3.33$ ppm).

For $[GaL^4Cl]$, the gCOSY spectrum revealed the resonances attributed to the pyridyl hydrogen and benzyl hydrogen atoms (Figure 2.13). The resonances at $\delta_H = 7.65$ and 7.43 ppm were assigned to the benzyl hydrogen atoms (Figure 2.13 red box). The resonance at $\delta_H = 8.28$ ppm involved in two spin systems and was assigned to the pyridyl hydrogen atoms furthest away from the nitrogen atom (Figure 2.13 green and orange box).

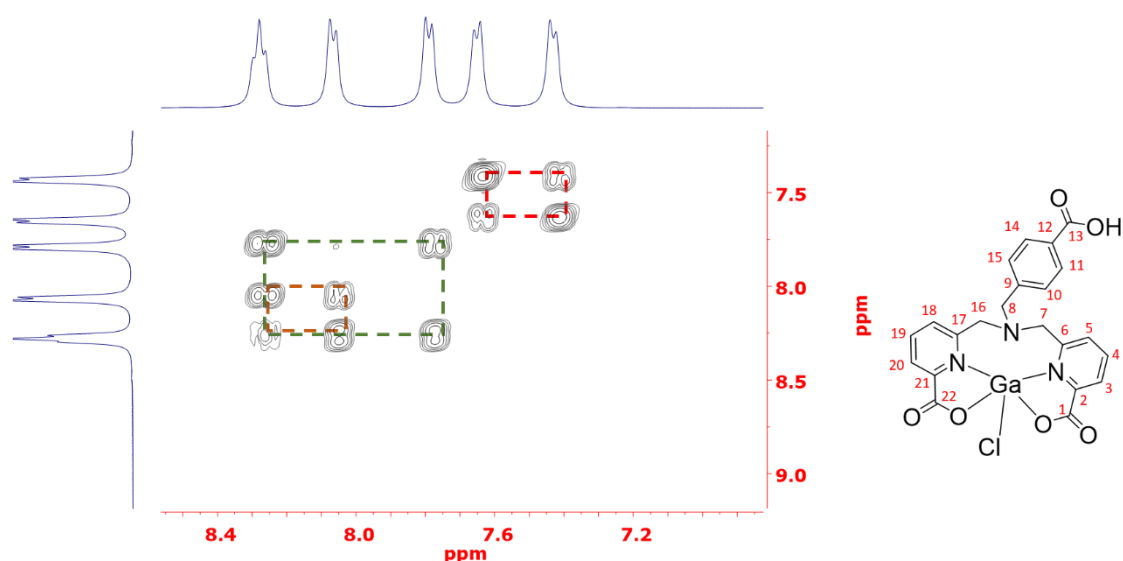


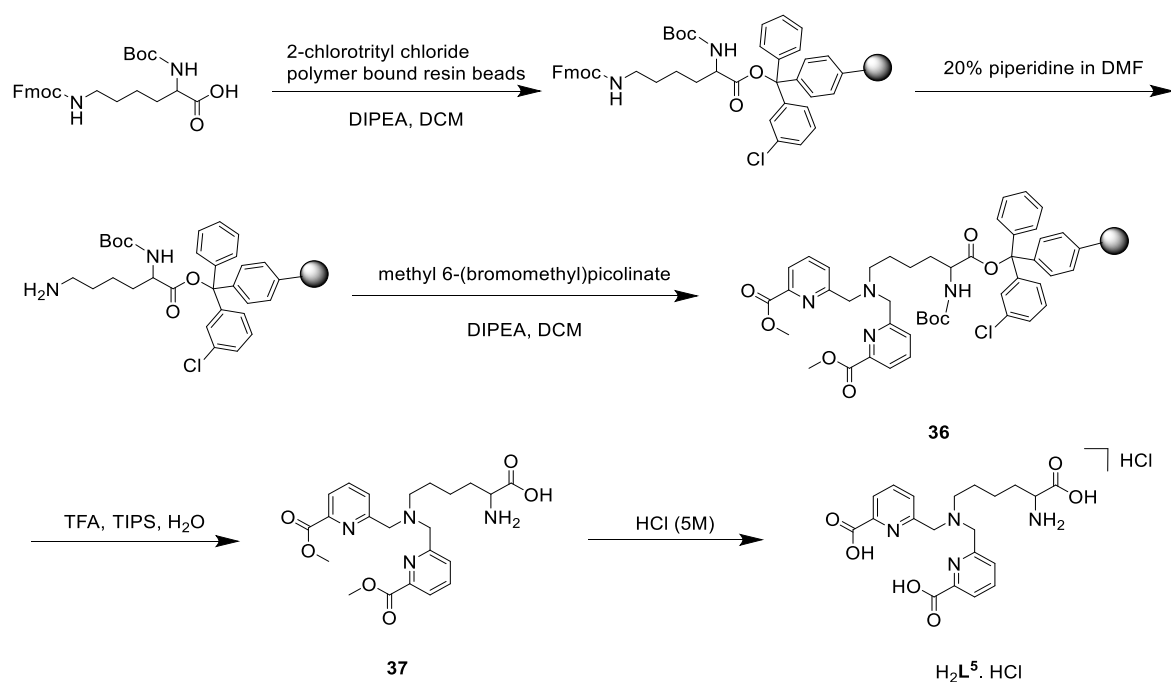
Figure 2.13. A section of the gCOSY NMR spectrum of $[GaL^4Cl]$ in d_6 -DMSO.

Table 2.4. The assignments of the ^1H NMR spectrum of $[GaL^4Cl]$.

Proton	Assignment	
PyrH	8.28	H4, H19
PyrH	8.07	H3, H20
PyrH	7.79	H5, H18
ArH	7.65	H11, H14
ArH	7.43	H10, H15
CH_2	4.19	H7, H16
Ar CH_2	4.04	H8

The incorporation of the appended methylbenzyl with a carboxylate functional group demonstrated the convenience of functionalisation at the central nitrogen atom. An alternative method to functionalise the ‘dpa’ ligand can be achieved by using the single amino acid chelate (SAAC) approach. The SAAC is a system of the inclusion of one amino acid to a chelate and that the chelate can then be inserted at any position along a peptide sequence.¹⁶⁵ Herein, a lysine residue was embedded to two dipicolinic acid molecules to form a functionalised dipyridylamine ligand using solid-phase peptide

synthesis (SPPS) (Scheme 2.6). Firstly, 2-chlorotrityl chloride polymer-bound resin beads (loading ~1 mmol/g) were suspended in a solution of Fluorenylmethyloxycarbonyl (Fmoc)-protected lysine residue in dichloromethane (DCM) and *N,N*-Diisopropylethylamine (DIPEA). The resin was washed to remove excess reagents and incubated with methanol in dichloromethane to deactivate any unreacted resin. The Fmoc protecting group was removed using 20% piperidine in *N,N*-dimethylformamide (DMF). A solution of methyl 6-(bromomethyl)picolinate in dichloromethane with DIPEA was added to the resin to react with the primary amine on the amino acid. The resin was washed again to remove the unreacted reagents. Cleavage of the product from the 2-chlorotrityl chloride polymer resin beads using an aqueous solution of trifluoroacetic acid (TFA) and triisopropylsilane (TIPS) gave compound (**37**). Finally, the methyl ester protecting groups were removed using acid to give $H_2L^5 \cdot HCl$.



Scheme 2.6. Synthesis of $H_2L^5 \cdot HCl$ using the solid-phase peptide synthesis. Black circle indicates polymer bound resin beads.

The SPPS strategy has an advantage of simplifying the purification process since it does not require purification using column chromatography. The SAAC system can also be incorporated within any peptide sequence of interest.

2.3 Stability of Ga^{3+} complexes under acidic conditions

The stability of the Ga^{3+} complexes was examined before undertaking the radiochemistry studies. The resonances in the ^1H NMR spectra of $[GaL^3Cl]$ and $[GaL^3(OH)]$ and the ^{19}F NMR spectrum of $[GaL^3F]$ were unshifted after a few weeks in solution indicating no decomposition. As trifluoroacetic acid (TFA) is a commonly used reagent in the buffer system of reversed-phase high-performance liquid chromatography (RP-HPLC), the susceptibility of $[GaL^3Cl]$ and $[GaL^3F]$ to acid was tested in the presence of TFA. The RP-HPLC traces of $H_2L^3 \cdot HCl$, $[GaL^3Cl]$, $[GaL^3(OH)]$ and $[GaL^3F]$ acquired under the same conditions (0 to 100% B in 15 min, A: 0.1% TFA; B: 0.1% TFA in acetonitrile) had the same retention times. This indicated that either the Ga^{3+} complexes and the ligand exhibit similar hydrophilicity or that the Ga^{3+} complexes decomposed under acidic conditions. Another study was done utilising ^1H NMR, where 0.1% of TFA was added to the solution of $[GaL^3(OH)]$ in d_6 -DMSO (Figure 2.14). New peaks were observed after 1-3 h of incubation, and after 24 h, the peaks in the ^1H NMR spectrum correlated to the peaks seen in the ^1H NMR spectrum of $H_2L^3 \cdot HCl$. The decomposition was most likely due to the cleavage of the Ga-O bonds by acid-catalysed dissociation, which led to the loss of Ga^{3+} and F^- .

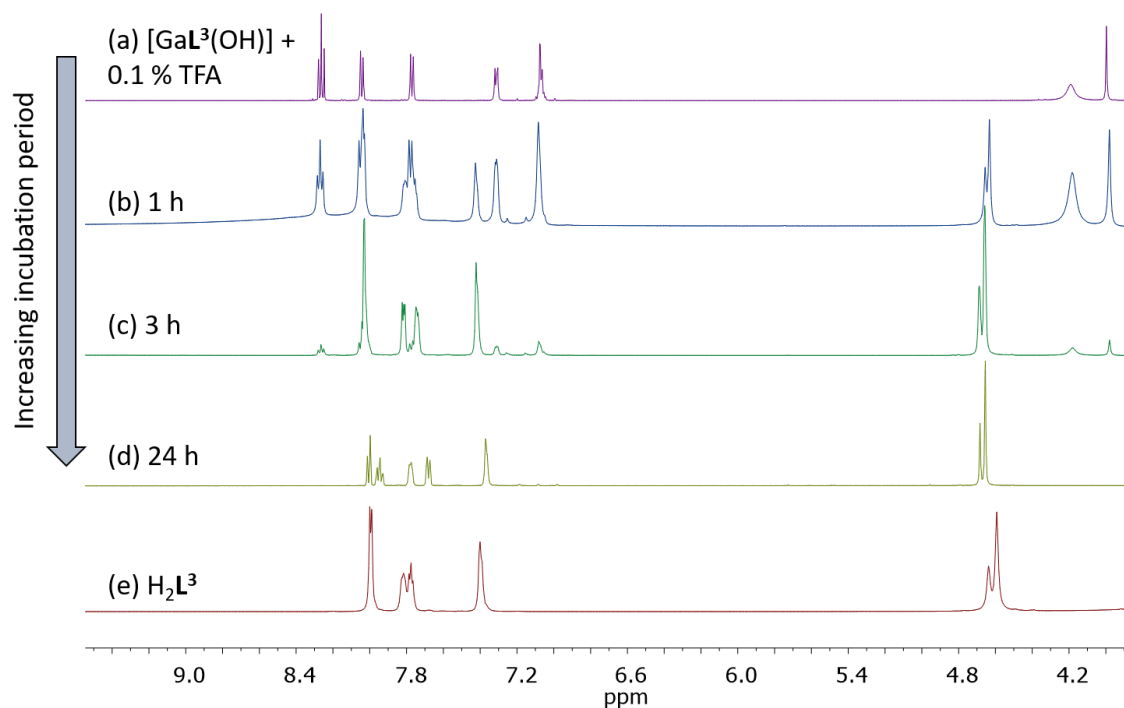


Figure 2.14. ^1H NMR spectrum of $[\text{GaL}^3(\text{OH})]$ and TFA (0.1%) in d_6 -DMSO upon addition (a), at 1 h (b), 3 h (c) and 24 h (d). ^1H NMR spectrum of $\text{H}_2\text{L}^3\cdot\text{HCl}$ (e).

Despite the instability of the Ga^{3+} complexes under acidic conditions, the ease of synthesis of the $[\text{GaL}^3\text{F}]$ in aqueous solution and the stability at neutral pH encouraged the pursuit of fluorine-18 labelling of $[\text{GaL}^3]^+$.

2.4 Synthesis and evaluation of $^{18}\text{F}[\text{GaL}^3\text{F}]$

A mixture of $^{18}\text{F}[\text{F}^-]/\text{H}_2\text{O}$ was obtained from a $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$ nuclear reaction (98% ^{18}O isotopic enrichment) on a 16.5 MeV cyclotron ($\sim 1,000$ - $1,500$ MBq/mL). Due to the susceptibility of $[\text{GaL}^3]^+$ to acid as evident from the ^1H NMR studies, the pH of the reaction solution was maintained between 4 and 7. Radiolabelling was attempted using sodium acetate buffer (pH 4.0) at room temperature and 80°C . The radio-RP-HPLC scintillation trace showed unlabelled $^{18}\text{F}[\text{F}^-]$ eluted at 2 min (Figure 2.15 red trace). A peak at 12.5 min was observed, which correlated to the peak in the UV trace in blue,

suggesting the formation of $[^{18}\text{F}][\text{GaL}^3\text{F}]$ with a low yield ($\sim 1\%$). Addition of organic solvent did not increase the radiochemical yield.

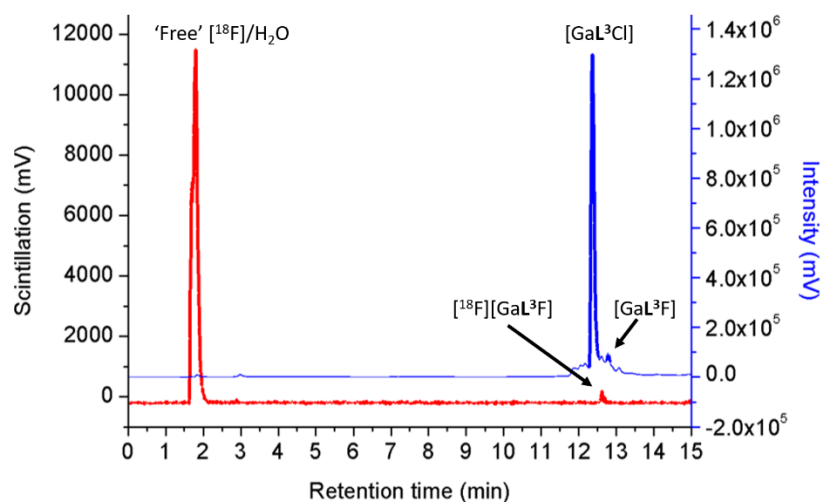


Figure 2.15. Radio-RP-HPLC trace (red) of the reaction mixture of $[\text{GaL}^3\text{Cl}]$ and $[^{18}\text{F}]/\text{H}_2\text{O}$ in aqueous buffer. 'Free' $[^{18}\text{F}]\text{F}^-/\text{H}_2\text{O}$ eluted at $R_t = 2$ minutes. The corresponding UV trace (λ 254 nm) (blue) of the non-radioactive analogue.

Despite the relatively simple preparation of non-radioactive $[\text{GaL}^3\text{F}]$ in high yields, the radioactive analogue, $[^{18}\text{F}][\text{GaL}^3\text{F}]$, could only be prepared in very low radiochemical yield presumably due to the significantly lower concentration of $[^{18}\text{F}]\text{F}^-$ (nanomolar) in the diluted acetate buffer solution. The radiolabelling was also attempted under anhydrous conditions using azeotropically dried $^{18}\text{F}^-$, kryptofix-222 as the phase transfer catalyst, K_2CO_3 , and acetonitrile as the organic solvent to eliminate the possibility of interference by the presence of water. Unfortunately, no improvement was observed using this method at various temperatures and reaction times.

2.5 Summary

Taken together, a family of picolinic acid derived acyclic chelators H_2L^3 , H_2L^4 and H_2L^5 were prepared. The SAAC approach using SPPS was useful in functionalising the backbone of the chelator with a single lysine residue. This methodology can be employed to further incorporate other amino acids or peptides. The new ligands formed complexes with Ga^{3+} and it was straightforward to prepare $\text{Ga}^{3+}\text{-F}^-$ complexes in organic and aqueous environments. Characterisation of Ga^{3+} complexes of H_2L^3 with three different anions, $[\text{GaL}^3(\text{OH})]$, $[\text{GaL}^3\text{Cl}]$ and $[\text{GaL}^3\text{F}]$ by X-ray crystallography revealed that in each case the ligand acted as tetradentate donor but with an additional weaker interaction to the tertiary nitrogen atom on the backbone of the chelator. The $\text{Ga}^{3+}\text{-F}^-$ bond was formed under mild conditions in aqueous media that is an advantage compared to the high temperature needed for the $\text{Al}^{3+}\text{-F}^-$ bond formation. The ease of synthesis of $[\text{GaL}^3\text{F}]$ encouraged further investigations with fluorine-18.

There was little success in radiolabelling either $[\text{GaL}^3(\text{OH})]$ or $[\text{GaL}^3\text{Cl}]$ with both aqueous and anhydrous $[^{18}\text{F}]\text{F}^-$. The nanomolar concentration of $[^{18}\text{F}]\text{F}^-$ in the diluted buffer solution may be incompatible for the radiolabelling of $[\text{GaL}^3]^+$. The limited stability of $[\text{GaL}^3\text{F}]$ at low pH (< 3) suggested that the radioactive analogue can also suffer from similar issues, suggesting the Ga^{3+} complexes with the acyclic ‘dpa’ chelator are not suitable for the incorporation of fluorine-18.

2.6 Method

General method

All reagents and solvents were obtained from standard commercial suppliers and were used as received. Nuclear magnetic resonance (NMR) spectra were acquired on a Varian FT-NMR 500 spectrometer or a Varian FT-NMR 400 spectrometer. ^1H NMR spectra were acquired at 400 or 500 MHz, $^{13}\text{C}\{^1\text{H}\}$ NMR spectra were acquired at 125.7 MHz and referenced to an internal solvent residue. $^{19}\text{F}\{^1\text{H}\}$ NMR spectra were acquired at 470.4 MHz. $^{19}\text{F}\{^1\text{H}\}$ NMR spectra were referenced (externally) to CF_3COOH . All spectra were recorded at 25 °C. Spectra were analysed on MestReNova software (MestreLab Research). High-resolution ESI-QTOF mass spectra were recorded on an Exactive Plus Orbitrap Infusion mass spectrometer (Exactive Series, 2.8 Build 268801, Thermo Fisher Scientific). The analysis was performed using Xcalibur 4.0.27.10 (Thermo Fisher Scientific). Elemental analysis for C, H and N was carried out by The Campbell Microanalytical Laboratory, The University of Otago, Dunedin, New Zealand.

UV-Visible spectra were acquired on a Shimadzu UV-1650 PC UV-Visible spectrophotometer (Shimadzu, Kyoto, Japan) from 800 nm to 250 nm. Fluorescence emission spectra were recorded on a Varian Cary Eclipse Fluorescence spectrometer. Emission and excitation spectra were recorded on a Varian CARY eclipse fluorescence spectrophotometer in quartz cuvettes. Analytical RP-HPLC traces were acquired using an Agilent (Santa Clara, CA) 1100 Series HPLC system with an SGE Analytical Science (Ringwood, VIC) ProteCol C18 HPL 125 column (4.6 x 150 mm, 5 μm , 120 Å) and were monitored at $\lambda = 220, 254$ and 280 nm. Agilent 1200 HPLC system equipped with an Alltech Hypersil BDS C18 analytical HPLC column (4.6 x 150 mm, 5 μm , 120 Å) with a flow rate of 1 mL/min and were monitored at $\lambda = 220, 254$ and 280 nm. Retention times

were recorded using a gradient elution of 5-100% B (0.1% TFA in acetonitrile) in A (0.1% TFA in Milli-Q water) over 30 min.

Single crystal X-ray crystallography data was collected and solved by Prof. Jonathan White or Prof. Paul Donnelly as follows: a typical crystal was mounted on a MiTeGen Micromount using high viscosity oil and cooled rapidly to 100(2) K in a stream of nitrogen gas using an Oxford Cryostream cooling device on a Rigaku XtaLAB Synergy diffractometer equipped with a HyPix detector using Cu-K α radiation (λ = 1.54184 Å) or Mo-K α radiation at 130 K. Raw frame data were reduced using CrysAlisPro. The structures were solved using SHELXT, and the refinement was carried out with SHELXL (version 2018/3) employing full-matrix least-squares on F^2 using the OLEX2 software package. All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were placed in geometrically calculated positions and refined using a riding model. Thermal ellipsoid plots were generated using ORTEP-3 integrated within the WINGX suite of program.

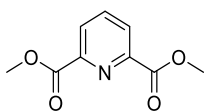
[^{18}F]/H₂O was obtained from the Peter MacCallum Cancer Centre Cyclotek, prepared using PETtrace 16.5 MeB cyclotron incorporating a high-pressure niobium target for the production of fluorine-18 (GE healthcare) as target wash.

Radio-RP-HPLC traces were acquired using a Shimadzu SPD-10ATvP HPLC system equipped with a Waters C column (4.6 x 150 mm, 5 μm) with a 1 mL/min flow rate. Chromatograms were recorded with a scintillation detector and a UV-vis detector (254 nm and 280 nm). Retention times were recorded using a gradient elution of (1) 0-95% B (acetonitrile) in A (Milli-Q water) over 15 min or (2) 0-100% B (0.05% TFA in acetonitrile) in A (0.05% TFA in Milli-Q water) over 20 min.

General method 1: Cleavage from the 2-chlorotrityl polymer-bound resin beads.

Cleavage was performed using TFA in the presence of triisopropylsilane (TIPS) as scavengers. A 1 mL solution containing H₂O (50 µL), TIPS (25 µL) and TFA (925 µL) was mixed with the resin beads (25 mg). The resin was filtered, and the filtrate was concentrated under nitrogen. The product was precipitated using diethyl ether, centrifuged, collected, redissolved in water: acetonitrile (50:50) and freeze-dried.

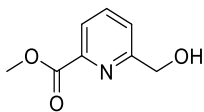
Dimethyl pyridine-2,6-dicarboxylate (31)



Dimethyl 2,6-pyridine-2,6-dicarboxylate was synthesized using an adapted literature protocol.¹⁶⁴

Pyridine-2,6-dicarboxylic acid (10.4 g, 62.0 mmol) and sulfuric acid (2 mL) were heated at reflux in methanol (50 mL) for 18 h. The solid that formed was collected by filtration then dissolved in dichloromethane and washed with saturated NaHCO₃ and water. The combined organic layers were dried over Na₂SO₄, and the solvent was removed under reduced pressure to give dimethyl pyridine-2,6-dicarboxylate as a colourless solid (9.12 g, 75%). HR-MS(ESI/O-TOF): [C₉H₉NO₄+H]⁺ *m/z* 196.061 (experimental), 196.061 (calculated). ¹H NMR (400 MHz CDCl₃): δ_H (ppm) 8.30 (d, *J*_{HH} = 0. Hz, 2H, Pyr*H*), 8.02 (t, 1H, Pyr*H*), 4.02 (s, 6H, CH₃). ¹³C{¹H} NMR (126 MHz, CDCl₃): δ_C (ppm) = 165.1 (C=O), 148.2 (PyrC), 138.4 (PyrC), 128.1 (PyrC), 53.2 (CH₃).

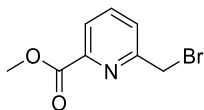
Methyl 6-(hydroxymethyl)picolinate (32)



Methyl 6-(hydroxymethyl)picolinate was synthesized using an adapted literature protocol.¹⁶⁴

To dimethyl 2,6-pyridine-2,6-dicarboxylate (5.1 g, 26 mmol) in dry methanol (100 mL) at 0 °C under nitrogen, was added sodium borohydride (1.8 g, 48 mmol). The reaction mixture was stirred for 4 h under nitrogen. The solution was diluted using dichloromethane (200 mL) and quenched using saturated NaHCO₃ (200 mL). The aqueous and organic layers were separated. The aqueous layer was extracted with chloroform (2 x 200 mL). The combined organic layers were dried over MgSO₄, filtered, and the solvents were removed under reduced pressure. The methyl 6-(hydroxymethyl)picolinate was purified by column chromatography (100% ethyl acetate) to give a colourless solid (1.89 g, 44%). HR-MS(ESI/O-TOF): [C₈H₉NO₃+H]⁺ *m/z* 168.066 (experimental), 168.066 (calculated). ¹H NMR (400 MHz, CDCl₃): δ_H (ppm) 8.03 (d, 1H, PyrH), 7.85 (t, 1H, PyrH), 7.52 (d, 1H, PyrH), 4.86 (s, 2H, CH₂), 4.00 (s, 3H, CH₃).

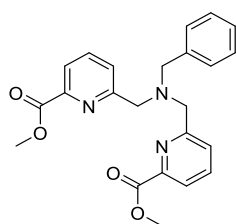
Methyl 6-(bromomethyl)picolinate (33)



Methyl 6-(bromomethyl)picolinate was synthesized using an adapted literature protocol.¹⁶⁴

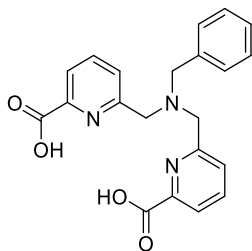
To methyl 6-(hydroxymethyl)picolinate (1.9 g, 11 mmol) in dry dichloromethane (80 mL) at 0 °C under nitrogen, was added phosphorus tribromide (1.2 mL, 14 mmol) dropwise. The reaction was stirred at 0 °C under nitrogen for 4 h. The reaction mixture was quenched using Na₂CO₃ in water (50 mL) and extracted using dichloromethane (2 x 100 mL). The combined organic layers were dried over MgSO₄, filtered and the solvent was removed under reduced pressure to give methyl 6-(bromomethyl)picolinate as a colourless solid (2.32 g, 89%). HR-MS(ESI/O-TOF): [C₈H₈BrNO₂+H]⁺ *m/z* 229.982 (experimental), 229.982 (calculated). ¹H NMR (400 MHz, CDCl₃): δ_H (ppm) 8.05 (d, 1H, PyrH), 7.87 (t, 1H, PyrH), 7.68 (d, 1H, PyrH), 4.64 (s, 2H, CH₂), 4.01 (s, 3H, CH₃).

Dimethyl 6,6'-(benzylazanediyl)bis(methylene)dipicolinate (34)



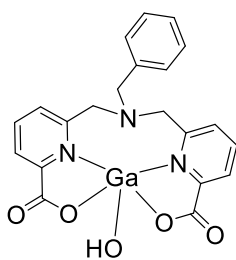
To a solution of methyl 6-(bromomethyl)picolinate (1.98 g, 8.61 mmol) in dry acetonitrile (50 mL) at 60 °C under nitrogen, was added benzylamine (0.44 mL, 4.0 mmol) and K₂CO₃ (1.9 g, 13 mmol). The solution was stirred for 18 h. K₂CO₃ was removed by filtration and washed with acetonitrile. The filtrate was collected and solvent was removed under reduced pressure to and was purified by column chromatography (100% dichloromethane to 3% methanol in dichloromethane) to afford dimethyl 6,6'-(benzylazanediyl)bis(methylene)dipicolinate as a yellow oil (1.5 g, 91%). HR-MS(ESI/O-TOF): [C₂₃H₂₃N₃O₄+H]⁺ *m/z* 406.177 (experimental), 406.177 (calculated). ¹H NMR (400 MHz, CDCl₃): δ_H (ppm) 7.88 (m, 2H, PyrH), 7.82 (m, 4H, PyrH), 7.38 (m, 2H, ArH), 7.30 (m, 2H, ArH), 7.22 (m, 1H, ArH), 3.98 (s, 6H, CH₃), 3.93 (s, 4H, CH₂), 3.70 (s, 2H, CH₂).

$H_2L^3 \cdot HCl$



Dimethyl 6,6'-(benzylazanediyl)bis(methylene)dipicolinate (1.4 g, 3.4 mmol) was dissolved in HCl (5 M) and heated at reflux for 18 h. A white solid that formed was collected by filtration and washed with cold water to give H_2L^3 as the HCl salt (0.98 g, 70%). HR-MS(ESI/O-TOF): $[C_{21}H_{19}N_3O_4+H]^+$ m/z 378.145 (experimental), 378.145 (calculated). ^1H NMR (500 MHz, DMSO- d_6): δ_H (ppm) 7.97 (d, 4H, $J = 4.3$ Hz, PyrH), 7.82 (d, 2H, $J = 4.0$ Hz, ArH), 7.77 (t, 2H, $J = 4.3$ Hz, PyrH), 7.38 (d, 3H, $J = 4.2$ Hz, ArH), 4.67 (s, 2H, CH_2), 4.61 (s, 4H, CH_2). $^{13}C\{^1H\}$ NMR (126 MHz, DMSO- d_6): δ_C (ppm) = 165.4 (C=O), 151.1 (C=O), 147.3, 138.8 (PyrC), 132.0 (ArC), 129.6 (ArC), 128.6 (ArC), 128.2 (PyrC), 124.3 (PyrC), 57.6 (CH_2), 56.0 (CH_2).

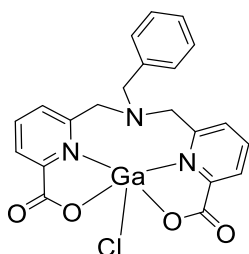
$[GaL^3(OH)]$



To a solution of $H_2L^3 \cdot HCl$ (0.51 g, 1.2 mmol) in anhydrous ethanol (50 mL) was added $Ga(NO_3)_3 \cdot 9H_2O$ (0.60 g, 1.4 mmol, 1.2 eq) in dry ethanol (6 mL). The reaction mixture was stirred for 2 h at 60 °C under nitrogen. The precipitate was collected by filtration, washed with cold ethanol and dried *in vacuo* to give $[GaL^3(OH)]$ as a colourless solid (0.45 g, 82%). HR-MS(ESI/O-TOF): $[C_{21}H_{17}GaN_3O_4]^+$ m/z 444.047 (experimental),

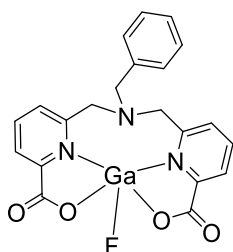
444.047 (calculated). ^1H NMR (500 MHz, DMSO- d_6): δ_{H} (ppm) 8.27 (t, 2H, $J = 7.7$ Hz, PyrH), 8.06 (d, 2H, $J = 7.2$ Hz, PyrH), 7.76 (d, 2H, $J = 7.7$ Hz, PyrH), 7.31 (dd, 2H, $J = 6.4, 2.9$ Hz, ArH), 7.08 (m, 3H, ArH), 4.18 (s, 4H, CH_2), 3.99 (s, 2H, CH_2). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ_{C} (ppm) = 163.0 (C=O), 154.2 (C=O), 146.6, 143.7 (PyrC), 134.2, 130.5 (ArC), 128.1 (ArC), 127.8 (PyrC), 127.5 (PyrC), 122.2 (PyrC), 58.6 (CH_2), 54.7 (CH_2). Anal. Calcd for $\text{C}_{21}\text{H}_{18}\text{GaN}_3\text{O}_5 \cdot 1.5\text{H}_2\text{O}$: C, 51.58; H, 4.33; N, 8.59. Found: C, 51.57; H, 3.25; N, 8.59.

[GaL³Cl]



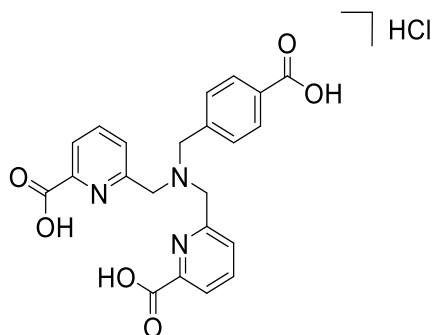
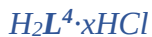
To a solution of $\text{H}_2\text{L}^3 \cdot \text{HCl}$ (0.20 g, 0.48 mmol) in dry ethanol (100 mL) was added GaCl_3 in pentane (1.9 mL, 0.96 mmol, 0.5 M). The reaction mixture was stirred for 2 h at 60 °C under nitrogen. A precipitate formed and was collected by filtration, washed with cold ethanol and dried *in vacuo* to give $[\text{GaL}^3\text{Cl}]$ as a colourless solid (0.072 g, 31%). HR-MS(ESI/O-TOF): $[\text{C}_{21}\text{H}_{17}\text{GaN}_3\text{O}_4]^+$ m/z 444.047 (experimental), 444.047 (calculated), 480.02 (experimental), 480.02416 (calculated). ^1H NMR (500 MHz, DMSO- d_6): δ_{H} (ppm) 8.26 (t, 2H, PyrH), 8.05 (d, 2H, PyrH), 7.77 (d, 2H, PyrH), 7.32 (dd, 2H, ArH), 7.07 (m, 3H, ArH), 4.19 (s, 4H, CH_2), 4.00 (s, 2H, CH_2). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ_{C} (ppm) = 162.6 (C=O), 153.9, 145.8, 143.9, 133.7, 130.7, 128.0, 127.4, 122.2, 58.6 (CH_2), 54.5 (CH_2). Anal. Calcd for $\text{C}_{21}\text{H}_{17}\text{ClGaN}_3\text{O}_4 \cdot \text{C}_2\text{H}_5\text{OH}$: C, 52.46; H, 4.40; N, 7.98. Found: C, 52.46; H, 3.68; N, 7.86.

[GaL³F]



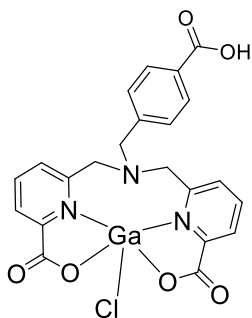
Method 1: To a solution of [GaL³(OH)] (0.10 g, 0.19 mmol) in water/acetonitrile mixture (10 mL, 1:1) was added dropwise aqueous potassium fluoride (0.012 g, 0.21 mmol, 60 μ L). The reaction was stirred at room temperature for 2 h. A colourless precipitate formed that was collected by filtration and washed with cold water (5 mL) to give [GaL³F] as a colourless crystal (0.053 g, 52%).

Method 2: A mixture of [GaL³Cl] (0.047 g, 98 μ mol), potassium fluoride (0.018 g, 0.31 mmol) and 18-crown-6 (0.026 g, 98 μ mol) in acetonitrile (10 mL) was heated to reflux for 3 h. The solution was allowed to cool to room temperature and the solvent was removed under reduced pressure. The residue was washed with cold acetonitrile and diethyl ether to afford [GaL³F] as a colourless solid (0.019 g, 42%). ¹H NMR (500 MHz, DMSO-d₆): δ_H (ppm) 8.18 (t, 2H, J = 7.5 Hz, PyrH), 8.12 (d, 2H, J = 7.3 Hz, PyrH), 7.65 (d, 2H, J = 7.4 Hz, PyrH), 7.41 (m, 2H, J = 7.3 Hz, ArH), 7.34 (m, 3H, J = 7.2 Hz, ArH), 3.98 (s, 4H, CH₂), 3.61 (s, 2H, CH₂). ¹³C{¹H} NMR (126 MHz, DMSO-d₆): δ_C (ppm) = 163.0 (C=O), 154.3 (C=O), 146.6, 143.7 (PyrC), 134.2, 130.5 (ArC), 128.1 (ArC), 127.8 (PyrC), 127.4 (PyrC), 122.2 (PyrC), 59.6 (CH₂), 54.7 (CH₂). ¹⁹F NMR (470 MHz, DMSO-d₆): δ_F (ppm) = -141.2 ppm. Anal. Calcd for C₂₁H₁₇FGaN₃O₄·5H₂O: C, 45.51; H, 4.91; N, 7.58. Found: C, 45.66; H, 4.89; N, 7.59.



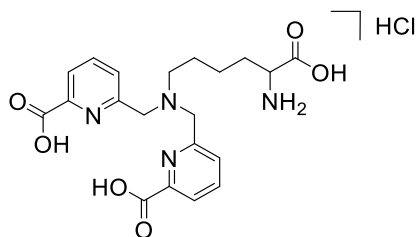
To a solution of methyl 6-(bromomethyl)picolinate (0.70 g, 3.0 mmol) in anhydrous acetonitrile (50 mL) at 60 °C under nitrogen, was added 4-(aminomethyl)benzoic acid (0.19 g, 1.3 mmol) and K_2CO_3 (1.4 g, 10 mmol). The solution was stirred for 18 h. K_2CO_3 was removed by filtration and washed with acetonitrile. The solvent was removed under reduced pressure to give yellow oil. The yellow oil was dissolved in HCl (5 M) and heated at reflux for 18 h. The colourless solid was collected by filtration and washed with cold water. Purification by semi-prep RP-HPLC yielded $H_2L^4 \cdot xHCl$ as a colourless solid (0.43 g, 81%). HR-MS(ESI/O-TOF): $[C_{22}H_{19}N_3O_6+H]^+$ m/z 422.135 (experimental), 422.135 (calculated). ^1H NMR (500 MHz, DMSO- d_6): δ_H (ppm) 8.04-7.96 (m, 4H), 7.92 (d, J = 8.1 Hz, 2H), 7.78 (dt, J = 8.6, 4.5 Hz, 4H), 4.24 (s, 6H, CH_2). $^{13}C\{^1H\}$ NMR (126 MHz, DMSO- d_6): δ_C (ppm) = 166.9 (C=O), 165.6 (C=O), 147.5, 138.7 (PyrC), 131.5 (PyrC), 129.3 (ArC), 127.7 (ArC), 124.1 (PyrC), 56.9 (CH_2), 56.7 (CH_2).

[GaL⁴Cl]



To a solution of H₂L⁴·xHCl (53 mg, 0.13 mmol) in anhydrous ethanol (50 mL) was added GaCl₃ in pentane (0.50 mL, 0.25 mmol, 0.5 M). The reaction mixture was stirred for 2 h at 60 °C under nitrogen. The precipitate was collected by filtration, washed with cold ethanol and dried *in vacuo* to give [GaL⁴Cl] as a colourless solid (32 mg, 49%). HR-MS(ESI/O-TOF): [C₂₂H₁₇GaN₃O₆]⁺ *m/z* 488.037 (experimental), 488.037 (calculated). ¹H NMR (500 MHz, DMSO-d₆): δ_H (ppm) 8.28 (t, 2H, *J* = 7.0 Hz, Pyr*H*), 8.07 (d, 2H, *J* = 6.9 Hz, Pyr*H*), 7.79 (d, 2H, *J* = 7.1 Hz, Pyr*H*), 7.65 (d, 2H, *J* = 6.9 Hz, Ar*H*), 7.43 (d, 2H, *J* = 6.9 Hz, Ar*H*), 4.19 (s, 4H, CH₂), 4.04 (s, 2H, CH₂). ¹³C{¹H} NMR (126 MHz, DMSO-d₆): δ_C (ppm) = 162.7 (C=O), 153.9, 145.9, 144.1, 130.9, 129.0, 127.7, 122.4, 57.8 (CH₂), 54.4 (CH₂). Anal. Calcd for C₂₂H₁₆ClGa₁N₃O₆·2H₂O: C, 47.22; H, 3.60; N, 7.51. Found: C, 47.76; H, 3.63; N, 6.56.

$H_2L^5 \cdot xHCl$



2-chlorotrityl polymer-bound resin beads (0.516 g) were suspended in DCM. Boc-Lys(Fmoc)-OH protected amino acid (0.241 g, 0.514 mmol) was dissolved in DCM (10 mL), and DIPEA (3 x) was added. The mixture was added to the resin beads and gently shaken overnight. The beads were washed with DCM (3 x 5 mL) to remove excess reagents and then incubated with methanol (5 mL) for 30 minutes to deactivate any unreacted resin. The resin was washed with DCM (3 x), DMF (3 x) and DCM (3 x) and allowed to dry. The Fmoc group was then cleaved with 20% piperidine in DMF (2 x 5 mL), stirring the resin for 2 x 10 min at RT, and then washed with DMF (3 x) and DCM (3 x) and allowed to dry. Methyl 6-(bromomethyl)picolinate (0.356 g, 3 eq, 1.54 mmol) was dissolved in DCM (5 mL) and DIPEA (3 eq.). The solution was added to the resin and left shaking overnight. Cleavage of compound (**37**) from the resin beads was performed using general method 1.

HR-MS(ESI/O-TOF): $[C_{22}H_{28}N_4O_6+H]^+$ m/z 445.209 (experimental), 445.209 (calculated). ^1H NMR (500 MHz, DMSO- d_6): δ_H (ppm) 7.86 (d, $J = 7.7$ Hz, 2H, PyrH), 7.79 (t, $J = 7.7$ Hz, 2H, PyrH), 7.45 (d, $J = 7.5$ Hz, 2H, PyrH), 4.68 (s, 4H, CH_2), 3.92 (s, 6H, CH_3), 3.86 (m, 1H, $-NH_2CH$), 3.12 – 3.03 (m, 2H, $-NH_2CH_2$), 1.90 – 1.29 (m, 6H, CH_2). $^{13}C\{^1H\}$ NMR (126 MHz, DMSO- d_6): δ_C (ppm) = 174.1, 171.0, 164.7, 162.4, 151.3, 147.2, 138.9, 128.7, 124.9, 56.3, 54.2, 53.3, 52.7, 51.9, 35.8, 30.8, 30.3, 29.5, 28.2, 23.0, 21.7.

The compound (**37**) dissolved in HCl (5 M, 15 mL) and heated at reflux for 18 h. A white solid that formed was collected by filtration and washed with cold water to give H_2L^5 as the HCl salt (0.14 g, 61%). HR-MS(ESI/O-TOF): $[\text{C}_{20}\text{H}_{24}\text{N}_4\text{O}_6+\text{H}]^+$ m/z 417.177 (experimental), 417.177 (calculated). ^1H NMR (500 MHz, DMSO- d_6): δ_{H} (ppm) 8.00 (d, $J = 6.8$ Hz, 4H, PyrH), 7.81 (d, $J = 8.6$ Hz, 2H, PyrH), 4.69 (s, 4H, CH_2), 3.85 (s, 1H, $-\text{NH}_2\text{CH}$), 3.32 (d, 2H, $-\text{NH}_2\text{CH}_2$), 1.92-1.21 (s, 6H, CH_2). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ_{C} (ppm) = 171.3, 165.7, 151.12, 147.84 139.4, 128.7, 124.8, 56.8, 54.7, 52.0, 34.5, 31.2, 29.8, 23.1, 22.0.

Attempted radiolabelling. [^{18}F][GaL ^{3} F]. Method A: To a solution of [GaL ^{3} Cl] (1.0 mg, 2.1 μmol) in sodium acetate buffer (1 mM, pH 4.0, 600 μL) was added a solution of $^{18}\text{F}[\text{F}^-]$ (500 MBq, ~ 25 nM) in water. The reaction was incubated for 30 min at 80 $^\circ\text{C}$. The radiolabelled complex was analysed by RP-HPLC (elution gradient 0-95% B (acetonitrile) in A (Milli-Q H_2O) over 15 min, 1 mL/min).

Method B: $^{18}\text{F}[\text{F}^-]$ (~ 500 MBq) trapped on the QMA cartridge was eluted with potassium bicarbonate (2.0 mg, 20 μmol) and [2,2,2]Kryptofix (6.3 mg, 16 μmol) in water:acetonitrile (1 mL, 1:4). The azeotropically dried $^{18}\text{F}[\text{F}^-]$ was removed from the iPHASE Flexlab module and was then dissolved in acetonitrile (400 μL) and a minimal amount of water (50 μL). To this was added [GaL ^{3} Cl] (1.7 mg, 3.5 μmol , 200 μL in acetonitrile) and the reaction was incubated for 30 min at 80 $^\circ\text{C}$. An aliquot of the diluted reaction mixture (100 μL , 2.08 MBq) was analysed by RP-HPLC (elution gradient 0-95% B (acetonitrile) in A (Milli-Q H_2O) over 15 min, 1 mL/min). [^{18}F][GaL ^{3} F] $R_t = 12.6$ min; unreacted [^{18}F] F^- $R_t = 2.0$ min (recovered 2.00 MBq, $\sim 96\%$).

Chapter 3 A Single Versatile Ligand for the Incorporation of Three Radionuclides for the Application of Alzheimer's Disease

3.1 The chemistry of copper-64 and gallium-68 and their commonly used chelators

In this chapter, a new ligand is prepared that has the potential to be radiolabelled with three different radionuclides, fluorine-18, copper-64 and gallium-68. The properties of the positron-emitting radioisotopes (copper-64 and gallium-68) are described. There are four different isotopes of copper (copper-60, copper-61, copper-62 and copper-64) that decay via positron emission.¹⁶⁶ Copper-64 is produced in a cyclotron by bombardment of an enriched nickel target $^{64}\text{Ni}(\text{p},\text{n})^{64}\text{Cu}$. It has a positron decay ratio of 19%, with 40% β^- emission and 41% electron capture, making it suitable for PET applications. The positron-emitting radionuclide copper-64 ($t_{1/2} = 12.7$ h) has a longer half-life compared to fluorine-18 and gallium-68. The longer half-life permits enough time for transportation of radiotracers from the radiolabelling sites to imaging facilities.

Gallium-68 (half-life = 67.7 min) is produced from a $^{68}\text{Ge}/^{68}\text{Ga}$ generator via the (p, 2n) reaction, decays by positron emission (89%) and electron capture (11%) (Figure 3.1). The cost-effective source of the isotope and the long half-life of germanium-68 (half-life = 270.8 days) makes gallium-68 an ideal choice for some PET imaging agents.

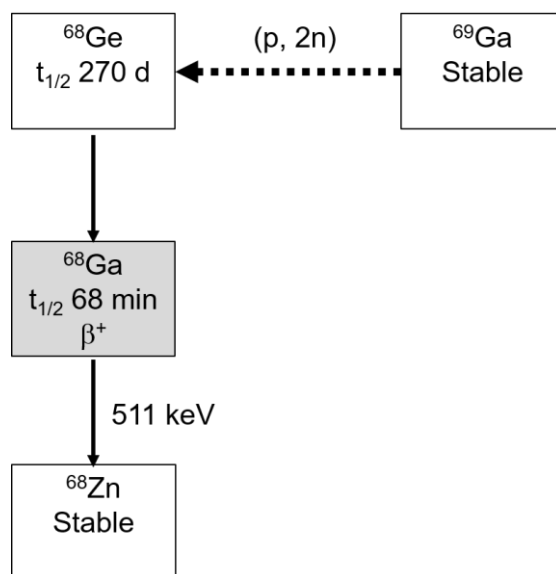


Figure 3.1. Production of gallium-68 by the decay of germanium-68.

The search for different chelators specific to different radionuclides can be simplified by using a chelate system that is capable of labelling with both copper-64 and gallium-68. The Ga^{3+} complex can be used to label with fluorine-18 through the formation of a $\text{Ga}^{3+}\text{-F}^-$ bond. In search of this universal chelator, a macrocyclic ligand featuring nitrogen and oxygen donor atoms is of interest. The commonly used bifunctional chelators for copper-64 and gallium-68 labelling are 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) and 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) (Figure 3.2). The radiolabelling with DOTA requires high temperatures above 100 °C whereas NOTA chelator can be labelled at room temperature.

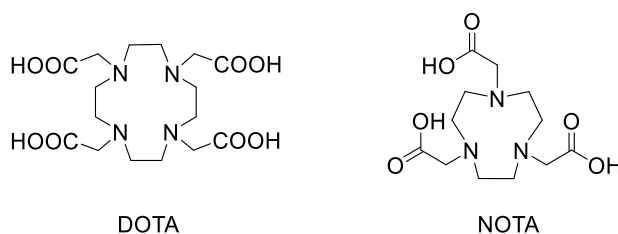


Figure 3.2. Commonly used bifunctional chelators, DOTA and NOTA for copper-64 and gallium-68 labelling.

The azamacrocyclic ligands exhibit the “macrocyclic effect” that leads to Cu^{2+} and Ga^{3+} complexes of enhanced thermodynamic stability and kinetic inertness when compared to acyclic ligands.¹⁶⁷ Consequently, this class of ligand is an attractive framework to generate bifunctional chelators for radiopharmaceutical applications. For example, $[\text{}^{68}\text{Ga}][\text{Ga-DOTATATE}]$ is an FDA approved PET imaging agent for neuroendocrine tumours (Figure 3.3).¹⁶⁸⁻¹⁶⁹

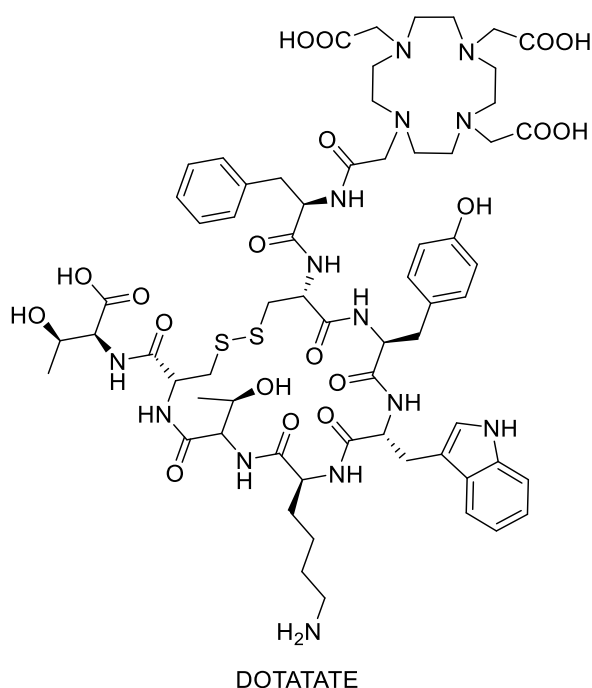


Figure 3.3. Chemical structure of DOTATATE.

The NOTA chelators are attractive candidates as the labelling temperature is more favourable than DOTA. As previously discussed in Chapter 1.6.4, the NOTA ligand is a triazacyclononane (TACN) derivative with three carboxylic acid groups on the side arms attached to the nitrogen atoms. The two main structural functionalisation to incorporate target molecules on this TACN-based model is either *via* one of the pendant carboxylate arms (Figure 3.4a) or built into the backbone of the chelator on the carbon atom (Figure 3.4b).

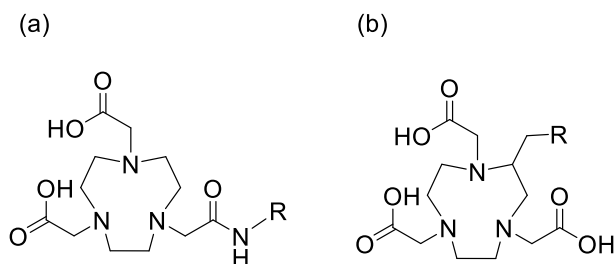


Figure 3.4. NOTA chelators with functionalisation via one of the pendant carboxylate arms (a) or built into the backbone of the chelator (b).

Functionalisation on one of the pendant groups to incorporate biomolecules has led to the development of bifunctional chelators for radiopharmaceutical applications. For example, a neurokinin 1 receptor (NK1R) antagonist attached to a NOTA ligand *via* one of the pendant arms can be labelled with either copper-64 (Figure 3.5 a) or gallium-67 (Figure 3.5 b) for *in vivo* targeting of NK1R-positive tumour xenografts.¹⁷⁰

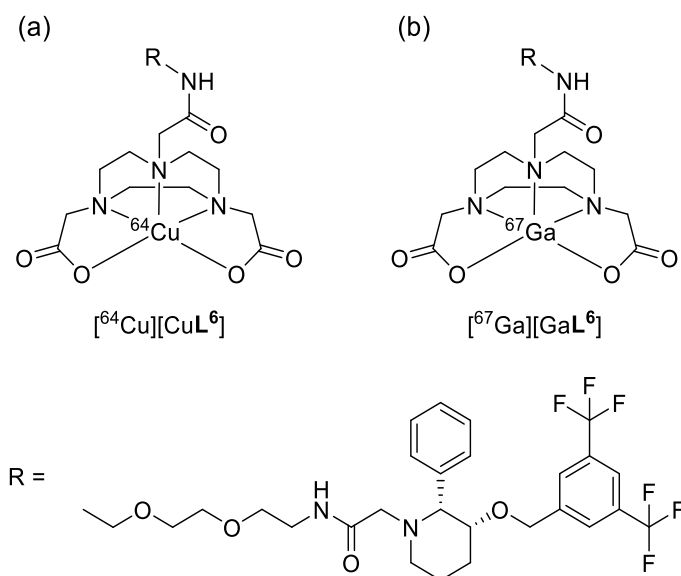


Figure 3.5. Chemical structure of a neurokinin 1 receptor (NK1R) antagonist attached to a NOTA ligand used to label two radionuclides, copper-64 and gallium-67.¹⁷⁰

The 1,4,7-triazacyclononane-1,4-diacetic acid (NODA) chelator has an N_3O_2 coordination with one less pendant carboxylic acid compared to the NOTA chelator. This chelator forms five-coordinate complexes with $^{64}\text{CuCu}^{2+}$ and $^{68}\text{GaGa}^{3+}$. For example, a glucagon-like peptide-1 agonist conjugated to a NODA chelator (L^7) is labelled with copper-64 and gallium-68 as potential PET imaging agents specific for glucagon-like peptide-1 receptor (Figure 3.6).¹⁷¹ The 39-amino acid glucagon-like peptide-1 agonist is used to target glucagon-like peptide-1 receptor to quantify pancreatic β cell mass for hyperglycemia monitoring in diabetic patients.

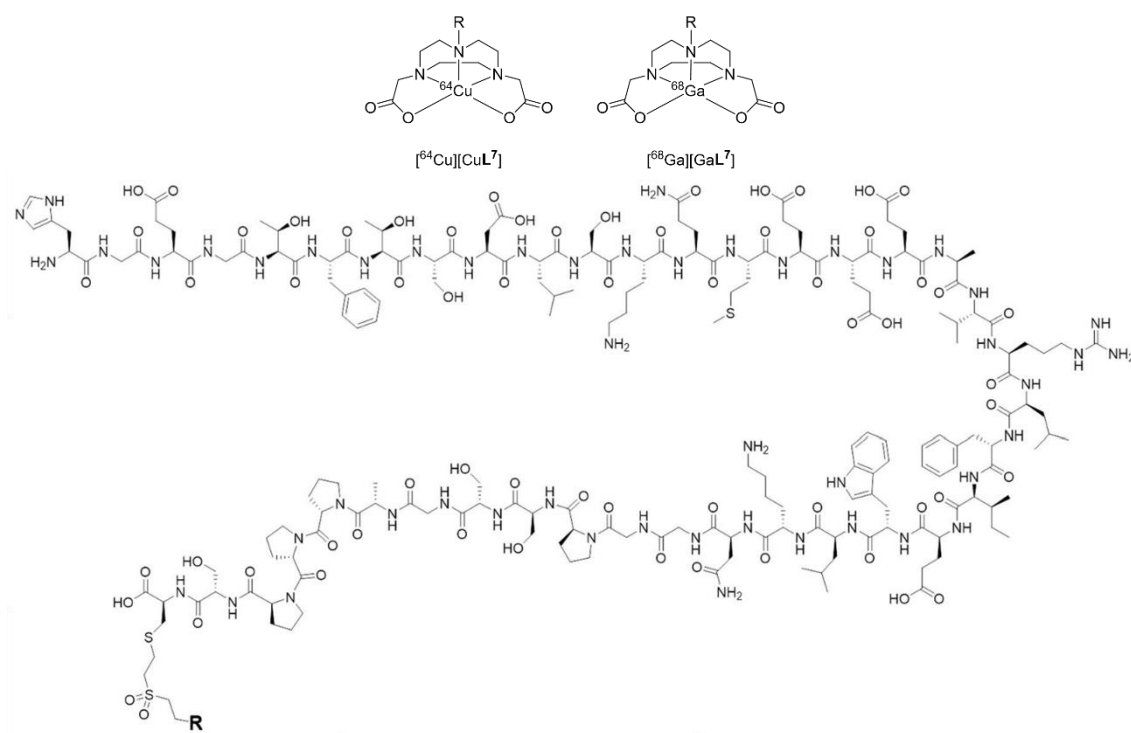


Figure 3.6. Chemical structure of a glucagon-like peptide-1 receptor agonists attached to a NODA chelator, L^7 used to label two radionuclides, copper-64 and gallium-68. Reprinted with permission from Bandara, N.; Zheleznyak, A.; Cherukuri, K.; Griffith, D. A.; Limberakis, C.; Tess, D. A.; Jianqing, C.; Waterhouse, R.; Lapi, S. E., *Mol. Imaging Biol.* **2016**, 18 (1), 90-98.¹⁷¹

Another example shows a NODA chelator conjugated to a Gastrin-releasing peptide receptor (GRPR) antagonist labelled with gallium-68 and fluorine-18 (via Al^{3+} complex) as potential imaging agent for prostate cancer (Figure 3.7).¹⁷²

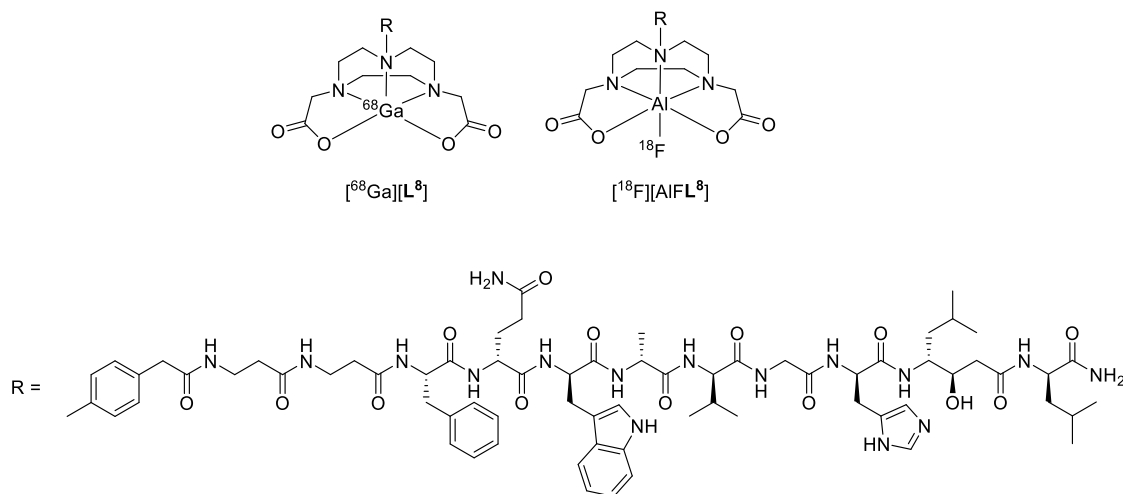


Figure 3.7. Chemical structure of NODA chelator conjugated to a GRPR antagonist, L^8 used to label two radionuclides, fluorine-18 and gallium-68.¹⁷²

These radiotracers proved the versatility of a TACN-based chelator to label with more than one radionuclide. The NODA chelator with one less pendant carboxylic acid compared to the NOTA chelator is more advantageous for our purpose of fluorine-18 labelling with a Ga^{3+} complex. This is because the presence of a third oxygen atom at close proximity can bind to the metal centre to form a six-coordinate complex. The absence of the oxygen atom removes any competition with the fluorine atom to bind to the metal centre. The work presented in this chapter shows the development of a single versatile ligand that incorporates a molecule that can target the amyloid plaque that is associated with Alzheimer's Disease.

3.2 Alzheimer's Disease

Alzheimer's Disease (AD) is the most common form of degenerative dementia, accounting for 60-70 % of cases. In 2016, it had affected over 43 million people worldwide and is predicted to grow to 106 million by 2050.¹⁷³⁻¹⁷⁴ It is clinically characterized by memory loss in the early stage which progresses to complete loss of cognition in the later stage. A complete understanding of the molecular nature of the disease remains elusive.

3.3 Protein aggregation and amyloid- β plaques

The amyloid cascade hypothesis proposes that the deposition of amyloid- β ($A\beta$), a molecular constituent of senile plaques is the initial pathological event in AD.¹⁷⁵⁻¹⁷⁷ The characteristic pathological hallmarks of AD feature the extracellular senile plaques constituted of the insoluble aggregated $A\beta$ plaques (Figure 3.8) and intracellular neurofibrillary tangles composed of hyperphosphorylated tubulin associating unit (tau) in the brain.¹⁷⁸⁻¹⁸⁰ The $A\beta$ peptide is identified as the main component of the senile plaques, and the sequence was discovered in 1984.¹⁸¹ Although the hypothesis has not been uniformly accepted, the amyloid cascade hypothesis has played a significant role in describing the pathogenesis of AD, and has since dominated the research in developing therapeutic approaches that target $A\beta$ plaques and potentially slow the onset of AD.¹⁸²

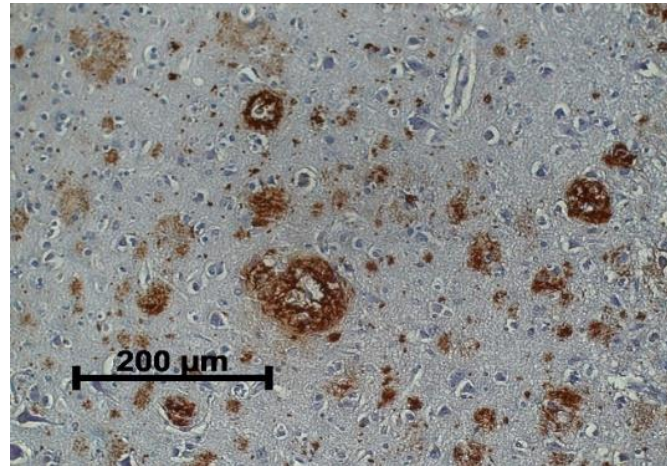


Figure 3.8. Human tissue section from the frontal cortex of an AD affected brain immunohistochemically stained with anti- $A\beta_{1-40/42}$ 1E8 antibody visualised at 10 x magnification.

The precise role of $A\beta$ plaques in AD is uncertain, though there is evidence of extensive $A\beta$ deposition in post-mortem analysis of AD patients that provides a definite diagnosis of the disease.¹⁸³⁻¹⁸⁵ The $A\beta$ peptide consists of 39-43 amino acid residues and is produced by cleavage of the amyloid precursor protein (APP), which is a transmembrane protein found in the body (Figure 3.9).¹⁸⁶ The genetic mutations in the APP gene were linked to early-onset familial AD (FAD).¹⁸⁷⁻¹⁸⁸

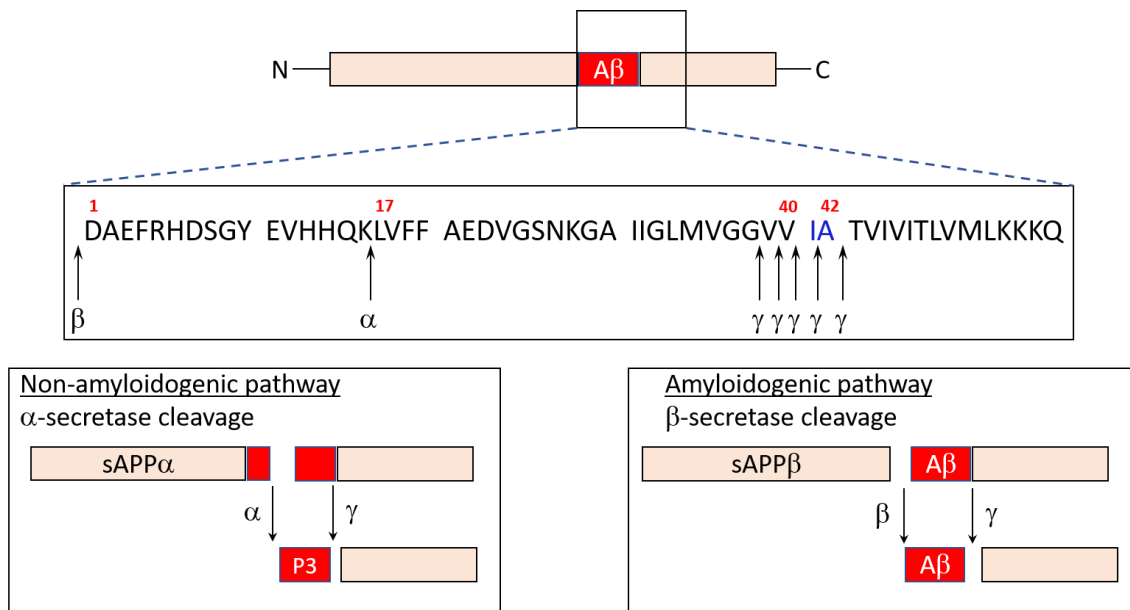


Figure 3.9. A schematic diagram showing the APP processing pathways and the sequence of the Aβ peptide.

APP is processed via two pathways, the amyloidogenic and the non-amyloidogenic pathway. The non-amyloidogenic pathway is cleaved by α -secretase, generating a soluble APP fragment of the N-terminal ectodomain (sAPP α) and the 83 amino-acid C-terminal membrane fragment (C83). This is then further cleaved by γ -secretase to produce the non-pathogenic P3 peptide (A $\beta_{17-40/42}$) and APP intracellular domain.¹⁸⁹⁻¹⁹⁰

The amyloidogenic pathway occurs when the APP is cleaved by β -secretase instead of α -secretase. Firstly, a β -secretase known as β APP cleavage enzyme (BACE-1) cleaves at ASP-1, resulting in the production of sAPP β and a second C-terminal membrane fragment. It is then cleaved by γ -secretase at various positions, resulting in A β peptides of various length, predominantly A β_{1-40} and A β_{1-42} .¹⁹¹⁻¹⁹² A $\beta_{1-40/42}$ contains two hydrophobic sections; between residues 17-21 and the C-terminal (residues 29-40 or 29-42) domain. The two additional hydrophobic residues (Ile41 and Ala42) at position 41 and 42 increases the toxicity of the A β peptide.¹⁹³

The $A\beta_{1-40/42}$ monomer is an intrinsically disordered peptide that adopts a variety of conformations in an aqueous environment.¹⁹⁴ Two monomers can interact to form dimers which can then form tetramers (Figure 3.10). The $A\beta_{1-42}$ oligomers undergo fibrillation, where the fibrils usually composed of two to six twisted protofibrils. These fibrils accumulate to form $A\beta$ plaques. There are some suggestions that the soluble oligomeric species formed before the formation of $A\beta_{1-42}$ fibrils are the toxic species.¹⁹⁵⁻¹⁹⁷

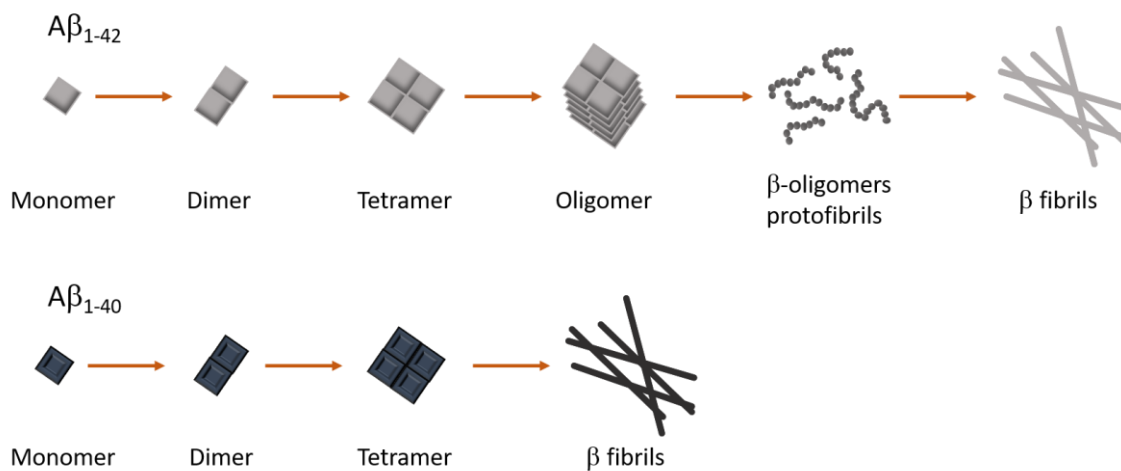


Figure 3.10. The stages of the formation of $A\beta_{1-40}$ and $A\beta_{1-42}$ fibrils from the cleavage of the APP by β -secretase and γ -secretase.

Solid-state nuclear magnetic resonance (ssNMR) spectroscopy revealed that $A\beta_{1-40}$ oligomers exhibit mixed parallel and antiparallel β -sheet structure, whereas the fibrils only have a parallel β -sheet structure.¹⁹⁸ These β -sheets are usually in pairs, creating a double-layered β -sheet. The amyloid fibril has repeating units of β -strands perpendicular to the long axes of the fibrils (Figure 3.11). Contrary to $A\beta_{1-40}$, the ssNMR spectroscopy showed that $A\beta_{1-42}$ has a triple parallel β -sheet segments.¹⁹⁹

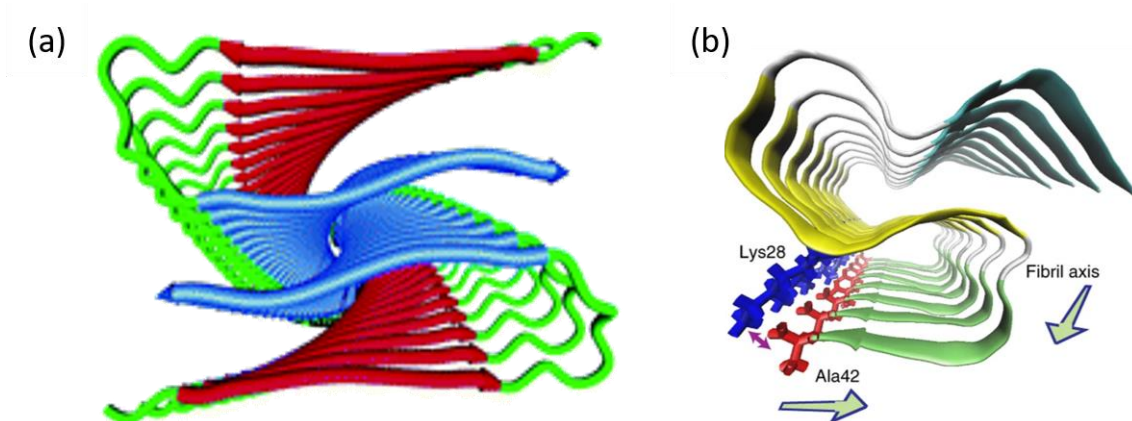


Figure 3.11. Molecular model of an $A\beta_{1-40}$ fibril, parallel to the z axis (a) and $A\beta_{1-42}$ fibril (b). Reprinted with permission from Petkova, A. T.; Yau, W.; Tycko, R. *Biochemistry*, 2006, 45, 498 and Xiao, Y.; Ma, B.; McElheny, D. et al., *Nat. Struct. Mol. Biol.*, 2015, 22, 499.

The parallel orientation of β -sheet structures gives rise to channels and hydrophobic pockets to which aromatic molecules can intercalate and bind *via* π - π interactions. As the misfolding aggregates of $A\beta_{1-42}$ fibrils are thought to be the causative agent of AD, the β -sheets assembling of $A\beta$ fibrils provided an insight into the structures that is useful in the design of PET imaging agents that target the fibrils.

3.4 Development of radiopharmaceuticals designed to bind to $A\beta$ plaques

Clinical diagnosis of AD relies on cognitive testing, which has limited accuracy, especially in the early stages.²⁰⁰ Challenges remain in earlier and differential diagnosis of AD from other forms of neurodegenerative dementia. The abnormally low uptake of [^{18}F]FDG is a consistent distribution pattern seen in AD patients' brains particularly in the lateral temporal-parietal cortex, posterior cingulate cortex and precuneus regions, reflecting an impairment in synaptic function (Figure 3.12c).²⁰¹⁻²⁰²

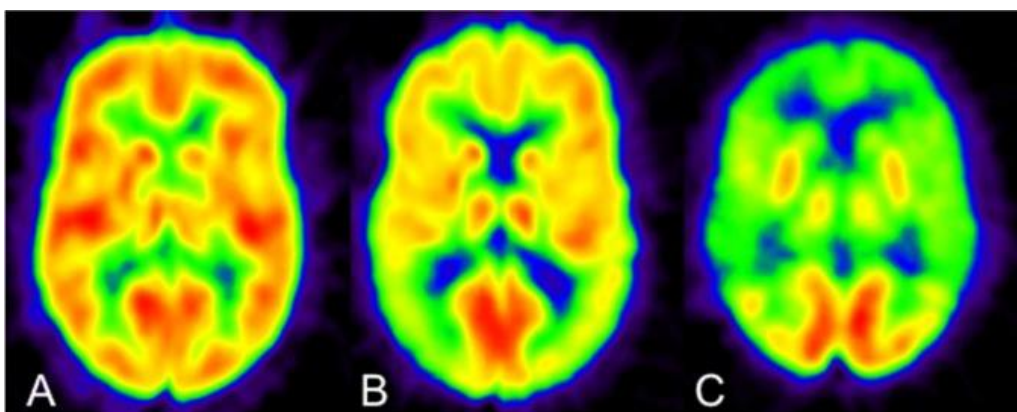


Figure 3.12. PET images of human brains with [^{18}F]FDG of a cognitively normal subject (A); of a patient with suspected early AD (B) and of a patient with clinically diagnosed AD (C). Reproduced with permission from Rice, L.; Bisdas, S. *Eur. J. Radiol.*, 2017, 94, 16.

There is a need for an accurate diagnosis of AD ante-mortem to assist in the design and evaluation of potential therapies. Molecular imaging modalities such as MRI and PET have been utilised for brain imaging based on the amyloid cascade hypothesis by using tracers that target the A β plaques as biomarkers for AD.²⁰³ Post-mortem studies reveal that A β plaques are most abundant in the frontal cortex, lateral parietal and temporal regions. In contrast, neurofibrillary tangles are present in the medial temporal areas, including the hippocampi.²⁰⁴ Radiotracers that can cross the blood-brain barrier and possess the ability to identify the burden of A β plaques in a non-invasive manner may assist our understanding of AD. Considerable effort has been put into developing amyloid plaque targeting imaging agents in the last two decades.

The molecules Congo Red and thioflavin T (2-(4-methylaminophenyl)-6-hydroxybenzothiazole, ThT) are amyloid-binding histological dyes that have been used to study the deposition of A β plaques *in vitro* (Figure 3.13).

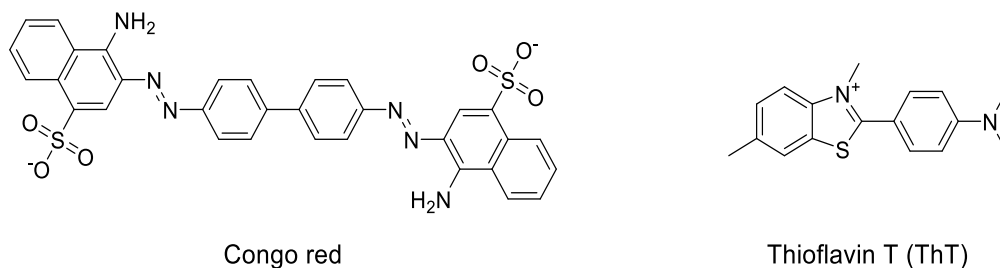


Figure 3.13. Chemical structures of Congo red and Thioflavin T (ThT).

It is proposed that these compounds contain aromatic molecules that interact with the β -sheets mainly *via* hydrophobic and π - π interactions.²⁰⁵⁻²⁰⁶ Simulated molecular dynamic-calculations show that Congo red sits diagonally across the top and the bottom of the fibril (Figure 3.14). The scale measures the binding energetic contributions of the molecule to the fibrils. The negative charges in Congo red also contribute to the electrostatic interactions between the molecule and the fibrils. The sulphate and amino groups interact through hydrogen bonding with the serine and asparagine side chains of the fibrils.²⁰⁷ The positive charge in ThT also prevents the molecule from entering into the groove. Many binding sites of ThT have been proposed but the most prominent are the two binding modes where one is parallel with the normal of the β -sheet plane, and one where ThT is positioned at the fibril ends. Despite the high affinity to A β fibrils, Congo red and ThT do not have the ability to cross the blood-brain barrier most likely due to the low lipophilicity (c log P of Congo Red = - 0.2, c log P of ThT = 0.16).

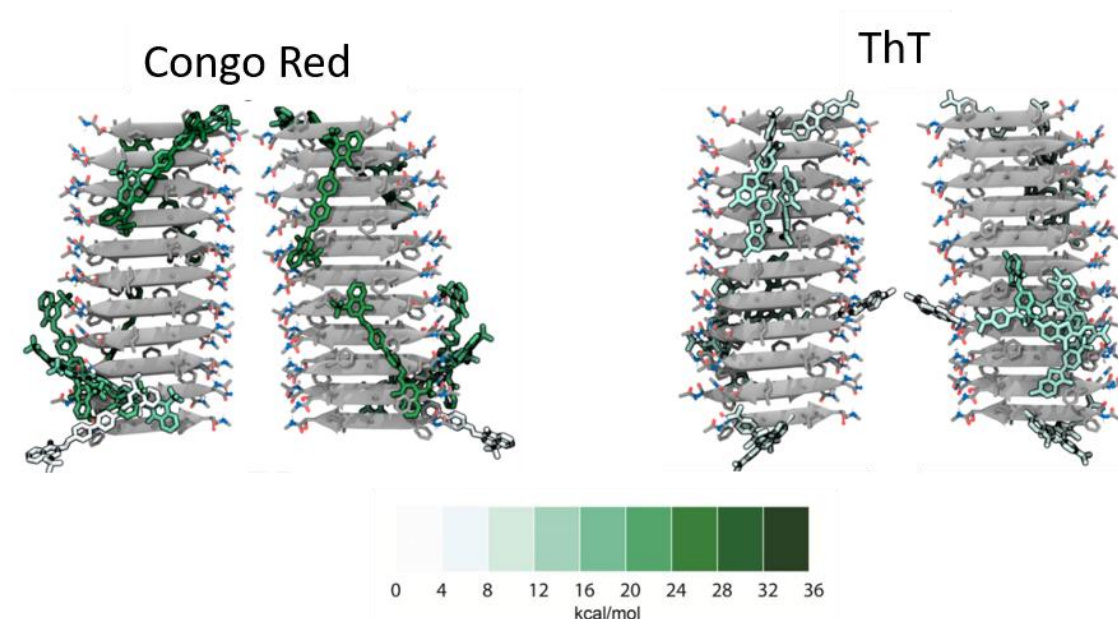


Figure 3.14. Representative structures of the interaction between the A β_{1-40} fibrils and Congo red (left) and ThT (right), as a measure of free energies (kcal/mol). Reprinted with permission from Skeby, K. K.; Soerensen, J.; Schioett, B. *J. Am. Chem. Soc.*, 2013, 135, 15114. Copyright (2013) American Chemical Society.

While these compounds did not meet the requirement to be used as amyloid plaque targeting imaging agent, they have provided crucial structural inspiration for the development of radioactive tracers that have been used for assessing the A β plaque burden in AD patients. The common characteristics of the amyloid plaque targeting groups are; low molecular weight, lipophilicity, planarity and have conjugated aromatic ring systems. The structures are designed to allow for interactions in the hydrophobic grooves parallel to the fibril axis as well as the side chains of the fibril to increase the binding affinity. A variety of benzothiazole, stilbene and styrylpyridine derivatives are excellent examples that possess this common structural motif which allows them to bind to A β plaques.^{78-79,}

The derivative of Congo Red

The compound Chrysamine G was designed based on the molecular structure of Congo Red where the amino-naphthalene sulfonate groups are replaced with salicylic acid moieties to increase the lipophilicity (Figure 3.15).²¹⁰⁻²¹¹ The compound reported higher lipophilicity as measured by octanol/PBS (pH 7.4) distribution coefficient (D) with log D value of 1.8 for Chrysamine G versus -0.2 for Congo Red but unfortunately showed no increase in uptake in brain in mouse models.²¹²

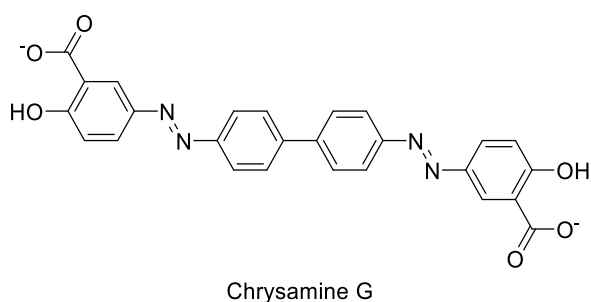


Figure 3.15. Chemical structures of Chrysamine G.

Benzothiazole and stilbene derivatives

Several other tracers have been developed that are able to pass through the blood-brain barrier. The benzothiazole-aniline derivatives, BTA-1 and 6-Me-BTA-1 were developed based on the structural motif of ThT, where the positively charged *N*-methyl group on the thiazolyl was removed (Figure 3.16). These derivatives, labelled with carbon-11 (half-life = 20 minutes), penetrate the blood-brain barrier and bind to A β fibrils with higher affinity compared to ThT.²¹³⁻²¹⁶ The carbon-11 labelled 2-(4-methylaminophenyl)-6-hydroxybenzothiazole or Pittsburgh compound B (PiB), [¹¹C]PiB crosses the blood-brain barrier, binds preferentially to A β fibrils but not to soluble A β or diffused amyloid plaques and shows increased retention in the frontal cortex of AD patients (Figure 3.16).²¹⁷⁻²¹⁹ These properties proved its potential as a promising PET imaging agent for the diagnosis of AD. One drawback is the high degree of non-specific grey matter binding of [¹¹C]PiB

in the brain, which results in lower signal-to-background ratio. Besides, the short half-life of carbon-11 may place practical limitations on the use of [^{11}C]PiB in clinical settings.

Due to the longer half-life, fluorine-18 labelled radiotracers were developed to extend the time for the transportation of tracers to medical facilities lacking generators or cyclotrons. The structurally related tracers [^{11}C]PiB and [^{18}F]flutemetamol have similar uptake in brain and affinity for A β .²⁰⁸ A common and distinct characteristic of [^{18}F]flutemetamol is the high degree of non-specific binding to white matter which also results in low signal-to-background ratio.

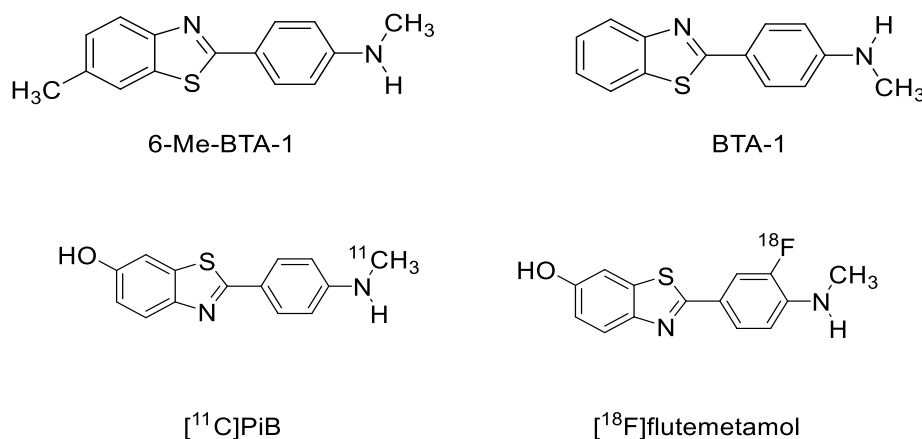


Figure 3.16. Chemical structures of imaging agents derived from benzothiazole motif.

Two other classes of structures that have shown potential to bind to amyloid plaques are the stilbene and styrylpyridine. The stilbene compound, *N*-[^{11}C]methylamino-4'-hydroxystilbene [^{11}C]SB-13 has a planar aromatic structure that binds to the β -sheets of A β fibrils *via* π - π interactions (Figure 3.17).²²⁰ The electron-donating groups such as -OH and -N(CH_3)₂ also increase the binding affinity for A β aggregates.

Further modification of the stilbene at the 4' position of the rings led to the development of the FDA-approved fluorine-18 imaging agent [^{18}F]florbetaben (AV-1) which incorporates an oligoethylene linker to separate the radioisotope from the targeting

moiety (Figure 3.17).⁷⁸ The length of the linker chain can be varied to modify the log *D* values to improve the blood-brain barrier permeability, with *n* = 3 giving the best uptake in brain. AV-1 has high specific binding to human post-mortem AD brain homogenates (*K_i* = 2.2 nM). Another FDA-approved imaging agent is [¹⁸F]florbetapir (AV-45) which replaces a phenyl ring on [¹⁸F]florbetaben with a pyridyl to form a styrylpyridine (Figure 3.17).⁷⁹ [¹⁸F]florbetapir exhibits high A β binding affinity, improved rapid brain penetration and clearance compared to the stilbene derivative.^{209, 221-223}

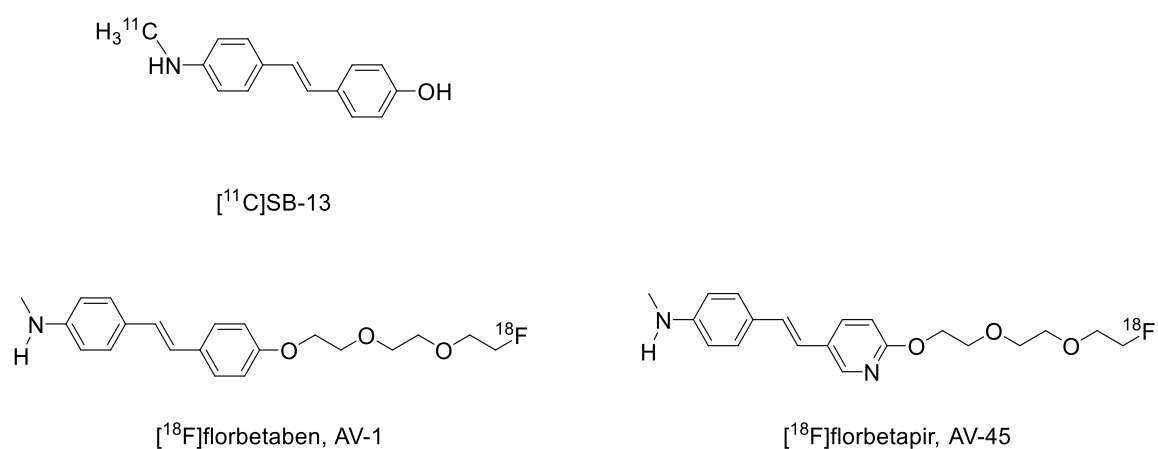


Figure 3.17. Chemical structures of stilbene and styrylpyridine derivative imaging agents.

Two notable imaging agents, [¹¹C]AZD2184 and [¹⁸F]AZD4694, developed based on the structural motif of [¹¹C]PiB showed promising results with lower white matter binding compared to [¹³C]PiB (Figure 3.18). The low non-specific binding of [¹⁸F]AZD4694 to white matter in transgenic mice shows improved specificity to A β which increases the signal-to-background ratio.^{86, 224} [¹⁸F]AZD4694 is currently undergoing phase III clinical trials for the diagnosis of AD.

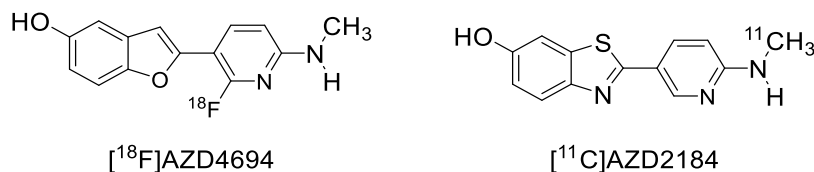


Figure 3.18. Chemical structures of $[^{18}\text{F}]\text{AZD4694}$ and $[^{11}\text{C}]\text{AZD2184}$.

Alternative to the conventional C- ^{18}F radiotracers, Cui and co-workers developed a fluorine-18 labelled Al^{3+} 2-phenylbenzothiazole complex conjugated to NODA via alkyl linkers. This radiotracer displayed insufficient blood-brain barrier permeability (brain = 0.3% ID/g at 2 min) for brain imaging applications but may find application in imaging cerebrovascular amyloid in cerebral amyloid angiopathy (CAA) (Figure 3.19).²²⁵

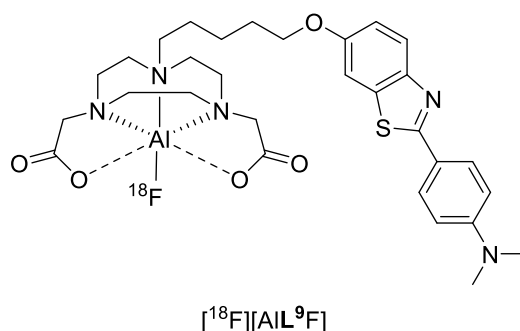


Figure 3.19. Chemical structure of a fluorine-18 labelled Al^{3+} NODA-2-phenylbenzothiazole complex.

Apart from fluorine-18 labelled PET imaging agents, copper-64 can be used to label bifunctional chelators conjugated to amyloid plaque targeting groups in the development of neuroimaging agents. For example, a bis(thiosemicarbazone) ligand with appending stilbene-based functional group (L^{10}) and a thiosemicarbazone-pyridylhydrazine ligand that incorporates a styryl-based plaque targeting functional group (L^{11}) were labelled with copper-64 with high radiochemical yields (Figure 3.20).²²⁶⁻²²⁷ Both $[\text{CuL}^{10}]$ and $[\text{CuL}^{11}]$ bind to $\text{A}\beta$ plaques with high affinity. The uptake in brain in wild-type mouse models was $1.7 \pm 0.6\%$ ID/g at 7 min post-injection for $[^{64}\text{Cu}][\text{CuL}^{10}]$ and $1.11 \pm 0.20\%$ ID/g at 5 min

post-injection for $[^{64}\text{Cu}][\text{CuL}^{11}]$. These $[^{64}\text{Cu}][\text{Cu}^{2+}]$ complexes are potential non-invasive diagnostic PET imaging agents and provided an insight into the strategy to incorporate plaque targeting group onto a Cu^{2+} complex.

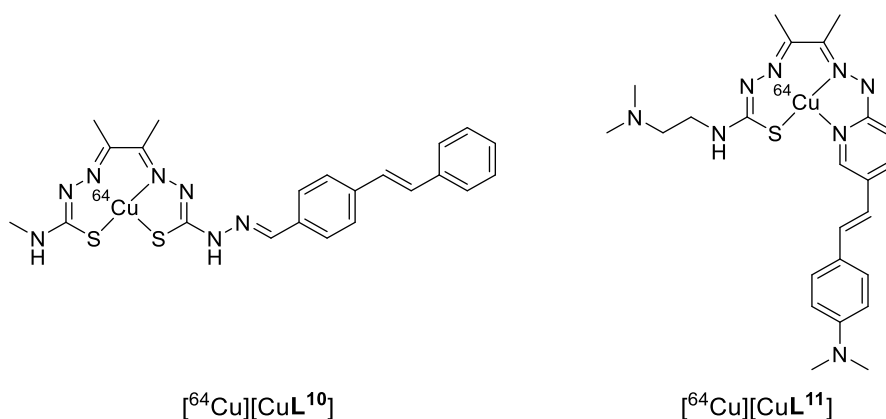
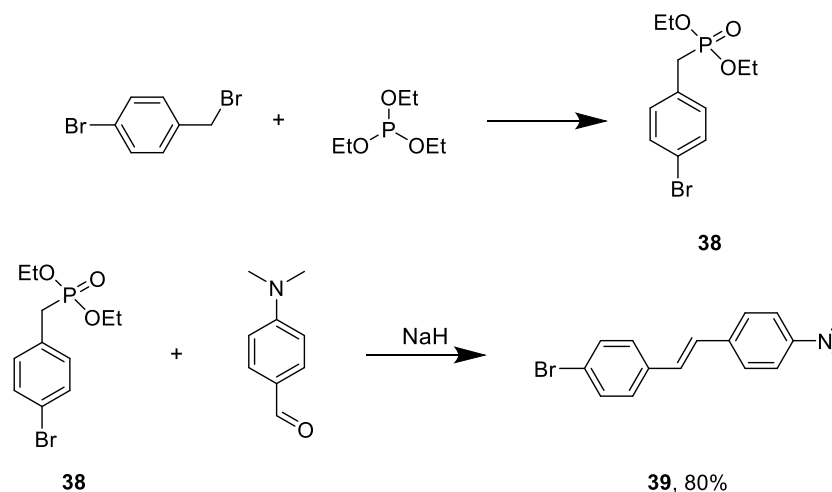


Figure 3.20. $[^{64}\text{Cu}]\text{Cu}^{2+}$ complexes with appended plaque targeting groups as neuroimaging agents.

Preliminary studies of $[^{11}\text{C}]\text{SB-13}$ and the $[^{64}\text{Cu}][\text{Cu}^{2+}]$ complexes, $[\text{CuL}^{10}]$ and $[\text{CuL}^{11}]$ were promising and sparked our interest in using stilbene derivatives as probes for the detection of amyloid plaques. Stilbene derivatives bind to $\text{A}\beta$ plaques and have less white matter binding than benzothiazole derivatives.²²⁸ Its selectivity for amyloid plaques over neurofibrillary tangles and Lewy bodies makes stilbene an attractive choice. The work presented in this chapter shows the development of a single versatile ligand that incorporates a molecule that can target the amyloid plaque that is associated with AD. Hence, we propose to prepare a NODA derivative featuring a pendant stilbene functional group. An alkyl linker between the ligand backbone and the stilbene targeting group hopefully minimises interference of the metal complex with the $\text{A}\beta$ plaques binding ability.

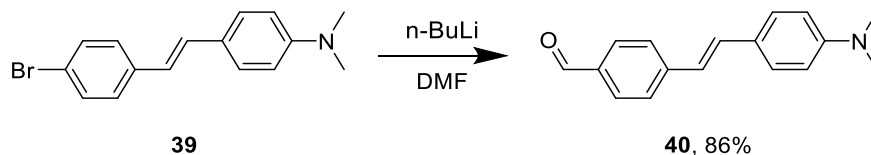
3.5 Synthesis of H_2L^{12} , $[GaL^{12}Cl]$ and $[GaL^{12}F]$ complexes and structural determination by nuclear magnetic resonance spectroscopy

A stilbene derivative with a bromomethyl functional group was needed in order to react with the secondary amine of the NODA ligand. Firstly, the compound (*E*)-4-(4-bromostyryl)-*N,N*-dimethylaniline (**39**) was synthesised according to the literature procedure by Wadsworth-Emmons-Horner reaction between diethyl-4-bromobenzylphosphate (**38**) and 4-*N,N*-dimethylaminobenzaldehyde (Scheme 3.1).²²⁹



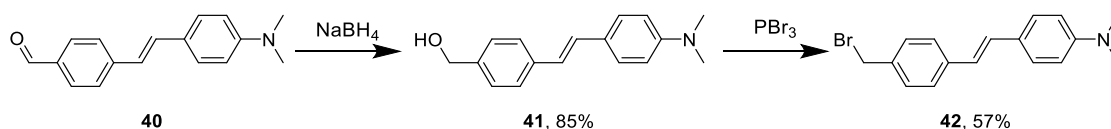
Scheme 3.1. Synthesis of (*E*)-4-(4-bromostyryl)-*N,N*-dimethylaniline (**39**).

The compound (*E*)-4'-dimethylamino-4-stilbenecarboxyaldehyde (**40**) was synthesised according to literature procedure by reacting (*E*)-4-(4-bromostyryl)-*N,N*-dimethylaniline (**39**) with *n*-butyl lithium and DMF in THF at -78 °C (Scheme 3.2).²²⁹



Scheme 3.2. Synthesis of (*E*)-4'-dimethylamino-4-stilbenecarboxyaldehyde (**40**).

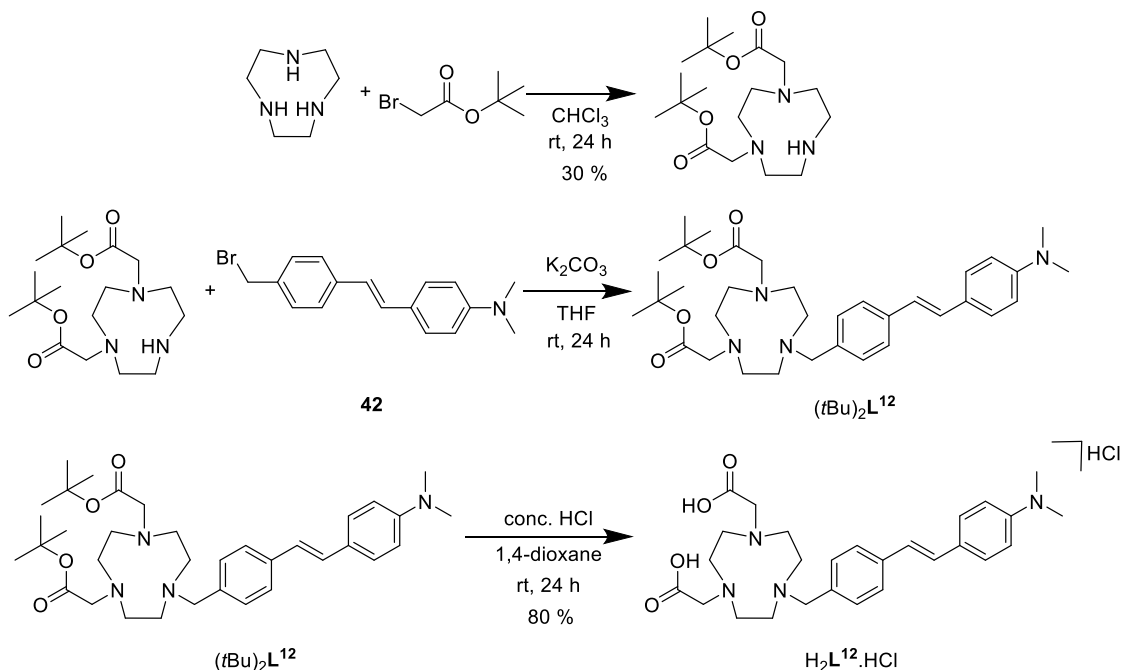
Reduction of (**40**) with sodium borohydride and methanol in THF gave the compound (*E*)-(4-(4-(dimethylamino)styryl)phenyl)methanol (**41**). Treatment of (**41**) with phosphorus tribromide gave (*E*)-4-(4-(bromomethyl)styryl)-*N,N*-dimethylaniline (**42**) (Scheme 3.3).



Scheme 3.3. Synthesis of (*E*)-4-(4-(bromomethyl)styryl)-*N,N*-dimethylaniline (**42**).

The ^1H NMR spectrum of compound (*E*)-4-(4-(bromomethyl)styryl)-*N,N*-dimethylaniline (**42**) showed similar peaks to (*E*)-4'-dimethylamino-4-stilbenecarboxyaldehyde (**40**) and (*E*)-(4-(4-(dimethylamino)styryl)phenyl)methanol (**41**) between $\delta_{\text{H}} = 7.7$ and 6.7 ppm. The resonances at $\delta_{\text{H}} = 7.44$, 7.35 and 6.81 ppm were assigned to the aromatic hydrogen atoms. The resonances at $\delta_{\text{H}} = 7.06$ and 6.91 ppm were assigned to the *CH*, with coupling constants of $J_{\text{HH}} = 16.2$ Hz and 16.3 Hz respectively, consistent with *E* (*trans*) configuration around the double bond. The resonance at $\delta_{\text{H}} = 4.52$ ppm was assigned to the hydrogen atoms next to the bromine.

The di-*tert*-butyl 1,4,7-triazacyclononane-1,4-dicarboxylate chelator was synthesised according to a literature procedure.¹³⁴ The di-substituted compound was obtained by reacting *tert*-butyl bromoacetate with 1,4,7-triazacyclononane-1,4-diacetic acid in chloroform, followed by organic extractions at different pH values. The compound (*t*Bu)₂**L**¹² was synthesised from the di-*tert*-butyl 1,4,7-triazacyclononane-1,4-dicarboxylate chelator with (**42**) in the presence of K_2CO_3 in THF. Deprotection of the *tert*-butyl esters using concentrated HCl in 1,4-dioxane yielded $\text{H}_2\text{L}^{12} \cdot x\text{HCl}$ as the hydrochloride salt (Scheme 3.4).



Scheme 3.4. Synthesis of ligand $\text{H}_2\text{L}^{12} \cdot x\text{HCl}$.

The ^1H NMR spectrum of $(t\text{Bu})_2\text{L}^{12}$ is shown in Figure 3.21. The peaks at $\delta_{\text{H}} = 7.06$ and 6.91 ppm with coupling constants $J_{\text{HH}} = 16$ Hz confirmed the presence of the *E* conformer of the stilbene, which is essential for the binding to $\text{A}\beta$ plaques. It is crucial that there is no *trans* to *cis* conversion as a result of ligand synthesis. Upon the alkylation reaction, the resonance at $\delta_{\text{H}} = 4.52$ ppm shifted upfield to $\delta_{\text{H}} = 3.67$ ppm, indicating the formation of $(t\text{Bu})_2\text{L}^{12}$. The singlet peak with resonance at $\delta_{\text{H}} = 2.98$ ppm was assigned to the hydrogen atoms of the dimethylamino group whereas the resonance at $\delta_{\text{H}} = 1.44$ ppm was assigned to the hydrogen atoms of the *tert*-butyl protecting group. The overlaid signals between $\delta_{\text{H}} = 2.81$ - 2.94 ppm gave rise to multiplets that were assigned to the hydrogen atoms of the CH_2 of the macrocycle.

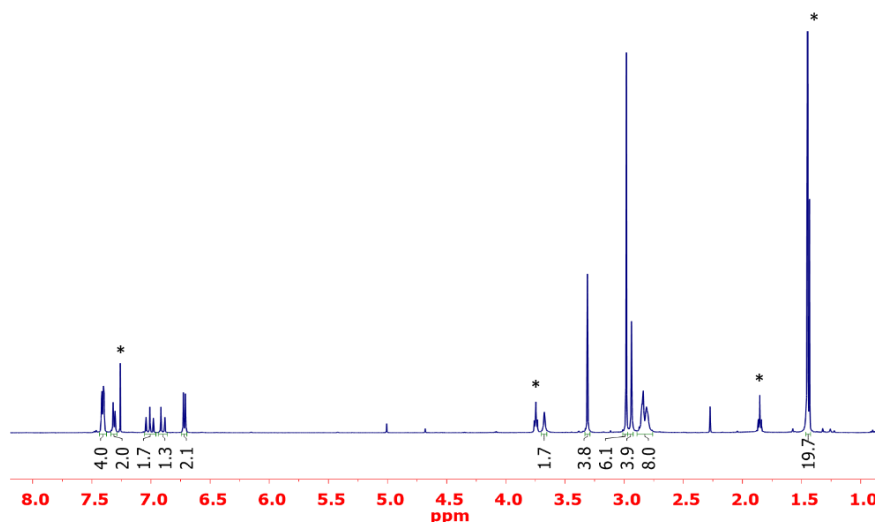


Figure 3.21. ^1H NMR spectrum of $(\text{tBu})_2\text{L}^{12}$ in CDCl_3 . Asterisks indicate solvent signals for CDCl_3 ($\delta_{\text{H}} = 7.26$ ppm), THF ($\delta_{\text{H}} = 3.76$ ppm and $\delta_{\text{H}} = 1.85$ ppm) and H_2O ($\delta_{\text{H}} = 1.50$ ppm).

The two-dimensional correlation spectroscopy (COSY) NMR spectrum allowed the assignment of hydrogen atoms (Figure 3.22). The resonance at $\delta_{\text{H}} = 7.44$ ppm was assigned to the aromatic hydrogen atoms at H4 and H9, which coupled to the resonances at $\delta_{\text{H}} = 7.31$ and 6.72 ppm. These two resonances were assigned to the aromatic hydrogen atoms H3 and H10, respectively.

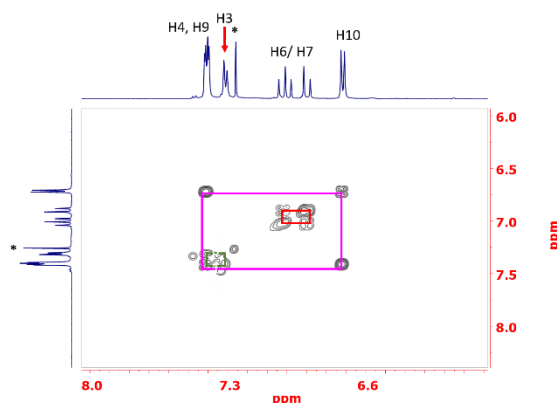
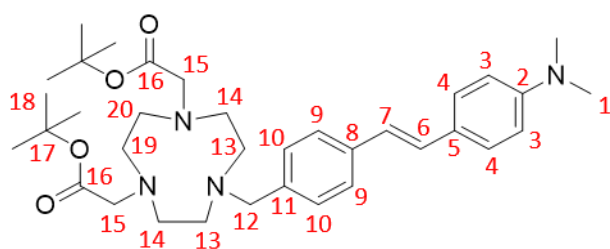


Figure 3.22. A portion of the COSY spectrum of $(\text{tBu})_2\text{L}^{12}$ in CDCl_3 . Asterisks indicate solvent signals for CDCl_3 ($\delta_{\text{H}} = 7.26$ ppm).

Table 3.1. The ^1H NMR assignment for $(t\text{Bu})_2\text{L}^{12}$.

Proton	Assignment	
ArH	7.41	H4, H9
ArH	7.31	H3
CH	7.05	H6/7
CH	6.90	H6/7
ArH	6.72	H10
ArCH_2	3.67	H12
NCH_2CO_2	3.31	H15
$\text{N}(\text{CH}_3)_2$	2.98	H1
CH_2	2.94	H13,14,19,20
CH_2	2.83	H13,14,19,20
$\text{C}(\text{CH}_3)_3$	1.44	H18



Assignment of the ^{13}C chemical shifts was achieved using $^{13}\text{C}\{^1\text{H}\}$ NMR and gradient heteronuclear single-quantum correlation spectroscopy (gHSQC). The carbon resonances at $\delta_{\text{C}} = 125.9$ and 127.6 ppm coupled to the aromatic hydrogen atoms at H4 and H9 ($\delta_{\text{H}} = 7.44$ ppm) (Figure 3.23 top).

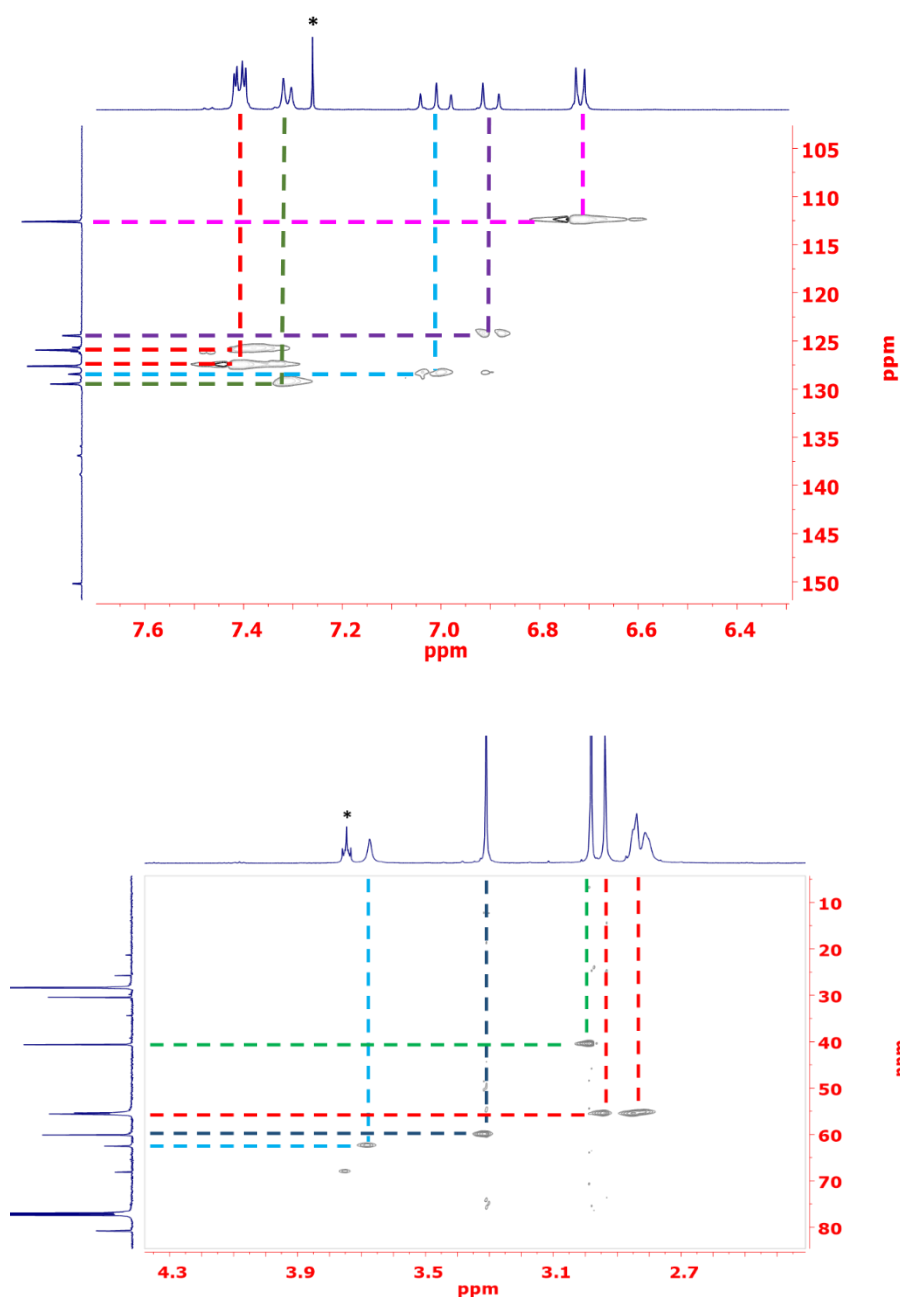


Figure 3.23. A portion of the gHSQC spectrum of $(\text{tBu})_2\text{L}^{12}$ in CDCl_3 . Asterisk indicates solvent signals for CDCl_3 ($\delta_{\text{H}} = 7.26$ ppm), THF ($\delta_{\text{H}} = 3.76$ ppm).

The ^1H NMR spectrum of $\text{H}_2\text{L}^{12} \cdot x\text{HCl}$ is shown in Figure 3.24. Again, the peaks at $\delta_{\text{H}} = 7.17$ ppm and $\delta_{\text{H}} = 6.99$ ppm with coupling constants 16 Hz confirmed that there is no *E* to *Z* conversion. The disappearance of the signal at $\delta_{\text{H}} = 1.44$ ppm indicated the removal of the *tert*-butyl group. The resonance at $\delta_{\text{H}} = 3.67$ ppm assigned to the ArCH_2 (H12) on $(t\text{Bu})_2\text{L}^{12}$ shifted downfield to $\delta_{\text{H}} = 4.29$ ppm. Upon the removal of the *tert*-butyl protecting group, the peaks assigned to the methylene hydrogen atoms shifted considerably. In contrast to $(t\text{Bu})_2\text{L}^{12}$, the signals attributed to the hydrogen atoms of the CH_2 of the macrocycle have split into individual peaks of multiplets at $\delta_{\text{H}} = 2.65 - 3.12$ ppm.

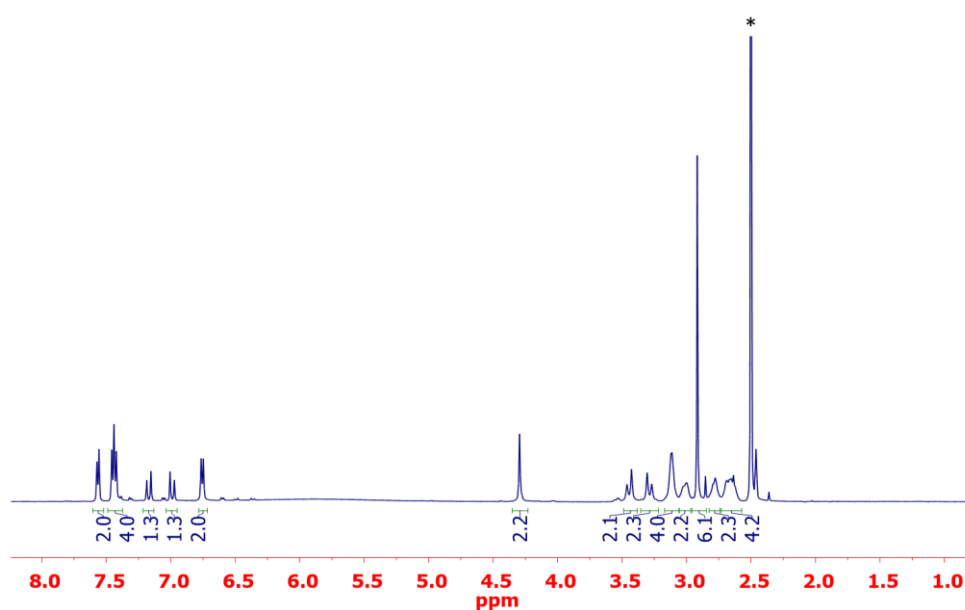


Figure 3.24. ^1H NMR spectrum of $\text{H}_2\text{L}^{12} \cdot x\text{HCl}$ in $d_6\text{-DMSO}$. Asterisk indicates solvent signals for $d_6\text{-DMSO}$ ($\delta_{\text{H}} = 2.50$ ppm).

The COSY spectrum identified the resonance at $\delta_{\text{H}} = 7.44$ ppm as the aromatic hydrogen atoms at H4 and H9, which coupled to the resonances at $\delta_{\text{H}} = 7.54$ and 6.75 ppm. These two resonances were assigned to the aromatic hydrogen atoms H3 and H10, respectively (Figure 3.25).

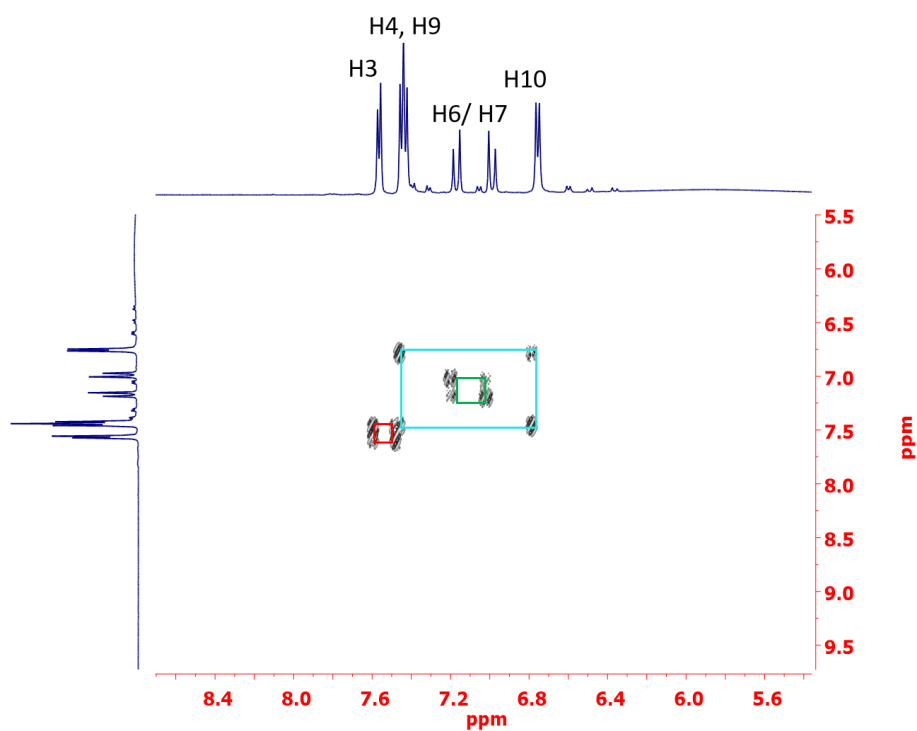
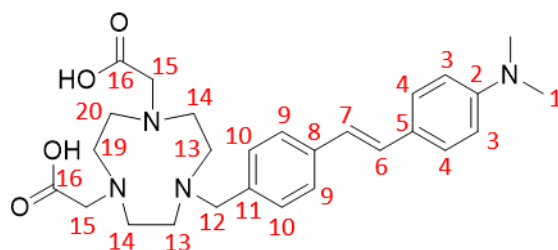


Figure 3.25. A portion of the COSY spectrum of $\text{H}_2\text{L}^{12} \cdot x\text{HCl}$ in d_6 -DMSO.

The summary of the ^1H NMR assignment for the ligand $\text{H}_2\text{L}^{12} \cdot x\text{HCl}$ is tabulated in Table 3.2.

Table 3.2. The ^1H NMR assignment for H_2L^{12} .

Proton	Assignment	
ArH	7.54	H3
ArH	7.44	H4/9
CH	7.17	H6/7
CH	6.99	H6/7
ArH	6.75	H10
ArCH_2	4.29	H12
NCH_2CO_2	3.46-3.43	H15
NCH_2CO_2	3.31-3.27	H15
CH_2	3.12	H14/19/20
CH_2	3.00	H14/19/20
$\text{N}(\text{CH}_3)_2$	2.92	H1
CH_2	2.79	H14/19/20
CH_2	2.67	H13



Within the gHSQC spectrum of $\text{H}_2\text{L}^{12}\cdot x\text{HCl}$, the multiplets at $\delta_{\text{H}} = 3.46\text{--}3.43\text{ ppm}$ and $\delta_{\text{H}} = 3.31\text{--}3.27\text{ ppm}$ resulted from 2 pairs of hydrogen atoms have the same carbon signal at $\delta_{\text{C}} = 54.7\text{ ppm}$ assigned to the methylene $\text{NCH}_2\text{CO}_2\text{H}$ (Figure 3.26). The gHSQC spectrum revealed the carbon signals attributed to the diaminomethyl ($\delta_{\text{H}} = 2.92\text{ ppm}$, $\delta_{\text{C}} = 40.4\text{ ppm}$) which was overlapping with the solvent peak ($\delta_{\text{C}} = 39.5\text{ ppm}$) in the $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum.

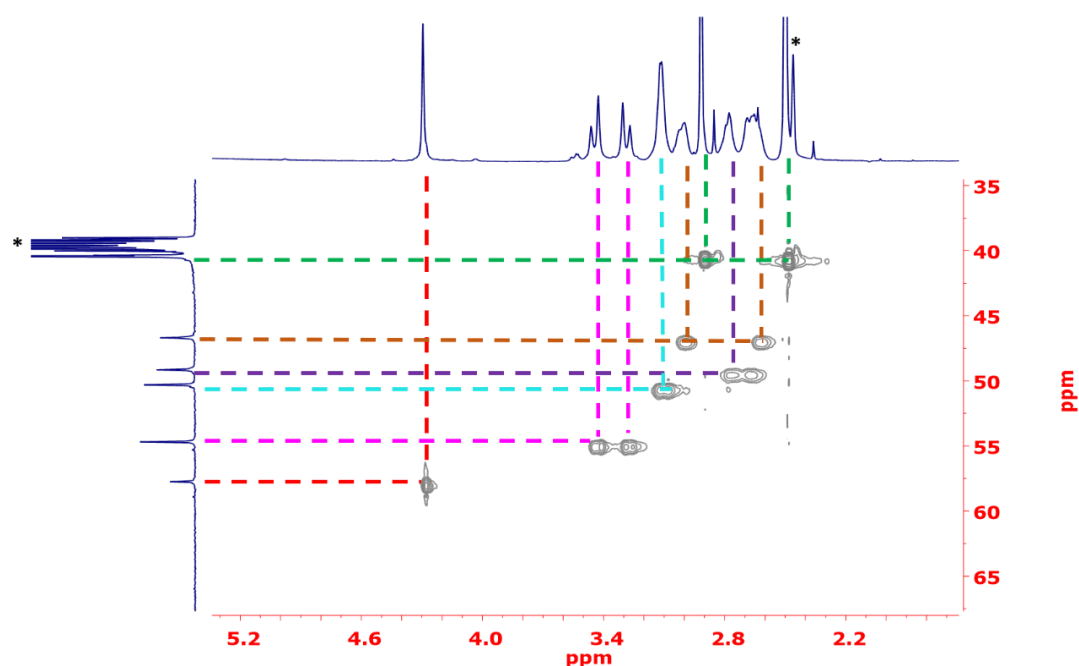
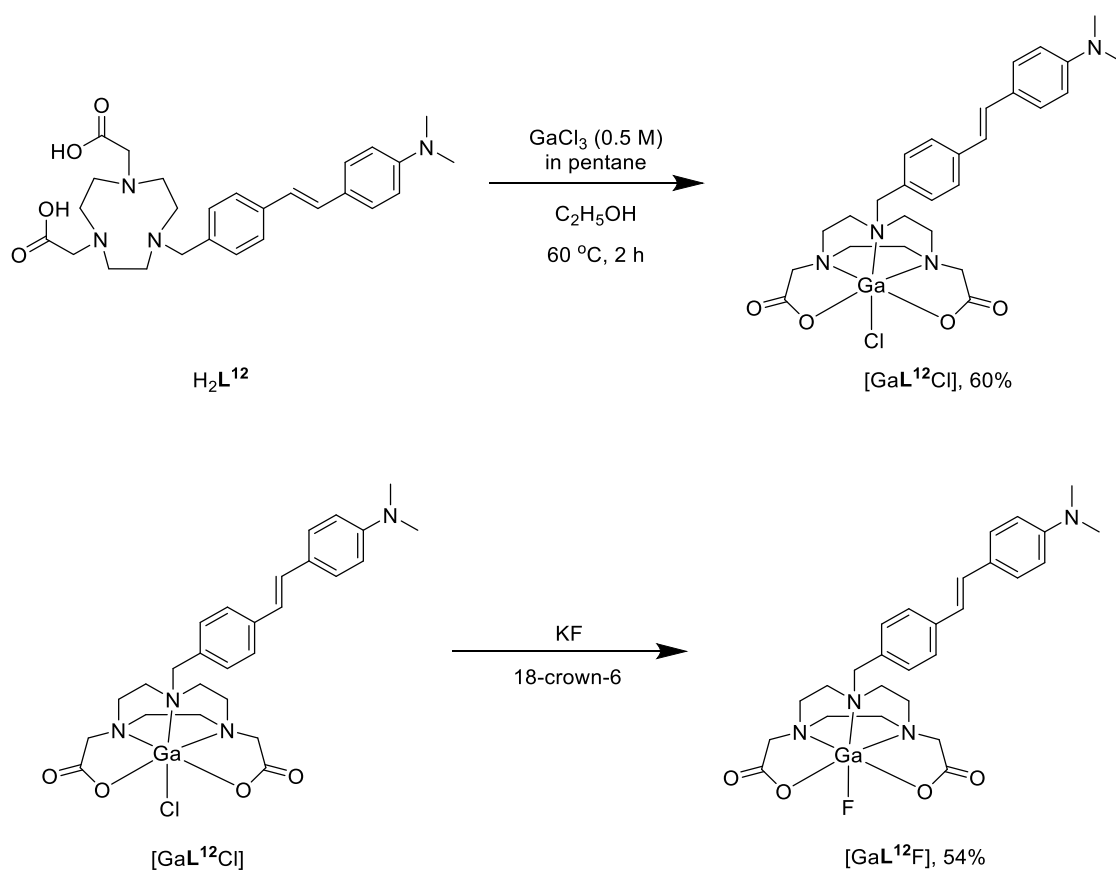


Figure 3.26. Portions of gHSQC spectrum of $\text{H}_2\text{L}^{12}\cdot x\text{HCl}$ in $d_6\text{-DMSO}$. Asterisk indicates solvent signals for $d_6\text{-DMSO}$ ($\delta_{\text{H}} = 2.50\text{ ppm}$).

The reaction of $\text{H}_2\text{L}^{12} \cdot x\text{HCl}$ and GaCl_3 in anhydrous ethanol under nitrogen at 60 °C yielded the Ga^{3+} complex $[\text{GaL}^{12}\text{Cl}]$ as a pale-yellow powder. Addition of KF into a solution of $[\text{GaL}^{12}\text{Cl}]$ in acetonitrile and 18-crown-6 produced $[\text{GaL}^{12}\text{F}]$ as a pale-yellow powder (Scheme 3.5).



Scheme 3.5. Synthesis of gallium complexes $[\text{GaL}^{12}\text{Cl}]$ and $[\text{GaL}^{12}\text{F}]$.

The *E* conformation of the stilbene group was observed at signals $\delta_{\text{H}} = 7.18$ and 6.99 ppm (Figure 3.27). The non-equivalent environment of the hydrogen atoms is different from the environment of the CH_2 hydrogen atoms of the ligand $\text{H}_2\text{L}^{12} \cdot x\text{HCl}$ with a singlet at $\delta_{\text{H}} = 4.29$ ppm. Upon coordination to Ga^{3+} , the methylene hydrogen atoms next to the benzene group are split into an AB quartet ($^2J_{\text{HH}} = 14.1$ Hz) ($\delta_{\text{H}} = 3.78$ and 4.34 ppm).

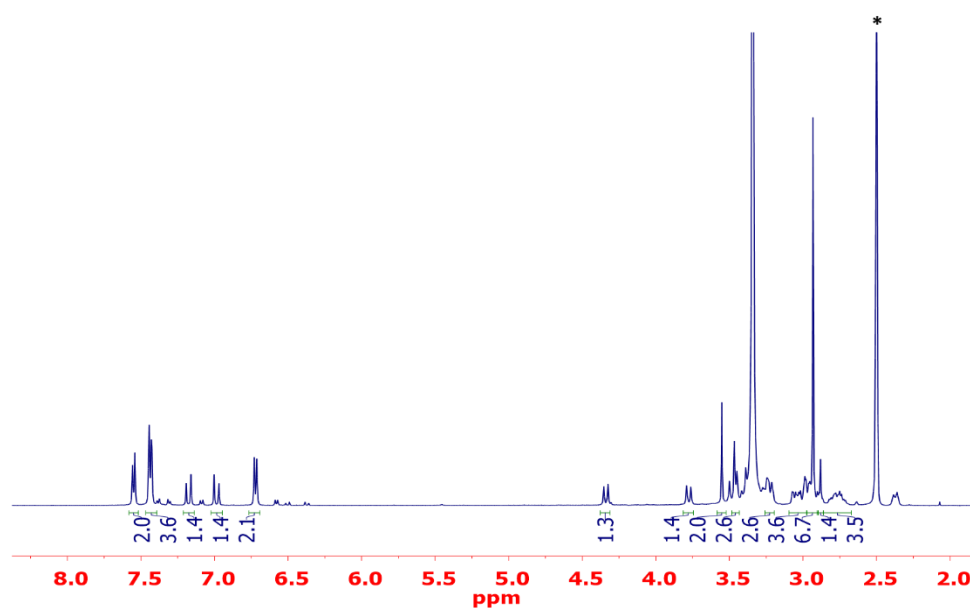
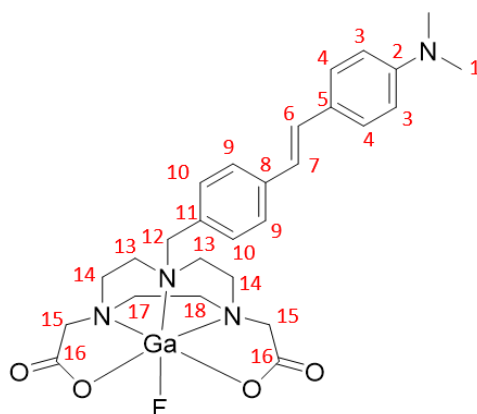


Figure 3.27. ^1H NMR spectrum of $[\text{GaL}^{12}\text{F}]$ in d_6 -DMSO. Asterisk indicates solvent signals for d_6 -DMSO ($\delta_{\text{H}} = 2.50$ ppm).

The summary of the ^1H NMR assignment for $[\text{GaL}^{12}\text{F}]$ is tabulated in Table 3.3.

Table 3.3. The ^1H NMR assignment for $[\text{GaL}^{12}\text{F}]$.

Proton	Assignment	
<i>ArH</i>	7.55	H4/9
<i>ArH</i>	7.44	H3,10
<i>CH</i>	7.18	H6/7
<i>CH</i>	6.99	H6/7
<i>ArH</i>	6.72	H4/9
<i>ArCH</i> ₂	4.34	H12
<i>ArCH</i> ₂	3.78	H12
<i>CH</i> ₂	3.55	H14/17/18
<i>NCH</i> ₂ <i>CO</i> ₂	3.46	H15
<i>NCH</i> ₂ <i>CO</i> ₂	3.24	H15
<i>CH</i> ₂	3.11-2.99	H14/17/18
<i>N(CH</i> ₃ <i>)</i> ₂	2.93	H1
<i>CH</i> ₂	2.89	H14/17/18
<i>CH</i> ₂	2.79	H13



The ^{19}F NMR spectrum was acquired to confirm the formation of $[\text{GaL}^{12}\text{F}]$. The spectrum revealed a single resonance at $\delta_{\text{F}} = -181$ ppm, confirming the formation of the $\text{Ga}^{3+}\text{-F}^-$ bond (Figure 3.28).

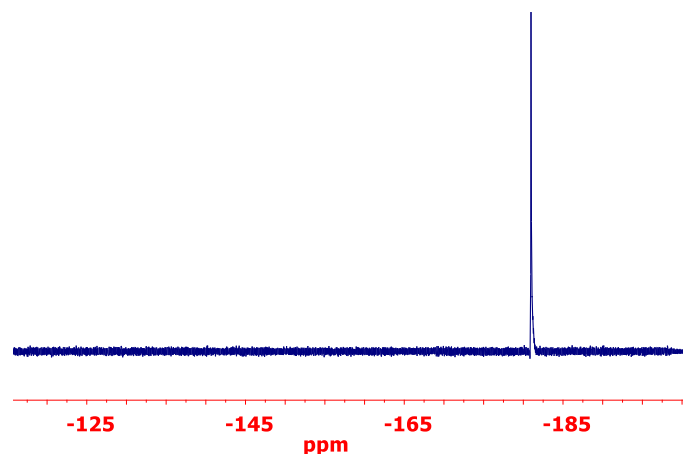


Figure 3.28. ^{19}F NMR spectrum of $[\text{GaL}^{12}\text{F}]$ in $d_6\text{-DMSO}$.

The stability of the Ga^{3+} complex in blood serum under physiological conditions was studied using ^{19}F NMR. The single peak at $\delta_{\text{F}} = -181$ ppm was unchanged after incubation of $[\text{GaL}^{12}\text{F}]$ in excess of human serum albumin at 37°C for 4 hours at pH 7, suggesting that the $\text{Ga}^{3+}\text{-F}^-$ bond was still intact and that $[\text{GaL}^{12}\text{F}]$ is stable under these conditions (Figure 3.29).

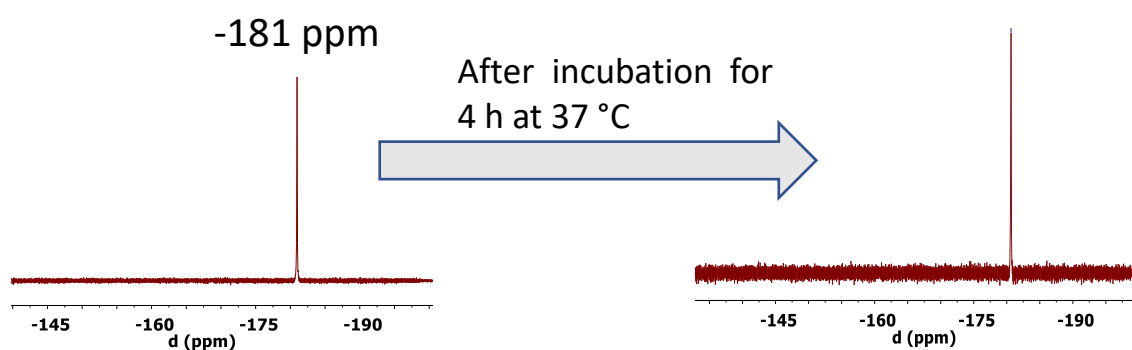


Figure 3.29. ^{19}F NMR spectrum of $[\text{GaL}^{12}\text{F}]$ in $d_6\text{-DMSO}$ before and after incubation with human serum albumin at 37°C .

3.6 Electronic spectroscopy studies of H_2L^{12} , $[GaL^{12}Cl]$ and $[GaL^{12}F]$

To assess the suitability of using epi-fluorescence microscopy to investigate the binding of compounds to A β plaques, the compounds were studied using UV-Visible and emission spectroscopy. The UV Visible spectra were acquired for $H_2L^{12} \cdot xHCl$, $[GaL^{12}Cl]$ and $[GaL^{12}F]$. The strong absorbance for $H_2L^{12} \cdot xHCl$ at $\lambda_{max} = 330$ nm is attributed to the π - π^* transition from the stilbene functional group. The absorption shifts to $\lambda_{max} = 363$ nm in $[GaL^{12}Cl]$ and $[GaL^{12}F]$ (Figure 3.30). The molar extinction coefficients for this transition are lower upon coordination to Ga^{3+} (Table 3.4).

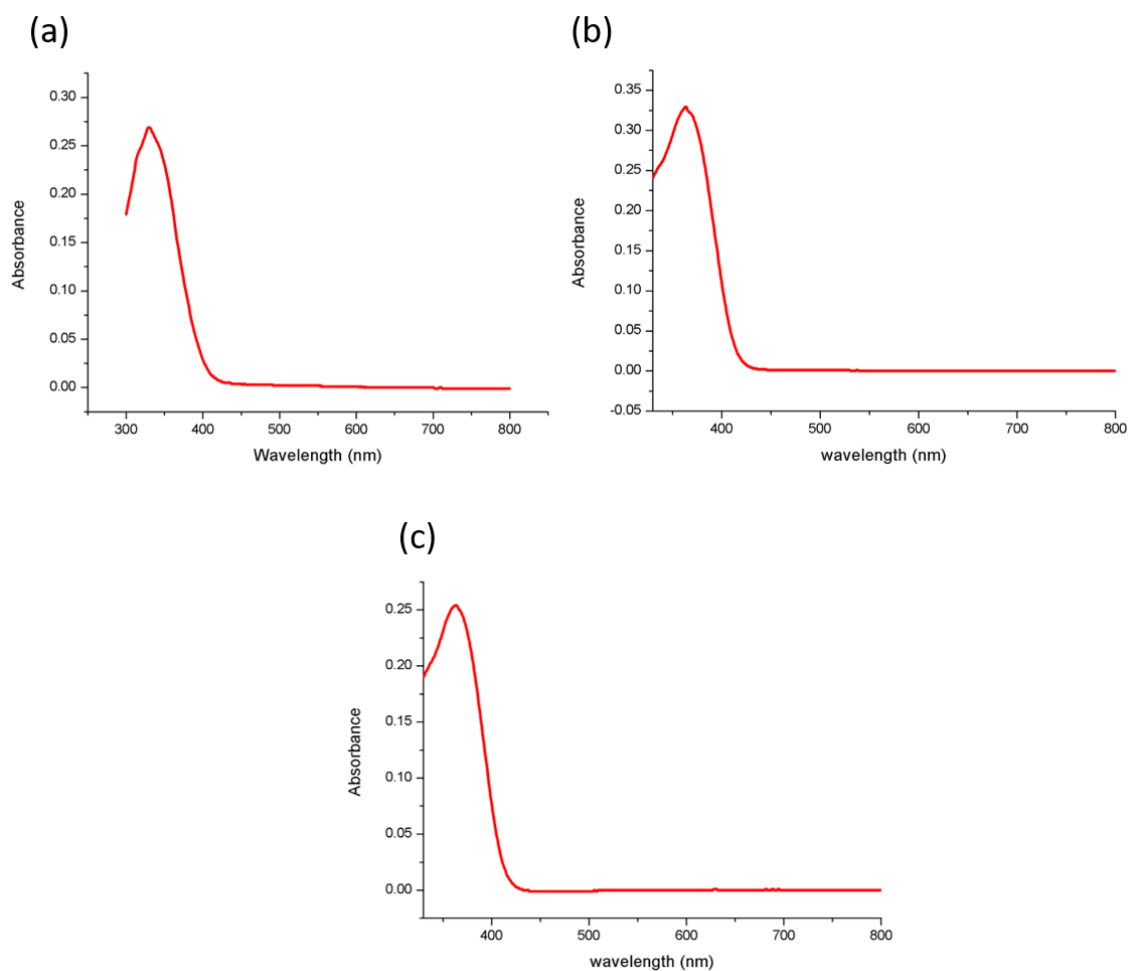


Figure 3.30. UV-vis spectra of $H_2L^{12} \cdot xHCl$ (a), $[GaL^{12}Cl]$ (b) and $[GaL^{12}F]$ (c).

Table 3.4. Summary of the λ_{max} and molar extinction coefficients for $\text{H}_2\text{L}^{12}\cdot\text{xHCl}$, $[\text{GaL}^{12}\text{Cl}]$ and $[\text{GaL}^{12}\text{F}]$.

	Concentration (μM)	λ_{max} (nm)	Molar extinction coefficient ($\times 10^3 \text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$)
$\text{H}_2\text{L}^{12}\cdot\text{xHCl}$	10	330	18
$[\text{GaL}^{12}\text{Cl}]$	27	363	12
$[\text{GaL}^{12}\text{F}]$	22	363	12

The fluorescence profiles for $\text{H}_2\text{L}^{12}\cdot\text{xHCl}$, $[\text{GaL}^{12}\text{Cl}]$ and $[\text{GaL}^{12}\text{F}]$ are similar ($\lambda_{\text{em}} \sim 470$ nm, $\lambda_{\text{ex}} \sim 360$ nm) but the coordination of Ga^{3+} leads to some quenching and the complexes are less fluorescent than the free ligand (Figure 3.31).

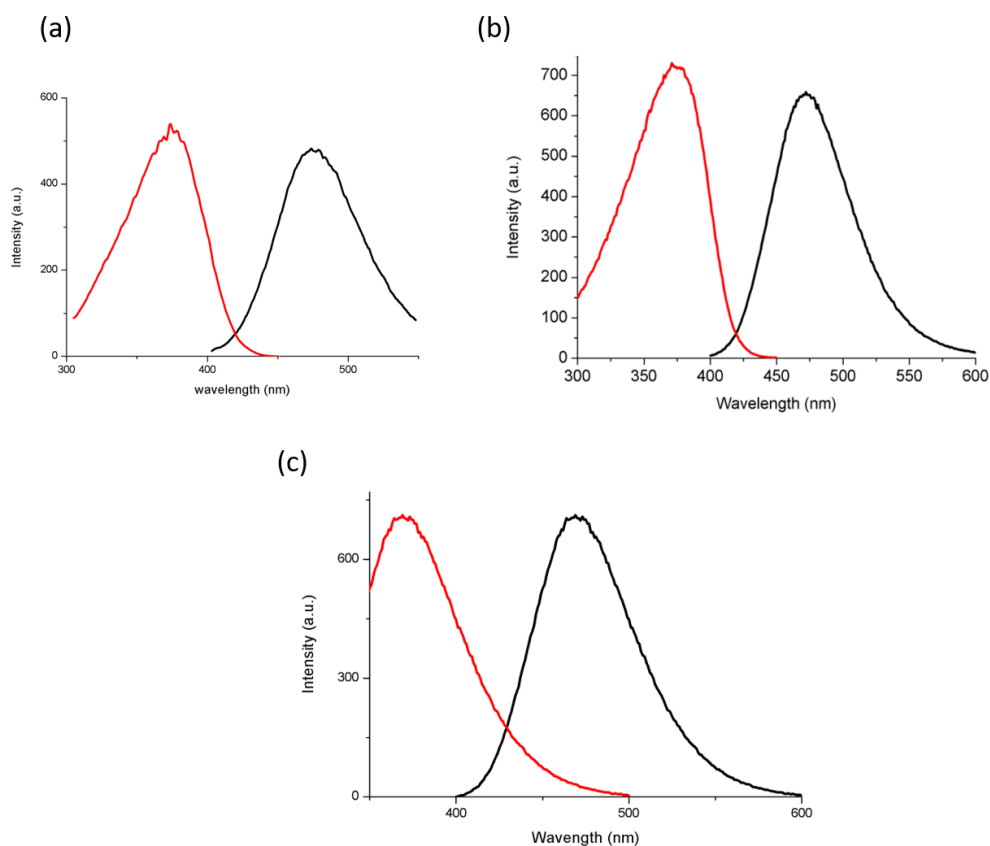


Figure 3.31. Emission (black) and excitation (red) spectra of $\text{H}_2\text{L}^{12}\cdot\text{xHCl}$ (1 μM) (a), $[\text{GaL}^{12}\text{Cl}]$ (10 μM) (b) and $[\text{GaL}^{12}\text{F}]$ (10 μM) (c).

3.7 Binding of H_2L^{12} and Ga^{3+} Complexes to $A\beta_{1-42}$ Plaques in Human Brain Tissue and Synthetic $A\beta_{1-40}$ Fibrils

The emission properties of the stilbene functional group allowed the detection of $H_2L^{12}\cdot xHCl$ using epi-fluorescent microscopy for *in vitro* studies into the $A\beta$ plaques binding. Human brain tissue sections (5 μm in diameter) collected from the frontal cortex of subjects with clinically diagnosed AD and age-matched control were treated with an amyloid₁₋₄₂ antibody (1E8) to selectively stain the $A\beta_{1-42}$ -containing plaques. The tissue sections were then treated with bovine serum albumin (2% in PBS) to prevent non-selective binding and with a solution of the compound (10 μM) in DMSO (15-30%) in PBS for 10 minutes.

Treatment of the human brain tissue sections collected from the frontal cortex of subjects with clinically diagnosed AD with $H_2L^{12}\cdot xHCl$ showed co-localisation of the compound on the treated human brain tissue identified using epi-fluorescence microscopy ($\lambda_{ex} = 359$ nm, $\lambda_{em} = 461$ nm) with adjacent tissue sections immunohistochemically stained with the $A\beta_{1-42}$ selective 1E8 antibody (Figure 3.32. a, c). $A\beta$ plaques are typically 30-50 μm in diameter so adjacent sections (5 μm) should contain same amyloid plaque patterns. Comparison of the 1E8 stained tissue with the tissue treated with $H_2L^{12}\cdot xHCl$ confirmed that $H_2L^{12}\cdot xHCl$ binds to amyloid plaques. The images are compared to samples from age-matched control subjects after treated with $H_2L^{12}\cdot xHCl$, which showed no non-specific binding (Figure 3.32. b, d).

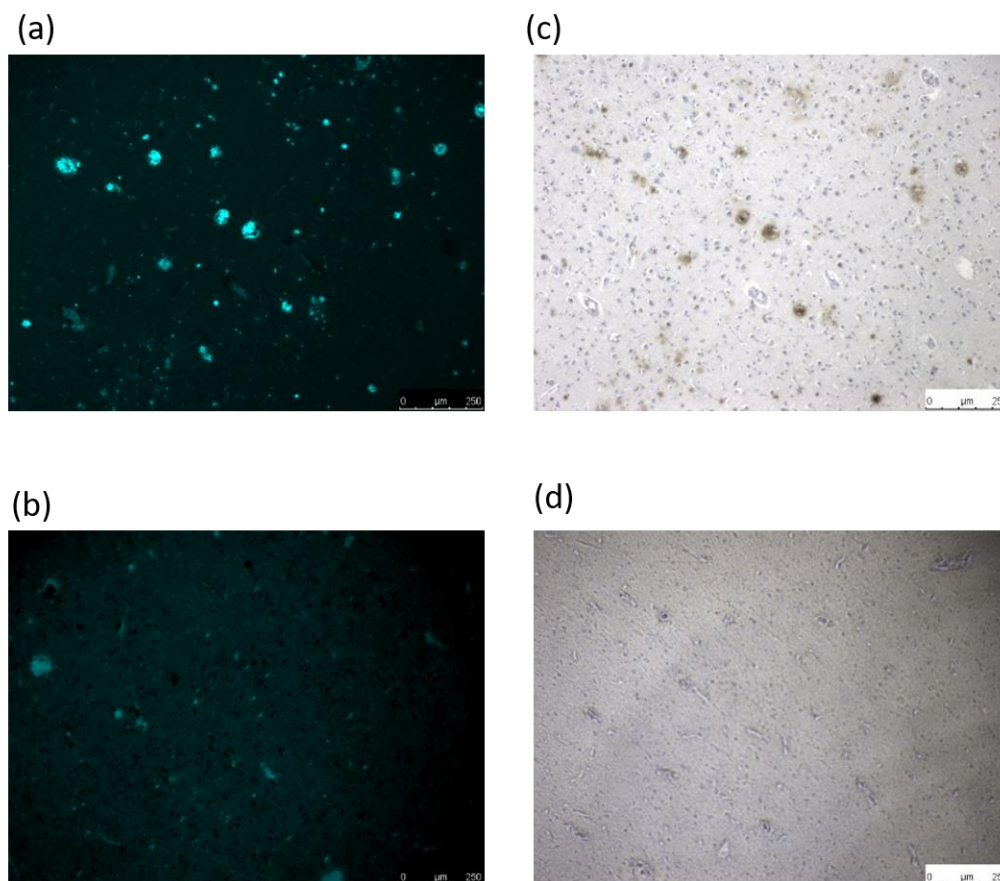


Figure 3.32. Human brain tissue sections from the frontal cortex of a subject with clinically diagnosed AD (a, c) and aged-matched control (b, d) at 10 x magnification. Images on the left are tissue section treated with $H_2L^{12} \cdot xHCl$ visualised with epi-fluorescent microscopy ($\lambda_{ex} = 359 \text{ nm}$, $\lambda_{em} = 461 \text{ nm}$) (a, b). Images on the right are adjacent tissue sections immunohistochemically stained with the $A\beta_{1-42}$ selective 1E8 antibody, visualised using bright-field microscopy (c, d).

The experiment was repeated using $[GaL^{12}Cl]$ and $[GaL^{12}F]$ under the same conditions. Unfortunately, there was no evidence of binding to $A\beta$ plaques when stained with the Ga^{3+} complexes (Figure 3.33). The low fluorescence intensity and low concentrations of $[GaL^{12}Cl]$ and $[GaL^{12}F]$ may have limited the detection of the binding to the amyloid plaques. It is likely that the presence of the Ga^{3+} ion reduced the binding affinity of the stilbene functional group for amyloid plaques.

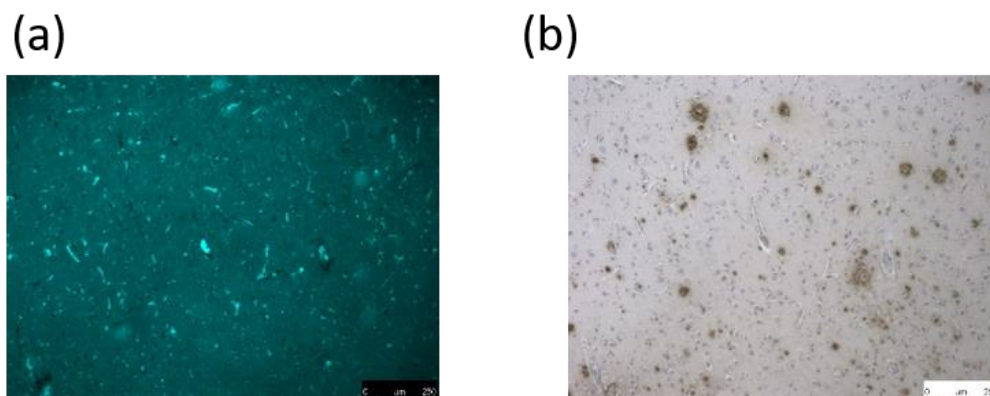


Figure 3.33. Human brain tissue sections from the frontal cortex of a subject with clinically diagnosed AD at 10 x magnification. Image on the left is tissue section treated with $[GaL^{12}Cl]$ visualised with epi-fluorescent microscopy ($\lambda_{ex} = 359 \text{ nm}$, $\lambda_{em} = 461 \text{ nm}$) (a). Image on the right is adjacent tissue section immunohistochemically stained with the $A\beta_{1-42}$ selective 1E8 antibody, visualised using bright-field microscopy (b).

Estimation of $A\beta_{1-40}$ Binding Affinities

The dye, ThT, exhibits enhanced fluorescence and a characteristic blue shift from $\sim 510 \text{ nm}$ in the free state to $\sim 480 \text{ nm}$ when it binds to β -sheet-rich structures such as $A\beta$ fibrils.²³⁰ A fluorescence competition assay against ThT can be employed to provide an estimation of the binding affinities of molecules to $A\beta$.²³¹ The affinity of an $A\beta$ plaque targeting molecule for $A\beta$ provides an indicator of how effective a tracer is as a potential imaging agent. Fibrils were prepared by dissolution of $A\beta_{1-40}$ with 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) to yield monomeric $A\beta_{1-40}$. HFIP is a polar compound with strong hydrogen bonding properties, and dissolves fibrils that are hydrogen-bond acceptors. After treatment with HFIP, the $A\beta_{1-40}$ was dissolved in NaOH (60 mM) and PBS (10x, pH 7.4) and incubated at 37°C for three days. $H_2L^{12}\cdot xHCl$, $[GaL^{12}Cl]$ and $[GaL^{12}F]$ at various concentrations were incubated with $A\beta_{1-40}$ and ThT of known concentrations before measuring the fluorescence intensities ($\lambda_{ex} = 440 \text{ nm}$; $\lambda_{em} = 480$

nm). Upon addition of increasing concentrations of $H_2L^{12} \cdot xHCl$ and $[GaL^{12}Cl]$, the fluorescence intensities decreased, indicating that the binding of the ThT to $A\beta_{1-40}$ decreased. The decrease in fluorescence intensity was plotted against concentrations using a one-phase decay model (Figure 3.34). Each compound showed a dose-dependent quenching of ThT fluorescence intensity with their IC_{50} values. The IC_{50} values were used to calculate the competitive inhibition constant, K_i values (Table 3.5) using the Cheng-Prusoff equation:

$$K_i = IC_{50} / \left(1 + \frac{[L]}{K_d} \right)$$

$$K_d(\text{ThT}) = 1.418 \mu\text{M}$$

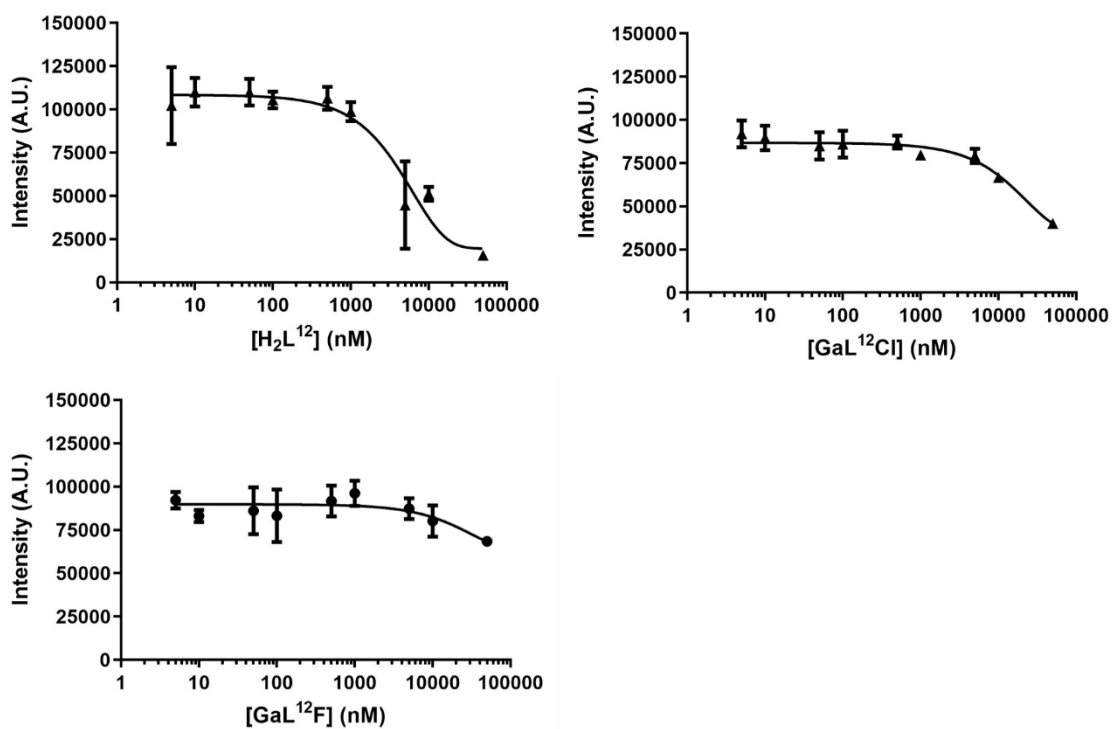


Figure 3.34. Plots of inhibition curve of $H_2L^{12} \cdot xHCl$, $[GaL^{12}Cl]$ and $[GaL^{12}F]$ against ThT and $A\beta_{1-40}$.

In this assay, the free ligand, $\text{H}_2\text{L}^{12} \cdot x\text{HCl}$ has a higher binding affinity ($K_i = 1.79 \mu\text{M}$) than the Ga^{3+} complexes. This difference in the K_i values may explain the lack of binding of $[\text{GaL}^{12}\text{Cl}]$ and $[\text{GaL}^{12}\text{F}]$ to A β plaque in the human brain tissue. It is possible that the proximity of the relatively hydrophilic azamacrocyclic metal complex and its associated solvation may impede the binding. Separating the plaque targeting pharmacophore by a spacer may improve the binding affinity.

Table 3.5. The K_i and IC_{50} values of $\text{H}_2\text{L}^{12} \cdot x\text{HCl}$, $[\text{GaL}^{12}\text{Cl}]$ and $[\text{GaL}^{12}\text{F}]$ based on the competitive binding experiment with ThT assay.

	K_i (μM)	IC_{50} (μM)
$\text{H}_2\text{L}^{12} \cdot x\text{HCl}$	1.8 ± 0.9	4.3
$[\text{GaL}^{12}\text{Cl}]$	6.5 ± 2.3	16
$[\text{GaL}^{12}\text{F}]$	8.8 ± 5.3	21

These K_i values suggest that all three compounds only have moderate affinities and are above the required 1 nM based on other reported A β binding tracers. The affinity values reported here are based on the competitive binding experiment with the ThT assay, which only involves the binding sites occupied by ThT on A β fibrils. In general, different dyes occupy different binding sites on the fibrils, and it should be noted that different molecules can bind to other sites than the ones bound by ThT.

Although H_2L^{12} and its Ga^{3+} complexes may not be suitable for A β plaque imaging, it serves as an excellent model compound to investigate the radiolabelling approaches with three different radionuclides.

3.8 Synthesis and evaluation of $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$, $[^{68}\text{Ga}][\text{GaL}^{12}\text{F}]$ and $[^{64}\text{Cu}][\text{CuL}^{12}]$.

Aqueous $[^{18}\text{F}]\text{F}^-/\text{H}_2\text{O}$ was obtained as target wash from the production of fluorine-18 by cyclotron incorporating a high-pressure niobium target. The synthesis of $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ was achieved by either introducing aqueous fluorine-18 into the pre-formed $[\text{GaL}^{12}\text{Cl}]$ in a buffered solution or addition of H_2L^{12} into a buffered solution of aqueous fluorine-18 and $\text{Ga}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$. Optimization of the radiolabelling conditions involved varying temperature, pH, reaction times and amount of precursor.

Keeping all other variables constant, the reaction was carried out at pH 3.5, 4.0, 4.5 and 7.4. The pH range was kept within 3 - 8 to avoid protonation of the carboxylate group that is coordinated to the Ga^{3+} metal centre at low pH and the formation of $\text{Ga}(\text{OH})_3$ at high pH. It is also important to avoid low pH that favours the formation of HF .²³² The radiochemical yield reported throughout this thesis is non-decay corrected and is estimated from the integration of peaks corresponding to the radioactive species in HPLC chromatographs. The radiochemical yield is the highest at pH 4.5 (sodium acetate buffer, 2 mM). There is no evidence of radiolabelling at pH 7.4 (phosphate-buffered saline). By increasing the reaction temperature from 60 to 80 °C, the rate of reaction significantly increased, which is reflected by the increased radiochemical yield (Figure 3.35).

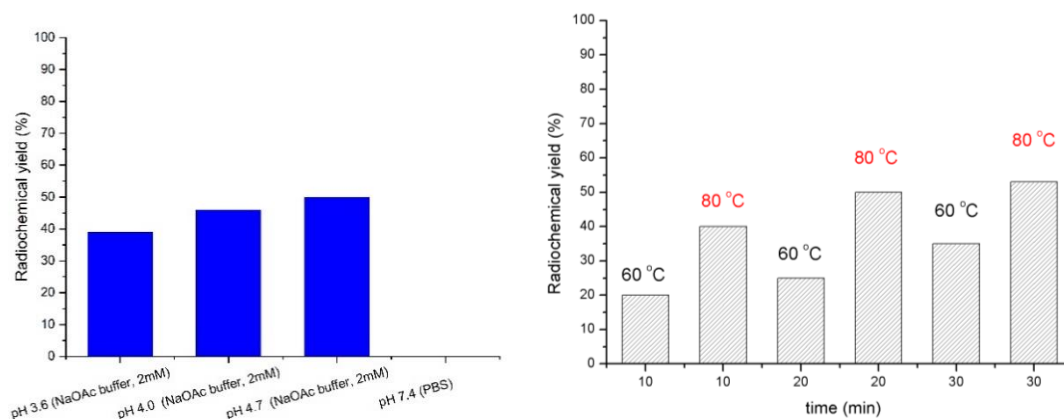
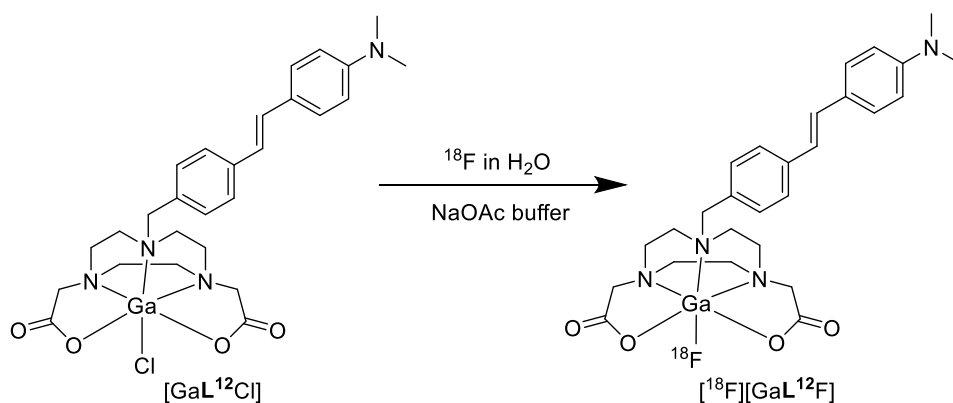


Figure 3.35. The graphs show the radiochemical yield of $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ with varying pH (left) and temperatures and reaction times at pH 4.0 (right).

Direct radiofluorination was performed by adding aqueous $[^{18}\text{F}]$ fluoride to $[\text{GaL}^{12}\text{Cl}]$ (10 $\mu\text{g}/\text{MBq}$) at pH 4.5 in sodium acetate buffer at 80 °C for 30 min with a radiochemical yield of 53% (Scheme 3.6). RP-HPLC analysis of the crude reaction mixture showed a peak at R_t 11.8 min, corresponding to $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ (Figure 3.36). The synthesised product $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ was confirmed by co-injection with the non-radioactive $[\text{GaL}^{12}\text{F}]$ and comparison of the HPLC profiles of absorbance at $\lambda = 254$ nm and detection of radioactivity. The crude fluorine-18 labelled product was purified using a solid-phase extraction column to give $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ with a radiochemical purity of 99%. The slight retention time difference between the product $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ and the $[\text{GaL}^{12}\text{F}]$ is attributed to the sequential detector configuration.



Scheme 3.6. The reaction scheme of the formation of $[^{18}\text{F}][\text{GaL}^9\text{F}]$.

The compound $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ was found to be stable in sodium acetate buffer (pH 4) at room temperature for at least 2 h, allowing sufficient time for purification.

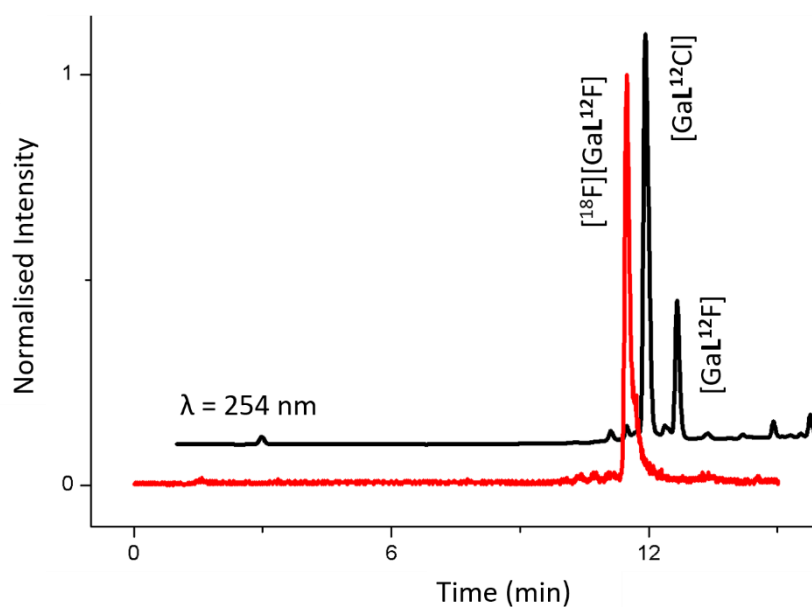
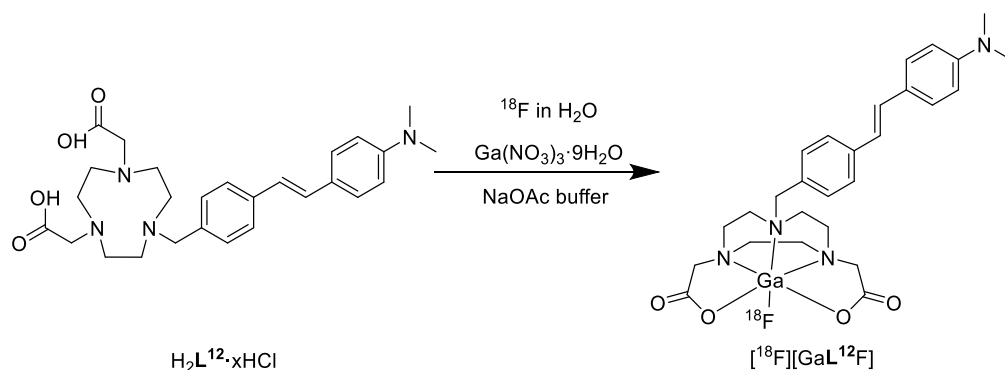


Figure 3.36. RP-HPLC chromatogram (black = UV absorbance, $\lambda = 254 \text{ nm}$ and red = radiation detection) of $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ after SPE purification. Retention time at 11.5 min.

Alternatively, $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ can be formed by the addition of $\text{H}_2\text{L}^{12}\cdot x\text{HCl}$ into a buffered solution of pre-formed $[^{18}\text{F}]\{\text{GaF}\}^{2+}$ using $\text{Ga}(\text{NO}_3)_3\cdot 9\text{H}_2\text{O}$ (Scheme 3.7).



Scheme 3.7. Alternative radiofluorination method of $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$.

As the $[^{18}\text{F}]\{\text{GaF}\}^{2+}$ species has to be formed immediately before reacting with $\text{H}_2\text{L}^{12}\cdot x\text{HCl}$, it is crucial to obtain the optimal concentration of $\text{Ga}(\text{NO}_3)_3\cdot 9\text{H}_2\text{O}$ required for a certain amount of radioactivity. This is because a high concentration of $\text{Ga}(\text{NO}_3)_3\cdot 9\text{H}_2\text{O}$ present in the reaction mixture does not necessarily increase the overall yield of the radiotracer. The yield of the formation of $[^{18}\text{F}]\{\text{GaF}\}^{2+}$ as a function of the concentration of $\text{Ga}(\text{NO}_3)_3\cdot 9\text{H}_2\text{O}$ in sodium acetate buffer (0.1 M, pH 4) was investigated (Figure 3.37). Different concentrations of $\text{Ga}(\text{NO}_3)_3\cdot 9\text{H}_2\text{O}$ in sodium acetate buffer (0.1 M, pH 4) were incubated with 37 MBq of $^{18}\text{F}/\text{H}_2\text{O}$ at room temperature for 10 - 20 minutes. A high yield of $> 99\%$ was obtained using 2 mM of $\text{Ga}(\text{NO}_3)_3\cdot 9\text{H}_2\text{O}$. Concentrations below 2 mM or higher than 3 mM produced $[^{18}\text{F}]\{\text{GaF}\}^{2+}$ in low yields ($< 30\%$). This is in contrast with the formation of $[^{18}\text{F}]\{\text{AlF}\}^{2+}$ where $\sim 90\%$ was formed using 0.3 - 20 mM of AlCl_3 .²³³ Despite that, labelling of the Al^{3+} complex with 2 mM of AlCl_3 gave the fluorine-18 labelled Al^{3+} complex with the highest radiochemical yield ($\sim 40\%$). Hence, the concentration of $\text{Ga}(\text{NO}_3)_3\cdot 9\text{H}_2\text{O}$ was kept to 2 mM for all subsequent synthesis.

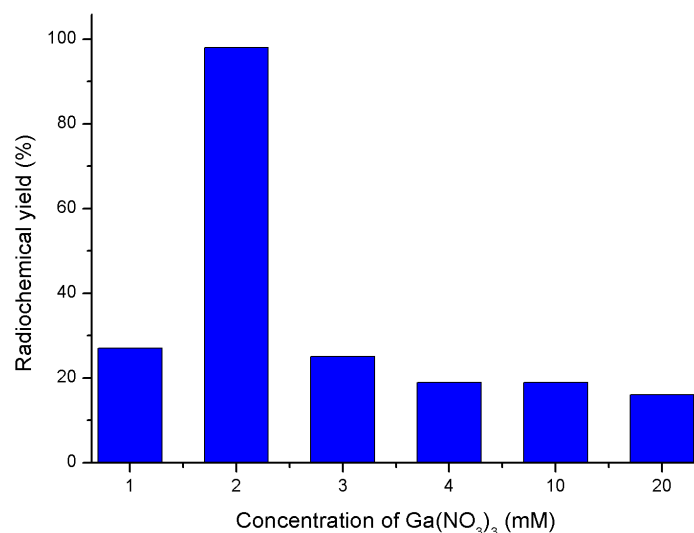


Figure 3.37. Percentage yield formation of $[^{18}\text{F}]\{\text{GaF}\}^{2+}$ at various concentration of $\text{Ga}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ in sodium acetate buffer (0.1 M, pH 4).

Addition of $\text{H}_2\text{L}^{12} \cdot x\text{HCl}$ (2 mM in DMSO stock solution) into a solution of $\text{Ga}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (2 mM in sodium acetate buffer) and aqueous $[^{18}\text{F}]$ fluoride (37 MBq) to form a 1:1 molar ratio of $\text{H}_2\text{L}^{12} \cdot x\text{HCl}$ and $\text{Ga}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ followed by incubation at 80 °C for 20 min formed $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ (Figure 3.38).

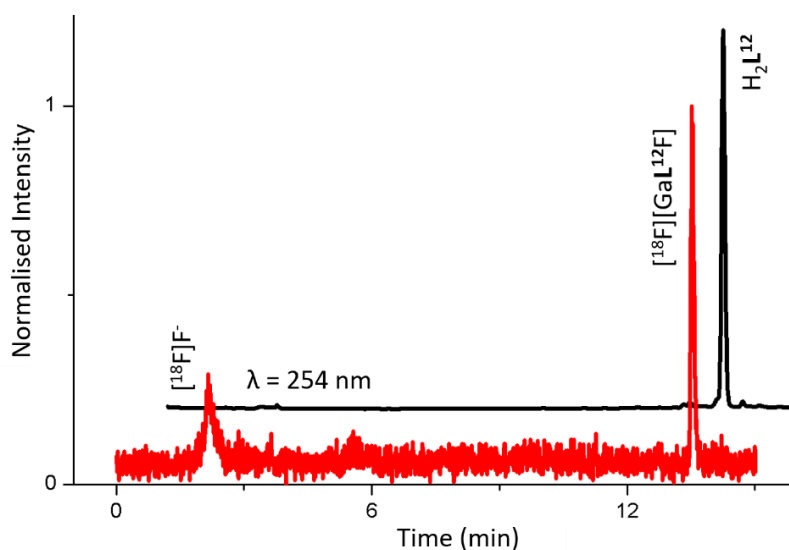


Figure 3.38. RP-HPLC chromatogram (red = radiation detection; black = UV detector at λ 254 nm) of the crude reaction mixture of $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$. Retention time at 13.7 min.

To assess the stability for further biological investigations, $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ was formulated in and incubated with cysteine (1 mM). The decreased radiochemical purity (70% to 52%) after 1.5 h of incubation showed the formation of more than one radioactive species in the presence of cysteine (Figure 3.39). The additional peaks at retention times of 12.7 and 13.1 min suggest complexes that are slightly more hydrophilic are being formed possibly due to the formation of ‘ternary’ complexes where cysteine is coordinating to Ga^{3+} .

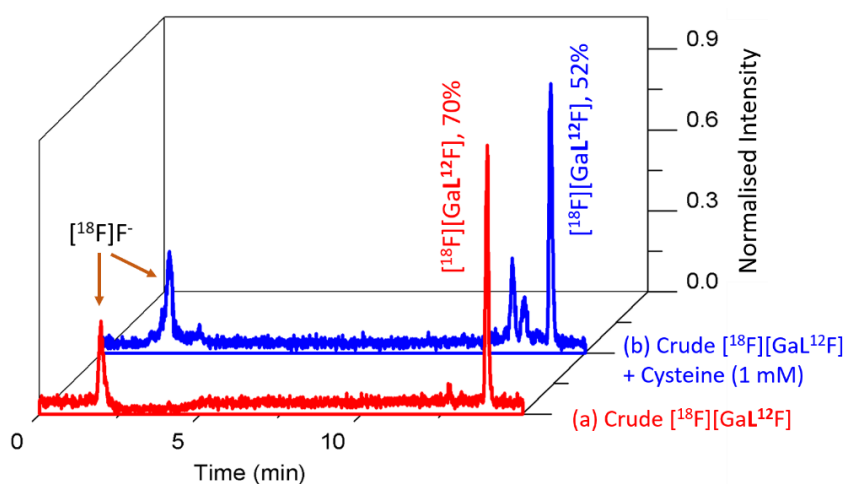


Figure 3.39. RP-HPLC chromatogram of radiation detection (red = crude reaction mixture of $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$; blue = crude reaction mixture of $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ + cysteine (1 mM).

Unfortunately, the stability of $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ decreased rapidly in phosphate buffered saline (PBS, pH 7.4). The radiochemical purity dropped to 50% within 20 minutes (Figure 3.40).

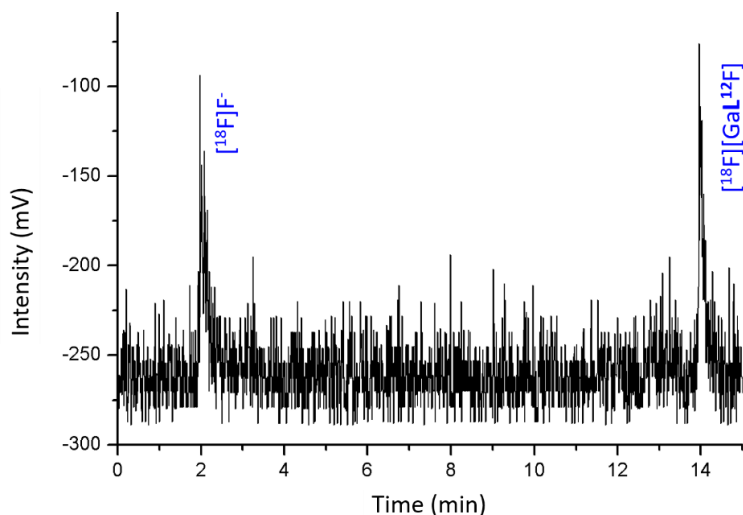
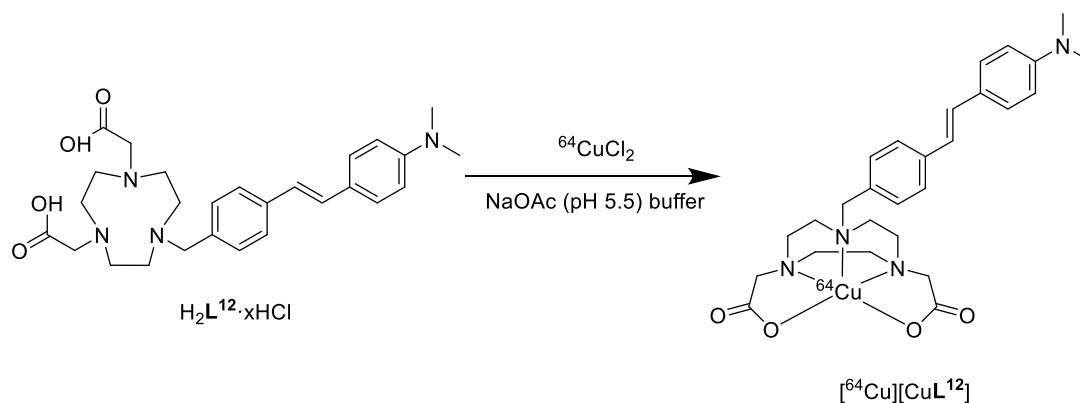


Figure 3.40. RP-HPLC chromatogram (radiation detection) of purified $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ incubated in PBS.

The $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ complex is stable in water and in 10% ethanol/acetate buffer (pH 6) but the stability reduced in PBS. The instability was most likely attributed to the presence of competing hydroxides and ions (HPO_4^{2-} and Cl^-) in the PBS solution, which have appreciable affinities for Ga^{3+} .

Synthesis of $[^{64}\text{Cu}][\text{CuL}^{12}]$ involved the addition of an aliquot of a DMSO stock solution of $\text{H}_2\text{L}^{12}\cdot\text{xHCl}$ (2 μL , 1 mg/mL) into a sodium acetate (pH 5.5) buffered solution of $[^{64}\text{Cu}][\text{CuCl}_2]$ (10 MBq) at room temperature. Incubation of the reaction mixture for 15 minutes yielded the product $[^{64}\text{Cu}][\text{CuL}^{12}]$ (Scheme 3.8). Analysis of the reaction mixture by RP-HPLC revealed a single peak at 16.0 minute with radiochemical purity of > 99% (Figure 3.41).



Scheme 3.8. Labelling of $H_2L^{12} \cdot xHCl$ with copper-64.

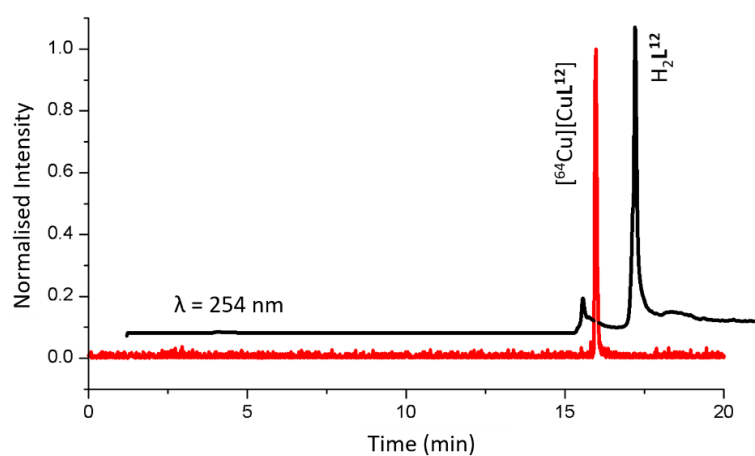


Figure 3.41. RP-HPLC chromatogram (red = radiation detection; black = UV detector at λ 254 nm) of the reaction mixture of $[^{64}Cu][CuL^{12}]$. Retention time at 16.0 min.

To study the stability of the copper-64 labelled radiotracer, $[^{64}\text{Cu}][\text{CuL}^{12}]$ was incubated in the presence of cysteine, histidine and EDTA for 1-2 h (Figure 3.42). It was evident that there was no release of free copper-64, suggesting that $[^{64}\text{Cu}][\text{CuL}^{12}]$ is stable and not susceptible to decomposition under these conditions.

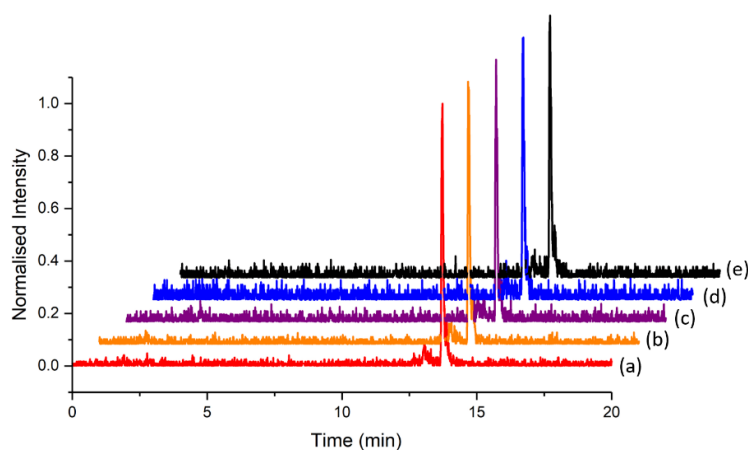


Figure 3.42. RP-HPLC chromatogram (radiation detection) of (a) $[^{64}\text{Cu}][\text{CuL}^{12}]$, (b) $[^{64}\text{Cu}][\text{CuL}^{12}]$ incubated with Cysteine (1 mM), (c) $[^{64}\text{Cu}][\text{CuL}^{12}]$ incubated with Histidine (1 mM), (d) $[^{64}\text{Cu}][\text{CuL}^{12}]$ incubated with EDTA (20 mM). (e) $[^{64}\text{Cu}][\text{CuL}^{12}]$ after 2 h.

Estimation of lipophilicity by log $D_{7.4}$ measurements

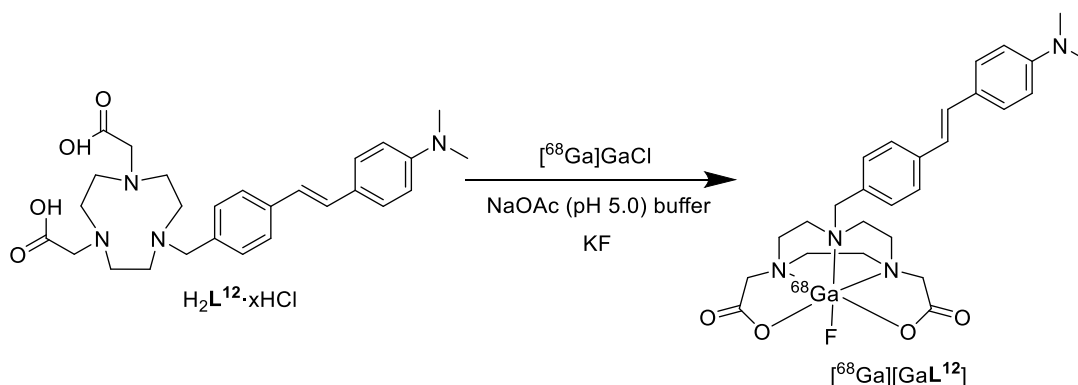
The lipophilicity of $[^{64}\text{Cu}][\text{CuL}^{12}]$ was tested using the shake-flask method by partitioning the compound in 1-octanol and PBS (pH 7.4) to obtain a log $D_{7.4}$ value. The log $D_{7.4}$ value provides a prediction of the absorption, distribution, metabolism and excretion rate of a compound.

The log $D_{7.4}$ value is obtained by using the following equation:

$$\text{Log } D_{7.4} = \text{Log}_{10} \frac{\text{cpm}_{\text{octanol}}}{\text{cpm}_{\text{PBS}}}$$

Compounds with log $D_{7.4}$ values in the range of 1-3.5 have a higher possibility to cross the blood-brain barrier.²³⁴ For $[^{64}\text{Cu}][\text{CuL}^{12}]$, the counts were obtained in triplicate, with an average log $D_{7.4}$ of 0.94. The relatively low log $D_{7.4}$ value suggested that $[^{64}\text{Cu}][\text{CuL}^{12}]$ has low lipophilicity, which may help partially explain the lack of binding to A β plaques in the human brain tissue.

Radiolabelling of $\text{H}_2\text{L}^{12} \cdot x\text{HCl}$ (4 μL , 0.033 mg/ μL) with gallium-68 (65 MBq) and KF was performed at pH 5. Initially, a radiochemical yield of 50% was achieved at room temperature (Scheme 3.9). The radiochemical yield increased with increasing reaction temperature, yielding a radiochemical yield of 77% at 80 °C. The crude mixture was purified using SPE cartridge and eluted with ethanol (Figure 3.43).



Scheme 3.9. Labelling of $\text{H}_2\text{L}^{12} \cdot x\text{HCl}$ with gallium-68.

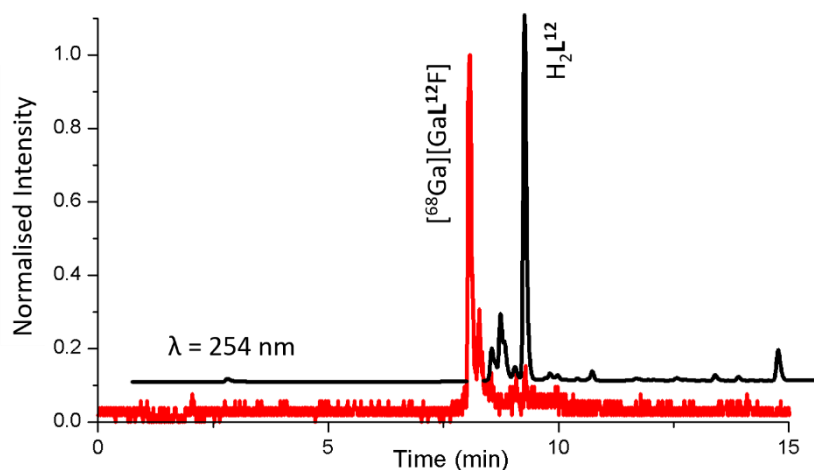


Figure 3.43. RP-HPLC chromatogram (red = radiation detection; black = UV detector at λ 254 nm) of [⁶⁸Ga][GaL¹²] after SPE purification. Retention time at 8.0 min.

All three radionuclides were incorporated into H₂L¹²·xHCl, proving the versatility of the chelate system. The ease of the copper-64 labelling without purification renders [⁶⁴Cu][CuL¹²] an excellent choice for further *in vivo* studies. The *in vivo* behaviour of the fluorine-18 labelled Ga³⁺-NODA complex is also explored for the first time.

3.9 *In vivo and ex vivo biodistribution studies of [¹⁸F][GaL¹²F] and [⁶⁴Cu][CuL¹²] in mouse models*

The biodistribution of [¹⁸F][GaL¹²F] and [⁶⁴Cu][CuL¹²] was investigated in healthy female C57Bl/6 mice (n=3). For the experiment, H₂L¹²·xHCl (2 µg) was used for every 10 MBq and the doses injected was 4 MBq per 100 µL. The radiotracers were administered intravenously in healthy mice, and the PET images were acquired at 5 min post-injection (Figure 3.44). The *ex vivo* biodistribution in the samples of interest was acquired at 2- and 60-min post-injection (Table 3.6). The radioactivity in the organs of interest was quantified and is expressed as the percentage of injected activity per gram of tissue (% IA/g). The uptake of [¹⁸F][GaL¹²F] and [⁶⁴Cu][CuL¹²] in the brain were $0.85 \pm 0.13\%$ IA/g and $0.71 \pm 0.03\%$ IA/g respectively at 2 min post-injection that reduced to

0.24 ± 0.02% IA/g for [¹⁸F][GaL¹²F] and 0.32 ± 0.01% IA/g for [⁶⁴Cu][CuL¹²] at 60 min post-injection. Fast brain clearance observed for both [¹⁸F][GaL¹²F] and [⁶⁴Cu][CuL¹²] suggesting that the radiotracers do not retain in the brain or bind non-specifically to white matter that could potentially lower the signal-to-background ratio.

The copper tracer, [⁶⁴Cu][CuL¹²], accumulated predominantly in the liver and kidneys. The uptake increased with time in the liver and kidney indicated excretion *via* the urinary and hepatobiliary pathways.

Table 3.6. Biodistribution measurements in normal mice (female C57Bl/6 mice) after intravenous injection of [¹⁸F][GaL¹²F] and [⁶⁴Cu][CuL¹²].

	[¹⁸ F][GaL ¹² F]		[⁶⁴ Cu][CuL ¹²]	
	2 min (% IA/g)	60 min (% IA/g)	2 min (% IA/g)	60 min (% IA/g)
Blood	15.7 ± 0.68	1.3 ± 0.18	13 ± 1.6	3.1 ± 0.68
Lungs	31.1 ± 6.45	2.5 ± 0.36	16 ± 2.7	7.9 ± 0.26
Heart	13 ± 0.86	1.0 ± 0.15	10 ± 0.71	2.5 ± 0.24
Liver	16 ± 1.6	6.0 ± 0.42	18 ± 2.6	27 ± 0.82
Kidneys	28.0 ± 2.1	4.3 ± 0.59	17 ± 1.6	34 ± 2.9
Muscle	4.5 ± 0.43	0.7 ± 0.06	2.3 ± 0.24	0.8 ± 0.05
Spleen	11 ± 1.2	1.5 ± 0.23	6.3 ± 1.2	3.6 ± 1.1
Bone	8 ± 0.9	27 ± 1.8	3.6 ± 0.45	2.0 ± 0.17
Brain	0.85 ± 0.13	0.2 ± 0.02	0.71 ± 0.03	0.32 ± 0.01

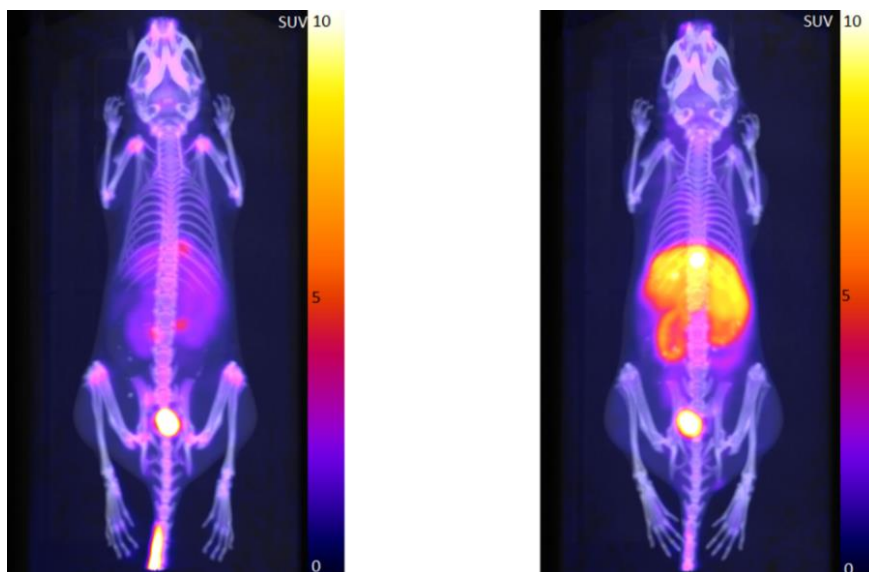


Figure 3.44. PET images reconstructed from images obtained at 5-min following administration of $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ (left) and $[^{64}\text{Cu}][\text{CuL}^{12}]$ (right) in healthy female C57Bl/6 mice.

There was a high uptake of $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ in the lungs and kidneys in the first 2 minutes post-injection which cleared rapidly. The initial uptake of $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ in bone was $8 \pm 0.9\%$ IA/g, which increased to $27 \pm 1.8\%$ IA/g at 60 min post-injection.

A saline solution of ‘free’ $[^{18}\text{F}]\text{F}^-$ (50 μCi) injected into the peritoneum of a mouse revealed that ‘free’ $[^{18}\text{F}]\text{F}^-$ in the body accumulates in calcium-rich bone and the bladder (Figure 3.45). Although the uptake of $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ in bone is not as high as the ‘free’ $[^{18}\text{F}]\text{F}^-$ in figure 3.45, the increase in uptake at 60-min post injection, particularly in the spine indicated the release of either $[^{18}\text{F}]\{\text{GaF}\}^{2+}$ or $[^{18}\text{F}]\text{F}^-$. The biodistribution of $[^{18}\text{F}]\text{F}^-$ following injection of $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ suggests that the $\text{Ga}^{3+}\text{-F}^-$ complex has a low stability *in vivo*.

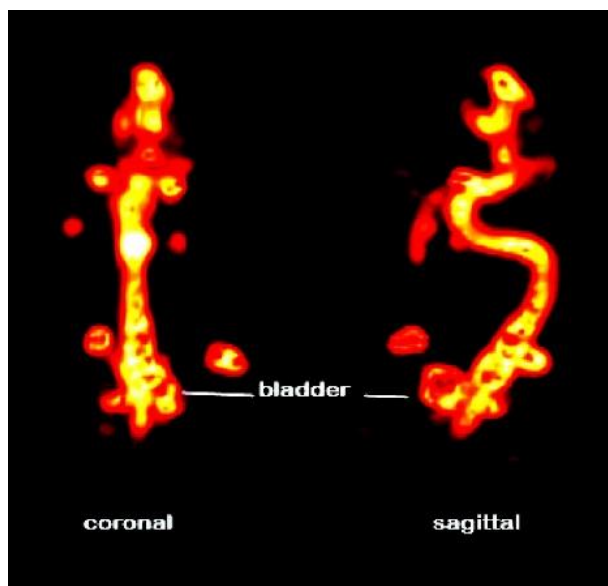


Figure 3.45. PET images reconstructed from images obtained at 60-min post-injection of a saline solution of free $[^{18}\text{F}]\text{F}^-$ (50 μCi) in mice (left: coronal; right: sagittal) with high localisation in the bladder and skeletal structures. Reproduced with permission from Ting, R.; Harwig, C.; Keller, U.; Perrin, D. M. et al. *J. Am. Chem. Soc.*, 2008, 130, 12045. Copyright (2008) American Chemical Society.

Although $[\text{GaL}^{12}\text{F}]$ is stable in aqueous solutions at pH 4 - 6 and in the presence of human serum albumin, the fluorine-18 analogue did not have sufficient stability *in vivo*. The instability of $[\text{GaL}^{12}\text{F}]$ can be attributed to the Ga^{3+} -carboxylate interaction that may have led to decomposition. Despite the formation of $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ with high radiochemical yield at temperature below 100 $^{\circ}\text{C}$, it appears to be less stable compared to $[^{18}\text{F}]\text{Al}^{3+}\text{-F}^-$ complexes.

3.10 Summary

A 1,4,7-triazacyclononane-1,4-diacetic acid (NODA) ligand with an appended stilbene functional group, $\text{H}_2\text{L}^{12} \cdot x\text{HCl}$, and its Ga^{3+} complexes, $[\text{GaL}^{12}\text{Cl}]$ and $[\text{GaL}^{12}\text{F}]$ were prepared. These compounds were designed to incorporate the amyloid-binding stilbene functional group for the detection of $\text{A}\beta$ aggregates in AD patients. The fluorescence properties of the stilbene functional group allowed the detection and visualisation of the interaction of $\text{H}_2\text{L}^{12} \cdot x\text{HCl}$ with amyloid plaques on human frontal cortex tissue using epifluorescence microscopy. The decreased emission intensities of $[\text{GaL}^{12}\text{Cl}]$ and $[\text{GaL}^{12}\text{F}]$ may be one of the reasons for the lack of binding to amyloid plaques. It is also possible that the coordination of $\text{H}_2\text{L}^{12} \cdot x\text{HCl}$ to Ga^{3+} reduced the binding affinity for amyloid plaques. The binding to $\text{A}\beta$ fibrils studied using the competition with ThT provided K_i values of $\text{H}_2\text{L}^{12} \cdot x\text{HCl} = 1.8 \pm 0.9 \mu\text{M}$, $[\text{GaL}^{12}\text{Cl}] = 6.5 \pm 2.3 \mu\text{M}$ and $[\text{GaL}^{12}\text{F}] = 8.8 \pm 5.3 \mu\text{M}$, which suggested that these compounds only bind to the amyloid fibrils with moderate affinity.

Despite the lack of ability as an $\text{A}\beta$ plaque imaging agent, H_2L^{12} can be exploited as a model compound to be radiolabelled with fluorine-18, copper-64 and gallium-68. The synthesis of $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ was achieved using either a one-pot method with H_2L^{12} and $\text{Ga}(\text{NO}_3)_3$ or the pre-formed $[\text{GaL}^{12}\text{Cl}]$. The labelled product was produced with a high radiochemical yield at elevated temperature and was readily purified to remove unlabelled fluorine-18. The fluorine-18 tracer showed high stability between pH 4 - 6 in sodium acetate solution but only 50% remained intact when formulated in PBS (pH 7.4). The instability was reflected by the increased uptake in bone at 60 min post-injection in mice. Although the formation of $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ with high radiochemical yield at temperature below 100°C is an advantage compared to the temperature ($> 100^\circ\text{C}$)

required to form $[^{18}\text{F}]\text{Al}^{3+}\text{-F}^-$ complexes, the $[^{18}\text{F}]\text{Ga}^{3+}\text{-F}^-$ system lacks the stability exhibited by the $[^{18}\text{F}]\text{Al}^{3+}\text{-F}^-$ system, particularly at pH 7.4.

The incorporation of three radionuclides; fluorine-18, gallium-68 and copper-64 into $\text{H}_2\text{L}^{12}\cdot\text{xHCl}$ proved the versatility of the chelator. The tracer $[^{64}\text{Cu}][\text{CuL}^{12}]$ was synthesised with high radiochemical yield without purification and showed excellent stability in the presence of EDTA, cysteine and histidine as well as *in vivo*. The low uptake in brain at 2 min post-injection of both tracers ($0.85 \pm 0.13\%$ IA/g for $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ and $0.71 \pm 0.03\%$ IA/g for $[^{64}\text{Cu}][\text{CuL}^{12}]$) suggests that these radioactive compounds can cross the blood-brain barrier, but they do not satisfy the requirement of a PET diagnostic neuroimaging agent for AD. The $[^{64}\text{Cu}][\text{CuL}^{12}]$ emerged as a promising candidate that may be more suited for other imaging applications where hydrophilicity is more favourable.

3.11 Method

General method

Paraffin preserved brain tissue blocks were provided by the Victorian Brain Bank Network. Both AD and HC brain tissue sections (8 μm) were deparaffined (xylene, 3 x 3 min) followed by rehydration (2 min soakings in 100%, 90%, 70% and 0% v/v ethanol/water). The hydrated sections were washed in phosphate-buffered saline (PBS, 5 min). Autofluorescence by the tissue was quenched by treatment with potassium permanganate (0.25% w/v PBS, 20 min) followed by washing with PBS (2 x 2 min). The brown coloured tissue was washed with potassium metabisulfite and oxalic acid (1% w/v PBS, 6 min). The tissue sections were then washed with PBS (3 x 2 min) and bovine serum albumin (BSA, 2% w/v in PBS, 10 min), rinsed with PBS and treated with compound (10 mM in DMSO in PBS, 10 min). The tissue sections were washed with BSA solution to remove non-bounded compounds. The sections were washed with PBS (3 x 2 min), dipped in distilled water and mounted with non-fluorescent mounting medium.

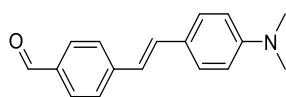
[^{18}F]/ H_2O was obtained from the Peter MacCallum Cancer Centre Cyclotek, prepared using PETtrace 16.5 MeB cyclotron incorporating a high-pressure niobium target for the production of fluorine-18 (GE healthcare) as target wash.

[^{64}Cu] was obtained as [^{64}Cu] CuCl_2 from RAPID Laboratories, Medical Technology and Physics, Sir Charles Gairdner Hospital (Perth, WA) as a formulation of 0.02 M HCl.

[^{68}Ga] was obtained as [^{68}Ga] GaCl_3 from Peter MacCallum Cancer Centre from a $^{68}\text{Ga}/^{68}\text{Ge}$ generator as a formulation of 0.1 M HCl.

Radio-RP-HPLC traces were acquired using a Shimadzu SPD-10ATvP HPLC system equipped with a Waters C column (4.6 x 150 mm, 5 μ m) with a 1 mL/min flow rate. Chromatograms were recorded with a scintillation detector and a UV-vis detector (254 nm and 280 nm). Retention times were recorded using a gradient elution of (1) 0-95% B (acetonitrile) in A (Milli-Q water) over 15 min or (2) 0-100% B (0.05% TFA in acetonitrile) in A (0.05% TFA in Milli-Q water) over 20 min.

4-((4-dimethylamino)styryl)benzaldehyde (**40**)

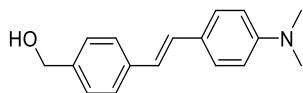


4-((4-dimethylamino)styryl)benzaldehyde was synthesized using an adapted literature protocol.²²⁹

To anhydrous and deoxygenated THF (5 mL) was added (*E*)-4-(4-bromostyryl)-*N,N*-dimethylaniline (0.68 g, 2.3 mmol) and the mixture was cooled to -78 °C. To the reaction mixture was added *n*-butyl lithium (1.2 mL, 2.5 M) dropwise and then DMF (5 mL). The mixture was stirred for 3.5 h at -78 °C. The reaction was diluted with diethyl ether (5 mL) then water (30 mL) to afford a bright yellow precipitate. The reaction mixture was extracted with dichloromethane (150 mL), washed with brine (100 mL), then water (3 x 100 mL). The organic phase was then dried with MgSO₄, filtered and the solvent was removed under reduced pressure to afford 4-((4-dimethylamino)styryl)benzaldehyde as an orange crystalline powder (0.49 g, 86%). HR-MS(ESI/O-TOF): [C₁₇H₁₇NO+H]⁺ *m/z* 252.139 (experimental), 252.139 (calculated). ¹H NMR (400 MHz; CDCl₃): δ_{H} 9.96 (s, 1H, O=CH), 7.84-7.82 (m, AA'BB', *J* = 8.2 Hz, 2H, ArH), 7.60 (m, AA'BB' 2H, *J* = 8.2 Hz, ArH), 7.45 (d, *J* = 8.8 Hz, 2H), 7.21 (d, ₃*J*_{HH} = 16.3, 1H, CH=CH), 6.94 (d, ₃*J*_{HH} = 16.3, 1H, CH=CH), 6.73-6.71 (m, AA'BB', *J* = 8.7 Hz, 2H, ArH), 2.99 (s, 6H, N(CH₃)₂).

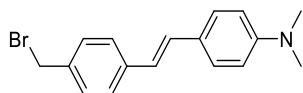
$^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, CDCl_3): δ_{C} (ppm) = 154.2, 95.0, 92.8, 90.7, 88.7, 85.2, 74.7, 2.85.

(E)-(4-(4-(diamethylamino)styryl)phenyl)methanol (**41**)



To a solution of 4-((4-dimethylamino)styryl)benzaldehyde (0.22 g, 0.85 mmol) in tetrahydrofuran (35 mL) was added sodium borohydride (0.080 g, 2.1 mmol) and methanol (50 μL). The reaction was stirred at room temperature for 5 h. The reaction was quenched with NH_4Cl (100 mL), extracted with dichloromethane (2 x 100 mL) and washed with water (100 mL). The organic layer was removed under reduced pressure and was purified by column chromatography (DCM:MeOH 2 to 5%) to give *(E)*-(4-(4-(diamethylamino)styryl)phenyl)methanol as a yellow powder (0.18 g, 85%). HR-MS(ESI/O-TOF): $[\text{C}_{17}\text{H}_{19}\text{NO}+\text{H}]^+$ m/z 254.154 (experimental), 254.154 (calculated). ^1H NMR (500 MHz, CDCl_3): δ_{H} 7.84-7.82 (m, AA'BB', J = 8.2 Hz, 2H, ArH), 7.60 (m, AA'BB' 2H, J = 8.2 Hz, ArH), 7.45 (d, J = 8.8 Hz, 2H), 7.21 (d, $^3J_{\text{HH}}$ = 16.3, 1H, CH=CH), 6.94 (d, $^3J_{\text{HH}}$ = 16.3, 1H, CH=CH), 6.73-6.71 (m, AA'BB', J = 8.7 Hz, 2H, ArH), 4.40 (s, 1H, CH_2), 2.99 (s, 6H, $\text{N}(\text{CH}_3)_2$).

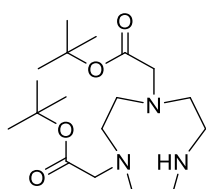
(E)-4-(4-(bromomethyl)styryl)-*N,N*-dimethylaniline (**42**)



To a solution of *(E)*-(4-(4-(diamethylamino)styryl)phenyl)methanol (0.15 g, 0.57 mmol) in dry dichloromethane (20 mL) was added phosphorus tribromide (0.070 mL, 0.74 mmol) dropwise at 0 $^{\circ}\text{C}$ under N_2 . The reaction was stirred at room temperature for 6 h. The reaction was quenched with saturated K_2CO_3 (50 mL), extracted with

dichloromethane (100 mL) and washed with water (3 x 100 mL). The organic layer was dried with MgSO₄, filtered and the solvent was removed under reduced pressure to afford (*E*)-4-(4-(bromomethyl)styryl)-*N,N*-dimethylaniline as a yellow powder (0.104 g, 57%). HR-MS(ESI/O-TOF): [C₁₇H₁₈BrN+H]⁺ *m/z* 316.070 (experimental), 316.070 (calculated). ¹H NMR (500 MHz, CDCl₃) δ_H (ppm) 7.44 (d, *J* = 8.2 Hz, 2H, Ar*H*), 7.41 (d, *J* = 8.7 Hz, 1H), 7.34 (d, *J* = 8.2 Hz, 2H, Ar*H*), 7.05 (d, *J* = 16.3 Hz, 1H, CH), 6.89 (d, *J* = 16.3 Hz, 1H, CH), 6.71 (d, *J* = 8.8 Hz, 2H, Ar*H*), 4.51 (s, 2H, CH₂), 3.01 (s, 6H, N(CH₃)₂).

1,4-bis(tert-butoxycarbonylmethyl)-1,4,7-triazanonane

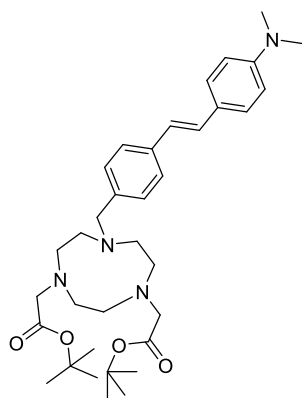


1,4-bis(*tert*-butoxycarbonylmethyl)-1,4,7-triazanonane was synthesized using an adapted literature protocol.¹³⁴

A solution of *tert*-butyl bromoacetate (0.53 g, 4.1 mmol) in CHCl₃ (15 mL) was added to triazacyclononane (1.4 mL, 9.0 mmol) in CHCl₃ (25 mL) dropwise. The mixture was stirred at room temperature for 24 h. The reaction mixture was filtered, and the solvent was removed under reduced pressure. The residue was treated with distilled water (15 mL) and 1M HCl to reach pH 3 followed by extraction with diethyl ether (50 mL x 2). The aqueous layer was adjusted to pH 8-9 using 1M NaOH and extracted with dichloromethane (25 mL x 2). The organic layer was removed under reduced pressure and the residue was treated with distilled water (5 mL). The resulting solution was adjusted to pH 10 using 1M NaOH and extracted with diethyl ether (30 mL x 2). The aqueous layer was adjusted to pH 8 using 1M HCl and extracted with dichloromethane

(25 mL x 2). The organic layer was removed under reduced pressure and dried in vacuo to give the disubstituted 1,4-bis(*tert*-butoxycarbonylmethyl)-1,4,7-triazanonane as a light-brown solid (0.3 g, 31%). HR-MS(ESI/O-TOF): $[\text{C}_{18}\text{H}_{35}\text{N}_3\text{O}_4+\text{H}]^+$ m/z 358.400 (experimental), 358.270 (calculated). ^1H NMR (400 MHz, CDCl_3) δ_{H} (ppm) 3.35 (s, 4H), 3.22 (t, $J = 5.5$ Hz, 4H), 3.03 (t, $J = 5.7$ Hz, 4H), 2.75 (s, 4H), 1.44 (s, 18H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, CDCl_3): δ_{C} (ppm) = 170.9, 82.3, 56.7, 51.9, 48.9, 44.8, 28.3.

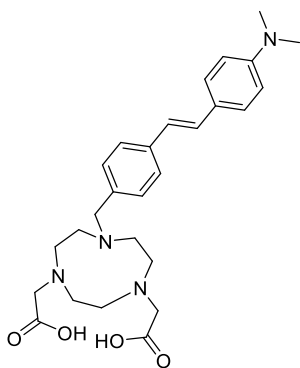
*(tBu)*₂**L**¹²



To a solution of 1,4-bis(*tert*-butoxycarbonylmethyl)-1,4,7-triazanonane (0.113 g, 0.316 mmol) and K_2CO_3 (0.3 g, 2 mmol) in THF (25 mL) was added (*E*)-4-(4-(bromomethyl)styryl)-*N,N*-dimethylaniline (0.100 g, 0.316 mmol) in THF dropwise. The mixture was stirred at room temperature for 24 h. The solution was filtered through a pad of celite and the solvent was removed under reduced pressure to give (*tBu*)₂**L**¹² as yellow oil. HR-MS(ESI/O-TOF): $[\text{C}_{35}\text{H}_{52}\text{N}_4\text{O}_4+\text{H}]^+$ m/z 593.406 (experimental), 593.407 (calculated). ^1H NMR (500 MHz, CDCl_3) δ_{H} (ppm) = 7.41 (dd, $J = 8.5, 3.1$ Hz, 4H, ArH), 7.31 (d, $J = 8.1$ Hz, 2H, ArH), 7.05 (t, $J = 15.6$ Hz, 1H, CH), 6.90 (d, $J = 16.3$ Hz, 1H, CH), 6.72 (d, $J = 8.8$ Hz, 2H, ArH), 3.75 (t, $J = 6.6$ Hz, 1H, ArCH₂), 3.67 (s, 1H, ArCH₂), 3.31 (s, 4H, NCH₂CO₂), 2.98 (s, 6H, N(CH₃)₂), 2.94 (s, 4H, CH₂), 2.83 (t, 8H, CH₂), 1.45-1.43 (s, 18H, (C(CH₃)₃)). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, CDCl_3): δ_{C} (ppm) = 171.7 (C=O),

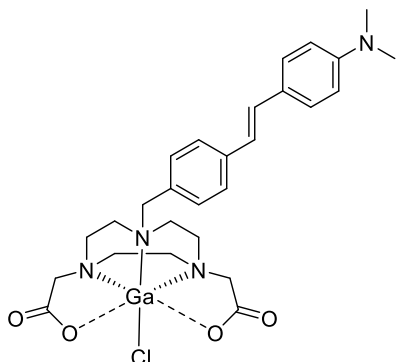
150.2 (C=O), 129.5 (ArC), 128.4 (ArC), 127.6 (ArC), 126.1 (ArC), 125.9 (ArC), 125.7 (ArC), 124.5 (CH), 112.6 (ArC), 80.8, 68.1 (ArCH₂), 62.5 (ArCH₂), 60.1 (NCH₂CO₂), 55.6 (CH₂), 55.4 (CH₂), 40.6 (N(CH₃)₂), 30.5 (C(CH₃)₃), 28.4 (C(CH₃)₃), 25.8.

H₂L¹²·xHCl



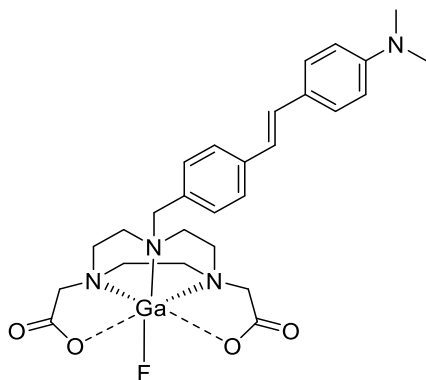
To a solution of (tBu)₂L¹² in 1,4-dioxane (20 mL) was added concentrated HCl (2 mL) dropwise. The mixture was left stirring at room temperature for 24 h. The solvent was removed under reduced pressure. The solid that formed was collected by filtration, washed with cold acetone and dried *in vacuo* to give H₂L¹²·xHCl as a light brown solid (0.13 g, 80%). HR-MS(ESI/O-TOF): [C₂₇H₃₆N₄O₄+H]⁺ *m/z* 481.281 (experimental), 481.281 (calculated). ¹H NMR (500 MHz, DMSO-*d*₆) δ_H (ppm) 7.57 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.49-7.37 (t, *J* = 8 Hz, 4H, Ar*H*), 7.17 (d, *J* = 16.4 Hz, 1H, CH), 6.99 (d, *J* = 16.4 Hz, 1H, CH), 6.76 (d, *J* = 8.6 Hz, 2H, Ar*H*), 4.31 (s, 2H, ArCH₂), 3.44 (d, *J* = 17.8 Hz, 2H, NCH₂CO₂), 3.29 (d, 2H, NCH₂CO₂), 3.10 (m, 4H, CH₂), 3.02 (m, 2H, CH₂), 2.92 (s, 6H, N(CH₃)₂), 2.79 (m, 2H, CH₂), 2.66 (m, 4H, CH₂). ¹³C{¹H} NMR (126 MHz, CDCl₃): δ_C (ppm) = 172.5 (C=O), 149.7 (C=O), 138.7, 130.7 (ArC), 130.2 (CH), 127.8 (ArC), 126.3 (ArC), 123.2 (CH), 119.4 (ArC), 117.0 (ArC), 114.7 (ArC), 112.9 (ArC), 57.8 (ArCH₂), 54.7 (NCH₂CO₂), 50.3 (CH₂), 49.2 (CH₂), 46.7 (CH₂), 40.4 (N(CH₃)₂).

[GaL¹²Cl]



To a solution of H₂L¹²·HCl (0.21 g, 0.40 mmol) in ethanol (20 mL) was added GaCl₃ in pentane (2.0 mL). The mixture was heated to 60 °C for 2 h. The solid that formed was collected by filtration, washed with cold ethanol and diethyl ether and dried *in vacuo* to give [GaL¹²Cl] as a pale-yellow solid (0.15 g, 60%). HR-MS(ESI/O-TOF): [C₂₇H₃₄GaN₄O₄]⁺ *m/z* 547.184 (experimental), 547.184 (calculated). ¹H NMR (500 MHz, DMSO-*d*₆) δ_H (ppm) 7.55 (d, *J* = 8.1 Hz, 2H, Ar*H*), 7.44 (d, *J* = 7.4 Hz, 4H, Ar*H*), 7.18 (d, *J* = 16.4 Hz, 1H, CH), 6.99 (d, *J* = 16.4 Hz, 1H, CH), 6.72 (d, *J* = 8.8 Hz, 2H, Ar*H*), 4.34 (d, *J* = 14.1 Hz, 1H, ArCH₂), 3.78 (d, *J* = 14.3 Hz, 1H, ArCH₂), 3.55 (s, 2H, CH₂), 3.46 (d, *J* = 8.8 Hz, 2H, NCH₂CO₂), 3.24 (d, *J* = 3.6 Hz, 2H, NCH₂CO₂), 3.11 – 2.99 (m, 4H, CH₂), 2.93 (s, 6H, N(CH₃)₂), 2.89 (m, 2H, CH₂), 2.79 (m, 4H, CH₂). ¹³C{¹H} NMR (126 MHz, DMSO-*d*₆): δ_C (ppm) = 170.9, 170.8, 138.2, 137.9, 132.7, 132.5, 130.9, 129.0, 127.7, 126.0, 125.9, 62.4, 61.3, 53.1, 51.8, 51.5, 51.2, 45.4.

[GaL¹²F]



To a solution of [GaL¹²Cl] (10 mg, 17 μ mol) in acetonitrile (20 mL) was added KF (1.0 mg, 17 μ mol) and 18-crown-6 (5.0 mg, 17 μ mol). The mixture was heated to reflux for 2 h. The solvent was allowed to cool to room temperature. The solid that formed was collected by filtration, washed with cold diethyl ether and dried *in vacuo* to give [GaL¹²F] as a pale-yellow solid (5.2 mg, 54%). HR-MS(ESI/O-TOF): [C₂₇H₃₄GaN₄O₄]⁺ *m/z* 547.143 (experimental), 547.184 (calculated). ¹H NMR (500 MHz, DMSO-d₆) δ _H (ppm) 7.55 (d, *J* = 8.1 Hz, 2H, Ar*H*), 7.44 (d, *J* = 7.4 Hz, 4H, Ar*H*), 7.18 (d, *J* = 16.4 Hz, 1H, CH), 6.99 (d, *J* = 16.4 Hz, 1H, CH), 6.72 (d, *J* = 8.8 Hz, 2H, Ar*H*), 4.34 (d, *J* = 14.1 Hz, 1H, ArCH₂), 3.78 (d, *J* = 14.3 Hz, 1H, ArCH₂), 3.55 (s, 2H, CH₂), 3.46 (d, *J* = 8.8 Hz, 2H, NCH₂CO₂), 3.24 (d, *J* = 3.6 Hz, 2H, NCH₂CO₂), 3.11 – 2.99 (m, 4H, CH₂), 2.93 (s, 6H, N(CH₃)₂), 2.89 (m, 2H, CH₂), 2.79 (m, 4H, CH₂). ¹³C{¹H} NMR (126 MHz, DMSO-d₆): δ _C (ppm) = 171.0, 170.8, 138.1, 137.9, 132.7, 132.5, 130.9, 128.9, 127.8, 126.1, 125.9, 62.6, 62.4, 61.34 53.1, 51.8, 51.7, 51.3, 45.4, 45.0. ¹⁹F NMR (470 MHz, DMSO-d₆): δ _F (ppm) = -180.7 ppm.

$[^{18}\text{F}][\text{GaL}^{12}\text{F}]$

An aliquot of $\text{Ga}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (1 μL , 2 mM stock solution) was added into a solution of $^{18}\text{F}^-$ (300 MBq) in sodium acetate buffer (300 μL). To this was added H_2L^{12} (7.0 μg , 1.4 μmol). The reaction was incubated for 30 min at 80 °C. The mixture was analysed by RP-HPLC (50% radiochemical yield). The reaction mixture was purified using a PD-10 column conditioned with water. Five 100 μL fractions were collected. Radiochemical purity was confirmed by RP-HPLC. The reaction mixture was diluted with sodium acetate. Six doses of 2 MBq in 100 μL fractions were drawn up into insulin syringes. Each dose was administered via tail vein injection into C57BI/6 mice. PET images were obtained at 5 min. Three mice were sacrificed at both 2- and 60-min. Collected organs of interest and samples of blood were weighed and measured for radioactivity in a Beckman well counter. The results were expressed as the percentage of the injected dose per gram (% IA/g) of blood or organs.

$[^{68}\text{Ga}][\text{GaL}^{12}\text{F}]$

An aliquot of $[^{68}\text{Ga}][\text{Ga}]$ (200 μL , 65 MBq) was eluted from an ITG generator using 0.05 M HCl. A solution of H_2L^{12} (130 μg , 2 $\mu\text{g}/\text{MBq}$) was added and the solution was left standing at 80 °C for 20 min. The mixture was analysed by RP-HPLC (70% radiochemical yield). The reaction mixture was purified using a PD-10 column conditioned with water. Five 100 μL fractions were collected. Radiochemical purity (>99%) was confirmed by RP-HPLC.

$[^{64}\text{Cu}][\text{CuL}^{12}]$

An aliquot of $[^{64}\text{Cu}][\text{CuCl}_2]$ (25 μL , 32 MBq) was buffered to pH 5.0 with sodium acetate buffer (0.1 M, 100 μL). A solution of H_2L^{12} (7.2 μg , 15 nmol, 1.5 $\mu\text{g}/10\text{ MBq}$) was added and the solution was left standing at ambient temperature for 20 min. The mixture was

analysed by RP-HPLC (>99% radiochemical yield). The reaction mixture was diluted with ethanol (147 μ L, 8%), DMSO (147 μ L, 8%) and sodium acetate (1421 μ L) to give a total volume of 1.84 mL of ~41 MBq. Six doses of 4.5 MBq in 100 μ L fractions were drawn up into insulin syringes. Each dose was administered via tail vein injection into C57BI/6 mice. PET images were obtained at 5 min. Three mice were sacrificed at both 2- and 60-min. Collected organs of interest and samples of blood were weighed and measured for radioactivity in a Beckman well counter. The results were expressed as the percentage of the injected dose per gram (% IA/g) of blood or organs.

Chapter 4 Tetraaza N4 core ligands as chelators

4.1 Introduction to porphyrins and tetraaza[14]annulene

The naturally occurring porphyrins are heterocyclic tetrapyrrolic ligands that are present in the active sites of biomolecules. The pigmented porphyrins derived their name from the Greek term “porphura” referring to the colour purple.²³⁵ The four pyrrolic rings are linked by four methene bridges to give a planar structure (Figure 4.1). An attractive feature of porphyrins with four pyrrolic nitrogen atoms includes the ability to bind to a great variety of metal ions. Nature uses porphyrin ligands to harness the potential of metal ions for applications ranging from catalysis, electron transfer and the transport of dioxygen.²³⁶⁻²⁴² For example, heme, a prosthetic group found in haemoglobin, consists of a tetrapyrrole porphyrin macrocycle containing a dianionic conjugated system coordinated to Fe^{2+} . The heme structure also includes two vinyl groups and two propionate side chains (Figure 4.1). The Fe^{2+} in heme groups binds to and transport oxygen in the body.²⁴³

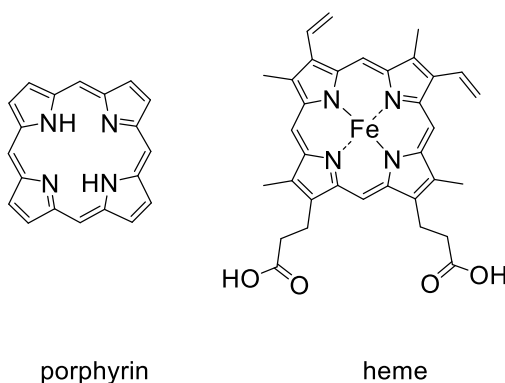
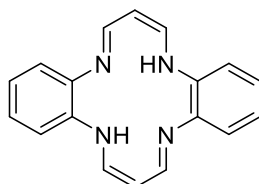


Figure 4.1. Chemical structures of porphyrin and heme.

Tetraaza[14]annulene ligands can be considered as synthetic analogues of porphyrins that share common characteristics with porphyrins (Figure 4.2). Both porphyrin and tetraaza[14]annulene are planar, with four nitrogen donor atoms and conjugated system of double bonds.



dibenzo[14]tetraazaannulene

Figure 4.2. Chemical structure of dibenzo[14]tetraazaannulene.

Porphyrin has a delocalised and aromatic ($4n+2$) system whereas tetraaza[14]annulene ligand has a Hückel anti-aromatic ($4n$) system. The negative ionic charges of tetraaza[14]annulene are delocalised over the 2,4-pentanediiimato section of the macrocycle. This is different to the porphyrins where the negative charges are delocalised over the entire macrocyclic framework. As tetraaza[14]annulene only has fourteen atom in the inner ring, it has a smaller central cavity (1.92 Å) compared to the sixteen-membered porphyrins (difference of ~ 0.1 Å). The difference in cavity size leads to shorter distances between the metal ion and the nitrogen atoms of tetraaza[14]annulene.

The 14-membered tetrazamacrocyclic can be prepared by a metal ion template reaction with imine formation as the ring-closing step. A variety of metal complexes with first-row *d*-transition metal cations have been prepared and their properties investigated. In general, these metal complexes are resistant to hydrolysis and demonstrate high kinetic inertness and thermodynamic stability ascribed to the “macrocyclic effect”. The repulsive interactions within the ligand may have contributed to shortening of the M-N distances.

The unusually large formation constants arise from the thermodynamic stability due to the high ligand field strength of the donor atoms and the “preorganised” macrocycle.²⁴⁴

The structure of dibenzotetraaza[14]annulene ligand as revealed by X-ray crystallography is essentially planar with a deviation of ~ 0.06 Å from planarity (Figure 4.3).²⁴⁵ The central cavity, which is the average distance from the nitrogen atoms to the mid-point of the four atoms, is approximately 1.92 Å. In the absence of a coordinating central metal ion, the distortion arises from the van der Waals repulsion of the hydrogen atoms of the NH groups. The N(2)-C(7) and N(4)-C(16) bond distances are 1.331(4) Å and 1.338(4) Å respectively. These two bonds are longer than the N(1)-C(18) and N(3)-C(9) bonds (1.297(4) Å and 1.299(4) Å). The difference in bond distances distinguishes the single (N(2)-C(7) and N(4)-C(16)) and double (N(1)-C(18) and N(3)-C(9)) bonds.

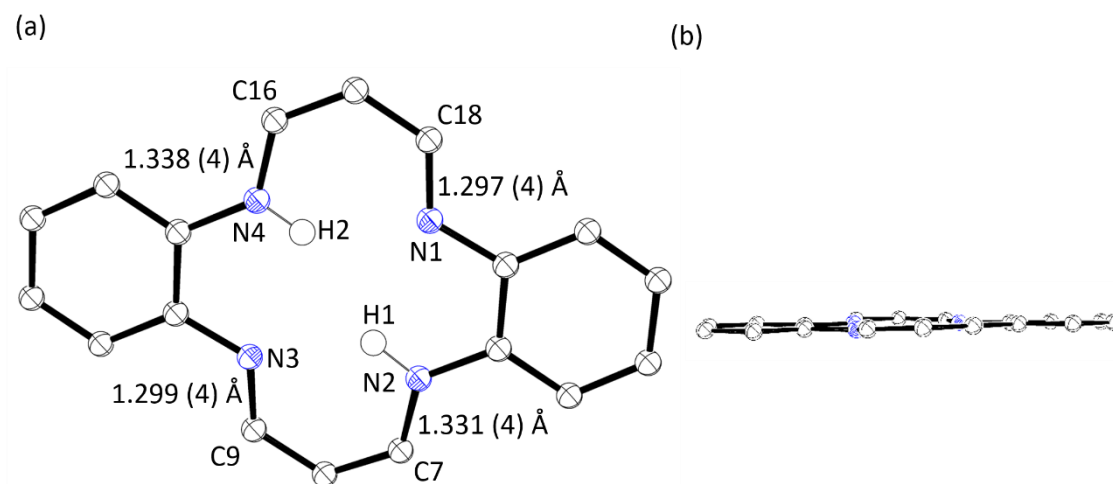


Figure 4.3. ORTEP representation (30% ellipsoid probability) of dibenzotetraaza[14]annulene and the side view showing the planarity. Hydrogen atoms attached to carbon atoms omitted for clarity.²⁴⁵

Interest in this area has been focused on functionalization of the macrocycle by adding and modifying substituents on the carbon atoms (positions R^1 , R^2 and R^3) of the dibenzotetraaza[14]annulene to alter the chemical properties and potential catalytic activities (Figure 4.4).

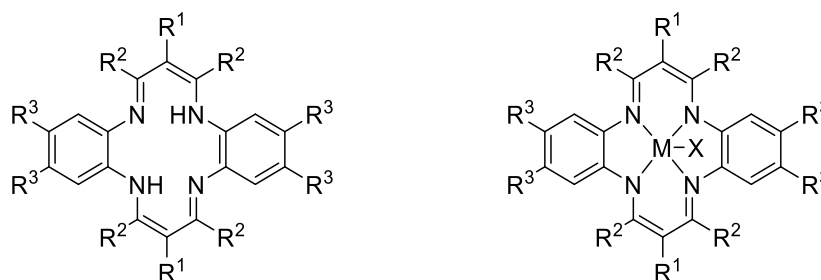


Figure 4.4. Chemical structure of taa and its metal complex with substituent positions on the carbon atoms at R^1 , R^2 and R^3 .

Substituents at positions R^1 and R^3 usually lead to macrocycles that remain planar (Figure 4.5).²⁴⁶⁻²⁴⁷ In each case, the single C-N bond (~ 1.33 Å) and double C-N bond (~ 1.30 Å) are distinguishable based on the bond distances.

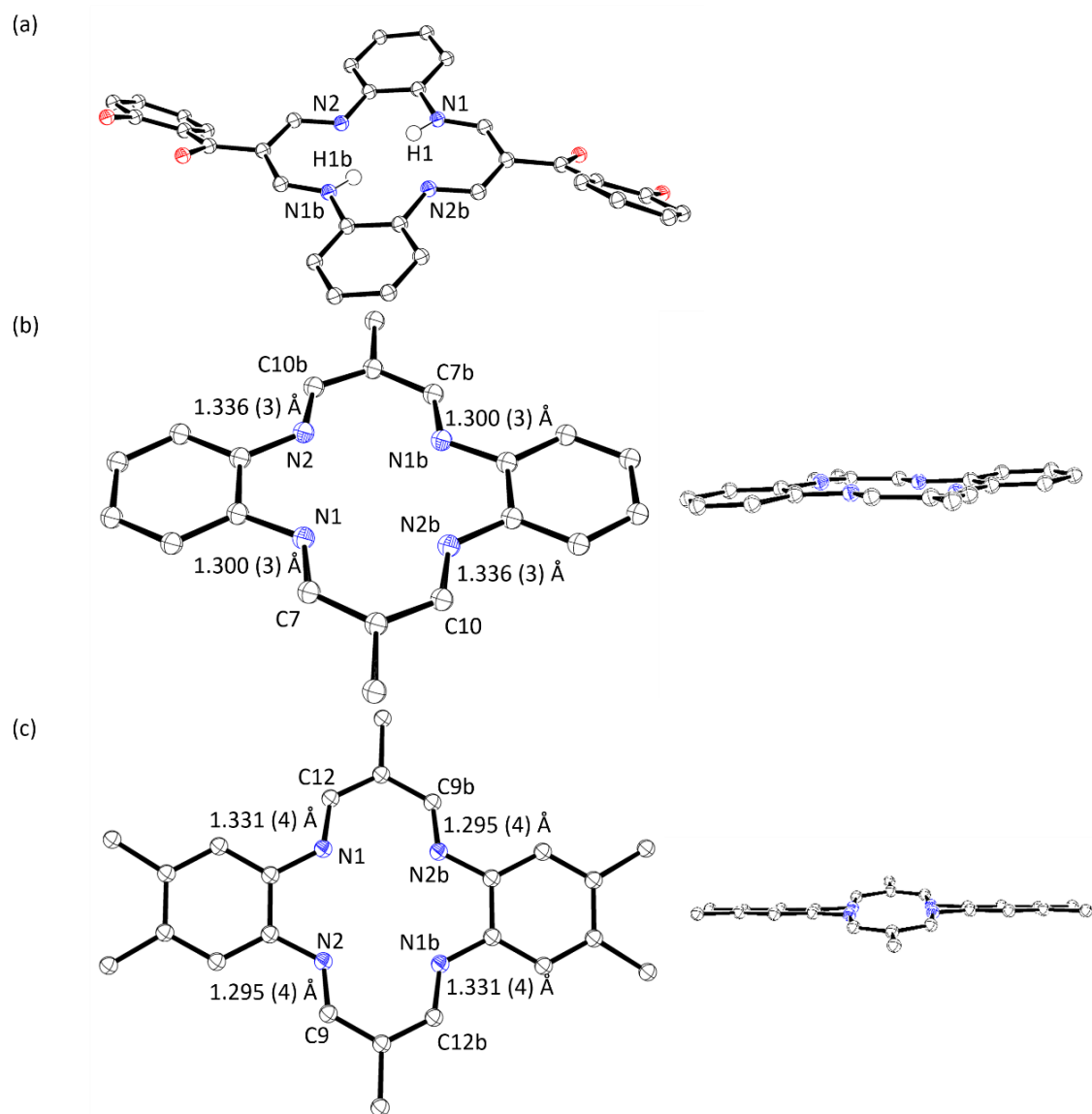


Figure 4.5. ORTEP representation (30% ellipsoid probability) of taa compounds with substituents at position R^1 (a and b) and at positions R^1 and R^3 (c) with their side views showing the planarity. Hydrogen atoms attached to carbon atoms and solvent atoms omitted for clarity.²⁴⁶⁻²⁴⁷

In the absence of substituent at the R^2 position, the planarity of the dibenzotetraaza[14]annulene ring is not significantly affected in the ligand, H_2L^{13} and its Co^{2+} complex, $[CoL^{13}]$ (Figure 4.6). For H_2L^{13} , the secondary amines N(2) and N(4) lies ~ 0.02 Å above the mean of the N_4 plane while N(1) and N(3) lies ~ 0.02 Å below the mean of the N_4 plane. The phenyl groups at position R^3 are rotated with respect to the N_4 plane at the C-C bond. Upon coordination to Co^{2+} , the nitrogen atoms are planar with near equal distance from Co^{2+} (~ 1.87 Å). There is less deviation from the N_4 plane as compared to the free ligand H_2L^{13} . The low-spin Co^{2+} has an ionic radius of 0.63 Å that fits in the ring without steric strain.¹²⁷

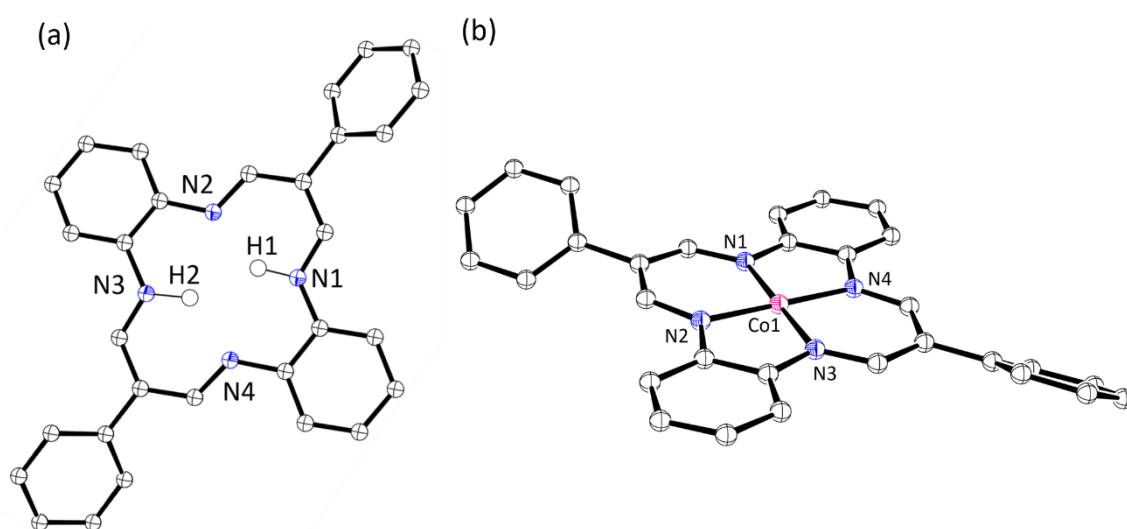


Figure 4.6. ORTEP representation (30% ellipsoid probability) of H_2L^{13} (a) and square planar $[CoL^{13}]$ (b) with no substituent at R^2 position. Hydrogen atoms attached to carbon atoms and solvent atoms omitted for clarity.²⁴⁸

The structure of an unsymmetrically substituted $[\text{NiL}^{14}]$ determined by X-ray crystallography revealed that Ni^{2+} sits in the N_4 plane (Figure 4.7a) but the pyridinium group is twisted about the $\text{C}(2)\text{-N}(5)$ bond out of the plane of the tetra[14]azaannulene by 43.8° (Figure 4.7b).

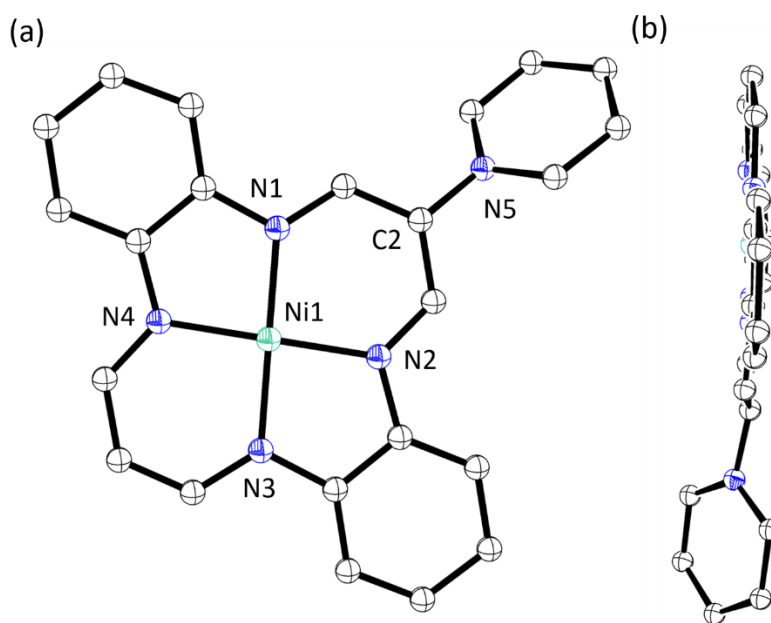


Figure 4.7. ORTEP representation (30% ellipsoid probability) of the square planar $[\text{NiL}^{14}]$ (a) and its side view (b). Hydrogen atoms attached to carbon atoms and solvent atoms omitted for clarity.²⁴⁹

The introduction of a substituent at R^2 position leads to distortions away from planarity. For example, replacing the hydrogen atoms in the R^2 position on the 1,3-dipropanediiminato linkage shown in the x-ray crystal structure of 1,8-dihydro-5,7,12,14-tetramethyldibenzo[*b,i*][1,4,8,11]tetraazacyclotetradeca-4,6,11,13-tetraene (H_2tmataa) with methyl groups significantly altered the conformation of the metal free ligand (Figure 4.8).²⁵⁰ The steric encumbrance between the methyl substituents and the hydrogen atoms of the phenyl rings contributes to the tendency for metal-free ligand to have the metal atom lying out of the N_4 plane.

Similar to the tetraaza[14]annulene ligand, the single and double -N bonds of H₂tmtaa are distinguishable based on the bond distances. The doubly bonded C(1)-N(1) and C(10)-N(3) are 1.317 (1) Å and 1.315 (6) Å respectively while the singly bonded C(3)-N(2) and C(12)-N(4) are 1.342 (3) Å and 1.342 (7) Å respectively.

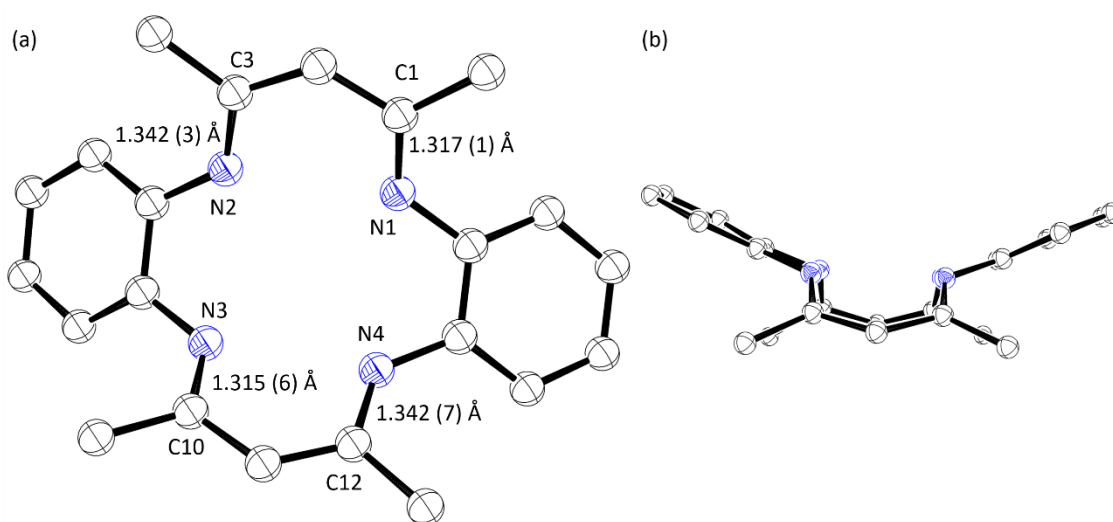


Figure 4.8. ORTEP representation (30% ellipsoid probability) of H₂tmtaa showed a saddled shape (side view) due to steric interactions. Hydrogen atoms attached to carbon atoms omitted for clarity.²⁵⁰

The smaller size of the central cavity of the tetraaza[14]annulene when compared to porphyrins often leads to metal complexes that are more kinetically inert. The average N₄ coordination cavity hole size is between 1.90 and 1.93 Å, which is about 0.1 Å less than that of the porphyrins.²⁵¹ When coordinated to metal ion of larger size, the metal tends to lie out of the N₄ plane of the tetraaza[14]annulene ring.

The tetraaza[14]annulene is less rigid compared to porphyrins, allowing metal complexes of tetraaza[14]annulene to adopt different conformations. The H₂tmtaa ligand forms four-coordinate
5,7,12,14-tetramethyldibenzo[*b,i*][1,4,8,11]tetraazacyclotetradecaheptaenatonickel(II), [Ni(tmtaa)]

and five-coordinate Al^{3+} complex, $[\text{Al}(\text{tmtaa})(\text{CH}_2\text{CH}_3)]$. The six-coordinated $[\text{Zr}(\text{tmtaa})\text{Cl}_2]$ and $[\text{Sn}(\text{tmtaa})\text{Cl}_2]$ exist in different geometries (Figure 4.9).

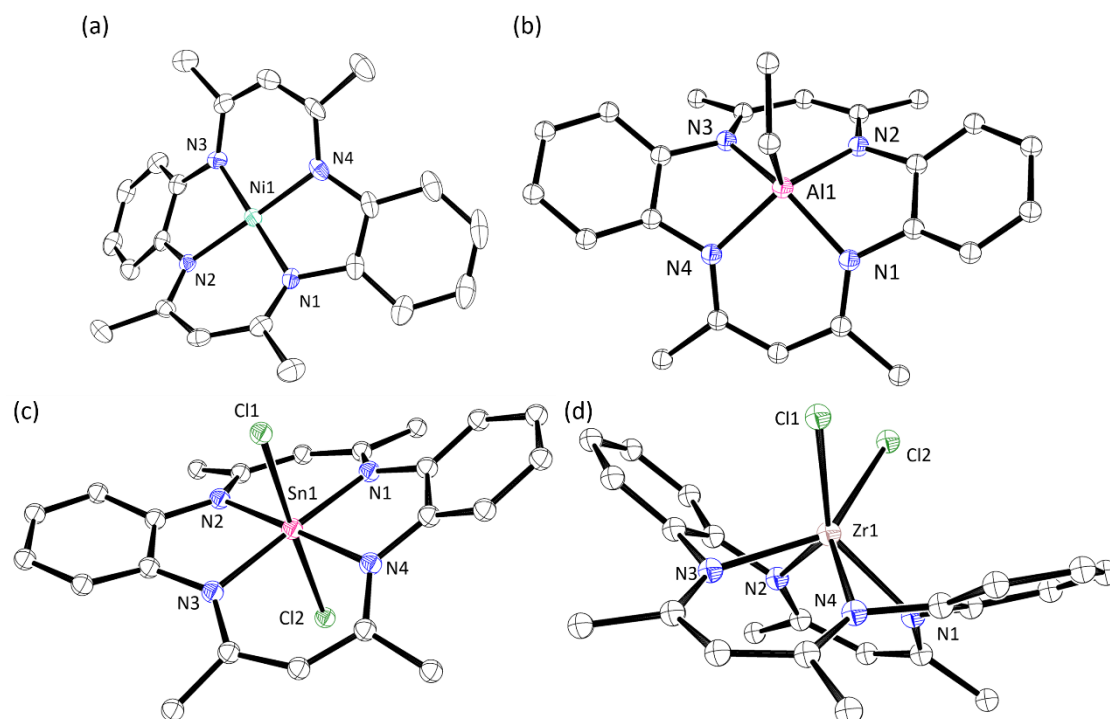


Figure 4.9. ORTEP representation (30% ellipsoid probability) of (a) square planar $[\text{Ni}(\text{tmtaa})]$, (b) square pyramidal $[\text{Al}(\text{tmtaa})(\text{CH}_2\text{CH}_3)]$, (c) trigonal prismatic $[\text{Zr}(\text{tmtaa})\text{Cl}_2]$ and (d) octahedral $[\text{Sn}(\text{tmtaa})\text{Cl}_2]$. Hydrogen atoms and solvent atoms omitted for clarity.²⁵²⁻²⁵⁵

Interestingly, the $[\text{Zr}(\text{tmtaa})\text{Cl}_2]$ complex exhibits a trigonal prismatic geometry with the two chlorine atoms adopted a *cis* arrangement with $\text{Cl}(1)\text{-Zr-Cl}(2) = 85.6(12)^\circ$.²⁵⁴ The Zr^{4+} sits above the plane which enforces a *cis* arrangement of the two Cl^- away from the tmtaa pocket. Unlike $[\text{Zr}(\text{tmtaa})\text{Cl}_2]$, both $[\text{Sn}(\text{tmtaa})\text{Cl}_2]$ and $[\text{Sn}(\text{omtaa})\text{I}_2]$ adopted *trans* dispositions with octahedral geometries.²⁵⁵⁻²⁵⁶

Owing to the ability to coordinate to an array of metal ion with varying oxidation states, the metal complexes of tetraaza[14]annulene have found some promising applications in biological system. As a synthetic analogue of porphyrin, a dibenzotetraaza[14]annulene

derivative of Fe^{3+} has interesting properties suitable as a biomimetic model for catalase.²⁵⁷⁻²⁵⁹ The water soluble $[\text{Fe}^{\text{III}}\text{L}^{15}](\text{BF}_4)$ acts as a catalase enzyme to control the intracellular level of hydrogen peroxide in living system. It is proposed that the free ligand H_2L^{15} chelates to Fe^{2+} in cells and subsequently converts two hydrogen peroxide molecules to an oxygen and two water molecules (Figure 4.10).²⁶⁰ The average yield of O_2 achieved is 87% in the presence of 5 μM of $[\text{Fe}^{\text{III}}\text{L}^{15}](\text{BF}_4)$, rendering it an efficient catalase.

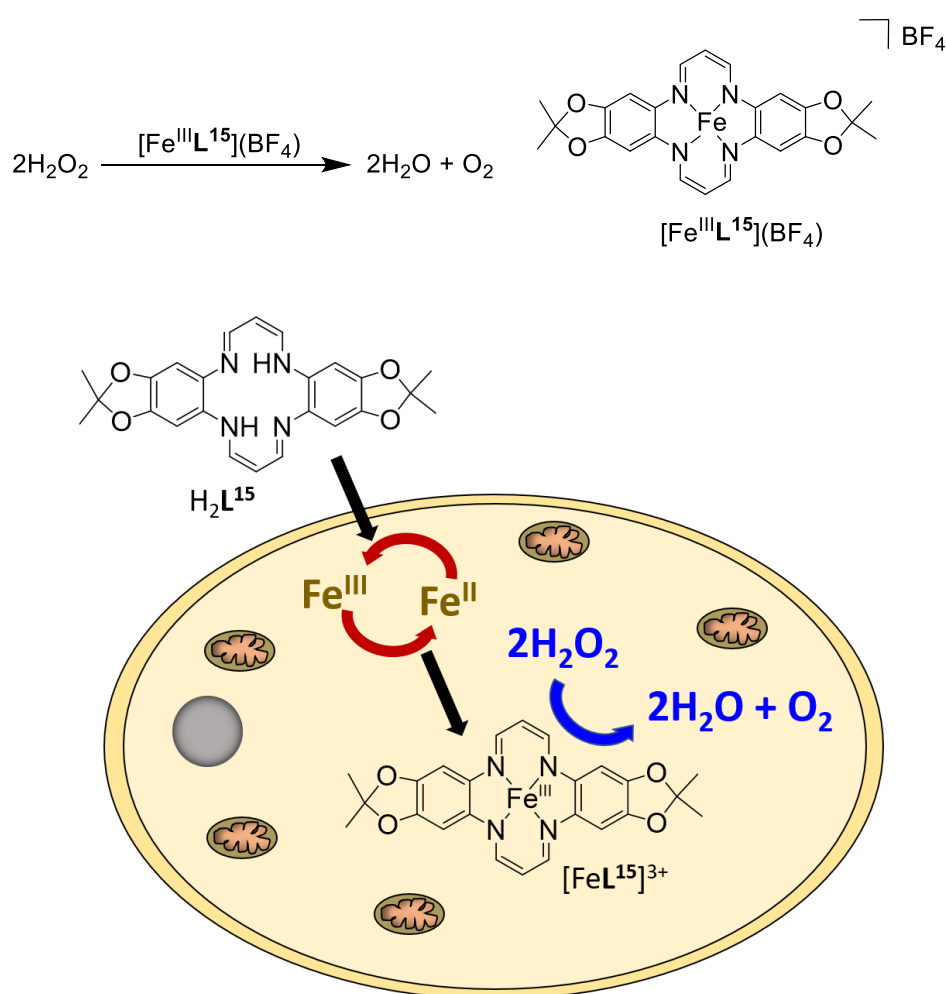


Figure 4.10. The proposed mechanism of catalase activity of $[\text{FeL}^{15}]^{3+}$ in cells. The free ligand binds to excess intracellular Fe^{2+} ion to form the active catalyst $[\text{FeL}^{15}]^{3+}$ before catalysing the decomposition of hydrogen peroxide.²⁶⁰

Several *meso*-substituted tetraaza[14]annulene ligands have the ability to act as DNA/RNA binding agents.²⁶¹⁻²⁶³ DNA or RNA are nucleic acids that can serve as target sites for drugs. The design of small molecules for sequence-selective binding to DNA/RNA has led to the adaptation of the tetraaza[14]annulene framework. The near planar conformation of tetraaza[14]annulene is an advantage for the ring to enter and stack between base pairs of double helical DNA, for either intercalation or groove binding interaction. It is proposed that compound **43** with pendant trimethylammonium groups allows easy insertion between base pairs of DNAs and does not hinder the interaction of tetraaza[14]annulene core with DNA *via* π - π stacking (Figure 4.11). Compound **44** has *o*-benzoyl moieties, which are almost perpendicular to the planar macrocycle (Figure 4.11). The pendant groups can have additional binding interaction within the groove of double stranded DNAs. A mixed mode of binding is observed for compound **44** with bulky groups which may involve minor or major groove binding, external electrostatic binding and π - π stacking. Nonetheless, bulky groups in close proximity to the tetraaza[14]annulene core can sterically hinder the insertion of the molecule between base pairs of DNA and thus preventing intercalation.

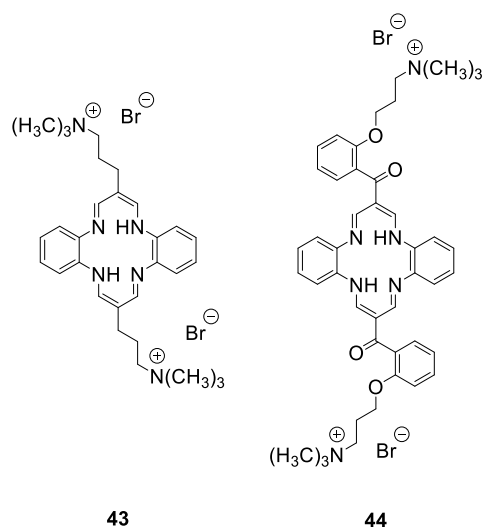
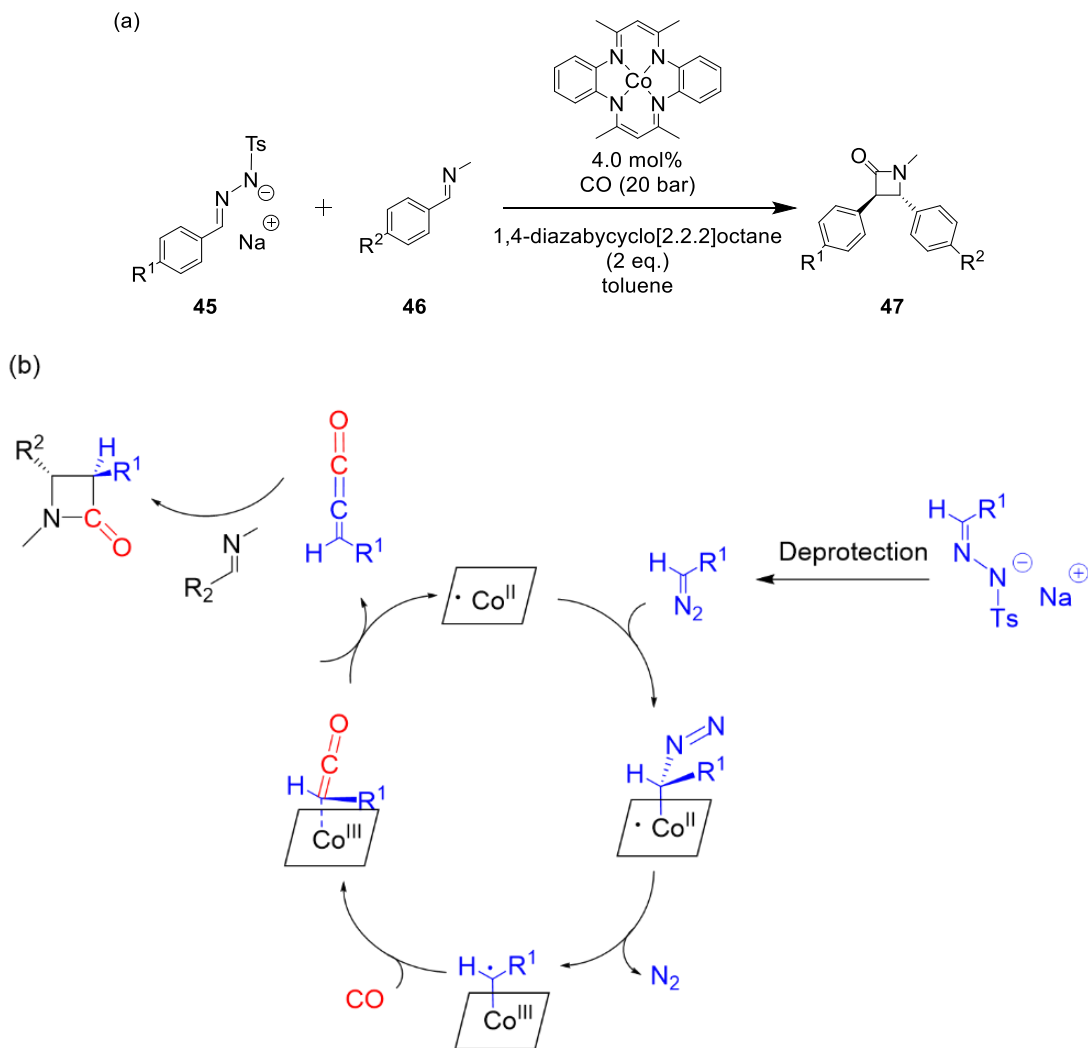


Figure 4.11. Chemical structures of two meso-substituted tetraaza[14]annulene ligands with different substituents.

The catalytic characteristics of metal complexes of tmtaa have been explored. The Co^{2+} and Ni^{2+} complexes of tmtaa serve as cheaper options as catalyst compared to the more commonly used platinum or iridium catalyst. For example, $[\text{Co}(\text{tmtaa})]$ can catalyse the carbonylation of carbene radical intermediates towards the synthesis of ketenes for the production of β -lactam (Scheme 4.1a). The precursor benzaldehyde tosylhydrazone sodium salt (**45**) is activated by $[\text{Co}(\text{tmtaa})]$ to afford the $[\text{Co}(\text{tmtaa})]$ -carbene radical which reacts with carbon monoxide to form the ketene. The ketene reacts with *N*-benzylidenemethanamine (**46**) to form β -lactam (**47**) *in situ* (Scheme 4.1b).



Scheme 4.1. General synthesis of the β -lactam (**47**) from benzaldehyde tosylhydrazone sodium salt (**45**) and *N*-benzylidenemethanamine (**46**) catalysed by $[\text{Co}(\text{tmtaa})]$ (a) and the proposed mechanism for the catalytic ketene synthesis (b).

There has been much interest in studying tetraaza[14]annulene and its tetramethyl derivatives with their metal complexes as mimics of porphyrins in applications ranging from enzyme mimics to DNA binders. Despite that, their use in nuclear medicine remains underexplored, especially compared to saturated analogues such as cyclam (Figure 4.12). The presence of C=N and C=C in tetraaza[14]annulene can provide a more rigid framework compared to cyclam, an advantage as chelator for imaging agents. The conjugation in unsaturated tetraaza[14]annulene can lead to metal complexes with

enhanced stability compared to saturated macrocycles such as cyclam. In addition, tetraaza[14]annulene ligands act as dianionic donors give complexes with a lower ionic charge than complexes with cyclam.

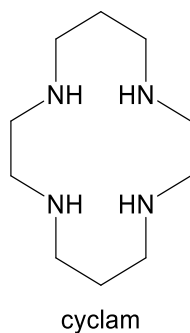
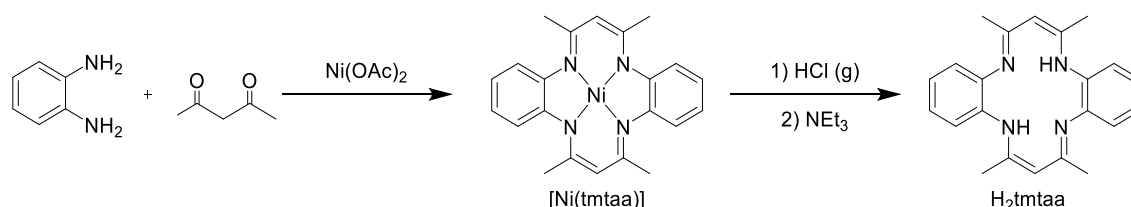


Figure 4.12. Chemical structure of cyclam.

The preparation of Ga^{3+} complexes containing tetramethyltetraaza[14]annulene ($[\text{Ga}(\text{CH}_3)(\text{tmtaa})]$ and $[\text{Ga}(\text{tmtaa})]\text{Cl}$) was first reported in 1991 but has not found many potential applications.^{252, 264-265} There is a possibility to apply the tetraaza[14]annulene ligand into the field of nuclear medicine. On this basis, we are inspired to explore the potential of the Ga^{3+} complex of tmtaa to be of use as fluorine-18 radiotracer for molecular imaging. The N_4 donor set is adopted to replace carboxylate donor groups to potentially increase stability of the $\text{Ga}^{3+}\text{-F}^-$ complex. We aim to study the coordination chemistry and properties of the $\text{Ga}^{3+}\text{-F}^-$ complex of tmtaa. It is also of our interest to decorate the chelate ring with appended substituents to enable chemical modifications of the chelator and its corresponding $\text{Ga}^{3+}\text{-F}^-$ complex.

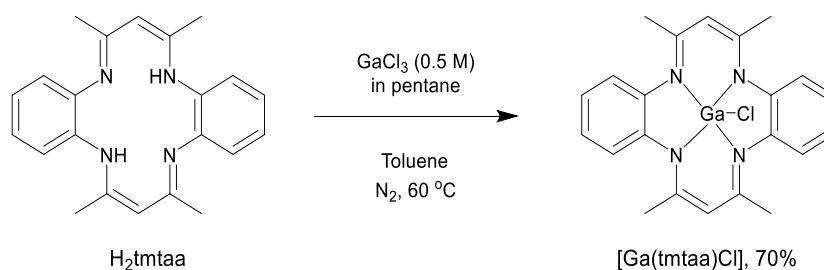
4.2 Synthesis of H_2tmtaa , $[Ga(tmtaa)Cl]$ and $[Ga(tmtaa)F]$, structural determination by nuclear magnetic resonance spectroscopy and characterisation by X-Ray Crystallography

A stepwise route is used in which $[Ni(tmtaa)]$ is first synthesised using a template synthesis.²⁶⁶ The synthesis involved *o*-phenylenediamine and 2,4-pentanedione with nickel (II) acetate tetrahydrate (Scheme 4.2). Removal of the metal ion under acidic conditions yielded the free ligand, H_2tmtaa .



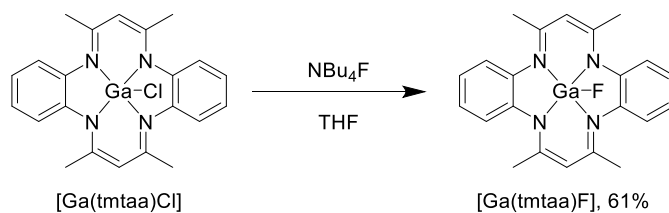
Scheme 4.2. Template synthesis of $[Ni(tmtaa)]$ and synthesis of H_2tmtaa .²⁶⁶

Group 13 metal ion was incorporated into the cavity of the ligand centre. The Ga^{3+} complex, $[Ga(tmtaa)Cl]$ was prepared by following literature procedures (Scheme 4.3).²⁶⁵



Scheme 4.3. Synthesis of $[Ga(tmtaa)Cl]$.²⁶⁵

The lipophilic $\text{Ga}^{3+}\text{-F}^-$ complex, $[\text{Ga}(\text{tmtaa})\text{F}]$ was synthesised using TBAF (NBu_4F) as the source of F^- ion (Scheme 4.4).



Scheme 4.4. Synthesis of $[\text{Ga}(\text{tmtaa})\text{F}]$.

The chloride complex, $[\text{Ga}(\text{tmtaa})\text{Cl}]$, and fluoride complex, $[\text{Ga}(\text{tmtaa})\text{F}]$, were characterised by NMR spectroscopy (Figure 4.13). Upon complexation to the metal there is a loss of the signal attributed to the $-\text{NH}$ groups ($\delta_{\text{H}} = 12.44$ ppm) consistent with deprotonation. The resonance assigned to the methyl group at $\delta_{\text{H}} = 2.10$ ppm shifted downfield to $\delta_{\text{H}} = 2.36$ ppm for $[\text{Ga}(\text{tmtaa})\text{Cl}]$ and $\delta_{\text{H}} = 2.33$ ppm for $[\text{Ga}(\text{tmtaa})\text{F}]$. The resonance at $\delta_{\text{H}} = 4.92$ ppm assigned to the $-\text{CH}_2$ on the 1,3-dipropanediiminato linkage also shifted downfield to $\delta_{\text{H}} = 5.11$ ppm for $[\text{Ga}(\text{tmtaa})\text{Cl}]$ and $\delta_{\text{H}} = 5.13$ ppm for $[\text{Ga}(\text{tmtaa})\text{F}]$.

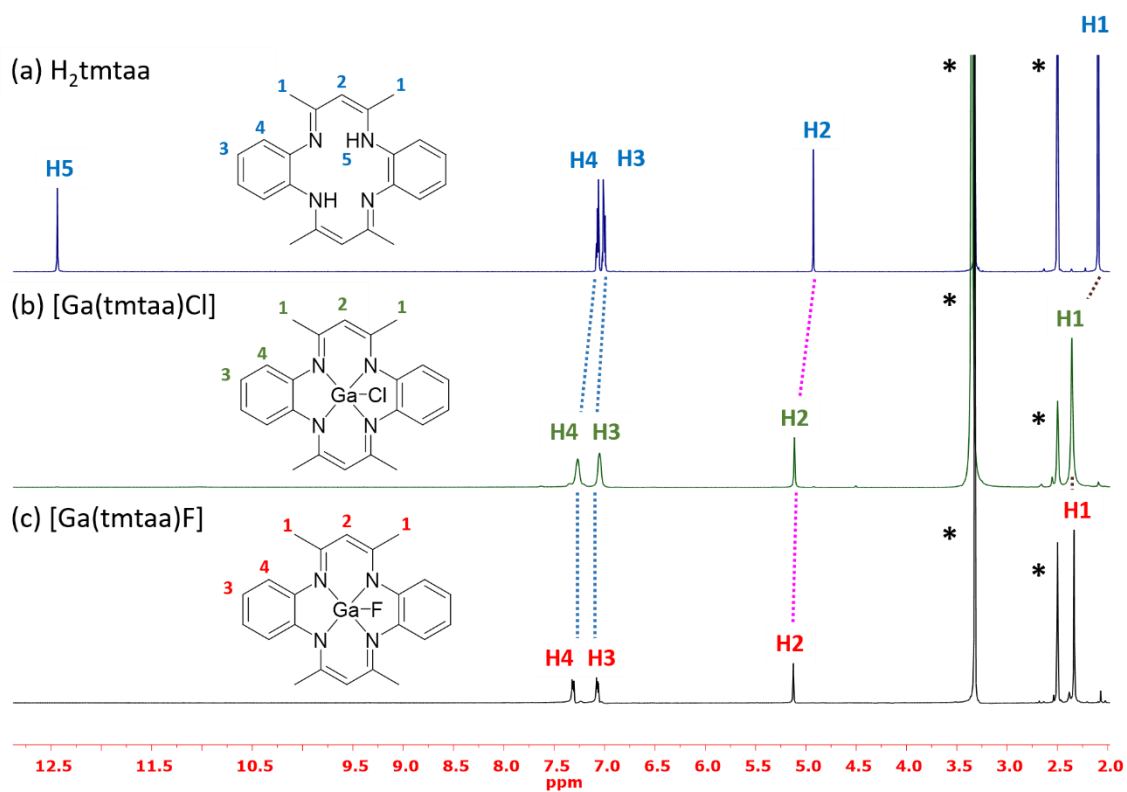


Figure 4.13. ^1H NMR spectra of H_2tmtaa (a), $[\text{Ga}(\text{tmtaa})\text{Cl}]$ (b) and $[\text{Ga}(\text{tmtaa})\text{F}]$ (c). Asterisk indicates solvent signals for $d_6\text{-DMSO}$ ($\delta_{\text{H}} = 2.50$ ppm) and H_2O ($\delta_{\text{H}} = 3.33$ ppm).

For comparison purposes, the $\text{Al}^{3+}\text{-F}^-$ complex was also synthesised. The ^{19}F NMR spectra revealed that the resonance has shifted from -105.46 ppm (F^- in TBAF) to a peak with resonance at $\delta_{\text{F}} = -194.2$ ppm for $[\text{Ga}(\text{tmtaa})\text{F}]$ (Figure 4.14b) and $\delta_{\text{F}} = -172.7$ ppm for $[\text{Al}(\text{tmtaa})\text{F}]$ (Figure 4.14c). The ^{19}F NMR spectrum of TBAF in THF in d_6 -DMSO revealed two peaks at $\delta_{\text{F}} = \sim -70$ ppm due to F^- . The bifluoride HF_2^- species has a characteristic doublet at $\delta_{\text{F}} = -144.1$ and -144.4 ppm (Figure 4.14a).²⁶⁷⁻²⁶⁸ There is also a residual impurity peak at $\delta_{\text{F}} = -148.9$ ppm. These peaks are not observed in the ^{19}F NMR spectra of $[\text{Ga}(\text{tmtaa})\text{F}]$ and $[\text{Al}(\text{tmtaa})\text{F}]$, further confirming the purity of the products.

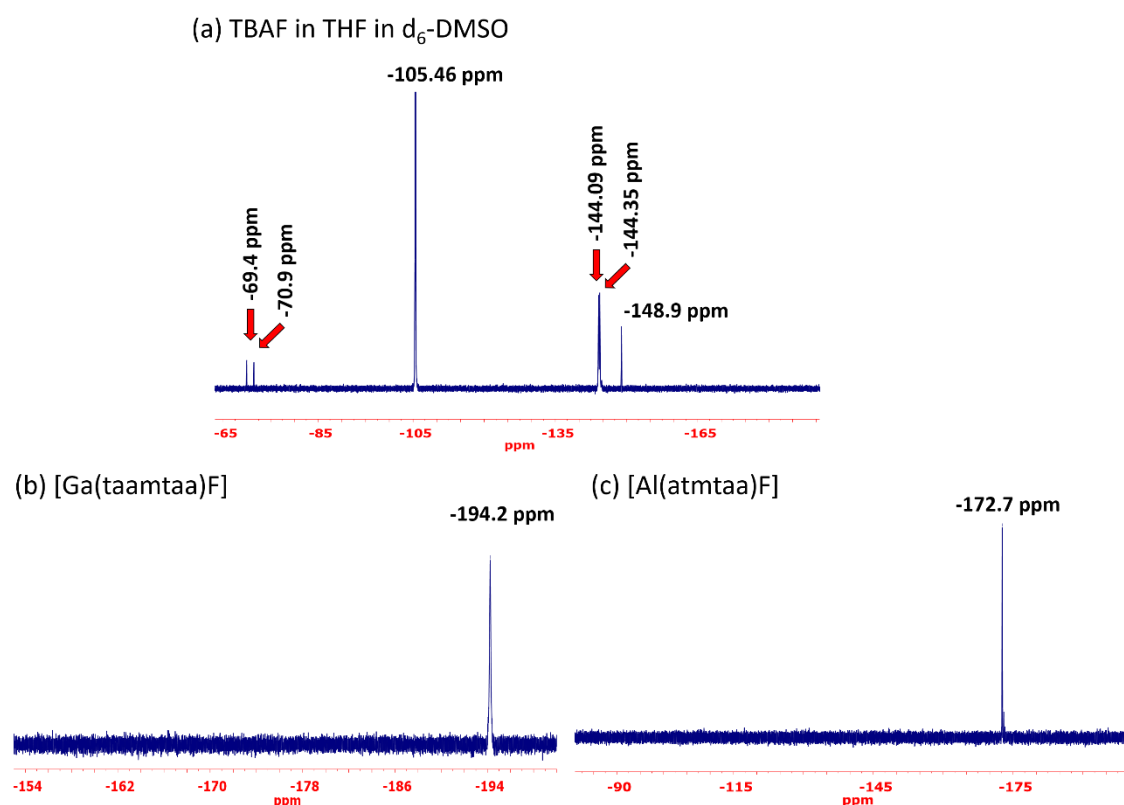


Figure 4.14. ^{19}F NMR spectrum of TBAF in THF (a), $[\text{Ga}(\text{tmtaa})\text{F}]$ (b) and $[\text{Al}(\text{tmtaa})\text{F}]$ (c) in d_6 -DMSO.

The X-ray crystal structure of $[\text{Ga}(\text{tmtaa})\text{F}] \cdot \text{CH}_2\text{Cl}_2$ depicts a five-coordinate square pyramidal geometry with the saddle-shape retained (Figure 4.15). The steric requirements of the methyl substituents imposed by the expected deformation of H_2tmtaa away from planarity and the Ga^{3+} ion coordinated symmetrically to the four planar N atoms sits 0.47 Å above the N_4 plane.

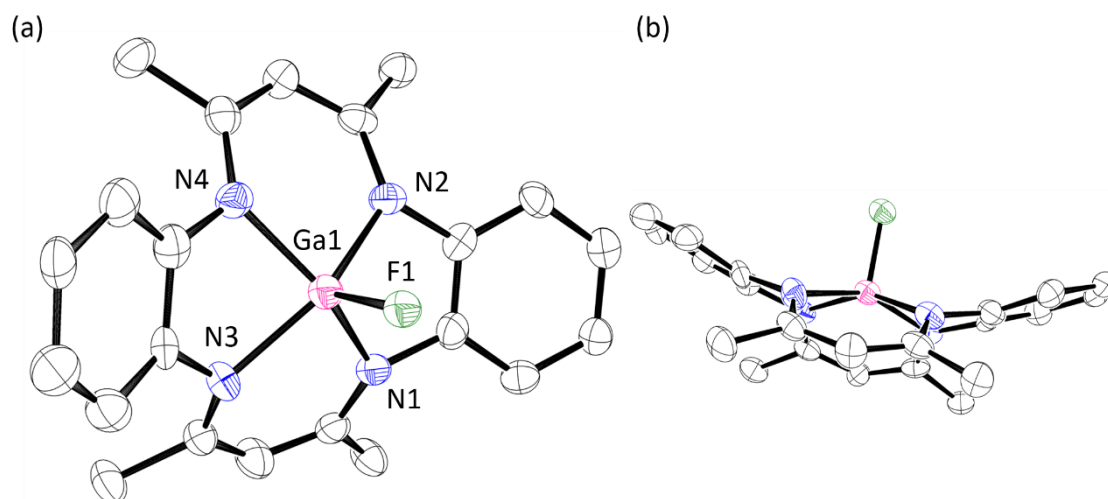


Figure 4.15. ORTEP representation (30% ellipsoid probability) of $[\text{Ga}(\text{tmtaa})\text{F}] \cdot \text{CH}_2\text{Cl}_2$ (a) and its side view (b). Hydrogen atoms and solvent atoms omitted for clarity.

The displacement of the metal centre from the equatorial plane of $[\text{Ga}(\text{tmtaa})\text{F}] \cdot \text{CH}_2\text{Cl}_2$ is similar to $[\text{Ga}(\text{tmtaa})\text{Cl}]$ (0.48 Å) but smaller compared to $[\text{Ga}(\text{tmtaa})(\text{CH}_3)]$ (0.65 Å).²⁶⁵ The bulkier CH_3 group at the axial position may have an increased steric strain with the tmtaa, which attributed to the large displacement from the plane. The bond distance of $\text{Ga}(1)\text{-F}(1)$ is 1.812(8) Å lies between 1.958(8) Å (Ga-C for $[\text{Ga}(\text{tmtaa})(\text{CH}_3)]$) and 2.222(2) Å (Ga-Cl for $[\text{Ga}(\text{tmtaa})(\text{CH}_3)]$). The value is within the range of acceptable bond lengths for $\text{Ga}^{3+}\text{-F}^-$ bond. The average bond lengths of Ga-N for $[\text{Ga}(\text{tmtaa})\text{F}]$ is 1.986 Å, are similar to $[\text{Ga}(\text{tmtaa})\text{Cl}]$ (1.98 Å) but shorter than the average value for $[\text{Ga}(\text{tmtaa})(\text{CH}_3)]$ (2.016 Å).

Table 4.1. Selected bond lengths (Å) of [Ga(tmtaa)F]·CH₂Cl₂.

	Bond lengths (Å)
Ga(1)-N(1)	1.974(9)
Ga(1)-N(2)	2.010(1)
Ga(1)-N(3)	1.990(1)
Ga(1)-N(4)	1.970(1)
Ga(1)-F(1)	1.812(8)

Upon metal complexation followed by the removal of two -NH hydrogen atoms, the average distance between N(1)-N(2) and N(3)-N(4) decreased from the average value of 2.706 Å for H₂tmtaa to ~2.605 Å for [Ga(tmtaa)F] (Figure 4.16 green line). The average distance between N(1)-N(3) and N(2)-N(4) increased from the average value of 2.68 Å for H₂tmtaa to ~2.85 Å for [Ga(tmtaa)F] (Figure 4.16 red line).

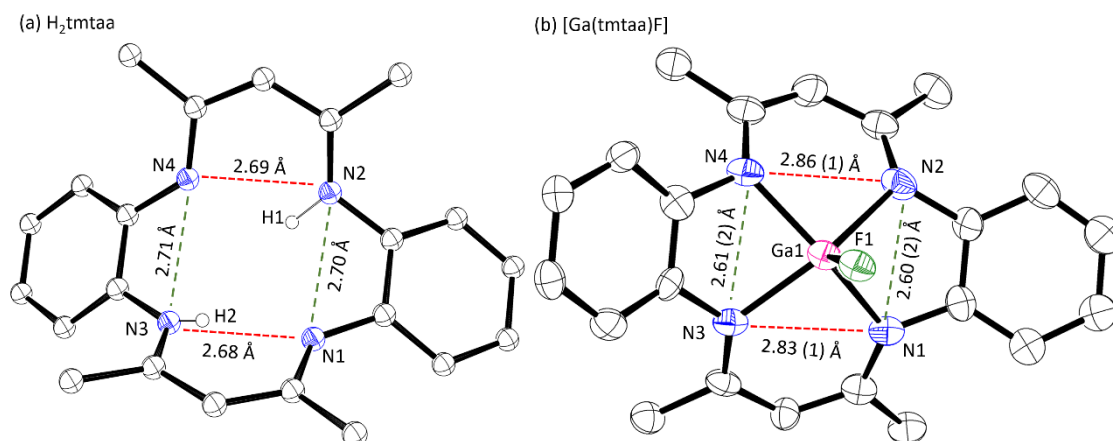


Figure 4.16. The distances between the nitrogen atoms of H₂tmtaa and [Ga(tmtaa)F].

This observation is mainly due to the re-direction of lone pairs on the nitrogen atoms out of the ring towards the Ga³⁺ ion and increased core size, which is consistent with the other two previously reported Ga³⁺ complexes, ([Ga(tmtaa)Cl] and [Ga(tmtaa)(CH₃)]).^{252, 265} Consequently, the N(1)-Ga(1)-N(2) and N(3)-Ga(1)-N(4) angles (81.7(4)° and 82.2(4)°)

of the *o*-phenylenediamine are smaller compared to the N(1)-Ga(1)-N(3) and N(2)-Ga(1)-N(4) angles (92.5(4)° and 90.5(5)°) involving the 2,4-pentanediiiminato chelate.

Table 4.2. Summary of the crystallographic data for [Ga(tmtaa)F].CH₂Cl₂.

	[Ga(tmtaa)F]·CH ₂ Cl ₂
Empirical formula	C ₂₃ H ₂₄ C ₁₂ FGaN ₄
MW	516.28
Colour	Orange
Space group	P21/c
<i>T</i> /K	100.00(10)
Crystal size/mm ³	0.06 x 0.05 x 0.05
Crystal system	Monoclinic
λ /Å	1.54184
<i>a</i> /Å	18.677(3)
<i>b</i> /Å	7.5709(8)
<i>c</i> /Å	17.193(3)
β /°	114.15(2)
α, γ /°	90
<i>V</i> /Å ³	2218.5(7)
<i>Z</i>	4
<i>D</i> _{calc} /Mg/m ³	1.545
μ /mm ⁻¹	4.137
<i>F</i> (000)	1056
Reflections measured	19482
Independent reflections	4494 [<i>R</i> _{int} = 0.3021]
final <i>R</i> indices [<i>I</i> > 2σ(<i>I</i>)]	<i>R</i> ₁ = 0.1292, <i>wR</i> ² = 0.3035
final <i>R</i> indices (all data)	<i>R</i> ₁ = 0.2213, <i>wR</i> ² = 0.3631
Goodness-of-fit on <i>F</i> ²	1.071

4.3 Electronic spectroscopy studies of H_2tmtaa , $[Ga(tmtaa)Cl]$ and $[Ga(tmtaa)F]$

The UV Visible spectra were acquired for H_2tmtaa , $[Ga(tmtaa)Cl]$ and $[Ga(tmtaa)F]$. The strong absorbance for H_2tmtaa at $\lambda_{max} = 340$ nm ($\epsilon \sim 55,900$ L mol⁻¹ cm⁻¹) due to the $\pi-\pi^*$ transition shifts to $\lambda_{max} = 362$ nm ($\epsilon \sim 55,100$ L mol⁻¹ cm⁻¹) for $[Ga(tmtaa)Cl]$ and $\lambda_{max} = 378$ nm for $[Ga(tmtaa)F]$ ($\epsilon \sim 51,400$ L mol⁻¹ cm⁻¹) (Figure 4.17). The molar extinction coefficients are tabulated in Table 4.3.

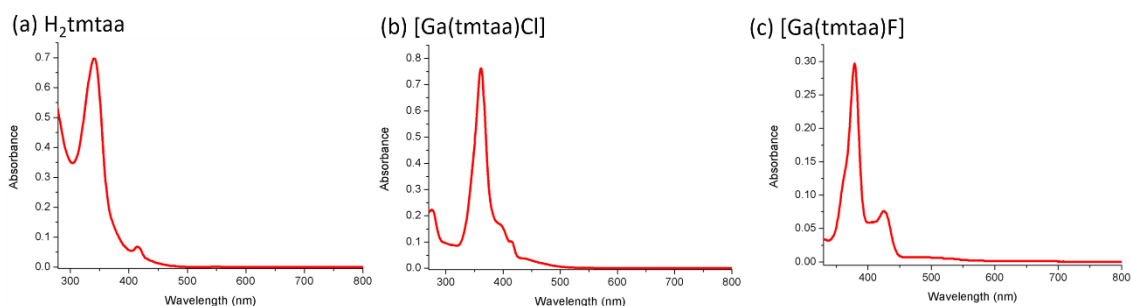


Figure 4.17. UV-vis spectra of H_2tmtaa (a), $[Ga(tmtaa)Cl]$ (b) and $[Ga(tmtaa)F]$ (c).

For $[Ga(tmtaa)F]$, a smaller peak at $\lambda_{max} = 426$ nm with a molar extinction coefficient, ϵ of 13,000 L mol⁻¹ cm⁻¹ is reflected by the intense orange colour observed of the product.

Table 4.3. Summary of the λ_{max} and molar extinction coefficients for H_2tmtaa , $[Ga(tmtaa)Cl]$ and $[Ga(tmtaa)F]$.

	Concentration (μM)	λ_{max} (nm)	Molar extinction coefficient, ϵ ($\times 10^3$ L $\cdot$ mol ⁻¹ $\cdot$ cm ⁻¹)
H_2tmtaa	13	340	56
$[Ga(tmtaa)Cl]$	14	362	55
$[Ga(tmtaa)F]$	5	378	51
		426	13

4.4 Stability studies against human serum albumin

To assess the suitability of $[\text{Ga}(\text{tmtaa})\text{F}]$ for imaging *in vivo*, the stability of $[\text{Ga}(\text{tmtaa})\text{F}]$ in human serum albumin at 37 °C was investigated by ^{19}F NMR spectroscopy. A ^{19}F NMR spectrum was acquired at two time points (2 h and 16 h) after addition of human serum albumin. The single peak at $\delta_{\text{F}} = -194$ ppm was unchanged after incubation for 2 hours, suggesting that the $\text{Ga}^{3+}\text{-F}^-$ bond was still intact and that $[\text{Ga}(\text{tmtaa})\text{F}]$ is stable under these conditions (Figure 4.18b). A shift to -117.9 ppm was observed after 16 h of incubation at 37 °C (Figure 4.18c).

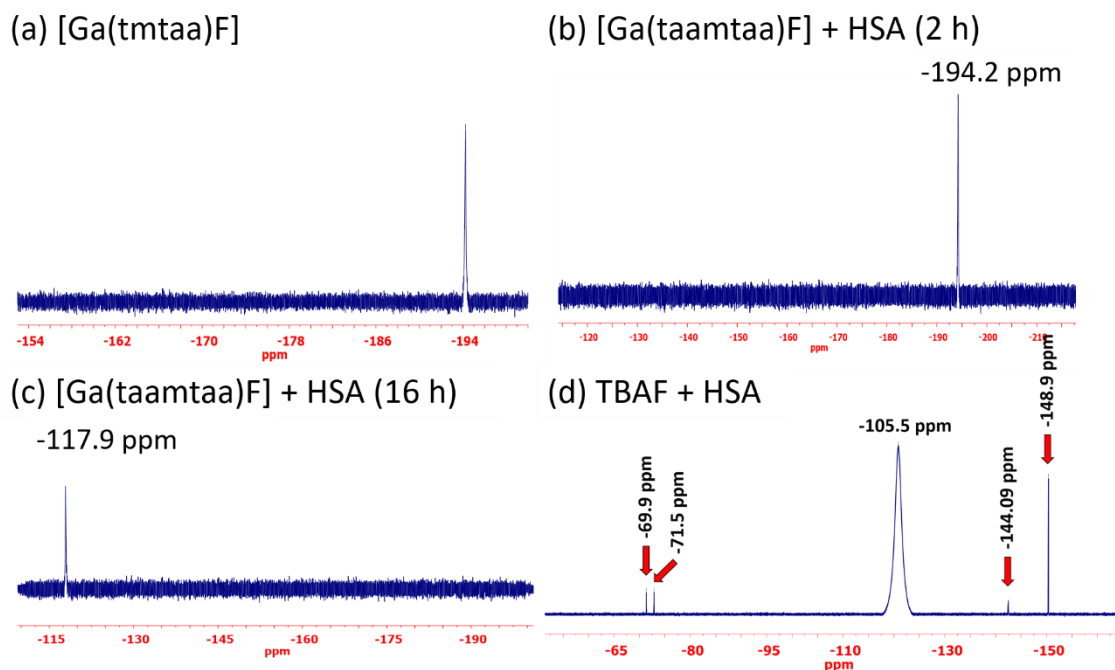


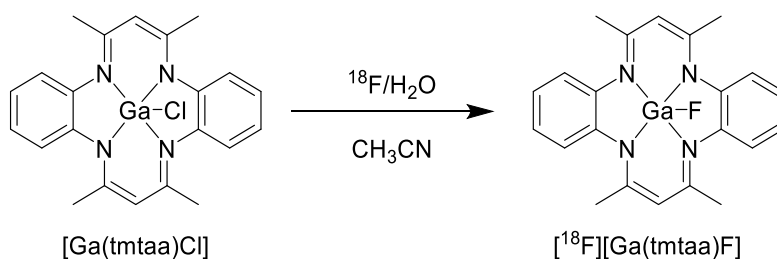
Figure 4.18. ^{19}F NMR spectrum of $[\text{Ga}(\text{tmtaa})\text{F}]$ (a), $[\text{Ga}(\text{tmtaa})\text{F}]$ incubated with human serum albumin for 2 h (b) and 16 h (c), TBAF in THF with human serum albumin in $d_6\text{-DMSO}$ (d).

The nature of the complex that gave rise to the signal at $\delta_{\text{F}} = -117.9$ ppm is not known but the spectrum is different to that of TBAF in human serum (Figure 4.18d).

The stability of $[\text{Ga}(\text{tmtaa})\text{F}]$ observed in the presence of human serum albumin suggested that the radioactive fluorine-18 analogue may also be stable. Hence, the potential of the Ga^{3+} complex of tetraaza[14]annulene to be radiolabelled with fluorine-18 was investigated.

4.5 Incorporation of Fluorine-18

A halide $\text{Cl}/^{18}\text{F}$ exchange reaction where $[\text{Ga}(\text{tmtaa})\text{Cl}]$ was reacted with an aqueous source of fluoride was investigated. An aqueous mixture of $[^{18}\text{F}]\text{F}^-$ in H_2O obtained from a $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$ nuclear reaction (98% ^{18}O isotopic enrichment) on a 16.5 MeV cyclotron ($\sim 1,000\text{--}1,500\text{ MBq/mL}$) was added to a mixture of $[\text{Ga}(\text{tmtaa})\text{Cl}]$ in acetonitrile at 80°C (Scheme 4.5). Analysis of the crude reaction mixture by RP-HPLC suggested formation of $[^{18}\text{F}][\text{Ga}(\text{tmtaa})\text{F}]$ in a radiochemical yield of $\sim 3\%$ eluted at $R_t = 12.6\text{ min}$ (Figure 4.19).



Scheme 4.5. Reaction scheme of $[^{18}\text{F}][\text{Ga}(\text{tmtaa})\text{F}]$.

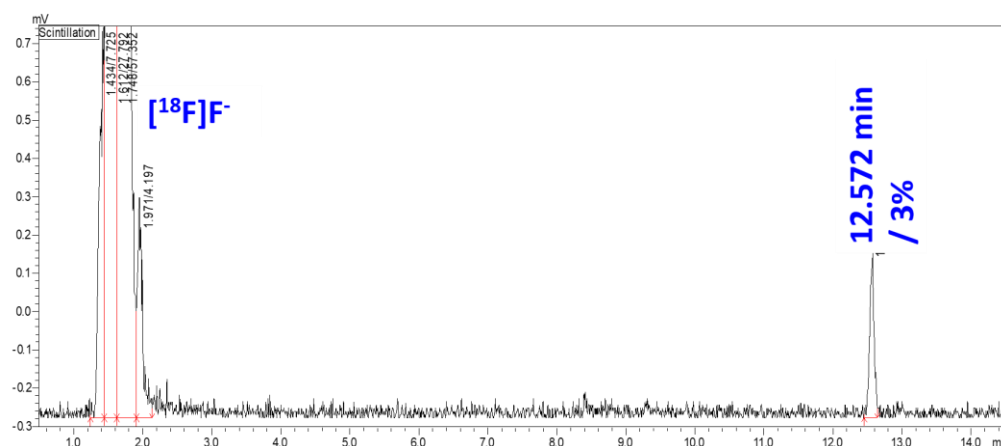
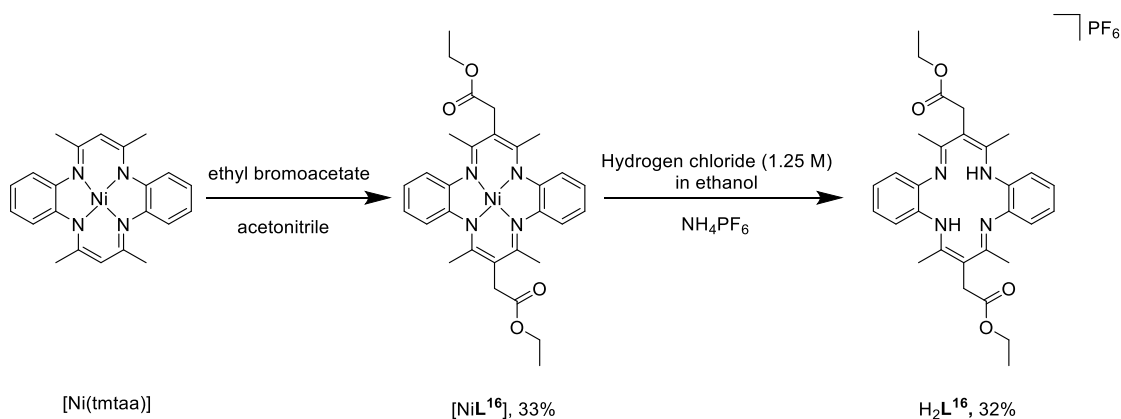


Figure 4.19. RP-HPLC (radiation detector) of the crude reaction mixture of $[^{18}\text{F}][\text{Ga}(\text{tmtaa})\text{F}]$.

Although $[\text{Ga}(\text{tmtaa})\text{F}]$ was formed in a low radiochemical yield, the success of this preliminary radiolabelling suggest that this particular approach warrants further investigations. It is likely that the use of an anhydrous source of $[^{18}\text{F}]\text{F}^-$ will lead to an increase in the radiochemical yield.

Alternatively, derivatives of $[\text{Ga}(\text{tmtaa})]^+$ with reduced lipophilicity and better solubility in water may be more favourable to be radiolabelled with fluorine-18. Herein, a synthetic route to functionalise the methine carbon atom with ethyl ester is described. Our interest is to incorporate biologically relevant molecules onto the Ga^{3+} complex *via* the ethyl ester before the incorporation of fluorine-18 to be used for imaging.

Functionalisation at the methine carbon atoms was achieved using the Ni^{2+} complex, $[(\text{Ni}(\text{tmtaa}))]$.²⁶⁶ The Ni^{2+} ion is used to protect the potentially nucleophilic nitrogen atoms to favour the substitution only at the methine carbon atoms. Addition of excess ethyl bromoacetate to the $[(\text{Ni}(\text{tmtaa}))]$ produced di-substituted $[\text{NiL}^{16}]$. The Ni^{2+} ion was removed under acidic conditions using hydrogen chloride in ethanol (Scheme 4.6).



Scheme 4.6. Synthesis of $[\text{NiL}^{16}]$ and H_2L^{16} .

The ethyl ester functional groups of H_2L^{16} can be used to form amide bonds with primary amines on targeting molecules or to add functional groups that improve water solubility.

4.6 Summary

In this chapter, a tetradentate azamacrocyclic ligand with N_4 donor set that forms hydrophobic Ga^{3+} complex was prepared. The tetramethyltetraaza[14]annulene macrocycle undergoes a double deprotonation in the presence of Ga^{3+} to serve as dianionic ligand. The structure of $[\text{Ga}(\text{tmtaa})\text{F}]$ determined by X-ray crystallography revealed that the five-coordinate Ga^{3+} complex has a square pyramidal geometry. The saddle-shape of $[\text{Ga}(\text{tmtaa})\text{F}]$ could, in principle, hinder an incoming nucleophilic ligand to coordinate to Ga^{3+} and disfavour the formation of a six-coordinate complex.

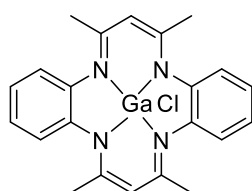
The ease of preparation of $[\text{Ga}(\text{tmtaa})\text{F}]$ and its stability in human serum albumin (at least 2 h) encouraged further investigations with fluorine-18. The radioactive complex, $^{18}\text{F}][\text{Ga}(\text{tmtaa})\text{F}]$ can be prepared by a halogen $\text{Cl}/^{18}\text{F}$ exchange reaction. Despite the relatively low radiochemical yield, the $\text{Ga}^{3+}\text{-}^{18}\text{F}^-$ bond was formed and initial studies suggest that replacing the aqueous source of fluoride with anhydrous $^{18}\text{F}]\text{F}^-$ may aid the radiolabelling in aprotic solvents.

The tetraaza[14]annulene macrocycle is a promising candidate that warrants further attention. Derivatives of H₂tmtaa with lower lipophilicity can be synthesised by functionalisation at the methine carbon atom. The ethyl ester functional group of H₂L¹⁶ allows further inclusion of targeting molecules that are useful for PET diagnostic applications. Future studies include investigation of more soluble variants of [Ga(tmtaa)F].

The preparation of [Al(tmtaa)F] was as straightforward as [Ga(tmtaa)F], and that encourages further investigation of the [¹⁸F]Al³⁺-F⁻ system as it may be a more stable system than the [¹⁸F]Ga³⁺-F⁻.

4.7 Method

[Ga(tmtaa)Cl]

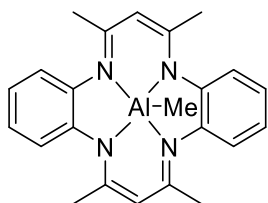


Modified from a literature procedure.²⁶⁵

To 1,8-dihydro-5,7,12,14-tetramethyldibenzo[*b,i*][1,4,8,11]tetraazacyclotetradeca-4,6,11,13-tetraene (50 mg, 0.15 mmol) in anhydrous toluene (20 mL) was added GaCl₃ (0.5 M) in pentane (1 mL). The solution was stirred under nitrogen at 60 °C for 2 h. The precipitate was collected by filtration, washed with diethyl ether to give [Ga(tmtaa)Cl] as an orangish yellow solid (45 mg, 70%). HR-MS(ESI/O-TOF): [C₂₂H₂₂ClGaN₄]⁺ *m/z* 446.079 (experimental), 446.079 (calculated). ¹H NMR (500 MHz, DMSO-*d*₆) δ_H (ppm) 7.25 (s, 4H, *ArH*), 7.04 (s, 4H, *ArH*), 5.11 (s, 2H, *CH*), 2.37 (s, 12H, *CH*₃). ¹³C{¹H} NMR

(126 MHz, DMSO- d_6): δ_C (ppm) = 163.0 (C-N), 137.5 (ArC-N), 123.7 (ArC), 122.5 (ArC), 99.3 (methine-C), 23.4 (CH_3).

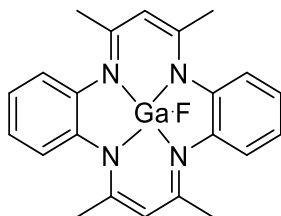
[Al(tmtaa)(CH₃)₃]



Modified from a literature procedure.²⁶⁹

To 1,8-Dihydro-5,7,12,14-tetramethyldibenzo[*b,i*][1,4,8,11]tetraazacyclotetradeca-4,6,11,13-tetraene (50 mg, 0.15 mmol) in anhydrous toluene (20 mL) was added $Al(CH_3)_3$ (2.0 M) in toluene (1 mL). The solution was stirred under nitrogen at 60 °C for 2 h. The precipitate was collected by filtration, washed with diethyl ether and water to give $[Al(tmtaa)(CH_3)]$ as an orangish yellow solid (45 mg, 70%). HR-MS(ESI/O-TOF): $[C_{23}H_{25}AlN_4+H]^+$ m/z 385.197 (experimental), 385.197 (calculated). ^1H NMR (500 MHz, DMSO- d_6) δ_H (ppm) 7.24 (s, 4H, ArH), 7.04 (s, 4H, ArH), 5.11 (s, 2H, CH), 2.30-2.25 (s, 15H, CH_3).

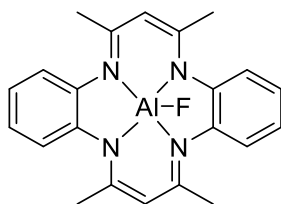
[Ga(tmtaa)F]



To $[Ga(tmtaa)Cl]$ (30 mg, 67 μ mol) in acetonitrile (10 mL) was added potassium fluoride (4.0 mg, 69 μ mol) and 18-crown-6 (18 mg, 69 μ mol). The reaction was heated at reflux for 2 h. The precipitate was collected by filtration, washed with cold diethyl ether to give

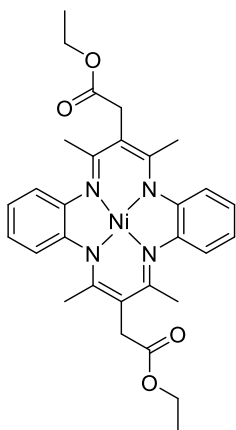
[Ga(tmtaa)Cl] as an orange solid (17 mg, 61%). HR-MS(ESI/O-TOF): [C₂₂H₂₂FGaN₄]⁺ *m/z* 430.108 (experimental), 430.108 (calculated). ¹H NMR (500 MHz, DMSO-d₆) δ_H (ppm) = 7.31 (dd, *J* = 5.9, 3.5 Hz, 4H, *ArH*), 7.07 (dd, *J* = 6.0, 3.4 Hz, 4H, *ArH*), 5.13 (s, 2H, methine-CH), 2.33 (s, 12H, CH₃). ¹³C{¹H} NMR (126 MHz, DMSO-d₆): δ_C (ppm) = 163.0 (C-N), 137.5 (ArC-N), 123.7 (ArC), 122.5 (ArC), 99.3 (methine-C), 23.4 (CH₃). ¹⁹F NMR (470 MHz, DMSO-d₆): δ_F (ppm) = -194.2 ppm.

[Al(tmtaa)F]



To [Al(tmtaa)(CH₃)] (63 mg, 0.16 mmol) in anhydrous THF (20 mL) was added tetrabutylammonium fluoride in THF (0.5 mL), stirred under nitrogen for 1 h. The mixture was filtered, and the filtrate was removed under reduced pressure. The residue was collected by filtration and washed with water to give [Al(tmtaa)F] as an orange solid (20 mg, 31%). HR-MS(ESI/O-TOF): [C₂₂H₂₂FAIN₄]⁺ *m/z* 389.172 (experimental), 389.172 (calculated). ¹H NMR (500 MHz, DMSO-d₆) δ_H (ppm) 7.24 (dd, *J* = 5.9, 3.5 Hz, 4H, *ArH*), 7.04 (dd, *J* = 6.0, 3.4 Hz, 4H, *ArH*), 5.04 (s, 2H, methine-CH), 2.35 (s, 5H, CH₃), 2.25 (s, 7H, CH₃). ¹⁹F NMR (470 MHz, DMSO-d₆): δ_F (ppm) = -172.7 ppm.

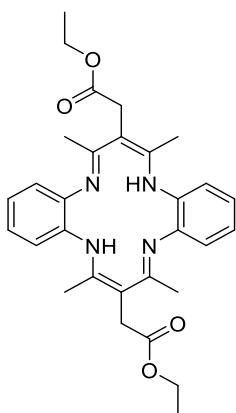
6,13-di-(ethyl-2-acetate)-5,7,12,14-tetramethyldibenzo[*b,i*][1,4,8,11]tetraazacyclotetradecahexanatonickel (II) complex.
[NiL¹⁶]



Modified from a literature procedure. The 6,13-di-(ethyl-2-acetate)-5,7,12,14-tetramethyldibenzo[*b,i*][1,4,8,11]tetraazacyclotetradecahexaenatonickel (II) complex was synthesized using an adapted literature protocol.²⁶⁶

To a solution of 5,7,12,14-tetramethyldibenzo[*b,i*][1,4,8,11]tetraazacyclotetradecahexaenatonickel(II), (0.53 g, 1.3 mmol) in anhydrous acetonitrile (50 mL) was added ethyl bromoacetate (3 mL). The mixture was heated to reflux for 24 h under nitrogen. The solvent was removed under reduced pressure. The residue was triturated with petroleum spirit, cooled to 4 °C overnight and filtered. The solid was purified by alumina column using dichloromethane. The green solution was collected, and the solvent was removed under reduced pressure to afford [NiL¹⁶] as a green solid (0.18 g, 23%). HR-MS(ESI/O-TOF): [C₃₄H₄₂NiN₄O₄+H]⁺ *m/z* 572.193 (experimental), 572.193 (calculated). ¹H NMR (500 MHz, DMSO-*d*₆) δ_H (ppm) 6.69 (m, 2H, ArH), 6.56 (m, 6H, ArH), 4.21 (q, 4H, CH₂CH₃), 3.32 (d, 4H, CH₂), 2.13-2.10 (s, 12H, CH₃), 1.30 (t, 6H, CH₂CH₃).

*1,8-dihydro-6,13-di-(ethyl-2-acetate)-5,7,12,14-tetramethyldibenzo[b,i][1,4,8,11]
tetraazacyclotetradeca-4,6,11,13-tetraene, H₂L¹⁶*



To [NiL¹⁶] (89 mg, 0.15 mmol) was added hydrogen chloride (1.25 M) in ethanol (4.4 mL) and heated to 80 °C. A solution of NH₄PF₆ (0.60 g) in water (10 mL) was added to the mixture, extracted with diethyl ether (2 x 35 mL). To this was added triethylamine (2 mL) or until the yellow colour was observed. The organic layer was washed with brine (3 x 70 mL), dried using MgSO₄ and removed under reduced pressure to give H₂L¹⁶ as an off-white solid (25 mg, 32%). HR-MS(ESI/O-TOF): [C₃₄H₄₂NiN₄O₄+H]⁺ *m/z* 517.3073 (experimental), 517.2815 (calculated). ¹H NMR (500 MHz, DMSO-*d*₆) δ_H (ppm) 12.3 (s, 2H, NH), 7.53 (m, 4H, ArH), 7.12 (m, 4H, ArH), 4.20 (q, 4H, CH₂CH₃), 2.41 (s, 6H, CH₃), 2.07 (s, 6H, CH₃), 1.22 (t, 6H, CH₂CH₃).

Chapter 5 Concluding Remarks

The positron-emitting radionuclide fluorine-18 is the most commonly used nuclide in diagnostic PET imaging agents largely due to the use of the glucose analogue [^{18}F]FDG. To realise the full potential of diagnostic PET molecular imaging, new tracers are required. One of the challenges in developing new PET tracers is that the incorporation of fluorine-18 into organic molecules often involves relatively challenging synthetic procedures. Recent advances have identified alternative methods of nucleophilic fluorination using aqueous [^{18}F] F^- and the Group 13 element Al^{3+} to form stable non-covalent [^{18}F] $\text{Al}^{3+}\text{-F}^-$ coordination complexes. Initial studies have demonstrated that such $\text{Al}^{3+}\text{-}^{18}\text{F}^-$ bonds are sufficiently stable for some imaging applications. A potential disadvantage of this approach is that the radiolabelling requires high temperatures to achieve sufficient radiochemical yields and this is incompatible with some heat-sensitive molecules.

The research in this thesis investigated the potential to use the Group 13 congener Ga^{3+} and $\text{Ga}^{3+}\text{-}^{18}\text{F}^-$ coordinate bond as an alternative to Al^{3+} that may allow radiolabelling at lower temperature. Three different families of ligand that could form coordinatively unsaturated complexes with Ga^{3+} were investigated to enable the incorporation of fluorine as monodentate ligand.

An acyclic ligand based on a picolinic acid scaffold was adopted to prepare H_2L^{3-5} in Chapter 2. These ligands were decorated with different functional groups on the backbone of the chelator. The five-coordinate Ga^{3+} complex, [GaL^3F] was readily formed in aqueous solution under mild conditions but the complex was sensitive to low pH (< 3). It was not possible to form [^{18}F][GaL^3F] in appreciable yields when working with diluted solutions of radioactive fluorine-18.

The limited stability of $[\text{GaL}^3\text{F}]$ and the difficulty in translating the simple non-radioactive chemistry to analogous chemistry with fluorine-18 encouraged an exploration of macrocyclic complexes of Ga^{3+} . Whilst the preparation of a $[\text{}^{18}\text{F}]\text{Ga}^{3+}\text{-F}^-$ complex with triazamacrocyclic ligand was first reported in 2015, the complex reported did not possess a targeting molecule for *in vivo* imaging application. In Chapter 3, a macrocyclic NODA chelator with an appended stilbene derivative, H_2L^{12} designed to bind to protein deposits associated with Alzheimer's disease (AD) was prepared.

The stilbene targeting motif allowed the visualisation of the binding of H_2L^{12} to $\text{A}\beta_{1-42}$ plaques in human brain tissue. The binding of H_2L^{12} to $\text{A}\beta_{1-40}$ fibrils was quantified using a competition assay and revealed a modest affinity ($K_i = 1.8 \pm 0.9 \mu\text{M}$) for $\text{A}\beta_{1-40}$ fibrils. Coordination of Ga^{3+} to L^{12} reduced the binding affinity, and there was no evidence of binding to $\text{A}\beta$ plaques in human brain tissue.

In contrast to $[\text{}^{18}\text{F}][\text{GaL}^3\text{F}]$, $[\text{}^{18}\text{F}][\text{GaL}^{12}\text{F}]$ can be formed in aqueous media at pH 4.0 at 80 °C. This difference can be attributed to the 'macrocyclic effect' of $[\text{}^{18}\text{F}][\text{GaL}^{12}\text{F}]$ that led to its enhanced stability. $[\text{}^{18}\text{F}][\text{GaL}^{12}\text{F}]$ is stable at pH 4-6 for at least 4 h but was unstable in phosphate buffered saline (pH 7.4) as only 50% remained intact after 0.5 h. The instability of $[\text{}^{18}\text{F}][\text{GaL}^{12}\text{F}]$ in PBS might reflect kinetic lability of the system in the presence of competing nucleophiles, chloride and hydroxide at pH 7.4.

One of the advantages of the chelator, H_2L^{12} is its ability to be labelled with two other radionuclides, copper-64 and gallium-68. The radiotracers, $[\text{}^{68}\text{Ga}][\text{GaL}^{12}\text{F}]$ and $[\text{}^{64}\text{Cu}][\text{CuL}^{12}]$ were formed at pH 4-5.5 in high radiochemical purity. The enhanced thermodynamic stability and kinetic inertness of $[\text{}^{64}\text{Cu}][\text{CuL}^{12}]$ made it an excellent choice for further biodistribution study in mouse models. The initial uptake of $[\text{}^{18}\text{F}][\text{GaL}^{12}\text{F}]$ and $[\text{}^{64}\text{Cu}][\text{CuL}^{12}]$ in the brain were $0.85 \pm 0.13\%$ IA/g and $0.71 \pm 0.03\%$

IA/g respectively. Whilst $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ and $[^{64}\text{Cu}][\text{CuL}^{12}]$ can cross the blood-brain barrier; this needs to be optimised as they do not satisfy the requirement of a PET diagnostic neuroimaging agent for AD. Notably, the in vivo PET images and biodistribution studies suggested that $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$ was unstable in vivo leading to a significant uptake of fluorine-18 in bone.

The versatility of the NODA derived chelator, L^{12} was demonstrated by effectively labelling the ligand with three different radionuclides. The $[^{64}\text{Cu}][\text{CuL}^{12}]$ emerged as a promising candidate for further investigations. The hydrophilic properties may suggest that the $[^{64}\text{Cu}]$ -NODA complex may be more suited for conjugation with peptides for cancer imaging purposes than for brain imaging applications.

To further explore the $\text{Ga}^{3+}\text{-}^{18}\text{F}^-$ approach for brain imaging applications, the azamacrocyclic ligand, tetramethyltetraaza[14]annulene were investigated as it forms more hydrophobic metal complexes. The five-coordinate complex, $[\text{Ga}(\text{tmtaa})\text{F}]$ does not rely on carboxylate donor groups that may have contributed to the lack of kinetic stability observed with $[^{18}\text{F}][\text{GaL}^3\text{F}]$ and $[^{18}\text{F}][\text{GaL}^{12}\text{F}]$. Whilst the radiochemical yield of $[^{18}\text{F}][\text{Ga}(\text{tmtaa})\text{F}]$ formed *via* halogen exchange reaction of $[\text{Ga}(\text{tmtaa})\text{Cl}]$ with fluorine-18 was relatively low, future investigations can explore various reaction conditions or other derivatives of $[\text{Ga}(\text{tmtaa})\text{F}]$. For example, functionalisation at the methine carbon atom to tune down the lipophilicity of H_2tmtaa could see future developments to include targeting molecules.

The $[^{18}\text{F}]\text{Al}^{3+}\text{-F}^-$ system utilising the tetraaza[14]annulene is worth investigating in future work as it may ultimately be more robust than the $[^{18}\text{F}]\text{Ga}^{3+}\text{-F}^-$ system. It was possible to prepare and characterise discrete $\text{Ga}^{3+}\text{-F}^-$ complexes with all three families of ligand. Translation of the synthetic chemistry that was developed for the non-radioactive $\text{Ga}^{3+}\text{-F}^-$

system to systems containing fluorine-18 remains challenging but the initial studies presented here will guide future investigations to improve radiolabelling with fluorine-18.

Although it is possible to form the $\text{Ga}^{3+}\text{-}^{18}\text{F}^-$ coordinate bonds at lower temperatures than the Al^{3+} analogues, the work presented here demonstrated that the $[\text{}^{18}\text{F}]\text{Ga}^{3+}\text{-F}^-$ complexes are less stable and this may limit their use as PET imaging agents. It would be of great interest to investigate the suitability of $[\text{}^{18}\text{F}]\text{Al}^{3+}\text{-F}^-$ complexes of these families of ligands described in this thesis for PET imaging applications.

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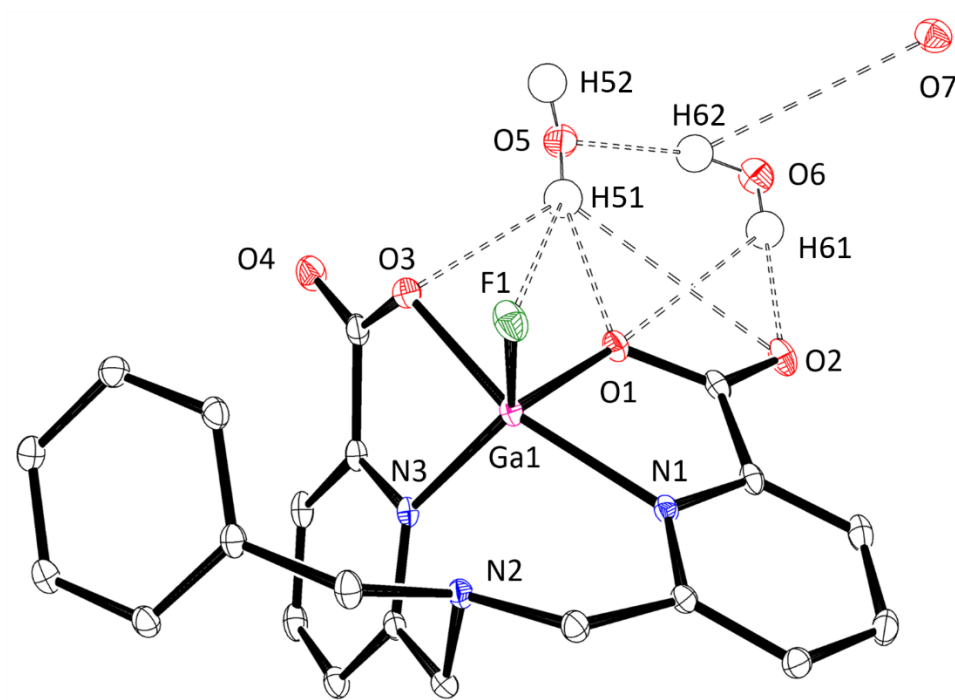
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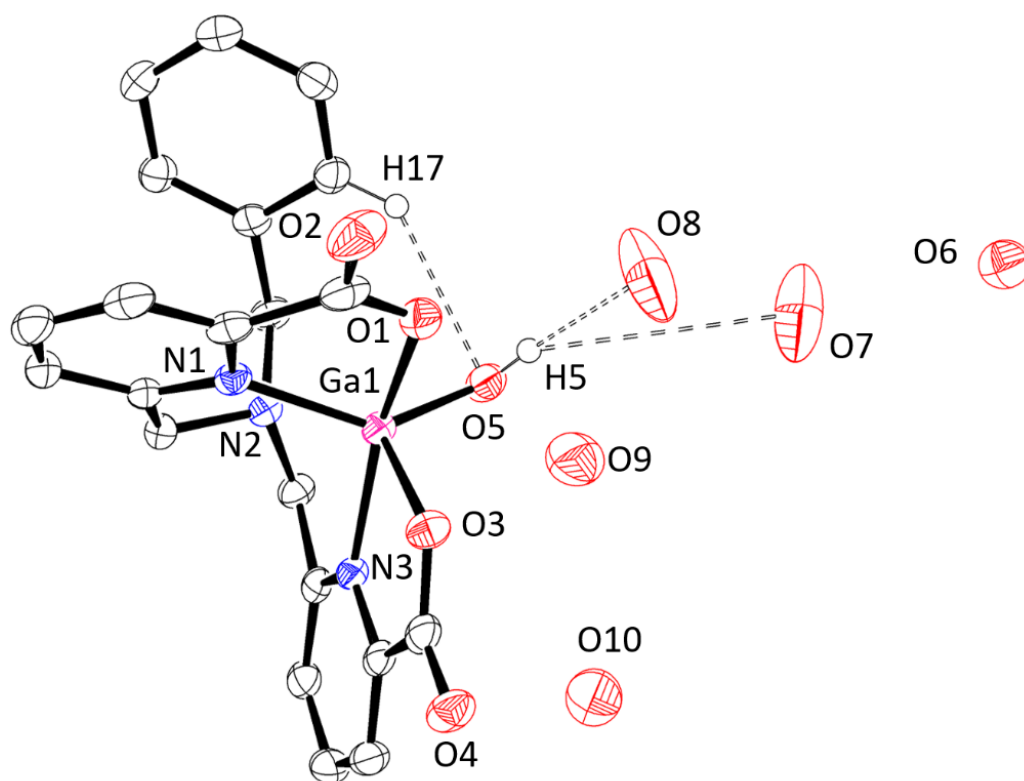
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Chapter 7 Appendix



Distance (Å)	[GaL ³ F]
F(1)-H(51)	4.16(3)
O(1)-H(51)	2.09(2)
O(2)-H(51)	3.62 (2)
O(3)-H(51)	2.60(2)
O(1)-H(61)	3.18(3)
O(2)-H(61)	1.95(3)
O(5)-H(62)	1.96(3)
O(7)-H(62)	3.32(3)



Distance (Å)	[GaL ³ (OH)]
O(5)–H(17)	2.575
O(5)–H(5)	0.819
O(7)–H(5)	3.601
O(8)–H(5)	1.992