THE UNIVERSITY OF HULL

Synthesis and characterisation of novel azamacrocyclic chelators for biomedical applications: biological activity and radiolabelling

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by

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Abstract

Azamacrocyclic derivatives that contain nitrogen donors have been used for decades in analytical, industrial and medical applications. They are good hosts for metal ions, anions, neutral molecules and organic cation guests. Azamacrocyclic complexes that are biologically active have been used in the identification of diseased tissues. Transition metal macrocyclic complexes have also received significant attention due to their pharmacological properties such as toxicity against bacterial and fungal growth. Many macrocyclic complexes have been reported to have anti-inflammatory properties.

A number of azamacrocyclic ligands have been used in the design of bifunctional chelators (BFCs) that have been utilised in molecular imaging. Incorporation of a positron emitting metal radioisotope such as ^{68}Ga requires a BFC to form a stable complex *in vivo* and for covalent bond formation (conjugation) with a targeting moiety. Macrocyclic complexes are essential to a number of biological systems and have shown affinity for the CXCR4 chemokine receptor. Studies confirmed that CXCR4 is an important factor in the migration, invasiveness, proliferation and metastasis of tumours and overexpression of CXCR4 has been shown in twenty three different human tumours including non-small cell lung cancer, ovarian cancer, prostate cancer, colorectal cancer and breast cancer.

In this work three different classes of azamacrocycles have been synthesised. The first group is a series of configurationally restricted copper(II), zinc(II) and nickel(II) mono-ring macrocycles bearing benzimidazole derivatised pendant arms. The antifungal activity has been determined for both ligands and their transition metal complexes. Most of the compounds tested showed a recognisable activity. The zinc(II) complexes of the ligands (side-bridged cyclen benzimidazole and side-bridged cyclen 4-nitrobenzyl benzimidazole) showed the highest antifungal activity of the compounds tested.

The second class of azamacrocyclic derivatives synthesised comprises five novel bifunctional chelators based on benzimidazole TACN and NO2A derivatives. Four ^{68}Ga complexes of the following ligands: NO2A benzimidazole, TACN tris 4-nitrobenzyl benzimidazole, NO2A 4-nitrobenzyl benzimidazole and NO2A 4-aminobenzyl benzimidazole, have been synthesised in radiochemical yields of 73%, 55%, 25% and 37% respectively at RT with a 5 minute reaction time.

The third type of azamacrocyclic ligands are C-functionalised bis-tetraazamacrocyclic derivatives to be used as CXCR4 antagonists. Four new C-functionalised bis-azamacrocycles that have an amino group to allow for subsequent conjugation were synthesised: 4-aminobenzyl-C-functionalised side-bridged bis-cyclam, 4-aminobenzyl-C-functionalised side-bridged bis-cyclam cyclen, 4-aminobenzyl-C-functionalised bis-cyclam and 4-aminobenzyl-C-functionalised bis-cyclam cyclen. Zinc(II), nickel(II) and copper(II) complexes of the 4-nitrobenzyl-C-functionalised side-bridged bis-cyclam were synthesised and characterised as CXCR4 antagonists. A selection of the synthesised compounds was biologically evaluated in a number of assays (displacement assays, anti-HIV assays, cytotoxicity assays and calcium(II) signalling assays) with the free ligands showing activity and the metal complexes also acxtive and significantly more potent.

Risk Assessment

All experiments were carried out in accordance with the University of Hull's Health and Safety guidelines. A full COSHH and risk assessment was carried out for each new experiment, signed by the undertaking student, supervisor (Prof. S. J. Archibald) and the departmental safety officer (Dr T. McCreedy) before any practical work started. Copies of each form were provided for reference to the departmental safety officer and supervisor. The COSHH forms carry the reference numbers AZ1-AZ29. Radiochemistry experiments were assessed using the PET Research Facility Risk Assessment Form**,** signed by the undertaking student, supervisor (Prof. S. J. Archibald) and the radiation protection supervisor (Prof. S. J. Archibald) before radiochemical experiments were carried out, with the forms carrying the reference numbers AZZ1-AZZ2.

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Abbreviations

λ_{max}	maximum wavelength
Asp	aspartate
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
ATSM	aromatic bis(thiosemicarbazonates)
β^-	electron (beta minus)
β^+	positron (beta plus)
BFC	bifunctional chelator
BMs	biomolecules
Bq	becquerel's
br	broad
bz	benzimidazole
CB	cross bridge
CB-DO2A	2,2'-(1,4,7,10-tetraazabicyclo[5.5.2]tetradecane-4,10-diyl)diacetic acid
CB-TE2A	1,4,8,11-tetraazabicyclo[6.6.2]hexadecane-4,11-diacetic acid
CB-TE2A-EtOH	2,2'-(1,4,8,11-tetraazabicyclo[6.6.2]hexadecane-4,11-diyl)diacetic acid compound with ethanol (1:1)
CC_{50}	the cytotoxic concentration required to reduce a population by 50%
CHN	carbon, hydrogen, nitrogen analysis
COSHH	control of substances hazardous to health
CT	computed tomography
δ	chemical shift

d	Doublet
2D	two dimensional
3D	three dimensional
DCM	Dichloromethane
DEDPA	1,2-[{6-(carboxylato-)pyridin-2-yl}methylamino]ethane
DME	1,2-dimethoxyethane
DMF	*N*,*N'*-dimethylformamide
DMSO	Dimethylsulfoxide
DNA	deoxyribose nucleic acid
DO2A	1,4,7,10-tetraazacyclododecane-1,7-diacetic acid
DO3A	1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid
DOTA or H_4DOTA	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic Acid
DOTATOC	consists of a disulfide-bridged octapeptide, D-Phe1-Cys2-Tyr3-D-Trp4- Lys5-Thr6-Cys7-Thr8-ol, connected to the metal chelator DOTA
DOTP or H8DOTP	1,4,7,1 0-tetraazacyclododecane-1,4,7,10-tetra(methylenephosphonic
DTPA	diethylene triamine pentaacetic acid
ε	molar absorptivity
EC	electron capture
EC_{50}	effective concentration required to reduce an effect by 50%
EDTA	ethylene diamine tetra-acetic acid
ESI	electrospray ionisation
EtOH	Ethanol
FDA	food and drug administration

FDG	2-deoxy-2-fluoro-D-glucose
GPCR	G-protein coupled receptor
h	Hour
HIV	human immunodeficiency virus
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
IC_{50}	concentration required to inhibit binding by 50%
It	isomeric transition
J	coupling constant
kDa	Kilodaltons
keV	Kiloelectronvolts
m	multiplet
M	Molar
mAb	monoclonal antibody
MeCN	Acetonitrile
MeOH	Metanol
mg	Milligram
MHz	Megahertz
min	Minute
ml	Millilitre
mM	Millimolar
mmol	Millimole
μM	Micromolar
μmol	Micromole
MRI	magnetic resonance imaging

MS	mass spectrometry
MT-4	meta trader 4
nm	nanometres
nM	Nanomolar
NE3TA	(4-carboxymethyl-7-[2-(carboxymethyl-amino)-ethyl]-[1,4,7]-trizonan-1-yl) Acetic Acid
NMR	nuclear magnetic resonance
NODAGA	1,4,7-triazacyclononane, 1-glutaric acid-4,7-acetic acid
NO2A	2,2'-(1,4,7-triazonane-1,4-diyl)diacetic acid
NOTA	1,4,7-triazacyclononane-1,4,7-triacetic acid
NOTGA	1,4,7-triazacyclononane-1,4,7-tris-(glutaric acid)
PCB-TE2A	2,2'-(1,4,8,11-tetraazabicyclo[6.6.3]heptadecane-4,11-diyl)diacetic acid
PET	positron emission tomography
ppm	parts per million
q	Quartet
quin	Quintet
RCY	radiochemical yield
RGD	arginylglycylaspartic acid
R_f	retention factor
RNA	ribose nucleic acid
RT	room temperature
s	Second
s	Singlet
SA-PET	small animal- positron emission tomography

SB	side bridge
SIV	simian immunodeficiency virus
SOD	superoxide dismutase
SPECT	single photon emission computed tomography
t	Triplet
$t_{1/2}$	half-life
TACN	1,4,7-triazacyclononane
TE2A	4,11-bis(carboxymethyl)-1,4,8,11-tetraazabicyclo[6.6.2] hexadecane
TE2A-EtOH	4,11-bis(carboxymethyl)-1,4,8,11 tetraazabicyclo[6.6.2] hexadecane compound with ethanol (1:1)
TETA	1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid
TETA-EtOH	1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid compound with ethanol (1:1)
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	Tetramethylsilane
TRAP	1,4,7-triazacyclononane-1,4,7-tris[(2-carboxyethyl) methylene phosphinic acid]
UK	united kingdom
UV	ultra-violet
UV-vis	ultra-violet-visible

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Chapter One

Introduction

1. Introduction

Medicinal inorganic chemistry is an offshoot of growing significance in both therapeutic and diagnostic medicine. Inorganic compounds have been used in medicine for many centuries, but frequently only in an experimental way with little effort to design the compounds to be used, and with little or no understanding of the molecular basis of their mechanism of action.[1] There is a crossover point between medicine and inorganic chemistry, including metal-based drugs,[2] metal sequestering or mobilizing agents,[3,4] metal-containing diagnostic aids,[5] and the medicinal recruitment of endogenous metal ions.[6,7]

For the treatment and understanding of diseases which are currently intractable, medicinal chemistry suggests the potential for the design of novel therapeutic and diagnostic agents.[8] Recently, utilizing a variety of chelating ligands to adjust and control the properties of metal ions in biological systems offers a chance to extend research impact in medicinal inorganic chemistry.[9] Even if the majority of recent pharmaceuticals are entirely organic compounds[10] an enormous influence on medicinal activity comes from inorganic chemistry, particularly the important role in the treatment of cancer.[11]

1.1. Chelating ligands

Ligands play a significant role in modifying the biological effects of metal-based drugs.[12] Chelation (from chela, the pincer-like claw of a crustacean) is the procedure that is used to describe the binding between ligand and metal ion. It gives an approach to modify a metal ion's properties; by binding ligands that are themselves active pharmaceutical agents it also offers a ways to control activity of an organic drug.[13] A single chemical entity can be developed in this approach and then used to modulate multiple targets concurrently and potentially deliver superior efficacy against complex diseases.[14] The oral bioavailability of metal ions can be influenced by the ligands, which can help in targeting specific tissues or enzymes. They can also deliver, bind or sequester a particular metal ion, depending on the requirements, for therapy or diagnosis.[7] Chelation is a good way to develop the properties of the metal ions and introducing substituents can modify the drug's properties and then act synergistically to improve the metal activity.[12] Substituents are added to the chelator to bind preferentially to membrane receptors (*e.g.* glycosyl moieties) or mimic naturally-occurring hormones (*e.g.* somatostatin-like oligopeptides).[15] Modification of ligands with designed target specificity can produce far-reaching diagnostic and therapeutic effects.[7] Multifunctional ligands that are designed for metal-based medicinal agents give many promising possibilities

and the resultant drugs may have a positive impact in areas of diagnosis and therapy by playing an essential role in reducing the potential toxicity of a metallodrug.[13]

1.1.1. Macrocycles

A cyclic compound containing a ring of at least nine atoms including at least three donor atoms oriented so as to bind to a central metal ion is called a macrocyclic ligand. Macrocyclic ring sizes tend to possess between 3x and 4x+1 atoms (where x= the number of donor atoms) in its chain. In this most simple form, a macrocyclic ligand would occupy three closest coordination sites on one side of a metal ion. However, cyclam or porphyrin type derivatives which contain larger rings can have a central cavity large enough for the transition metal ions to fit into the plane of the macrocycle.[16] Macrocyclic derivatives that contain a number of nitrogen donors have been used for a long time in analytical, industrial and medical applications.[17] Due to their various suitable properties they are good hosts for metal ions, anions, neutral molecules and organic cation guests and they are interesting ligand systems for further investigation.[18] There are many factors controlling the properties of macrocyclic complexes including the size of the macrocycles, the type of donor atoms and the complexation performance of the anions involved in coordination.[19] Ligands that are complementary in geometry and size to the targeted metal ion can be called preorganised[20] and during complexation they do not have to reorganize themselves around the metal ion, making the chelates more thermodynamically stable.[21,22] The 12 membered tetraazamacrocyclic derivatives based on cyclen with pendant arms on the nitrogen atoms, such as H_4DOTA, H_8DOTP and many other related derivatives, are well-known azamacrocyclic compounds used in medical applications and are preorganised chelators, see Figure *1*. The donor atoms that are bound to the metal ion are included in the cage arrangement and form complexes that are thermodynamically very stable[23] and at the same time in many cases kinetically inert.[24] Another example of macrocyclic ligands are the more flexible cyclam derivatives H_4TETA and H_8TETP, see Figure *1*, that have many applications including formation of complexes with $^{64/67}$Cu.[20]

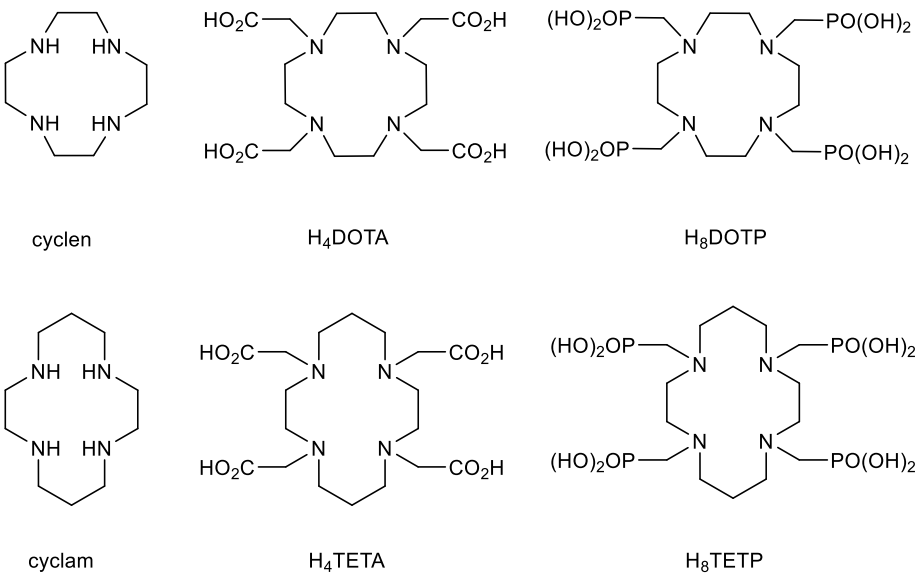


Figure 1 - Chemical structures of cyclen and cyclam along with selected common derivatives thereof.[20]

1.1.1.1. The chelate and macrocyclic effect

The chelate effect gives an explanation for the enhanced stability of a complex containing polydentate ligands compared to one containing (analogous) monodentate ligands. It is the increase of free energy when a metal ion is bonded to a bidentate or polydentate ligand and that leads to more stable complexes compared to unidentate ligands. A similar concept can apply to the macrocyclic effect to illustrate the property of coordinating macrocyclic molecules. Cabbiness and Margerum introduced this term firstly in 1969,[25] when they observed increasing in the stability of a copper(II) complex coordinated to Curtis' macrocycle[26] compared to similar non-cyclic tetraamine ligands. The preorganisation of macrocyclic ligands is responsible for this stability along with the affinity between the ligand and the metal ion.

1.1.1.2. Configurationally restricted macrocycles

Wainwright and Hancock synthesised ethyl bridged monomacrocycles first in the 1980s,[27,28] followed by Weisman and Wong in the 1990s.[29] Configurationally restricted macrocycles can be obtained when the macrocyclic skeleton has an ethylene bridge either between adjacent or opposite nitrogens. A cross bridge (CB) structure is formed when opposite nitrogen bonded and a side bridge (SB) structure formed by connecting adjacent nitrogens, see Figure 2.

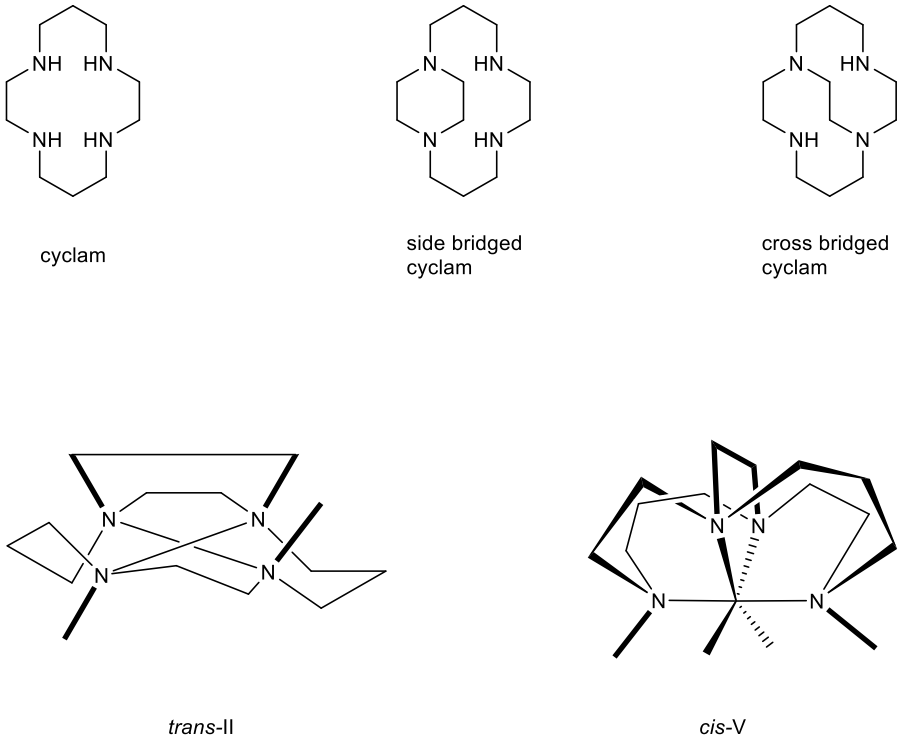
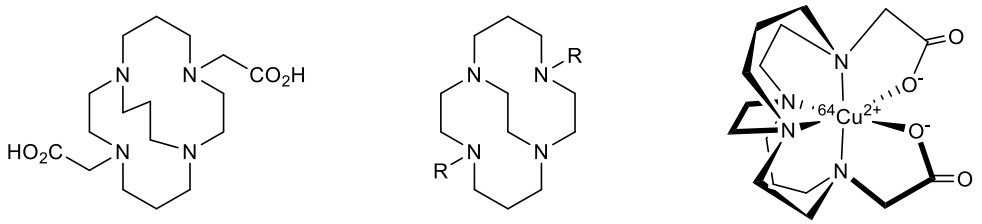


*Figure 2 - Configuration of cyclam with the addition of an ethylene bridge between adjacent (SB, trans-*II*), when mono-alkylated and non-adjacent (CB, cis-*V*) nitrogen atoms. (Reproduced from Curr. Med. Chem.)*[30]

Several parameters including basicity, ring size, flexibility, steric strain and importantly configuration can be controlled by structurally reinforcing the skeleton. When an ethylene bridge is introduced in the skeleton the configuration locks and allows only one configuration; mono-N-alkylated SB compounds force the structure to adopt a *trans*-II configuration[31] or have *trans*-IV in bis-N-alkylated.[32] A *cis*-V configuration can be adopted in CB compounds,[33] see Figure 2. A new family of rigid chelators were produced (ligands L^1-L^4), see Figure 3.[34,35] These restrictions in configuration have been exploited by Wadas *et al.*[36] and our group[37] to form stable complexes for chelation to metal ions and subsequent use *in vivo*, most often with copper-64 radiopharmaceuticals in molecular imaging. Recently Pandya *et al.* have successfully synthesised a new type of bridged cyclam derivative PCB-TE2A in an efficient way by using propyl bridge instead of ethyl bridge which also forms a *cis*-V configuration as expected.[38]



PCB-TE2A

L^1: R = H
L^2: R = CH_2COO^-
L^3: R = $CH_2CON(C_2H_5)_2$
L^4: R = $C_2H_4CONH_2$

$^{64}CuL^2$

Figure 3 - Cyclam derivatives containing a cross-bridge between two opposite nitrogen atoms.[35,38]

1.2. Molecular Imaging

The term molecular imaging defines the procedure of imaging a receptor or organ with radiolabelled molecule, often framed as a radiopharmaceutical,[39] allowing the relevant information in the diagnosis and treatment of patients to be obtained.[40] As a result it is possible to diagnose patients that are suffering from or susceptible to specific diseases and so, new chemotherapeutics have been designed to target specific disease biomarkers and by this approach, treatment saves healthy tissue and takes full advantage of the therapeutic effectiveness of the drugs.[41] There are different types of imaging techniques including: positron emission tomography (PET), magnetic resonance imaging (MRI), computed tomography (CT) and single-photon emission computed tomography (SPECT), all of which can be used in hospitals to scan patients. For pre-clinical research, smaller, less expensive analogues are also used.[42] Diagnostic imaging is a non-invasive technique that is used to evaluate the disease states and screen the effects of treatment. Usually the two nuclear medicine modalities of diagnostic imaging, single photon emission computed tomography (SPECT) or positron emission tomography (PET) are used in combination with CT. These modalities offer complementary images and sensitivity in deep tissue.[43]

1.2.1. Positron Emission Tomography (PET)

Positron emission tomography (PET) is a non-invasive molecular imaging modality with good resolution, high sensitivity and accurate quantification which can be used to address questions central to drug development by offering a new kind of 'precision pharmacology'.[44] By using PET it is possible to check if a new chemical object reaches a target tissue (like the brain) in sufficient amounts to be pharmacologically active through biodistribution studies with drug molecules containing positron-emitting radioisotopes.[44,45] PET participates in the clinical management of a patient by introducing advanced functional information on the degree of disease and therapy reaction and can also be used for therapy preparation.[46] The physical and chemical properties, obtainability and the timescale of studying biological processes determine the selection of PET radionuclide.[47]

1.2.1.1. Basic principles of PET

PET detects the 511-keV photons that are produced from positron annihilation (β^+ decay) by unstable proton-rich radionuclides.[48] The two ways that these nuclei can achieve stability are by electron capture or by emission of a positron (a particle with a positive charge and the same mass and spin as an electron). In this decay route, positrons emitted are released with kinetic energy determined by the properties of each radionuclide. By electrostatic interactions with the nearest charged units (protons and electrons), the positron that is emitted from the nucleus loses its kinetic energy, as it moves through the nearby environment. The positron slows when the kinetic energy is transferred to its environment, and finally associates with a close electron to give an ultra-short-lived unit called positronium. Two high-energy 511-keV annihilation photons are generated due to the quick annihilation of the positronium and are emitted in opposite directions (nearly 180° apart) because of preservation of energy and momentum, and move at the speed of light (30 cm/ns). Many types of radiation detectors can be used to detect gamma rays to determine the location of the annihilation photons. To detect the two emitted photons by PET scanners, a chain of pairs of contrasting radiation detectors can be used which are set in a geometric shape that approximates a circle in 2D and a cylinder in 3D. The opposing detectors recognise photons (a coincident event) within a limited span of time (nanoseconds); this is called a line of response (LOR). The mathematical tomographic image will then be formed using algorithms after the events are combined and transform them into images that represent slices through the subject in the plane of the detector ring,[46,49] see Figure 4.

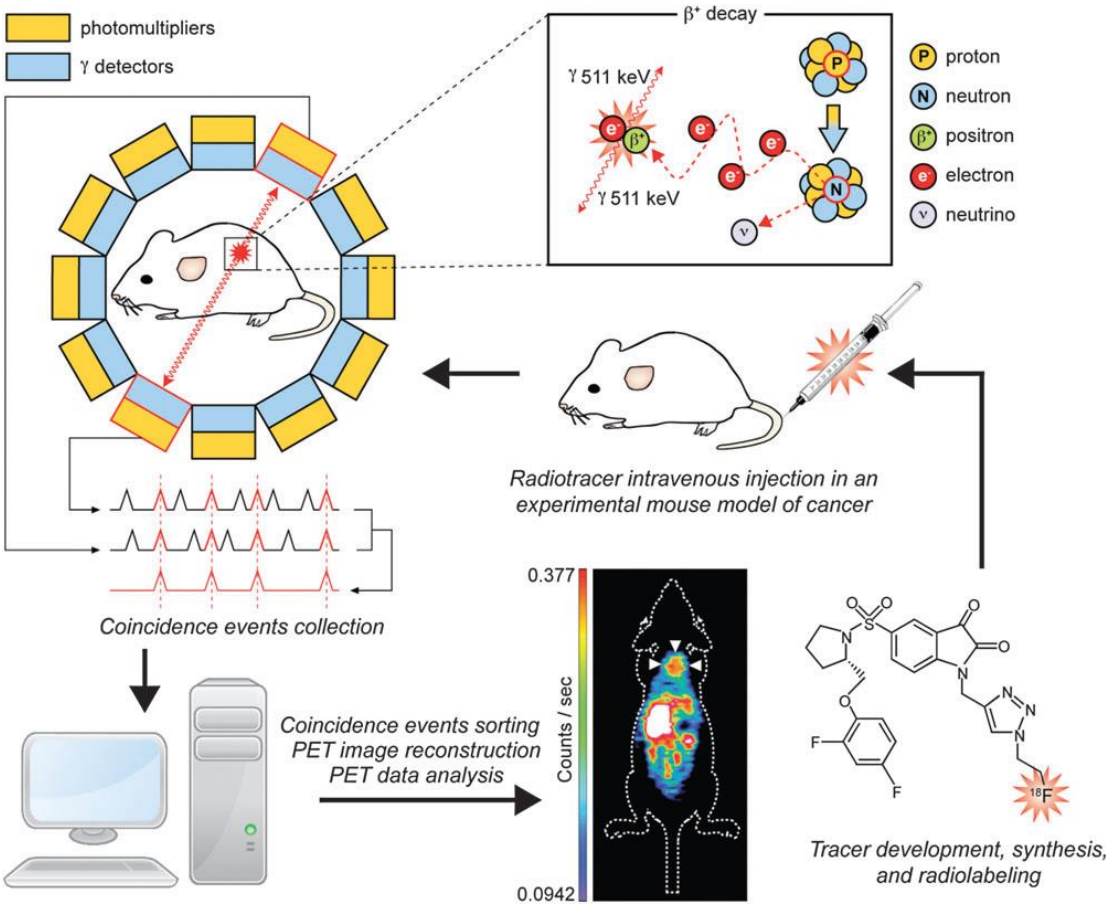


Figure 4 - Small animal PET imaging: A typical simplified SA-PET imaging procedure. (Reproduced from Integr. Biol.)[46]

1.3. Radiopharmaceuticals

Radiopharmaceuticals are radioactive drugs and have two fundamental components; a radionuclide that emits an appropriate ionising radiation when it decays, and a pharmaceutical ligand that binds to the radionuclide and delivers it to the target organ or tissue. Routinely they are used in nuclear medicine departments for the diagnosis or therapy of various diseases. Most of them are small organic or inorganic compounds with a defined composition. Also macromolecules such as monoclonal antibodies and antibody fragments may be used in formation of radiopharmaceuticals. They can be divided into two primary classes depending on their medical applications: diagnostics and therapeutics. According to their biodistribution characteristics they can also be classified: those that the chemical and physical properties determine their biodistribution (non target-specific radiopharmaceuticals) and those that have

distribution determined by their receptor binding or other biological interactions (target-specific radiopharmaceuticals).[39,50,51]

1.3.1. Isotopes and macrocycle selection in radiopharmaceuticals

There are two types of isotopes that have been reported, diagnostic isotopes and therapeutic isotopes (radioimmunotherapy). A minimal interaction of the radiation with tissue and a maximum interaction with an external detector is preferable in molecular imaging.[52] Radioisotopes that are gamma-emitting and positron emitting emit photons of sufficient energy and intensity to allow the necessary resolution for tumour imaging. By using a conventional Anger camera, single photon emission can be detected externally giving reasonable resolution (1 cm) and by tomographic methods improved information may be obtained. The PET technique, as already discussed, gives improved resolution (3 mm).[52-54] As much energy as possible is required in radioimmunotherapy, which should be delivered to the target site in order to provide a sterilising dose of radiation that will cleave cellular DNA. Either β or α-emitters, with little or no gamma component are appropriate isotopes. The half-life of the radionuclide for both imaging and therapy should be sufficiently long to allow transportation to the tumour site.[52] Diagnostic radiopharmaceuticals are usually used at very low concentrations (nano-molar to pico-molar range) without have any pharmacological effect.[43] Radioisotopes have other features common to imaging and therapeutic: (a) stable daughter isotope should be produced by the decay; (b) the isotope should be cheap; (c) readily available, preferably from a generator, in a carrier-free form, *i.e.* does not have other stable isotopes that came from the given element; (d) excellent radiochemical and chemical purity; (e) it must have chemical properties that allow the radionuclide to be attached to the target molecule.[51,52] Because of their wide range of nuclear properties (half-life, type of radiation, particle energy, ease of isolation, coordination chemistry) metallic radionuclides (such as ^{99m}Tc, ^{186}Re, ^{64}Cu, ^{111}In, ^{68}Ga, ^{90}Y), see Table 1, provide significant advantages over their non-metal counterparts and thus can be chosen for the properties that best fit the intended application.[13] In addition, the use of metallic radionuclides offers many opportunities for the design and development of new target-specific radiotracers.[55] Due to its ideal nuclear properties ($t_{1/2}$ = 6.01 h, γ = 142.7 keV) and easy isolation as $Na^{99m}TcO_4$ from a ^{99}Mo generator, ^{99m}Tc is the most commonly used isotope in nuclear medicine (85% of all procedures). Either monodentate or acyclic ligands are usually bound to it.[9,13] A number of technetium complexes of the TACN macrocycle were prepared and characterized.[56] Technetium in the oxidation states III, IV and VII forms few compounds. The unusual high

stability is technetium(VII) trioxo complex. The high valent technetium oxo TACN compounds which was reported by Alberto and co-workers to come up with a new labelling strategy whereby a molecule can be attached to this core via a [3 + 2] cycloaddition with an alkene to form a glycolato complex with a technetium(V) centre. Therefore biological molecules could be tagged with an alkene for following reaction with this complex.[57]

Radionuclide	Half life	Production	βmax(KeV)	Decay mode
^{13}N	9.97 min	Cyclotron	1198 KeV	β^+ 100%
^{15}O	2.03 min	Cyclotron	1732 KeV	β^+ 100%
^{11}C	20 min	Cyclotron	960 KeV	β^+ 98%
^{18}F	110 min	Cyclotron	634 KeV	β^+ 97%
^{68}Ga	68 min	Generator	1899 KeV	β^+ 90%
^{64}Cu	12.6 hours	Cyclotron	653 KeV	β^+ 19% EC 41% β^- 40%
^{86}Y	14.7 hours	Cyclotron	2335 KeV	β^+ 33% EC 67%
^{89}Zr	78.4 hours	Cyclotron	897 KeV	β^+ 23% EC 77%
^{124}I	4.17 days	Cyclotron	1535 KeV	β^+ 23% EC 77%
^{99m}Tc	6.01 hours	Generator	140 KeV	It 99.9%
^{111}In	67.39 hours	Cyclotron	171 KeV	EC 100%
^{67}Ga	78.28 hours	Cyclotron	93 KeV	EC 100%

Table 1 - Characteristics of radionuclides frequently used in PET and SPECT techniques. β^+=Positron (beta plus), β^-=Electron (beta minus) EC=Electron cupture, It=Isomeric transition.[36,47]

1.3.2. Metal ion radionuclides

There are two types of radionuclides used in PET techniques, metal ion and non-metal ion radionuclides, each have advantages and disadvantages. Metal ion radionuclides generally have longer half-lives which are preferable when radiolabelling biomolecules,[36] although this might also be seen as a disadvantage as this would mean longer radiation exposure to the patient. Another advantage of the long half-lives is that it allows production and transport of some isotopes. The fact that the radioactive decay of metal isotopes is not usually by pure positron emission can be seen as either an advantage or a disadvantage.

1.3.3. Radiometals for PET

Because of its improved sensitivity and image resolution, PET has been considered as a superior imaging modality.[43] Copper radionuclides offer a selection of diagnostic (^{60}Cu, ^{61}Cu, ^{62}Cu, and ^{64}Cu) and therapeutic (^{64}Cu and ^{67}Cu) isotopes. The diagnostic nuclides are positron-emitters with a wide range of half-lives (10 min to 12.7 h).[58] The short half-life (9.7 min) of ^{62}Cu allows repeated doses without causing a considerable radiation load on the patient.[51] ^{68}Ga is a generator-produced PET radionuclide which decays to the stable daughter isotope ^{68}Zn with a half-life of 68 min. Current examples show that small molecules, peptides and antibody fragments are ideal biomolecules (BMs) for the development of ^{68}Ga-based PET pharmaceuticals.[43,51,55] ^{89}Zr has a half-life of 78.5 h with β^+ emission. Owing to its long half-life; it is a desirable isotope for ^{89}Zr-labeling of large biomolecules.[55] Another generator-produced PET radionuclide with a very short half-life (75 s) is ^{82}Rb. It is mainly used in the PET studies of myocardial perfusion.[43] ^{86}Y has half-life ($t_{1/2}$ 14.2 h) and due to the high positron energy and additional γ emission the spatial resolution and image quality will be affected. The derivatives DOTA and DTPA have been used to develop immunoconjugates for ^{86}Y-immuno-PET.[43,59]

1.3.3.1. Copper-64

The intermediate half-life (12.7 hours) of ^{64}Cu offers an opportunity for production and delivery across different sites and also allows its use with a wide range of biomolecules including antibodies[43,60]

The low percent of β^+ decay (18% β^+) of ^{64}Cu will have a negative effect on the resolution of the image and when the isotope decays to a gamma product by other decay pathways can cause both unnecessary radiation to patients and make image reconstruction more challenging. This can be considered as an advantage in some cases especially when it decays by 40% β^-

and can therefore be used as a simultaneous diagnosis/treatment isotope. Generally the oxidation state of copper in aqueous environments is 2+ and shows Jahn-Teller distortion, as expected for the $3d^9$ electron configuration. Copper(II) is a moderate/hard Lewis acid and prefers donors such as nitrogen.[36,61] A common coordination number of copper is six.[62] The electron configuration of copper(I) is $3d^{10}$ and is therefore much more labile to ligand exchange, therefore kinetic inertness of the copper(II) chelate complex and avoiding reduction to copper(I) are of primary importance for *in vivo* stability.[43,63,64]

Tetraazamacrocycles based on cyclam and cyclen such as DOTA, TETA, CB-TE2A and propyl CB-TE2A are considered as the most common chelators for ^{64}Cu. Transmetallation might occur in many of macrocyclic copper(II) complexes which suffer from low *in vivo* stability.[65,66] As mentioned in section 1.1.1.2, introduction of an ethylene bridge in macrocycles led to increased stability of the macrocyclic complexes especially the cross-bridged cyclam system,[16] however the necessity of high temperature to form such complexes affects it's use with temperature sensitive biomolecules.

Recently, a useful study of eight BFCs for ^{64}Cu for labelling of antibodies run by Donnelly, Blower and co-workers, investigates a range of variables.[67] By taking account of all the properties assessed, the studies revealed NOTA and a sarcophagine ligand to be among the best, outperforming the commonly used DOTA and these chelators are recommended for use in further studies. An important development in ^{64}Cu chelation chemistry appears when propyl CB-TE2A was synthesized[38] as the chelator radiolabels at room temperature whilst remaining highly stable in a similar way to ethyl bridged cyclam although further *in vivo* validation is required. A carbon-functionalised CB-TE2A derivative has been developed by Archibald and co-workers to allow for bioconjugation whilst not compromising coordination number.[68] Another study on a CB-TE2A derivative in which one acetate arm was changed with a phosphonate derivative has carried out by Weisman, Wong and co-workers improved ^{64}Cu complexation at room temperature.[69] Recently Camus *et al.*[70] synthesised three BFCs with great interest especially for biomedical applications (C-functionalised cyclam derivatives) TE2A-EtOH, TETA-EtOH and CBTE2A-EtOH with their Cu(II) complexes.

1.3.3.2. Gallium-68

Two main reasons, the relatively short half-life (68 min) and is produced by radioactive decay of a parent isotope, make ^{68}Ga a highly interesting metallic PET radioisotope. Due to the ideal characteristics for generator production, with a half-life of 271 days, ^{68}Ge as a parent isotope,

decays to ^{68}Ga to give a daily supply of the isotope.[36,71] Recently efforts have been focused on developing a commercial system for continuous production of ^{68}Ga for laboratory use over one to two years.[72,73] Generators for ^{68}Ga are now commercially available and also in clinical trials but expansion in the area of generator production is on-going with improvements required in the areas of HCl elution and possible metal ion impurities issues.[36] However, these restrictions do not influence the overall advantage that ^{68}Ga can be produced on site from a long term generator without the need for a cyclotron or daily isotope delivery, a characteristic that could have significant future clinical applications.[64]

As mentioned above an ideal short half-life of ^{68}Ga makes it suitable for radiochemistry, being long enough to perform synthetic procedures but not so long to cause radiosynthetic handling challenges which are a concern for longer lived isotopes. The unnecessary patient dose, one of the clinical issues, are not a major factor with a 68 minute half-life; this is also can be overcome by ^{68}Ga decaying by 89% positron, avoiding large percentages of more harmful decay types that the patient may receive with other radiometals. Targeting molecules such as small molecules, peptides and antibody fragments which localise relatively quickly are exploited in normal application of ^{68}Ga.[74,75]

The oxidation state of ^{68}Ga in aqueous solution at physiological pH is III and generally eluted from a ^{68}Ge/^{68}Ga generator using 0.1M hydrochloric acid.[36,76,77] To avoid the formation of insoluble $Ga(OH)_3$ and soluble $Ga(OH)_4^-$, the majority of synthetic procedures are performed in the presence of weakly coordinating ligands such as citrate, acetate or oxalate.[78] Generally bifunctional chelators (BFCs) are used to incorporate ^{68}Ga into a targeting vector. The formation of radiolabelled complexes using mild conditions is a vital factor for a useful BFC to obviate degradation of the linked biomolecule and should be carried out as rapidly as possible due to the short half-life of the radioisotope. As a hard Lewis acid, gallium(III) binds to hard Lewis base donor atoms such as nitrogen and oxygen, which are present in routinely used BFCs and generally gallium(III) forms six coordinate complexes.[79,80] Studies showed that acyclic BFCs are generally less kinetically inert and thermodynamically stable compared to their macrocyclic counterparts but have the advantage of faster metal ion binding kinetics.[15,81] Gallium(III) may form sufficiently stable complexes with acyclic chelators for *in vivo* use compared to copper(II).[82] Clinically, ^{68}Ga is acquiring increased interest driven mainly by the use of DOTA-TOC, DOTA-TATE and DOTA-NOC,[83-85] DOTA-peptide derivatives which are neoendocrine tumour targeting agents. Many ^{68}Ga peptide conjugates at different stages of clinical trials[75,86] were first explored by Maecke and co-workers[87] and later

by Macke *et al.*[88,89] then recently expanded into further clinical trials by Baum and co-workers.[90-92]

1.4. Bifunctional chelators (BFCs)

The bifunctional chelator (BFC) concept is an extremely important in the targeting of radiopharmaceuticals to different tissue types or a tumour.[15] A BFC generally includes three parts: binding unit, ligand framework, and a conjugation group see Figure 5.

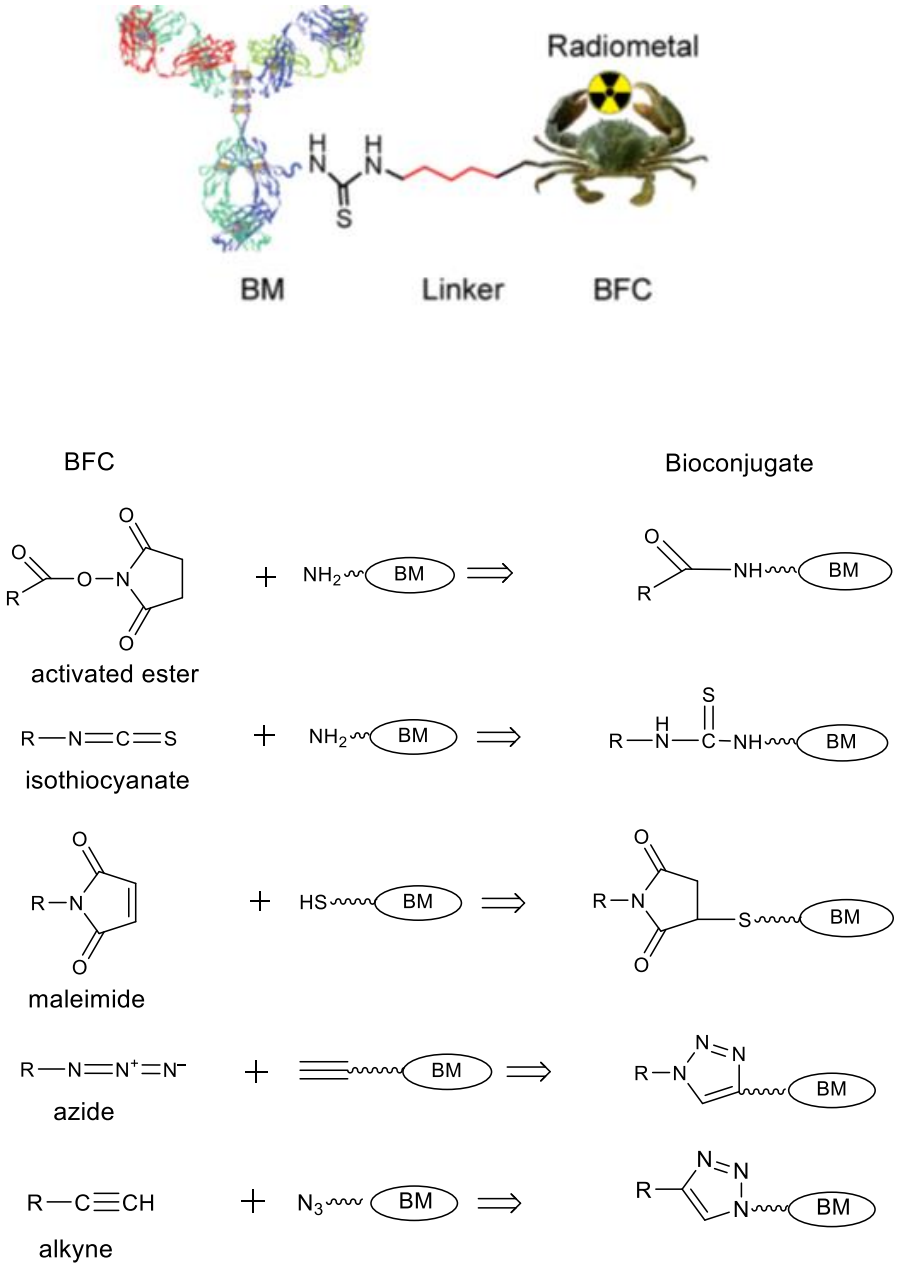


Figure 5 - Radiopharmaceutical design: schematic diagram of the bifunctional chelator (BFC) approach and different bioconjugation strategies. R is the chelator part of the BFC. (Reproduced from Dalton Trans.)[43]

A radiopharmaceutical having a BFC consists of the following elements: a targeting biomolecule (BM), the BFC, radionuclide, and a linker. To adjust the pharmacokinetic properties of the radiopharmaceutical, a linker can be used. It can be a hydrocarbon chain to increase lipophilicity, a peptide sequence to improve the hydrophilicity and renal clearance, or a poly (ethyleneglycol) to reduce the extraction by hepatocytes.[20,50]

To bind a radionuclide and a targeting molecule to form a radiopharmaceutical a BFC should be used.[93] For an ideal BFC there are several requirements.[15] It should form a stable complex and coordinate to the radionuclide in a high yield. The agent must be suitable for the nature and oxidation state of a radionuclide and should avoid any accidental changes in its redox potential. It is essential to carefully choose a suitable BFC, specific conditions may be required for the conjugation reaction with the targeting molecule: pH, temperature, reaction time. When synthesising radiopharmaceuticals targeting specific receptors, the stereochemistry of a BFC is important.[93] In order to keep the metal chelate intact under physiological conditions the BFC must form a metal complex with high thermodynamic stability and kinetic inertness at neutral pH. Free metal ions produced as a result of decomposition of the metal chelate (such as ^{90}Y) may deposit in the bone and cause bone marrow toxicity or at least give lower sensitivity. The minimum number of isomers is required for the BFC forming a metal complex.[15,51,94]

A number of fundamental criteria have to be included in the design of bifunctional chelating agents for clinical applications. Fundamental coordination chemistry criteria such as: (1) charge; (2) matching cavity size of the chelating agent with the ionic radius of the radionuclide; (3) providing the appropriate chelate denticity or number of donor binding groups; and (4) providing donor binding groups of appropriate chemical character are all key elements. Two extra properties are also important to consider: the rate at which the metal complex forms and the rate of dissociation.[59]

There are two methods that are used to incorporate radiometals to receptor-specific molecules. To make a stable attachment of a radiometal and a BM the most fitting approach is to use a suitable bifunctional chelate (BFC), which can seize the radiometal firmly and simultaneously form a stable conjugation with the active groups of the BM see Figure 5. Active groups are either naturally present (*e.g.*, $–NH_2$, –SH, *etc.*) in BMs or are synthetically introduced (*e.g.*, $–N_3$, –C≡CH, *etc.*) into them. The BFC is first conjugated to the BM, see Figure 5, and then the radiometal is added to the conjugate in this approach.[43,95,96] A number of target-specific

radiopharmaceuticals, either commercially existing or under clinical trials have been developed in this way. The receptor binding affinity could be retained by a careful design and attachment of the radiometal chelate, a key advantage of this approach.[50] The second method is integrated approach which includes replacement of part of a known high affinity receptor ligand with an 'unnatural' metal chelate in such a way that there are minimal changes in size, conformation, and receptor binding affinity, see Figure 6.

The radiometal chelate is a fundamental component of the receptor binding motif. In this approach, individual parts are not active in receptor binding. All parts are arranged through metal chelation in a way that the whole metal complex becomes a high affinity receptor ligand.[50,51] This approach unfortunately, often results in more synthetically challenging target molecules with relatively low receptor binding affinity.[97] It appears that, changing of the C–C or C–heteroatom bonds with M–N or M–O or M–S bonds has considerable impact on the size and conformation of the receptor ligand, which are significant for the receptor binding as in complex ^{64}Cu-AMD3465.[51,98] For high molecular weight species where impact of the size of the metal complex can be minimized this approach may be appropriate.[43]

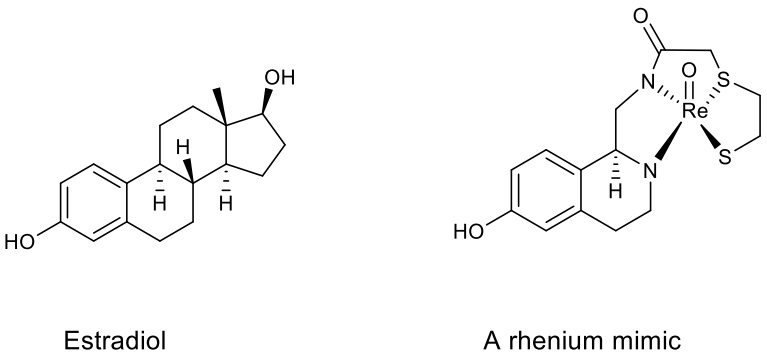


Figure 6 - Chemical structures of ligands that used in an integrated approach.[43]

To reduce the overall structural variations (shape and size) of the BM by the metal chelate a linker is often used to keep the radiometal chelate away from targeting BM. It is also used to facilitate radiopharmaceutical movement in the body (pharmacokinetics), incorporating distribution and removal. Coordination chemistry and bioconjugation play vital roles in radiopharmaceutical design. Common bifunctional chelating groups for radiometals are illustrated in Figure 7.[43,58,99]

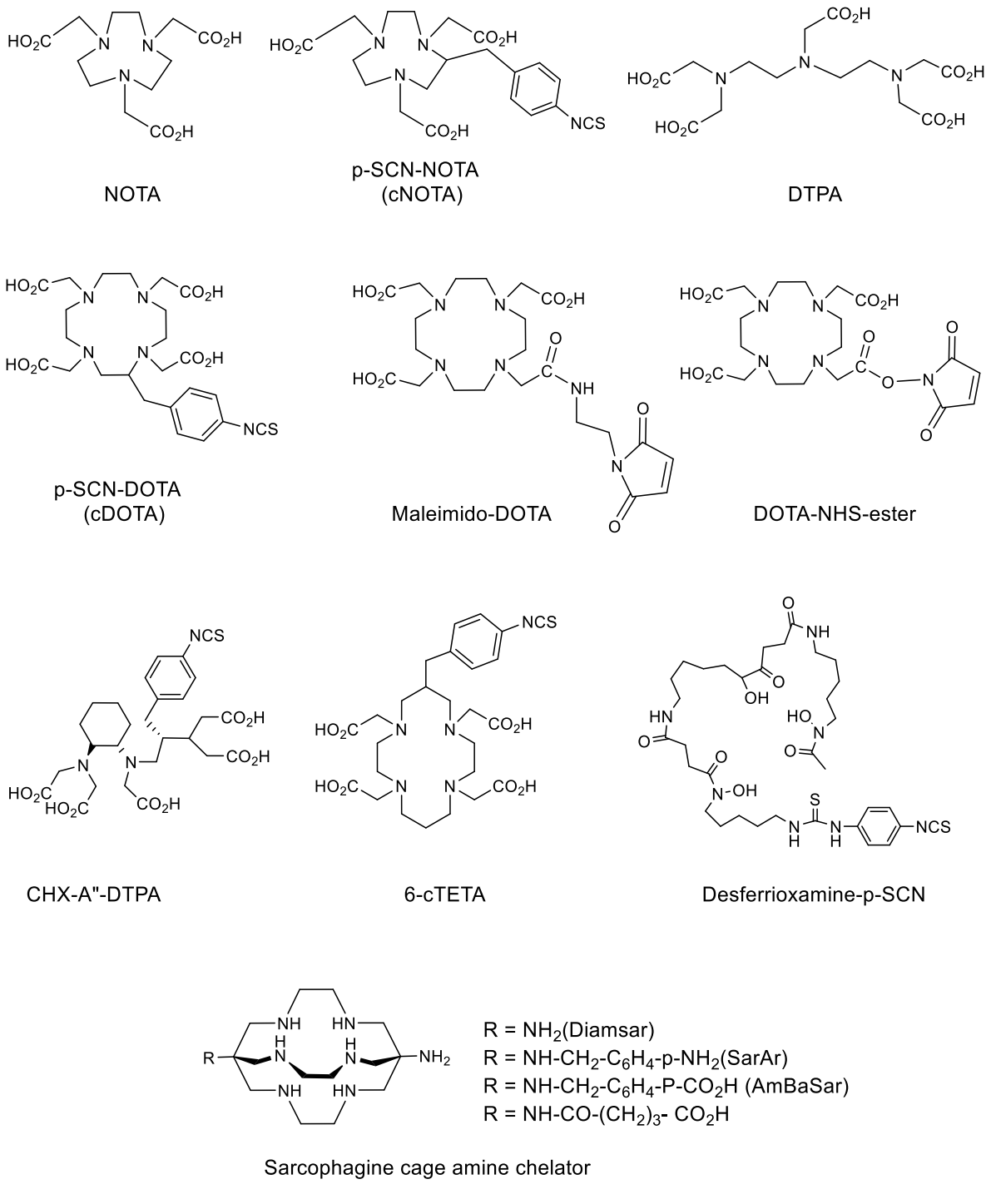


Figure 7 - Selected BFCs often employed in conjugate labelling strategies.[43]

1.5. Biomedical applications of macrocyclic metal complexes

Due to their ability to offer a wide variety of donor atoms, ionic charges, coordination numbers and geometry of the resultant complexes macrocyclic ligands are significantly attractive in the search for designing of new medicines.[100-105] As well as the chemistry and the biological activities of macrocyclic ligands and their complexes have attracted the attention of both inorganic and bioinorganic chemists.[106]

1.5.1. MRI contrast agents

Magnetic resonance imaging (MRI) contrast agents are a clinically important example of the applications of macrocyclic chemistry. MRI is a non-radioactive, non-invasive medical imaging technique used in medical diagnoses by providing a three-dimensional image of internal organs and tissues.[107,108] The role of contrast agents in MRI is to accelerate the relaxation of water protons in proximity to the compound.[109]

Gadolinium(III) is an ideal metal ion used in MRI contrast agents because it has seven unpaired electrons make it highly paramagnetic and also has relatively slow electronic relaxation. Due to the toxicity of the free gadolinium(III) ion it should be chelated with a ligand to form highly stable complexes. Usually only one or two coordination sites on the metal ion should be available for a water molecule to bind through the designing the chelator to encapsulate the metal ion.[109,110] The stability of the complexes which are used as MRI contrast agents is very important due to the toxicity of metal released through dissociation or transmetalation.[109] A large majority of gadolinium(III) based MRI contrast agents based on cyclen,[111] forming highly stable complexes due to small cavity size of cyclen and the ability to bond pendent arms by nitrogen atoms. The most commonly used clinical MRI contrast agent is Gd-DOTA (Dotarem).[112] Also Gd-HP-DO3A[113] (ProHance) was shown to be a good MRI contrast agent. Both gadolinium complexes showed higher stability than a complex of gadolinium with DTPA.[114] Another MRI contrast agent is gadolinium(III) DO3A complex bearing a pendent β-glucuronic acid moiety, synthesised by Meade and co-workers.[115] Also a pair of structurally similar iron complexes of TACN derivatives have been identified and their ability to be used in MRI application was investigated.[116] Linked azamacrocycles have also been used as MRI contrast agents after complexation with gadolinium(III) and gave higher relaxvity compared with analogous monoazamacrocyclic complexes.[117] Many gadolinium(III) complexes have been commercialised with trade names, Dotarem, ProHance, Gadovist and Omniscan as magnetic resonance imaging (MRI) contrast agents for diagnostics in daily practice, see Figure 8.[118]

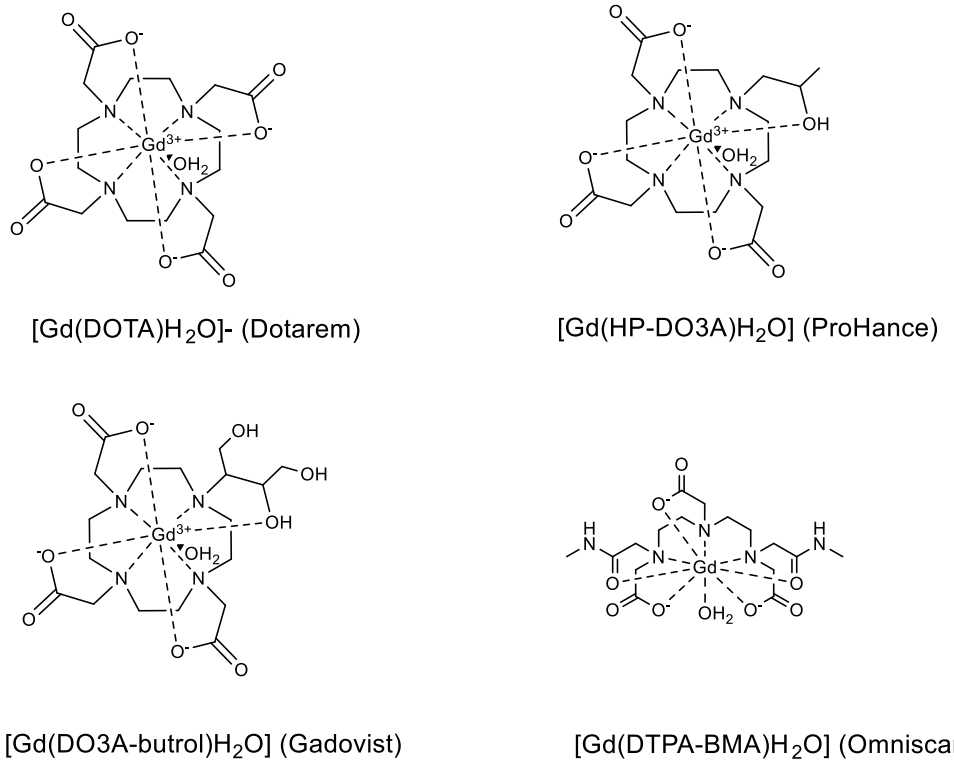


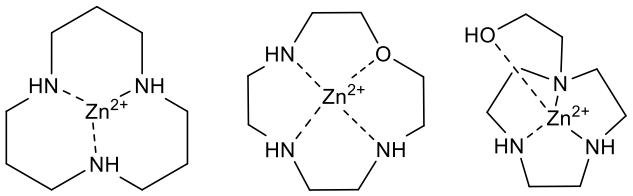
Figure 8 - Chemical structures of commercial MRI contrast agents of gadolinium complexes.[118]

1.5.2. Hydrolysis of biological molecules

There is increasing interest in the biomimetic hydrolysis of RNA and DNA in medicine and biotechnology.[119] Cleaving nucleic acids efficiently in a non-degradative way, with high levels of selectivity for site or structure will provide many applications such as the manipulation of genes, the planning to produce the structural probes and the designing of new therapeutics.[120] Hydrolytic enzymes, such as nucleases and proteases cleave the two vital biopolymers, nucleic acids and polypeptides. These enzymes are responsible for the biological processes which comprises biopolymer degradation/digestion, nucleic acid and protein modification, pathological defence and repair of DNA.[121] Many complexes of macrocycles can be used as hydrolytic enzyme mimics. Copper(II), zinc(II), and the lanthanide ions can form complexes with small molecules to obtain hydrolytic enzyme mimics.[120,122]

The properties of the chelator (orientation and donor type) play a significant role on the catalytic process. Catalysts for ester hydrolysis of carboxy and phosphate can be formed by reacting triazamacrocycles with first row transition metals (mainly copper(II) and zinc(II)) with particular significance to RNA as a target.[123,124] Three complexes of zinc(II) L^5-L^7 have

been synthesised and showed ability to cleave the RNA analogue 2-hydroxypropyl-4-nitrophenyl phosphate(HpPNP), see Figure 9.[125] The use of dinuclear or trinuclear systems can offer a method to improve the catalytic rate. The bis-macrocyclic propyl bridged 1,5,9-triazacyclododecane ligand have formed dinuclear zinc(II) methoxide complex which revealed a surprisingly high catalytic activity for the cleavage of model RNA systems in methanol, mainly in comparison to the activity under aqueous conditions.[126,127] Also the complex of bis-macrocyclic propyl bridged 1,5,9-triazacyclododecane with copper(II) showed activity as a hydrolytic cleavage catalyst.[128] The macrocyclic ligand, 1,7-dimethyl cyclen, have been synthesised and showed ability to hydrolytically cleave DNA in the absence of metal ions.[129]



L^5 L^6 L^7

Figure 9 - Chemical structures of zinc(II) complexes(L^5-L^7).[125]

The formation of hydrolysis catalysts can be achieved by using larger ring macrocyclic complexes. Complexes of monoimidazolium armed cyclen ligand with zinc(II) L^8, cobalt(II) L^9 and copper(II) L^{10}, see Figure 10, have been prepared and their plasmid DNA cleavage properties investigated. The cobalt(II) complex showed the high activity and all complexes showed DNA cleavage due to the presence of the imidazolium positive charge activating the phosphate ester.[130]

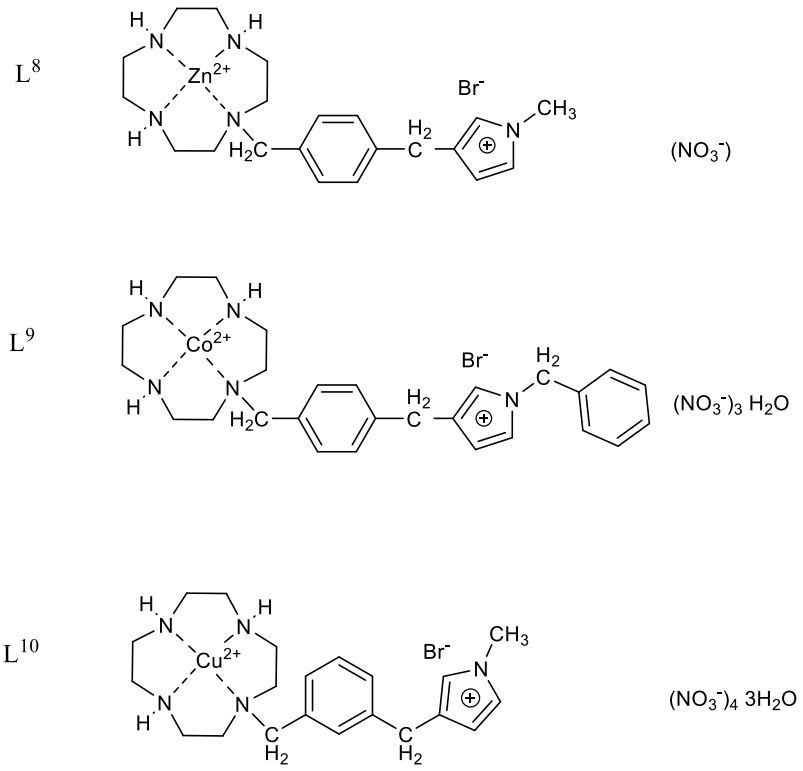


Figure 10 - Chemical structures of zinc(II), cobalt(II) and copper(II) complexes (L^8-L^{10}) used as hydrolysis catalysts.[130]

1.5.3. Radioimmunotherapy

Recently macrocycles have increasingly been used as chelators in radiopharmaceuticals and the formation of bifunctional chelators (BFCs) became more widespread in the 1980s and 1990s.[131] The importance of the BFC explained through the role in the targeting of radiopharmaceuticals to different tissue or a tumour.[132] Also macrocyclic polyamines could be used in cancer via radioimmunotherapy.[129] Macrocyclic ligands also play an important role in the field of nuclear medicine by forming stable metal complexes with various radionuclei. Tetraaza complexes formed between macrocyclic amines and radioactive rhodium-105 have been used in therapeutics.[133,134] Because of the very stable complexes formed with a variety of trivalent radionuclides, DOTA and its derivatives play an important role in clinical applications. DOTA and its derivatives were successfully conjugated to a number of somatostatin analogues and this led to radiopharmaceuticals that have good pharmacological parameters.[93] The conjugated DO3A chelator forms a stable complex with ^{111}In which is used as a SPECT imaging substitute for therapeutic radiolanthanides.[9] Another example of the use

of lanthanides in radioimmunotherapy is Zevalin which is a kit for the preparation of a radiolabelled infusion of the active substance ibritumomab tiuxetan. Zevalin is radiolabelled by mixing it with a solution of radioactive yttrium-90 chloride. The radiolabelled medicine is used to treat a cancer of the lymph tissue.[135]

Complexes of TACN based ligands with lanthanides have been synthesised and tested to be used in radioimmunotherapy.[136] The complexes of ^{153}Sm or ^{166}Ho with the 13-membered tetraazamacrocycle with pendant methylphosphonate arms were studied for use as a therapeutic agent.[23]

1.5.4. Cytotoxic drugs

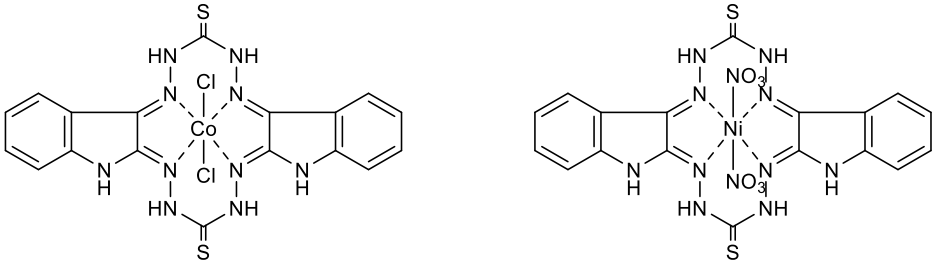
By studying the properties of macrocyclic ligands and their transition metal complexes researchers found many of those possess cytotoxic activities to tumours through binding DNA, crosslinking DNA, cleaving DNA or depleting the endogenous ATP levels of the tumours cells.[129] TACN derivatives chelators were used to deplete iron in cellular systems in an attempt to cause toxicity to cancer cells, particularly targeting the transferrin receptor and ribonucleotide reductase.[137] A cobalt complex, $[Co(III)(cyclen)Cl_2]Cl$, has been found to be selectively cytotoxic to human leukaemia cells.[138]

Also a novel bifunctional chelator NE3TA was produced to allow conjugation of dyes and tracking of the chelator uptake by the cells.[137] To this chelator, Chong and co-workers attached bile acid to target these chelators to colon cancer cells which overexpress the bile acid transporter.[139] The results showed the ability of these compounds to be used as targeted clinical agents with higher cytotoxicities observed than for current clinical iron chelating agents. Essential disease developments pathways can be interrupted by use of metal ion chelation. Researchers have discovered a link between copper and the normal functioning of prion proteins.[140] Bismacrocyclic ligands in recent years have been found to possess high activity as anti-HIV agents, xylyl-bicyclam blocks entry of HIV into cells.[141] Also two linked bridged azamacrocycles have been prepared, and their Cu^{2+} and Zn^{2+} complexes tested for anti-HIV activity.[142-144] Monomacrocyclic complexes of cyclam have been used as anti-cancer drugs. Lipophilic derivatives of cyclam have been prepared by using isopropyl and isobutyl as pendants which displayed inhibition of tumour cell growth.[145] Macrocyclic ligands of cyclen type have been studied to be used as therapeutic drugs. New cyclen complexes of manganese(II) have been synthesised and the results showed antimicrobial and anti-inflammatory activities.[146]

1.6. Macrocyclic complexes as antimicrobial agents

Recently microbial infections become more common than in the first half of the century. During last two decades the applications of the known antifungal and antibacterial agents became more limited because of development of microbial resistance. This situation explains the need for development of new, effective and safe antimicrobial agents.[147] Macrocyclic complexes that are biologically active have been used in the identification of diseased and normal tissues. Because of their pharmacological properties such as toxicity against bacterial and fungal growth, transition metal macrocyclic complexes have received a great attention.[148,149]

Many macrocyclic complexes have been reported to have anti-inflammatory actions.[146] Sharma and co-workers synthesised a new series of macrocyclic complexes such as L^{11} and L^{12}, see Figure 11, by [2+2] condensation of thiocarbohydrazide and isatin in the presence of divalent metal salts in methanolic medium and tested *in vitro* antimicrobial activities against some Gram-positive bacteria. All of the complexes of the tested series possessed good antibacterial activity against Gram-positive bacteria (*B. subtilis*) and antifungal activity against mold (*A. niger* and *A. flavus*).[150]

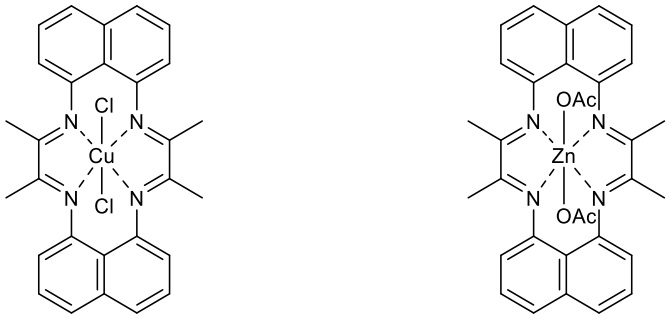


L^{11} L^{12}

Figure 11 - Proposed chemical structure of macrocyclic complexes L^{11} and L^{12}.[150]

Another series of macrocyclic complexes such as L^{13} and L^{14} have been produced, see Figure 12. Antibacterial activity was examined *in vitro* for all the synthesised macrocyclic complexes against some pathogenic bacterial strains, *viz Bacillus cereus, Salmonella typhi, Escherichia*

coli and *Staphylococcus aureus*. The minimum inhibitory concentration (MIC) values were compared with the MIC shown by the standard antibiotics Linezolid and Cefaclor. Promising results were obtained to the most of synthesised complexes.[151]

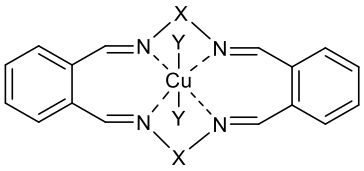


L^{13} L^{14}

Figure 12 - Chemical structures of macrocyclic complexes L^{13} and L^{14}.[151]

Also a series of new 14-membered octaazamacrocyclic complexes have been synthesised and tested for their *in vitro* antimicrobial activities against some bacterial strains *viz*. *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive bacteria), *Escherichia coli* and *Pseudomonas aeruginosa* (Gram-negative bacteria) and *Aspergillus niger*, *Aspergillus flavus* (molds), *Candida albicans*, and *Saccharomyces cerevisiae* (yeasts) for assessment of antibacterial and antifungal actions of the synthesised compounds. The results compared with standard antibiotic (Ciprofloxacin) and the standard antifungal drug (Amphotericin-B). All the compounds of the tested series showed good antibacterial activity against Gram-positive bacteria (*B. subtilis*) but were not effective against Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and yeast (*C. albicans*, *S. cerevisiae*).[152]

A new family of tetraazamacrocyclic Cu(II) complexes, see Figure 13, have been synthesised and biological activity of all these complexes against Gram-positive and Gram-negative bacteria was compared against the standards Linezolid and Cefaclor. Because of the presence of thio group in the coordinated ligands the results of half of synthesised 12 complexes showed activity against both Gram-positive and Gram-negative bacteria.[153]



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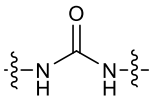
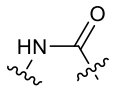
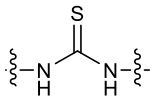
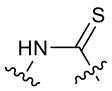
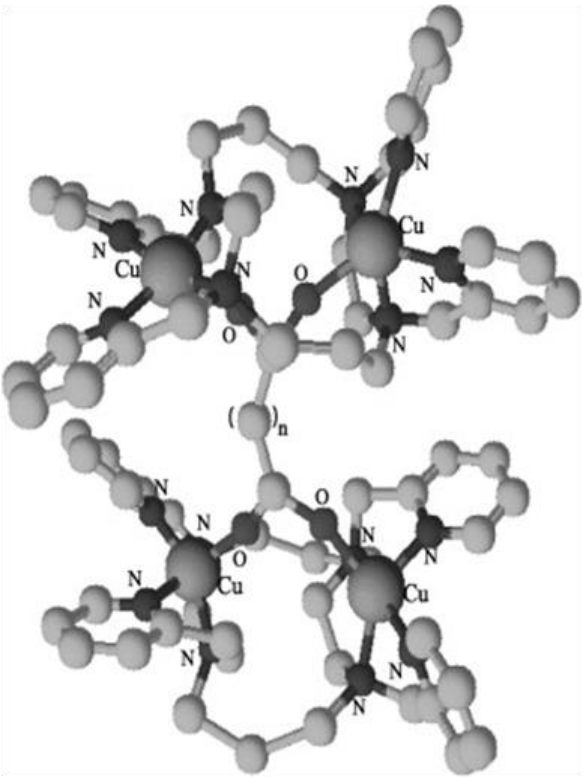
	Cl^- or NO_3^-	(HBOADO)
	Cl^- or NO_3^-	(TBACD)
	Cl^- or NO_3^-	(HBOADT)
	Cl^- or NO_3^-	(DBACDT)
	Cl^- or NO_3^-	(TBAHD)
	Cl^- or NO_3^-	(OBACI)

Figure 13 - Proposed chemical structures of a new family of tetraazamacrocyclic Cu(II) complexes which have showed activity against both gram-positive and gram-negative bacteria.[153]

Novel macrocyclic binuclear Cu(II), Ni(II) and VO(II) Schiff base complexes have been synthesised by template condensation of binucleating Schiff base ligand with metal salt and *o*-phthalaldehyde. The antibacterial activity of prepared complexes that occurred due to presence of several azomethine groups which coordinate with metal ions were tested by the disc diffusion technique against Gram-negative bacterial such as *Klebsiella pneumoniae*, *Escherichia coli* and Gram-positive bacterial like *Staphylococcus aureus*. The results revealed that the Cu(II) complexes have higher activity than the free ligand and the other complexes.[154]

Four new cationic Cu(II) complexes with *N, N', N'', N'''*-tetrakis(2-pyridylmethyl)-1,4,8,11-tetraazacyclotetradecane (tpmc) and aliphatic dicarboxylic acids: pentanedioic (glutaric acid=glutH_2), hexanedioic acid (adipic acid=adipH_2) and decanedioic acid (sebacic acid=sebH_2), see Figure 14, have been prepared and all four complexes were tested *in vitro* antimicrobial and cytotoxic activity along with free primary and secondary ligands, Cu(II) salt

and solvent controls. The results of antibacterial and cytotoxic activity for the complexes were better than the corresponding controls.[155]



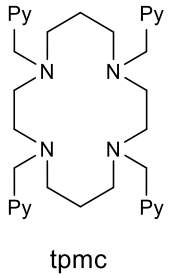
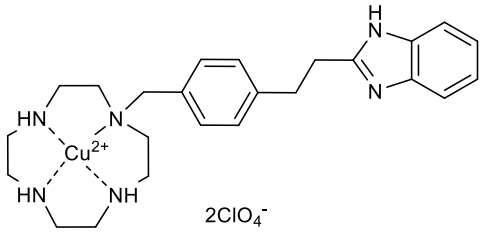


Figure 14 - The geometry of complex cation $[Cu_4 L (tpmc)_2]^{6+}$; n = 2–4 or 8 within all four complexes, where L = succ/glut/adip or seb.[155]

Shuo Li *et al* synthesised and characterised a series of novel complexes of copper(II) (L^{15}, L^{17}) and zinc(II) (L^{16}, L^{18}) ligands containing 1,4,7,10-tetraazacyclododecane and azoles, see Figure 15. Bioactive assays for some target compounds displayed good and broad spectrum antimicrobial activities with relative low minimum inhibitory concentration (MIC) values against most of tested strains.[156]



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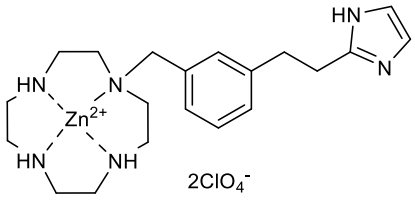
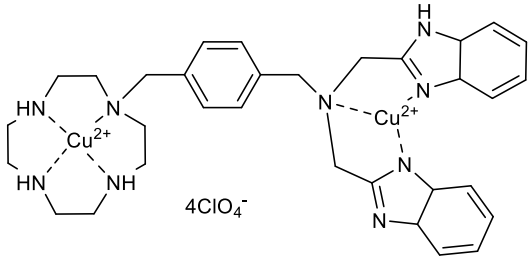
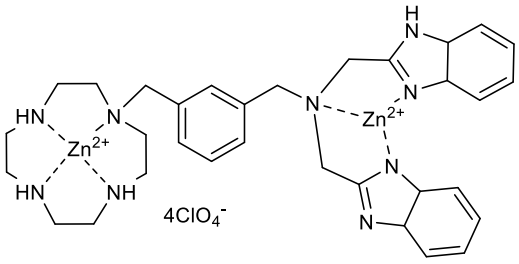


L[16]



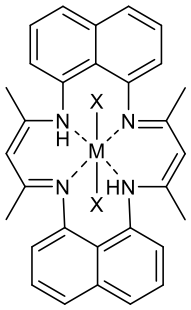
L[17]



L[18]

Figure 15 - Chemical structures of novel mononuclear (L^{15}-L^{16}) and dinuclear (L^{17}-L^{18}) cyclen complexes bearing azole pendants with copper(II) and zinc(II).[156]

Recently, Rathi and Singh have produced a new series of 16-membered tetraazamacrocyclic complexes with Co(II), Ni(II) and Cu(II), see Figure 16. All of the complexes were examined for antimicrobial activity against the Gram-positive (*Bacillus subtilis*), Gram-negative (*E. coli)* bacteria and yeast (*S. cerevisiae* and *Candida albicans*). The results displayed some interesting antibacterial activity. However, these complexes were not effective against Gram negative bacteria (i.e. *P. aeruginosa).*[157]



where M = Co(II), Ni(II), Zn(II) and Cu(II)
X = Cl^- , NO_3^- , OAc^-

Figure 16 - Chemical structures of 16-membered tetraazamacrocyclic complexes.[157]

Recently Hubin *et al.* synthesised several new metal complexes of cross-bridged cyclam and cyclen derivatives with iron(II), copper(II) L^{19}, manganese(II) L^{20}, nickel(II), cobalt(II), and zinc(II), see Figure 17, and tested their activity against strains of *Plasmodium falciparum* (protozoan parasite) to act as antimalarial drugs. Superior and encouraging results were obtained.[158]

L^{19} L^{20}

Figure 17- Chemical structure of complexes of CB cyclam derivatives with Cu(II) L^{19} and Mn(II) L^{20}.[158]

1.7. Research aims

The aim of this thesis is to use knowledge of organic chelator design and coordination chemistry to develop functionalised tetraazamacrocycle derivatives for use in various biomedical applications. This can be split into three specific aims centred around the results chapters presented herein:

Aim 1 – Development of transition metal complexes of benzimidazole cyclen derivatives as potential antifungal agents (Chapter 2).

Benzimidazole based precursors can be synthesised and attached to a macrocyclic backbone to form a library benzimidazole cyclen derivatives. This can be followed by complex formation of benzimidazole cyclen derivatives with copper(II), nickel(II) and zinc(II). Antifungal activity assays can be conducted on all synthesised ligands and complexes.

Aim 2 – Synthesis and studies of benzimidazole TACN derivatives as bifunctional chelators with gallium-68 (Chapter 3)

Benzimidazole precursors developed in chapter 2 can be further exploited by conjugation to TACN derivatives to form a library of hexadentate triazamacrocycles. This can be followed by ^{68}Ga radiosynthesis optimisation and radiolabelling of a selection of chelators using a range of conditions.

Aim 3 – Design of bifunctional bis-tetraazamacrocycles as CXCR4 antagonists (Chapter 4)

Bioconjugation of tetraazamacrocyclic based CXCR4 antagonists is currently challenging and requires a novel bottom-up structural redesign. In this chapter, the aim is to use a C-functional cyclam derivative to form high affinity bis-macrocycle CXCR4 antagonists with an aniline group for further conjugation. This chapter focuses on the synthesis of a C-functionalised cyclam based precursor which can be attached to different types of macrocyclic backbone to form a range of C-functionalised bis-cyclam derivatives. This can be followed by complex formation of selected C-functionalised bis-cyclam derivatives with copper(II), nickel(II) and zinc(II) to givre high affinity CXCR4 targeting derivatives. The assessment of the compounds produced will be carried using in vitro biological assays to determine their applicability as CXCR4 antagonists.

Chapter Two

Development of benzimidazole functionalised macrocycles as antifungal agents.

2. Development of macrocyclic complexes as antifungal agents.

2.1. Aims

This chapter reports the synthesis, transition metal complex formation and biological applications of benzimidazole cyclen derivatives as antifungal agents. The first step is synthesis of benzimidazole based precursors, see *section* 2.4, which are then conjugated to a macrocyclic backbone, see *sections* 2.6.2 and 2.6.3, to form benzimidazole cyclen derivatives, see Figure 18. This was followed by complex formation of benzimidazole cyclen derivatives with Cu(II), Ni(II) and Zn(II) see *section* 2.6.5. Finally the antifungal activity has been investigated for the chelators and their complexes see *section* 2.7.

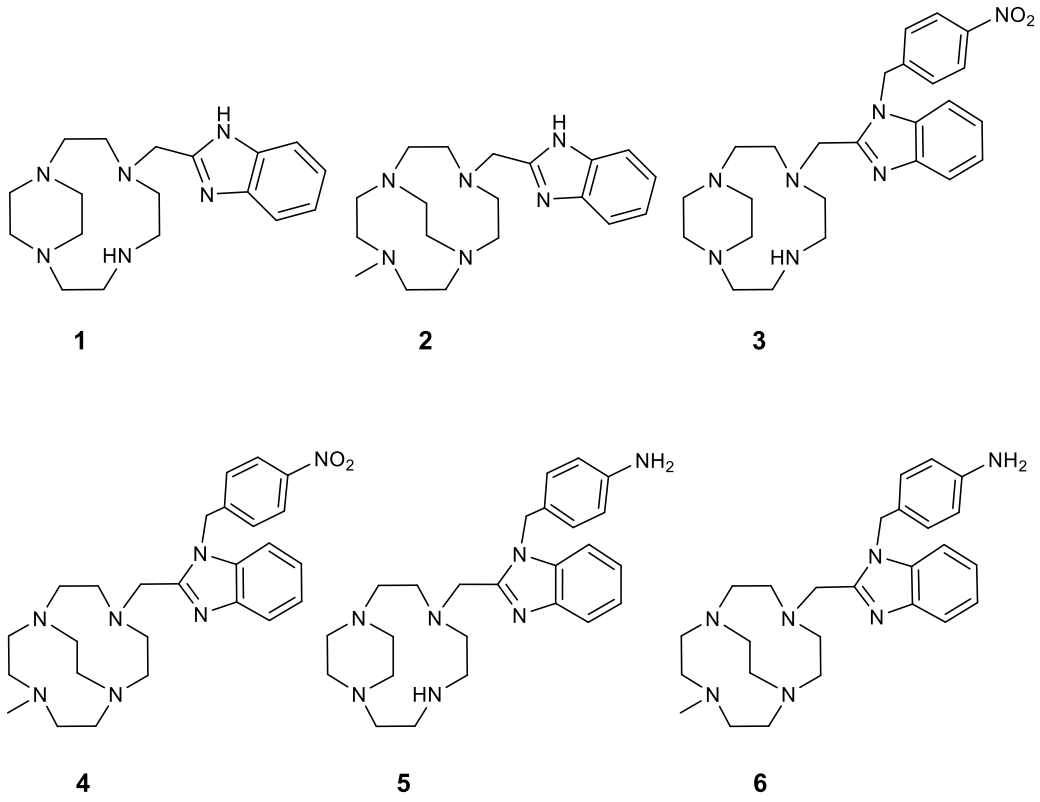


Figure 18 - Chemical structures of the target benzimidazole cyclen derivatives.

2.2. Fungal causing disease

Fungi spread everywhere in the world. There are approximately 1.5 million different species of fungi. Three hundred of those are known to cause diseases to humans. Fungi that are present in the environment often cause diseases. They can live in a variety of different places such as soil, trees and plants and also live on various indoor surfaces and on human skin.[159,160]

2.2.1. Types of diseases caused by fungus

2.2.1.1. Aspergillosis

The fungus *Aspergillus,* a common mould (a type of fungus), causes Aspergillosis and usually people with weakened immune systems or lung diseases are more susceptible to this ailment. There are around 180 species of *Aspergillus*, 40 of them are known to cause infections in humans. Several types of health problems such as allergic reactions, lung infections, and infections in other organs are also caused by *Aspergillus*. The human *Aspergillus* infections are generally caused by *Aspergillus fumigatus*.[161] The drug itraconazole is frequently used in treatment and sometimes corticosteroids may also be used.[162]

2.2.1.2. Blastomycosis

Blastomyces dermatitidis is a fungus that lives in decomposing organic matter, such as leaves, wood and moist soil, and is the main cause of blastomycosis. A person inhales airborne, microscopic fungal spores from the environment making them susceptible to lung infection; though, inhalation of the spores alone is not enough for many people to get sick. Blastomycosis has symptoms similar to flu, and if it is not treated, the infection can sometimes become a serious problem, particularly if it transfers from the lungs to other organs. There is no vaccine currently available to prevent blastomycosis. A variety of respiratory, eye, and skin lesions can be developed by contact with *Blastomyces dermatitidis.*[163,164] The antifungal medicine itraconazole is usually used to treat people with mild or moderate infections.[165]

2.2.1.3. Candidiasis

Yeasts that belong to the genus *Candida* are responsible for this type of fungal infection. More than twenty species have been discovered and can cause infection in humans. Mucous membranes and skin are the places where these species can live without causing infection but this may develop in overgrowth. In the mouth or throat the developed candidiasis is called thrush. *Candida* species can enter the bloodstream and spread to internal organs. White patches or plaques on the tongue and other oral mucous membranes are the most common

symptom of oral thrush.[166-168] Topical treatments such as clotrimazole troches and nystatin suspension can be used for oral candidiasis (in mouth and throat) and for oropharyngeal infections, fluconazole or itraconazole may be required.[169]

2.2.1.4. Valley fever

Sometimes called coccidioidomycosis, the fungus *Coccidioides* causes this infection. The soil in Central and South America and the southwestern United States and parts of Mexico are the common places where this species lives. By breathing in the microscopic fungal spores from the air, some people can get the infection, however most people who breathe in the spores do not become sick. Infectious people usually recover in weeks to months dependent on the person's immune system but some people require medicines whilst others may become seriously ill. Sixty percent of people who are in contact with the fungus never get symptoms. Symptoms of the infection comprise tiredness, cough, fever and headache among others. Skin infection may occur in unusual cases through a cut, wound or splinter. People suffering from extreme valley fever require courses of fluconazole lasting three to six months.[170-172]

2.2.1.5. Fungal eye infections

Eye injury can cause fungal eye infections, although many different kind of fungi, bacteria and viruses could also cause eye infections. Fungal eye infections are very rare but they can be severe. The types of fungi that cause eye infection are *Fusarium, Aspergillus* and *candida.* Several days to weeks after the fungi enter the eye the symptoms will appear. The symptoms are similar to other types of eye infection which include eye pain, eye redness and sensitivity to light. Fungal eye infections cannot generally transfer from one person to another. The treatment of eye infections is dependent on the type of fungus, the harshness of the infection and the affected eye part, it usually comprises antifungal eye drops or an orally administered antifungal drug.[173-175]

2.2.1.6. Fungal nail infections

Fungal nail infections cause the nail to become discolored, dense, and more likely to crack and break. They are common infections in the toenail or fingernail, more commonly in toenails, and are usually not harmful. Older adults and people with diabetes or with weakened immune system are more likely to get nail infections. Nail infections require the use of antifungal drugs; oral terbinafine and azole can be used in their treatment.[176-179]

2.2.1.7. Histoplasmosis

The fungus *Histoplasma* causes histoplasmosis. Soil that contains large amounts of bird or bat droppings is a suitable place for *Histoplasma* to inhabit. People who breathe in the microscopic fungal spores from the air can get histoplasmosis. The symptoms of histoplasmosis are cough, fever and fatigue. For some people who have weakened immune system the infection develops to be more serious. For histoplasmosis in the lungs and chronic histoplasmosis, the treatment can incorporate the use of itraconazole but most histoplasmosis does not need an antifungal drug.[180,181]

2.2.1.8. Ringworm

The infection can cause a circular rash (shaped like a ring) and is called ringworm, it is usually red and itchy. It is a skin infection caused by fungus and can affect anyone. The fungi can live on surfaces and skin or may live in household materials such as towels, clothing and bedding. Dermatophytosis or tinea is the medical name for ringworm. Forty different species of fungi can cause ringworm. Any part of the body can be affected by this infection. The general symptoms are ring rash, itchy skin, red cracked skin and hair loss. The infection can be spread by person, animal and through the environment. The treatment depends on the location of infection and how serious the infection is, and clotrimazole, miconazole, ketoconazole and terbinafine are frequently used for treatment.[182-185]

2.2.1.9. Mucormycosis

Mucormycosis is an infrequent infection caused by organisms called Mucoromycotina. The soil and decaying organic compounds such as compost piles, leaves and rotten wood are the preferable habitats. The sinuses or lungs are the common parts susceptible to Mucormycosis. Fever, headache and sinus pain are the symptoms of sinus infection whereas fever and cough are observed for lung infection. Skin infection causes blisters or ulcers. Antifungal medicine such as posaconazole and amphotericin B is used with most cases.[186-189]

2.2.1.10. Other fungal diseases

Alongside the types of fungal diseases that mentioned above, there are many other types of infections caused by different species of fungus these include:

C. gattii infection, *Pneumocystis* pneumonia (PCP), Sporotrichosis, *Exserohilom*, *Cladosporium* and *C. neoformans* infection.

2.2.1.11. Cryptococcus neoformans (C. neoformans) infection

Cryptococcus neoformans is a fungus which lives in plant and animals, see Figure 19. It is commonly found in the faeces of pigeons. It grows as a yeast (unicellular) and replicates through budding.[190] The infection caused by this yeast is called cryptococcosis. After breathing in the microscopic fungus, infection can occur but most people will not get infected even though they have been exposed to the fungus. It is very rare in healthy people but may infect people who have weakened immune systems. The lungs or the central nervous system (spinal cord and the brain) are usually affected by cryptococcosis[191] but other parts of the body can also be affected.[190,192] The symptoms in lung infections are cough, shortness of breath, chest pain and fever whilst in the brain include headache, fever, neck pain, nausea and vomiting, sensitivity to light and confusion or changes in behavior.[193-196]

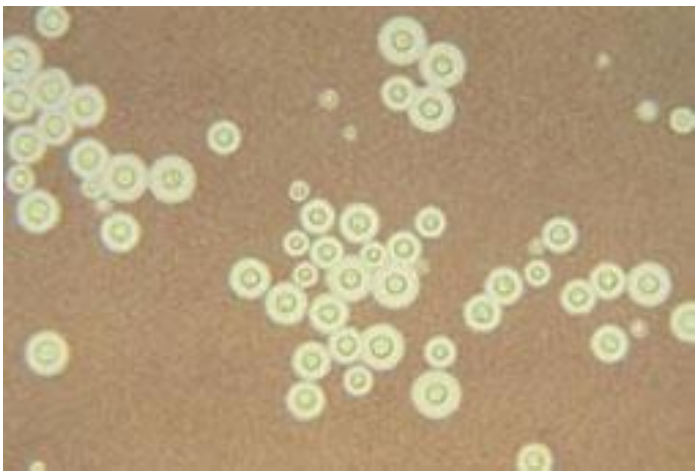


Figure 19 - Microscope image of Cryptococcus neoformans.

The treatment of cryptococcosis last six months or longer, by taking antifungal drugs, dependent on the parts of the body that are affected and the state of the infection. For the mild to moderate cases fluconazole is usually suffcient for treatment but amphotericin B in combination with flucytosine is used for severe lung infection or infection of the central nervous system.[197,198]

2.3. Benzimidazole and derivatives in medicinal chemistry

In terms of their medicinal applications benzimidazole and derivatives of this heterocycle are very important. Many research groups have studied the medicinal properties of the flexible

heterocyclic pharmacophore benzimidazole.[199] Many commercially available drugs including proton pump inhibitors,[200] antihelmintics,[201] antidopaminergics,[202] antipsychotics,[203] ionodilators[204] and anticancers[205] contain a benzimidazole ring, see Figure 20. Derivatives of benzimidazole such as thiabendazole, cambendazole, parbendazole, mebendazole, albendazole, flubendazole are very popular anti-helminth drugs used to cure people and animals of gastrointestinal worm infections,[206] see Figure 21.

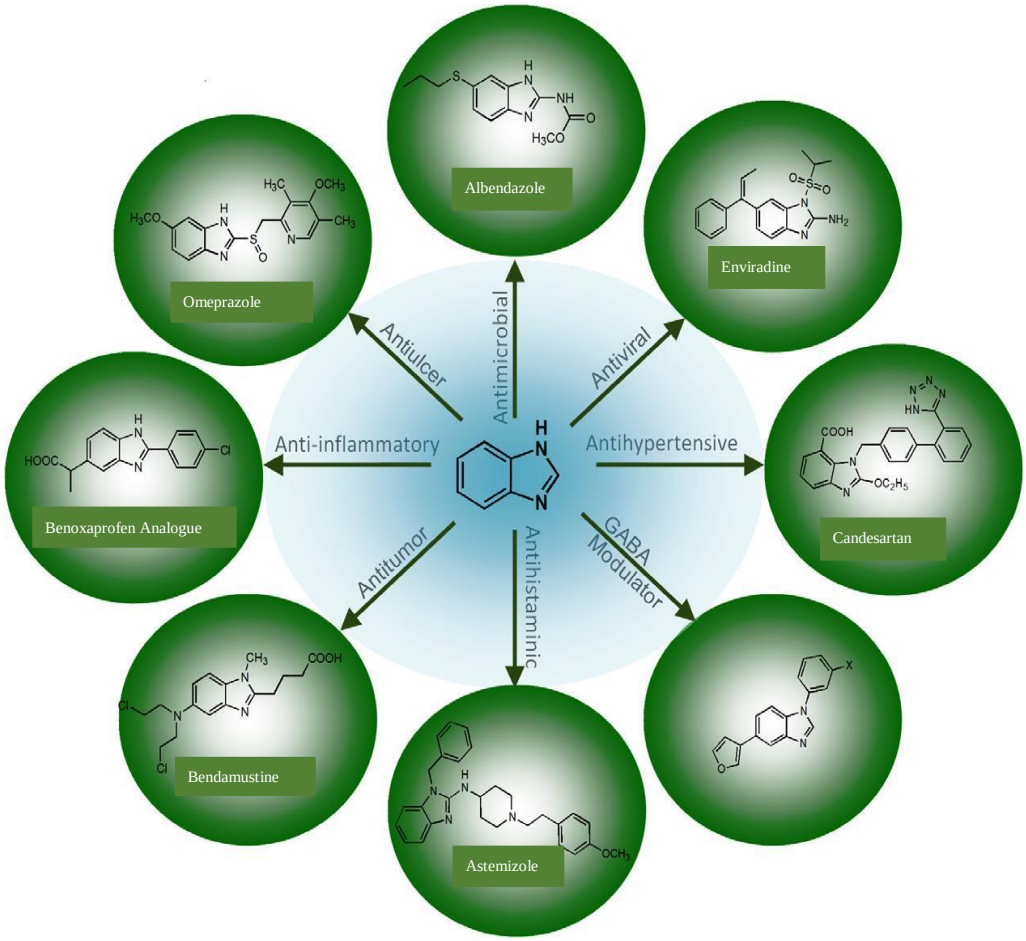


Figure 20 - Medicinal chemistry applications of the benzimidazole core structure.[207]

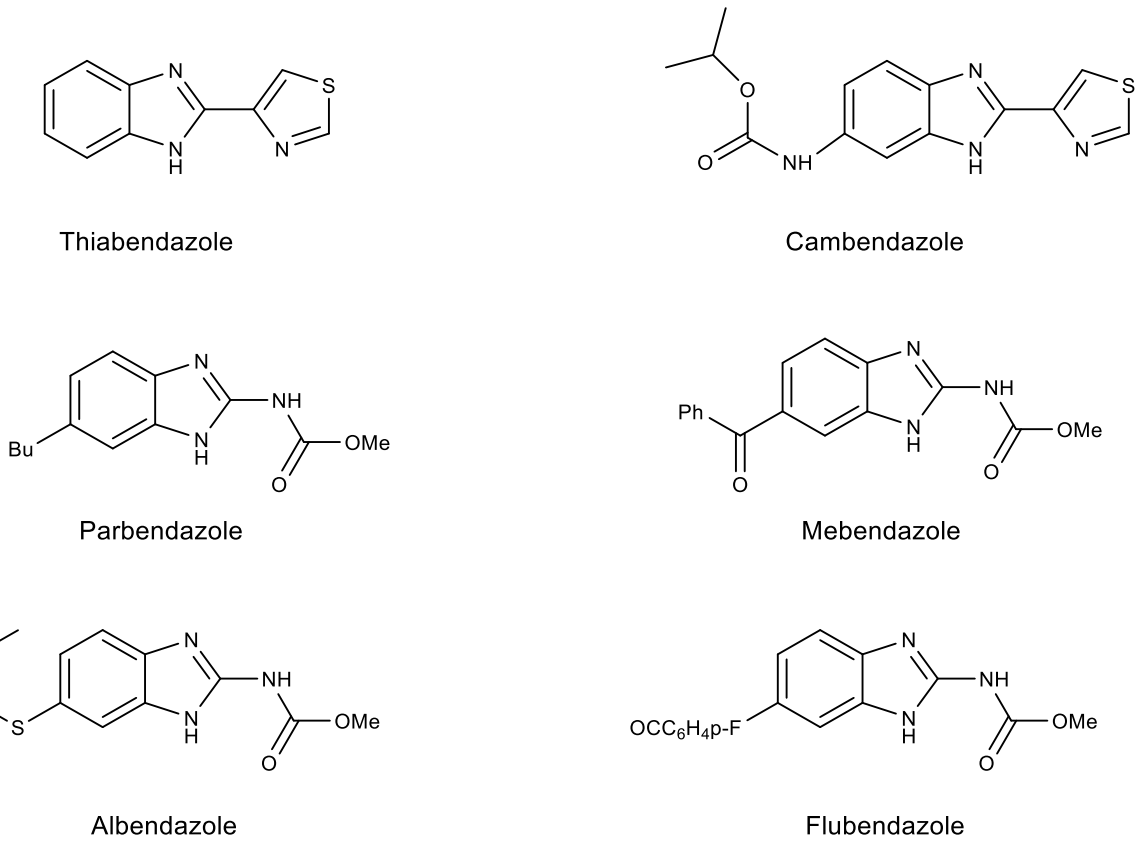
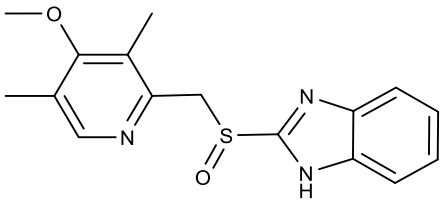


Figure 21 - Examples of benzimidazole derivatives as anti-helminth drugs.

Due to an electron rich nitrogen atom present in the imidazole ring, benzimidazole and its derivatives can act as excellent ligands for transition metal ions which can form complexes with benzimidazoles and some have shown anti-cancer activity.[208] Cu(II) complexes of 2-substituted benzimidazole have the ability to mimic copper, zinc superoxide dismutase (Cu, Zn-SOD) which is an antioxidant enzyme that protects cells from the toxicity of superoxides by dismutation into oxygen and hydrogen peroxide. The lower superoxide dismutase activity of tumour cells, as compared to the normal cells, may partially explain why Cu(II) complexes of 2-substituted benzimidazole can target tumour cells easily and have the ability to act as anticancer agents.[209] Omeprazole is very important and commonly used benzimidazole derivative, see Figure 22, which acts as a proton pump inhibitor. It is widely used in treatment of dyspepsia, gastroesophageal reflux, laryngopharyngeal reflux and peptic ulcer disease. Due to its existence as a racemate, it is converted to achiral products under acidic conditions in the stomach, which on reaction with cysteine residue of H^+/K^+ ATPase, inhibit the production of gastric acid. Omeprazole acts as a competitive inhibitor for certain enzymes that are associated with cytochrome P450 and hence also interacts with other drugs that involve

cytochrome P450 metabolism. Examples include diazepam and warfarin. There are several side effects associated with its use such as headache, diarrhoea, nausea, reduction in vitamin B12 and sleep deprivation. Its prolonged use may also cause inflammation of the kidney (tubulointerstitial nephritis).[210-213]



Omeprazole

Figure 22 - Chemical structure of proton pump inhibitor omeprazole.

Some benzimidazole derivatives have shown activity against *Entamoeba* (a genus of Amoebozoa found as internal parasites of animals) indicating anti-protozoal efficacy of the drugs. 2-Methoxycarbonylamino derivatives of benzimidazole have shown improved antiprotozoal activities against some protozoan parasites such as *Giardia lamblia* and *Entamoeba histolytica* in comparison the standards of metronidazole and albendazole.[214] Also two groups of benzimidazole derivatives, namely 5,6-dinitro and 2-trifluromethyl derivatives, are known for use as antihelminth drugs.[215] Nitrogen and halogen substituted derivatives of benzimidazole and their analogues show potential chemotherapeutic activity against *Stenotrophomonas maltophilia* which is a Gram-negative opportunistic pathogen. Some analogues are highly effective against *Trichomonas vaginalis* which is an anaerobic, flagellated protozoan parasite, and are known to act by the same mechanism as metronidazole, via reduction of the nitro group by ferrodoxin. Benzimidazole D-ribonucleosides such as 2,5,6-trichloro-(1-β-D-ribofuranosyl) benzimidazole (TCRB) as well as its bromo homologue (BDCRB) have been found to be potent and selective inhibitors of human cytomegalovirus (HCMV). These benzimidazole ribonucleoside derivatives are biologically stable and orally active.[216] Also lansoprazole, a derivative of benzimidazole, acts as a proton pump inhibitor for stomach and hence is useful for treatment of gastroduodenal diseases.[217] A number of the derivatives of benzimidazole that have sulfide groups display inhibitory activities against *Helicobacter spp*.[218] Das *et al.* conducted a study on the *N*-linked benzimidazole against

selected strains; the results showed antibacterial activities comparable to *C*-linked benzimidazole.[219] A series of 1,2,4-triazalo[2,3-a] benzimidazoles synthesised by Mohamed *et al*. displayed antibacterial as well as the antifungal activities.[220]

Transition metal coordination compounds of benzimidazole derivatives have promising therapeutic potential. Complexes with 2-substituted benzimidazoles have cytotoxic[221-223] antiviral[222] and antiamoebic activity.[224] Many studies have also revealed that the presence of metals connected with the benzimidazole increases the activity of these compounds against a number of microorganisms. A benzimidazole derived copper(II) complex studied by Arjmand *et al.* was found to be active against *S. aureus* , *E. coli* and *A. Niger*.[225] The biological activity of complexes formed by the reaction of zinc(II) chloride with 1-benzylbenzimidazole derivatives showed that the Zn(II) complexes were more effective than the non-coordinated ligands against all the tested bacteria, including *Bacillus cereus*, *Staphylococcus aureus*, *Sarcina lutea* and *Pseudomonas aeruginosa*.[226] A series of Co(II) and Zn(II) complexes of benzimidazole derivatives have been synthesised and tested against various bacteria. The complex $[Zn(bz)_2Cl_2]0.5H_2O$ displayed antibacterial activity against *Micrococcus luteus* whereas complex$[Co(bz)_2Br_2]$ showed no growth inhibition. Also, both complexes showed antibacterial activity against *E.Coli.*[227]

Sánchez-Guadarrama O *et al*. have synthesised series of complexes of cobalt(II), copper(II) and zinc(II) with 2-methylbenzimidazole, 2-phenylbenzimidazole, 2-chlorobenzimidazole, 2-benzimidazolecarbamate and 2-guanidinobenzimidazole. Their cytotoxic activity was evaluated and the results showed that the zinc(II) and copper(II) compounds had significant cytotoxic activity.[228] A complex of silver(I) with benzimidazole could be useful as an antimicrobial agent. Özdemir *et al*. have synthesised some novel benzimidazole complexes of silver and tested them against a series of bacteria and fungi. The complexes exhibited good activity against the selected bacteria.[229]

2.4. Synthesis of benzimidazole cyclen precursors

Benzimidazoles are present naturally and are an essential part of the structure of vitamin B_{12} with binding to cobalt(II).[230] In addition to the roles of benzimidazole based compounds as pharmaceuticals (HIV inhibitors[231] and as anticancer and antimicrobial agents[232-234]) they can be used as ligands in luminescent lanthanide complexes.[235,236]

In addition to the basic properties of benzimidazole derivatives in medicinal chemistry that mentioned in *section 2.3* benzimidazoles have some features, see Figure 23, which make them a suitable choice for this project:

1. Available nitrogen atom to coordinate to a metal centre
2. The potential to add a reactive group to attach it to a chelator
3. The second reactive nitrogen available for bioconjugation or radiolabelling

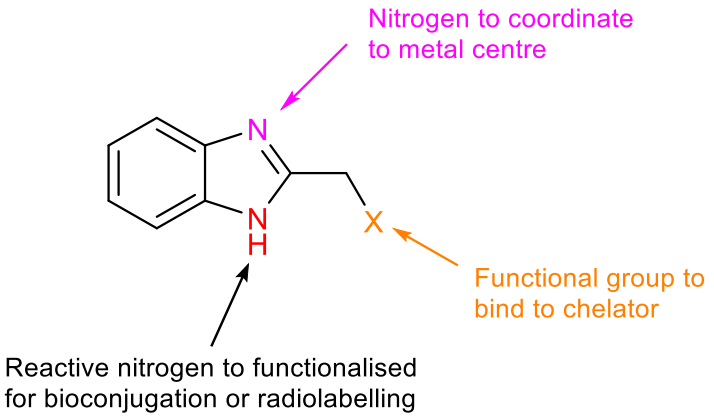
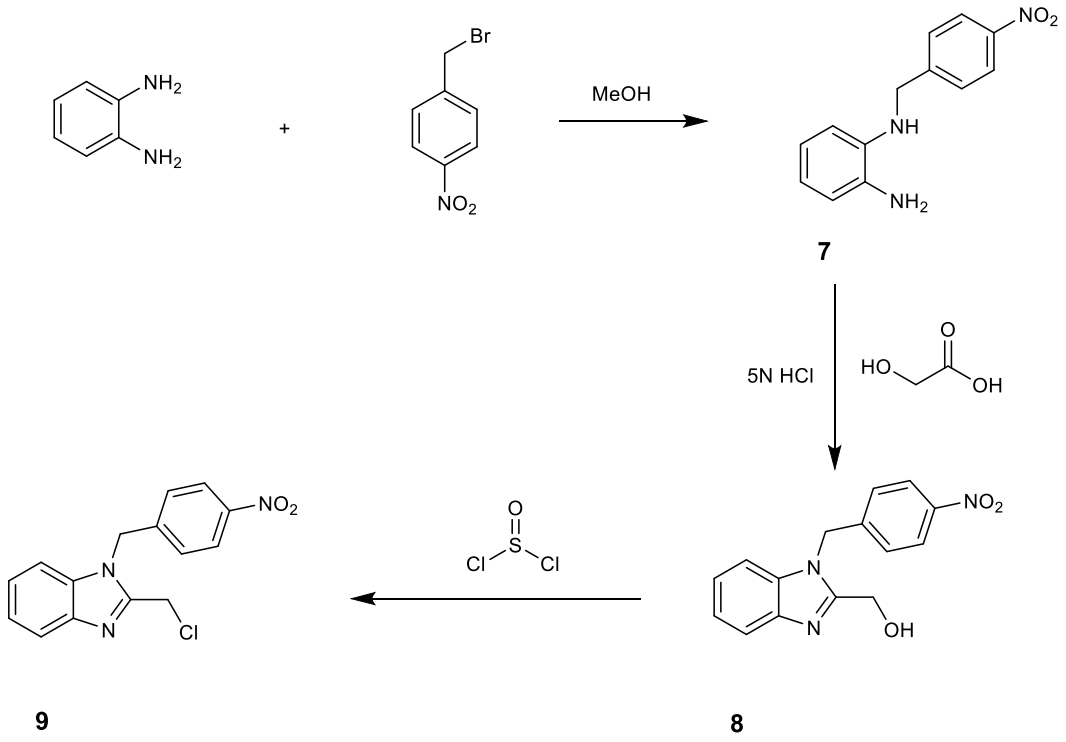


Figure 23 - Representation of the chemical structure of a benzimidazole precursor.

2.4.1. Synthesis of N-functionalised benzimidazole derivatives

The synthesis in this section was carried out following literature methods previously[237] developed in our group via an initial alkylation of *o*-phenylenediamine with a para substituted benzyl halide to form **7**, this is followed by a cyclisation step to form the benzimidazole **8** and finally conversion of the hydroxyl group into a chloride to offer a reactive site for chelator attachment **9**, see Scheme 1.



Scheme 1 - Reactions to synthesise N-functionalised benzimidazole precursors.[237]

The initial alkylation was performed by addition of the 4-nitrobenzylbromide in methanol drop-wise to a five-fold excess of *o*-phenylenediamine in methanol over 30 minutes. The reaction was stirred at room temperature for 4 hours and then the solvents were removed. TLC analysis of N1-(4-nitrobenzyl)benzene-1,2-diamine (**7**) showed multiple products with similar R_f values and so a recrystallisation step was performed, followed by silica gel column chromatography to give **7** in a 65% yield.

Cyclisation reaction of **7** was performed by dissolving the compound in 5M HCl, adding a 1.5 molar excess of glycolic acid and then heating to reflux for 60 hours. A slight modification in

the work up was made by cooling to 0 $^{\circ}$C and then making the solution basic with sodium hydroxide to cause the product to precipitate. The solid was collected by filtration and washed with water and diethyl ether to yield a light brown solid which was dried via Schlenk line to give **8** in a 94% yield compared with a 52% yield before the adopted modification.

The hydroxyl group can then easily be converted to the chloride by treatment with thionyl chloride to give **9** in a 92% yield. There is a clear downfield shift observed in the ^{1}H NMR of the CH_2 peak, from 4.86 ppm to 6.05 ppm for **9** due to the increased electron withdrawing properties of the chloride compared with the hydroxyl group.[238] Isolation of the desired product was confirmed using MS analysis. The target compound **9** was synthesised in a three step reaction in an overall yield of 50%.

2.5. Synthesis of macrocyclic chelators

2.5.1. Synthetic strategy

The synthesis of novel macrocyclic compounds which have an optimised structure for metal binding sites and functional groups that can be modified or reacted was explored. Optimisation includes restricting the configuration of the macrocycles by introducing an ethylene bridge to produce cross bridged (CB) and side bridged (SB) compounds and also complexation with transition metal ions; copper(II), nickel(II) and zinc(II).

2.5.2. Previous chelator design strategies

Macrocyclic ligands play important roles in biological systems, they are easily derivatised and form stable complexes with metal ions, such factors have initiated a broad spectrum of research activities and their applications range from industry to medicine to biomedical imaging.

Cyclen; a twelve-membered, N4-donor macrocyclic ring and its larger counterpart cyclam; a fourteen-membered, N4-donor macrocyclic ring have been utilised in drug design. Their carbon skeletons are rigid enough to provide strong metal binding sites and orient functional groups stereoselectively but they are flexible enough to accommodate the structural changes needed to interact with biological targets.[239]

Functionalisation of macrocyclic ligands can be achieved through carbon or nitrogen atoms; this coupled with the wide range of pendant arms available has led to a huge array of related but structurally different macrocyclic ligands. There number of advantages to the addition of a pendant arm to a macrocyclic skeleton. The number of donor atoms can be increased for

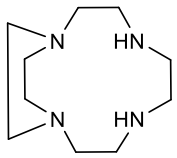
coordination to a metal centre. In this respect pendant arms can facilitate complexation reactions, directing the metal ion into the macrocyclic cavity. Also additional functionality is added by incoporation of a pendant arm for conjugation to other molecules of interest. Another important opportunity can be modification of the properties e.g. altering and improving the biological activity. They can also increase the stability of the overall compound. These advantages are an important part of macrocyclic design and the following section gives selected relevant examples from the last twenty years of monomacrocyclic ligands with N-functionalised skeletons.

2.5.2.1. N-functionalised monomacrocycles

Much of the research effort into functionalised macrocycles involve N-functionalisations, some examples of structurally rigidified monomacrocycles bearing a functional pendant arm will be discussed.

2.5.2.1.1. SB monomacrocycles bearing functional pendant arms

The first group to take steps to rigidify the carbon skeleton of azamacrocycles were Wainwright and Hancock[27,28,240] publishing the structural reinforcement of cyclen using 1,2-dibromoethene (L^{21}) in 1982,[241] see Figure 24. Addition of an ethylene bridge between adjacent nitrogen atoms forms a piperazine ring which of the four conformations it can adopt (chair, boat, twist and half-boat) is most stable in the chair conformation.



L^{21}

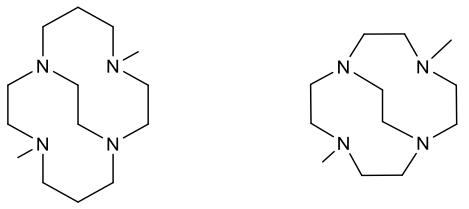
Figure 24 - Chemical structure of a SB macrocycle published by Wainwright L^{21}.[241]

Kowallick *et al.* prepared and studied a structure of three ligands (reinforced tetraazamacrocycles) and their metal complexes with copper(II) and nickel(II).[242] New types of side bridged macrocyclic have since been synthesised by Bernier *et al.*[243] Archibald and co-workers have been at the forefront of this research, synthesising three SB ligands with

functional pendant arms a nitrophenyl SB monocyclam ligand and published this work along with an aminophenyl derivative.[244] A carboxylic acid functionalised ligand was also published in the same year.[245] Plutnar *et al.* utilised the chemistry outlined by Archibald to synthesise an unsymmetrically substituted SB ligand bearing a nitrophenyl and a phosphonate pendant arm hoping that this mixed arm species would improve complexation parameters.[246] A mixed arm species was also synthesised by Boswell *et al*., who attached a phosphonate arm to Archibald's ligand in 72% overall yield.[247]

2.5.2.1.2. CB monomacrocycles bearing functional pendant arms

The first group who published the synthesis of a CB cyclam compound L^{22} were Weisman and Wong.[29] A few years later Bencini published the analogous CB cyclen compound L^{23},[248] see Figure 25. Attachment of an ethylene bridge between opposite nitrogen atoms forces the resulting macrocycle into a 'clamshell' structure and restricts the configuration of subsequent metal complexes to *cis*-V, see Figure 2.



L^{22} L^{23}

Figure 25 - Chemical structures of rigidified macrocycles published by Weisman L^{22} and Bencini L^{23}.[29,248]

The advantages of incorporating a CB into the macrocyclic framework have been explained by several articles, with Busch terming them 'ultra rigid' ligands[249] but relatively few CB compounds have been published with functional pendant arms since Wong and co-workers detailed the synthesis of CB-TE2A in 2000, along with two other CB derivatives with functional arms; a diester L^{24} and a diamide L^{25},[250] see Figure 26. Wong and co-workers also synthesised an analogous CB cyclen derivative of CB-TE2A; CB-DO2A,[250] see Figure 26. The *in vivo* stability of the ^{64}Cu complexes of CB-TE2A and CB-DO2A has been evaluated by Boswell *et al.* and it has been found that they have higher stability constants than ^{64}Cu-

TETA and ^{64}Cu-DOTA.[34] Wong and co-workers went on to synthesise copper(II) and zinc(II) complexes of the diamide L^{25}, along with its analogous cyclen derivative L^{26}.[251]

In 2007 Weisman and Wong evaluated how pendant arm length would influence kinetic inertness and resistance to reduction of subsequent copper(II) complexes by synthesising two new CB ligands bearing propylacetate pendant arms L^{27} and L^{28}.[252] Despite comparable acid inertness to CB-TE2A and CB-DO2A, the authors concluded that L^{27} and L^{28} showed poorer clearance properties *in vivo*, behaviour thought to be a consequence of their higher reduction potentials. Recently, the same group have published the synthesis of a mixed arm species, a phosphonate/acetate species; L^{29}.[253] The phosphonate arm was shown to improve radio-labelling conditions while the carboxyl arm provides a scaffold to facilitate further bioconjugation (albeit by sacrificing a coordinating arm).

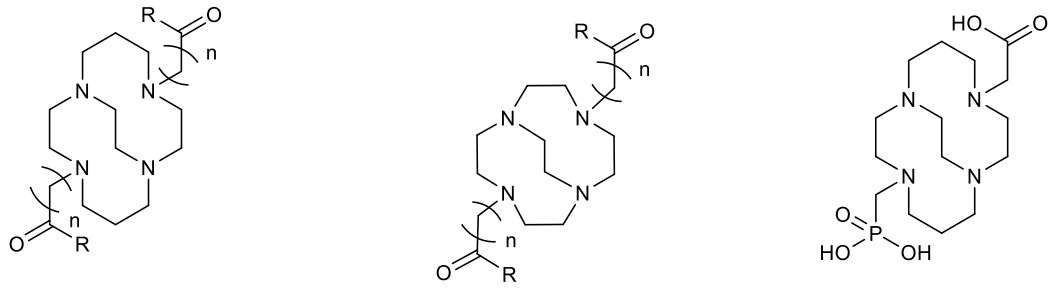


Figure 26 - Chemical structures of CB monomacrocycles bearing functional pendant arms; CB-DO2A and L^{24}-L^{29}, synthesised by Weisman, Wong and co-workers.[252,253]

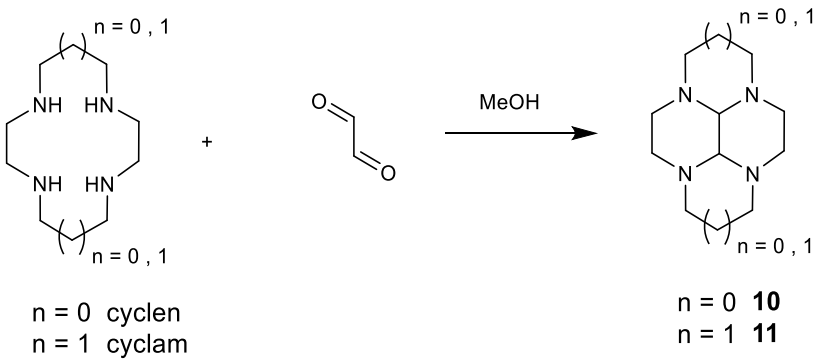
2.6. Synthesis of monomacrocyclic ligands

Mono-ring azamacrocyclic compounds potentially offer some useful properties such as antimicrobials, antimalarials and antifungals. Despite often not being as effective inhibitors as their multi-ring counterparts in some applications they are much simpler to synthesise. Azamacrocycles are not generally orally bioavailable but the monomacrocycles, with their lower molecular weight and decreased molecular charge in comparison to multi-ring

macrocycles have the potential to show improved pharmacological properties such as biodistribution and clearance.[254]

The precursors (cyclen and cyclam) for producing configurationally restricted tetraazamacrocycles were purchased to provide the macrocyclic skeleton to which modifications can be made. 1,4,7,10-Tetraazacyclododecane (cyclen) can be synthesised using the Stetter-Richman-Atkins method but this route has disadvantages such as (a) it is not "atom-economic", i.e. it requires tosylations and subsequent detosylations; (b) the cyclization requires large amounts of dry DMF; and (c) it is labour intensive.[255]

Cyclen and cyclam can be bridged with glyoxal to produce the bisaminal macrocycle (**10** and **11**), which can then be functionalised and derivatised to produce structurally rigidified macrocycles. The reaction to synthesise bridged cyclen (**10**) and bridged cyclam (**11**) was adapted from a method proposed by Handel and Le Baccon,[27] see Scheme 2. The reaction is temperature dependent and the mixture must be kept below –10 °C during glyoxal addition to prevent polymerisation. The mixture is treated with diethyl ether at a later stage as a further precautionary measure to extract the desired product from any polymer formed. ^{1}H NMR spectroscopy confirmed that there was no polymerisation of the product and showed peaks equivalent to eighteen protons, with the expected splitting patterns and mass spectrometry gave the correct peak for the molecular ion. The analogous reaction was performed to synthesise bridged cyclam **11**, see Scheme 2. NMR data confirmed that a pure compound was obtained. Both compounds were synthesised in good yields (>90%) providing two precursors from which a range of configurationally restricted macrocycles can be produced.



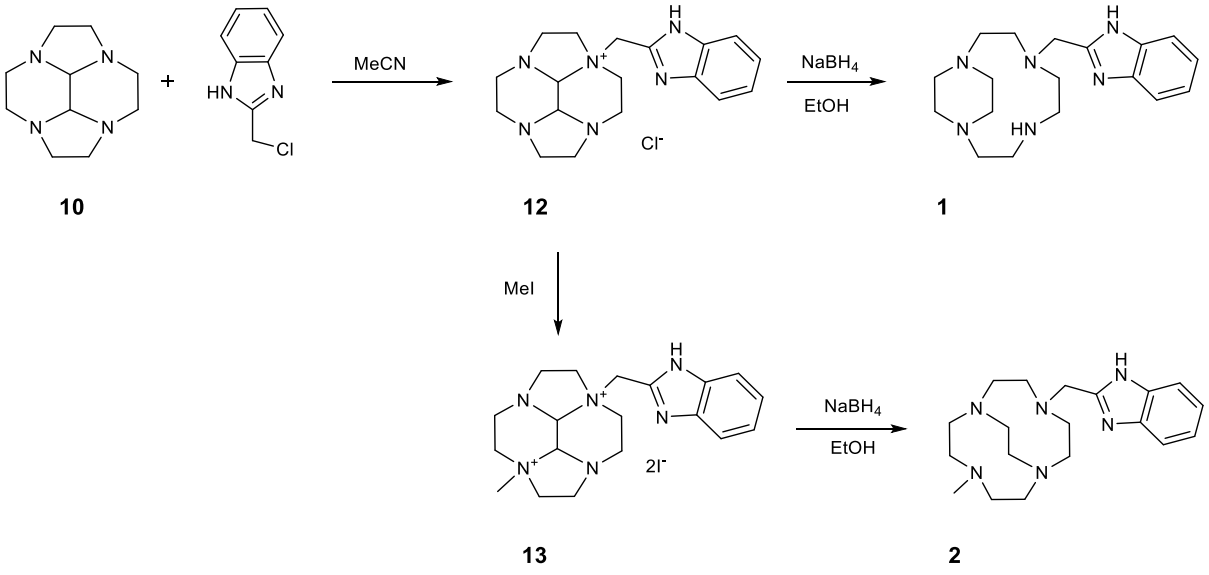
*Scheme 2 - Synthetic pathway to produce bridged cyclen (**10**) and bridged cyclam (**11**).*

2.6.1. Configurationally restricted monomacrocycles

According to modern coordination chemistry principles, ligands of increased rigidity impart higher kinetic stability to their complexes, and such a property facilitates the exploitation of transition metal ions in biomimicry, catalysis, and numerous other areas.[256] The formation of side-bridges (SB) in a macrocyclic ligand (cyclen and cyclam) provides a suitable method for reducing the propensity of the macrocycle for binding to a metal ion in a cis(folded)-conformation rather than the trans(planar)-conformation.[241] The addition of a cross-bridge (CB) restricts the macrocycle to cis(folded)a conformation having all four nitrogen lone pairs convergent on a cleft or cavity for complexation of metal ions.[29]

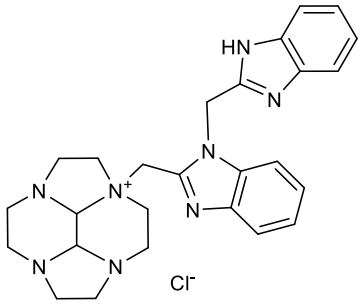
2.6.2. Synthesis of novel SB and CB benzimidazole cyclen derivatives

The ability to produce hetero-substituted cyclen derivatives is important because this allows the physical properties of the compounds to be manipulated, tailoring them to the desired application. Rigidifying the cyclen skeleton to produce either CB or SB compounds has received considerable attention from many research groups, not just because they are relatively easy to synthesise but they have also displayed promising results *in vitro*.[29,256] Bridged cyclen (**10**) was used to synthesise analogous mono-ring SB and CB compounds, see Scheme 3.



Scheme 3 - Synthetic route to produce SB and CB benzimidazole cyclen compounds.

Chloromethylbenzimidazole was used as a pendant arm to form compounds **1** and **2** following the strategies mentioned in *section* 2.3. An important step in the preparation of functionalised macrocycles is selective mono *N*-alkylation. Several routes for mono *N*-alkylation have been reported. The use of direct *N*-alkylation methods has been attempted, but a large excess of costly polyazacrowns are needed.[257] Wong and co-workers developed a convenient method to synthesise mono N-alkylated cyclam and cyclen.[258] They observed that previous routes have a number of issues such as requiring a three N-site protection, mono functionalisation and a deprotection step. These multistep routes often use more specialised reagents and harsh conditions which give compounds in low yields. The major advantages of the methodology used in this work are mild conditions and low ratio of macrocycle to alkylating agent. The route followed involves formation of a glyoxal bridge, followed by selective alkylation of a single nitrogen which is favoured due to the folded nature of the bridged cyclen causing only two of the nitrogens to be oriented with the lone pairs pointing out of the formed cavity in a suitable orientation for reaction.[259] In addition, selection of a suitable solvent causes the quaternerised amino product to precipitate out of solution and it therefore cannot react beyond a single substitution.[260] Our methodology allows for selective monoalkylation of bridged cyclen using a large amount of dry MeCN (*ca.* 400 ml per gram of macrocycles) as the solvent, the reaction stirred at room temperature for 18 hours and a slight excesses of bridged-cyclen **10** [1 : 0.8] added to avoid 'self-reaction **12a**' of benzimidazole that may be formed, see Figure 27. The product was obtained in good yield and MS analysis confirms the presence of **12** and the absence of peaks for any doubly substituted benzimidazole derivatives, see Figure 27.



12a

*Figure 27 - Self-reaction product **12a**.*

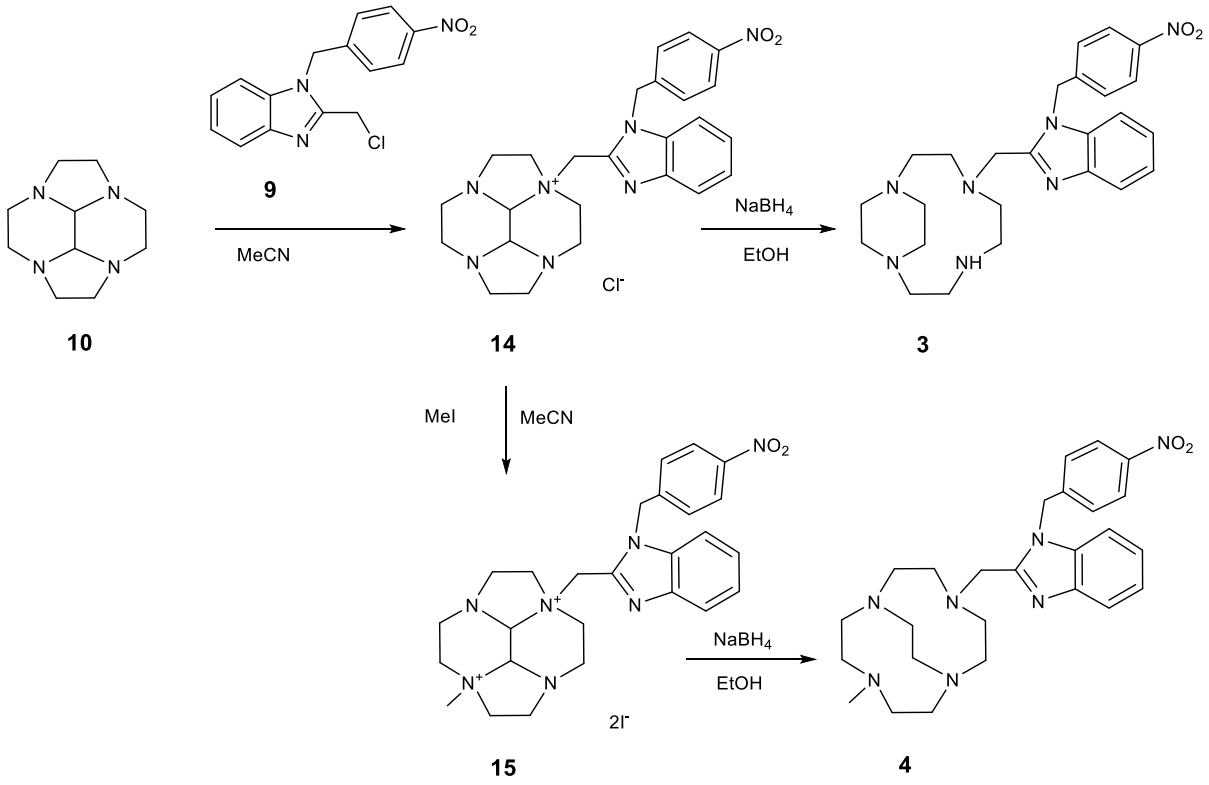
In order to synthesise cross-bridged **2**, the methyl group was introduced into bisaminal salt **12**. MeI is an excellent substrate for S_N2 substitution reactions and sterically open for attack by nucleophiles. Also iodide is a good leaving group, therefore MeI was used for the alkylation. MeCl and MeBr are weaker alkylating agent and they are gaseous, thus harder to handle. Methylation of macrocyclic ligand **12** was performed following established literature preparations.[29] The bisaminal salt **13** was synthesised in a 100% yield and characterised by ^{1}H and ^{13}C NMR, CHN and HRMS analysis.

After addition of the arm and methylation, reduction of the macrocyclic ligands was conducted to obtain the configurationally restricted **1** and **2**. Introduction of the ethylene side bridge into bisaminal salt **12** to form compound **1** was achieved in a simple reduction over a short time through reductive ring cleavage by using the reducing agent $NaBH_4$ in EtOH following the procedure reported by Weisman *et al*.[29,241] The incorporation of an ethylene cross bridge cannot be enforced by simple nucleophilic substitution, or by reductive ring opening from the bisaminal intermediate, **12**, as both methods result in the side bridged macrocycle.[27,261] One of drawbacks of cross bridge systems compared to their side bridged counterparts is the length of time of their synthesis. The reductive ring cleavage step for the cross bridged analogue takes 14 days compared to a couple of hours for its side bridged relative. There are different routes capable of reducing a bisaminal salt **12** to a side bridge **1** or to a cross bridge **2** but the simplest and most widely used is $NaBH_4$ in 95% EtOH at room temperature.[27] The methodology of Weisman *et al.* uses reductive ring cleavage to open a bissubstituted bisaminal salt intermediate **13** giving a pure product.[262]

All compounds in Scheme 3 were produced in reasonable yields and characterised using a variety of analytical techniques. Compounds **1** and **2** were synthesised for the first time and gave clean NMR spectra and CHN analysis confirmed the purity of these compounds. ^{13}C NMR data also confirmed a successful reaction due to the disappearance of the aminal peaks. Side-bridged (SB) benzimidazole cyclen **1** and cross-bridged (CB) benzimidazole cyclen **2** were synthesised in 88% and 80% yields from their bridged intermediates **12** and **13** respectively.

2.6.3. Synthesis of 4-nitrobenzyl benzimidazole cyclen derivatives

This procedure involved direct alkylation of bridged-cyclen **10** with the previously synthesised N-functionalised benzimidazole derivative **9**, see *section* 2.4. This offers a method to synthesise the N-functionalised benzimidazole cyclen derivatives with a reactive arm for further conjugation reactions, see Scheme 4.



Scheme 4 - Synthetic route to produce SB and CB 4-nitrobenzyl benzimidazole cyclen compounds.

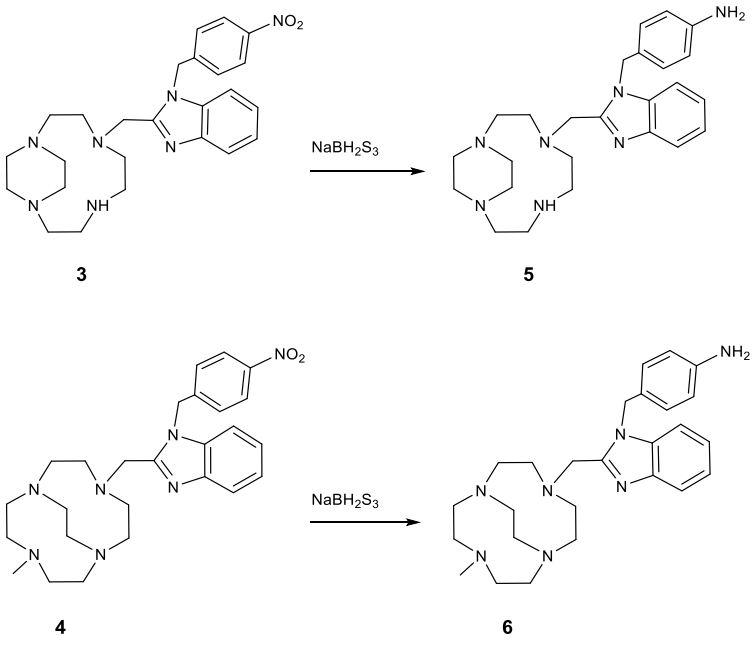
Bridged cyclen **10** was reacted with 2-(chloromethyl)-1-(4-nitrobenzyl)-1H-benzo[d] imidazole (**9**) in dry acetonitrile to form compound **14** by direct alkylation. An excess of benzimidazole can be used as there is no possibility of 'self-reaction' on the imidazole. Compound **3** was synthesised by reduction using $NaBH_4$ as the reducing agent. Compound **4** was prepared by methylation of compound **14** and then reduction of the product **15** by $NaBH_4$. The compounds **3** and **4** were produced in good yields, 92% and 72% respectively. Both compounds were fully characterised by NMR and MS analysis.

2.6.4. Synthesis of 4-aminobenzyl benzimidazole cyclen derivatives

The aim of synthesising compounds **3** and **4** was to use them as a precursor to prepare 4-aminobenzyl benzimidazole cyclen derivatives to be ready for reaction as bifunctional chelators.

To provide a site for conjugation reactions the nitro group of **3** and **4** can be transformed into a more reactive amine group. Palladium on carbon under a H_2 atmosphere has been used in previous work in this area, however it has also been reported that these conditions can cause debenzylation of molecules of this type.[263,264] Lalancet *et al.* reported an alternative method for the reduction of nitrobenzyl groups by use of sulfurated borohydride ($NaBH_2S_3$).[265] This procedure has been carried out successfully in our group previously.[238]

The synthetic procedure was carried out following a minor modification of a literature procedure[238] by using a 20 fold excess of $NaBH_4$, see Scheme 5. $NaBH_2S_3$ is prepared and used *in situ* by stirring together S and $NaBH_4$ in dry THF for 1 hour under argon before addition of **3** or **4** in dry THF and then heating under reflux for 18 hours. The crude products from the reactions to prepare **5** and **6** were then purified via column chromatography to yield the desired pure products in 81% and 64% yields respectively. The shift of peaks in the aromatic region in ^{1}H NMR is characteristic of a nitro reduction and confirmed isolation of **5** and **6**.



*Scheme 5 - Reduction of the nitro group to give amino benzimidazole cyclen compounds **5** and **6**.*

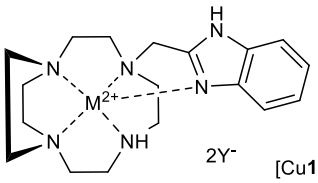
2.6.5. Novel metal-containing monomacrocycles

Tetraazamacrocycles are ideally set up to complex metal ions, in particular transition metal ions with a 2+ charge. The chemistry in sections 2.6.2-2.6.4, describes a novel series of configurationally restricted monomacrocyclic compounds (six ligands), complex formation with various metal ions produced a novel series of metal containing monomacrocycles.

Copper(II), nickel(II) and zinc(II) were the transition metal ions of choice since previous studies have shown improved the biological activity when macrocycles have been complexed with these metals, see *section* 2.3. Copper can adopt various oxidation states and coordination geometries. Its radioisotopes also make it a metal of interest since such compounds would be useful diagnostic and therapeutic probes. Nickel(II) also displays some interesting coordination properties. It can either form diamagnetic square planar complexes or expand its coordination sphere to octahedral through incorporation of additional donors. Zinc(II) does not undergo redox chemistry in biological systems due to its full shell of d-electrons.

Different salts of copper(II), nickel(II) and zinc(II) were used to make a novel series of such compounds, see Figure 28. Standard preparations were followed for all the complexation reactions.[143,250] All of the complexes (thirty two complexes) were purified *via* size exclusion chromatography (sephadex LH20) which can give up to a 30% loss in product resulting in a wide range of yields (51% – 100%). Complexes were characterised by MS (showing appropriate isotopic distribution for the metal centres present), CHN analysis and where applicable UV-vis spectroscopy. Different types of anions have been used due to the properties of the anion can have an effect on solubility and toxicity.[266]

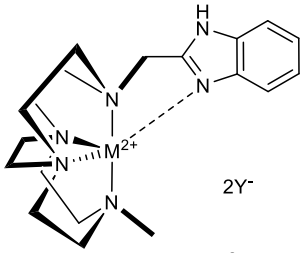
It is much harder to insert a metal ion into a CB cavity rather than a SB cavity due to the decreased structural flexibility, as can be seen from the reaction conditions employed. Nickel(II) and zinc(II) CB complexes are more difficult to synthesise because protons abstracted from water or solvent molecules compete effectively with metal ions to be in the CB cavity, copper(II) is able to compete more effectively. CHN data for a range of the metal complexes described shows successful insertion of the metal ion into the cavity to form pure compounds; solvent molecules (H_2O and MeOH) were noted. Previous x-ray studies on complexes of cyclen substituted with a benzimidazole arm[267] and complexes of cyclam substituted with a benzimidazole arm[268] showed that the metal ion adopts a square –based pyramidal geometry. The coordination sphere of the metal centre can consists of the four macrocyclic nitrogen atoms and the imine nitrogen atom of the benzimidazole.



2Y$^-$

[M**1**]Y$_2$

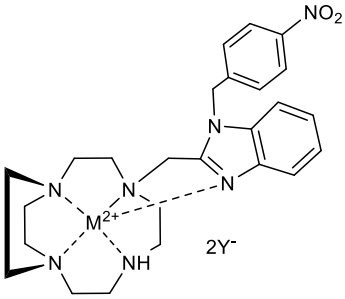
[Cu**1**]Y$_2$	$M^{2+} = Cu^{2+}$,	$Y^- = CH_3CO_2^-$
[Cu**1**]Y$_2$	$M^{2+} = Cu^{2+}$,	$Y^- = NO_3^-$
[Zn**1**]Y$_2$	$M^{2+} = Zn^{2+}$,	$Y^- = CH_3CO_2^-$
[Zn**1**]Y$_2$	$M^{2+} = Zn^{2+}$,	$Y^- = NO_3^-$
[Zn**1**]Y$_2$	$M^{2+} = Zn^{2+}$,	$Y^- = Cl^-$
[Ni**1**]Y$_2$	$M^{2+} = Ni^{2+}$,	$Y^- = CH_3CO_2^-$
[Ni**1**]Y$_2$	$M^{2+} = Ni^{2+}$,	$Y^- = NO_3^-$
[Ni**1**]Y$_2$	$M^{2+} = Ni^{2+}$,	$Y^- = ClO_4^-$



2Y$^-$

[M**2**]Y$_2$

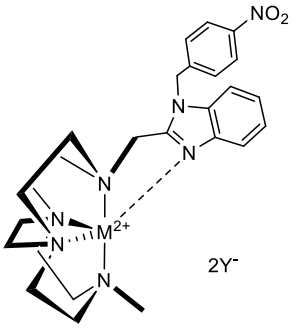
[Cu**2**]Y$_2$	$M^{2+} = Cu^{2+}$,	$Y^- = CH_3CO_2^-$
[Cu**2**]Y$_2$	$M^{2+} = Cu^{2+}$,	$Y^- = NO_3^-$
[Zn**2**]Y$_2$	$M^{2+} = Zn^{2+}$,	$Y^- = CH_3CO_2^-$
[Zn**2**]Y$_2$	$M^{2+} = Zn^{2+}$,	$Y^- = NO_3^-$
[Ni**2**]Y$_2$	$M^{2+} = Ni^{2+}$,	$Y^- = CH_3CO_2^-$
[Ni**2**]Y$_2$	$M^{2+} = Ni^{2+}$,	$Y^- = NO_3^-$



2Y$^-$

[M**3**]Y$_2$

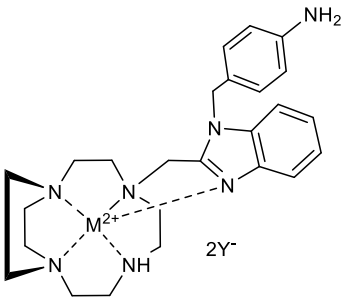
[Cu**3**]Y$_2$	$M^{2+} = Cu^{2+}$,	$Y^- = CH_3CO_2^-$
[Cu**3**]Y$_2$	$M^{2+} = Cu^{2+}$,	$Y^- = NO_3^-$
[Zn**3**]Y$_2$	$M^{2+} = Zn^{2+}$,	$Y^- = CH_3CO_2^-$
[Zn**3**]Y$_2$	$M^{2+} = Zn^{2+}$,	$Y^- = NO_3^-$
[Ni**3**]Y$_2$	$M^{2+} = Ni^{2+}$,	$Y^- = CH_3CO_2^-$
[Ni**3**]Y$_2$	$M^{2+} = Ni^{2+}$,	$Y^- = NO_3^-$



2Y$^-$

[M**4**]Y$_2$

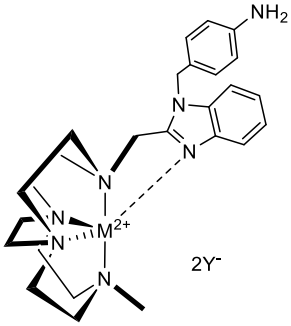
[Cu**4**]Y$_2$	$M^{2+} = Cu^{2+}$,	$Y^- = CH_3CO_2^-$
[Cu**4**]Y$_2$	$M^{2+} = Cu^{2+}$,	$Y^- = NO_3^-$
[Zn**4**]Y$_2$	$M^{2+} = Zn^{2+}$,	$Y^- = CH_3CO_2^-$
[Zn**4**]Y$_2$	$M^{2+} = Zn^{2+}$,	$Y^- = NO_3^-$
[Ni**4**]Y$_2$	$M^{2+} = Ni^{2+}$,	$Y^- = CH_3CO_2^-$
[Ni**4**]Y$_2$	$M^{2+} = Ni^{2+}$,	$Y^- = NO_3^-$



2Y$^-$

[M**5**]Y$_2$

[Cu**5**]Y$_2$	$M^{2+} = Cu^{2+}$,	$Y^- = CH_3CO_2^-$
[Zn**5**]Y$_2$	$M^{2+} = Zn^{2+}$,	$Y^- = CH_3CO_2^-$
[Ni**5**]Y$_2$	$M^{2+} = Ni^{2+}$,	$Y^- = CH_3CO_2^-$



2Y$^-$

[M**6**]Y$_2$

[Cu**6**]Y$_2$	$M^{2+} = Cu^{2+}$,	$Y^- = CH_3CO_2^-$
[Zn**6**]Y$_2$	$M^{2+} = Zn^{2+}$,	$Y^- = CH_3CO_2^-$
[Ni**6**]Y$_2$	$M^{2+} = Ni^{2+}$,	$Y^- = CH_3CO_2^-$

*Figure 28 - Representative chemical structures of copper(II), zinc(II) and nickel(II) complexes of monomacrocyclic ligands **1**, **2**, **3**, **4**, **5** and **6**. (Note: the coordination spheres of the metal ions may also include additional bound water or solvent molecules to give six coordinate species)*

It is also possible to have additional bound solvent or water molecules (or counter anions if they can be sterically accommodated around the metal centre) to give six coordinate species. It is challenging to determine this in solution and there may be an equilibrium of different species. However, the colours of the complexes can give information on the coordination geometry; for example the nickel(II) complexes are orange if square planar and only bound to the four nitrogens and green or brown (as in this work) if five or six coordinate with coordination to the pendant arm. This evidence supports the coordination of the pendant arm

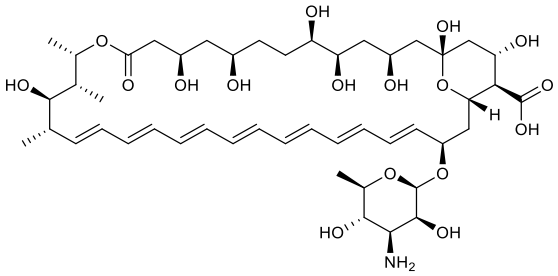
2.7. Antifungal activity of benzimidazole, 4-nitrobenzyl benzimidazole, 4-aminobenzyl benzimidazole cyclen derivatives

The investigation of compounds that have superior properties which can be used against the infections caused by fungi and other microbes is of high current interest.[147] The requirement for new antimicrobial compounds is ongoing because of the ability of the microorganisms to develop resistance to these drugs.[269]

The prepared side-bridged and cross bridged tetraazamacrocyclic ligands **1, 2, 3**, **4**, **5** and **6**, see Figure 18, and their complexes with copper(II), nickel(II) and zinc(II) were verified to work as antifungal agents. Then full set of compounds was submitted for screening with a collaborator at the University of Mississippi. The effect of metal coordination on the ligands was investigated for impact on the antifungal activity.

Both the free ligands and their metal complexes were tested for *in vitro* antifungal activity against C. *neoformans*, C. *krusei*, C. *albicans* and A. *fumigatus*. A full data set has been obtained. Amphotericin B was used as a reference which is a polyene antifungal agent first isolated by Gold *et al*. from *Streptomyces nodosus* in 1955.[270] Despite its high toxicity, amphotericin B has been the bedrock of systemic antifungal therapy for many years, however fungal resistance has been observed especially in candidiasis cases. It has high *in vitro* activity against many species of fungi. *Histoplasma capsulatum, Coccidioides immitis, Candida species, Blastomyces dermatitidis, Rhodotorula, C. neoformans, Mucor mucedo, and Aspergillus fumigatus* are all inhibited by concentrations of amphotericin B ranging from 0.03 to 1.0 µg/mL *in vitro*, see Figure 29. Infusional toxicity, nephrotoxicity and low blood potassium are adverse side effects associated with amphotericin B. The mechanism of action of the drug is mediated by the complex formed between the polyene amphotericin B and ergosterol which disrupts the fungal plasma membrane and results in increased membrane

permeability, the leakage of the cytoplasmic contents and eventually death of the fungal cell.[271]



Amphotericin B

Figure 29 - Chemical structure of Amphotericin B.[272]

Some of compounds under investigation have shown activity against *C. neoformans*. As shown in Tables 2A and 2B, zinc(II) complexes of ligands **1** and **2** demonstrated a higher antifungal activity compared to copper(II) and nickel(II) complexes of the same ligands. The nickel(II) complex of ligand **5** showed a reasonable activity. The assay data also shows that the macrocyclic ligands, especially SB 4-nitrobenzyl benzimidazole cyclen **3**, have have higher activity than their complexes. This may be due to the benzimidazole unit bonded to macrocyclic frame being 'free' to interact rather than coordinated to the metal centre. The CB macrocycless and their complexes displayed lower antifungal activity in comparison to the rest of compounds under study.

The activity of ligand **3** is higher than ligand **1** despite the fact that they both possess the same 'cyclen' macrocyclic frame this difference may be due to presence of extra benzyl arm in ligand **3**.The results from the antifungal activity assays of the novel macrocyclic derivatives matched with those previously obtained by Chunquan *et al.*[273] who synthesised a group of benzoheterocyclic derivatives (contains benzimidazole, benzoxazole, benzothiazole, quinazolin-4-one and carboline) and tested their activity against *C. neoformans*. Their benzimidazole derivatives displayed a reasonable activity although lower than the macrocyclic derivatives in this work. Tetrazole and triazole based derivatives bearing an ethyl chain linked with an aryl-piperazine were synthesised by Ram *et al.*[274] and their activity against *C. neoformans* was examined. Some of the compounds showed a good antifungal activity similar to that of synthesised macrocyclic derivatives.

Compounds	Minimum inhibitory concentration* (µM)			
	C. neoformans	C. krusei	C. albicans	A. fumigatus
[**1**]	17.52	>20	>20	>20
[Cu**1**](OAc)$_2$	>20	>20	>20	>20
[Cu**1**](NO$_3$)$_2$	>20	>20	>20	>20
[Zn**1**](OAc)$_2$	>20	>20	>20	>20
[Zn**1**](NO$_3$)$_2$	>20	>20	>20	>20
[Zn**1**]Cl$_2$	5.61	>20	>20	>20
[Ni**1**](OAc)$_2$	>20	>20	>20	>20
[Ni**1**](NO$_3$)$_2$	>20	>20	>20	>20
[Ni**1**](ClO$_4$)$_2$	>20	>20	>20	>20
[**2**]	15.06	>20	>20	>20
[Cu**2**](OAc)$_2$	>20	>20	>20	>20
[Cu**2**](NO$_3$)$_2$	>20	>20	>20	>20
[Zn**2**](OAc)$_2$	10.81	>20	>20	>20
[Zn**2**](NO$_3$)$_2$	>20	>20	>20	>20
[Ni**2**](OAc)$_2$	>20	>20	>20	>20
[Ni**2**](NO$_3$)$_2$	>20	>20	>20	>20
[**3**]	4.07	16.12	>20	>20
[Cu**3**](OAc)$_2$	>20	>20	>20	>20
[Cu**3**](NO$_3$)$_2$	>20	>20	>20	>20
[Zn**3**](OAc)$_2$	>20	>20	>20	>20
[Zn**3**](NO$_3$)$_2$	>20	>20	>20	>20
Amphotericin B	0.21	>20	>20	>20

Table 2A - MIC (µM) of some selected ligands and complexes against tested fungi.

MIC= Minimum inhibitory concentration, – =Waiting data. {[1**] – [Zn**3**](NO$_3$)$_2$}.*

Compounds	Minimum inhibitory concentration* (µM)			
	C. neoformans	C. krusei	C. albicans	A. fumigatus
[Ni**3**](OAc)$_2$	7.91	>20	>20	>20
[Ni**3**](NO$_3$)$_2$	>20	>20	>20	>20
[**4**]	16.60	>20	>20	>20
[Cu**4**](OAc)$_2$	>20	>20	>20	>20
[Cu**4**](NO$_3$)$_2$	>20	>20	>20	>20
[Zn**4**](OAc)$_2$	>20	>20	>20	>20
[Zn**4**](NO$_3$)$_2$	>20	>20	>20	>20
[Ni**4**](OAc)$_2$	>20	>20	>20	>20
[Ni**4**](NO$_3$)$_2$	>20	>20	>20	>20
[**5**]	>20	>20	>20	>20
[Cu**5**](OAc)$_2$	>20	>20	>20	>20
[Cu**5**](NO$_3$)$_2$	>20	>20	>20	>20
[Zn**5**](OAc)$_2$	>20	>20	>20	>20
[Zn**5**](NO$_3$)$_2$	>20	>20	>20	>20
[Ni**5**](OAc)$_2$	14.04	>20	>20	>20
[Ni**5**](NO$_3$)$_2$	>20	>20	>20	>20
[**6**]	>20	>20	>20	>20
[Cu**6**](OAc)$_2$	>20	>20	>20	>20
[Cu**6**](NO$_3$)$_2$	>20	>20	>20	>20
[Zn**6**](OAc)$_2$	>20	>20	>20	>20
[Zn**6**](NO$_3$)$_2$	>20	>20	>20	>20
[Ni**6**](OAc)$_2$	>20	>20	>20	>20
[Ni**6**](NO$_3$)$_2$	>20	>20	>20	>20
Amphotericin B	0.21	>20	>20	>20

Table 2B - MIC (µM) of some selected ligands and complexes against tested fungi.

MIC= Minimum inhibitory concentration, – =Waiting data. {[Ni3**](OAc)$_2$ – [Ni**6**](NO$_3$)$_2$}.*

The mechanism of action of the benzimidazole derivatives to give the antifungal activity is thought to occur via blocking of the synthesis of ergosterol, a major component of fungal cytoplasmic membranes which plays a hormone-like role in fungal cells and stimulates growth. Hence, the net effect of benzimidazole is inhibition of the fungal growth.[275]

Complex formation of the prepared ligands with transition metals led to increase the antifungal activity of complexes compared to free ligands. A study was introduced by Claudio *et al.*[276] where *in vitro* antifungal activity of nine ruthenium dithiocarbamate compounds and their corresponding free ligands was investigated and compared with amphotericin B and fluconazole where four different fungal species including *C. neoformans* have been used. In this study promising results were obtained and confirmed that complexation has positive effect on the antifungal activity (16-64 µg/ml) where the efficacy of complexes was more than their corresponding free ligands (16-128 µg/ml) and this result is consistent with our study where the complexes of zinc(II) with ligands **1** and **2** and nickel(II) with ligand **5** showed higher antifungal activity than free corresponding ligands.

Also a study conducted by Zishen *et al.*[277] in which two new Schiff bases, N-4-hydroxysalicylidene-glycylglycine, N-O-vanillal-glycylglyeine and their manganese(II), cobalt(II), nickel(II) and copper(II) complexes have been synthesised and their antifungal activity were tested against *C. neoformans*. Results of some of the complexes exhibit strong inhibitory action towards *C. neoformans* compared to weak activity of free corresponding ligands which again matches with present study.

Another study introduced by Jian *et al.*[278] where two cobalt(II) and copper(II) complexes of valine-derived Schiff bases have been synthesised and their antifungal activity were examined against *C. neoformans*. Results obtained are also consistent with current study and displayed that the complex of copper(II) had a wide and remarkable inhibitory effect against *C. neoformans*.

Terpolymer of 2-amino-6-nitro-benzothiazole-ethylenediamine-formaldehyde has been synthesised and complexes of this ligand with copper(II), nickel(II) and zinc(II) also prepared and the antifungal activity of free ligand and its complexes were tested against different fungal species including *C. neoformans*. High activity was observed to the metal complexes

(Inhibition zone 13-15 mm) compared to their ligand (Inhibition zone 11 mm) which may be strongly dependent on the central metal ions and coordination numbers metal chelates. The higher activity owing to the metal ions shared with the donor atoms (N and S) of the thiazole ring is existing in the ligand and the π-electron delocalization over the chelate ring. This effect rises the lipophilic character of the metal ion, which facilitate the permeation through the lipid layers of the fungal membrane.[279]

This partial set of results indicates that the choice of the metal ion which is coordinated to a biologically active molecule plays a vital role in the biological activity and may result in an improvement of the activity of the resulting molecule which is dependent on the identity of the metal ion.

2.8. Conclusions

This chapter outlines the successful synthesis of a series of novel SB and CB N-functionalised macrocycles (six ligands). New SB and CB cyclen bearing benzimidazole derivatives pendant arms **1** and **2** were synthesised. Their copper(II), zinc(II) and nickel(II) complexes (fourteen complexes) have also been synthesised and characteraised by using NMR, MS and CHN techniques. Two other novel SB and CB N-functionalised cyclen bearing 4-nitrobenzyl benzimidazole derivatives pendant arms, **3** and **4**, have been prepared. The initial step in chelator synthesis was the production of the benzimidazole unit **9**. The benzimidazole derivatives were then reacted with azamacrocycles to form two different benzimidazole cyclen derivatives **3** and **4**. Their copper(II), zinc(II) and nickel(II) complexes (twelve complexes) have also been synthesised and fully characterized. The last two novel ligands that have been synthesised were SB and CB N-functionalised cyclen bearing 4-aminobenzyl benzimidazole derivatives pendant arms (**5** and **6**) which were obtained by reduction of the nitro group to amino throughout using S/$NaBH_4$ as a reducing agent. These two new ligands have reactive functional groups available for bioconjugation. Copper(II), zinc(II) and nickel(II) complexes (six complexes) have also been synthesised and fully characterized for ligands **5** and **6**. Monomacrocycles potentially offer improved pharmacological properties therefore ligands and their complexes have been tested their ability to use as antifungal agent. Some of compounds involved in this test showed a recognisable activity. Compared to copper(II) and nickel(II) complexes of the ligands **1** and **3**, the zinc(II) complexes generally showed a higher antifungal activity. Also the antifungal activity of ligand **3** was higher than all ligands involved in study. Nickel(II) complex with ligand **5** which has amino group showed sensible antifungal activity.

Chapter Three

Synthesis and ^{68}Ga radiolabelling of TACN derivatives

3. Synthesis and ^{68}Ga radiolabelling of TACN derivatives

3.1. Aims

This chapter reports the synthesis and ^{68}Ga radiolabelling of triazamacrocycles for use as bifunctional chelators in positron emission tomography. The initial step utilises benzimidazole precursors synthesised in chapter two, see *section 2.4.1.*, which were then conjugated to a macrocyclic backbone, see *section 3.3,* to form TACN derivatives. This was followed by optimisation of the ^{68}Ga radiolabelling procedure, see *section 3.4* and labelling of a selection of chelators under the conditions determined see *section 3.5*.

3.2. Developments in ^{68}Ga chelator design

In the past few years research into ^{68}Ga chelator design has been focused on the development of stable acyclic complexes or macrocyclic complexes that form rapidly at room temperature to improve properties for imaging applications.

3.2.1. Cyclen-based chelators

A common choice for ^{68}Ga complexation are cyclen (1,4,7,10-tetraazacyclododecane) based BFCs because of the prevalent use of DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) based ligands, see Figure 30, in various applications, such as radioimmunotherapy, which are dependent on the metal ion selection.[280] Therefore, many research groups already have proficiency in bioconjugation of DOTA derivatives to different biomolecules; the simple variation of the stable metal to ^{68}Ga is a natural transition into PET radiochemistry.[281] Complex formation with DOTA is generally slow and usually requires conventional or microwave heating, due to the preorganisation of DOTA ligand, restricting its use to non-heat sensitive biomolecules.[282,283]

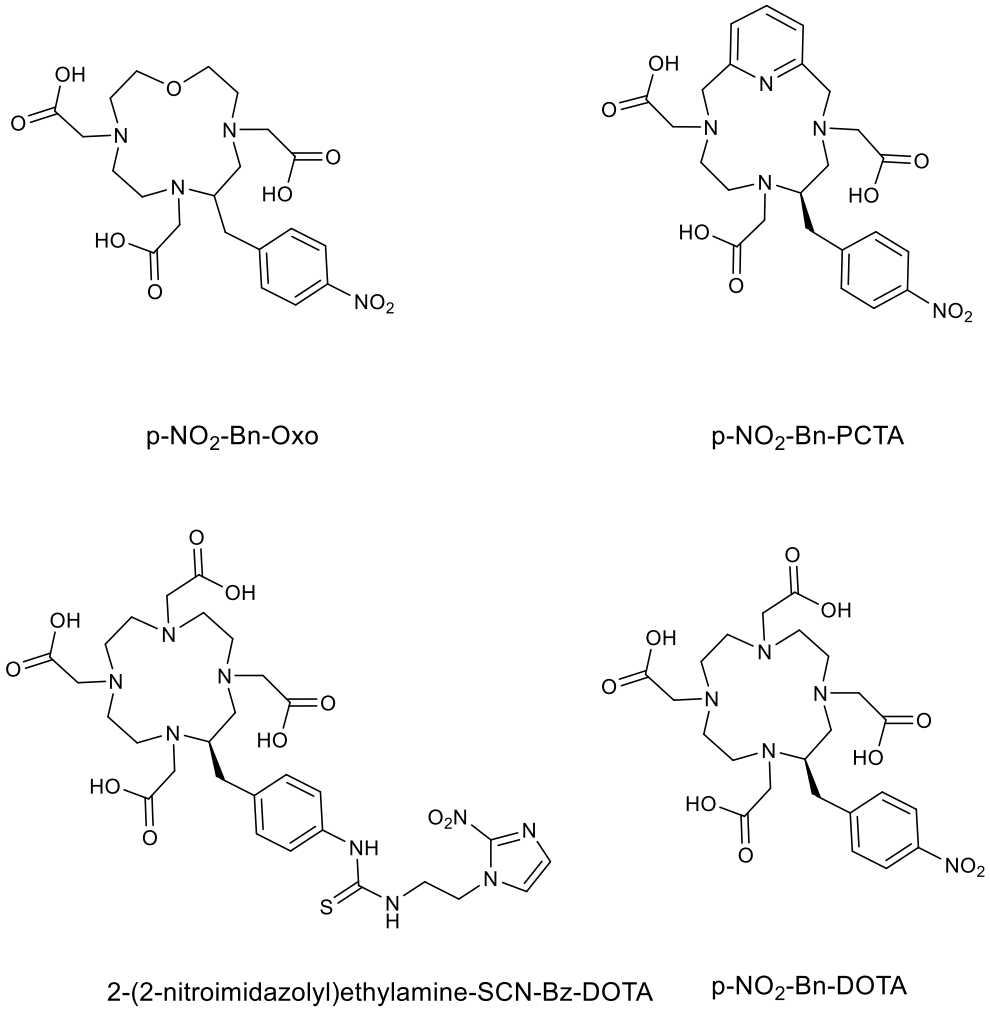


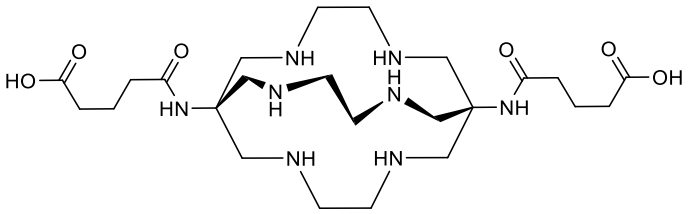
Figure 30 - Chemical structures of C-functionalised cyclen derivatives used for ^{68}Ga radiolabelling.[284,285]

As expected the C-functionalised ligand (p-NO_2-Bn-DOTA), which needs a high temperature for efficient complexation with ^{68}Ga, showed a good radiochemical yield (RCY) 93% after 10 minutes at 80 °C or 88% after 30 minutes at RT.[284] Another derivative of C-functionalised cyclen 2-(2-nitroimidazoly)ethylamine-SCN-Bz-DOTA also gave a high RCY >98% after 10 minutes at 100 °C.[285] Recently, researchers found similar macrocycles p-NO_2-Bn-Oxo and p-NO_2-Bn-PCTA which illustrate much more favourable complexation characteristics, with both giving higher (<98%) RCYs at RT after a reaction time of 5 mins and showing analogous complexation characteristics, but the oxo derivative did not display the kinetic inertness which is found for the parent DOTA and also shows poorer *in vivo* characteristics when conjugated to RGD peptides. However p-NO_2-Bn-PCTA shows both high kinetic inertness and favourable *in vivo* biodistribution characteristics.[286] The comparison study between p-NO_2-

Bn-PCTA and a NOTA derivative for small peptide *in vivo* imaging has shown that they possess very similar properties and are both effective BFCs for *in vivo* ^{68}Ga imaging.[287]

3.2.2. Other types of macrocyclic ligands

A sarcophagine derivatised chelator which is more routinely used with ^{64}Cu has been studied by Donnelly and co-workers to determine the applicability for use with ^{68}Ga, see Figure 31.[288] Sarcophagine based chelators form highly stable complexes in which gallium(III) does not dissociate in the presence of transferrin, a common mechanism for demetallation *in vivo*. Good *in vivo* characteristics have been obtained when two RGD peptides were conjugated with the ligand. To transform labelling to more general procedures compatible with a wider range of biomolecules, additional work needs to be carried out. This chelator group requires harsh conditions for complex formation (85 °C, 30 mins) which are not suited to proteins and antibodies. The radiolabelling reactions and stability have also been studied for a novel series of tricarboxylate ligands based on 6-amino-perhydro-1,4-diazepine.[289] Good radiochemical synthesis characteristics (≥ 96% RCY, 3 mins reaction time) were observed in a pH range between 4.0 to 6.8 and also no demetallation in the presence of transferrin or iron(III) over 2h occurred with these chelators.



Derivative of $(NH_2)_2$sar

Figure 31 - Chemical structure of a sarcophagine derivative for ^{68}Ga complexation.[288]

3.2.3. Non-macrocyclic ligands (acyclic)

Acyclic chelators, when compared to macrocyclic ligands, for ^{68}Ga generally have faster kinetics involved in complex formation and lower *in vivo* stability, therefore the recent work in the area is focused on the design of novel chelators which keep the kinetic advantages and address the stability problem. A gallium compound with the aliphatic bis(thiosemicarbazonate) has been synthesised and radiolabelled under aqueous conditions in >95% radiochemical purity,[288] see Figure 32. Chelators based on the ATSM type thiosemicarbazone structure, which has previously been used for ^{64}Cu peptide labelling,[290,291] have been developed by Pascu and co-workers.[288] To allow the ligand to be used as a multimodal PET/optical agent with ^{68}Ga the backbone of the ligand has been modified by the addition of aromatic groups and also the introduction of other functional groups to improve aqueous solubility, Radiolabelling has been achieved by transmetallation of the zinc(II) complex with the reactions requiring elevated temperatures for good RCYs. A novel chelator CP256,[81] has been developed by Blower and co-workers which shows RCYs of 98-100% after 5 minutes reaction time at RT. The reaction also proceeds to ca. 75% RCY at concentrations as low as 1 μM, a concentration at which DOTA shows negligible conversion even at 100 °C for 30 minutes. ^{67}Ga stability studies demonstrated that the CP256 complex is highly stable for an acyclic chelator and the results showed no transchelation after 4 hours with 130-fold excess of iron(II).

Recently gallium formed complexes of high thermodynamic stability with DFO (desferrioxamine) and has proven useful for conjugation to peptides and small molecules.[292] DEDPA derivatives have been developed by Orvig and co-workers and they initially synthesised three versions of the chelator; one with no reactive groups, a bis-N-functionalised derivative and a C-functionalised derivative, with all three ligands showing quantitative radiolabelling after 10 minutes at RT and 97% of the complex remained intact after transferrin challenge experiments. Bis-N functionalised and C-functionalised derivatives were tested as BFCs by conjugating the chelator to RGD,[293] with excellent results obtained. The C-functionalised mono-RGD conjugate showed high stability (92% after 2 hours) as the N-functionalised bis-RGD conjugate showed much lower stability (73% after 2 hours). A di-azide derivative was also synthesised by the group which can be used via copper(I)-catalysed azide-alkyne cycloaddition (CuAAC) reactions for bioconjugation and showed flexibility in forming complexes with a range of radiometals.[294]

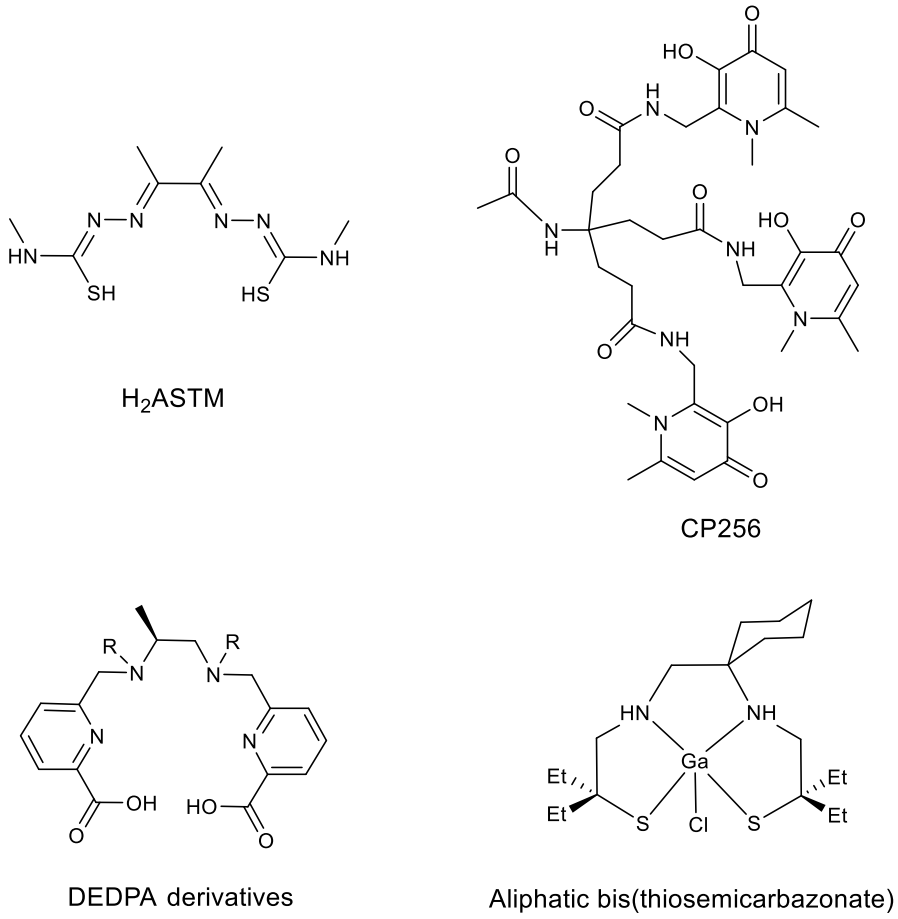


Figure 32 - Chemical structures of some acyclic chelators for ^{68}Ga radiolabelling.[288]

3.2.4. TACN based chelators

Chelators based on TACN (1,4,7-triazacyclononane) such as NOTA (1,4,7-triazacyclononane -1,4,7-triacetic acid), see Figure 33, show favourable characteristics for ^{68}Ga radiolabelling. Due to the cavity size and properties, TACN based chelators generally exhibit fast complexation reaction kinetics, high *in vivo* stability and high selectivity towards gallium(III).[63,71,295] Recently researchers interested in ligand design have focused on studying and optimising chelators N-functionalised with three pendant arms containing sites for both coordination and conjugation, see Figure 33.[296-298] Trimeric bioconjugate species which retain the coordinating atoms have been obtained by following this method with the aim of signal amplification through multivalency.[299,300] Two interesting molecules have been synthesised, an RGD trimer and TRAP $(RGD)_3$, and showed a 10-20 fold increase in specific activity compared to both NODAGA-RGD and DOTATOC, although to achieve this, temperatures of 95 $^{\circ}$C are required. Also, in comparison with ^{68}Ga-NODAGA-RGD and ^{18}F-galacto-RGD *in vivo* experiments showed higher affinity for the trimeric derivatives.[301] Tri-glutaric acid

(NOTGA), a similar system, which has also been developed by the same group, was used to synthesise mono-, bis- and trimeric bioconjugates in order to directly compare the effects of variation in the number of RGD biomolecules per construct.[302] Compared to the monomeric version the trimeric bioconjugate showed a signal amplification leading to a 54% increase in tumour uptake. Also the effect of different isomers on radiosynthesis has been studied[302] and showed that a diastereomic mixture has no negative effect on biological activity for RGD conjugates. Notni and co-workers found that the phosphonate donating groups are much less affected by transmetallation compared to acetate donors when they studied the influence of metal ion exchange processes.[303]

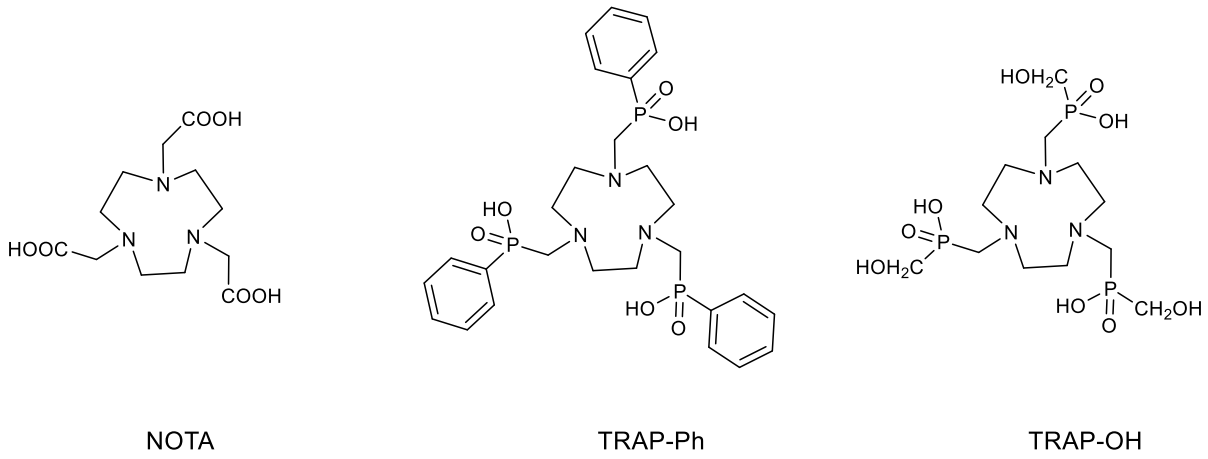


Figure 33 - Chemical structures of NOTA, TRAP-Ph and TRAP-OH.[297]

3.2.5. Conjugation reactions

Bioconjugation is a chemical strategy to form a stable covalent link between two molecules, at least one of which is a biomolecule. Current advances in the understanding of biomolecules allowed their application to numerous fields including medicine and materials. Synthetically modified biomolecules can have varied functionalities, such as tracking cellular events, disclosing enzyme function, determining protein biodistribution, imaging specific biomarkers, and supplying drugs to targeted cells.[304,305] Bioconjugation is an essential strategy that bonds these modified biomolecules with different substrates.

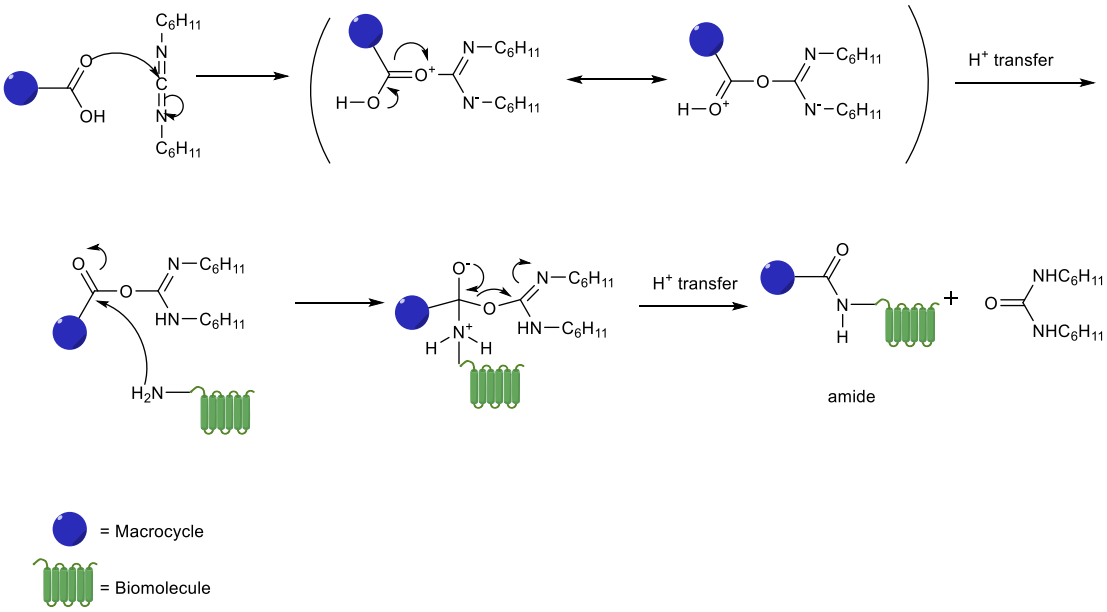
Synthesis of bioconjugates includes a variety of challenges, ranging from the simple and nonspecific use of a fluorescent dye marker to the complex design of antibody drugs conjugates. As a result, different bioconjugation reactions have been developed to chemically

modify proteins. Common types of bioconjugation reactions are coupling of lysine amino acid residues, coupling of cysteine residues, coupling of tyrosine residues, modification of tryptophan residues, and modification of the N- and C- terminus.[306]

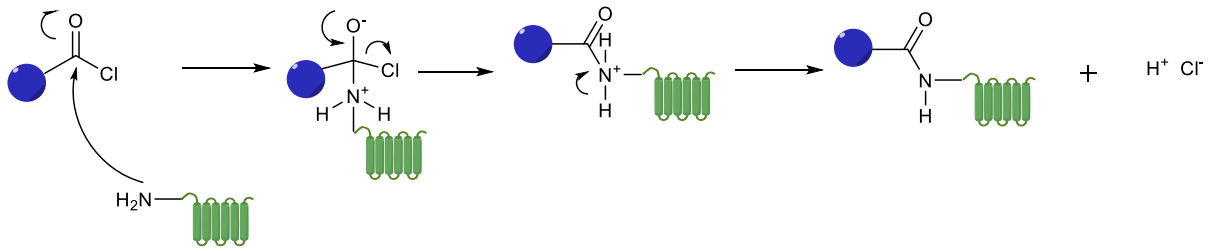
3.2.5.1. Amide formation

An appropriate conjugation strategy is the formation of amide bonds between macrocyclic BFCs and biological targeting groups.[307] Amides may be formed straight from terminal acid functionalities via a peptide coupling reaction; using a coupling agent such as dicyclohexylcarbodiimide (DCC), or via activation of the acid through conversion to the acyl chloride.

Use of either of these strategies requires the targeting moiety to have an available amine in order to permit the amide formation to occur through the mechanisms shown in Scheme 6 and Scheme 7, below. The diimide coupling agent achieves coupling by producing a nucleophilically active intermediate, and has best results using primary amines as nucleophiles, as shown in scheme 6. Use of the acyl chloride will encourage amide coupling with more hindered amine sites, but will couple most rapidly with primary amines.



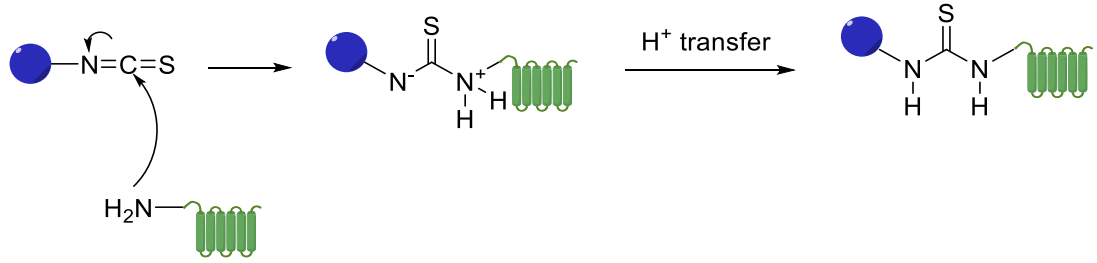
Scheme 6 - Mechanism of amide formation using DCC, a diimide coupling agent, to encourage the conjugation of a carboxylic acid and a primary amine.[308]



Scheme 7 - Mechanism of amide formation between an acyl chloride and an amine.[308]

3.2.5.2. Thiourea formation

As a primary conjugation strategy, amide formation is not specific to molecules having terminal amine groups. Another possible way of producing a link to a terminal amine group is by using an isothiocyanate functionality, which gives a thiourea linkage between the BFC and the targeting agent, see Scheme 8.



Scheme 8 - Mechanism of thiourea formation between isothiocyanate and amine functionalities.[308]

Isothiocyanate functionalities may be synthesised from primary amine groups by reaction with thiophosgene,[68] which gives the terminal N=C=S system, the carbon of which is activated towards nucleophilic attack by another primary amine.

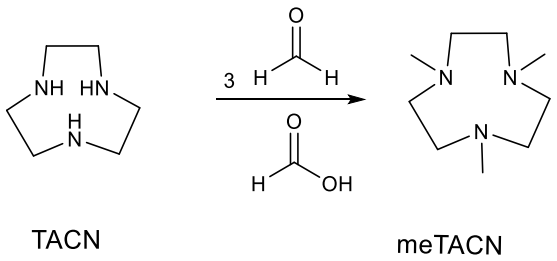
3.3. Synthesis of macrocyclic chelators

3.3.1. Synthetic methodology to form N-functionalised TACN derivatves

The selective functionalisation of macrocycles has become an area of increased interest over past few years. The heightened interest in the regulation of the properties of metal-ligand

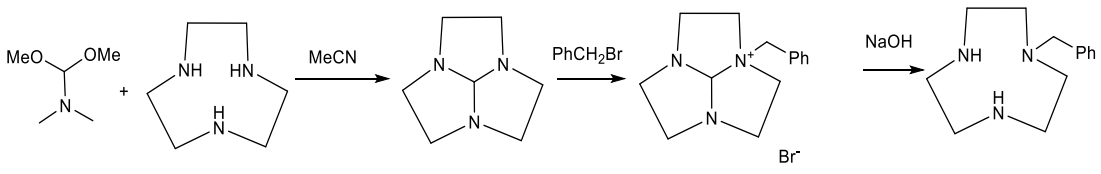
complexes led to an increasing desire to synthesise ligands with specific differing pendants.[309] The need to produce multi substituted azamacrocyclic ligands in higher yields and in fewer steps has increased. Many applications of azamacrocyclic ligands require functionalisation of the parent ligand.[310,311]

The use of TACN as starting material is a simple way to add pendent arms on to the nitrogen atoms. The addition of a desired group before the cyclization step to form *N*-alkylated derivatives is a one of the procedures that is used to synthesise N-functionalised TACN.[312-314] Specific protection can be achieved of one or two nitrogen atoms of TACN by tosylation,[315] carbamation,[316] or pH adjusted sulfomethylation,[317] comprising additional purification and/or deprotection steps. Also the formation of a single *N*-formyl group covered as a tricyclic orthoamide and following reaction of the orthoamide functional groups is another way to prepare *N*-functionalised TACN. As an example, this can be achieved through the condensation of formaldehyde in a mixture of TACN and formic acid, meTACN was synthesised using this methodology, see Scheme 9.[318]



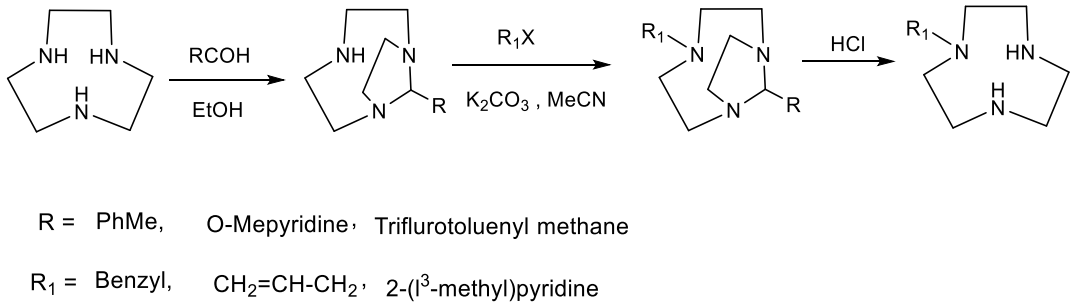
Scheme 9 - Synthesis of meTACN.[318]

Mono- or disubstitution of TACN derivatives can also be achieved by condensation of formaldehyde in a mixture of TACN and formic acid, see Scheme 10.[319] Hence, with one equivalent of $(CH_3)_2NCH(OCH_3)_2$, followed by addition of one equivalent of benzylbromide and hydrolytic elimination of the formyl group, the monobenzyl TACN was obtained.



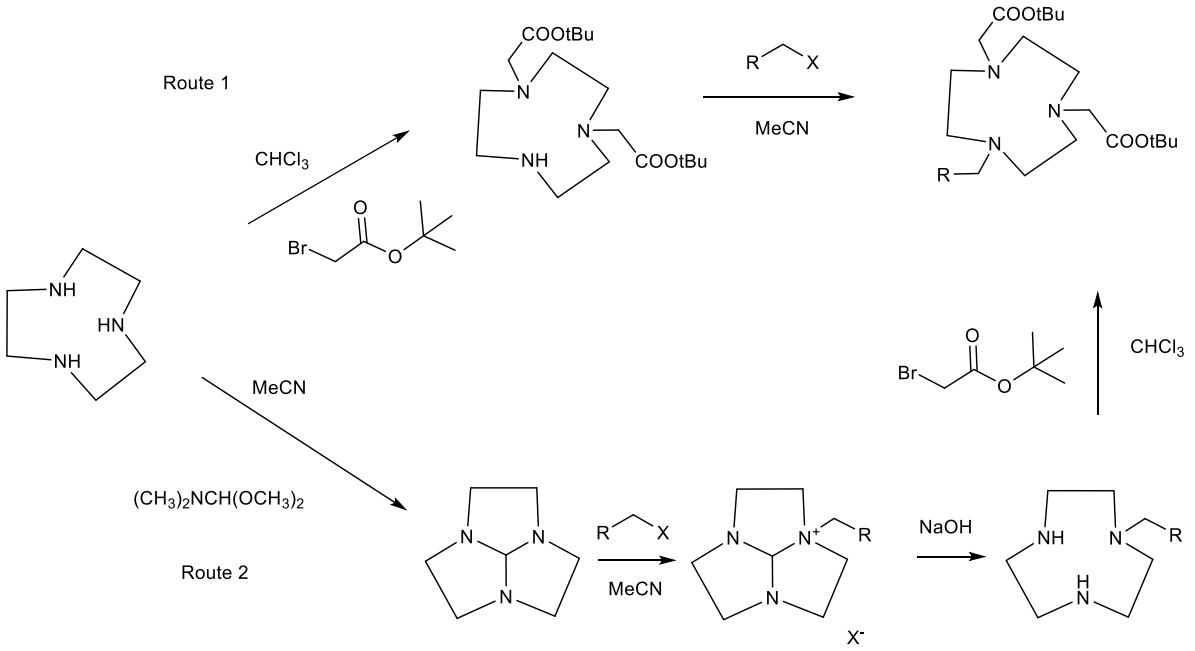
Scheme 10 - Synthesis of monobenzyl TACN.[319]

Tripier and co-workers have reported a new method by using mild and fast conditions of aminal formation with substituted aromatic aldehydes see Scheme 11.[320] The role of aminal in this procedure is to act as a protecting group which allows the functionalisation with halogenoalkanes. A series of TACN derivatives which have one arm can be obtained by hydrolysis with hydrochloric acid.



Scheme 11 - Synthesis of monosubstituted TACN derivatives.[320]

The diversity of procedures that are discussed above offer an opportunity to manipulate in the nature of substituents on the nitrogen atoms. Di-*tert*-butyl 2,2'-(1,4,7-triazonane-1,4-diyl) diacetate (NO2AtBu) functionalised derivatives are generally synthesised using one of two possible routes, see Scheme 12. Route 1 involves selective di alkylation of the TACN ring with *tert*-butyl ester protected carboxylic acids, followed by functionalisation of the remaining secondary amine with a different reactive arm. Route 2 involves formation of formyl group, followed by selective alkylation of a single nitrogen.[259,321] In addition, selection of a suitable solvent causes the amino product to precipitate out of solution and it therefore cannot react beyond a single substitution.[260] After hydrolytic removal of the formyl group the remaining secondary amines can be alkylated in a similar way as route 1 to form the desired product.

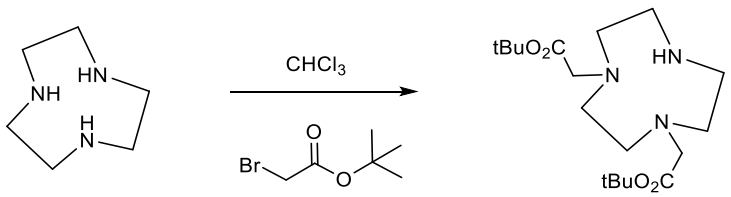


Scheme 12 - Two routes for the synthesis of NO2A derivatives.

The advantage of route 2 is that it gives the mono-substituted TACN in high purity, and so no chromatographic separation is required, but it has the disadvantage of low overall yields due to the increased number of steps and the variability in yield dependent on pendant arm selection. A short two-step synthesis with little loss of TACN is the key advantage in route 1 but it has the problem of forming mixed arm products in the first reaction which can be challenging to purify. Route 1 was selected as the preferred choice for this work and route 2 was not attempted.

3.3.2. Synthesis of di-*tert*-butyl 2,2'-(1,4,7-triazanonane-1,4-diyl)diacetate (NO2AtBu)

The synthesis of di-*tert*-butyl 2,2'-(1,4,7-triazonane-1,4-diyl)diacetate (NO2AtBu) (**16**) was carried out following a literature procedure,[322] in which *tert*-butylbromoacetate in a two-fold excess in chloroform is added drop-wise into a stirred solution of TACN in chloroform, see Scheme 13. The reaction mixture is stirred for 24 hours, then filtered and the solvents are removed.



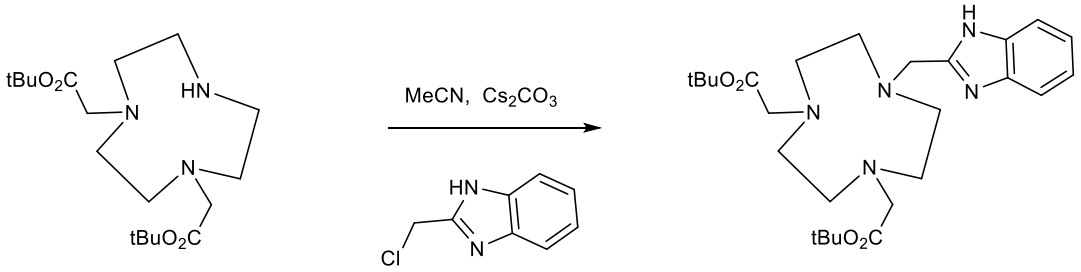
16

*Scheme 13 - Synthesis of NO2AtBu **16**.*

This reaction forms a mixture of multiple arm products, due to selectivity being controlled only by stoichiometry, then the desired compound is separated by pH control via addition of NaOH or HCl and extraction to give di-*tert*-butyl 2,2'-(1,4,7-triazonane-1,4-diyl)diacetate (NO2AtBu) (**16**). Yields are relatively low (48%) for this reaction and although ^{1}H and ^{13}C NMR of the product showed it to be relatively pure, MS confirmed slight contamination with tri substituted TACN.

3.3.3. Synthesis of di-*tert*-butyl 2,2'-(7-((1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane-1,4-diyl)diacetate

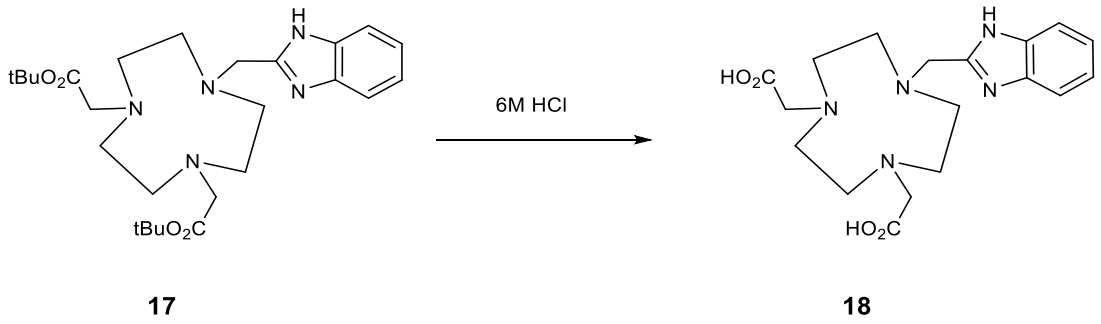
The secondary amine of the compound NO2AtBu **16** is available for alkylation. To synthesise benzimidazole NO2AtBu **17** in high purity, a stoichiometric amount of NO2AtBu **16** and 2-(chloromethyl)-benzimidazole have been used, see Scheme 14. Cs_2CO_3 was used as a base, with caesium(I) being too large to form a macrocyclic complex with the metal ion within the macrocyclic cavity. A large amount of dry acetonitrile (*ca.* 1150 ml per gram of macrocycle) was used as a solvent to give high dilution conditions and the reaction was stirred at RT for 18 hours. MS analysis confirms the synthesis of compound **17** and the absence of peaks for any doubly substituted benzimidazole.



16 **17**

*Scheme 14 - Synthesis of benzimidazole NO2AtBu **17**.*

A common method used in deprotection of NO2AtBu derivatives is the acid catalysed hydrolysis of esters which is performed by heating the reaction mixture to reflux in the presence of acid. Deprotection of **17** was initially carried out by dissolving the compound in 6M HCl and heating the reaction mixture to reflux for 18 hours, see Scheme 15. After removing the solvents, ether was added and decanted multiple times to dissolve and remove any ether soluble impurities. After deprotection of the *tert*-butyl arms to form the NO2A derivative, the NMR spectra of 2,2'-(7-((1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazonane-1,4-diyl) diacetic acid (**18**) showed a clear indication of the purity of benzimidazole NO2AtBu derivative and demonstrated that a pure product **17** had been formed.



17 **18**

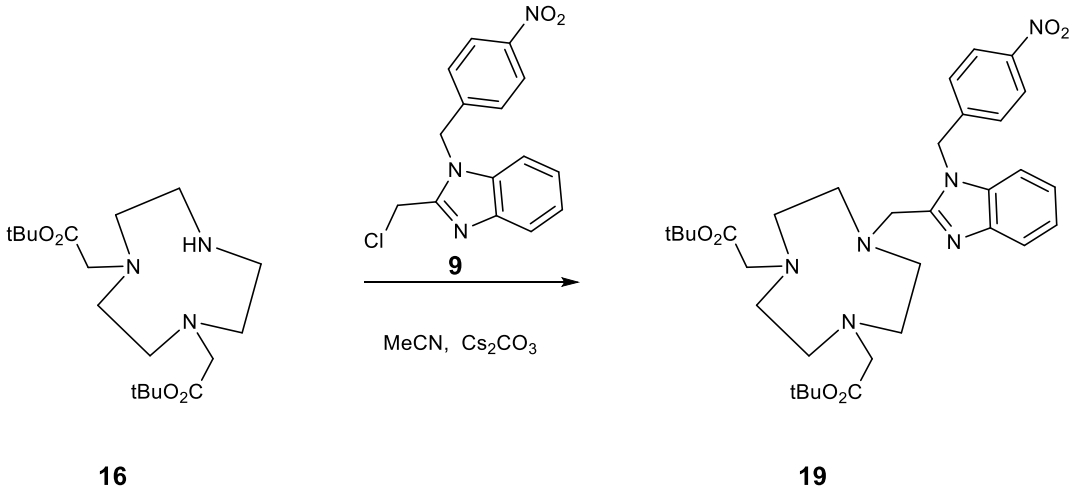
*Scheme 15 - Deprotection reaction to form benzimidazole NO2A **18**.*

The reaction can be tracked by ^{1}H NMR through disappearance of the 'boc' peak at 1.45 ppm. Another way to follow the reaction is to use TLC which offers useful *in situ* information due to the large difference in polarity between starting material and product. At this stage of the scheme using NMR as a tool to determine purity is much easier compared with analysis of the protected version **17** because the HCl completely protonates the imidazole, giving a characteristic pattern for the aromatic region and showing that no 'self-reaction' has occurred. Compound **18** was fully characterised by NMR and MS. Also HPLC analysis showed the high purity (89%) of the compound.

In an attempt to decrease the reaction time and to obtain the compound in the same purity, the ester hydrolysis was successfully achieved by following a new procedure reported in our group[323] using a microwave reactor through heating the reaction at 130 $^{\circ}$C which gave the desired compound and reduced the reaction time from 18 hours to 10 minutes. For molecules in this class, this method is the recommended deprotection method due to the very rapid high yielding conversion.

3.3.4. Synthesis of di-*tert*-butyl 2,2'-(7-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl) methyl)-1,4,7-triazanonane-1,4-diyl)diacetate

The synthesis of N-functionalised benzimidazole NO2AtBu has been carried out by the direct alkylation of compound NO2AtBu **16** with the previously synthesised N-functionalised benzimidazole derivatives **9**, see *section* 2.4. This procedure offers a method to synthesise an N-functionalised benzimidazole NO2AtBu derivative with a reactive arm for bioconjugation, see Scheme 16.

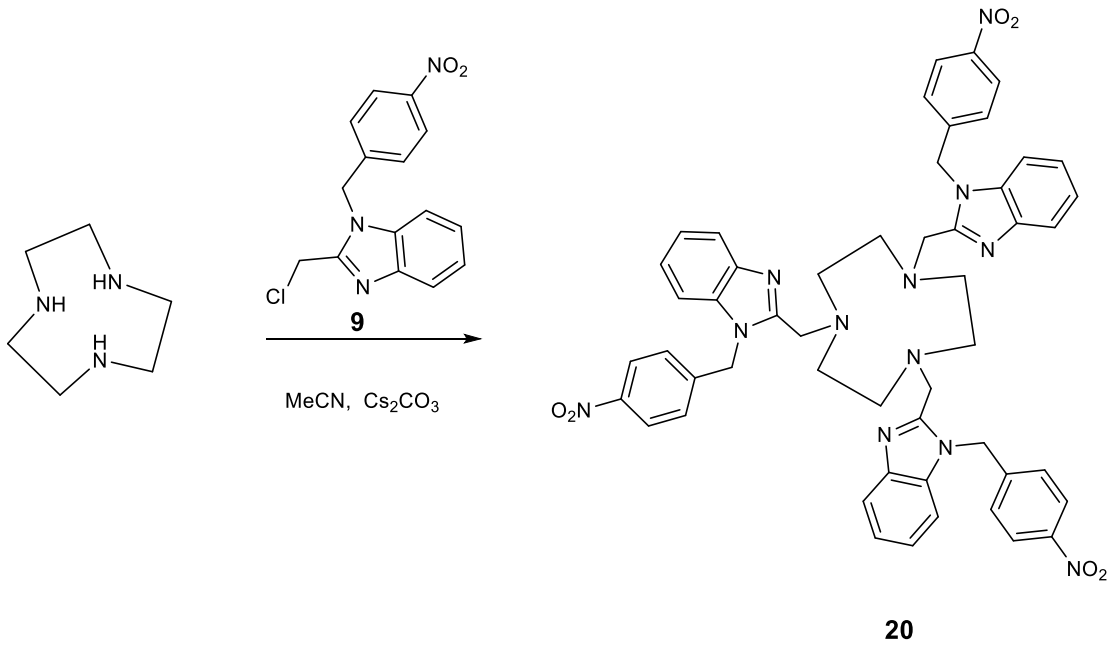


*Scheme 16 - Synthesis of N-functionalised benzimidazole NO2AtBu derivatives **19**.*

NO2AtBu **16** was reacted with 2-(chloromethyl)-1-(4-nitrobenzyl)-1H-benzo[d]imidazole (**9**) by adding the benzimidazole derivative **9** in a 1:1 ratio to a stirred solution of NO2AtBu **16** in dry acetonitrile, followed by the addition of a four-fold excess of caesium carbonate. Due to there being no possibility of 'self-reaction' on the imidazole, an excess of benzimidazole could be used. The reaction was stirred at RT for 24 hours. Reaction completion was confirmed by TLC, then the mixture was filtered. After washing with MeCN the crude solid obtained was purified by column chromatography using DCM/MeOH (9:1) as eluent. This method gave the desired compound; di-*tert*-butyl 2,2'-(7-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7 triazanonane -1,4-diyl)diacetate (**19**) in a 65% yield and the compound was fully characterised by NMR, elemental analysis (CHN) and MS analysis.

3.3.5. Synthesis of 1,4,7-tris((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane

The direct alkylation of TACN with the previously synthesised N-functionalised benzimidazole derivatives **9** has been used to synthesise tris-substituted TACN derivative **20**, see Scheme 17. By following this procedure a tris N-functionalised benzimidazole TACN derivative with a three reactive arms for bioconjugation has been obtained.

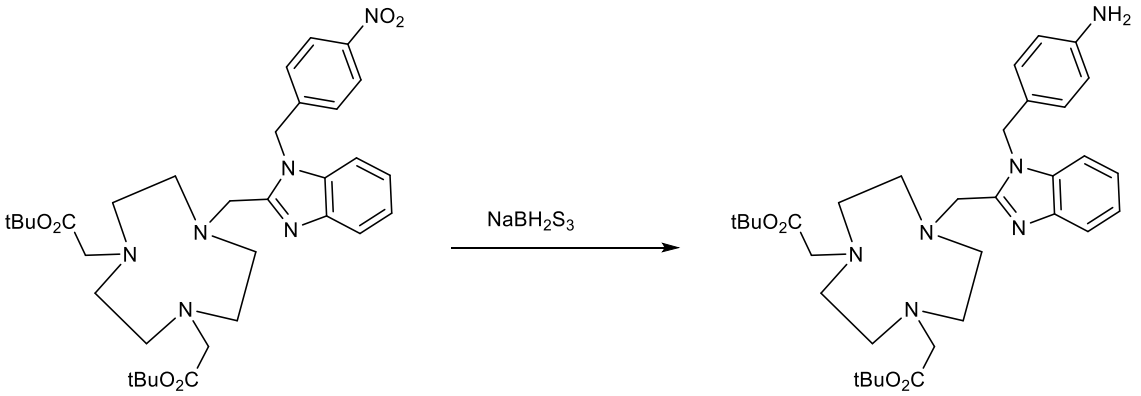


*Scheme 17 - Synthesis of tris N-functionalised benzimidazole TACN derivative **20**.*

A three-fold excess of 2-(chloromethyl)-1-(4-nitrobenzyl)-1H-benzo[d]imidazole (**9**) in dry acetonitrile was added dropwise to a stirred solution containing TACN and a four-fold excess of caesium carbonate in dry acetonitrile. The reaction mixture was stirred at RT for 48 hours. TLC was used to confirm reaction completion, the mixture was then filtered. After washing with MeCN the crude solid was obtained. Because the selectivity is controlled by stoichiometry, this reaction forms a mixture of multiple arm products which then need to be separated by silica gel column chromatography to give 1,4,7-tris((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane (**20**). Yields are relatively low (62%) for this reaction. Identification and the purity of the product was confirmed by ^{1}H, ^{13}C NMR, elemental analysis (CHN) and MS.

3.3.6. Synthesis of di-*tert*-butyl 2,2'-(7-((1-(4-aminobenzyl)-1H-benzo[d]imidazol-2-yl) methyl)-1,4,7-triazanonane-1,4-diyl)diacetate and 4,4',4''-((2,2',2''-((1,4,7-triazanonane-1,4,7-triyl)tris(methylene))tris(1H-benzo[d]imidazole-2,1-diyl))tris(methylene))trianiline

To provide a site for conjugation reactions the nitro groups of compounds **19** and **20** can be converted into more reactive amine groups. Palladium on carbon under a H_2 atmosphere has been used as a reducing agent for related molecules but it is also reported that these conditions can cause debenzylation of molecules of this type.[264,324] Lalancet *et al.* reported an alternative method for the reduction of nitrobenzyl groups which involves the use of a $NaBH_2S_3$.[265] This method was adapted after minor modification to use a twenty–fold excess of $NaBH_4$, see Scheme 18 and 19.



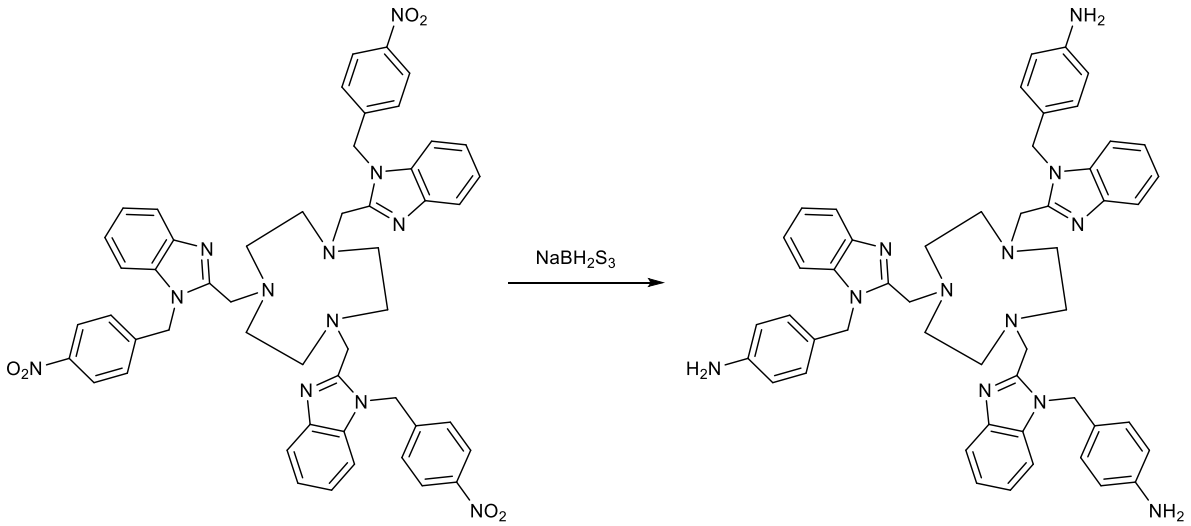
19 **21**

*Scheme 18 - Reduction of the nitro group to amino to synthesis compound **21**.*

By stirring together S and $NaBH_4$ in dry THF for 1 hour under argon, $NaBH_2S_3$ was prepared and used *in situ* before addition of compound **19** in dry THF. The reaction mixture was then heated under reflux for 18 hours. After cooling the reaction was purified via column chromatography to yield the desired product **21** in 40% yield.

A multivalent ligand includes of multiple copies of binding groups conjugated to a scaffold, permitting the simultaneous binding to multiple sites or receptors. To increase the binding affinity, avidity and specificity of the ligand to the receptor many research groups have successfully designed and synthesised multivalent ligands.[325]

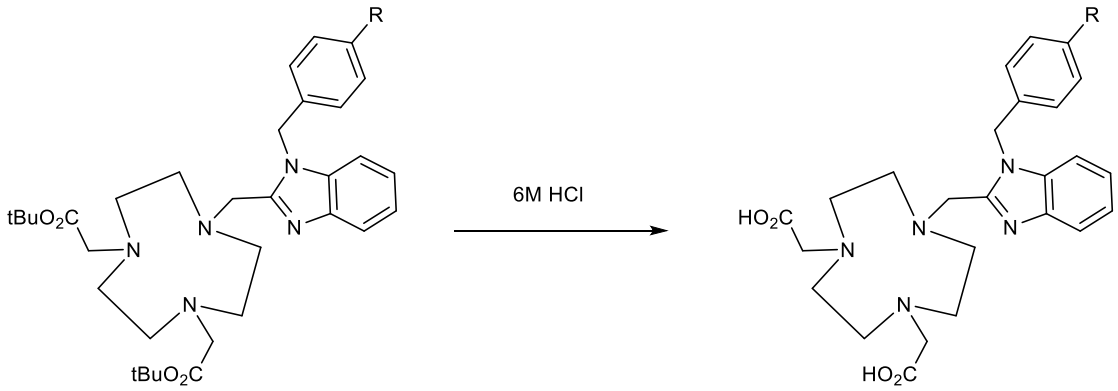
A central unit that could be used to form a multivalent ligand **22** has been synthesised by followed the same procedure which was used to prepare compound **21** see Scheme 19. The crude solid was purified via flash column alumina and the product characterised by ^{1}H NMR and MS. Compound **22** offer three amino groups arms which are preferable for bioconjugation and molecular imaging applications. The ligand **22** has the potential to be a useful BFC for PET imaging by utilising the three amino groups for bioconjugation.



*Scheme 19 - Reduction of the nitro groups to amino group to synthesise compound **22**.*

3.3.7. Acid hydrolysis of ester to remove the *tert*-butyl protecting groups

The removal of the *tert*-butyl protecting groups to form the diacid is the final step in bifunctional chelator synthesis. The microwave irradiation method has been used, see *section* 3.3.3. Deprotection of protected ligands **19** and **21** gave, respectively, the diacids **23** and **24** in near quantitative yields, see Scheme 20.



R = NO_2 **19**
R = NH_2 **21**

R = NO_2 **23**
R = NH_2 **24**

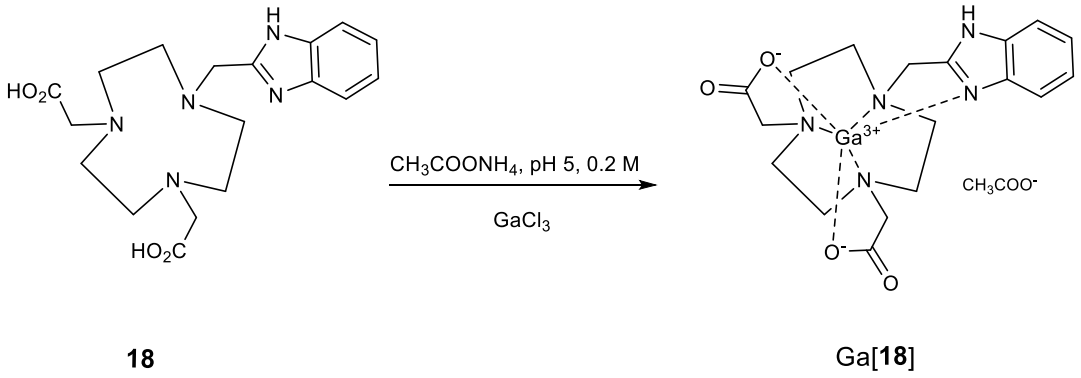
Scheme 20 - Derivatives of benzimidazole NO2A diacid formed by hydrolysis.

All chelates have been successfully synthesised and the structures confirmed by using NMR and MS analysis. In addition, analytical HPLC showed that the purity of ligands is acceptable for further metal complexation reactions.

3.4. Formation of the 'cold' $^{69/71}$Ga complex

There are two reasons for the synthesis of 'cold' $^{69/71}$Ga complex of the prepared chelators, firstly this was to determine the conditions for the formation of $^{69/71}$Ga complexes that can be translated to ^{68}Ga radiolabelling. Secondly, 'cold' standards of $^{69/71}$Ga complexes are needed for HPLC identification of radiolabelled species.

Initially, a test reaction was performed using compound **18** on a relatively small scale to see if the proposed methods for complex formation were valid. The reaction conditions chosen to synthesise the $^{69/71}$Ga complex [Ga**18**] used ammonium acetate buffer at pH 5 to avoid the formation of hydroxides which reduces the yield, see Scheme 21. The reaction was carried out for 18h at 95 $^{\circ}$C and was then purified using amberlite XAD16N, eluting first with water to remove inorganic salts, followed by water:acetonitrile (9:1) to elute the compound in a 75% yield.[326]

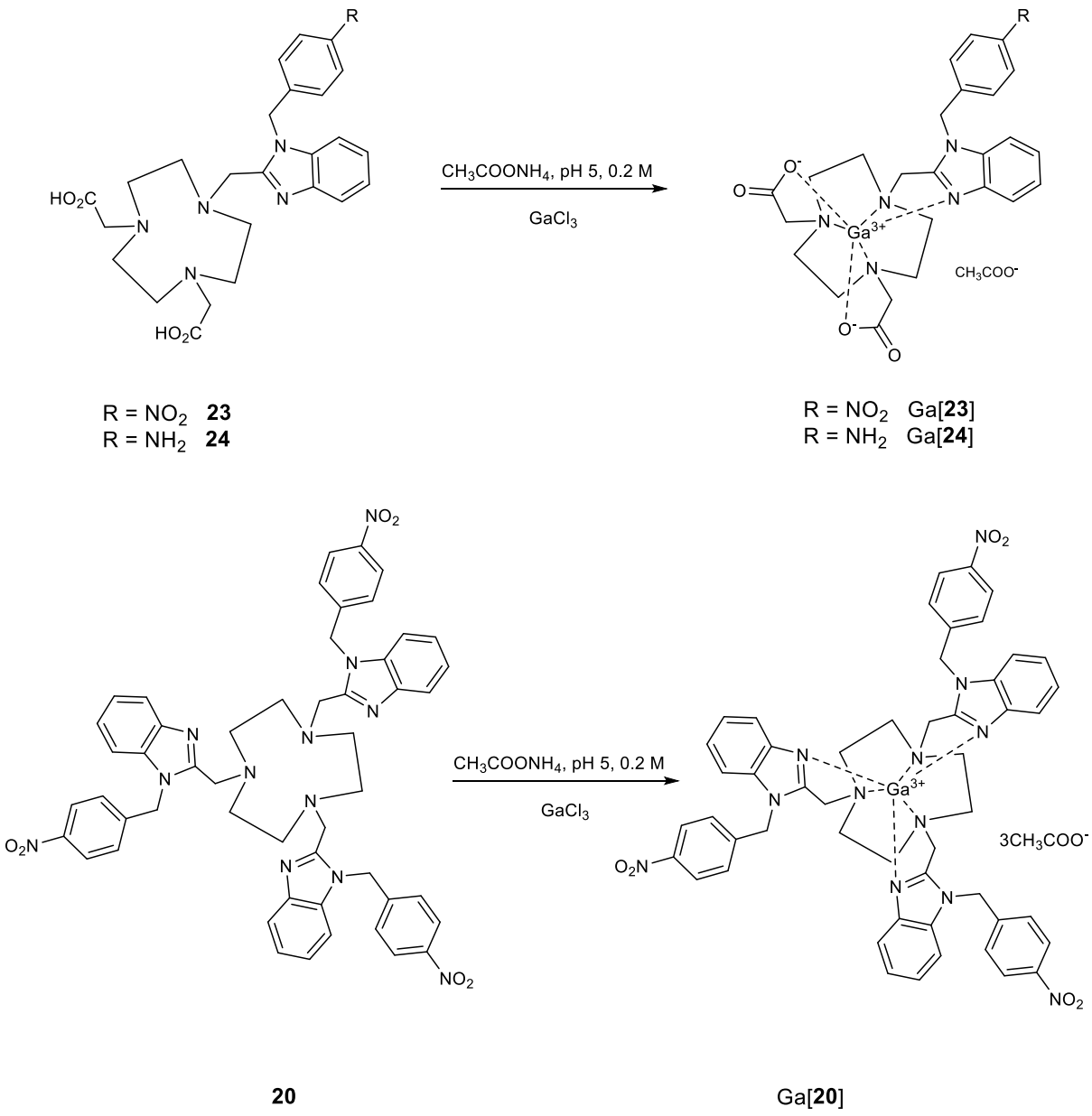


18 Ga[**18**]

*Scheme 21 - $^{69/71}$Ga complex of ligand **18**.*

Mass spectrometry was used to confirm the structure of the $^{69/71}$Ga complex. Elemental analysis and HPLC were employed to determine the purity of the compound. HPLC comparison of the ligand **18** with the complex gives the cold standard for radiochemistry, with a shift of the product peak retention time from 7.39 mins for **18** to 6.44 mins for [Ga**18**].

To synthesise $^{69/71}$Ga complexes of chelators **20, 23** and **24,** the same procedures, see Scheme 22, have been applied on a small scale to produce HPLC standards and allow confirmation of their identity using MS technique. All four reaction mixtures showed a complete disappearance of the ligand peak when tested by HPLC. The retention time of each complex was recorded and MS was used to confirm the identity of the desired complex in all cases.



*Scheme 22 - [69/71]Ga complex of ligands **23, 24** and **20**.*

3.5. Radiolabelling of 18, 20, 23 and 24 with ^{68}Ga

After successfully synthesising the cold standards, the chelators could be radiolabelled and analysed. Chelator **23** was selected for initial method development.

3.5.1. Use of chelator 23 to develop the method for radiolabelling

Some general indications of the best conditions for successful synthesis have been obtained through research in the literature on ^{68}Ga radiolabelling of NOTA and TACN type chelators. Blower and co-workers through a study to evaluate a new chelator, carried out the

radiolabelling reaction with a series of different concentrations for both the novel chelator and as a comparison also investigated the labelling of standard chelators under the same conditions, see Figure 34.[327]

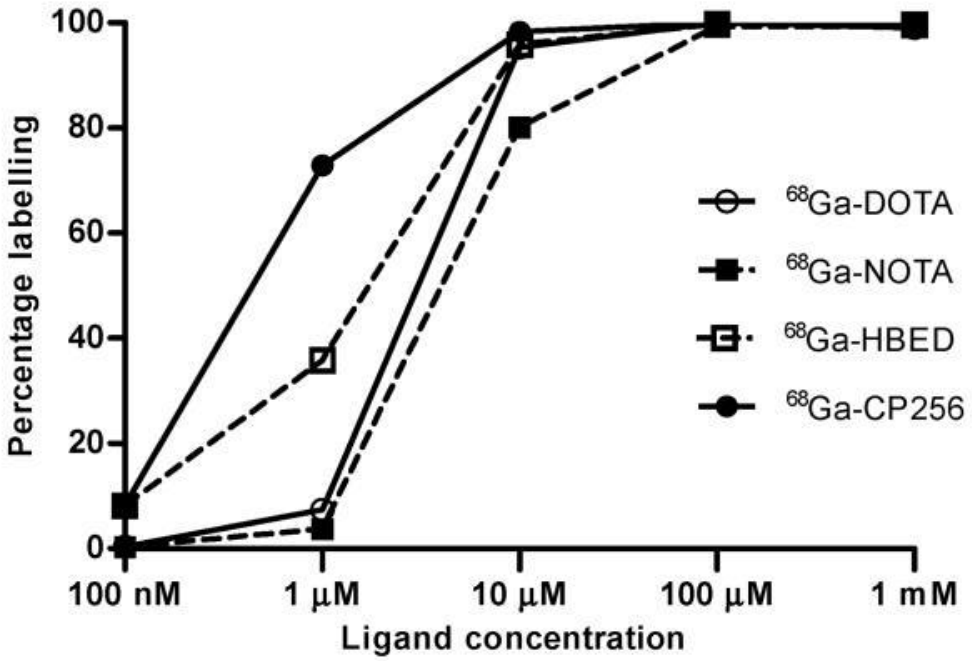
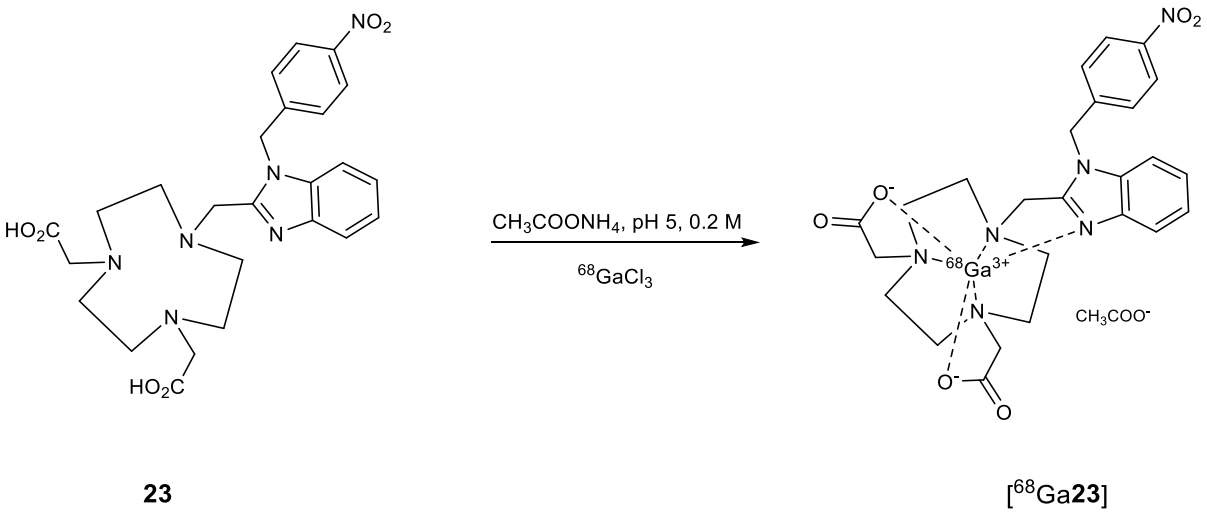


Figure 34 - Ligand concentration versus *radiolabelling yield for ^{68}Ga-NOTA (pH 3.6, 10 min, RT) and a further three ^{68}Ga complexes.*[327]

This study showed the possibility of radiolabelling of NOTA at RT and pH 3.5 for 10 minutes at 10 µM ligand concentration. However, decreasing the concentration to 1 µM gave 4% yields.

Velikyan *et al*. conducted a study which showed the radioactivity incorporation (RAI) for ^{68}Ga-NOTA was >95% within less than 10 min at RT, pH 3.5 and 10 μM.[295] Also optimal conditions for radiolabelling a range of chelators were obtained by Ferreira *et al*.[284] Their results showed that p-NO_2-Bn-NOTA was radiolabeled at >95% yield at RT for 5 minutes at 1 µM ligand concentration and pH between 3 and 5. In the initial labelling reactions similar conditions to those reported have been selected. **23** was radiolabelled with ^{68}Ga using 200 µl of a 2 mM solution in 0.2 M ammonium acetate buffer at pH 5, see Scheme 23. Firstly, the reaction was carried out at RT, with HPLC analysis performed after 5, 25 and 80 minutes.



23 [^{68}Ga**23**]

*Scheme 23 - ^{68}Ga radiolabelling of **23** to form [^{68}Ga**23**].*

The first concentration utilised was 10 μM of chelator but this concentration was not sufficient for radiolabelling with ^{68}Ga, the concentration was increased incrementally up to 2 mM which was sufficient for radiolabelling. Using these conditions, HPLC analysis showed that after 5 minutes reaction time, 73% ^{68}Ga had been incorporated to form [^{68}Ga**23**], see Figure 35. Over the following time points there was no variation up to 55 minutes. After this initial result, the reaction temperature was increased to 90 $^{\circ}$C in an attempt to improve the RCY. This modification gave a yield of 59% after 5 minutes which showed little improvement between 5 and 55 minutes, see *section* 6.7.1.

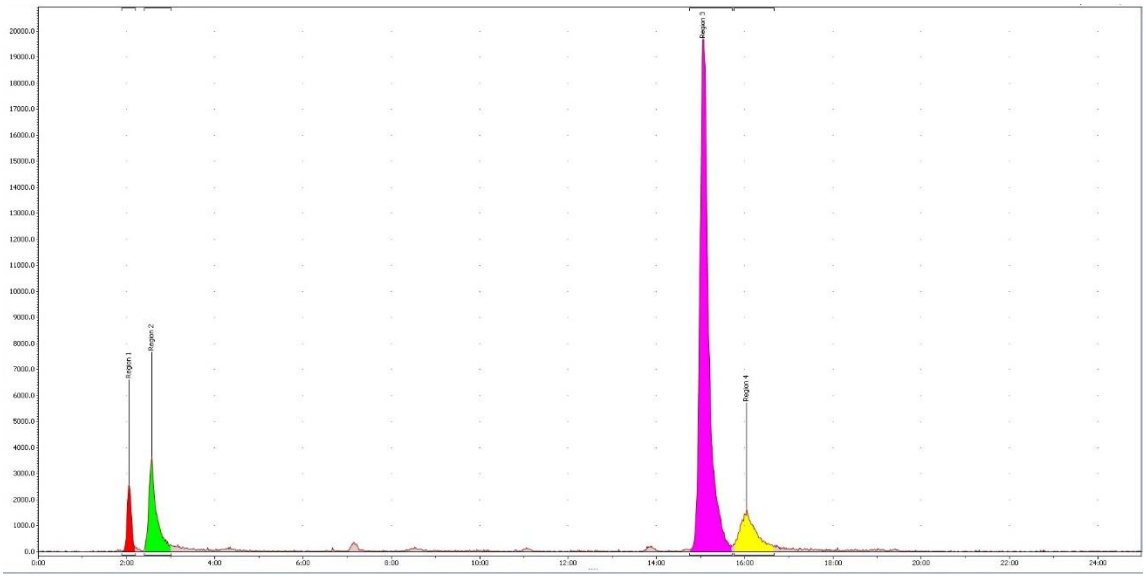
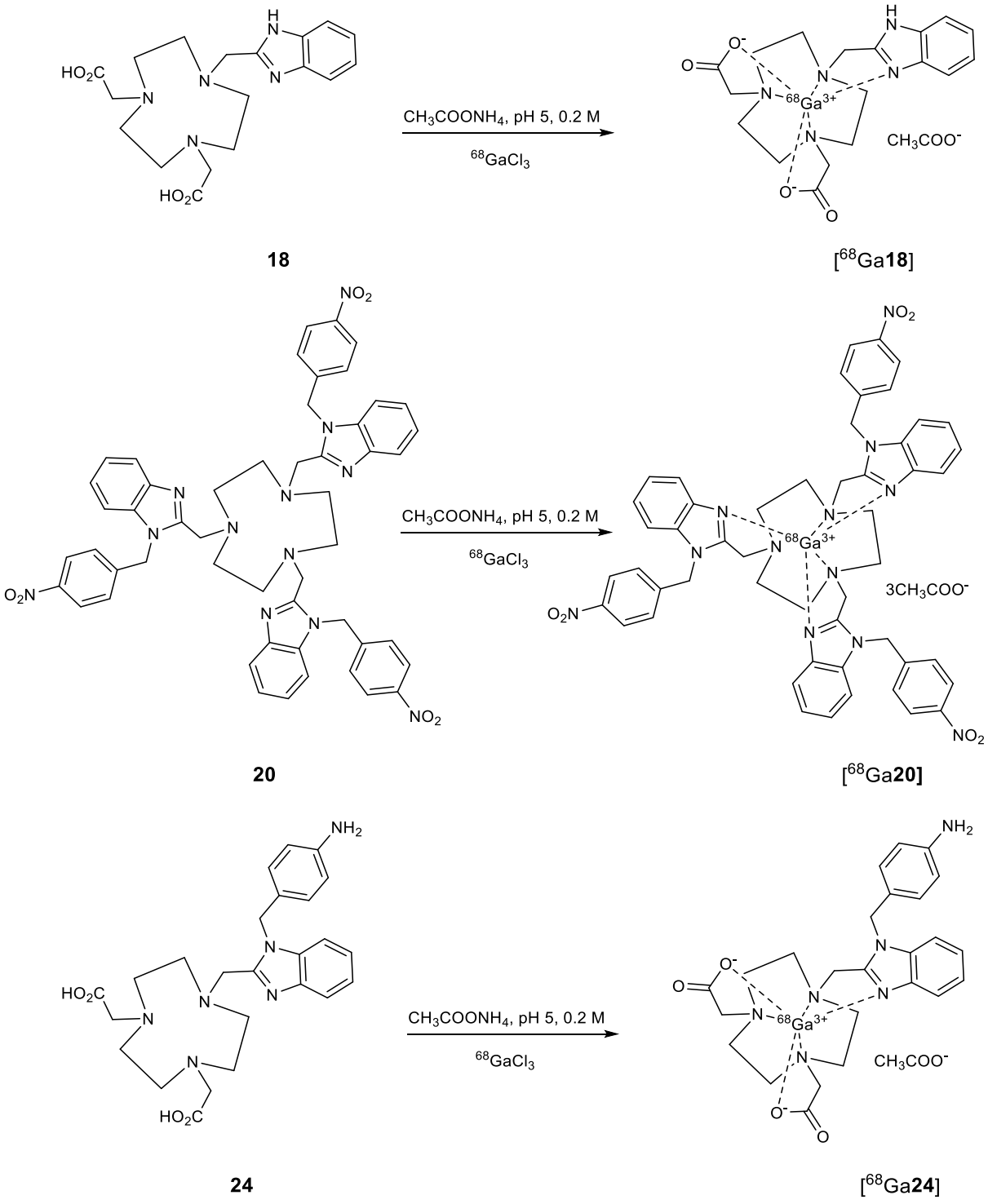


*Figure 35 - HPLC Chromatogram of [^{68}Ga**23**].*

3.5.2. Complexation of TACN and NO2A derivatives with ^{68}Ga

The preferred reaction conditions for this class of chelators would be at room temperature with only a 5 minute reaction time with a concentration of 2 mM. This method did not give 100% RCY, but a number of reasons make these conditions preferred including the very short reaction time and being at room temperature as they are applicable to most biomolecules. The same reaction conditions as used in the developed method were applied, with only minor changes in the chelator concentration, were applied to ^{68}Ga radiolabel **18** (0.6 mM), **20** (1 mM) and **24** (25 µM) to form [^{68}Ga**18**], [^{68}Ga**20**] and [^{68}Ga**24**] respectively, see Scheme 24. The different chelators displayed a range of results, see Figure 36. **18** showed a RCY of 55% after 5 minutes. However, **20** and **24** gave RCYs of 25% and 37% respectively. The radiolabelling study showed that increasing reaction time had no positive effect on the formation of the ^{68}Ga complex, see *section* 6.7.2-6.7.4. The results of radiochemical yield for all compounds were not sufficiently high for the application. This may due to the problem of metal ion contamination from the ^{68}Ge/^{68}Ga generators. In recent years two research groups studied this phenomena and how increasing concentration of other metal ions besides ^{68}Ga affects RCY of different bifunctional chelators.[303,328] In their study Chakravarty *et al*. chose metal ions (Fe, Cu, Al, Zn, Sn and Ti) which were commonly present in ^{68}Ga elution.[328] For most of chelators under study they found that the presence other metal ions led to a decrease

the radiochemical yield and for some chelators the decrease was more than 50%. Šimeček *et al*. also demonstrated that increasing metal ion contamination had a crucial effect on RCY.[303]



18 [^{68}Ga**18**]

20 [^{68}Ga**20**]

24 [^{68}Ga**24**]

Scheme 24 - ^{68}Ga radiolabelling of **18**, **20** and **24**.

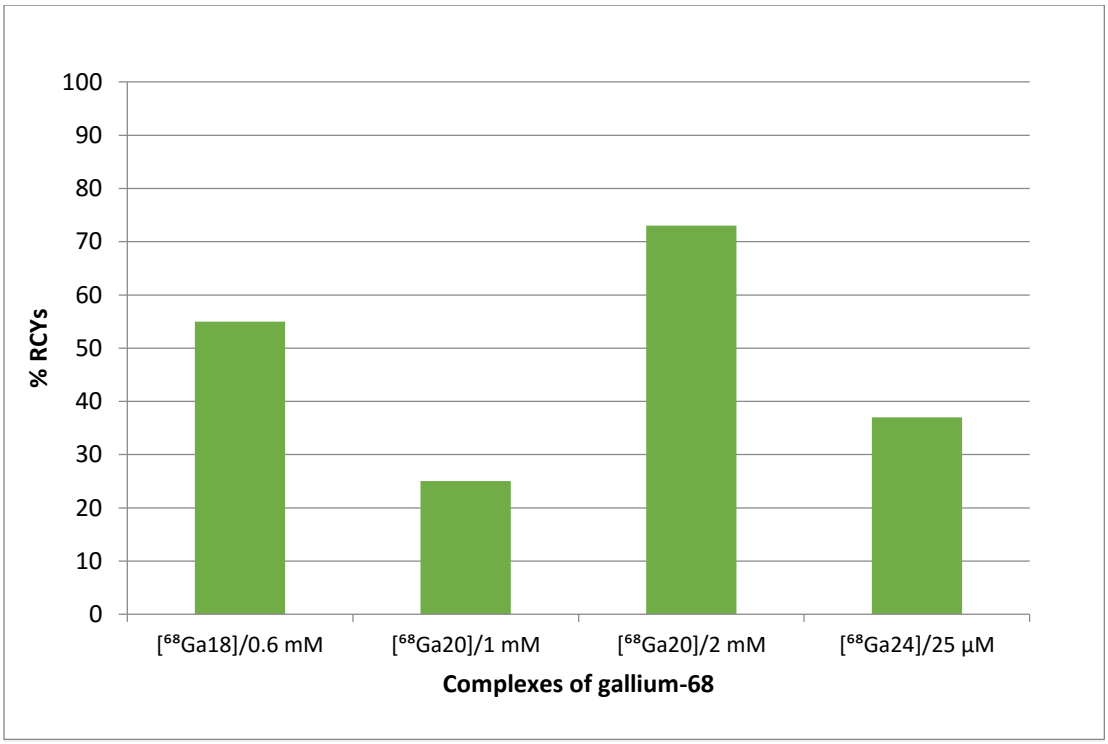


Figure 36 - Radiochemical yields of ^{68}Ga complexes.

3.6. Conclusions

Five novel chelators have been synthesised and fully characterised. Optimised labelling conditions for ^{68}Ga complex formation to four ligands were developed, see Figure 37. The synthesis of chelators started with preparation of the benzimidazole unit which was previously discussed see *section* 2.4. Five different benzimidazole TACN and NO2A derivatives were prepared by attaching the benzimidazole derivatives to azamacrocycles. Two of them have reactive functional groups available for bioconjugation. The strategy described in section 3.2.5 can be used to conjugate chelators **22** and **24** to biological molecules.

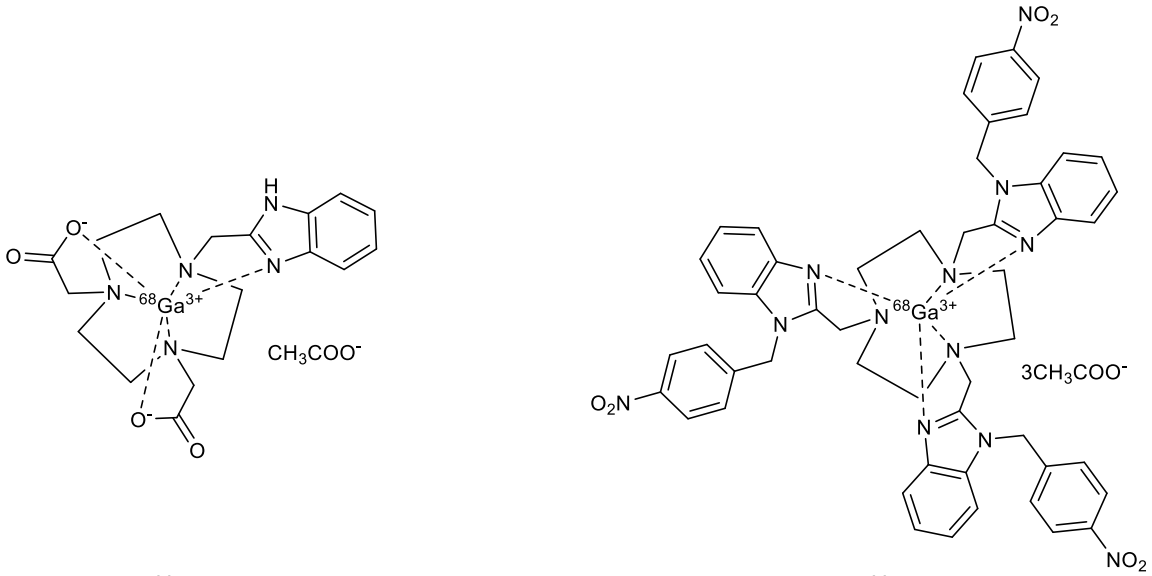


Figure 37 - Synthesised ^{68}Ga BFCs.

To demonstrate the possibility of ^{68}Ga radiolabelling for positron emission tomography (PET) $^{69/71}$Ga complexes of four ligands were synthesised and characterised. ^{68}Ga radiochemical synthesis of **23** was been achieved under varying conditions to optimise the protocol. Three ^{68}Ga complexes of chelators **18**, **20** and **24** have been synthesised. The radiochemical yields were 73%, 55%, 25% and 37% respectively, achieved at 2 mM, 0.6 mM, 1 mM and 25 μM

respectively at room temp with a 5 minute reaction time. This area of research requires further exploration for improvement in a number of areas. To raise RCYs, the ^{68}Ga radiochemistry need further study. The effect of metal ion contamination should be given priority and to avoid introduction of other metal ions (such as iron) modifications must be made. ^{68}Ga complex formation require further studies to optimise the concentration that give maximum RCY for each chelator and to determine the stability *in vitro* and *in vivo.*

Chapter Four

Synthesis, characterisation and biological evaluation of C-functionalised bis-azamacrocyclic derivatives as CXCR4 antagonists

4. Synthesis, characterisation and biological evaluation of C-functionalised bis-azamacrocyclic derivatives as CXCR4 antagonists

4.1. Aims

The aim of this chapter is to synthesise C-functionalised bis-tetraazamacrocyclic derivatives for application as CXCR4 chemokine receptor antagonists. The first step is the synthesis of a C-functionalised cyclam based precursor, see *section 4.5.1*. which can be combined with different types of linker and other macrocycles to form C-functionalised bis-cyclam derivatives. This was followed by complexation of selected C-functionalised bis-cyclam derivatives with copper(II), nickel(II) and zinc(II). Flow cytometry and anti HIV assays have been conducted for some of prepared compounds to assess their utility as CXCR4 chemokine receptor antagonists.

4.2. Chemokines and chemokine receptors

Cytokines are small proteins having molecular weight 8-30 kDa which play a significant role in biological process to send messages to both neighbouring and distant cells.[30] Chemokines are a subfamily of cytokines which control the movement of cellular migration in various types of leukocytes and modulate their trafficking through the relevant chemokine receptors that are present on these cells.[329,330] The human chemokine system is complex and there are 46 chemokines and 18 chemokine receptors that have been identified.[244] Chemokine receptors have multiple functions in normal cells including embryogenesis, angiogenesis and wound healing and participate in many diseases including cancer, AIDS, multiple sclerosis, asthma, allergic disorders, arthritis and inflammatory bowel disease. The overexpression of chemokine receptors was identified in some of these disease conditions and in most of them progression can be addressed by small molecular receptor antagonists.[30,329,331]

4.2.1. The nature of chemokine signalling

Chemokines begin their biological function through binding to the cell surface receptor that belongs to the G-protein coupled receptor (GPCR) super family.[332] A series of intracellular events initiate when the chemokine binds to the receptor mediated by the receptor associated heterotrimeric G proteins, triggering various secondary messenger signalling pathways, which lead to the activation, not only of chemotaxis, but also a wide range of functions in different leukocytes, such as degranulation, phagocytosis or a respiratory burst.[332,333] The conformation of GPCRs shows there are seven transmembrane domains with three intracellular and three extracellular connecting loops that transduce signals via G-proteins. Hydrophilic amino acids

play a pivotal role in the structure of loops which are perpendicular to the plasma membrane. A disulfide bond links highly conserved cysteines in extracellular loop 1 and 2. The effect of an intracellular C-terminal chain which has serine and threonine residues is important for G-protein activation and these residues act as phosphorylation sites for receptor regulation and desensitisation.[334,335] The extracellular N-terminal domain has an overall negative charge which is thought to be necessary for ligand binding.[336]

4.2.2. CXCR4 chemokine receptor

Chemokines can be divided depending on the conserved cysteine motif into four subfamilies C, CC, CXC, and CX3C, among which the CC and CXC chemokines have been widely investigated.[337] One of the most important members of the chemokine receptor family is CXCR4 and it is considered an essential component of the signalling pathways in the body.[336] Studies confirmed that CXCR4 is an important factor in the migration, invasiveness, proliferation and metastasis of tumours and showed overexpression of CXCR4 in a minimum of twenty three different human tumours including non-small cell lung cancer, ovarian and prostate cancer and colorectal cancer but are most prominent in breast cancers.[338,339]

CXCR4 contains 352 amino acids with a negative charge at physiological pH due to the high ratio of acidic amino acids such as aspartate, see Figure 38**.** Its interaction with its natural ligand CXCL12 make it an multifunction molecule involved in many biological processes such as directing the trafficking of stem cells expressing CXCR4, as required in embryo development.[30,340] As mentioned above, high overexpression of CXCR4 in breast cancer led it to be a candidate as a supreme biomarker for tumour imaging. High levels of CXCR4 point to the potential for metastatic spread and therefore the need for severe treatment.[341] By using mouse models, researchers found blocking the CXCR4 receptor led to reduced breast cancer cell invasiveness and metastasis.[342]

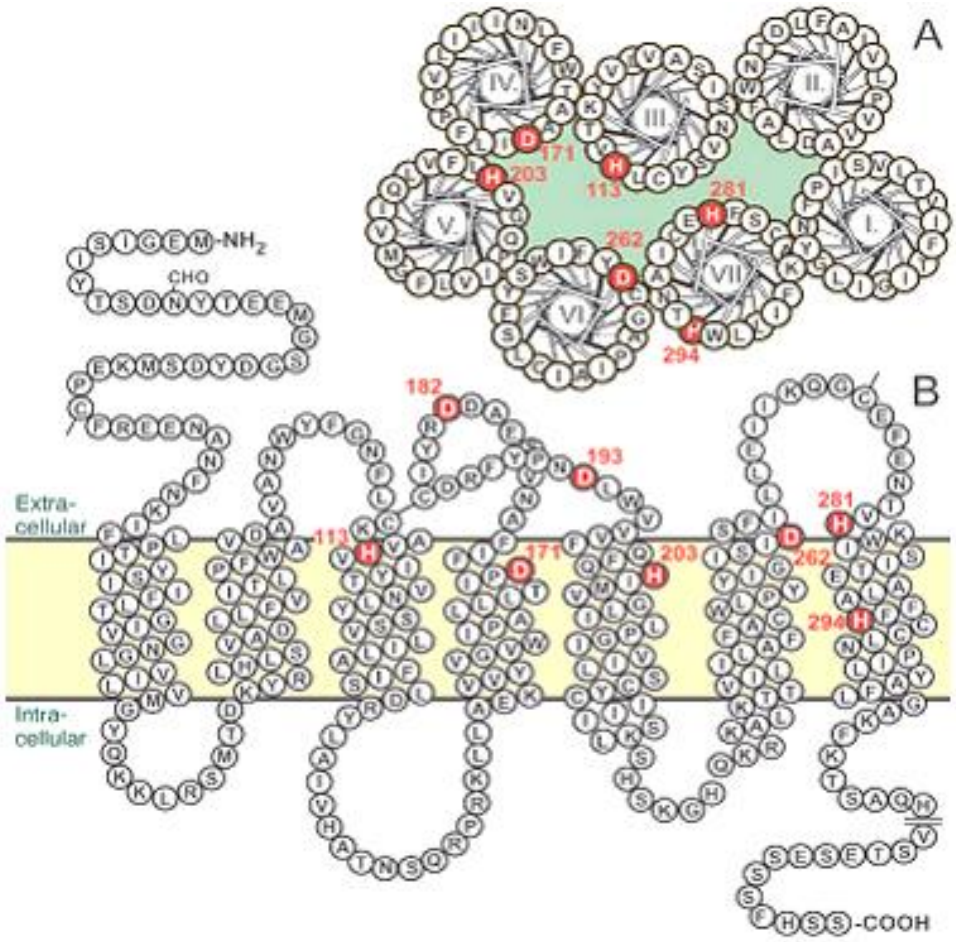


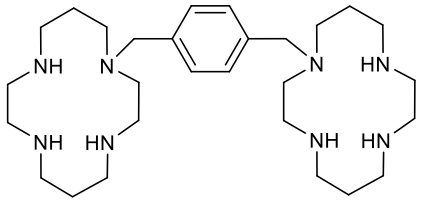
Figure 38 - Amino acid sequence and membrane organization of the chemokine receptor CXCR4. (Reproduced from Nat Rev Drug Discov)[343]

The interactions between the chemokines and chemokine receptors are promiscuous. However CXCL12 is the sole natural ligand of CXCR4 and this interaction between the receptor-ligand pair can initiate metastasis to distant organs.[17] Chemokines are secreted by target organs and work as attractants to metastasising cancer cells.[339] CXCL12 is expressed constitutively in a number of tissues and may elucidate why tumours employ this receptor-ligand pair for metastasis.[331] As mentioned previously, the CXCR4/CXCL12 signalling axis participates in the migration of embryonic cells in the usual growth and development processes. These migrational signals seem to be replicated in tumour progression and metastasis. Therefore, interfering with this gradient by blocking the CXCR4 receptor could give a method for detecting and diagnosing cancers and potentially a valuable method for preventing metastasis.[344] Previous studies showed that small molecule receptor antagonists can be used to mediate the progression of cancers.[345] CXCR4 antagonists are reported as potential

therapeutic and so they are used for the treatment of HIV infection, cancer, and rheumatoid arthritis.[346]

4.3. Tetraazamacrocycles as small molecule CXCR4 antagonists

Macrocyclic complexes are essential to a number of biological processess such as the mechanism of photosynthesis and the transport of oxygen in mammalian and other respiratory systems[347] and have displayed a significant affinity for the CXCR4 receptor.[345] The investigation into macrocyclic structures as CXCR4 antagonists and attempts to link with cancer disease began with their initial success as anti-HIV agents.[348] As an effective HIV inhibitor by blocking the virus' entry into the cell, AMD3100 was discovered in the 1990s and licensed for use in 2009, see Figure 39.[349]



AMD3100

Figure 39 - Chemical structure of AMD3100.

The possibility of using macrocyclic structures for disease control and therapy has been investigated by many groups. A series of specific interactions have been found in the binding mechanism for the macrocyclic compounds with the CXCR4 receptor. The current theory introduced by Gerlach *et al.* is that macrocycles form a series of direct hydrogen bonds with carboxylic acid groups that contribute significantly to the stability of the binding interaction.[350]

4.3.1. Monomacrocyclic CXCR4 antagonists

Many groups of researchers,[245,246] have shown considerable interest in monomacrocycles due to their relative ease of synthesis and the possibility to improve pharmacological properties compared with their bismacrocyclic counterparts because of reduced molecular charge.[98] For example, the small molecule antagonist AMD3465 showed a 10 times higher affinity for the CXCR4 receptor compared to AMD3100.[98] AMD3389, see Figure 40, has the same affinity as AMD3100 for the CXCR4 receptor and was shown to have a similar binding mode.[98,350]

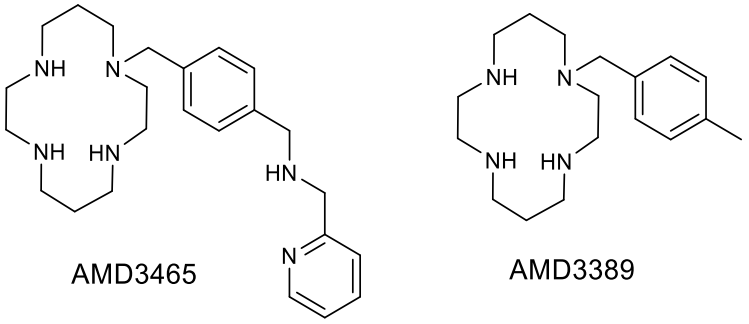


Figure 40 - Chemical structures of AMD3465 and AMD3389.[98,350]

4.3.2. Metal complexes of monomacrocycles

Azamacrocycles are metal chelating agents and studies have confirmed their ability to complex to a wide range of metal ions especially first row transition metal ions.[351] Pendent arms play a significant role in the formation of complexes and can induce and propagate metal complex formation by forming initial binding interactions with the metal ions.[347] The complexation of ligand AMD3100 with Ni^{2+} or Zn^{2+} leads to an increase in the affinity for the CXCR4 receptor compared to the free ligand. Hence, the complexation of AMD3100 with Zn^{2+} increased the affinity of AMD3100 toward the CXCR4 receptor ten fold.[352] It is proposed that the active drug on administration of AMD3100 is actually a complex of AMD3100 with Zn^{2+} ($[Zn_2AMD3100]^{4+}$) which is formed in the body after administration of the ligand as a pro-drug.[353] This result showed that the formation of metal complexes of macrocyclic compounds may offer a method to increase binding to the CXCR4 receptor and prompts the need for further study. The main factor which determines the affinity is the coordination bond. The electronic configuration of the metal ions is a key factor in differentiating the binding affinities but generally the formation of coordinate bonds between the macrocycle and aspartate residues led to an improvement in the affinity.

Conformational changes occur when the metal binds with azamacrocycles such as cyclam.[143,254,354] Through the coordination the four nitrogen atoms become chiral and the N-H bonds reside above or below the ligand plane to produce six possible configurations, see Figure 41. Because of its lower strain energy the *trans*-III is the most prevalent configuration[347] although the new studies led to a proposal that a *cis*-V configuration is most favourable for binding to the CXCR4 receptor. A number of cyclam complexes such as

$[Zn_2AMD3100]^{4+}$ which have shown a high affinity for the CXCR4 receptors adopt a *cis*-V configuration in the solid state. Due to the formation of coordinate bonds metal ions permit stronger interactions at the CXCR4 receptor and also a more stable interaction can be obtained because of the folded geometry that the macrocycle adopts. Studies have indicated that the stability will increase the residence time of macrocycles at the CXCR4 receptor. By using a highly rigid copper(II) complex with a bismacrocycle Archibald and co-workers explained that the residence time at the CXCR4 receptor will be extend when compared to that of AMD3100.[355] This may be advantageous if macrocycles are to be used as diagnostic and therapeutic agents targeting chemokine receptors.

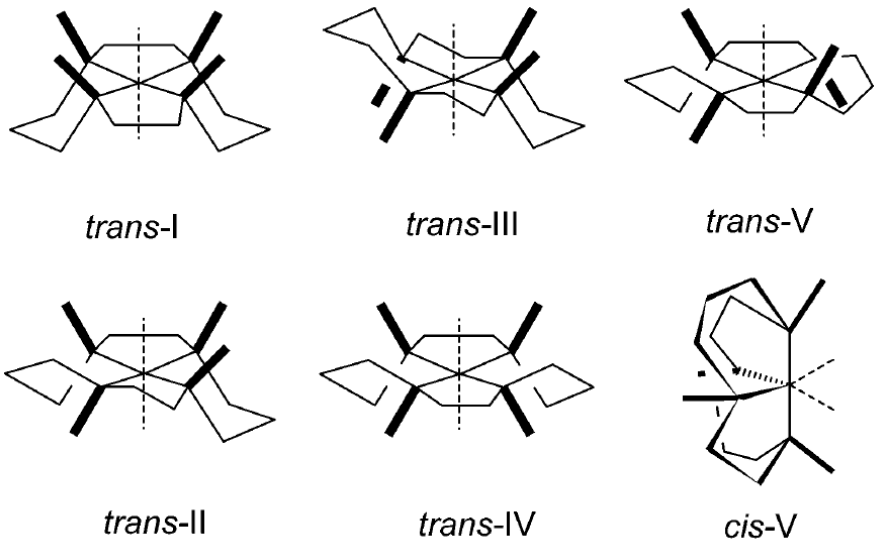


Figure 41 - The six possible configurations of metal cyclam complex.[254]

4.3.3. Bismacrocycles

Bismacrocycles are a group of macrocycles that consist of two macrocyclic rings connected by an aliphatic or aromatic linker which have the ability to work as CXCR4 antagonists through additional interactions with the receptor. They generally have a higher affinity for the CXCR4 receptor than monomacrocyclic compounds.[356] Despite a range of such molecules being synthesised, to date only AMD3100 has been licensed for clinical use, see Figure 42.

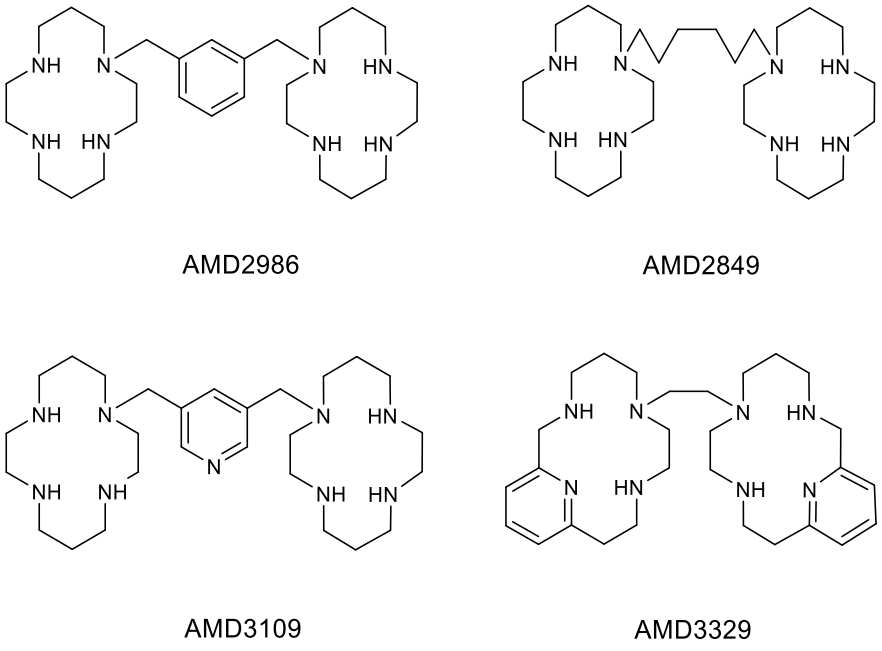
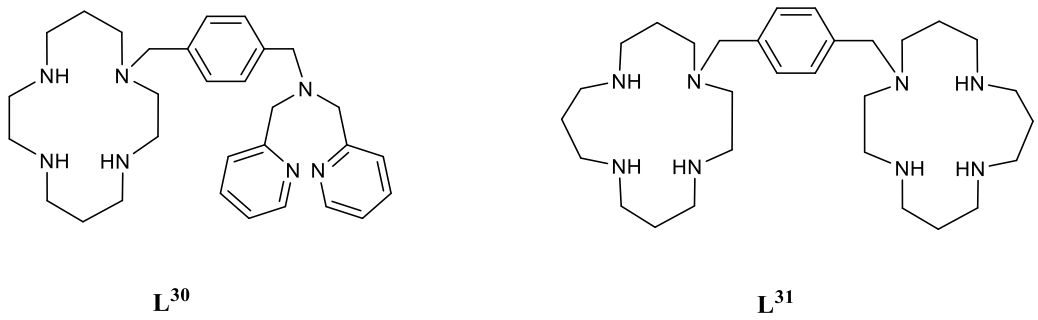


Figure 42 - Chemical structures of some bismacrocycles.[356]

Bismacrocycles can be connected together in three ways; fused, mechanically interlocked or linked.[357] The aspartate residues on the CXCR4 receptor's surface play a vital role in the binding to bismacrocycles and mutagenesis studies revealed that several aspartate residues can interact with bismacrocycles and the key role in binding with macrocycles involves the aspartate residues in positions 171 and 262.[350,358,359] These results explain that an increase in the number of macrocyclic rings led to an increased number of interactions with the aspartate residues thus an increase in the affinity to the CXCR4 receptor. To study the effect of the type and length of linkers on the activity, Este *et al.* conducted a study by choosing several of biscyclam analogues that have linkers which are different in the type and chain length.[352] The results showed that the fused macrocycles were inactive and macrocycles with aliphatic linkers gave low activity whilst the aromatic linker led to high activity. Also the length of linker between the macrocycles and aromatic ring affected the activity with an increase in the aliphatic length causing reduced activity. In general, the inclusion of substituents on the linker decreased the activity with a few exceptions. Another study conducted by Bridger *et al.* to test the activity of 12 to 16 membered bismacrocycles linked by aromatic groups with either *para* or *meta* substitution as anti HIV-1 and HIV-2 agents.[360] The results obtained revealed an increase in the activity from 12 membered rings to 14 membered rings for both the *para* and *meta* substituted bismacrocycles and the activity was reduced for ring sizes that are more than

14 membered. The toxicity results showed inverse properties to the anti HIV activity, toxicity decreased on going from 12 to 14 membered rings whilst increasing for above 14 membered rings, the role of symmetry on the activity was investigated and it was found there was no effect on the activity. Whilst exploring the effect of substitution pattern the group found high activity in 12 and 13 membered rings in the *meta* substituted derivative whilst the *para* substitution pattern is suitable for 14 membered rings. Another noteworthy result from this study is that the electron donating and withdrawing groups on *para* linker did not affect the activity of bismacrocycles and the toxicity of bismacrocyclic compounds is increased considerably in the presence of halogen substituents.

The activity of the bismacrocyclic compounds is also affected negatively by bulky groups substituted in the linker. They found the best linker is aromatic group with methylene groups to link the two macrocycles. In an attempt to explain the relation between different types of bismacrocycles (aza containing compounds) and the ability of these compounds to act as CXCR4 antagonists, Tanaka *et al.*[361] selected cyclam, cyclen, dipicolylamine, homo-cyclam, 2-(pyridin-2-yl)-N-(pyridin-2-ylmethyl)ethan-1-amine, 2-(pyridin-2-yl)ethan-1-amine and di(pyridin-2-yl)amine linked by *para*- or –*meta*-xylene then determined their percentage inhibitions values. They observed that the compounds which have pyridine-groups, cyclen or one of each displayed low inhibition and high inhibition was observed for the compounds linked by a *para*-xylene linker in comparison with the *meta*-xylene linked compounds. The compounds L^{30} and L^{31} showed the highest values, see Figure 43.



L^{30} **L^{31}**

*Figure 43 - Chemical structures of bismacrocycles **L^{30}** and **L^{31}**.*[361]

The percentage inhibition was decreased from 94% to 32% on changing the cyclam to cyclen in ligand L^{30}. This study also found the compounds with two 14 or two 15 membered rings have the highest inhibition values. The least active compound was bis-*para*-linked cyclen. This study also indicated that the type of the linker affect the biscyclam macrocycle's anti-HIV activity and these results matched with those obtained by Este *et al*.[352,361]

4.4. Positioning of the side chain for the additional applications of macrocycles

Recently in medicine there has been an increase in applications for macrocycles that can be functionalised in order to give the chelator two purposes, for example to chelate a metal ion and contain a functional group capable of linkage to a biomolecule. Functionalisation of bis-macrocycles to introduce a way to investigate the relationship between changes in structure and the affinity toward CXCR4 receptors is of interest. Such a study may require the introduction of an imaging component such as an optical probe or PET imaging agent. Usually the basic structure of the tetraazamacrocycle can be adjusted by two methods; by connecting pendant arms at the ring donor atoms or through functionalisation of the carbon backbone.[15] Additional donor atoms for complexation with radionuclide or for bioconjugation can be obtained by substitution at nitrogen atoms. Attachment of pendant arms to the carbon backbone of macrocyclic ring offer a way to keep the two functions (site of biomolecule and metal chelation) intact through a spacer on the carbon backbone, leaving all of the ring nitrogen atoms available for additional pendant coordinating arms. To investigate the effect of the position of side chain at the carbon backbone of macrocycles Kukis *et al.* studied the biological stability of some C-functionalised macrocyclic complexes. They found that the 6-C-functionalised macrocyclic complexes are more stable than 2-C-functionalised macrocyclic complexes.[362] This result was explained by the effect of the side chain position on the flexibility of macrocyclic ring which led to a decrease in the kinetic stability.

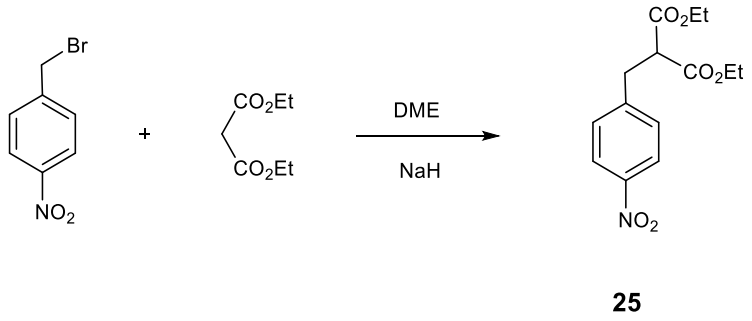
4.5. Synthesis of C-functionalised bismacrocycles ligands

After the target receptor has been identified the next step is designing ligands which have high attraction and ideal *in vivo* properties to target this receptor and assessing their properties using biological assays.

4.5.1. Modified procedure to synthesise C-functionalised cyclam precursor for C-functionalised bis-azamacrocycles in high yield.

Several different synthetic procedures have been reported for selective C-functionalisation of macrocycles comprising the use of metal cations as a template, high dilution method, protection/deprotection systems, functionalisation on carbon atoms and bisaminal intermediates.[312,363] In addition to providing a site for conjugation to biomolecules the C-functionalisation method offers a way to avoid the multistep synthesis of a selected pendant arm. Although tetraazamacrocycles are now commercially available the incorporation of functionalisation at 6-position of cyclam is still synthetically challenging. Most procedures to prepare C-funtionalised macrocyclic derivatives include the use of C-functionalised precursor before the cyclisation. The most commonly used methods that have been used to synthesise such compounds are dependent on the Richman and Atkins cyclisation which includes protecting groups (*p*-toluenesulfonyl).[312] The disadvantages of this method are that the deprotection step requires harsh conditions, it is not atom-economic and it is labour intensive. The bisaminal template method has also been used to synthesise different C-functionalised tetraazamacrocycles. In a variation on the standard bisaminal template method some researchers[363-365] have developed another procedure in which 2,3 butanedione was used as the organic template due to the mild conditions used in deprotection.[366] One-pot techniques have been used to prepare protected C-functionalised macrocycles through reaction of a biselectrophile with the tricyclic bisaminal derivative. Another group have used dihalogenated or ditosylated ethane or propane derivatives that incorparate a wide range of functional groups, such as alcohol or vinyl groups, to synthesise C-functionalised macrocycles.[364] Our group has introduced an efficient procedure to prepare C-functionalised cyclam derivatives through a high yielding cyclisation step which includes the use of commercially available and cheap starting materials, the capability to yield gram quantities of the desired compounds C-functionalised cyclam macrocycles, and finally, offer simple procedure to synthesise a range of selectively N-substituted derivatives.[365] Recently a new class of C-functionalised oxo-cyclam bisaminals have been synthesised through the cyclisation of the linear tetraamine with functionalised-α,β-unsaturated esters.[70] The procedure selected in this work to synthesise 6-C-functionalised cyclam precursor comprises the condensation of the linear tetraamine with substituted malonic esters. The selection of this procedure is to avoid one of the drawbacks of other methods such as the use of column chromatography. This method is a modification of the procedure previously reported by Moran *et al.*[367] The first step in this procedure is the synthesis of a substituted malonic ester diethyl 2-(4-nitrobenzyl)malonate (**25**) which was

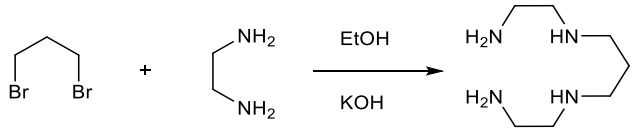
prepared by following the method published by Moreau *et al.*[368] The condensation of 4-nitrobenzyl bromide and diethyl malonate was carried out in dry DME in the presence of NaH, see Scheme 25. Pure product **25** was obtained after recrystallisation (hexane/Et_2O 50:50) in 84% yield.



25

*Scheme 25 - Synthesis of diethyl 2-(4-nitrobenzyl) malonate (**25**).*[368]

The second starting material, the linear tetraamine N^1,$N^{1'}$-(propane-1,3-diyl)bis(ethane-1,2-diamine) was synthesised by the method of Hamilton *et al.*[369] An excess of 1,2-ethylenediamine was added to 1,3-dibromopropane in refluxing ethanol to give **26** in a 60% yield after distillation, see Scheme 26.

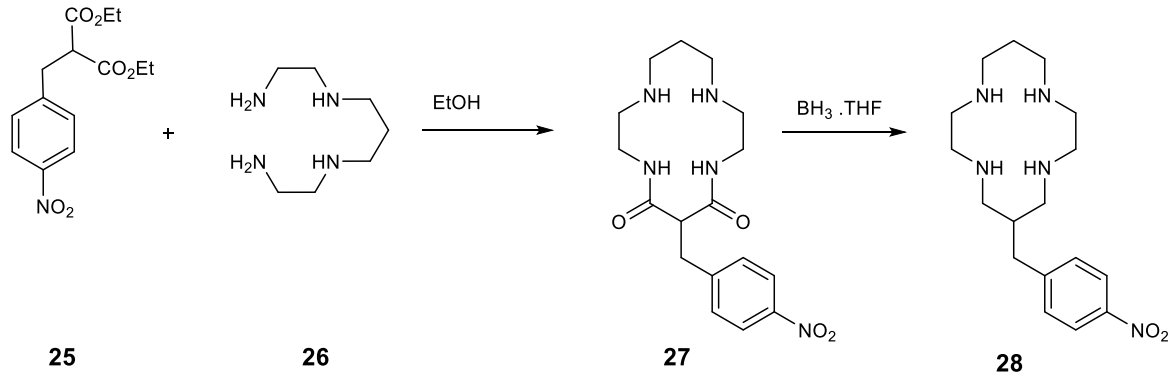


26

*Scheme 26 - Synthesis of N^1,$N^{1'}$-(propane-1,3-diyl)bis(ethane-1,2-diamine)(**26**).*[369]

The next step is to prepare the precursor C-functionalised cyclam is the treatment of 2-(4-nitrobenzyl) malonate (**25**) with linear tetraamine N^1,$N^{1'}$-(propane-1,3-diyl)bis(ethane-1,2-diamine) (**26**). A modification was made to the procedure published by Moran *et al.*[367] by changing the solvent from methanol to ethanol, reducing the reaction time to 17 days and reduced the cooling time at 4 $^{\circ}$C to 2 h. This modified procedure leads to an increase in the yield to 80% compared to a 41% yield stated in the literature.[368] This allows synthesis of large

amounts of precursor to use in the production of a series of different types of C-functionalised macrocycles. The product 6-(4-nitrobenzyl)-1,4,8,11-tetraazacyclotetradecane-5,7-dione (**27**) was obtained by refluxing the mixture of **25** and **26** in dry ethanol for 17 days, see Scheme 27.



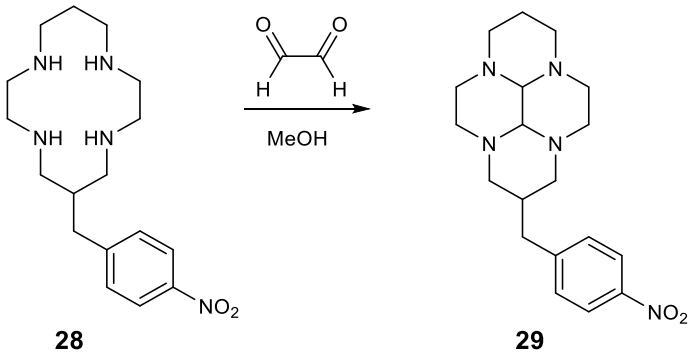
*Scheme 27 - Synthesis of C-functionalised cyclam compounds **27** and **28**.*

The final steps to synthesise the C-functionalised cyclam precursor, 6-(4-nitrobenzyl)-1,4,8,11-tetraazacyclotetradecane (**28**), are dissolving compound **27** in THF then reducing it using BH_3.THF under reflux for 72 h in a dry nitrogen atmosphere. 6M HCl solution was then added and final product **28** separated by basic extraction in an 82% yield. The disadvantage of this synthetic scheme is that the reaction takes a long time due to the slow formation of **27**.

4.5.2. Use of bisaminal route to synthesise C-functionalised cyclam bisaminal precursor

The bisaminal methodology is one of the most influential synthetic procedures in the field of macrocycle synthesis. Bisaminals are simply prepared in high yields by the condensation of aldehydes or ketones with a tetraamine. This route also offers a way for selectively functionalising systems which can be controlled by selection of the α-dicarbonylated species to govern the reactivity of the bisaminal intermediates for selective alkylation. The mono[370] and di[364] alkylation of several macrocycles was achieved by using bisaminal route that led to various selectively functionalised derivatives in addition to their configurationally restricted analogues.

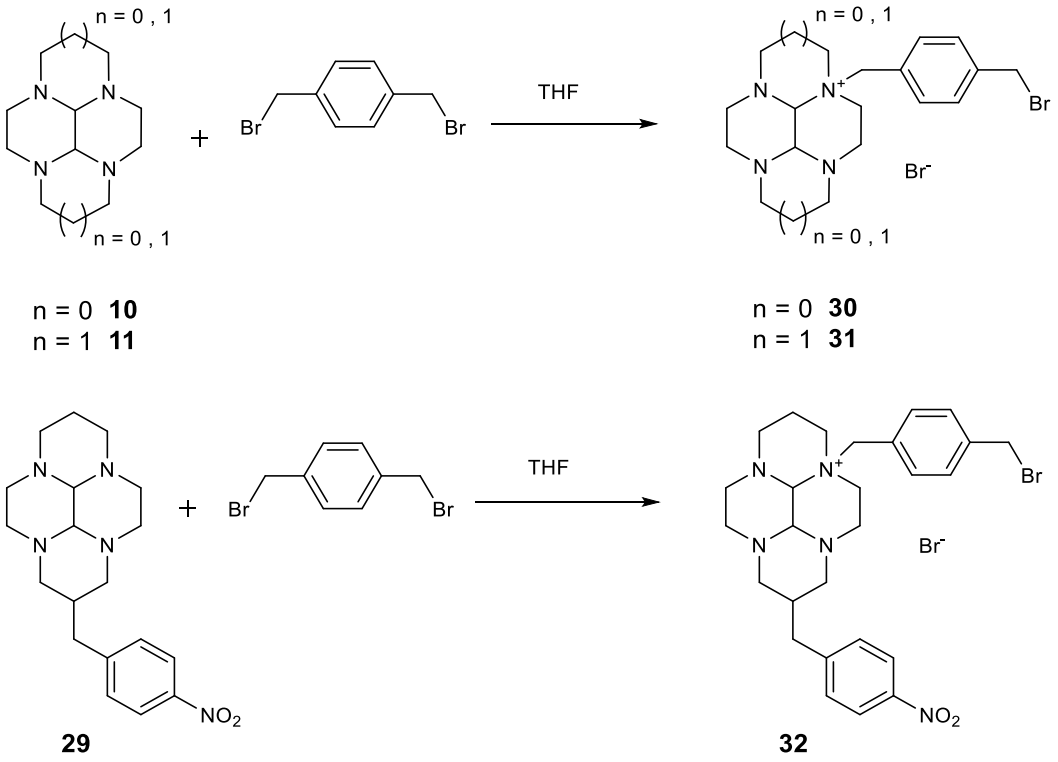
The use of glyoxal to form bisaminals was introduced by Weisman *et al.*[250,259,371] Our group exploited this procedure to synthesise a C-functionalised cyclam bisaminal precursor for selective mono or di alkylation.[365] The product 7-(4-nitrobenzyl)decahydro-1H,6H-3a,5a,8a,10a-tetraazapyrene (**29**) was obtained by following a literature procedure[372] through reaction of the deprotected 6-C-functionalised cyclam **28** with the dicarbonyl glyoxal in cold methanol to give the bisaminal **29** in a yield of 98%, see Scheme 28.



*Scheme 28 - Synthesis of C-functionalised cyclam bisaminal **29**.*

4.5.3. Preparation of a novel mono-N-substituted-C-functonalised cyclam precursor to synthesise C-functionalised bis-azamacrocycles

The procedure used to synthesise C-functionalised cyclam precursor was similar to the method used by Le Baccon *et al.*[373] Three different quaternary salts **30**, **31** and **32** were prepared for use in obtaining a series of C-functionalised bis-azamacrocyclic quaternary salts for comparison. All quaternary salts **30**, **31** and **32** were synthesised by direct alkylation. Bridged cyclen **10** was reacted with α,ˉα-dibromo-p-xylene in dry THF to form **30**. The same reaction was carried out with bridged cyclam **11** with α,ˉα-dibromo-p-xylene in dry THF to prepare **31**. **32** is prepared by following the same procedure; reaction α,ˉα-dibromo-p-xylene with C-functionalised cyclam bisaminal **29** in dry THF, see Scheme 29.

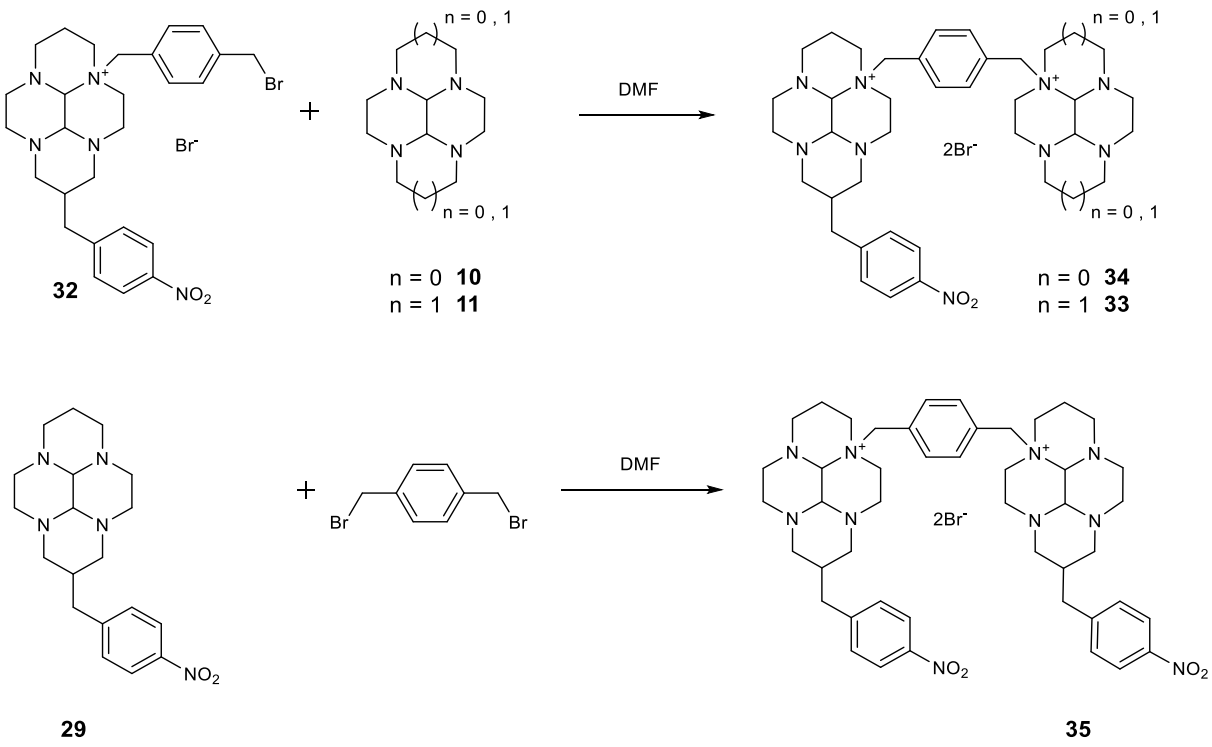


*Scheme 29 - Synthesis of quaternary salts **30**, **31** and **32**.*

The novel quaternary salt **32** offers a precursor for the synthesis of a range of C-functionalised bis-azamacrocycles.

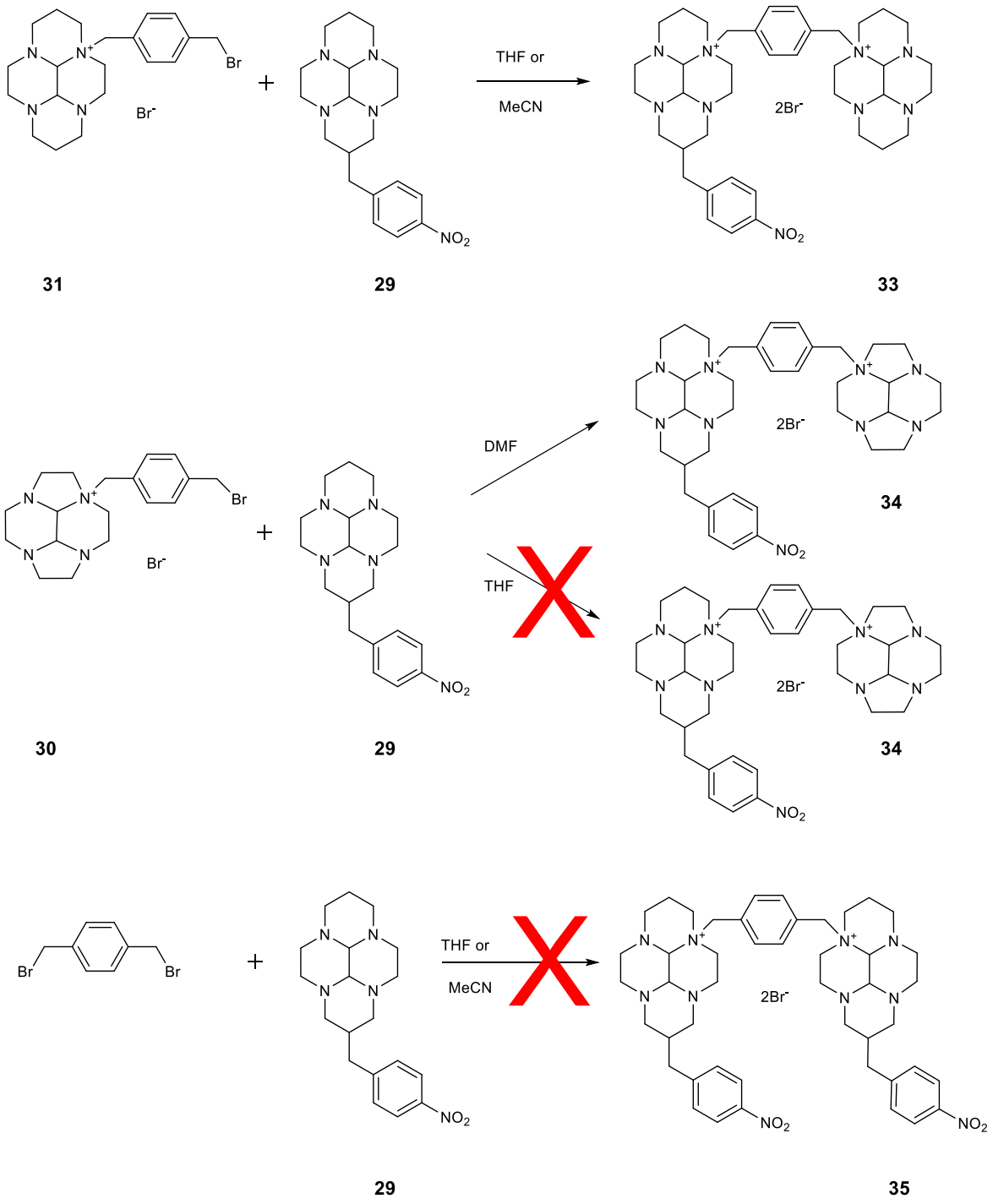
4.5.4. C-functionalised bis-azamacrocyclic quaternary salts

Three novel quaternary salts of C-functionalised bis-azamacrocycles **33**, **34**, and **35** have been synthesised by following a minor modification of a literature procedure,[373] see Scheme 30. Due to the insolubility of the starting material **32** in MeCN, dry DMF was used in the preparation of **33** and **34**. Excess amounts of macrocycles were not used because of the difficult nature of purification and all reactions were carried out at RT to avoid the formation of di substituted products that could occur at high temperature.[260]

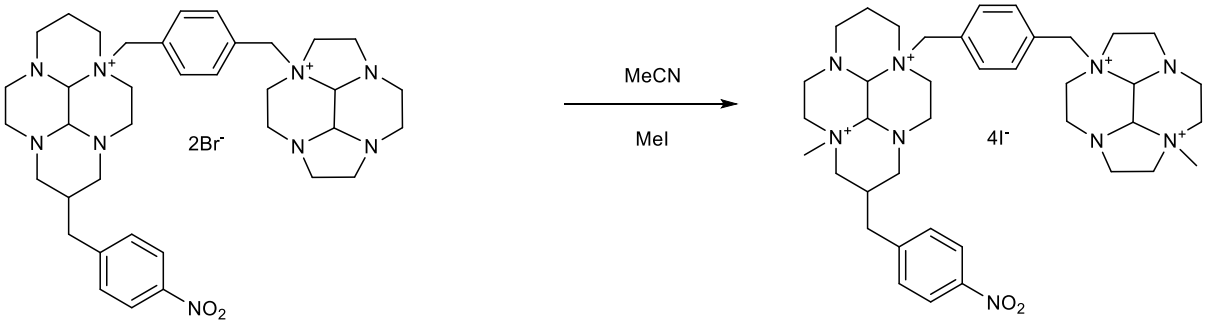


*Scheme 30 - Preferred routes to synthesise quaternary salts **33**, **34**, and **35**.*

For comparison and optimisation of reaction conditions, different methods have been tried to make the compounds **33**, **34** and **35**. The best procedure to give a high yield in the synthesis of a quaternary salt **33** is the reaction of **11** with **32** in dry DMF at RT. The yield was 81% compared with 62% from using the starting materials **29** and **31** in dry THF at RT and 42% by using dry MeCN as solvent to form **33**, see Scheme 31. Compound **34** was obtained in high yield 82% by following the same method as for compound **33** in which starting material **10** was reacted with **32** in dry DMF at RT (compared to 65% by using the starting materials **29** and **30** in dry DMF at RT and no product isolated by using dry THF as solvent). A suitable method to prepare the quaternary salt **35** is the reaction of starting material **29** with α,ˉα-dibromo-p-xylene in dry DMF at RT, which gives a 99% yield. No product was isolated by using either dry THF or dry MeCN as solvent, see Scheme 31. Methylation of quaternary salt **34** to form compound **36** was carried out by suspending the quaternary salt **34** in MeCN and then adding methyl iodide. Compound **36** was isolated in a 77% yield, see Scheme 32. All quaternary salts **33**, **34**, and **35** have been characterised by using MS and ^{1}H NMR. Compound **36** gave a ^{1}H NMR spectrum that contained many complex, broadened and overlapping peaks that was unclear.



*Scheme 31 - Alternative routes to synthesise quaternary salts **33**, **34** and **35**.*

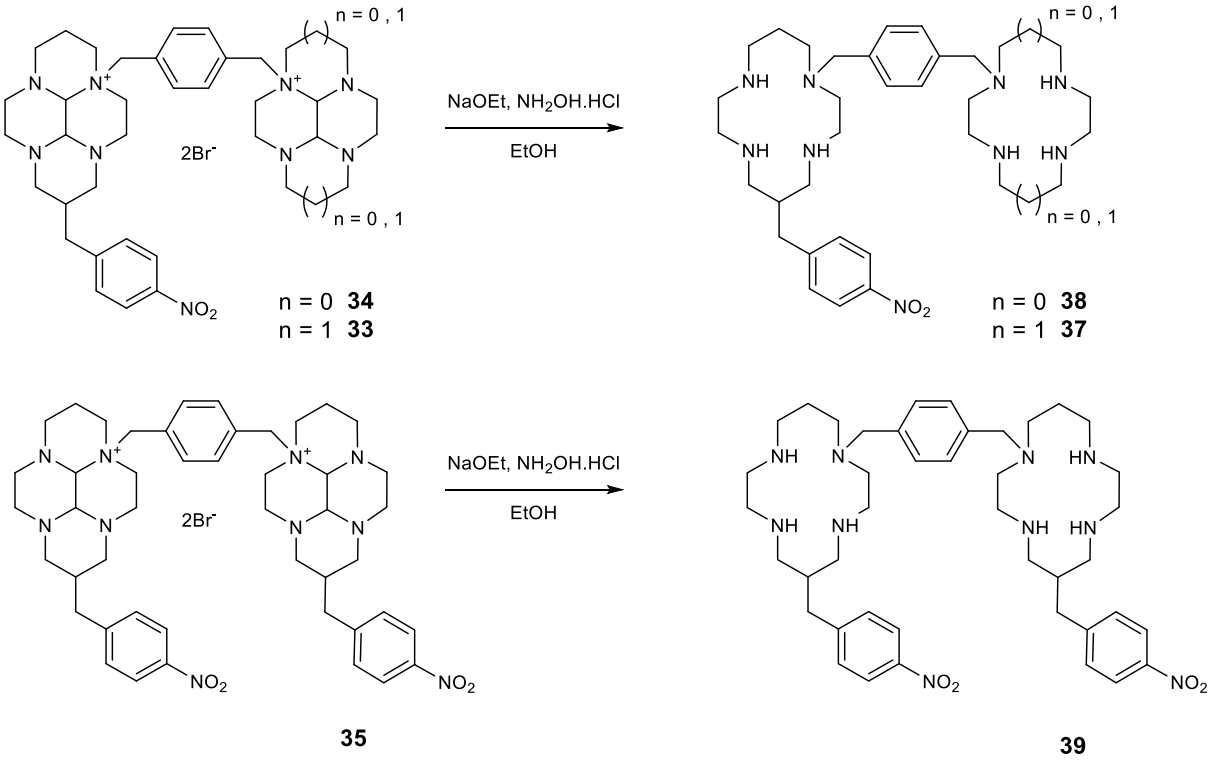


34 **36**

*Scheme 32 - Methylation of quaternary salt **34** to form compound **36**.*

4.5.5. Synthesis of novel AMD3100 analogues with C-functionalised bis-azamacrocycles

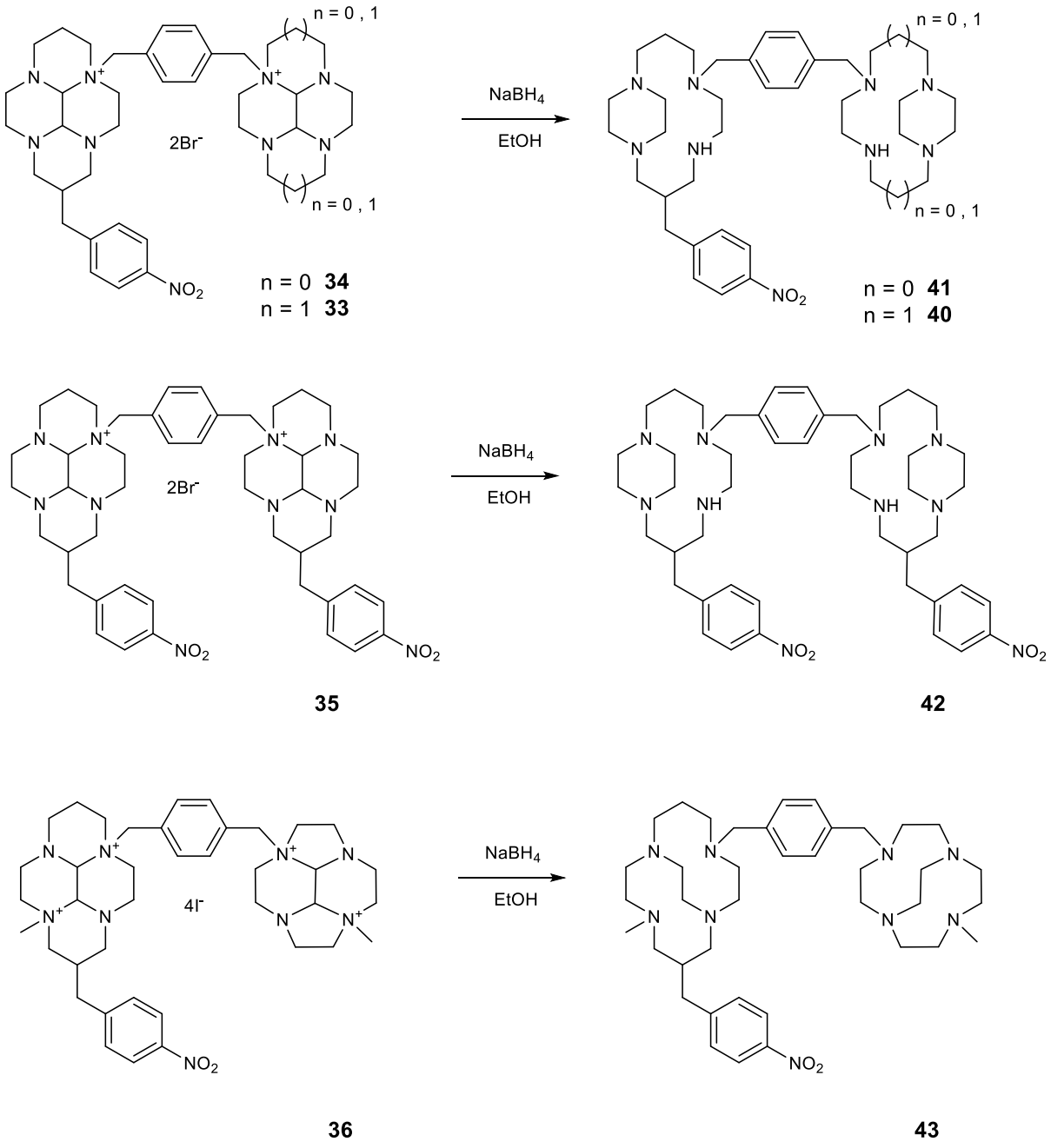
A protection-deprotection route was used to prepare AMD3100 in the first reported synthesis by Fabrizzi and co-workers which used cyclam as starting material with a relatively low yield 30%.[374] Yang *et al*. reported an improved route by using a trifluoroacetyl protecting group and the overall yield was over 70%.[375] A facile route to synthesise of AMD3100 was introduced by Handel and co-workers in which they exploited the bis-aminal route. The bis-aminal bridge was cleaved using a combination of hydroxylamine hydrochloride and sodium ethoxide. The overall yield was over 80%.[243] In this work three novel AMD3100 analogues of C-functionalised bis-azamacrocycles, **37**, **38** and **39**, see Scheme 33, were synthesised by adapting Handel and co-workers method. The yield for compounds **37**, **38** and **39** were 93%, 64% and 33% respectively. All compounds have been characterised by MS and ^{1}H, ^{13}C NMR. The NMR spectra showed the disappearance of the C_{aminal} and H_{aminal} peaks.



*Scheme 33 - Synthetic routes to produce AMD3100 analogues **37**, **38** and **39**.*

4.5.6. C-functionalised bis-azamacrocycles: reduction step

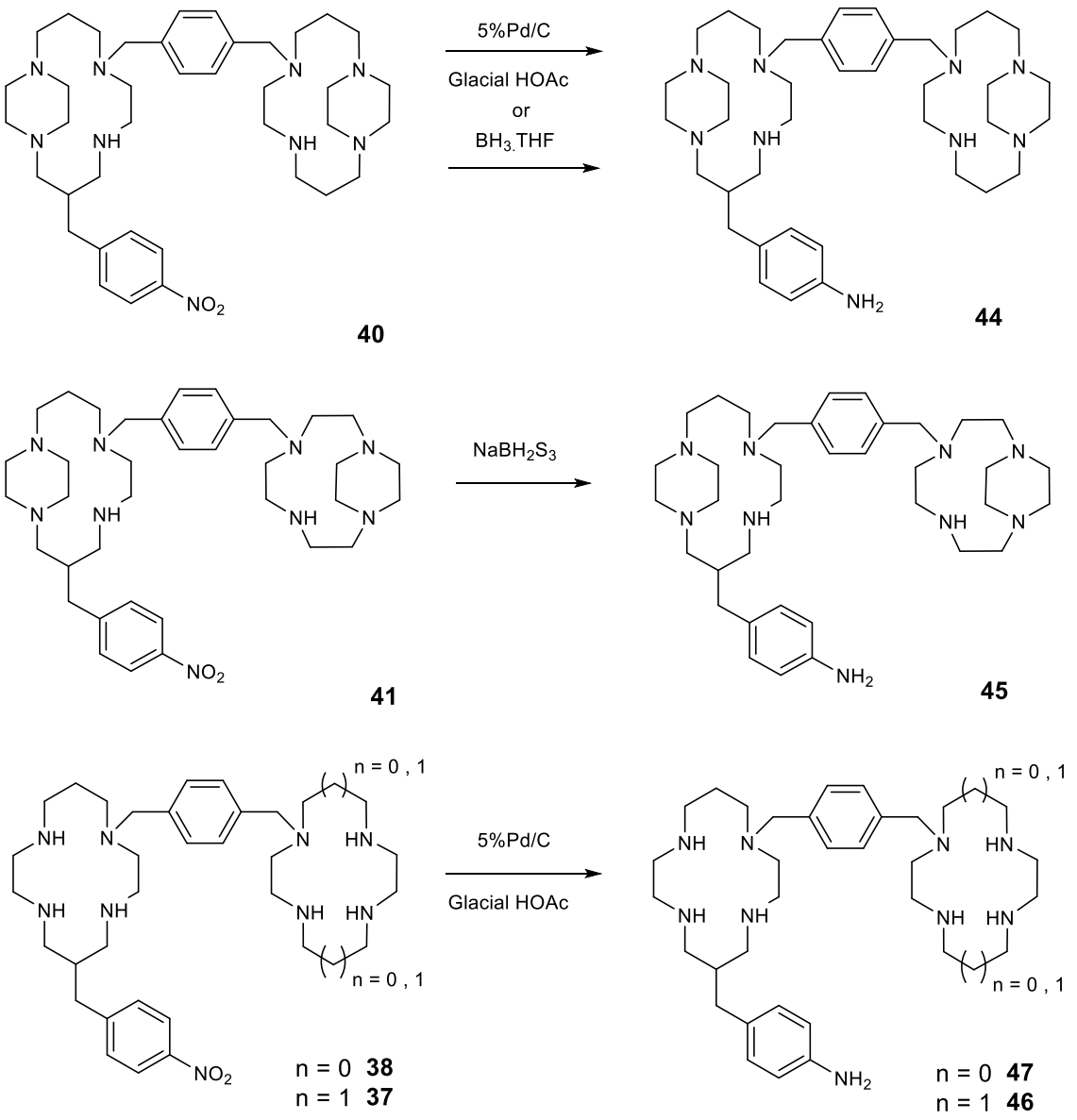
The isolation of final side bridged C-functionalised bis-azamacrocycles products have been achieved by following the literature procedure.[143] Reduction by using $NaBH_4$ in an ethanolic solution is slow (14 days) to prepare the cross-bridged (CB) derivative but affords a single, desired product and the same reagent can be used successfully to synthesise side-bridged (SB) macrocycles in a short time (1 hours). The SB and CB C-functionalised bis-azamacrocyclic products **40-43** were isolated, see Scheme 34, and with a significant variation in yields, typically around 40%, with the exception of **40** where a 100% yield was obtained. These ca. 40% yields obtained were less than those reported by Valks *et al*. and Wong *et al*. where more than 70% was obtained.[143,250] The lower yield may due to the short time used for this reduction. ^{13}C NMR spectrum has been used to confirm the isolation of the appropriate products, **40**, **41**, **42** and **43**, see Scheme 34, which showed the disappearance of the C_{aminal} peaks, although a small amount of impurity was observed from the reduction process. In spite of the overall low yield and long reaction time required to form CB derivatives, four novel structurally reinforced C-functionalised (**40**, **41**, **42** and **43**) bis-azamacrocycles have been successfully synthesised and effectively reduced by using $NaBH_4$.



*Scheme 34 - Synthesis of SB and CB C-functionalised bis-azamacrocycles **40**, **41**, **42** and **43** via reduction with $NaBH_4$.*

4.5.7. Modification of nitro group of C-functionalised bis-azamacrocycles

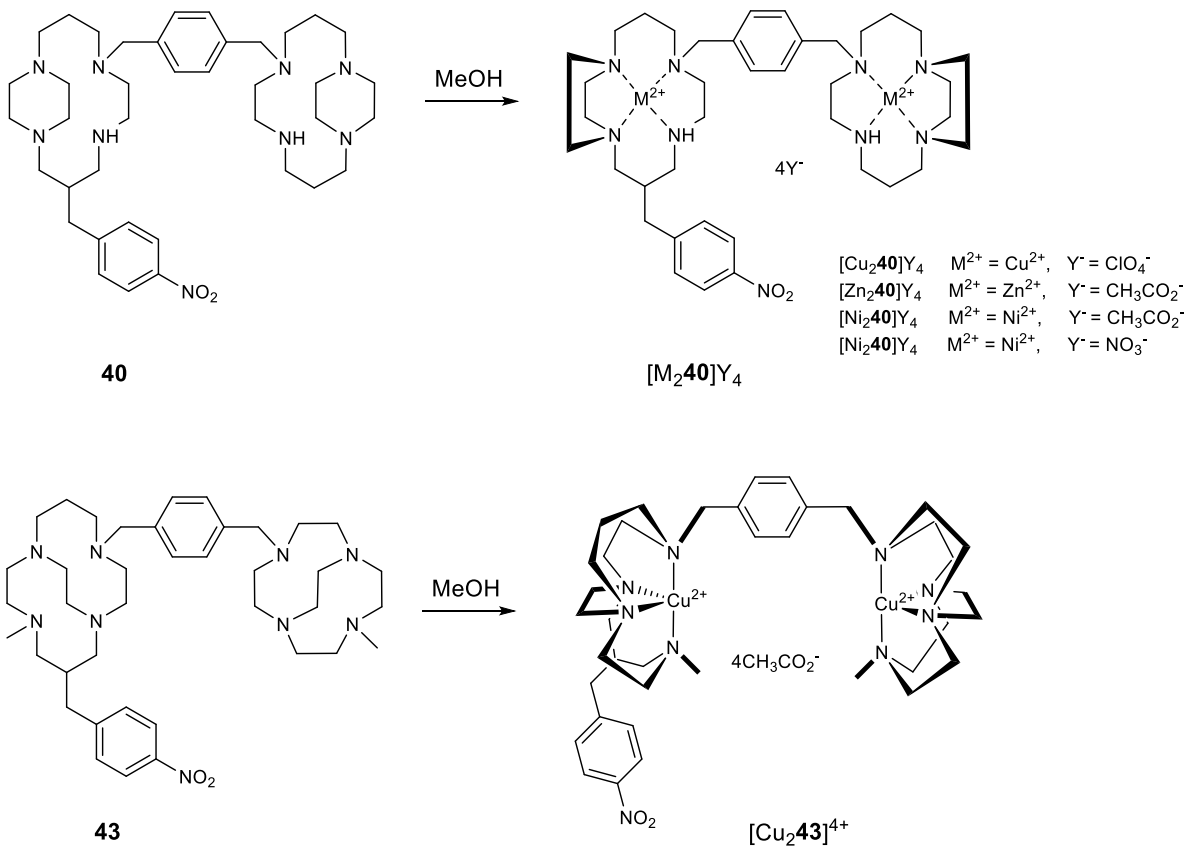
An attempt to modify compounds **37**, **38**, **40** and **41** for facile conjugation the nitro group was reduced to a primary amine, see Scheme 35. This will permit attachment to other molecules, such as fluorescent dyes or radiolabelled groups for molecular imaging. Different routes have been used to reduce the nitro group to an amine, see *section* 2.6.4. In this work three different routes have been tried to reduce the nitro group; the first procedure to reduce compounds **37**, **38** and **40** used 5% palladium on activated carbon to control the extent of reduction and prevent debenzylation, see Scheme 35. Compounds **37**, **38** and **40** were successfully reduced after 4 h in glacial acetic acid and quantitative yields were achieved. The specific shift of peaks in the aromatic region in ^{1}H NMR is characteristic of a nitro reduction and confirmed the formation of **44**, **46** and **47**, and MS characterisation was also consistent. This method was unsuccessful with compound **41** and so an alternative route was attempted by using sulfurated borohydride in THF, a similar method to that which was successful in *section* 2.6.4. The reaction product was isolated and analysed showing the characteristic ^{1}H NMR aromatic peak shift to demonstrate the formation of **45**. Reduction of compound **40** by this method was unsuccessful and so a third method was attempted using BH_3.THF as the reducing agent. Compound **40** was successfully reduced via this method; using BH_3.THF and heating to reflux for 1 week. Product **44** was characterised via ^{1}H NMR, which showed the expected aromatic peak shift, and MS.



*Scheme 35 - Reduction of nitro group to primary amine to form C-functionalised bis-azamacrocyclic derivatives **44**, **45**, **46** and **47**.*

4.6. Synthesis of metal complexes of C-functionalised bis-azamacrocycles

Two of the prepared C-functionalised bis-azamacrocycles ligands, **40** and **43**, were selected for transition metal complexation. Ligand **40** was reacted with copper(II) perchlorate, zinc(II) acetate, nickel(II) acetate and nickel(II) nitrate while the ligand **43** was reacted with copper(II) acetate, see Scheme 36. Acetate salts were chosen to synthesise most of complexes as this counter ion forms water soluble complexes which have no known toxicity effects. Also the acetate salt was selected for consistency in most of complexes to confirm that any differences detected between compounds in the biological assessment were due to the macrocycle structure only and not because of counter anion.



Scheme 36 - Synthesis of C-functionalised bis-azamacrocycle copper(II), zinc(II) and nickel(II) complexes. The coordination number for the copper(II) complex is likely to be 5 or 6 with bound solvet and/or counter anions..

Metal complexation of CB macrocycles requires longer reaction times because of the reduced flexibility of the chelator. Increased reaction times were employed to make sure metal ion encapsulation occurred in both SB and CB macrocycles. In general, high yields were obtained (60-90%) although $[Ni_2\mathbf{40}](NO_3)_4$ gave relatively low yield 50%. Solvent molecules were noted in the elemental analysis for complex $[Ni_2\mathbf{40}](CH_3CO_2)_4$ however this is consistent with previously reported crystallographic data for related compounds which often shows the association of solvent molecules within the unit cell.

4.7. Biological evaluation of synthesised C-functionalised bis-azamacrocycles

The affinity and activity of macrocyclic compounds for CXCR4 in cell lines known to overexpress the receptor was determined by using biological assays. A series of different types of biological assays were used in this work.

4.7.1. Displacement (antibody competition) binding assay

The displacement assay is a method used for initial screening of the macacrocycles before more in depth assays are conducted. It was carried out using Jurkat cells, a human leukaemia cell line that overexpresses the CXCR4 receptor, normal white blood cells express about 10,000 copies of the CXCR4 receptor on the cell surface whilst Jurkat cells express up to 140,000 copies. The macrocycle under study competes for binding to the CXCR4 receptor against a CXCR4 specific mAb tagged with a fluorescent label. The percentage of inhibition is measured by detection of the label. A low level of displacement of the macrocycle by the mAb correlates with a high percentage and indicates that the macrocycle has high affinity for the CXCR4 receptor. A low percentage of inhibition means high fluorescence reading due to a high degree mAb attachment via displacement of the macrocycle from the CXCR4 receptor. The principles of the assay comprise incubation of the macrocycle under study with Jurkat cells at a saturating concentration, then excess macrocycle is removed and the mAb added. There is then a second incubation period followed by removal of the excess mAb. Flow cytometry is a technique which detects the amount of fluorescently labelled mAb there is bound to each cell (each individual cell is analysed in the flow chamber) of which 10,000 are counted per sample and the percentages of inhibition can be measured from this data. The assay limits can be determined by using negative and positive controls. The negative control has a mAb that will not bind to the CXCR4 receptor and hence should give zero fluorescence; the positive control contains only CXCR4 specific mAb generating a reading for 100% fluorescence (the maximum value).

4.7.2. Anti-HIV activity

The ability of macrocycle to prevent infection of HIV can be determined by using anti-HIV assays. The main cell culture used is MT-4 (NL4.3WT), a human T cell leukaemia cell line. An AMD3100 resistant MT-4 cell line also exists and offers a tougher test for macrocycles as there are mutations in the CXCR4 chemokine receptor. Anti-HIV activity and cytoxicity measurements can be determined by evaluating the viability of the cells against different concentrations of macrocycles under study in cells that have been exposed to HIV-1, HIV-2 or SIV.[376] The HIV virus can enter cells utilising either the CXCR4 or CCR5 chemokine

receptor hence the X4 HIV-1 viral strain was used which utilises exclusively the CXCR4 receptor for viral cell entry. The process includes culturing the cells for five days and counting the number of viable cells by using the tetrazolium-based colourimetric method defined by Pauwels *et al.* and De Clercq and co-workers [377,378] The cells were infected with HIV as described by Schols *et al.*[379]

4.7.3. Calcium signalling assay

This assay can provide information which can be used to conclude if the macrocycles are having an antagonistic or agonistic effect by exploiting the calcium(II) flux downstream effect. On stimulation of CXCR4 by its natural ligand CXCL12 several downstream effects occur, the flux in intracellular calcium(II) is a one of them. To study how efficiently macrocycles block the natural signalling, calcium(II) signalling studies are used. A set of different concentrations of the compound under study is incubated with CXCR4 expressing U87.CD4.CXCR4 cells and on adding CXCL12 the amount of calcium(II) released should be lower than normal because the receptors are blocked. To calculate the amount of calcium(II) released a calcium sensitive fluorescent dye is used and a reduced fluorescence correlates with a reduced calcium(II) flux. This data was used to obtain IC_{50} values which are defined as the concentration needed to reduce the calcium(II) flux to 50% of the normal level.[380]

4.7.4. C-functionalised bis-azamacrocycles as CXCR4 antagonists

Zinc(II), nickel(II) and copper(II) coordinated C-functionalised bis-azamacrocycles were evaluated as CXCR4 antagonists, alongside with the corresponding free ligand in displacement assays against a CXCR4 specific mAb. This data is presented in Table 3. In general all C-functionalised bis-azamacrocycles (free ligands and metal complexes) were confirmed as binding to the CXCR4 receptor by flow cytometry displacement studies. The first point to note regarding the percentage inhibitions is that the free chelators (C-functionalised bis-azamacrocycles **37**-**47** (except **38**)) are significantly poorer at competing for the receptor than the metal complexes. This supports the plan to include the metal ions in the macrocyclic cavities. The benefit from having a metal ion present more than outweighs any decrease in H-bonding potential. Configurationally restricted ligands have a more rigid shape which can slow the kinetics of complex formation and dissociation; such properties are important when these complexes are to be used *in vivo*.[381] Generally, free chelators containing one bridged cyclen macrocycle showed lower inhibition than C-functionalised bis-azamacrocycles containing two bridged cyclam macrocycles. Also presence of bridged unit appeared to impact percentage inhibition for the free chelators. The metal free chelator **38** (C-

functionalised bis-azamacrocycles AMD3100 analogue) showed higher inhibition than the other metal-free SB or CB C-functionalised bis-azamacrocycle chelators tested. The compounds where both macrocycles are functionalised via the carbon atom gives lower inhibition than compounds containing only one C-functionalised macrocycle. The change of the functional group from nitro to amine seemed not to influence percentage inhibition for the free chelators in this case but our group has previously observed higher inhibition from an amine functionalised macrocycle.[31] Inhibition of CXCR4 by zinc(II), nickel(II) and copper(II) complexes of C-functionalised bis-azamacrocycle **40** were comparable, all displayed more than 90% inhibition see Table 3 and this assay does not differentiate between compounds as has been observed in the literature previously where zinc(II) complexes were frequently found to have the highest activity.[31,143,144,382] The assay is showing full inhibition for all of the metal complexes (within error), hence a more rigorous and challenging assay is required to differentiate between them.

Sample	Av[a] % inhibition Jurkat	Anti-HIV activity IC_{50}[b]/μM MT-4 (NL4.3WT)	Cytotoxicity CC_{50}[c]/μM MT-4 (NL4.3WT)	Calcium signalling IC_{50}[b]/μM U87.CD4.CXCR4
37	**–**	**3.30**	**74.38**	**>7.80**
38	**97**	**0.40**	**14.7**	**0.55**
39	**32**	**1.77**	**43.55**	**>6.46**
40	**59**	**1.03**	**–**	**>1.00**
$[Cu_2 40](ClO_4)_4$	**99**	**0.11**	**–**	**0.37**
$[Zn_2 40](CH_3CO_2)_4$	**92**	**0.05**	**–**	**0.28**
$[Ni_2 40](CH_3CO_2)_4$	**100**	**0.045**	**–**	**0.48**
$[Ni_2 40](NO_3)_4$	**94**	**–**	**–**	**–**
41	**–**	**3.42**	**15.18**	**>7.55**
42	**38**	**3**	**42.94**	**>6.05**
43	**17**	**11.90**	**61.56**	**>7.24**
$[Cu_2 43](CH_3CO_2)_4$	**–**	**0.14**	**55.07**	**–**
44	**41**	**6.27**	**60.44**	**>7.57**
45	**31**	**11.20**	**63.93**	**>7.91**
46	**–**	**>70**	**164**	**–**
47	**32**	**>172**	**172**	**–**
AMD3100		**0.025**	**>1.98**	**0.0006**

Table 3 - Biological assay data produced for C-functionalised bis-azamacrocycles and metal complexes. [a] *Experiments were run in either duplicate or triplicate and the results averaged.* [b] *Concentration required to reduce the level of* Ca^{2+} *ions observed during a 'normal' signalling process by 50% (IC_{50}) in U87.CD4.CXCR4 cells.* [c] *Concentration required to reduce cell viability by 50% (CC_{50}) in MT-4 (NL4.3WT) cells. – = Awaiting data.*

Results from the displacement assay confirmed that functionalisation on carbon atom of bismacrocycle frame did not significantly disrupt the affinity toward CXCR4 receptors while the functionalisation on phenyl moiety on AMD3100 has previously been shown to decrease the percentage inhibition in derivatives of AMD3100 studied by Poty *et al*.[383]

Further assays were conducted to determine if C-functionalised bis-azamacrocycles are efficient CXCR4 antagonists as the inhibition data only shows the capability to disrupt antibody binding. Anti-HIV assays indicate distinct differences between the complexes. Nickel(II) and zinc(II) complexes are significantly more active than copper(II) complexes in all cases, see Table 2, these findings are supported by the literature.[31,143,144,382] Zinc(II), nickel(II) and copper(II) complexes of C-functionalised bis-azamacrocycle **40** showed lower anti-HIV activity in comparison to AMD3100, IC_{50} values of 52 nM, 45 nM and 116 nM vs 25 nM respectively. Significantly lower activity was observed for the free chelator **40** as was expected. The copper(II) complex of C-functionalised bis-azamacrocycle **43** was much more potent in its anti-HIV activity than free chelator **43**.

Calcium(II) signalling studies were conducted on the free ligands and complexes. CXCL12 signal blocking calcium flux studies showed that all the 'free' ligands; **37-47** were considerably less active than AMD3100. The free ligands trend in potency observed in the anti-HIV activity data was reproduced in this assay. Compound **38** was the most potent ligand.

The difference in potency between the free ligands may be explained by their overall shapes. The long, straight shape of **38** may cover more of the receptor surface preventing CXCL12 from binding better than the rest of the ligands. Calcium(II) signalling studies to complexes of ligand **40** displayed greater affinity toward the CXCR4 receptor than than free ligand. The observed trend in potency for the metal complexes in the anti-HIV activity data was changed in this assay and the trend was zinc(II) > copper(II) > nickel(II). $[Zn_2\mathbf{40}](CH_3CO_2)_4$ was the most potent complex, IC50: 280 nM. Note that all of the C-functionalised bis-azamacrocycles (free ligands and metal complexes) showed lower cytotoxicity compared to AMD3100. In conclusion, C-functionalised bis-azamacrocycle **38** was seen to have the highest potency as a CXCR4 antagonist of the compounds tested (although its metal complexes were not investigated in these assays). The most active anti-HIV agent was also **38** which showed better activity and lower toxicity compared to other ligands.

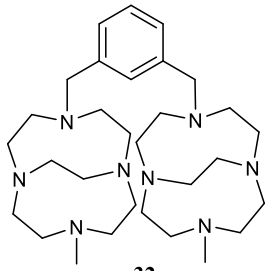
The results obtained in this study in which the transition metals complexes of C-functionalised bis-azamacrocycles showed higher affinity for CXCR4 receptor than their corresponding free ligands are consistent with the concept introduced by Gerlach *et al.*[384] where they found that incorporation of zinc(II) or other transition metals into the macrocyclic rings of AMD3100 enhances the binding affinity to the CXCR4 receptor 6-36-fold. They explained that the increased binding affinity of the metal ion substituted AMD3100 is obtained through enhanced interaction of one of the cyclam ring systems with the carboxylate group of Asp 262. This is attributed to via a strong concomitant interaction of one of the oxygens directly with the metal ion and the other oxygen to one of the nitrogens of the cyclam ring through a hydrogen bond.

Results summarised in Table 2 are supported by the study conducted by Valks *et al.*[143] where a zinc(II) complex of a configurationally restricted bismacrocyclic cyclam was shown to be a highly potent CXCR4 antagonist and only one configuration was obtained in solution. *In vitro* study of this zinc(II) complex showed high activity against HIV infection (EC_{50} = 2.5 nM).

Our group synthesised and studied the affinity toward CXCR4 receptors of many of azamacrocyclic ligands and their complexes. Khan *et al.* synthesised the copper(II) complex of a novel rhodamine-azamacrocycle and found that the metal complex competes for receptor binding more effectively than the free protonated chelator, supporting the idea that a direct coordination interaction with the receptor from the metal centre not only enhances but activates binding.[244] Smith synthesised metal complexes of a *meta*-substituted CB biscyclen compound L^{32}, see Figure 44, which were found to have a high affinity for the CXCR4 receptor *in vitro* studies (IC_{50}; $[Cu_2L^{32}]^{4+}$ = 3.6 nM, $[Zn_2L^{32}]^{4+}$ = 3.4 nM).[382]

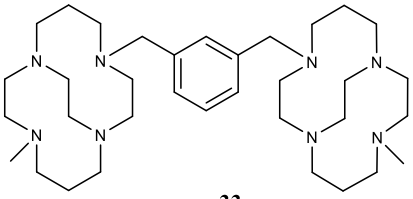
Also Smith *et al.* synthesised a nickel(II) complex of *meta*-linked CB bis-macrocycle, L^{33}, as a CXCR4 antagonist.[31] $[Ni_2L^{33}]^{4+}$ along with $[Ni_2L^{34}]^{4+}$, a previously published bis-macrocycle synthesised by McRobbie *et al.*, were tested as anti-HIV-1 agents.[144] IC_{50} values were calculated following calcium signalling assays and compared with AMD3100.[31] $[Ni_2L^{34}]^{4+}$ was found to be the most effective CXCR4 antagonist with an IC_{50} value of 14 nM, this is comparable to AMD3100. Cytotoxicity assays revealed that all of the nickel(II) complexes have a CC_{50} of more than 125 μM. Anti-HIV assays were conducted using an X4 strain which exploits the CXCR4 receptor for viral cell entry. The *para*-complex, $[Ni_2L^{34}]^{4+}$, was more active than $[Ni_2L^{33}]^{4+}$ but less active than AMD3100, EC_{50} values of 74 nM, 398

nM and 11 nM respectively were observed showing that the nickel(II) complexes have high anti-HIV-1 activity and low cytotoxicity, see Figure 44.



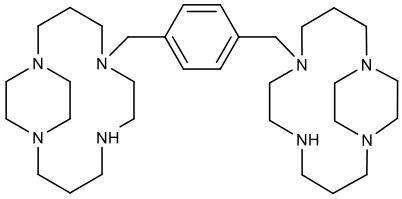
L^{32}

$[Zn_2L^{32}]^{4+}$: IC_{50} : 3.4 nM
$[Cu_2L^{32}]^{4+}$: IC_{50} : 3.6 nM



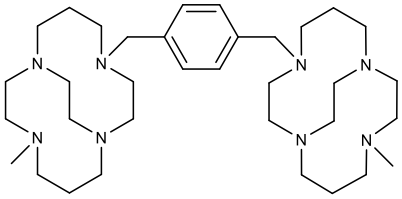
L^{33}

$[Ni_2L^{33}]^{4+}$: IC_{50} : 194 nM
EC_{50} : 398 nM



L^{34}

$[Ni_2L^{34}]^{4+}$: IC_{50} : 14 nM
EC_{50} : 74 nM
$[Zn_2L^{34}]^{4+}$: EC_{50} : 2.5 nM
$[Cu_2L^{34}]^{4+}$: EC_{50} : 26 nM



L^{35}

$[Cu_2L^{35}]^{4+}$: EC_{50} : 4.3 nM

Figure 44 - Chemical structures of bis-macrocycles synthesised by Archibald and co-workers alopng with their biological activity.[31, 35, 382] The metal ions are bound in the cavities of the tetraazamacrocycles.

Our group also synthesised and assessed $[Cu_2L^{35}]^{4+}$ as an anti-HIV-1 agent and found it had greater activity than AMD3100 when tested on HIV-1 infected MT-4 cells.[355] The EC_{50} value for $[Cu_2L^{35}]^{4+}$ was 4.3 nM. The increase in activity was attributed to a longer receptor residence time due to coordinate bonds forming with the copper(II) complex as opposed to weaker hydrogen bonds detected with AMD3100. However, the increase is also thought to be due to the optimisation of the configuration because complex formation of AMD3100 with copper(II) leads to a decrease in activity. The macrocyclic structure favours the metal ion forming coordination bonds with oxygen atoms from the carboxylate groups on the aspartate residue when bound to the CXCR4 protein. All of these results were consistent with those

obtained in this study, see Figure 44. More detailed and robust conclusions will be drawn once all the biological data has been obtained.

4.8. Conclusions

A novel route to synthesise a series of C-functionalised bis-azamacrocycles has been developed. Three types of AMD3100 related C-functionalised bis-azamacrocycles **37**, **38** and **39** were successfully synthesised. The nitro group was efficiently reduced to the amine in the synthesised AMD3100 analogues and two new C-functionalised bis-azamacrocycles **46** and **47** were obtained. Another three novel of side bridged C-functionalised bis-azamacrocycles **40**, **41** and **42** have been prepared and two new C-functionalised bis-azamacrocycles **44** and **45** were produced by reducing the nitro group to amine in the compounds **40** and **41**. A novel cross bridged C-functionalised bis-azamacrocycle **43** was synthesised. Zinc(II), nickel(II) and copper(II) complexes of C-functionalised bis-azamacrocycle **40** were synthesised and characterised. The copper(II) complex of ligand **43** was successfully synthesised. Most of synthesised compounds were biologically evaluated in a number of assays with the aim of determining the optimum structure for CXCR4 antagonism.

Most of C-functionalised bis-azamacrocycles and zinc(II), nickel(II) and copper(II) complexes of ligand **40** and copper(II) complexes of ligand **43** were tested through displacement assays, anti-HIV assays, cytotoxicity assays and calcium(II) signalling assays. Both the free ligands and metal complexes showed a quantifiable inhibitory percentage although the metal complexes in general were significantly higher. More than 90% inhibition of anti-CXCR4 mAb binding was achieved with zinc(II), nickel(II) and copper(II) complexes of ligand **40**. Generally free ligands containing one bridged cyclen macrocycle showed lower inhibition than C-functionalised bis-azamacrocycles containing two bridged cyclam macrocycle. Ligand **38** showed higher inhibition than the other ligands with a high potency. Functionalisation of both macrocycles via the carbon atoms lowered inhibition relative to a single functionalised macrocycle. All free ligands and metal complexes of ligand **40** showed lower cytotoxicity relative to AMD3100. Zinc(II), nickel(II) and copper(II) complexes of ligand **40** showed lower anti-HIV activity in comparison to AMD3100, IC_{50} values of 52 nM, 45 nM and 116 nM vs 25 nM respectively. The copper(II) complex of the C-functionalised bis-azamacrocycle **43** displayed lower anti-HIV activity than free ligand **43**.

Calcium(II) signalling assay studies of the complexes of ligand **40** demonstrate higher affinity towards the CXCR4 receptor than the free ligand. Robust conclusions can be drawn once the full set of the biological data has been obtained.

Chapter Five

Conclusions and future work

5.1. Conclusions

5.1.1. Overview

This work focuses on the synthesis and applications of three different classes of macrocycles. The three 'results' chapters of this work discuss in detail the main achievements and the applications of each type of macrocycle. Chapter two includes the synthesis of a group of cyclen benzimidazole derivatives and their complexes with copper(II), nickel(II) and zinc(II). This chapter demonstrates the development of several novel cyclen benzimidazole ligands through structural rigidification and functional group modification, as improved chelating ligands. Some of the selected ligands and complexes were tested as anti-fungal drugs.

Chapter three reports the synthesis of bifunctional chelators based on the macrocyclic backbone TACN and NO2A functionalised with benzimidazole arms which potentially offer advantages over related NOTA derivatives. Several benzimidazole bifunctional chelators were produced and ^{68}Ga radiolabelling carried out to show potential in PET applications. The development of PET imaging agents has received a lot of attention in recent years for a wide range of applications. The incorporation of ^{68}Ga(III) into the macrocycle cavity is attractive due to the 68 minute half-life of the radioisotope allowing reasonable radiochemistry reaction and purification times, and suitable timescales for imaging studies to allow full clearance of the tracer from the bloodstream.

Chapter four highlights the synthesis of novel C-functionalised bis-azamacrocycles for use as CXCR4 expressing cancer diagnosis and therapeutic agents. Macrocycles have been extensively used as chelating agents in the literature, but transmetallation and toxicity has limited their clinical application in some cases. The structural reinforcement and functionalisation present in the compounds discussed in this work can counteract these limitations. The development of therapeutic agents for CXCR4 expressing cancers has also received considerable interest as a result of its association to metastasis which is responsible for the most of deaths in cancer patients. The well-known strong-interaction between macrocyclic ligands and the CXCR4 chemokine receptor has attracted many research groups and led to the development of novel CXCR4 antagonists. The propensity of macrocyclic ligands to bind to the chemokine receptor CXCR4, which is identified to be overexpressed on at least 23 different human cancers means that these compounds have the potential to be targeted probes. This chapter outlines the synthesis of novel C-functionalised bis-azamacrocycles which were structurally reinforced and some of them coordinated to transition metals to provide optimum interactions with the CXCR4 receptor. A series of novel C-

functionalised bis-azamacrocycles and selected metal complexes were biologically evaluated to determine their capability in CXCR4 antagonism for the diagnosis and therapy of CXCR4 expressing cancers.

5.1.2. Main achievements

The capability to synthesise hetero-substituted cyclen derivatives is significant because this permits the physical properties of the compounds to be manipulated, tailoring them to the desired application. Rigidifying the cyclen skeleton to prepare configurationally restricted compounds, has received considerable interest from many research groups, not just because they are relatively easy to synthesise but also because they have shown promising results *in vitro*. In chapter two bridged cyclen **10** was used to synthesise configurationally restricted mono-ring compounds. Benzimidazole has a nitrogen donor for coordination and a second heterocyclic N-position for bioconjugation. In this chapter, the synthesis of bifunctional chelators was carried out. This was initially achieved by synthesising N-functionalised benzimidazole derivatives to provide a reactive arm for bioconjugation. A benzimidazole precursor with a nitro functional group present **9** was synthesised in a three step reaction sequence, with alkylation followed by cyclisation, and lastly chlorination in an overall yield of 50%. Attachment of the N-functionalised benzimidazole derivative **9** along with another benzimidazole derivative, 2-(chloromethyl)benzimidazole, to bridged cyclen **10** was achieved to form macrocyclic bifunctional chelators with five donor atoms and a potential site for bioconjugation. Synthesis of benzimidazole cyclen derivatives (**1**, **2**) was challenging due to 'self-reaction' at the imidazole NH position. In an attempt to overcome this, a large amount of dry acetonitrile (*ca.* 400 ml per gram) was used as the solvent, the reaction stirred at room temperature for 8 days and a slight excesses of bridged-cyclen **10** [1 : 0.8] included to avoid 'self-reaction product **12a**' of benzimidazole that may be formed. The successful synthesis of a series of novel, configurationally restricted, N-functionalised cyclen compounds bearing a benzimidazole or benzimidazole derivative pendant arm (**1**, **2**, **3**, **4**, **5**, **6**) and their copper(II), zinc(II) and nickel(II) complexes was achieved. Configurational restriction was obtained through addition of an ethylene bridge between opposite nitrogen atoms to form cross bridged (CB) compound or between adjacent nitrogen atoms to form side bridged (SB) compound. A pendant arm; benzimidazole or a benzimidazole derivative was attached to one of the nitrogen atoms on the macrocyclic skeleton and sulphur activated sodium borohydride was found to reduce the nitro group to an amine group in compounds **3** and **4** in yields of 81% and 64% respectively. Monomacrocycles may offer improved pharmacological properties therefore

some of ligands and complexes were selected to test their ability for use as antifungals. Most of compounds involved in this test showed a recognisable activity. Ligand **3** and [Zn**1**]Cl_2 have the highest anti-fungal activity of the series tested (MIC= 4.07 μM) and (MIC= 5.61 μM) respectively.

Five novel bifunctional chelators based on the macrocyclic backbone TACN and NO2A and functionalised with benzimidazole arms (**18**, **20**, **22**, **23**, **24**) have been synthesised and fully characterised with details of this work included in chapter three. The synthesis and radiolabelling of bifunctional chelators was carried out. N-functionalised benzimidazole derivative **9** and 2-(chloromethyl)benzimidazole were used as arms to form macrocyclic bifunctional chelators with six donor atoms and with a potential site for bioconjugation. The nitro derivatives were converted into a primary amine (**22**, **24**) to give a useful reactive group for bioconjugation by reduction using sulphur activated sodium borohydride. Three chelators were finally deprotected to give two acetate arms for metal complexation (**18**, **23**-**24**), with this reaction optimised under microwave irradiation to give reaction times of 10 minutes.

$^{69/71}$Ga complexes of the four ligands were synthesised as a proof of principle and HPLC traces of the complexes were compared with the ligands. Radiolabelling of BFCs with ^{68}Ga was developed and one BFC was chosen for ^{68}Ga radiolabelling optimisation using different conditions. The best conditions were using a 2 mM concentration of chelator at room temperature for 5 minutes, increasing the reaction time offered no advantage in radiochemical yield achieved for any of the conditions attempted. The optimised conditions were then used to radiolabel the remaining three chelators and synthesise four ^{68}Ga complexes. This study confirms that the benzimidazole TACN and benzimidazole NO2A derivatives can be synthesised and successfully radiolabelled with ^{68}Ga. The group of chelators have wider potential applications than the related NOTA chelator due to the introduction of the benzimidazole unit. Ligand **24** is proposed as the lead compound for future study as it has an amine group which is ideal for bioconjugation.

A novel route to synthesise a series of new types of C-functionalised bis-azamacrocycles, **37**-**47**, containing a reactive group for functionalisation has been outlined in chapter four. The synthesis of the novel C-functionalised bis-azamacrocycles offers the opportunity for use in imaging applications. Except compounds **39** and **42**, the C-functionalised bis-azamacrocycles were isolated by reacting equimolar amounts of mono-substituted mono-macrocycle quaternary salts together before reduction with $NaBH_4$ to isolate **37**-**47** in good yield. In

general the nitro group was reduced to an amine to provide the reactive site for bioconjugation. The synthesis of this type of bismacrocyclic derivative containing an amine group has not been previously reported. Copper(II), nickel(II) and zinc(II) complexes of the C-functionalised bis-azamacrocycle **40** were synthesised to improve the interaction with the CXCR4 receptor and the biological profile of these complexes *in vitro* was determined. As symmetry is not required for high affinity these compounds may show improved interaction with the CXCR4 receptor.[360] Excluding free ligand **38**, displacement assays showed that the free ligands were generally poorer (31%-59% inhibition) at competing for binding to the CXCR4 receptor against a CXCR4 specific mAb, compared to the metal complexes which all showed >90% inhibition. Poorer inhibition was generally observed for C-functionalised macrocycles containing one of the bridged-cyclen macrocycles. Anti-HIV activity data revealed that compound **38** was highly potent compared to the rest of the C-functionalised bis-azamacrocycles. Further data is required to fully understand the biological activity of the remaining C-functionalised bis-azamacrocyclic compounds.

5.2. Future work

5.2.1. Short-term goals

Much can be done to rapidly extend the research reported in chapter two, including:

- Preparing the metal complexes of ligands **5** and **6** with copper(II) nitrate, nickel(II) nitrate and zinc(II) nitrate.
- Studying the antifungal activity for the all of the complexes that have been prepared to determine the full picture of the relationship between structure of the complexes and the antifungal activity.
- Determination of the HPLC conditions for the analysis of ligands **5** and **6**.
- Synthesis of the cold gallium(III) complexes of ligands **5** and **6** and radiolabelling ligands **5** and **6** with ^{68}Ga after obtaining the HPLC conditions for the cold gallium(III) complexes.

Chapter three reports the synthesis and radiolabelling of TACN-based chelators with ^{68}Ga but other aspects and applications can be realised, including:

- A full study (including HPLC conditions) of both cold and hot gallium(III) and copper(II) complexes of the chelator **22**, with its amine functional group which is ideal for bioconjugation.

- A study of both cold and hot metal complexes of the chelators **20**, **23** and **24** with the following metal ions and respective applications. $^{64}Cu/^{86}Y$ (PET), ^{111}In (SPECT), and $^{90}Y/^{177}Lu$ (radioimmunotherapy). This data would validate a chelator system in which varying the metal ion gives access to the widest range of applications.

Further useful studies for chapter four would be:

- Synthesis of the metal complexes of C-functionalised bis-azamacrocycles **37-39** and **41-47** with copper(II), nickel(II) and zinc(II).
- Evaluate the binding affinity of the prepared complexes toward CXCR4 chemokine receptors by studying them using flow cytometry and calcium signalling assays.

5.2.2. Long-term goals

Chapter two:

- Synthesise the metal complexes of ligands **1**, **2**, **3**, **4**, **5** and **6** with manganese(II) and iron(II) and study the antifungal activity for these complexes.
- Radiolabel the ligands **5** and **6** with ^{64}Cu after obtaining the HPLC analysis conditions for the cold copper(II) complexes.

Chapter three:

- After synthesis of the BFCs in chapter three and the formation of ^{68}Ga complexes has been successfully achieved as a proof of principle. More effort must now be dedicated to this area to improve this system for *in vivo* use.
- To achieve this a range of studies need to be completed. Initially, the complex must be shown to be stable and non-toxic *in vitro* for the time of a simulated *in vivo* study (ca. 4 hours) to decide if demetallation could occur *in vivo*, consequently causing a background signal increase.
- This should be followed by a specific activity study, carried out so as to conclude how much of the chelator can be radiolabelled at the lowest concentration. This is essential for *in vivo* use when the targeted moiety has a relatively low target concentration, therefore causing receptor saturation. Next, the partition coefficient (log P) of the ^{68}Ga complexes should be determined. This information gives insight

into the BFC's properties *in vivo*. If this study showed acceptable results a biodistribution would be obtained in an animal study to evaluate the *in vivo* characteristics of the complex, stability and excretion pathways. This can be combined with a metabolite study to conclude if the complex remains intact.

- The reactive amine groups of the BFC can be evaluated, initially to elucidate if the arm is still reactive enough for conjugation but also to illustrate that the modification does not have a damaging effect on the radiolabelling properties. Initially this could be performed with a small, well characterised biomolecule such as biotin, which can easily be evaluated *in vitro* to show that biotin conjugation to the BFC does not affect its ability to bind to streptavidin. If these criteria are met then other targeting molecules can be evaluated with a view to *in vivo* targeted imaging.

Chapter four:

- Optimising the yields of **41**, **42** and **43**, possibly by increasing the reaction time for **41** and **42** and by carrying out further extraction cycles to **43**.
- Synthesise a series of tris-SB-C-functionalised macrocycles by using the quaternary salts of C-functionalised bis-azamacrocycles. Alternatively it would be interesting to determine whether a tris-macrocycle made up of three cyclam macrocycles, or tris-macrocycles containing cyclen in the terminal positions, would have a strong effect on affinity. Another set of compounds to explore could be tris-CB-C-functionalised macrocycles, see Figure 45.
- Prepare a series of metal complexes of the ligands in Figure 45 and to study their affinity toward CXCR4 receptor by using flow cytometry and the calcium signalling assay.
- Study the ^{64}Cu radiolabelling of the highest affinity CXCR4 antagonist of the C-functionalised bis-azamacrocycle copper(II) complexes.

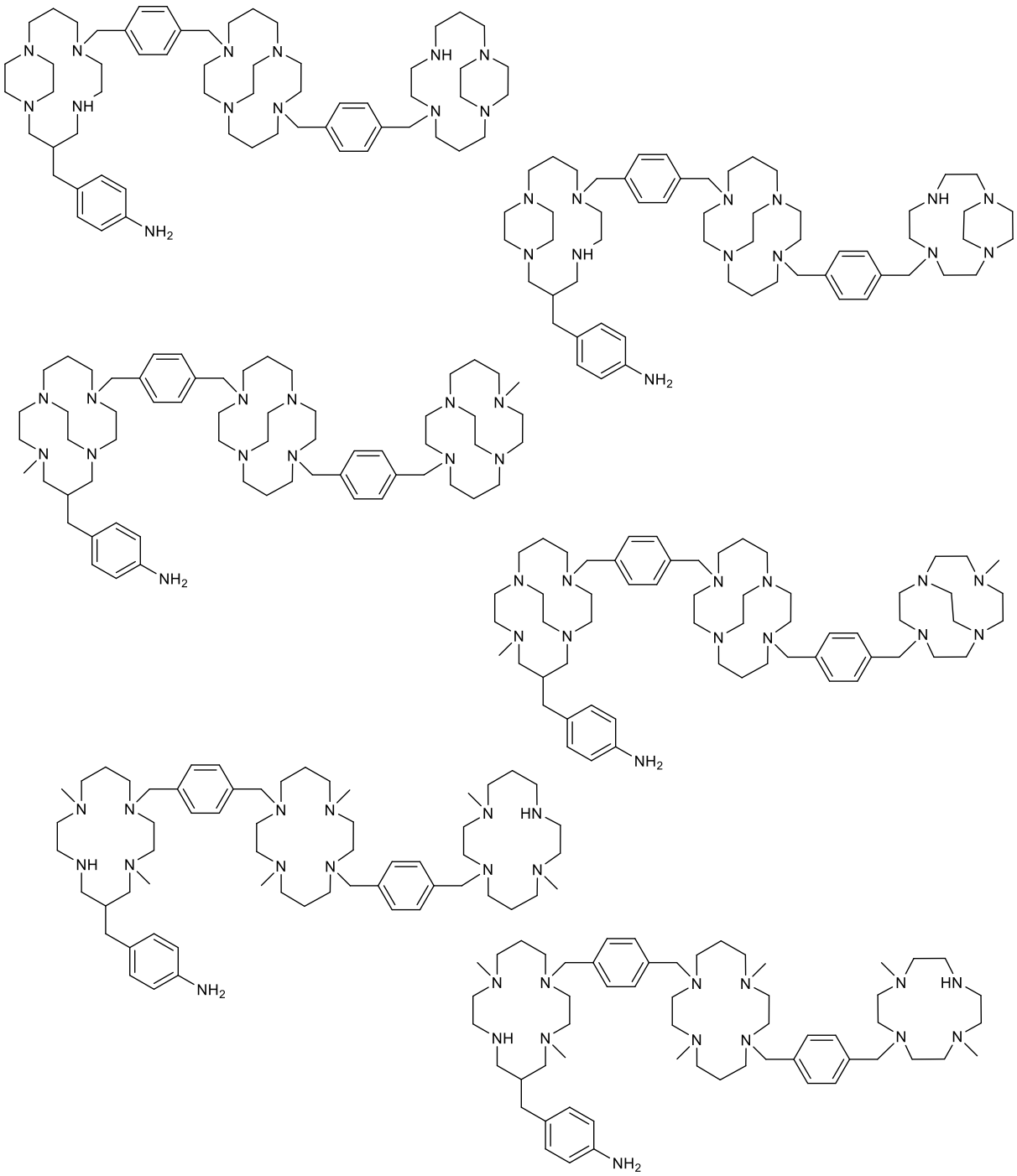


Figure 45 - Chemical structure of potential tris-CB-C-functionalised cyclam and tris-SB-C-functionalised cyclen macrocycles.

Chapter Six

Experimental

6.1. General methods

The compounds (**7**[237], **8**[237], **9**[237], **10**[27], **11**[27], **16**[322], **25**[368], **26**[369], **27**[367], **28**[367], **29**[372], **30**[373] **and 31**[373]) have been previously synthesised. ^{1}H NMR and ^{13}C NMR spectra were obtained using a Jeol JNM-LA400 spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Different types of solvents were used for the analysis and referenced against standard internal TMS or residual non-deuterated solvent signal. All NMR solvents were purchased from Cambridge Isotopes Ltd or Goss chemicals Ltd. Chemical shifts (δ) are indicated in parts per million, ppm, for ^{1}H NMR and ^{13}C NMR spectra, coupling constants (J) reported in Hertz (Hz). Splitting patterns are labelled as s: singlet, d: doublet, dt: double triplet, t: triplet, q: quartet, quin: quintet, m: multiplet, td: triple doublet and br: broad signal. Electrospray MS was performed at the University of Hull using a Finnegan MAT 900 XLT system. Accurate mass spectrometry measurements (HRMS) were recorded using a LQT Orbitrap XL at the EPSRC National Mass Spectrometry Service Centre at the University of Swansea. An Agilent 8453E UV-vis diode array spectrometer was used to obtain UV-vis spectra by using quartz cells. CHN analyser EA1108 (Carlo Erba) was used to record CHN data and all compounds were within the limit of 0.4% of the expected percentages. Rotary evaporation was used to remove bulk solvent via a Buchi RE 111 evaporator equipped with a diaphragm vacuum pump, trace solvent was removed on a Schlenk line equipped with an oil pump. Reactions were achieved at room temperature RT unless otherwise stated. All metal complexes were purified *via* size exclusion chromatography using sephadex LH20, which was pre-soaked in MeOH for three hours before use.

6.1.1. Materials

Reagents for chemical reactions were purchased from Fisher, Sigma-Aldrich, Acros, CheMatech and Strem. Chemicals were used as obtained without further purification. Solvents used were of general purpose grade and used as received. Acetonitrile (MeCN), dichloromethane (DCM), tetrahydrofuran (THF), ethanol (EtOH) and methanol (MeOH) were dried over 3 Å molecular sieves, following activation at 300 $^{\circ}$C for 18 h, according to literature methods.[385]

6.2. Procedure used for ^{68}Ga preparation

^{68}Ga must be formulated to be in a method suitable for the radiochemical reaction. $^{68}GaCl_3$ is eluted from a 20mCi Eckert & Ziegler IGG100 ^{68}Ge/^{68}Ga generator in 5 ml of 0.1 M HCl. This is then loaded on a phenomenex strata X-C solid-phase extraction cartridge. The sample is then eluted using an acetone: 0.1 M HCl solution (98:2) to give the desired amount of

activity for the reaction. The solution was dried using a heating block at 90 °C with a flow of compressed air.

6.4. High performance liquid chromatography (HPLC)

High performance liquid chromatography was carried out using either an Agilent 1200 series or an Agilent 1100 series using a Phenomenex Gemini 5 µ C18 110 Å, 150 x 4.60 mm column at 1 ml/min. Both equipped with a UV detector (series G1314A) and a NaI radiodetector. Data was recorded using Lablogic Laura (version 4.1.13.91).

6.5. Standard HPLC methods

Method 1: (a) acetonitrile 0.1% TFA (b) ammonium acetate buffer (0.2 M, pH 5). Gradient from 100% (b) to 40% (b) over 14 minutes follow by returning to 100% (b) after 15 minutes.

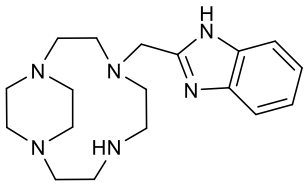
Method 2: (a) acetonitrile 0.1% TFA (b) ammonium acetate buffer (0.2 M, pH 5). Gradient from 100% (b) to 0% (b) over 20 minutes follow by returning to 100% (b) after 25 minutes.

Method 3: (a) acetonitrile 0.1% TFA (b) ammonium acetate buffer (0.2 M, pH 5). Gradient from 95% (b) to 40% (b) over 17 minutes follow by returning to 95% (b) after 20 minutes.

Method 4: (a) acetonitrile 0.1% TFA (b) ammonium acetate buffer (0.2 M, pH 5). Gradient from 50% (b) to 0% (b) over 20 minutes follow by returning to 50% (b) after 22 minutes.

6.6. Synthetic procedures

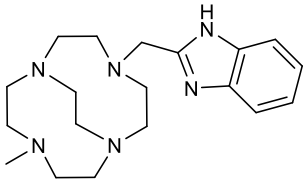
6.6.1. Synthesis of 4-((1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[8.2.2] tetradecane (1)



1

N-(2-Methylbenimidazolyl)cis-13-1,4,7,10-tetraazatetracyclo[5.5.2.$0^{4,14}$ $0^{10,13}$]tetradecane chloride (**12**) (0.48 g, 1.33 mmol) was dissolved in EtOH (100 ml) then $NaBH_4$ (0.25 g, 6.65 mmol) was added over 20 min. The solution was stirred at RT for 30 min and then heated to reflux for 1 h. After this time H_2O (10 ml) was added to quench the reaction. The solution was then concentrated *in vacuo* and H_2O (20 ml) was added. The pH was increased to 14 by addition of KOH pellets, and the aqueous layer was extracted with DCM (3 x 100 ml). The DCM fraction was dried over anhydrous $MgSO_4$ and evaporated to yield a dark orange solid (0.4 g, 88%). ^{1}H NMR (CD_3OD): δ 2.2 (m, 6H, CH_2), 2.3 (m, 8H, CH_2), 2.71 (m, 2H, CH_2), 2.87-3.34 (m, 2H, CH_2), 4.01 (s, 2H, CH_2), 5.47 (m, 2H, CH_2), 7.20 (m, 2H, CH-Ar), 7.56 (m, 2H, CH-Ar); ^{13}C NMR (CD_3OD): δ 43.83 (N-CH_2), 44.47 (N-CH_2), 45.94 (N-CH_2), 48.80 (N-CH_2), 49.72 (N-CH_2), 49.81 (N-CH_2), 50.45 (N-CH_2), 51.37 (N-CH_2), 53.03 (N-CH_2), 55.42 (N-CH_2), 63.24 (CH_2), 114.55 (CH-Ar), 114.75 (CH-Ar), 122.28 (CH-Ar), 122.48 (CH-Ar), 138.15 (C-Ar), 138.35 (C-Ar), 152.80 (C-Ar), Elemental analysis: (%) calc. for $C_{18}H_{28}N_6$(L)+3.5H_2O, C 55.22 H 9.01 N 21.47. Found: C 54.83 H 9.16 N 21.86. HRMS (ESI) calc. 329.2448; found, 329.2455 $[M+H]^+$.

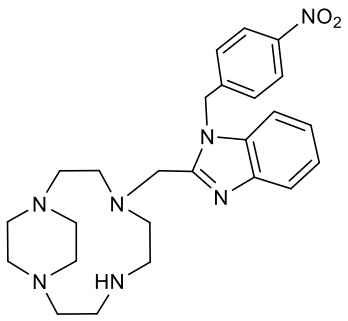
6.6.2. Synthesis of 4-((1H-benzo[*d*]imidazol-2-yl)methyl)-10-methyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (2)



2

8a-((1H-Benzo[d]imidazol-2-yl)methyl)-4a-methyldodecahydro-2a,4a,6a,8a-tetraazacyclo penta[fg]acenaphthylene-4a,8a-diium (**13**) (2.00 g, 3.36 mmol) was dissolved in EtOH (120 ml) and $NaBH_4$ (5.00 g, 132.7 mmol) was added in small portions over period of 1 h. The solution was stirred for 14 days. Water (80 ml) was added to quench the reaction and solvents were removed *in vacuo*. The residue was taken up in water (100 ml) and the pH was increased to 14 by addition of KOH pellets. The basic solution was extracted with DCM (4 x 100 ml), the combined organic extracts were dried over Na_2SO_4 and evaporated *in vacuo* to yield a dark orange (0.104 g, 80%). ^{1}H NMR (CD_3OD): δ 2.31-2.53 (m, 2H, CH_2), 2.60-2.76 (m, 4H, CH_2), 2.80-2.96 (m, 4H, CH_2), 3.00-2.15 (m, 4H, CH_2), 3.21 (s, 3H, CH_3), 3.34-3.53 (m, 2H, CH_2), 3.54-3.72 (m, 4H, CH_2), 4.10 (s, 2H, CH_2), 7.15 (m, 2H, CH-Ar), 7.59 (m, 2H, CH-Ar); ^{13}C NMR (CD_3OD): δ 42.42 (N-CH_3), 50.82 (N-CH_2), 52.42 (N-CH_2), 53.42 (N-CH_2), 55.62 (N-CH_2), 55.82 (N-CH_2), 57.42 (N-CH_2), 59.62 (N-CH_2), 60.42 (N-CH_2), 62.32 (N-CH_2), 64.61 (N-CH_2), 69.81 (CH_2), 115.57 (CH-Ar), 119.02 (CH-Ar), 130.99 (CH-Ar), 139.58 (CH-Ar), 152.58 (C-Ar), 158.22 (C-Ar), 165.77 (C-Ar); Elemental analysis: (%) calc. for $C_{19}H_{30}N_6$(L)+2.6H_2O, C 58.62 H 9.11 N 21.59. Found: C 58.80 H 9.13 N 21.20; HRMS (ESI) calc. 343.2605; found, 343.2608 $[M+H]^+$.

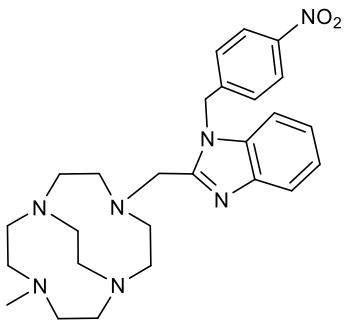
6.6.3. Synthesis of 4-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10 tetraazabicyclo[8.2.2]tetradecane (3)



3

8a-((1-(4-Nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)decahydro-1H-2a,4a,6a,8a-tetraazacyclopenta[fg]acenaphthylen-8a-ium chloride (**14**) (2.00 g, 4 mmol) was dissolved in EtOH (100 ml) then $NaBH_4$ (0.75 g, 20 mmol) was added over 20 min. The solution was stirred at RT for 30 min and then heated to reflux for 1 h. After this time H_2O (10 ml) was added to quench the reaction. The solution was then concentrated *in vacuo* and H_2O (30 ml) was added. The pH was increased to 14 by addition of KOH pellets and the aqueous layer was extracted with DCM (3 x 100 ml).The DCM fractions were dried over anhydrous $MgSO_4$ and evaporated to yield a light green solid (1.6 g, 92%). ^{1}H NMR (CD_3OD): δ 2.13-3.06 (m, 20H, CH_2), 4.21 (s, 2H, CH_2), 5.90 (s, 2H, CH_2-CAr), 7.16-7.38 (m, 5H, CH-Ar), 7.61-7.73 (m, 1H, CH-Ar), 8.05-8.23 (m, 2H, CH-Ar); ^{13}C NMR: δ 46.13 (CH_2), 47.49 (CH_2), 47.67 (CH_2), 47.94 (CH_2), 49.76 (CH_2), 50.40 (CH_2), 50.95 (CH_2), 52.91 (CH_2), 53.37 (CH_2), 55.41 (CH_2), 56.62 (CH_2), 57.55 (CH_2), 77.03 (CH_2-CAr), 110.25 (CH-Ar), 118.70 (CH-Ar), 122.78 (CH-Ar), 123.52 (CH-Ar), 123.80 (CH-Ar), 127.33 (CH-Ar), 135.21 (C-Ar), 141.43 (C-Ar), 143.84 (C-Ar), 147.65 (C-Ar), 152.10 (C-Ar). HRMS (ESI) calc. 464.2768; found, 464.2758 $[M+H]^+$.

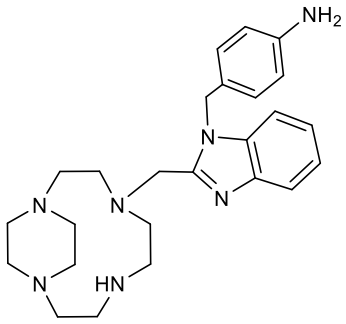
6.6.4. Synthesis of 4-methyl-10-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (4)



4

4a-Methyl-8a-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)dodecahydro-2a,4a,6a,8a-tetraazacyclopenta[fg]acenaphthylene-4a,8a-diium (**15**) (2.40 g, 3.30 mmol) was dissolved in EtOH (120 ml) and $NaBH_4$ (4.99 g, 132.00 mmol) was added in small portions over period of 1 hour. The solution was stirred for 14 days. Water (80 ml) was added to quench the reaction and solvents were removed *in vacuo*. The residue was taken up in water (100 ml) and the pH was increased to 14 by addition of KOH pellets. The basic solution was extracted with DCM (4 x 100 ml), the combined organic extracts were dried over (Na_2SO_4) and evaporated *in vacuo* to yield a dark red solid (1.157 g, 72%). ^{1}H NMR ($CDCl_3$): δ 2.28 (s, 3H, CH_3), 2.31-3.11 (m, 20H, CH_2), 5.20 (s, 2H, CH_2), 5.37 (s, 2H, CH_2-CAr), 7.19 (m, 5H. CH-Ar), 7.73 (m, 1H, CH-Ar), 8.14 (m, 2H, CH-Ar); ^{13}C NMR ($CDCl_3$): δ 43.45 (CH_3), 44.32 (CH_2), 46.55 (CH_2), 50.16 (CH_2), 53.28 (CH_2), 55.13 (CH_2), 56.27 (CH_2), 56.43 (CH_2), 56.76 (CH_2), 56.96 (CH_2), 57.66 (CH_2), 59.27 (CH_2), 59.53 (CH_2-CAr), 109.59 (CH-Ar), 120.17 (CH-Ar), 122.57 (CH-Ar), 123.37 (CH-Ar), 124.37 (CH-Ar), 126.96 (CH-Ar), 135.55 (C-Ar), 142.54 (C-Ar), 143.74 (C-Ar), 147.53 (C-Ar). 152.33 (C-Ar). MS (ESI): 478.4 $[(M+H)]^+$.

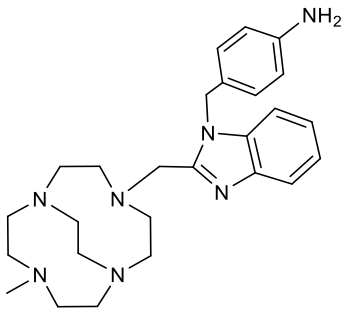
6.6.5. Synthesis of 4-((2-(1,4,7,10-tetraazabicyclo[8.2.2]tetradecan-4-ylmethyl)-1H-benzo[d]imidazol-1-yl)methyl)aniline (5)



5

$NaBH_4$ (280 mg, 7.6 mmol) and S_8 (480 mg, 15.2 mmol) were suspended in dry THF (10 ml) and stirred under argon for 1 hour. 4-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl) methyl)-1,4,7,10-tetraazabicyclo[8.2.2]tetradecane (**3**) (180 mg, 0.38 mmol) in THF (10 ml) was added and the reaction was heated under reflux overnight then left to cool. The resulting solution was washed with 5% NaOH solution (5 x 20 ml), the organic layer was dried (Na_2SO_4), filtered and concentrated *in vacuo* to yield a crude brown solid (156 mg). The crude solid was purified via column chromatography, eluting with DCM : MeOH (95:5) to yield a brown solid (133 mg, 81%). ^{1}H NMR (CD_3OD): δ 2.20-3.27 (m, 16H, CH_2), 3.32-3.89 (m, 4H, CH_2), 4.27 (s, 2H, CH_2), 5.48 (s, 2H, CH_2-CAr), 6.53-6.71 (m, 2H, CH-Ar), 6.72-6.97 (m, 2H, CH-Ar), 7.14-7.32 (m, 2H, CH-Ar), 7.44 (m, 1H, CH-Ar), 7.60-7.71 (m, 1H, CH-Ar); ^{13}C NMR (CD_3OD): δ 45.31 (CH_2), 46.54 (CH_2), 48.88 (CH_2), 49.10 (CH_2), 50.00 (CH_2), 50.11 (CH_2), 51.44 (CH_2), 54.12 (CH_2), 55.57 (CH_2), 56.46 (CH_2), 58.69 (CH_2), 61.48 (CH_2-CAr), 110.65 (CH-Ar), 115.27 (CH-Ar), 118.38 (CH-Ar), 122.42 (CH-Ar), 123.05 (CH-Ar), 124.99 (CH-Ar), 127.35 (C-Ar), 135.41 (C-Ar), 141.48 (C-Ar), 147.69 (C-Ar), 151.92 (C-Ar), HRMS (ESI) calc. 434.3027; found, 434.3026 $[M+H]^+$.

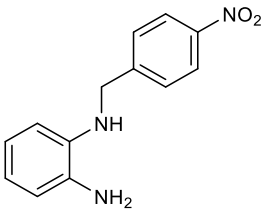
6.6.6. Synthesis of 4-((2-((10-methyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradecan-4-yl)methyl)-1H-benzo[d]imidazol-1-yl)methyl)aniline (6)



6

$NaBH_4$ (830 mg, 22.2 mmol) and S_8 (1.42 g, 44.4 mmol) were suspended in dry THF (10 ml) and stirred under argon for 1 h. 4-methyl-10-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (**4**) (500 mg, 1.11 mmol) in dry THF (10 ml) was added and the reaction was heated under reflux overnight then left to cool. The resulting solution was washed with 5% NaOH solution (5 x 20 ml), the organic layer was dried (Na_2SO_4), filtered and concentrated *in vacuo* to yield a brown solid (313 mg, 64%). ^{1}H NMR (CD_3OD): δ 2.21 (s, 3H, CH_3), 2.71-2.77 (m, 4H, CH_2), 2.84-3.06 (m, 16H, CH_2), 4.14 (s, 2H, CH_2), 5.39 (s, 2H, CH_2-CAr), 6.66-6.70 (m, 2H, CH-Ar), 6.84 (m, 1H, CH-Ar), 7.00-7.14 (m, 1H, CH-Ar), 7.16-7.33 (m, 2H, CH-Ar), 7.42-7.52 (m, 1H, CH-Ar), 7.61-7.71(m, 1H, CH-Ar); ^{13}C NMR (CD_3OD): δ 29.61 (CH_3), 34.08 (CH_2), 42.46 (CH_2), 42.79 (CH_2), 42.90 (CH_2), 46.36 (CH_2), 51.61 (CH_2), 52.95 (CH_2-CAr), 53.85 (CH_2), 54.18 (CH_2), 110.29 (CH-Ar), 115.29 (CH-Ar), 118.38 (CH-Ar), 122.29 (CH-Ar), 124.83 (CH-Ar), 127.29 (CH-Ar), 128.51 (C-Ar), 135.63 (C-Ar), 143.36 (C-Ar), 147.71 (C-Ar), 151.99 (C-Ar). HRMS (ESI) calc. 448.3183; found, 448.3177 $[M+H]^+$.

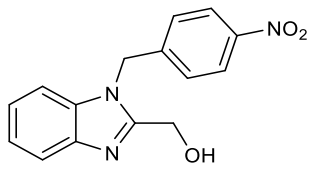
6.6.7. Synthesis of N^1-(4-nitrobenzyl) benzene-1, 2-diamine (7)[237]



7

The synthesis procedure was completed following literature method.[237] 4-nitrobenzyl bromide (15 g, 0.069 mol) in MeOH (600 ml) was added drop wise to a stirred solution of o-phenylenediamine (37.53 g, 0.36 mol) in MeOH (900 ml) over 30 minutes. The reaction stirred for 4 hours, concentrated *in vacuo* and recrystallized from EtOH (500 ml) to give crude solid which was purified via column chromatography, eluting with DCM to yield a orange/red solid (10.5 g, 65%). ^{1}H NMR (CD_2Cl_2): δ 3.44 (br s, 2H, NH_2), 3.48 (br s, 1H, NH), 4.47 (s, 2H, CH_2-CAr), 6.49 (m, 1H, CH-Ar), 6.67-6.75 (m, 3H, CH-Ar), 7.56 (d, 2H, CH-Ar, J = 8.8 Hz), 8.18 (d, 2H, CH-Ar, J = 8.8 Hz); ^{13}C NMR (CD_2Cl_2): δ 48.06 (CH_2), 112.27 (CH-Ar), 117.00 (CH-Ar), 119.50 (CH-Ar), 120.81 (CH-Ar), 124.03 (CH-Ar), 128.36 (CH-Ar), 134.90 (C-Ar), 137.25 (C-Ar), 147.22 (C-Ar), 148.09 (C-Ar). MS (ESI): 243 $[M]^+$.

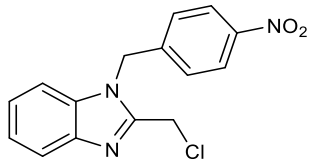
6.6.8. Syntheis of (1-(4-nitrobenzyl)-1*H*-benzo[d]imidazol-2-yl)methanol (8)[237]



8

The synthesis procedure was completed following literature method.[237] N^1-(4-nitrobenzyl) benzene-1, 2-diamine (**7**) (10 g, 0.041 mol) and glycolic acid (4.68 g, 0.061 mol) were dissolved in 5 N HCl (200 ml) and heated under reflux for 60 h. Cooled to 0 $^{\circ}$C and made basic with 5% NaOH solution (~1500 ml) resulting in a light brown precipitate which was filtered and washed with water (400 ml) and diethyl ether (4 x 300 ml) to yield a light brown solid which was dried under Schlenk line to give a light brown solid (11 g, 94%). ^{1}H NMR (CD_3OD): δ 4.86 (s, 2H, CH_2), 5.74 (s, 2H, CH_2-CAr), 7.23-7.32 (m, 3H, CH-Ar), 7.38 (m, 2H, CH-Ar), 7.66 (m, 1H, CH-Ar), 8.15 (m, 2H, CH-Ar); ^{13}C NMR (CD_3OD): δ 46.06 (CH_2), 56.62 (CH_2-CAr), 110.13 (CH-Ar), 118.56 (CH-Ar), 122.77 (CH-Ar), 122.97 (CH-Ar), 123.57 (CH-Ar), 123.59 (CH-Ar), 127.56 (C-Ar), 127.76 (C-Ar), 143.74 (C-Ar), 143.94 (C-Ar), 147.61 (C-Ar). MS (ESI): 283 $[M]^+$.

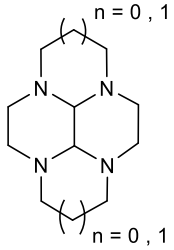
6.6.9. Synthesis of 2-(chloromethyl)-1-(4-nitrobenzyl)-1*H*-benzo[d]imidazole (9)[237]



9

The synthesis procedure was completed following literature method.[237] Thionyl chloride (110 ml) was added to (1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl) methanol (**8**) (11 g, 0.038 mol) and the reaction stirred under argon for 24 h. Thionyl chloride was removed *in vacuo.* The solid was redissolved in a minimum amount of MeOH and precipitated using excess of Et_2O which was left to settle then decanted to leave a light brown solid (10.5 g, 92%). ^{1}H NMR (CD_3OD): δ 5.45 (s, 2H, CH_2-CAr), 6.05 (s, 2H, CH_2), 7.55-7.76 (m, 5H, CH-Ar), 7.90 (m, 1H, CH-Ar), 8.25 (m, 2H, CH-Ar); ^{13}C NMR (CD_3OD): δ 32.65 (CH_2), 48.53 (CH_2-CAr), 113.69 (CH-Ar), 115.08 (CH-Ar), 124.39 (CH-Ar), 127.84 (CH-Ar), 128.14 (CH-Ar), 128.59 (CH-Ar), 131.02 (C-Ar), 132.74 (C-Ar), 140.92 (C-Ar), 148.66 (C-Ar), 149.44 (C-Ar). MS (ESI): 301 $[M]^+$.

6.6.10. Synthesis of cis-13-1, 4, 7, 10-tetraazatetracyclo [5.5.1.$0^{4,14}0^{10,13}$] tetradecane (10)[27] and decahydro-1*H*,6*H*-3a,5a,8a,10a-tetraazapyrene (11)[27]



n = 0 **10**
n = 1 **11**

General procedure A

The macrocycle was dissolved in MeOH and cooled to –10 ˚C. A cold (0 ˚C) aqueous solution of glyoxal was added dropwise over 90 min. The clear solution was stirred at –10 ˚C for 30 min then at RT for 3 h. The solvent was removed *in vacuo* and the crude solid was redissolved in diethyl ether. The filtrate was dried ($MgSO_4$), filtered and solvent removed *in vacuo*.

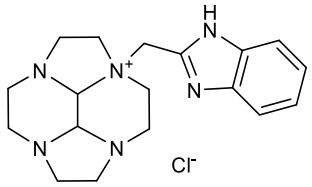
cis-13-1, 4, 7, 10-Tetraazatetracyclo [5.5.1.$0^{4,14}0^{10,13}$] tetradecane (10)[27]

Amounts: 1,4,7,10-Tetraazacyclododecane (cyclen) (4.18 g, 24.3 mmol), MeOH (150 ml), glyoxal (40% w/w, 3.52 g, 60.7 mmol), Et_2O (150 ml). To yield a cream solid (4.40 g, 93%). ^{1}H NMR ($CDCl_3$): δ 2.49 (m, 4H, N-CH_2), 2.63 (br s, 4H, N-CH_2), 2.87-2.96 (m, 8H, N-CH_2), 3.07 (s, 2H, N-CH,(H_{aminal})); ^{13}C NMR ($CDCl_3$): δ 50.29 (N-CH_2), 51.06 (N-CH_2), 76.68 (N-CH, (C_{aminal})). HRMS (ESI) calc. 195.1604; found, 195.1605 $[M + H]^+$.

decahydro-1H,6H-3a,5a,8a,10a-Tetraazapyrene (11)[27]

Amounts: 1,4,8,11-tetraazacyclotetradodecane (5g, 25mmol), MeOH (250 ml), aqueous glyoxal solution (40 % w/w, 3.63 g, 62.58 mmol), diethyl ether (200 ml). To yield a white solid (5.3 g, 96%). ^{1}H NMR ($CDCl_3$): δ 1.23 (m, 2H, N-β-CH_2), 2.07-2.38 (m, 8H, N-α-CH_2), 2.72 (d, 2H, N-β-CH_2, J = 10.6 Hz), 2.90-2.97 (m, 6H, N-α-CH_2), 3.08 (s, 2H, CH_{aminal}), 3.53 (t, 2H, N-α-CH_2, J = 10.5 Hz). ^{13}C NMR ($CDCl_3$): δ 19.29 (N-β-CH_2), 44.47 (N-α-CH_2), 52.20 (N-α-CH_2), 54.07 (N-α-CH_2), 55.76 (C_{aminal}). MS (ESI): 223 $[(M+H)]^+$.

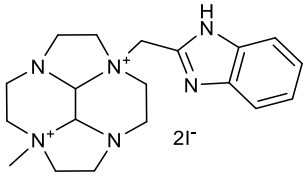
6.6.11. Synthesis of N-(2-methylbenimidazolyl) cis-13-1,4,7,10-tetraazatetracyclo [5.5.2.$0^{4,14}$ $0^{10,13}$] tetradecane chloride (12)



12

2-(Chloromethyl)-benzimidazole (0.41 g, 2.46 mmol) was dissolved in acetonitrile (60 ml) and stirred. Cis-13-1,4,7,10- tetraazatetracyclo[5.5.2.$0^{4,14}$ $0^{10,13}$] tetradecane (**10**) (0.59 g, 2.95 mmol) was dissolved in MeCN (60 ml) and added dropwise. The reaction was stirred at RT for 18 h. The reaction mixture was evaporated to yield a light orange solid (0.8 g, 90%). ^{1}H NMR (CD_3OD): δ 2.51 (m, 2H, CH_2), 2.66-3.16 (m, 10H, CH_2), 3.67-3.93 (m, 4H, CH_2), 4.06 (s, 2H, CH_2), 5.03 (m, 1H, CH_{aminal}), 5.20 (m, 1H, CH_{aminal}), 7.24 (m, 2H. CH-Ar), 7.56 (m, 2H, CH-Ar); ^{13}C NMR (CD_3OD): δ 42.66 (N-CH_2), 43.48 (N-CH_2), 48.79 (N-CH_2), 51.45 (N-CH_2), 51.61 (N-CH_2), 53.90 (N-CH_2), 59.01 (N-CH_2), 59.42 (N-CH_2), 75.57 (CH_2), 78.43 (C_{aminal}), 83.34 (C_{aminal}), 121.98 (CH-Ar), 122.13 (CH-Ar), 122.87 (CH-Ar), 123.00 (CH-Ar), 123.82 (C-Ar), 125.25 (C-Ar), 141.60 (C-Ar), HRMS (ESI) calc. 325.2135; found 325.2135 $[M - Cl^-]^+$.

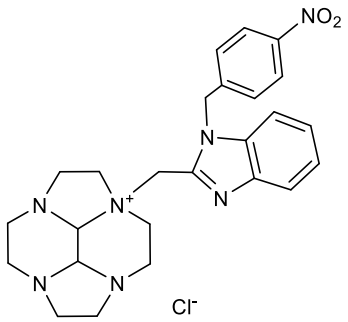
6.6.12. Synthesis of 8a-((1H-benzo[d]imidazol-2-yl)methyl)-4a-methyldodecahydro-2a,4a,6a,8a-tetraazacyclopenta[fg]acenaphthylene-4a,8a-diium (13)



13

N-(2-Methylbenimidazolyl) cis-13-1,4,7,10- tetraazatetracyclo[5.5.2.$0^{4,14}$ $0^{10,13}$]tetradecane chloride (**12**) (4.00 g, 11.00 mmol), was suspended in dry MeCN (100 ml) under nitrogen. Iodomethane (59.00 ml, 134.8 g, and 950.00 mmol) was added dropwise. The brown suspension was left to stir for 10 days. A second portion of iodomethane (30 ml, 475.00 mmol) was added after 5 days. Excess iodomethane was removed by flowing nitrogen through the suspension for 30 minutes. The suspension was filtrated then Et_2O (100 ml) was added and the solvent was removed *in vacuo* to yield pale yellow crystals (6.6 g 100%). ^{1}H NMR (CD_3OD): δ 2.08 (s, 3H, CH_3), 2.51-3.18 (m, 8H, CH_2), 3.6-4.02 (m, 8H, CH_2), 4.70 (s, 2H, CH_2), 5.07 (m, 1H, CH), 5.19 (m, 1H, CH_2), 7.32 (m, 2H, CH-Ar), 7.70 (m, 2H, CH-Ar); ^{13}C NMR (CD_3OD): δ 43.33 (N-CH_2), 45.40 (N-CH_2), 47.14 (N-CH_2), 54.11 (N-CH_2), 58.99 (N-CH_2), 65.11 (CH_2), 74.80 (C_{aminal}), 77.84 (C_{aminal}), 100.71 (CH-Ar), 111.51 (CH-Ar), 123.87 (CH-Ar), 131.93 (CH-Ar), 140.22 (C-Ar), 140.65 (C-Ar), 142.42 (C-Ar); Elemental analysis: (%) calc. for $C_{19}H_{28}I_2N_6$(L)+5H_2O, C 33.35 H 5.60 N 12.28. Found: C 33.76 H 5.11 N 11.96. HRMS (ESI) calc. 371.2554; found, 371.2558 $[M\text{-}2I^- + CH_3O^-]^+$.

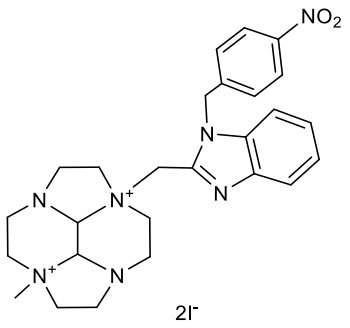
6.6.13. Synthesis of 8a-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)decahydro-1H-2a,4a,6a,8a-tetraazacyclopenta[fg]acenaphthylen-8a-ium chloride (14)



14

2-(Chloromethyl)-1-(4-nitrobenzyl)-1H-benzo[d]imidazole (**9**) (3 g, 9.9 mmol) was dissolved in MeCN (500 ml) and stirred. Bridged cyclen (**10**) (1.92 g, 9.9 mmol) was dissolved in MeCN (30 ml) and added dropwise. The reaction was stirred at RT. for 18 h. The reaction mixture was evaporated and the resulting precipitate was washed with Et_2O (2 x 100 ml) to yield a light brown solid (4.4 g, 90%). ^{1}H NMR (CD_3OD): δ 2.58-2.75 (m, 1H, CH_2), 2.79-3.28 (m, 8H, CH_2), 3.35-3.65 (m, 4H, CH_2), 3.82 (bs, 1H, CH_2), 3.97-4.08 (m, 1H, CH_2), 4.09-4.35 (m, 2H, CH_2), 4.57 (m, 1H, CH_2), 5.00 (s, 1H, CH_{aminal}), 5.26-5.50 (m, 1H, CH_{aminal}), 5.74-5.97 (m, 2H, CH_2), 7.19-7.55 (m, 5H, CH-Ar), 7.64-7.89 (m, 1H, CH-Ar), 8.07-8.28 (m, 2H, CH-Ar); ^{13}C NMR (CD_3OD): δ 42.9 (CH_2), 43.76 (CH_2), 48.96 (CH_2), 49.31 (CH_2), 51.86 (CH_2), 52.48 (CH_2), 58.82 (CH_2), 63.93 (CH_2), 64.12 (CH_2), 71.15 (CH_2-CAr), 82.82 (C_{aminal}), 110.79 (C_{aminal}), 119.77 (CH-Ar), 119.86 (CH-Ar), 122.85 (CH-Ar), 123.29 (CH-Ar), 123.82 (CH-Ar), 124.35 (CH-Ar), 127.52 (C-Ar), 135.09 (C-Ar), 142.32 (C-Ar), 143.29 (C-Ar), 147.69 (C-Ar). HRMS (ESI) calc. 460.2455; found 460.2446 $[M - Cl^-]^+$.

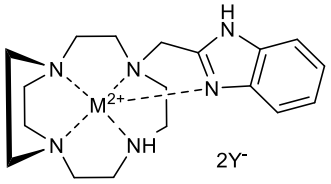
6.6.14. Synthesis of 4a-methyl-8a-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl) dodecahydro-2a,4a,6a,8a-tetraazacyclopenta[fg]acenaphthylene-4a,8a-diium (15)



15

8a-((1-(4-Nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)decahydro-1H-2a,4a,6a,8a-tetraazacyclopenta[fg]acenaphthylen-8a-ium chloride (**14**) (1.80 g, 3.60 mmol), was suspended in dry MeCN (100 ml) under nitrogen. Iodomethane (19.30 ml, 44.00 g, and 310.00 mmol) was added dropwise. The brown suspension was left to stir for 10 days. A second portion of iodomethane (9.65 ml, 155.00 mmol) was added after 5 days. Excess iodomethane was removed by flowing nitrogen through the suspension for 30 minutes. Diethyl ether (100 ml) was added and the precipitate was filtered off and washed with Et_2O (100 ml) to yield brown crystals (2.60 g 100%). ^{1}H NMR ($(CD_3)_2SO$): δ 2.08 (s, 3H, CH_3), 2.85-3.23 (m, 4H, CH_2), 3.45-4.34 (m, 14H, CH_2), 4.39-4.65 (m, 2H, CH_2), 4.94 (m, 1H, CH_{aminal}), 5.23-5.67 (dd, 1H, CH_{aminal}, J=15.9 Hz), 5.94 (br s, 2H, CH_2-CAr) 7.33 (m, 2H, CH-Ar,), 7.46 (m, 3H, CH-Ar), 7.78 (d, 1H, CH-Ar, J=6.7 Hz), 8.21 (d, 2H, CH-Ar, J=7.8 Hz); ^{13}C NMR $(CD_3)_2SO$): δ 15.72 (CH_3), 40.03 (CH_2), 43.30 (CH_3), 47.08 (CH_2), 47.21 (CH_2), 47.58 (CH_2), 52.62 (CH_2), 57.28 (CH_2), 58.92 (CH_2), 59.05 (CH_2), 63.96 (CH_2), 65.09 (CH_2), 65.47 (CH_2-CAr), 77.69 (CH_2), 77.81 (C_{aminal}), 79.07 (C_{aminal}), 111.95 (CH-Ar), 120.26 (CH-Ar), 123.66 (CH-Ar), 124.42 (CH-Ar), 124.54 (CH-Ar), 128.57 (CH-Ar), 135.25 (C-Ar), 142.43 (C-Ar), 144.19 (C-Ar), 144.44 (C-Ar), 147.59 (C-Ar). HRMS (ESI) calc. 506.2875; found, 506.2867 $[M\text{-}2I^- + CH_3O^-]^+$.

6.6.15. Synthesis of metal complexes of ligand 4-((1H-benzo[*d*]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[8.2.2]tetradecane (1)



[M**1**]Y_2

[Cu**1**]Y_2	$M^{2+} = Cu^{2+}$,	$Y^- = CH_3CO_2^-$
[Cu**1**]Y_2	$M^{2+} = Cu^{2+}$,	$Y^- = NO_3^-$
[Zn**1**]Y_2	$M^{2+} = Zn^{2+}$,	$Y^- = CH_3CO_2^-$
[Zn**1**]Y_2	$M^{2+} = Zn^{2+}$,	$Y^- = NO_3^-$
[Zn**1**]Y_2	$M^{2+} = Zn^{2+}$,	$Y^- = Cl^-$
[Ni**1**]Y_2	$M^{2+} = Ni^{2+}$,	$Y^- = CH_3CO_2^-$
[Ni**1**]Y_2	$M^{2+} = Ni^{2+}$,	$Y^- = NO_3^-$
[Ni**1**]Y_2	$M^{2+} = Ni^{2+}$,	$Y^- = ClO_4^-$

General method B

The ligand was dissolved in MeOH (10 ml), to which a methanolic (5 ml) solution of the metal ion was added dropwise. The mixture was heated to 60 $^{\circ}$C for 30 minutes under nitrogen and then stirred for 2 h at RT under nitrogen. The resulting solution was concentrated *in vacuo* ~ 5 ml then purified via size exclusion chromatography.

4-((1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[8.2.2]tetradecane copper(II) acetate [Cu1]$(CH_3COO)_2$

Amounts: 4-((1H-benzo[*d*]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[8.2.2] tetradecane (**1**) (200 mg, 0.6 mmol); copper(II) acetate monohydrate (130 mg, 0.65 mmol); to yield a blue solid (270 mg, 90%). Elemental analysis: (%) Calc. for $C_{22}H_{34}CuN_6O_4$(M)+5H_2O: C 44.10, H 7.23, N 14.03. found: C 43.68, H 6.81, N 14.40. HRMS calc. for $C_{18}H_{27}CuN_6$: 390.1588[(M-2$CH_3CO_2^-$-H)]$^{2+}$, found 390.1594 [(M-2$CH_3CO_2^-$-H)]$^{2+}$, (100; λ_{max} (MeOH)/nm 607 (ε/dm^3 mol^{-1} cm^{-1} 226).

4-((1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[8.2.2]tetradecane copper(II) nitrate [Cu1]$(NO_3)_2$

Amounts**:** 4-((1H-benzo[*d*]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[8.2.2] tetradecane (**1**) (200 mg, 0.6 mmol); copper(II) nitrate trihydrate (159 mg, 0.66 mmol) to yield a blue solid (210 mg, 70%). HRMS calc. for $C_{18}H_{27}CuN_6$: 195.5829 [(M-2NO_3^-)]$^{2+}$, found 195.5830 [(M-2NO_3^-)]$^{2+}$; λ_{max} (MeOH)/nm 585 (ε/dm^3 mol^{-1} cm^{-1} 200.8).

4-((1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[8.2.2]tetradecane zinc(II) acetate [Zn1]$(CH_3COO)_2$

Amounts: 4-((1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[8.2.2] tetradecane (**1**) (200 mg, 0.6 mmol); zinc acetate (121 mg, 0.66 mmol) to yield a light brown solid (170 mg, 56%). HRMS calc. for $C_{18}H_{27}ZnN_6$: 391.1583 $[(M\text{-}2CH_3CO_2^-\text{-}H)]^{2+}$, found 391.1580 $[(M\text{-}2CH_3CO_2^-\text{-}H)]^{2+}$.

4-((1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[8.2.2]tetradecane zinc(II) nitrate [Zn1]$(NO_3)_2$

Amounts: 4-((1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[8.2.2] tetradecane (**1**) (200 mg, 0.6 mmol); zinc nitrate hexahydrate (196 mg, 0.66 mmol) to yield a light brown solid (60 mg, 51%). HRMS calc. for $C_{18}H_{28}ZnN_6$: 391.1583 $[(M\text{-}H\text{-}2NO_3^-)]^{2+}$, found 391.1584 $[(M\text{-}H\text{-}2NO_3^-)]^{2+}$.

4-((1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[8.2.2]tetradecane zinc(II) chloride [Zn1]Cl_2

Amounts: 4-((1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[8.2.2] tetradecane (**1**) (200 mg, 0.6 mmol); zinc chloride (890 mg, 0.65 mmol) to yield a cream solid (228 mg, 82%).^{1}H NMR (CD_3OD): δ 2.37-2.84 (m, 6H, CH_2), 2.89-3.17 (m, 12H, CH_2), 4.01 (s, 2H, CH_2), 4.81 (m, 2H, CH_2), 7.33 (m, 2H, CH-Ar), 7.63 (m, 2H, CH-Ar); HRMS calc. for $C_{18}H_{28}ZnN_6$: 427.1350 $[(M\text{-}Cl^-)]^+$, found 427.1343 $[(M\text{-}Cl^-)]^+$.

4-((1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10tetraazabicyclo[8.2.2]tetradecane Nickel(II) acetate [Ni1]$(CH_3COO)_2$

Amounts: 4-((1H-benzo[*d*]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[8.2.2] tetradecane (**1**) (200 mg, 0.6 mmol); nickel(II) acetate tetrahydrate (164 mg, 0.66 mmol) to yield a light yellow solid (260 mg, 86%). Elemental analysis: (%) Calc. for $C_{22}H_{34}NiN_6O_4(M)+ 3H_2O$: C, 47.25; H, 7.21; N, 15.03. Found: C, 47.28; H, 7.00; N, 14.99. HRMS calc. for $C_{18}H_{27}NiN_6$: 385.1640 $[(M\text{-}2CH_3CO_2^-\text{-}H)]^{2+}$, found 385.1645 $[(M\text{-}2CH_3CO_2^-\text{-}H)]^{2+}$; λ_{max} (MeOH)/nm 483(ε/dm^3 mol^{-1} cm^{-1} 7.22).

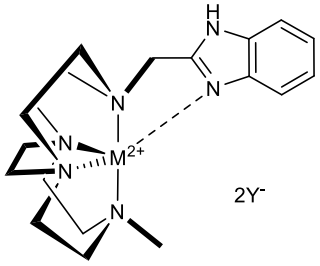
4-((1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[8.2.2]tetradecane nickel(II) nitrate [Ni1]]$(NO_3)_2$

Amounts: 4-((1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[8.2.2] tetradecane (**1**) (200 mg, 0.6 mmol); nickel(II) nitrate hexahydrate (190 mg, 0.65 mmol); to yield a grey solid (250 mg, 90%). HRMS calc. for $C_{18}H_{28}NiN_6$: 448.1602 $[(M\text{-}NO_3^-)]^+$, found 448.1523 $[(M\text{-}NO_3^-)]^+$; λ_{max} (MeOH)/nm 436 (ε/dm^3 mol^{-1} cm^{-1} 112).

4-((1H-benzo[*d*]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[8.2.2]tetradecane nickel(II)perchlorate [Ni1]$(ClO_4)_2$

Amounts: 4-((1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[8.2.2] tetradecane (**1**) (200 mg, 0.6 mmol); nickel(II) perchlorate hexahydrate (240 mg, 0.65 mmol); to yield a brown solid (218 mg, 77%). Elemental analysis: (%) Calc. for $C_{18}H_{28}Cl_2NiN_6O_8$(M)+H_2O+ 0.5CH_3OH: C 35.83, H 5.20, N 13.55. found: C 35.44, H 5.26, N 13.51. HRMS calc. for $C_{18}H_{28}NiN_6$.: 193.0859 $[(M\text{-}2ClO_4^-)]^{2+}$, found: 193.0863 $[(M\text{-}2ClO_4^-)]^{2+}$; λ_{max} (MeOH)/nm 433 (ε/dm^3 mol^{-1} cm^{-1} 109).

6.6.16. Synthesis of metal complexes of ligand 4-((1H-benzo[d]imidazol-2-yl) methyl)- 10-methyl-1,4,7,10-tetraazabicyclo [5.5.2] tetradecane (2)



[M**2**]Y_2

[Cu**2**]Y_2	$M^{2+} = Cu^{2+}$,	$Y^- = CH_3CO_2^-$
[Cu**2**]Y_2	$M^{2+} = Cu^{2+}$,	$Y^- = NO_3^-$
[Zn**2**]Y_2	$M^{2+} = Zn^{2+}$,	$Y^- = CH_3CO_2^-$
[Zn**2**]Y_2	$M^{2+} = Zn^{2+}$,	$Y^- = NO_3^-$
[Ni**2**]Y_2	$M^{2+} = Ni^{2+}$,	$Y^- = CH_3CO_2^-$
[Ni**2**]Y_2	$M^{2+} = Ni^{2+}$,	$Y^- = NO_3^-$

General method C

The ligand was dissolved in degassed anhydrous MeOH (15 ml), an anhydrous methanolic (5 ml) solution of the metal ion was added dropwise and the mixture was heated under reflux under nitrogen for 24 h. The resulting solution was concentrated *in vacuo* to ~5 ml and purified via size exclusion chromatography.

4-((1H-benzo[*d*]imidazol-2-yl)methyl)-10-methyl-1,4,7,10-tetraazabicyclo[5.5.2] tetradecane copper(II) acetate [Cu2]($CH_3COO)_2$

Amounts**:** 4-((1H-benzo[d]imidazol-2-yl)methyl)-10-methyl-1,4,7,10-tetraazabicyclo [5.5.2] tetradecane (**2**) (765 mg, 0.156 mmol); copper(II) acetate monohydrate (35 mg, 0.173 mmol) to yield a blue solid (130 mg, 82%). HRMS calc. for $C_{23}H_{36}CuN_6O_4$: 405.3 $[M\text{-}2CH_3CO_2^-]^{2+}$, found 405.2 $[M\text{-}2CH_3CO_2^-]^{2+}$; λ_{max} (MeOH)/nm 656 (ε/dm^3 mol^{-1} cm^{-1} 922.8).

4-((1H-benzo[d]imidazol-2-yl)methyl)-10-methyl-1,4,7,10-tetraazabicyclo[5.5.2] tetradecane copper(II) nitrate [Cu2]($NO_3)_2$

Amounts**:** 4-((1H-benzo[d]imidazol-2-yl)methyl)-10-methyl-1,4,7,10-tetraazabicyclo [5.5.2] tetradecane (**2**) (100 mg, 0.3 mmol); copper(II) nitrate trihydrate (77 mg, 0.32 mmol) to yield a blue solid (130 mg, 82%). Elemental analysis: (%) Calc. for $C_{19}H_{30}CuN_8O_6$(M)+$3H_2O$: C 39.07, H 6.21, N 19.18. found: C 39.20, H 5.66, N 18.90. HRMS calc. for $C_{19}H_{29}CuN_6$: 404.1739 $[(M\text{-}2NO_3^-\text{-}H)]^{2+}$, found 404.1744 $[(M\text{-}2NO_3^-\text{-}H)]^{2+}$; λ_{max} (MeOH)/nm 656 (ε/dm^3 mol^{-1} cm^{-1} 922.8).

4-((1H-benzo[d]imidazol-2-yl)methyl)-10-methyl-1,4,7,10-tetraazabicyclo[5.5.2] tetradecane zinc(II) acetate [Zn2]($CH_3COO)_2$

Amounts: 4-((1H-benzo[d]imidazol-2-yl)methyl)-10-methyl-1,4,7,10-tetraazabicyclo [5.5.2] tetradecane (**2**) (100 mg, 0.3 mmol); zinc(II) acetate (58 mg, 0.32 mmol) to yield a light brown solid (150 mg, 93%). HRMS calc. for $C_{19}H_{30}ZnN_6$: 405.1734 $[(M\text{-}2CH_3CO_2^-\text{-}H)]^{2+}$ found 405.1735 $[(M\text{-}2CH_3CO_2^-\text{-}H)]^{2+}$.

4-((1H-benzo[d]imidazol-2-yl)methyl)-10-methyl-1,4,7,10-tetraazabicyclo[5.5.2] tetradecane zinc(II) nitrate [Zn2]($NO_3)_2$

Amounts: 4-((1H-benzo[d]imidazol-2-yl)methyl)-10-methyl-1,4,7,10-tetraazabicyclo [5.5.2] tetradecane (**2**) (100 mg, 0.3 mmol); zinc(II) nitrate hexahydrate (95 mg, 0.32 mmol) to yield a light yellow solid (149 mg, 94%). HRMS calc. for $C_{19}H_{29}ZnN_6$: 405.1740 $[(M\text{-}H\text{-}2NO_3^-)]^{2+}$, found 405.1741 $[(M\text{-}H\text{-}2NO_3^-)]^{2+}$.

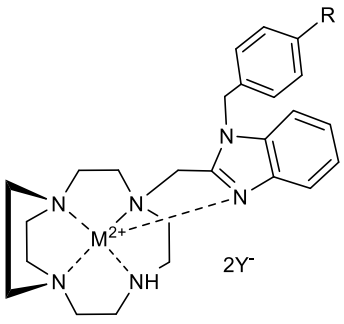
4-((1H-benzo[d]imidazol-2-yl)methyl)-10-methyl-1,4,7,10-tetraazabicyclo[5.5.2] tetradecane nickel(II) acetate [Ni2]$(CH_3COO)_2$

Amounts: 4-((1H-benzo[d]imidazol-2-yl)methyl)-10-methyl-1,4,7,10-tetraazabicyclo [5.5.2] tetradecane (**2**) (100 mg, 0.3 mmol); nickel(II) acetate tetrahydrate (79 mg, 0.32 mmol); to yield a light brown solid (130 mg, 83%). Elemental analysis: (%) Calc. for $C_{23}H_{36}NiN_6O_4.(M)+2.5H_2O+CH_3OH$: C, 48.34; H, 7.61; N, 14.09. Found: C, 48.43; H, 7.35; N, 14.10. HRMS calc. for $C_{19}H_{29}NiN_6$: 200.0937 $[(M-2CH_3CO_2^-)]^{2+}$, found 200.0934 $[(M-2CH_3CO_2^-)]^{2+}$; λ_{max} (MeOH)/nm 485 (ε/dm^3 mol^{-1} cm^{-1} 31.21).

4-((1H-benzo[d]imidazol-2-yl)methyl)-10-methyl-1,4,7,10-tetraazabicycl[5.5.2] tetradecane nickel(II) nitrate [Ni2]$(NO_3)_2$

Amounts: 4-((1H-benzo[d]imidazol-2-yl)methyl)-10-methyl-1,4,7,10-tetraazabicyclo [5.5.2] tetradecane (**2**) (100 mg, 0.3 mmol); nickel(II) nitrate hexahydrate (93 mg, 0.32 mmol) to yield a brown solid (157 mg, 100%). Elemental analysis: (%) Calc. for $C_{19}H_{30}NiN_8O_6.(M)+4H_2O$: C 38.21, H 6.41, N 18.76. found: C 38.31, H 5.95, N 18.95. HRMS calc. for $C_{19}H_{29}NiN_6$: 399.1802 $[(M-2NO_3^-H)]^{2+}$, found 399.1796 $[(M-2NO_3^-H)]^{2+}$; λ_{max} (MeOH)/nm 485 (ε/dm^3 mol^{-1} cm^{-1} 15.07).

6.6.17. Synthesis of metal complexes of ligands 4-((1-(4-nitrobenzyl)-1H-benzo[d] imidazol-2-yl) methyl)-1, 4, 7, 10 tetraazabicyclo[8.2.2]tetradecane (3) and 4-((2-(1,4,7,10-tetraazabicyclo [8.2.2] tetradecan-4-ylmethyl)-1H-benzo[d]imidazol-1-yl)methyl)aniline



3 R=NO_2 [M**3**]Y_2
5 R=NH_2 [M**5**]Y_2

[Cu**3**]Y_2	$M^{2+} = Cu^{2+}$,	$Y^- = CH_3CO_2^-$
[Cu**3**]Y_2	$M^{2+} = Cu^{2+}$,	$Y^- = NO_3^-$
[Zn**3**]Y_2	$M^{2+} = Zn^{2+}$,	$Y^- = CH_3CO_2^-$
[Zn**3**]Y_2	$M^{2+} = Zn^{2+}$,	$Y^- = NO_3^-$
[Ni**3**]Y_2	$M^{2+} = Ni^{2+}$,	$Y^- = CH_3CO_2^-$
[Ni**3**]Y_2	$M^{2+} = Ni^{2+}$,	$Y^- = NO_3^-$
[Cu**5**]Y_2	$M^{2+} = Cu^{2+}$,	$Y^- = CH_3CO_2^-$
[Zn**5**]Y_2	$M^{2+} = Zn^{2+}$,	$Y^- = CH_3CO_2^-$
[Ni**5**]Y_2	$M^{2+} = Ni^{2+}$,	$Y^- = CH_3CO_2^-$

General method B

4-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10 tetraazabicyclo [8.2.2] tetradecane copper(II) acetate [Cu3]($CH_3COO)_2$

Amounts; 4-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10tetraazabicyclo [8.2.2] tetradecane (**3**) (100 mg, 0.21 mmol); copper (II) acetate monohydrate (46 mg, 0.23 mmol) to yield a blue solid (130 mg, 96%). HRMS calc. for $C_{29}H_{39}CuN_7O_6$: 263.0990 [(M-$2CH_3CO_2^-$)]$^{2+}$, found 263.0990 [(M-$2CH_3CO_2^-$)]$^{2+}$.

4-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10 tetraazabicyclo [8.2.2] tetradecane copper(II) nitrate [Cu3]($NO_3)_2$

Amounts: 4-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10 tetraazabicyclo [8.2.2]tetradecane (**3**) (100 mg, 0.21 mmol); copper (II) nitrate trihydrate (55 mg, 0.23 mmol) to yield a dark blue solid (130 mg, 100%). HRMS calc. for $C_{25}H_{33}CuN_9O_8$: 263.0990 [(M-$2NO_3^-$)]$^{2+}$, found 263.0991 [(M-$2NO_3^-$)]$^{2+}$.

4-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10 tetraazabicyclo [8.2.2] tetradecane zinc(II) acetate [Zn3]($CH_3COO)_2$

Amounts; 4-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10 tetraazabicyclo [8.2.2]tetradecane (**3**) (100 mg, 0.21 mmol); zinc(II) acetate (42 mg, 0.23 mmol) to yield a yellow solid (127 mg, 94%). HRMS calc. for $C_{29}H_{39}ZnN_7O_6$: 588.2031 [(M-$CH_3CO_2^-$)]$^{2+}$, found 588.1899 [(M-$CH_3CO_2^-$)]$^{2+}$.

4-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10 tetraazabicyclo [8.2.2] tetradecane zinc(II) nitrate [Zn3]($NO_3)_2$

Amounts; 4-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10 tetraazabicyclo [8.2.2]tetradecane (**3**) (100 mg, 0.21 mmol); zinc(II) nitrate hexahydrate (68 mg, 0.23 mmol) to yield a creamy solid (120 mg, 87%). HRMS calc. for $C_{25}H_{33}ZnN_9O_8$: 526.1904 [(M-$2NO_3^-$ -H)]$^+$, found 526.1895 [(M-$2NO_3^-$-H)]$^+$.

4-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10 tetraazabicyclo [8.2.2] tetradecane nickel (II) acetate [Ni3]($CH_3COO)_2$

Amounts; 4-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10 tetraazabicyclo [8.2.2]tetradecane (**3**) (100 mg, 0.21 mmol); nickel(II) acetate tetrahydrate (57 mg, 0.23 mmol) to yield a light yellow solid (134 mg, 100%). HRMS calc. for $C_{29}H_{39}NiN_7O_6$: 520.1966 $[(M-2CH_3CO_2^-H)]^+$, found 520.1954 $[(M-2CH_3CO_2^-H)]^+$.

4-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10 tetraazabicyclo [8.2.2] tetradecane nickel (II) nitrate [Ni3]($NO_3)_2$

Amounts; 4-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10 tetraazabicyclo [8.2.2]tetradecane (**3**) (100 mg, 0.21 mmol); nickel(II) nitrate hexahydrate (66 mg, 0.23 mmol) to yield a light yellow solid (135 mg, 100%). HRMS calc. for $C_{25}H_{33}NiN_9O_8$: 520.1965 $[(M-2NO_3^-H)]^+$, found 520.1972 $[(M-2NO_3^-H)]^+$.

4-((2-(1,4,7,10-tetraazabicyclo[8.2.2]tetradecan-4-ylmethyl)-1H-benzo[d] imidazol-1-yl)methyl)aniline copper(II) acetate [Cu5]($CH_3COO)_2$

Amounts: 4-((2-(1,4,7,10-tetraazabicyclo[8.2.2]tetradecan-4-ylmethyl)-1H-benzo[d] imidazol -1-yl)methyl)aniline (**5**) (15 mg, 0.034 mmol); copper(II) acetate monohydrate (7.4 mg, 0.037 mmol) to yield a light blue solid (14 mg, 75%). HRMS calc. for $C_{27}H_{38}CuN_7O_2$: 555.2378 $[(M-CH_3CO_2^-)]^+$, found 555.2371 $[(M-CH_3CO_2^-)]^+$.

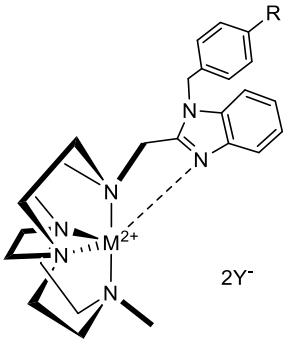
4-((2-(1,4,7,10-tetraazabicyclo[8.2.2]tetradecan-4-ylmethyl)-1H-benzo[d] imidazol-1-yl)methyl)aniline zinc(II) acetate [Zn5]($CH_3COO)_2$

Amounts: 4-((2-(1,4,7,10-tetraazabicyclo[8.2.2]tetradecan-4-ylmethyl)-1H-benzo[d] imidazol -1-yl)methyl)aniline (**5**) (15 mg, 0.034 mmol); zinc acetate (6.7 mg, 0.037 mmol) to yield a light yellow solid (10 mg, 50%). MS (ESI): for $C_{29}H_{41}ZnN_7O_4$: 556.2 $[(M-CH_3CO_2^-)]^+$, found 556.1 $[(M-CH_3CO_2^-)]^+$.

4-((2-(1,4,7,10-tetraazabicyclo[8.2.2]tetradecan-4-ylmethyl)-1H-benzo[d] imidazol-1-yl)methyl)aniline nickel(II) acetate [Ni5]$(CH_3COO)_2$

Amounts: 4-((2-(1,4,7,10-tetraazabicyclo[8.2.2]tetradecan-4-ylmethyl)-1H-benzo [d] imidazol -1-yl)methyl)aniline (**5)** (27 mg , 0.062 mmol); nickel(II) acetate tetrahydrate (17 mg, 0.068 mmol) to yield a light yellow solid (36 mg, 97%). HRMS calc. for $C_{29}H_{41}N_7NiO_4$: 550.2435 $[(M\text{-}CH_3CO_2^-)]^+$, found 550.2422 $[(M\text{-}CH_3CO_2^-)]^+$.

6.6.18. Synthesis of metal complexes of ligands 4-methyl-10-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (4) and 4-((2-((10-methyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradecan-4-yl)methyl)-1H-benzo[d] imidazol-1-yl)methyl)aniline (6)



4 R=NO_2 [M**4**]Y_2
6 R=NH_2 [M**6**]Y_2

[Cu**4**]Y_2 $M^{2+} = Cu^{2+}$, $Y^- = CH_3CO_2^-$
[Cu**4**]Y_2 $M^{2+} = Cu^{2+}$, $Y^- = NO_3^-$
[Zn**4**]Y_2 $M^{2+} = Zn^{2+}$, $Y^- = CH_3CO_2^-$
[Zn**4**]Y_2 $M^{2+} = Zn^{2+}$, $Y^- = NO_3^-$
[Ni**4**]Y_2 $M^{2+} = Ni^{2+}$, $Y^- = CH_3CO_2^-$
[Ni**4**]Y_2 $M^{2+} = Ni^{2+}$, $Y^- = NO_3^-$
[Cu**6**]Y_2 $M^{2+} = Cu^{2+}$, $Y^- = CH_3CO_2^-$
[Zn**6**]Y_2 $M^{2+} = Zn^{2+}$, $Y^- = CH_3CO_2^-$
[Ni**6**]Y_2 $M^{2+} = Ni^{2+}$, $Y^- = CH_3CO_2^-$

General method C

4-methyl-10-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane copper(II) acetate [Cu4]$(CH_3COO)_2$

Amounts; 4-methyl-10-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (**4**) (50 mg, 0.10 mmol); copper (II) acetate monohydrate (22 mg, 0.11 mmol) to yield a dark green solid (62 mg, 95%). HRMS calc. for $C_{30}H_{41}CuN_7O_6$: 270.1069 $[(M\text{-}2CH_3CO_2^-)]^{2+}$, found 270.1075 $[(M\text{-}2CH_3CO_2^-)]^{2+}$

4-methyl-10-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo [5.5.2]tetradecane copper(II) nitrate [Cu4]$(NO_3)_2$

Amounts; 4-methyl-10-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (**4**) (50 mg, 0.10 mmol); copper (II) nitrate trihydrate (26.5 mg, 0.11 mmol) to yield a green solid (61 mg, 92%). HRMS calc. for $C_{26}H_{35}CuN_9O_8$: 602.2021 $[(M\text{-}NO_3^-)]^+$, found 602.2003 $[(M\text{-}NO_3^-)]^+$.

4-methyl-10-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane zinc(II) acetate [Zn4]$(CH_3COO)_2$

Amounts; 4-methyl-10-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (**4**) (50 mg, 0.10 mmol); zinc (II) acetate (20 mg, 0.11 mmol) to yield a brown solid (56 mg, 84%). MS (ESI): for $C_{30}H_{41}ZnN_7O_6$: 600.2 $[(M\text{-}CH_3CO_2^-)]^+$, found 600.1 $[(M\text{-}CH_3CO_2^-)]^+$.

4-methyl-10-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo [5.5.2]tetradecane zinc(II) nitrate [Zn4]$(NO_3)_2$

Amounts; 4-methyl-10-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (**4**) (50 mg, 0.10 mmol); zinc(II) nitrate hexahydrate (32.7 mg, 0.11 mmol) to yield a yellow solid (63 mg, 95%). MS (ESI): for $C_{26}H_{35}ZnN_9O_8$: 604.2 $[(M+H\text{-}NO_3^-)]^+$, found 604.2 $[(M+H\text{-}NO_3^-)]^+$.

4-methyl-10-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo [5.5.2]tetradecane nickel(II) acetate [Ni4]$(CH_3COO)_2$

Amounts; 4-methyl-10-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)1,4,7,10 tetraazabicyclo[5.5.2]tetradecane (**4**) (50 mg, 0.1 mmol); nickel(II) acetate tetrahydrate (27 mg, 0.11 mmol) to yield a light brown solid (65 mg, 100%). MS (ESI): for $C_{30}H_{41}NiN_7O_6$: 267.6 $[(M\text{-}2CH_3CO_2^-)]^{2+}$, found 267.7 $[(M\text{-}2CH_3CO_2^-)]^{2+}$.

4-methyl-10-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7,10-tetraazabicyclo [5.5.2]tetradecane nickel(II) nitrate [Ni4]$(NO_3)_2$

Amounts; 4-methyl-10-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)1,4,7,10 tetraazabicyclo[5.5.2]tetradecane (**4**) (50 mg, 0.1 mmol); nickel(II) nitrate hexahydrate (31.9 mg, 0.11 mmol) to yield a light brown solid (66 mg, 100%). %). HRMS calc. for $C_{26}H_{35}NiN_9O_8$: 267.6103 $[(M-2NO_3^-)]^{2+}$, found 267.6109 $[(M-2NO_3^-)]^{2+}$.

4-((2-((10-methyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradecan-4-yl)methyl)-1H-benzo [d]imidazol-1-yl)methyl)aniline copper(II) acetate [Cu6]$(CH_3COO)_2$

Amounts**:** 4-((2-((10-methyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradecan-4-yl) methyl)-1H-benzo[d]imidazol-1-yl)methyl)aniline (**6**) (25 mg, 0.056 mmol); copper(II) acetate monohydrate (12 mg, 0.061 mmol) to yield a light blue solid (16 mg, 53%). HRMS calc. for $C_{30}H_{43}CuN_7O_4$: 569.2334 $[(M-CH_3CO_2^-)]^+$, found 569.2520 $[(M-CH_3CO_2^-)]^+$.

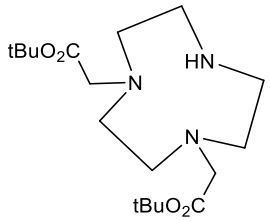
4-((2-((10-methyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradecan-4-yl)methyl)-1H-benzo [d]imidazol-1-yl)methyl)aniline zinc(II) acetate [Zn6]$(CH_3COO)_2$

Amounts**:** 4-((2-((10-methyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradecan-4-yl) methyl)-1H-benzo[d]imidazol-1-yl)methyl)aniline (**6**) (25 mg, 0.056 mmol); zinc(II) acetate (11 mg, 0.061 mmol) to yield a light yellow solid (26 mg, 74%). HRMS calc. for $C_{30}H_{43}ZnN_7O_4$: 570.2530 $[(M-CH_3CO_2^-)]^+$, found 570.2524 $[(M-CH_3CO_2^-)]^+$.

4-((2-((10-methyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradecan-4-yl)methyl)-1H-benzo [d]imidazol-1-yl)methyl)aniline nickel(II) acetate [Ni6]$(CH_3COO)_2$

Amounts**:** 4-((2-((10-methyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradecan-4-yl) methyl)-1H-benzo[d]imidazol-1-yl)methyl)aniline (**6**) (25 mg, 0.056 mmol); nickel (II) acetate tetrahydrate (15 mg, 0.061 mmol) to yield a light yellow solid (30 mg, 88%). HRMS calc. for $C_{30}H_{43}NiN_7O_4$: 504.2386 $[(M-2CH_3CO_2^-)]^{2+}$, found 504.2369 $[(M-2CH_3CO_2^-)]^{2+}$.

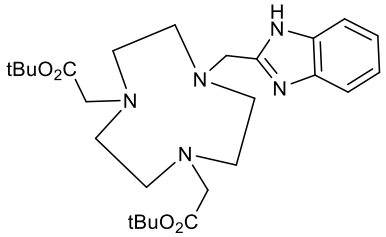
6.6.19. Synthesis of di-*tert*-butyl 2,2'-(1,4,7-triazanonane-1,4-diyl)diacetate(NO2AtBu) (16)[322]



16

The synthesis of compound **16** was carried out following a literature procedure.[322] A solution of *tert*-butyl bromoacetate (3.32 g, 17.03 mmol) in $CHCl_3$ (25 ml) was added to triazacyclononane (TACN) (1.00 g, 7.74 mmol) in $CHCl_3$ (25 ml) slowly over 40 min. The resulting mixture was stirred at RT for 24 h. After monitoring the completion of the starting material using thin layer chromatography (DCM/MeOH; 9/1), reaction mixture was filtered, and the filtrate was evaporated. The residue was treated with deionized (DI) water (15 ml) and the resulting solution was adjusted to pH=3 using 1M HCl and extracted with Et_2O (50 ml × 2). Organic layer was evaporated and dried to obtain trisubstituted product. The aqueous layer was then adjusted to pH=8 using 1M NaOH and extracted with DCM (25 ml × 2). Organic layer was evaporated and resulting residue was treated with DI water (5 ml). Solution pH was adjusted to 10 using 1 M NaOH, and extracted with Et_2O (30 ml × 2). Organic layer was evaporated and dried. Hexane (5 ml) was added to resulting solution and kept in a freezer for 6 hours. Disubsubstituted product was obtained as solid and evaporation of decanted hexane layer gave trisubstituted product. Aqueous layer was further adjusted to pH=8 using 1 M HCl and extracted with DCM (25 ml × 2). Organic layer collected was evaporated and dried to obtain required disubstituted product **16**. Overall yield: (1.32 g, 48%). ^{1}H NMR ($CDCl_3$): δ 1.42 (s, 18H, CH_3), 2.73 (s, 4H, CH_2-N), 2.96 (m, 4H, CH_2-N), 3.05 (m, 4H, CH_2-N), 3.32 (s, 4H, CH_2); ^{13}C NMR ($CDCl_3$): δ 28.25 (C ($\underline{C}H_3)_3$), 45.33 (CH_2), 49.97 (CH_2), 52.14 (CH_2), 55.27 (CH_2), 57.06 (CH_2), 81.77 (CH_2), 171.11 (CO); MS (ESI): 358.4 $[(M+H)]^+$.

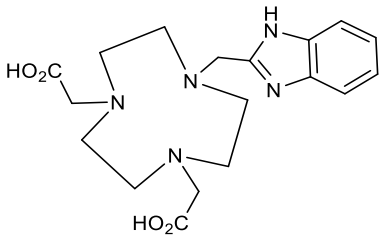
6.6.20. Synthesis of di-*tert*-butyl 2,2'-(7-((1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane-1,4-diyl)diacetate (17)



17

di-*tert*-butyl 2,2'-(1,4,7-Triazonane-1,4-diyl)diacetate(NO2AtBu) (**16**) (0.30 g, 0.84 mmol) was dissolved in dry MeCN (150 ml), 2-chloromethylbenzimidazole (0.13 g, 0.84 mmol) and Cs_2CO_3 (1.07 g, 3.30 mmol) were added and the reaction was stirred for 18 h. The resulting mixture was filtered and concentrated in *vacuo*. The precipitate was purified by column chromatography using eluent DCM:MeOH (95:5) to yield a light yellow solid (0.30 g, 75%). ^{1}H NMR (CD_2Cl_2): δ 1.45 (s, 18H, CH_3), 2.84-3.04 (m, 12H, CH_2), 3.46 (m, 4H, CH_2), 4.10 (s, 2H, CH_2), 7.21 (m, 2H, CH-Ar), 7.53 (m, 2H, CH-Ar). HRMS (ESI) calc. 488.3231 found 488.3237 $[(M+H)]^+$ and 546.3297 found 546.3294 $[(M+CH_3COO)]^+$.

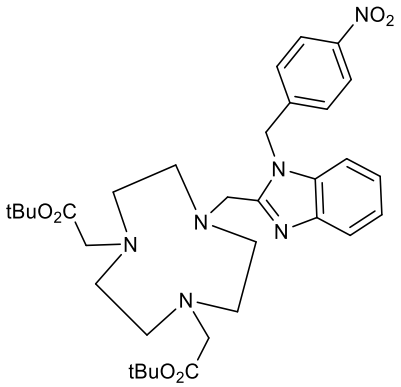
6.6.21. Synthesis of 2,2'-(7-((1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane-1,4-diyl)diacetic acid (18)



18

di-*tert*-butyl 2,2'-(7-((1H-Benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane-1,4-diyl) diacetate (**17**) (50 mg, 0.10 mmol) was dissolved in (5 ml) 6N HCl and heated using microwave irradiation at 230 °C for 12 mins then concentrated by *vacuo* to give a light yellow solid (37 mg, 100%). ^{1}H NMR (CD_3OD): δ 2.85-3.63 (m, 12H, CH_2), 3.09-4.23 (m, 4H, CH_2), 4.55 (s, 2H, CH_2), 7.62 (m, 2H, CH-Ar), 7.81 (m, 2H, CH-Ar); ^{13}C NMR (CD_3OD): δ 49.54(CH_2), 49.68 (CH_2), 50.25 (CH_2), 50.97 (CH_2), 51.11 (CH_2), 56.06 (CH_2), 64.89 (CH_2), 78.33 (CH_2), 113.75 (CH-Ar), 122.67 (CH-Ar), 123.88 (CH-Ar), 126.51 (CH-Ar), 126.65 (C-Ar), 130.81 (C-Ar), 139.12 (C-Ar), 172.95 (C=O), HRMS (ESI) calc. 376.1985 found 376.1982 $[(M+H)]^+$. HPLC: Method 1 – retention time = 7:39 mins.

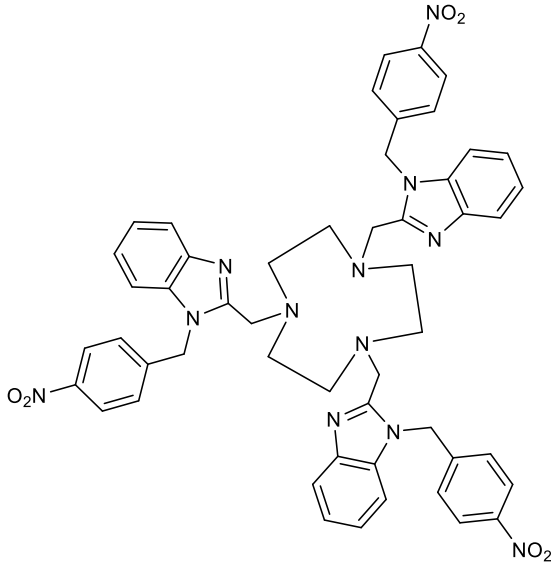
6.6.22. Synthesis of di-*tert*-butyl 2,2'-(7-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane-1,4-diyl)diacetate (19)



19

di-*tert*-butyl 2,2'-(1,4,7-Triazanonane-1,4-diyl)diacetate(NO2AtBu) (**16**) (0.50 g, 1.40 mmol) was dissolved in dry MeCN (70 ml), 2-(chloromethyl)-1-(4-nitrobenzyl)-1H-benzo[d]imidazole (**9**) (0.42 g, 1.40 mmol) and Cs_2CO_3 (1.79 g, 5.50 mmol) were added and the reaction was stirred for 24 h. Reaction completion was confirmed by TLC, and the mixture was filtered. The solid obtained was washed with MeCN and the residual solvent was evaporated. The crude compound obtained was purified by silica column chromatography using DCM/ MeOH (9:1) to produce a light orange solid (0.56 g, 65%). ^{1}H NMR (CD_2Cl_2): δ 1.42 (s, 18H, CH_3), 2.72-3.01 (m, 12H, CH_2), 3.31 (s, 4H, CH_2), 4.01 (br s, 2H, CH_2), 5.80 (s, 2H, CH_2-CAr), 7.17-7.35 (m, 5H, CH-Ar), 7.71 (m, 1H, CH-Ar), 8.13 (d, 2H, CH-Ar, J = 8.8 Hz); ^{13}C NMR (CD_2Cl_2): δ 28.26 (C ($\underline{C}H_3)_3$), 46.67 (CH_2), 54.68 (CH_2), 55.26 (CH_2), 59,66 (CH_2), 66.38 (CH_2), 89.21 (CH_2), 108.78 (CH-Ar), 109.64 (CH-Ar), 120.08 (CH-Ar), 122.62 (CH-Ar), 123.33 (CH-Ar), 124.18 (CH-Ar), 127.28 (C-Ar), 135.61 (C-Ar), 142.39 (C-Ar), 144.08 (C-Ar), 147.47 (C-Ar), 159.75 (C=O), 174.72 (C=O). Elemental analysis: (%) calc. for $C_{33}H_{46}N_6O_6$(L)+1.9H_2O, C 60.33 H 7.64 N 12.79 Found: C 60.61 H 7.37 N 12.72. HRMS (ESI) calc. 623.3539 found 623.3552 $[(M+H)]^+$.

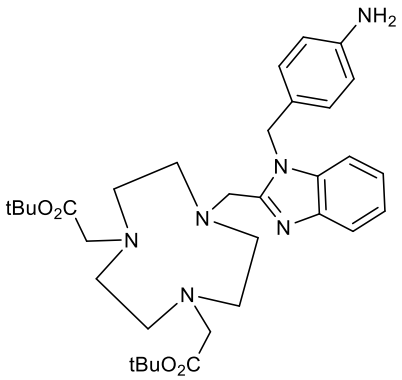
6.6.23. Synthesis of 1,4,7-tris((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane (20)



20

A mixture of triazacyclononane (TACN) (0.10 g, 0.77 mmol) and Cs_2CO_3 (1.00 g, 3.08 mmol) was stirred in dry MeCN (50 ml). A solution of 2-(chloromethyl)-1-(4-nitrobenzyl)-1H-benzo[d]imidazole (**9**) (0.69 g, 2.31 mmol) in dry MeCN (20 ml) was added dropwise to this mixture with vigorous stirring at RT and the reaction mixture was left to stir for 48 h. Reaction completion was confirmed by TLC, and the mixture was filtered. The solid obtained was washed with MeCN, and the residual solvent was evaporated. The crude compound obtained was purified by column Chromatography using MeOH/DCM (5:95) to produce a light orange pure solid (0.44 g, 62%). ^{1}H NMR ($CDCl_3$): δ 1.94-3.27 (m, 14H, CH_2), 3.75 (s, 4H, CH_2), 5.46 (s, 4H, CH_2), 5.50-5.67 (m, 2H, CH_2), 7.07 (dd, 6H, CH-Ar, J=8.2, 2.9 Hz), 7.10-7.55 (m, 10H, CH-Ar), 7.75 (d, 2H, CH-Ar, J=7.8 Hz), 7.98 (m, 1H, CH-Ar), 8.05 (dd, 4H, CH-Ar, J=8.7, 2.8 Hz), 8.15 (m, 1H, CH-Ar); Elemental analysis: (%) calc. for $C_{51}H_{48}N_{12}O_6$(L)+2.4CH_2Cl_2+2H_2O, C 55.06, H 4.91, N 14.43. found: C 54.71, H 4.82, N 14.78 HRMS (ESI) calc. 941.3842 found 941.3836 $[(M+OH)]^+$. HPLC: Method 4 – retention time = 8:53 mins.

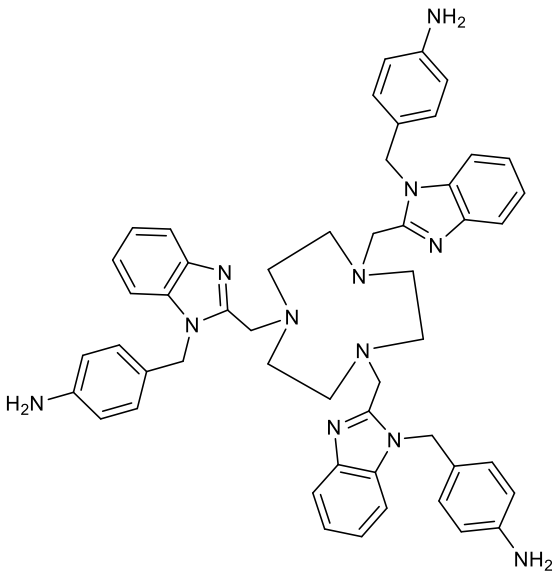
6.6.24. Synthesis of di-*tert*-butyl 2,2'-(7-((1-(4-aminobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane-1,4-diyl)diacetate (21)



21

$NaBH_4$ (0.18 g, 4.8 mmol) and S_8 (0.30 g, 9.6 mmol) were suspended in dry THF (10 ml) and stirred under argon for 1 hour. Di-*tert*-butyl 2,2'-(7-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane-1,4-diyl)diacetate (**19**) (0.15 g, 0.24 mmol) in dry THF (10 ml) was added and the reaction was heated under reflux overnight then left to cool. The resulting solution was washed with 5% NaOH solution (5 x 20 ml), the organic layer was dried (Na_2SO_4), filtered and concentrated *in vacuo* to yield a crude yellow solid (116 mg). The crude solid was purified via column chromatography, eluting with DCM:MeOH (95:5) to yield a light yellow solid (56 mg, 40%). ^{1}H NMR (CD_3OD): δ 1.40 (s, 18H, CH_3), 2.74-2.93 (m, 12H, CH_2), 3.29 (m, 2H, CH_2), 3.80 (s, 2H, CH_2), 5.59 (s, 2H, CH_2-CAr), 6.64 (d, 2H, CH-Ar, J=8.6 Hz), 6.88 (d, 2H, CH-Ar, J=8.4 Hz), 7.21 (m, 2H, CH-Ar), 7.37 (m, 1H, CH-Ar), 7.62 (m, 1H, CH-Ar); ^{13}C NMR (CD_3OD): δ 27.13 (C ($\underline{C}H_3)_3$), 46.76 (CH_2), 54.95 (CH_2), 55.38 (CH_2), 59.33 (CH_2), 69,76 (CH_2), 80,66 (CH_2), 110.55 (CH-Ar), 115.31 (CH-Ar), 118.28 (CH-Ar), 122.07 (CH-Ar), 122.80 (CH-Ar), 125.56 (CH-Ar), 127.47 (C-Ar), 135.74 (C-Ar), 141.26 (C-Ar), 143.85 (C-Ar), 147.25 (C-Ar), 152.55 (C=O), 171.49 (C=O). MS (ESI): 593.5 $[(M+H)]^+$.

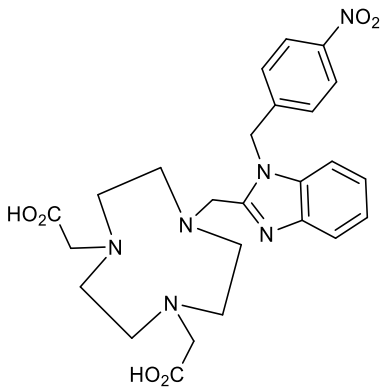
6.6.25. Synthesis of 4,4',4''-((2,2',2''-((1,4,7-triazanonane-1,4,7-triyl)tris(methylene))tris (1H-benzo[d]imidazole-2,1-diyl))tris(methylene))trianiline (22)



22

$NaBH_4$ (122 mg, 3.24 mmol) and S_8 (200 mg, 6.48 mmol) were suspended in dry THF (10 ml) and stirred under argon for 1 hour. 1,4,7-tris((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane (**20**) (50 mg, 0.054 mmol) in dry THF (10 ml) was added and the reaction was heated under reflux overnight then left to cool. The resulting solution was washed with 5% NaOH solution (5 x 20 ml), the organic layer was dried (Na_2SO_4), filtered and concentrated *in vacuo* to yield a crude yellow solid (45 mg). The crude solid was purified via flash column alumina, eluting was DCM with increased gradient of MeOH from 0→5 % to yield a light yellow solid (0.035 mg, 77%). ^{1}H NMR ($CDCl_3$): δ 1.26 (m, 2H, CH_2), 2.14-2.31 (m, 8H, CH_2), 2.32-2.91 (m, 8H, CH_2), 5.04 (s, 2H, CH_2), 5.09-5.49 (m, 4H, CH_2), 6.37-6.83 (m, 9H, CH-Ar), 6.99 (s, 7H, CH-Ar), 7.12-7.44 (m, 8H, CH-Ar), 7.65-8.10 (m, 2H, CH-Ar); HRMS (ESI) calc. 835.4673 found 835.4662 $[(M+H)]^+$.

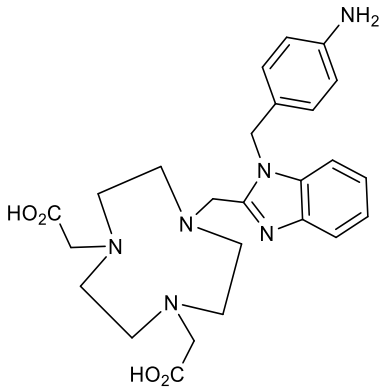
6.6.26. Synthesis of 2,2'-(7-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane-1,4-diyl)diacetic acid (23)



23

di-*tert*-butyl 2,2'-(7-((1-(4-Nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7 triazanonane -1,4-diyl) diacetate (**19**) (0.56 g, 0.89 mmol) was dissolved in (10 ml) 6 N HCl and heated using microwave irradiation at 230 °C for 12 minutes then concentrated by *vacuo* to give a light yellow solid (0.45 g, 100%). ^{1}H NMR (D_2O): δ 2.71 (br s, 4H, CH_2), 2.94 (m, 4H, CH_2), 3.05 (s, 4H, CH_2), 3.72 (s, 4H, CH_2), 4.40 (s, 2H, CH_2), 5.71 (s, 2H, CH_2-CAr), 7.23 (d, 2H, CH-Ar, J=8.4 Hz), 7.42 (m, 2H, CH-Ar), 7.53 (d, 1H, CH-Ar, J=8.2 Hz), 7.60 (d, 1H, CH-Ar, J=8.0 Hz), 7.92 (d, 2H, CH-Ar, J=8.2 Hz); ^{13}C NMR (D_2O): δ 49.54(CH_2), 49.68 (CH_2), 50.97 (CH_2), 51.11 (CH_2), 56.10 (CH_2), 64.89 (CH_2), 113.75 (CH-Ar), 119.77 (CH-Ar),122.67 (CH-Ar), 123.88 (CH-Ar), 126.51 (CH-Ar), 126.65 (CH-Ar), 130.81 (C-Ar) 137.95 (C-Ar), 145.75 (C-Ar), 149.13 (C-Ar), 151.73 (C-Ar), 167.31 (C=O), 172.95 (C=O), Elemental analysis: (%) calc. for $C_{25}H_{30}N_6O_6$.4HCl(L)+H_2O, C 44.52 H 5.38 N 12.46 Found: C 44.63 H 5.61 N 12.30; MS (ESI): 511.4 $[(M+H)]^+$. HPLC: Method 2 – retention time = 15:53 mins.

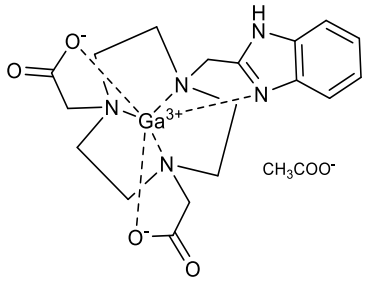
6.6.27. Synthesis of 2,2'-(7-((1-(4-aminobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane-1,4-diyl)diacetic acid (24)



24

2,2'-(7-((1-(4-Aminobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane-1,4-diyl) diacetate (**21**) (53 mg, 0.089 mmol) was dissolved in 5 ml 6 N HCl and heated using microwave irradiation at 230 $^{\circ}$C for 12 mins then concentrated by *vacuo* to give a light orange solid (42 mg, 100%). ^{1}H NMR (D_2O): δ 2.69 (br s, 4H, CH_2), 2.93 (br s, 4H, CH_2), 3.04 (s, 4H, CH_2), 3.35 (s, 2H, CH_2), 3.70 (br s, 2H, CH_2), 4.39 (s, 2H, CH_2-CAr), 5.64 (s, 4H, CH_2), 7.23 (s, 4H, CH-Ar), 7.43 (m, 2H, CH-Ar), 7.59 (d, 2H, CH-Ar, J=8.8 Hz); ^{13}C NMR (D_2O): δ 47.80 (CH_2), 48.12 (CH_2), 49.26 (CH_2), 49.76 (CH_2), 50.91 (CH_2), 55.37 (CH_2), 112.99 (CH-Ar), 114.24 (CH-Ar), 124.00 (CH-Ar), 126.66 (CH-Ar), 126.98 (CH-Ar), 127.47 (CH-Ar), 128.81 (C-Ar), 129.76 (C-Ar), 130.24 (C-Ar), 132.17 (C-Ar), 134.70 (C-Ar), 151.02 (C=O), 172.81 (C=O); Elemental analysis: (%) calc. for $C_{25}H_{32}N_6O_4$(L).5HCl+10H_2O, C 35.62 H 6.82 N 9.97 Found: C 35.67 H 5.04 N 8.58; MS (ESI): 482.3 $[M+H]^+$. HPLC: Method 3 – retention time = 8:20 mins.

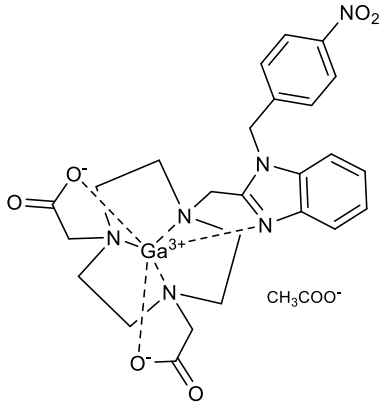
6.6.28. Synthesis of $^{69/71}$Ga complex of 2,2'-(7-((1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane-1,4-diyl)diacetic acid (Ga[18])



Ga[**18**]

2,2'-(7-((1H-Benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane-1,4-diyl)diacetic acid (**18**) (10 mg, 26.6 μmol) was dissolved in ammonium acetate buffer (pH 5, 0.2 M, 10 ml). To this, gallium(III) chloride (5 mg, 26.6 μmol) was added. The reaction was heated to 95 °C for 18 h then concentrated *in vacuo*. The crude solid was redissolved in water (1 ml) and purified using an Amberlite XAD16N column, eluting with water (10 ml) then H_2O: MeCN (9:1, 10 ml) to yield a light creamy solid (9 mg, 75%). Elemental analysis: (%) calc. for $C_{20}H_{26}GaN_5O_6$(M)+2CH_3OH+2H_2O, C 43.87, H 6.36, N 11.63. found: C 43.19, H 6.00, N 12.04. MS (ESI): 443.2 $[(M\text{-}CH_3COO^-+H)]^+$. HPLC: Method 1 – retention time = 6.44 mins.

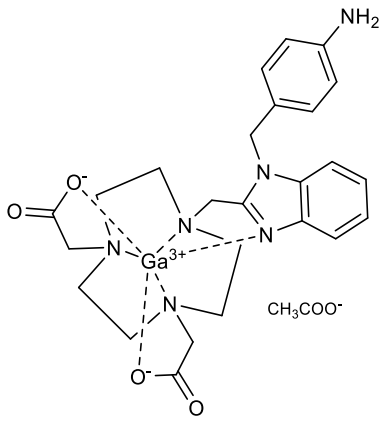
6.6.29. Synthesis of $^{69/71}$Ga complex of 2,2'-(7-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane-1,4-diyl)diacetic acid (Ga[23])



Ga[**23**]

2,2'-(7-((1-(4-Nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane-1,4-diyl) diacetic acid (**23**) (10 mg, 19.6 μmol) was dissolved in ammonium acetate buffer (pH 5, 0.2 M, 10 ml). To this, gallium(III) chloride (3.4 mg, 19.6 μmol) was added. The reaction was heated to 95 °C for 18 h then concentrated *in vacuo*. The crude solid was redissolved in water (1 ml) and purified using an Amberlite XAD16N column, eluting with water (10 ml) then H_2O: MeCN (9:1, 10 ml) to yield a light creamy solid (10 mg, 83%). MS (ESI): 577.3 $[(M\text{-}CH_3COO^-)]^+$. HPLC: Method 2 – retention time = 14.44 mins.

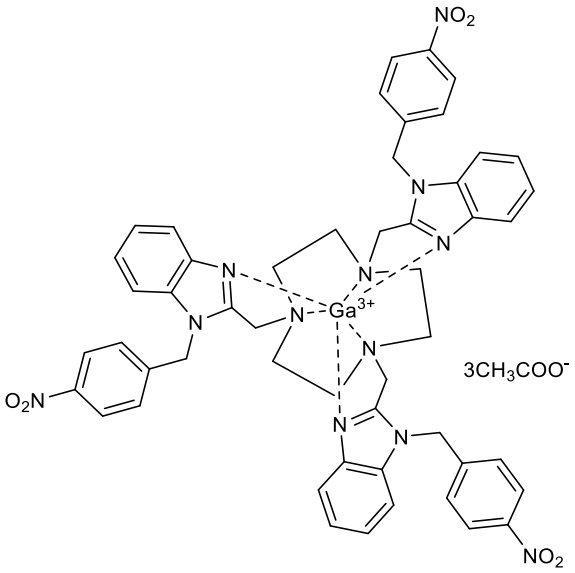
6.6.30. Synthesis of $^{69/71}$Ga complex of 2,2'-(7-((1-(4-aminobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane-1,4-diyl)diacetic acid (Ga[24])



Ga[**24**]

2,2'-(7-((1-(4-Aminobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane-1,4-diyl) diacetic acid (**24**) (3 mg, 6.24 μmol) was dissolved in ammonium acetate buffer (pH 5, 0.2 M, 5 ml). To this, gallium(III) chloride (1.1 mg, 6.24 μmol) was added. The reaction was heated to 95 $^{\circ}$C for 18 h then concentrated *in vacuo*. The crude solid was redissolved in water (1 ml) and purified using an Amberlite XAD16N column, eluting with water (10 ml) then H_2O: MeCN (9:1, 10 ml) to yield a light creamy solid (3 mg, 83%). MS (ESI): 550.2 [(M-CH_3COO^-+3H)]$^+$. HPLC: Method 3 – retention time = 7.57 mins.

6.6.31. Synthesis of $^{69/71}$Ga complex of 1,4,7-tris((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane (Ga[20])

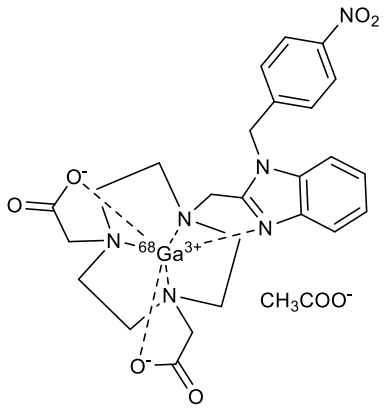


Ga**[20]**

1,4,7-tris((1-(4-Nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane (**20**) (14 mg, 15 μmol) was dissolved in a minimum volume of dry MeCN (1 ml) and ammonium acetate buffer (pH 5, 0.2 M, 4 ml). To this, gallium(III) chloride (3.1 mg, 15 μmol) was added. The reaction was heated to 95 °C for 18 h then concentrated *in vacuo*. The crude solid was redissolved in water (1 ml) and purified using an Amberlite XAD16N column, eluting with water (10 ml) then H_2O: MeCN (9:1, 10 ml) to yield a light yellow solid (1 mg, 63%). HRMS (ESI) calc. 331.1020 found 331.0998 $[(M\text{-}3CH_3COO^-)]^{3+}$. HPLC: Method 4 – retention time = 5.04 mins.

6.7. TACN and NO2A derivatives radiolabelling

6.7.1. Synthesis of ^{68}Ga complex of 2,2'-(7-((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane-1,4-diyl)diacetic acid (^{68}Ga[23])



[^{68}Ga**23**]

Method 1

2,2'-(7-((1-(4-Nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane-1,4-diyl) diacetic acid (**23**) (200 µl, 2 mM) in ammonium acetate buffer (0.2 M, pH 5) was added to formulated $^{68}GaCl_3$ (67 MBq) and the reaction was mixed at RT using a vortex stirrer over 80 mins.

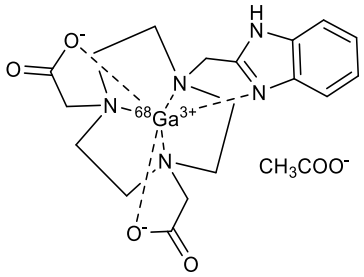
Radio-HPLC	**% RCY**
Time (mins)	**2 Mm**
5	73.17
30	73.82
55	73.92
80	73.78

Method 2

2,2'-(7-((1-(4-Nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane-1,4-diyl) diacetic acid (**23**) (200 µl, 2 mM) in ammonium acetate buffer (0.2 M, pH 5) was added to formulated $^{68}GaCl_3$ (74 MBq) and and the reaction was heated at 90 $^{\circ}$C over 80 mins.

Radio-HPLC	% RCY
Time (mins)	2 mM
5	59.12
30	60.86
55	63.37
80	61.46

6.7.2. Synthesis of ^{68}Ga complex of 2,2'-(7-((1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane-1,4-diyl)diacetic acid (^{68}Ga[18])



[^{68}Ga**18**]

Method 1

2,2'-(7-((1H-Benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane-1,4-diyl)diacetic acid (**18**) (200 µl, 0.6 mM) in ammonium acetate buffer (0.2 M, pH 5) was added to formulated $^{68}GaCl_3$ (55 MBq) and the reaction was mixed at RT using a vortex stirrer over 60 mins.

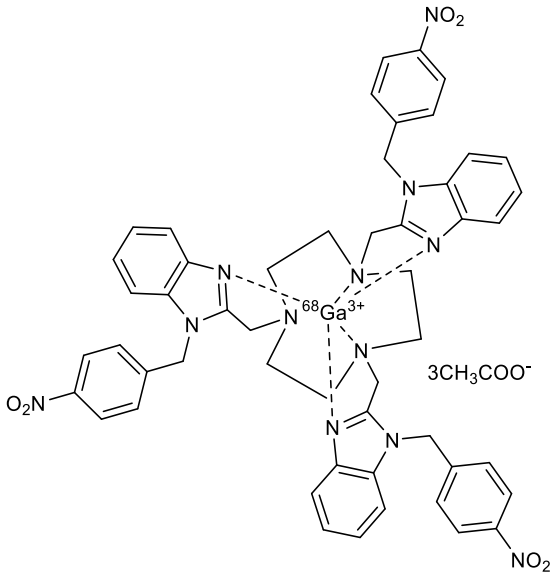
Radio-HPLC	% RCY
Time (mins)	0.6 mM
5	55.66
20	56.82
40	58.01
60	57.57

Method 2

2,2'-(7-((1H-Benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane-1,4-diyl)diacetic acid (**18**) (200 µl, 0.6 mM) in ammonium acetate buffer (0.2 M, pH 5) was added to formulated $^{68}GaCl_3$ (150 MBq) and the reaction was heated at 90 °C over 60 mins.

Radio-HPLC	% RCY
Time (mins)	0.6 mM
5	53.89
20	56.51
40	56.72
60	55.21

6.7.3. Synthesis of ^{68}Ga complex of 1,4,7-tris((1-(4-nitrobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane (^{68}Ga[20])

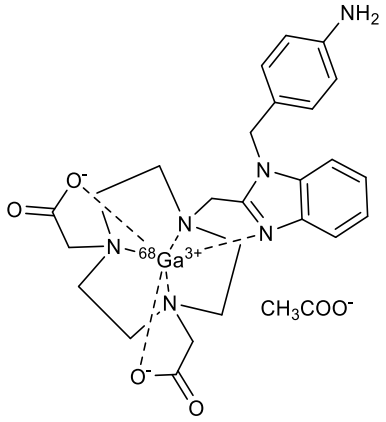


[^{68}Ga**20**]

1,4,7-tris ((1-(4-Nitrobenzyl) -1H-benzo [d]imidazol-2-yl) methyl) -1,4,7-triazanonane (**20**) (200 µl, 1 mM) in a mixture of (ammonium acetate buffer (0.2 M, pH 5): dry MeCN, 8:2) was added to formulated $^{68}GaCl_3$ (71 MBq) and the reaction was mixed at RT using a vortex stirrer over 85 mins.

Radio-HPLC	**% RCY**
Time (mins)	**1 mM**
10	25.42
35	41.12
60	41.34
85	38.77

6.7.4. Synthesis of ^{68}Ga complex of 2,2'-(7-((1-(4-aminobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane-1,4-diyl)diacetic acid (^{68}Ga[24])



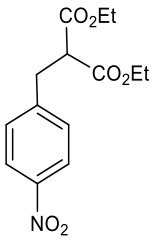
[^{68}Ga**24**]

2,2'-(7-((1-(4-Aminobenzyl)-1H-benzo[d]imidazol-2-yl)methyl)-1,4,7-triazanonane-1,4-diyl) diacetic acid (**24**) (200 µl, 25 µM) in ammonium acetate buffer (0.2 M, pH 5) was added to formulated $^{68}GaCl_3$ (73 MBq) and the reaction was mixed at RT using a vortex stirrer over 70 mins.

Radio-HPLC	**% RCY**
Time (mins)	**25 µM**
10	37.58
30	39.32
50	40.47
70	38.89

6.8. Synthesis of the precursors for C-functionalised bis-azamacrocycles

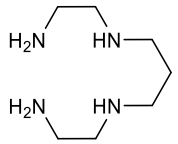
6.8.1. Synthesis of diethyl 2-(4-nitrobenzyl) malonate (25)[368]



25

The synthetic procedure was carried out following a literature procedure.[368] Diethyl malonate (36.6 ml, 38.61 g, 241.08 mmol) was added slowly to a stirring suspension of NaH (60% dispersion in mineral oil, 6.00 g, 150 mmol) in dry DME (dimethoxymethane) (250 ml) cooled to 0 $^{\circ}$C in an ice bath. The mixture was stirred under N_2 for 0.5 h until effervescence had ceased. 4-Nitrobenzyl bromide (25.92 g, 120 mmol) in dry DME (50 ml) was added dropwise via cannula. The resulting mixture was allowed to warm to RT and stirred for 1 h. The resulting orange suspension was poured into a separating funnel charged with EtOAc (300 ml) and sat.aq.NH_4Cl (250 ml). The aqueous layer was then extracted with EtOAc (5 x 100 ml) and the combined organic extracts were washed with distilled H_2O and dried over anhydrous $MgSO_4$. The solvent was reduced on the rotary evaporator to yield a solid which was then recrystallized (hexane/Et_2O 50:50) affording yellow needles (28.1 g, 85%). ^{1}H NMR ($CDCl_3$): δ 1.30 (m, 6H, CH_3), 3.30 (d, 2H, CH_2, J=7.7 Hz), 3.63 (t, 1H, CH, J=7.7 Hz), 4.13 (m, 4H, CH_2), 7.36 (d, 2H, CH-Ar, J=6.8 Hz), 8.12 (d, 2H, CH-Ar, J=6.8 Hz); ^{13}C NMR ($CDCl_3$): δ 13.98 (CH_3) 34.28 (CH_2), 53.08 (CH), 61.80 (CH_2), 123.73 (CH-Ar), 129.80 (CH-Ar), 145.60 (C-Ar), 168.23 (C-Ar); HRMS (ESI) calc. 296.1129; found, 296.1132 $[M+H]^+$.

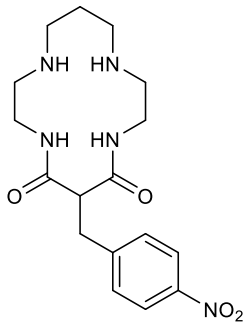
6.8.2. N^1,$N^{1'}$-(propane-1,3-diyl)bis(ethane-1,2-diamine) (26)[369]



26

The synthetic procedure was carried out following a literature procedure.[369] To a solution of 1,2-ethanediamine (62 g, 1.24 mol) in EtOH (200 ml) heated to 70 °C, 1,3-dipromopropane (52.7 g, 0.26 mol) was added dropwise with stirring. After complete addition the reaction mixture was heated to reflux for 2 h. the mixture was allowed to cool to RT and KOH pellets (31 g) were added slowly. On complete addition the mixture stood at RT overnight. The precipitate of KBr was filtrated off and the solvent removed to yield red oil which was distilled under *vacuo* at 123 °C as clear oil. (25 g, 60%). ^{1}H NMR ($CDCl_3$): δ 1.38 (br s, 6H, NH), 1.45 (quin., 2H, CH_2-β-N, J = 6.9 Hz), 2.40-2.58 (m, 8H, CH_2-α-N), 2.67 (m, 4H, CH_2). HRMS (ESI) calc. 161.1761; found, 161.1760 $[M+H]^+$.

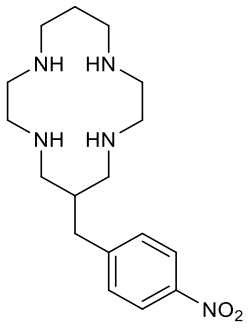
6.8.3. Synthesis of 6-(4-nitrobenzyl)-1,4,8,11-tetraazacyclotetradecane-5,7-dione (27)[367]



27

The synthetic procedure was carried out following a minor modification of a literature procedure.[367] To a solution of 20.65 g (128.85 mmol) of N,N'-bis(2-aminoethy1)-1,3 propanediamine **(26)** in 500 ml of dry EtOH was added 32.62 g (110.50 mmol) of diethyl 2-(4-nitrobenzyl)malonate (**25**). The solution was refluxed for 17 days. The solvent was removed under reduced pressure until a precipitate began to form, and the mixture was cooled to 4 °C for 2 h before the precipitate was collected. The volume of the filtrate was again reduced until a precipitate formed and the resulting solid was filtered after cooling. Additional product could be precipitated by the addition of Et_2O. Yield: 32.14 g (80%) as a tan solid. ^{1}H NMR (CD_3OD): δ 1.56-1.73 (dd, 2H, CH_2-β-N, J=10.6, 5.9 Hz), 2.47-2.78 (m, 8H, CH_2-α-N), 2.97-3.05 (m, 2H, CH_2- α -N), 3.25 (br d, 2H, CH_2-CAr, J=7.5 Hz), 3.48 (m, 1H, CH-β-N), 3.60 (m, 2H, CH_2-α-N), 7.47 (m, 2H, CH-Ar), 8.12 (m, 2H, CH-Ar); ^{13}C NMR (CD_3OD): δ 27.90 (CH_2-β-N), 33.71 (CH_2-CAr), 38.42 (CH_2-α-N), 40.46 (CH_2-α-N), 49.34 (CH_2-α-N), 57.02 (CH-β-N), 123.18 (CH-Ar), 129.87 (CH-Ar), 146.89 (C-Ar), 147.12 (C-Ar), 169.77 (C=O); HRMS (ESI) calc. 364.1979, found 364.1977 $[M+H]^+$.

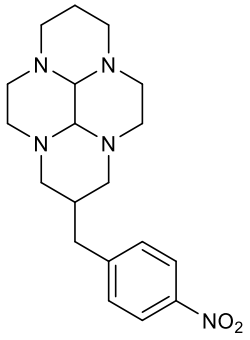
6.8.4. Synthesis of 6-(4-nitrobenzyl)-1,4,8,1 l-tetraazacyclotetradecane (28)[367]



28

The synthetic procedure was carried out following a literature procedure.[367] To a three-necked flask equipped with a dropping funnel and condenser was added 10.00 g (27.5 mmol) of 6-(4-nitrobenzyl)-l,4,8,1 l-tetraazacyclotetradecane-5,7-dione (**27**). The flask was flushed with N_2, and 50 ml of dry THF was added. The solid was dissolved by stirring at 0 °C for 30 mins. To this solution was added dropwise (220 ml) of 1.0 M BH_3.THF at 0 °C. The solution was stirred for 30 mins at 0 °C and then warmed to reflux and refluxed for 24 h. The solution was cooled and the excess borane destroyed by the slow addition of water until the evolution of gas ceased. The solvent was removed under reduced pressure and the residue taken up in 120 ml of 6 M HC1. The resulting solution was refluxed for 3 h followed by stirring at RT for 24 h. The solvent was removed, and the residue was taken up in 50 ml of H_2O. The pH was adjusted to 11.5 using concentrated aqueous NH_3. The solution was extracted five times with 200 ml of chloroform. The combined $CHCl_3$ extracts were dried over Na_2SO_4, and the solvent was removed under reduced pressure to yield a yellowish solid (7.63 g, 82%). ^{1}H NMR ($CDCl_3$): δ 1.69-1.73 (m, 2H, CH_2-β-N), 1.89-2.16 (m, 1H, CH-β-N), 2.17-4.14 (m, 18H, CH_2-α-N x 8, CH_2-CAr x 1), 7.28 (m, 2H, CH-Ar), 8.08 (m, 2H, CH-Ar); ^{13}C NMR ($CDCl_3$): δ 29.23 (CH_2-β-N), 38.74 (CH_2-CAr), 40.72 (CH_2-α-N), 49.25 (CH_2-α-N), 50.78 (CH_2-α-N), 55.52 (CH_2-α-N), 61.92 (CH-β-N), 123.87 (CH-Ar), 129.84 (CH-Ar), 146.51 (C-Ar), 148.64 (C-Ar); MS (ESI): 336.3 $[(M+H)]^+$.

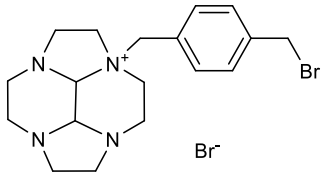
6.8.5. Synthesis of 7-(4-nitrobenzyl)decahydro-1*H*,6*H*-3a,5a,8a,10a-tetraazapyrene (29)[372]



29

The synthetic procedure was carried out following a literature procedure.[372] To a solution of 6-(4-nitrobenzyl)-1,4,8,11-tetraazacyclotetradecane (**28**) (1.56 g, 4.65 mmol) in MeOH (150 ml) at -5 °C was added glyoxal (0.53 ml, 4.65 mmol) in MeOH (20 ml) dropwise. After complete addition the mixture was stirred for 3 h. The mixture was then concentrated *in vacuo* and the residue taken up in DCM, the DCM solution was then filtered concentrated *in vacuo* to yield a light yellow solid (1.62 g, 98%). ^{1}H NMR ($CDCl_3$): δ 0.68-1.44 (m, 2H, CH_2-β-N), 1.52-3.60 (m, 21H, CH_2-α-N x 8, CH-β-N x 1, CH_{aminal} x 2, CH_2-CAr x 1), 7.23 (m, 2H, CH-Ar), 8.08 (m, 2H, CH-Ar); ^{13}C NMR ($CDCl_3$): δ 19.67 (CH_2-β-N), 19.94 (CH-β-N), 29.12 (CH_2-CAr), 30.28 (CH_2-α-N), 36.97 (CH_2-α-N), 37.25 (CH_2-α-N), 38.33 (CH_2-α-N), 38.73 (CH_2-α-N), 40.67 (CH_2-α-N), 41.35 (CH_2-α-N), 49.21 (CH_2-α-N), 50.77 (C_{aminal}), 55.52 (C_{aminal}), 123.69 (CH-Ar), 129.84 (CH-Ar), 147.46 (C-Ar), 148.64 (C-Ar); HRMS (ESI) calc. 358.2238, found 358.2235 $[M+H]^+$.

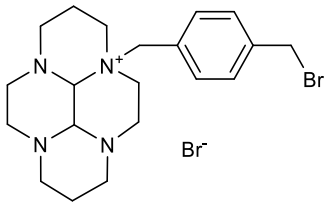
6.8.6. Synthesis of 8a-(4-(bromomethyl)benzyl)decahydro-2*H*-2a,4a,6a,8a-tetraazacyclopenta [fg]acenaphthylen-8a-ium bromide (30)[373]



30

The synthetic procedure was carried out following a minor modification of a literature procedure.[373] A solution of α, α-dibromo-p-xylene (1.35 g, 5.14 mmol) in 10 ml of dry THF was added dropwise to a THF solution of cyclen glyoxal (**10)** (1.00 g, 5.14 mmol, 15 ml) and the mixture was stirred for 24 h at RT. The precipitate was filtered off, washed with dry THF and dried *in vacuo* to give a white solid (2.286 g, 97%). ^{1}H NMR (D_2O): δ 2.30-2.50 (m, 1H, CH_2-N), 2.60-2.88 (m, 2H, CH_2-N), 2.90-3.55 (m, 14H, CH_2-N), 3.64-3.80 (m, 2H, CH_{aminal}), 3.90-4.17 (m, 2H, CH_2-CAr), 4.38-4.61 (m, 1H, CH_2-N), 7.30-7.46 (m, 1H, CH-Ar), 7.50-7.63 (m, 3H, CH-Ar); ^{13}C NMR (D_2O): δ 12.35 (CH_2-N), 19.61 (CH_2-Br), 45.50 (CH_2-N), 47.12 (CH_2-N), 49.50 (CH_2-N), 53.62 (CH_2-N), 54.70 (CH_2-N), 56.00 (CH_2-CAr), 56.87 (CH_2-N), 65.10 (CH_2-N), 65.43 (C_{aminal}), 88.50 (C_{aminal}), 125.76 (CH-Ar), 126.52 (CH-Ar), 127.06 (C-Ar), 127.49 (C-Ar); MS (ESI): 377.2 $[(M-Br)]^+$; HRMS (ESI) calc. 377.1336 found 377.1335 $[M-Br]^+$.

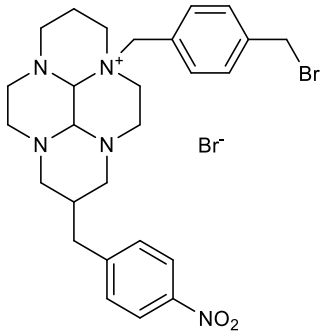
6.8.7. Synthesis of 3a-(4-(bromomethyl)benzyl)dodecahydro-6*H*-3a,5a,8a,10a-tetraazapyren-3a-ium bromide (31)[373]



31

The synthetic procedure was carried out following a minor modification of a literature procedure.[373] A solution of α, α-dibromo-p-xylene (2.36 g, 9 mmol) in 30 ml of dry THF was added dropwise to a THF solution of cyclam glyoxal (**11**) (2.00 g, 9 mmol) in 30 ml and the mixture was stirred for 3 days at RT. The precipitate was filtered off, washed with dry THF and dried *in vacuo* to give a white solid (3.03 g, 68%). ^{1}H NMR ($CDCl_3$): δ 1.33 (d, 1H, CH_2-β-N, J=13.1 Hz), 1.71 (m, 1H, CH_2-β-N), 2.14 (m, 4H, CH_2-α-N), 2.30 (d, 1H, CH_2-β-N, J=11.2 Hz), 2.43-2.61 (m, 2H, CH_2-β-N), 2.74-3.04 (m, 6H, CH_2-α-N), 3.13 (m, 1H, CH_2-α-N), 3.33-3.47 (m, 2H, CH_2-α-N), 3.52 (m, 1H, CH_2-α-N), 3.99 (m, 1H, CH_2-α-N), 4.09 (s, 1H, CH_2-α-N), 4.21 (m, 1H, CH_{aminal}), 4.44 (s, 2H, CH_2-CAr) 4.49 (br s, 1H, CH_{aminal}), 5.52 (m, 1H, CH_2-CAr), 5.63 (m, 1H, CH_2-Ar), 7.32 (s, 1H, CH-Ar), 7.34 (d, 1H, CH-Ar, J=2.2 Hz), 7.64 (d, 2H, CH-Ar, J=8.2 Hz); ^{13}C NMR ($CDCl_3$): δ 19.01 (CH_2-β-N), 19.33 (CH_2-β-N), 25.68 (CH_2-Br), 32.43 (CH_2-α-N), 42.83 (CH_2-α-N), 46.93 (CH_2-α-N), 49.49 (CH_2-α-N), 52.30 (CH_2-α-N), 54.46 (CH_2-α-N), 58.32 (CH_2-α-N), 60.22 (CH_2-α-N), 68.06 (CH_2-CAr), 70.00 (C_{aminal}), 80.91 (C_{aminal}), 126.79 (CH-Ar), 129.60 (CH-Ar), 134.48 (C-Ar), 140.21 (C-Ar); HRMS (ESI) calc. 405.1649 found 405.1646 $[M\text{-}Br]^+$.

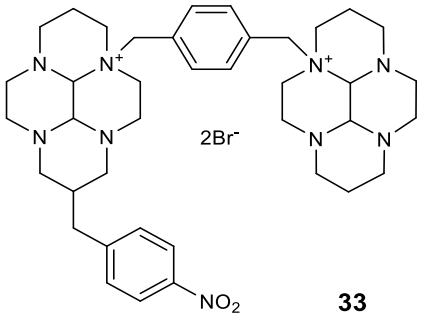
6.8.8. Synthesis of 3a-(4-(bromomethyl)benzyl)-7-(4-nitrobenzyl)dodecahydro-6*H*-3a,5a,8a,10a-tetraazapyren-3a-ium bromide (32)



32

A solution of α, ˉα –dibromo-p-xylene (0.73 g, 2.79 mmol) in 30 ml of dry THF was added dropwise to a THF solution of 7-(4-nitrobenzyl)decahydro-1*H*,6*H*-3a,5a,8a,10a-tetraazapyrene (**29**) (1.00 g, 2.79 mmol) in 30 ml and the mixture was stirred for 6 days at RT. The precipitate was filtered off, washed with dry THF and dried *in vacuo.* Light yellow solid was obtained (1.41 g, 80%). ^{1}H NMR ($(CD_3)_2SO$): δ 1.08 (m, 1H, CH-β-N), 2.33 (m, 2H, CH_2-CAr), 2.55-2.82 (m, 10H, CH_2-α-N x4, CH_2-β-N x1), 3.09-3.24 (m, 6H, CH_2-α-N), 4.16 (m, 1H, CH_{aminal}), 4.32 (m, 1H, CH_{aminal}), 4.63 (m, 2H, CH_2-CAr), 5.14 (m, 2H, CH_2-CAr), 7.49 (m, 4H, CH-Ar), 8.16 (m, 4H, CH-Ar). HRMS (ESI) calc. 540.1969 found 540.1982 $[M-Br]^+$.

6.8.9. Synthesis of 10a-(4-((dodecahydro-1H-3a,5a,8a,10a-tetraazapyren-3a-ium-3a-yl)methyl)benzyl)-7-(4-nitrobenzyl)dodecahydro-1H-3a,5a,8a,10a-tetraazapyren-10a-ium (33)



33

Method 1 (preferred method)

To a solution of 3a-(4-(bromomethyl)benzyl)-7-(4-nitrobenzyl)dodecahydro-6H-3a,5a,8a,10a-tetraazapyren-3a-ium bromide (**32**) (1.00 g, 1.60 mmol) in 40 ml of dry DMF, (0.35 g, 1.60 mmol) of decahydro-1*H*,6*H*-3a,5a,8a,10a-tetraazapyrene (**11**) was added and the mixture was stirred for 5 days at RT. The solvent was reduced and precipitate was washed with Et_2O (3 x 20 ml) to give a light yellow solid (1.08 g, 81%). ^{1}H NMR (D_2O): δ 1.25-1.29 (m, 2H, CH_2-β-N), 1.57-1.60 (m, 2H, CH_2-β-N), 1.69 (m, 2H, CH_2-β-N), 1.90-2.16 (m, 6H, CH_2-α-N), 2.22-2.34 (m, 4H, CH_2-α-N), 2.37-2.52 (m, 2H, CH_2-CAr), 2.77-3.19 (m, 18H, CH_2-α-N), 3.25-3.60 (m, 8H, CH_2-α-N), 3.96-4.08 (m, 2H, CH_2-CAr), 4.17 (m, 2H, CH_{aminal}), 4.44 (s, 2H, CH_2-CAr), 4.86-4.91 (m, 2H, CH_{aminal}), 7.33 (m, 4H, CH-Ar), 7.42 (m, 4H, CH-Ar); ^{13}C NMR (D_2O): δ 17.71 (CH_2-β-N), 18.51 (CH_2-β-N), 24.11 (CH_2-β-N), 25.10 (CH_2-CAr), 32.49 (CH-β-N), 42.08 (CH_2-α-N), 46.47 (CH_2-α-N), 48.07 (CH_2-α-N), 51.07 (CH_2-α-N), 51.27 (CH_2-α-N), 51.84 (CH_2-α-N), 51.93 (CH_2-α-N), 53.02 (CH_2-α-N), 53.90 (CH_2-α-N), 59.90 (CH_2-α-N), 60.19 (CH_2-α-N), 60.78 (CH_2-α-N), 62.16 (CH_2-α-N), 62.46 (CH_2-α-N), 63.14 (CH_2-α-N), 67.86 (CH_2-α-N), 67.96 (CH_2-CAr), 69.73 (CH_2-CAr), 69.83 (C_{aminal}), 81.63 (C_{aminal}), 82.03 (C_{aminal}), 86.65 (C_{aminal}), 124.71 (CH-Ar), 125.49 (CH-Ar), 127.95 (CH-Ar), 129.92 (CH-Ar), 133.46 (C-Ar), 133.56 (C-Ar), 133.75 (C-Ar), 134.15 (C-Ar). HRMS (ESI) calc. 762.3813 found 762.3811 $[M\text{-}Br]^+$.

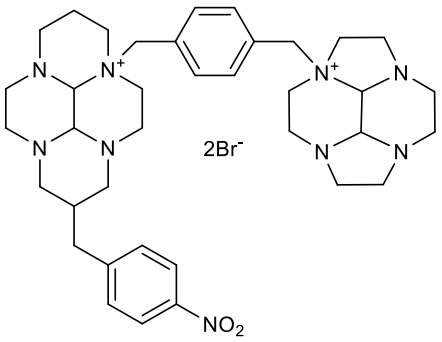
Method 2

To a suspension of compound (**31**) (0.6 g, 1.23 mmol) in 20 ml of dry THF (0.44 g, 1.23 mmol) of compound (**29**) in 20 ml of dry THF was added dropwise and the mixture was stirred for 14 days at RT. The precipitate was filtered and washed with THF and dried in *vacuo* to give a light yellow solid (0.63 g, 62%). ^{1}H NMR (D_2O): δ 1.25-1.29 (m, 2H, CH_2), 1.57-1.60 (m, 2H, CH_2), 1.69 (s, 2H, CH_2), 1.90-2.16 (m, 6H, CH_2), 2.22-2.34 (m, 4H, CH_2), 2.37-2.52 (m, 2H, CH_2), 2.77-3.19 (m, 18H, CH_2), 3.25-3.60 (m, 8H, CH_2), 3.96-4.08 (m, 2H, CH_2), 4.17 (s, 2H, CH_2), 4.44 (s, 2H, CH_2), 4.86-4.91 (m, 2H, CH_2), 7.33 (m, 4H, CH-Ar), 7.42 (m, 4H, CH-Ar). MS (ESI): 762.2 $[(M-Br)]^{+}$.

Method 3

To a suspension of compound (**31**) (0.27 g, 0.56 mmol) in 10 ml of dry MeCN (0.20 g, 0.56 mmol) of compound (**11**) in 10 ml of dry MeCN was added dropwise and the mixture was stirred for 14 days at RT. The precipitate was filtered and washed with dry MeCN and dried in *vacuo* to give a light yellow solid (0.20 g, 42%). ^{1}H NMR (D_2O): δ 1.25-1.29 (m, 2H, CH_2), 1.57-1.60 (m, 2H, CH_2), 1.69 (s, 2H, CH_2), 1.90-2.16 (m, 6H, CH_2), 2.22-2.34 (m, 4H, CH_2), 2.37-2.52 (m, 2H, CH_2), 2.77-3.19 (m, 18H, CH_2), 3.25-3.60 (m, 8H, CH_2), 3.96-4.08 (m, 2H, CH_2), 4.17 (s, 2H, CH_2), 4.44 (s, 2H, CH_2), 4.86-4.91 (m, 2H, CH_2), 7.33 (m, 4H, CH-Ar), 7.42 (m, 4H, CH-Ar). HRMS (ESI) calc. 341.7312 found 341.7313 $[M-2Br]^{2+}$.

6.8.10. Synthesis of 10a-(4-((decahydro-1*H*-2a,4a,6a,8a-tetraazacyclopenta[fg] acenaphthylen-8a-ium-8a-yl)methyl)benzyl)-7-(4-nitrobenzyl)dodecahydro-1*H*-3a,5a,8a,10a-tetraazapyren-10a-ium (34)



34

Method 1 (preferred method)

To a solution of 3a-(4-(bromomethyl)benzyl)-7-(4-nitrobenzyl)dodecahydro-6H-3a,5a,8a,10a-tetraazapyren-3a-ium bromide (**32**) (1.00 g, 1.6 mmol) in 40 ml of dry DMF , (0.31 g, 1.6 mmol) of cis-13-1, 4, 7, 10-tetraazatetracyclo [5.5.1.$0^{4,14}0^{10,13}$] tetradecane **(10)** was added and the mixture was stirred for 3 days at RT. The solvent was reduced and precipitate was washed with Et_2O (3 x 20 ml) to give a light yellow solid (1.07 g, 82%). ^{1}H NMR ($(CD_3)_2SO$): δ 1.23 (m, 1H, CH-β-N), 1.38-2.41 (m, 8H, CH_2-β-N x 1, CH_2-N x 3), 2.54-2.82 (m, 8H, CH_2-N), 2.84-3.36 (m, 14H, CH_2-N), 3.56-3.96 (m, 6H, CH_2-N x 2, CH_2-CAr x 1), 3.97-4.55 (m, 4H, CH_2-CAr), 4.57-4.90 (m, 2H, CH_{aminal}), 4.92-5.11 (m, 1H, CH_{aminal}), 5.15-5.43 (m, 1H, CH_{aminal}), 7.25-7.85 (m, 5H, CH-Ar), 7.95 (m, 1H, CH-Ar), 8.05-8.28 (m, 2H, CH-Ar). HRMS (ESI) calc. 734.3500 found 734.3498 $[M-Br]^+$.

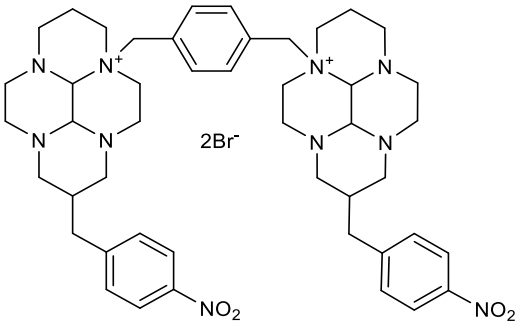
Method 2

To a suspension of compound (**30**) (1.02 g, 2.23 mmol) in 20 ml of dry DMF (0.80 g, 2.23 mmol) of compound (**29**) in 5 ml of dry DMF was added dropwise and the mixture was stirred for 12 days at RT. The solvent was removed then (20 ml) of $CHCl_3$ was added and the mixture was left to stir 10 minutes then precipitate was filtered and washed with $CHCl_3$ (20 ml x 2) and dried *in vacuo* to give a light brown solid (1.19 g, 65%). MS (ESI): 734.4 $[(M-Br)]^+$.

Method 3

To a suspension of compound (**30**) (0.25 g, 0.56 mmol) in 10 ml of dry THF (0.20 g, 0.56 mmol) of compound (**29**) in 10 ml of dry THF was added dropwise and the mixture was stirred for 14 days at RT. The precipitate was filtered and washed with dry THF and dried in *vacuo* to give a creamy solid (0.28 g). The desired product was not isolated using this synthetic procedure**.**

6.8.11. Synthesis of 10a',10a-(1,4-phenylenebis(methylene))bis(7'-(4-nitrobenzyl)dodecahydro-1'H-3a',5a',8a',10a'-tetraazapyren-10a'-ium) (35)



35

Method 1 (preferred method)

α,ˉα–dibromo-p-Xylene (0.16 g, 0.63 mmol) was added to stirred solution of 7-(4-nitrobenzyl) decahydro-1*H*,6*H*-3a,5a,8a,10a-tetraazapyrene (**29**) (0.50 g, 1.39 mmol) in dry DMF (10 ml). The mixture was stirred at RT for 3 days after that time the solution was concentrated by *vacuo* then washed with Et_2O (3 x 20 ml) and dried *in vacuo* to yield a light brown solid (0.614 g, 99%). ^{1}H NMR ($(CD_3)_2SO$): δ 1.18-1.48 (m, 2H, CH_2-β-N), 1.56-1.84 (m, 2H, CH_2-β-N), 1.92-2.35 (m, 6H, CH_2-α-N x 2, CH-β-N x 2) , 2.72 (m, 8H, CH_2-α-N), 2.90 (m, 10H, CH_2-α-N x 3, CH_2-CAr x 2), 3.28-3.53 (m, 16H, CH_2-α-N x 7, CH_{aminal} x 2), 4.01-4.60 (m, 4H, CH_2-CAr), 4.70-4.87 (m, 1H, CH_{aminal}), 5.11-5.43 (m, 1H, CH_{aminal}), 7.38-7.79 (m, 6H, CH-Ar), 7.90-8.02 (m, 2H, CH-Ar , 8.04-8.39 (m, 4H, CH-Ar). HRMS (ESI) calc. 409.2472 found 409.2472 $[M\text{-}2Br]^{2+}$.

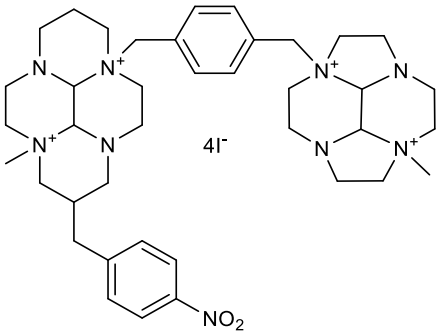
Method 2

α,ˉα–dibromo-p-Xylene (88 mg, 0.33 mmol) and 7-(4-nitrobenzyl) decahydro-1*H*,6*H*-3a,5a,8a,10a-tetraazapyrene (**29**) (300 mg, 0.84 mmol) were suspended in dry THF (30 ml) and stirred under nitrogen (N_2) at RT for 7 days. A precipitate was collected by filtration, washed with dry THF (50 ml) and dried *in vacuo* to yield a light brown solid (0.16 g).The desired product was not isolated using this synthetic procedure**.**

Method 3

α,ˉα–dibromo-p-Xylene (0.13 g, 0.51 mmol) was added to a stirred suspension of 7-(4-nitrobenzyl) decahydro-1*H*,6*H*-3a,5a,8a,10a-tetraazapyrene (**29**) (0.41 g, 1.14 mmol) in dry MeCN (20 ml). The mixture was stirred at RT for 7 days. The precipitate was collected by filtration, washed with dry MeCN (50 ml) and dried *in vacuo* to yield a light yellow solid (0.38 g). The desired product was not isolated using this synthetic procedure**.**

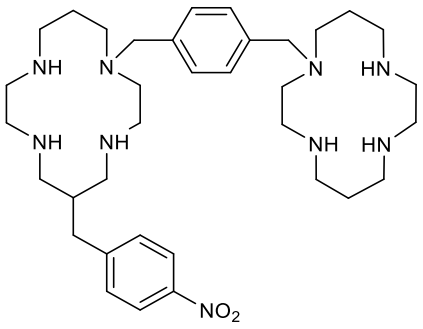
6.8.12. Synthesis of 5a-methyl-10a-(4-((4a-methyldodecahydro-2a,4a,6a,8a tetraazacyclopenta[fg]acenaphthylen-4a,8a-diium-8a-yl)methyl)benzyl)-7-(4-nitrobenzyl)tetradecahydro-3a,5a,8a,10a-tetraazapyrene-5a,10a-diium (36)



36

10a-(4-((decahydro-1H-2a,4a,6a,8a-Tetraazacyclopenta[fg]acenaphthylen-8a-ium-8a-yl) methyl)benzyl)-7-(4-nitrobenzyl)dodecahydro-1H-3a,5a,8a,10a-tetraazapyren-10a-ium (**34**) (0.30 g, 0.37 mmol), was suspended in dry MeCN (50 ml) under nitrogen. Iodomethane (2 ml, 4.54 g, 32 mmol) was added dropwise. The suspension was left to stir for 10 days. A second portion of iodomethane (1 ml, 2.27 g, 16 mmol) was added after 5 days. Excess iodomethane was removed by flowing nitrogen through the suspension for 30 mins. The creamy solid was collected by filtration, washed with Et_2O (100 ml) and dried *in vacuo* to yield a light yellow solid (0.34 g, 77%). $^{1}H,^{13}C$ NMR ($(CD_3)_2SO$) was not clear. MS (ESI): 372.3 $[(M\text{-}4I^{-} +2CH_3O^{-})]^{2+}$. The compound was used in further reactions.

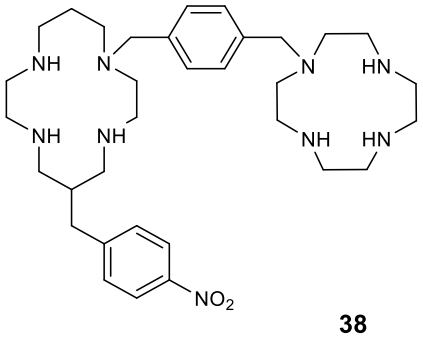
6.8.13. Synthesis of 1-(4-((1,4,8,11-tetraazacyclotetradecan-1-yl)methyl)benzyl)-6-(4-nitrobenzyl)-1,4,8,11-tetraazacyclotetradecane (37)



37

10a-(4-((dodecahydro-1H-3a,5a,8a,10a-Tetraazapyren-3a-ium-3a-yl)methyl)benzyl)-7-(4-nitrobenzyl) dodecahydro-1H-3a,5a,8a,10a-tetraazapyren-10a-ium (**33**) (100 mg, 0.11 mmol), EtONa (320 mg, 4.72 mmol) and hydroxylamine hydrochloride (330 mg, 4.72 mmol) were dissolved in dry MeOH (100 ml) and stirred at reflux under nitrogen for 4 h and then at RT overnight. The clear solution was concentrated *in vacuo* and NaOH solution (10 M, 25 ml) was added. The crude product was extracted from DCM (8 x 50 ml). The combined organic fractions were dried over ($MgSO_4$), filtered and evaporated *in vacuo* to yield a light yellow solid (70 mg, 93%). ^{1}H NMR ($CDCl_3$): δ 0.75-1.06 (br s, 1H, CH-β-N), 1.09-1.42 (m, 4H, CH_2-β-N), 1.53-1.86 (m, 6H, CH_2-α-N), 2.01-2.17 (m, 2H, CH_2-β-N), 2.18-2.27 (m, 2H, CH_2-α-N), 2.28-2.90 (m, 24H, CH_2-α-N), 2.93-3.06 (m, 2H, CH_2-CAr), 3.46-3.65 (m, 4H, CH_2-CAr), 7.10-7.50 (m, 6H, CH-Ar), 8.01 (m, 2H, CH-Ar); ^{13}C NMR ($CDCl_3$): δ 14.19 (CH_2-β-N), 19.68 (CH_2-β-N), 22.74 (CH_2-β-N), 24.28 (CH_2-β-N), 25.11 (CH_2-β-N), 28.01 (CH_2-β-N), 29.75 (CH_2-α-N), 31.98 (CH_2-α-N), 44.86 (CH-ß-N), 46.24 (CH_2-α-N), 48.55 (CH_2-α-N), 49.59 (CH_2-CAr), 50.44 (CH_2-α-N), 52.58 (CH_2-α-N), 54.45 (CH_2-α-N), 56.15 (CH_2-α-N), 57.58 (CH_2-α-N), 58.51 (CH_2-CAr), 62.42 (CH_2-CAr), 115.27 (CH-Ar), 123.69 (CH-Ar), 126.97 (CH-Ar), 127.97 (CH-Ar), 129.85 (C-Ar), 130.87 (C-Ar), 131.72 (C-Ar), 146.55 (C-Ar). MS (ESI): 660.6 $[(M+Na)]^+$.

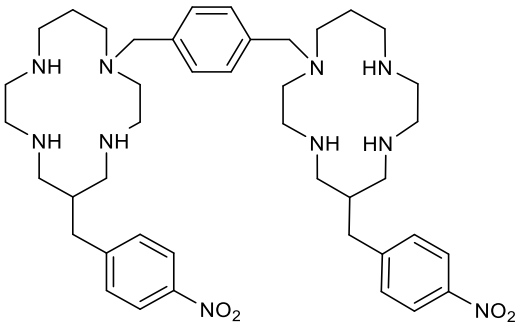
6.8.14. Synthesis of 1-(4-((1,4,7,10-tetraazacyclododecan-1-yl)methyl)benzyl)-6-(4-nitrobenzyl)-1,4,8,11-tetraazacyclotetradecane (38)



38

10a-(4-((decahydro-1*H*-2a,4a,6a,8a-Tetraazacyclopenta[fg]acenaphthylen-8a-ium-8a-yl) methyl)benzyl)-7-(4-nitrobenzyl)dodecahydro-1*H*-3a,5a,8a,10a-tetraazapyren-10a-ium (**34**) (0.20 g, 0.24 mmol), EtONa (0.79 g, 11.52 mmol) and hydroxylamine hydrochloride (0.80 g, 11.52 mmol) were dissolved in dry MeOH (100 ml) and stirred at reflux under nitrogen for 4 h and then at RT overnight. The clear solution was concentrated *in vacuo* and NaOH (10 M, 25 ml) was added. The crude product was extracted from DCM (8 x 50 ml). The combined organic fractions were dried ($MgSO_4$), filtered and evaporated *in vacuo* to yield a light yellow solid (93 mg, 64%). ^{1}H NMR ($CDCl_3$): δ 0.85 (m, 2H, CH_2-β-N), 1.08-1.28 (m, 4H, CH_2-N), 1.50-1.82 (m, 5H, CH_2-N x 2, CH-β-N x 1), 1.91-2.24 (m, 4H, CH_2-N), 2.97-3.67 (m, 24H, CH_2-N x 10, CH_2-CAr x 2), 4.25-4.48 (m, 2H, CH_2-CAr), 7.26-7.35 (m, 6H, CH-Ar), 8.11 (m, 2H, CH-Ar); ^{13}C NMR ($CDCl_3$): δ 18.20 (CH_2-β-N), 29.75 (CH-β-N), 29.81 (CH_2-CAr), 45.41 (CH_2-N), 45,53 (CH_2-N), 45.57 (CH_2-N), 45.67 (CH_2-N), 45.74 (CH_2-N), 45.76 (CH_2-N), 45.84 (CH_2-N), 51.38 (CH_2-N), 51.44 (CH_2-N), 51.50 (CH_2-N), 51.80 (CH_2-N), 53.71 (CH_2-N), 59.45 (CH_2-N), 59.85 (CH_2-N), 66.15 (CH_2-N), 70.42 (CH_2-N), 90.14 (CH_2-CAr), 93.60 (CH_2-CAr), 122.26 (CH-Ar), 123.68 (CH-Ar), 126.93 (CH-Ar), 128.97 (CH-Ar), 129.37 (C-Ar), 129.98 (C-Ar), 143.20 (C-Ar), 151.53 (C-Ar). MS (ESI): 632.4 $[(M+Na)]^+$.

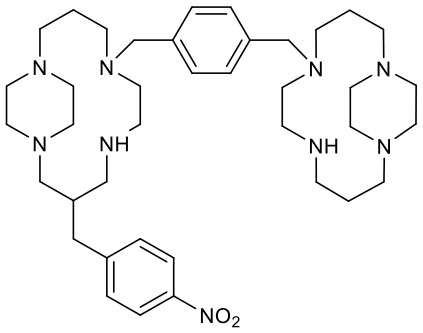
6.8.15. Synthesis of 1,4-bis((6-(4-nitrobenzyl)-1,4,8,11-tetraazacyclotetradecan-1-yl)methyl)benzene (39)



39

10a',10a-(1,4-Phenylenebis(methylene))bis(7'-(4-nitrobenzyl)dodecahydro-1'H-3a',5a',8a',10a'-tetraazapyren-10a'-ium) (**35**) (250 mg, 0.25 mmol), EtONa (0.82 g, 12 mmol) and hydroxylamine hydrochloride (0.84 g, 12 mmol) were dissolved in dry MeOH (100 ml) and stirred at reflux under nitrogen for 4 h and then at RT overnight. The clear solution was concentrated *in vacuo* and NaOH solution (10 M, 25 ml) was added. The crude product was extracted from DCM (8 x 50 ml). The combined organic fractions were dried over ($MgSO_4$), filtered and evaporated *in vacuo* to yield a light yellow solid (89 mg, 33%). ^{1}H NMR ($CDCl_3$): δ 0.69-1.03 (m, 1H, CH-β-N), 1.09-1.26 (m, 2H, CH_2-β-N), 1.53-1.74 (m, 2H, CH_2-β-N), 1.82 (d, 1H, CH-β-N, J=5.6 Hz), 2.02-2.72 (m, 20H, CH_2-α-N x 9, CH_2-CAr x 1), 2.77 (d, 2H, CH_2-Ar, J=12.6 Hz), 2.85 (d, 2H, CH_2-CAr, J=5.3 Hz), 2.91 (d, 2H, CH_2-α-N, J=11.6 Hz), 2.98 (d, 1H, CH_2-α-N, J=2.9 Hz), 3.05 (m, 1H, CH_2-α-N), 3.10 (m, 2H, CH_2-α-N), 3.26-3.73 (m, 10H, CH_2-α-N x 4, CH_2-CAr x 1), 6.91-7.56 (m, 8H, CH-Ar), 7.89-8.25 (m, 4H, CH-Ar); ^{13}C NMR ($CDCl_3$): δ 19.67 (CH_2-β-N), 19.93 (CH_2-β-N), 29.75 (CH-β-N), 30.29 (CH-β-N), 36.66 (CH_2-Ar), 38.33 (CH_2-CAr), 41.35 (CH_2-α-N), 44.69 (CH_2-α-N), 45.77 (CH_2-α-N), 52.58 (CH_2-α-N), 54.26 (CH_2-α-N), 56.10 (CH_2-α-N), 58.51 (CH_2-α-N), 61.74 (CH_2-α-N), 123.13 (CH-Ar), 123.76 (CH-Ar), 129.60 (CH-Ar), 129.77 (CH-Ar), 131.08 (C-Ar), 131.75 (C-Ar), 146.47 (C-Ar), 147.45 (C-Ar). MS (ESI): 795.5 $[(M+Na)]^+$.

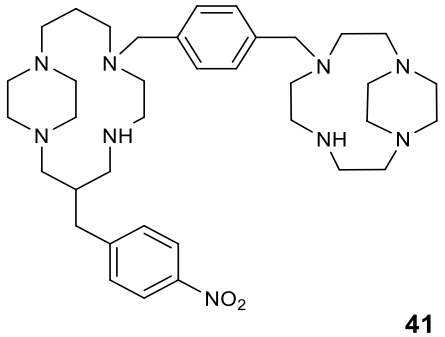
6.8.16. Synthesis of 8-(4-((1,5,8,12-tetraazabicyclo[10.2.2]hexadecan-5-yl)methyl)benzyl) -3-(4-nitrobenzyl)-1,5,8,12-tetraazabicyclo[10.2.2]hexadecane (40)



40

10a-(4-((dodecahydro-1H-3a,5a,8a,10a-Tetraazapyren-3a-ium-3a-yl)methyl)benzyl)-7-(4-nitrobenzyl)dodecahydro-1H-3a,5a,8a,10a-tetraazapyren-10a-ium (**33**) (0.2 g, 0.23 mmol) was dissolved in EtOH (100 ml) and cooled to 0 $^{\circ}$C. $NaBH_4$ (0.34 g, 9.20 mmol) was added slowly and the clear solution was stirred under nitrogen for 30 mins at RT then heated to reflux for 30 mins. After this time the solution was allowed to cool to RT concentrated *in vacuo* and the residue was taken up in purified water (75 ml). The pH was increased to 14 by addition on KOH pellets and extracted with DCM (5 x 50 ml). The organic extracts were combined, dried over anhydrous ($MgSO_4$), filtered and concentrated *in vacuo* to yield a light yellow solid (0.14 g, 88%). ^{1}H NMR ($CDCl_3$): δ 1.30-1.85 (m, 6H, CH_2-β-N), 2.22 (m, 4H, CH_2-α-N), 2.28-2.80 (m, 28H, CH_2-α-N), 2.84-3.07 (m, 8H, CH_2-α-N), 3.10 (m, 1H, CH-β-N), 3.14-3.32 (m, 4H, CH_2-CAr), 3.44-3.57 (m, 2H, CH_2-CAr), 7.05-7.43 (m, 6H, CH-Ar), 8.11 (m, 2H, CH-Ar). Elemental analysis: (%) calc. for $C_{39}H_{63}N_9O_2$(L)+2CH_2Cl_2+H_2O C, 60.61; H, 8.81; N, 15.52 Found: C, 60.82; H, 8.61; N, 15.32. HRMS (ESI) calc. 690.5177 found; 690.5173 $[M+H]^+$.

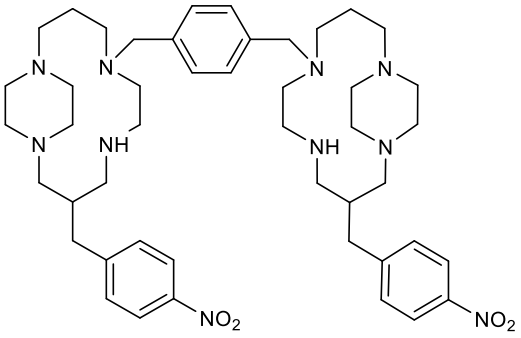
6.8.17. Synthesis of 8-(4-((1,4,7,10-tetraazabicyclo[8.2.2]tetradecan-4-yl)methyl)benzyl)-3-(4-nitrobenzyl)-1,5,8,12-tetraazabicyclo[10.2.2]hexadecane (41)



41

10a-(4-((decahydro-1H-2a,4a,6a,8a-Tetraazacyclopenta[fg]acenaphthylen-8a-ium-8a-yl) methyl)benzyl)-7-(4-nitrobenzyl)dodecahydro-1H-3a,5a,8a,10a-tetraazapyren-10a-ium (**34**) (0.50 g, 0.61 mmol) was dissolved in EtOH (60 ml) and cooled to 0 $^{\circ}$C. $NaBH_4$ (0.92 g, 24.4 mmol) was added slowly and the clear solution was stirred under nitrogen for 30 mins at RT then heated to reflux for 30 mins. After this time the solution was allowed to cool to RT, concentrated *in vacuo* and the residue was taken up in purified water (75 ml). The pH was increased to 14 by addition on KOH pellets and extracted with DCM (5 x 50 ml). The organic extracts were combined, dried over anhydrous ($MgSO_4$), filtered and concentrated *in vacuo* to yield a light yellow solid (0.18 g, 45%). ^{1}H NMR ($CDCl_3$): δ 0.78-1.84 (m, 6H, CH_2-β-N x 1, CH_2-N x 2), 1.86-3.12 (m, 33H, CH-β-N x 1, CH_2-N x 16), 3.14-3.94 (m, 10H, CH_2-CAr x 3, CH_2-N x 2), 6.40-6.79 (m, 1H, CH-Ar), 6.84-7.57 (m, 5H, CH-Ar), 7.90-8.30 (m, 2H, CH-Ar); ^{13}C NMR ($CDCl_3$): δ 18.18 (CH_2-β-N), 18.39 (CH_2-N), 29.77 (CH-β-N), 31.40 (CH_2-N), 36.68 (CH_2-N), 37.49 (CH_2-N), 46.38 (CH_2-N),48.27 (CH_2-N), 50.50 (CH_2-N), 50.71 (CH_2-N), 51.32 (CH_2-N), 52.13 (CH_2-N), 53.15 (CH_2-N), 53.35 (CH_2-N), 53.55 (CH_2-N), 54.97 (CH_2-N), 55.23 (CH_2-N), 56.41 (CH_2-N), 57.21 (CH_2-N), 57.62 (CH_2-CAr), 58.43 (CH_2-N), 59.65 (CH_2-N), 59.85 (CH_2-N), 63.11 (CH_2-CAr), 67.78 (CH_2-CAr), 123.71 (CH-Ar), 123.78 (CH-Ar), 128.77 (CH-Ar), 129.29 (CH-Ar), 129.84 (C-Ar), 129.91 (C-Ar), 129.97 (C-Ar), 130.01 (C-Ar). HRMS (ESI) calc. 662.4870 found; 662.4859 $[(M+H)]^+$.

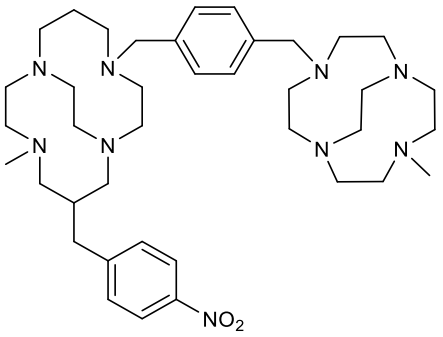
6.8.18. Synthesis of 1,4-bis((10-(4-nitrobenzyl)-1,5,8,12-tetraazabicyclo[10.2.2] hexadecane-5-yl)methyl)benzene (42)



42

10a',10a-(1,4-Phenylenebis(methylene))bis(7'-(4-nitrobenzyl)dodecahydro-1'H-3a',5a',8a',10a' -tetraazapyren-10a'-ium) (**35**) (0.35 g, 0.35 mmol) was dissolved in EtOH (100 ml) and cooled to 0 °C. $NaBH_4$ (0.26 g, 7 mmol) was added slowly and the clear solution was stirred under nitrogen at RT for 4 h then heated to reflux over night. After this time the solution was allowed to cool to RT, concentrated *in vacuo* and the residue was taken up in purified water (50 ml). The pH was increased to 14 by addition on KOH pellets and extracted with DCM (5 x 50 ml). The organic extracts were combined, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to yield a light yellow solid (0.116 g, 41%). ^{1}H NMR ($CDCl_3$): δ 1.10-1.85 (m, 4H, CH_2-β-N), 2.08-2.79 (m, 30H, CH_2-α-N x 12, CH-β-N x 2, CH_2-CAr x 2), 2.84-3.12 (m, 8H, CH_2-α-N), 3.14-3.88 (m, 12H, CH_2-α-N x 4, CH_2-CAr x 2), 6.50-7.25 (m, 4H, CH-Ar), 7.28-7.45 (m, 4H, CH-Ar), 7.93-8.27 (m, 4H, CH-Ar); ^{13}C NMR ($CDCl_3$): δ 13.92 (CH_2-β-N), 19.30 (CH_2-β-N), 23.27 (CH-β-N), 25.03 (CH-β-N), 30.88 (CH_2-CAr), 31.35 (CH_2-CAr), 40.00 (CH_2-α-N), 48.31 (CH_2-α-N), 49.48 (CH_2-α-N), 49.59 (CH_2-α-N), 53.22 (CH_2-α-N), 53.45 (CH_2-α-N), 54.62 (CH_2-α-N), 54.97 (CH_2-α-N), 55.79 (CH_2-α-N), 58.60 (CH_2-α-N), 60.00 (CH_2-α-N), 60.94 (CH_2-α-N), 68.42 (CH_2-α-N), 92.05 (CH_2-α-N), 96.61 (CH_2-α-N), 100.00 (CH_2-CAr), 104.45 (CH_2-CAr), 123.51 (CH-Ar), 123.63 (CH-Ar), 123.75 (CH-Ar), 123.78 (CH-Ar), 129.60 (CH-Ar), 129.83 (CH-Ar), 129.95 (C-Ar), 130.06 (C-Ar), 137.32 (C-Ar), 141.88 (C-Ar), 143.40 (C-Ar), 148.78 (C-Ar). MS (ESI): 825.7 $[(M+H)]^+$.

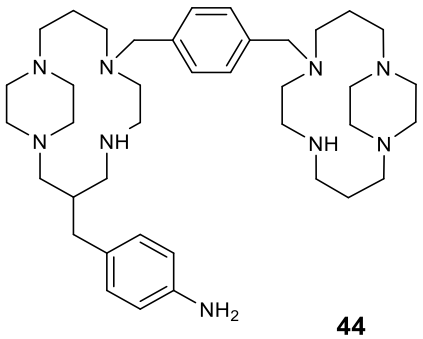
6.8.19. Synthesis of 4-methyl-11-(4-((10-methyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradecan -4-yl)methyl)benzyl)-6-(4-nitrobenzyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (43)



43

5a-Methyl-10a-(4-((4a-methyldodecahydro-2a,4a,6a,8a tetraazacyclopenta[fg]acenaphthylen-4a,8a-diium-8a-yl) methyl)benzyl)-7-(4-nitrobenzyl) tetradecahydro-3a,5a,8a,10a-tetraaza pyrene-5a,10a-diium (**36**) (0.31 g, 0.26 mmol) was dissolved in EtOH (100 ml) and $NaBH_4$ (0.4 g, 10.4 mmol) was added in small portions over period of 1 h. The solution was stirred for 14 days. Water (70 ml) was added to quench the reaction and solvents were removed in *vacou*. The residue was taken up in water (100 ml). The pH was increased to 14 by addition on KOH pellets and extracted with DCM (5 x 100 ml), the combined organic extracts were dried over ($MgSO_4$) and evaporated in *vacuo* to yield a light yellow solid (0.071 g, 40%). ^{1}H NMR ($CDCl_3$): δ 1.09-1.88 (m, 1H, CH-β-N), 1.90-2.50 (m, 14H, CH_2-N x 2, CH_3-N x 2, CH_2-β-N x 1, CH_2-Ar x 1), 2.54-3.03 (m, 20H, CH_2-N), 3.06-3.41 (m, 10H, CH_2-N), 3.43-3.95 (m, 10H, CH_2-N x 3, CH_2-CAr x 2), 6.97-7.61 (m, 6H, CH-Ar), 7.91-8.29 (m, 6H, CH-Ar); ^{13}C NMR ($CDCl_3$): δ 20.03 (CH_2-β-N), 21.62 (CH_2-N), 26.38 (CH-β-N), 29.70 (CH_3-N), 33.57 (CH_2-N), 38.33 (CH_3-N), 38.45 (CH_2-N), 43.45 (CH_2-N), 43.94 (CH_2-N), 44.18 (CH_2-N), 47.72 (CH_2-N), 50.16 (CH_2-N), 50.77 (CH_2-N), 51.62 (CH_2-N), 52.96 (CH_2-CAr), 53.33 (CH_2-N), 55.65 (CH_2-N), 56.13 (CH_2-N), 59.06 (CH_2-N), 59.91 (CH_2-N), 60.52 (CH_2-N), 61.87 (CH_2-N), 62.84 (CH_2-N), 64.06 (CH_2-N), 65.28 (CH_2-N), 67.60 (CH_2-CAr), 71.62 (CH_2-CAr), 115.28 (CH-Ar), 115.65 (CH-Ar), 123.58 (CH-Ar), 123.94 (CH-Ar), 127.97 (C-Ar), 129.31 (C-Ar), 130.16 (C-Ar), 146.63 (C-Ar). MS (ESI): 345.8 $[(M+2H)]^{2+}$.

6.8.20. Synthesis of 4-((8-(4-((1,5,8,12-tetraazabicyclo[10.2.2]hexadecan-5-yl)methyl) benzyl)-1,5,8,12 tetraazabicyclo[10.2.2]hexadecan-3-yl)methyl)aniline (44)



44

Method 1 (preferred method)

8-(4-((1,5,8,12-Tetraazabicyclo[10.2.2]hexadecan-5-yl)methyl)benzyl)-3-(4-nitrobenzyl)-1,5,8,12-tetraazabicyclo[10.2.2]hexadecane (**40**) (0.089 g, 0.12 mmol) and 5% Pd/C (0.30 g) in glacial acetic acid (50 ml) were placed under an atmosphere of H_2 for 4 h. After this time the flask was flushed with N_2 and the solution filtered through diatomaceous earth and washed with HOAc (6 x 20 ml). The filtrate was *vacuo* down to yield a light yellow solid (0.079 g, 100%). ^{1}H NMR ($CDCl_3$): δ 1.09-1.43 (m, 4H, CH_2-β-N), 1.72-1.89 (m, 2H, CH_2-β-N), 1.90-2.07 (m, 16H, CH_2-α-N), 2.10-2.22 (m, 1H, CH-β-N), 2.29-2.55 (m, 4H, CH_2-α-N), 2.54-3.11 (m, 20H, CH_2-α-N), 3.14-3.47 (m, 4H, CH_2-CAr), 3.48-3.77 (m, 2H, CH_2-CAr), 6.47-6.70 (m, 1H, CH-Ar), 6.76-6.99 (m, 1H, CH-Ar), 7.02-7.45 (m, 4H, CH-Ar), 8.01-8.21 (m, 2H, CH-Ar); ^{13}C NMR ($CDCl_3$): δ 12.57 (CH_2-β-N), 12.64 (CH_2-β-N), 13.91 (CH_2-β-N), 14.01 (CH_2-β-N), 19.07 (CH_2-β-N), 19.21 (CH_2-β-N), 22.08 (CH_2-α-N), 22.22 (CH_2-α-N), 23.45 (CH-β-N), 25.10 (CH_2-α-N), 25.19 (CH_2-α-N), 36.51 (CH_2-CAr), 36.62 (CH_2-α-N), 37.27 (CH_2-α-N), 40.03 (CH_2-α-N), 43.98 (CH_2-α-N), 44.39 (CH_2-α-N), 44.53 (CH_2-α-N), 46.44 (CH_2-α-N), 46.58 (CH_2-α-N), 46.72 (CH_2-α-N), 49.39 (CH_2-α-N), 51.60 (CH_2-α-N), 53.09 (CH_2-α-N), 53.56 (CH_2-α-N), 53.66 (CH_2-α-N), 55.25 (CH_2-α-N), 61.93 (CH_2-α-N), 75.59 (CH_2-CAr), 75.73 (CH_2-CAr), 112.13 (CH-Ar), 123.49 (CH-Ar), 123.90 (CH-Ar), 129.24 (CH-Ar), 129.79 (C-Ar), 131.02 (C-Ar), 146.21 (C-Ar), 146.90 (C-Ar). HRMS (ESI) calc. 660.5436 found; 660.5431 $[M+H]^+$.

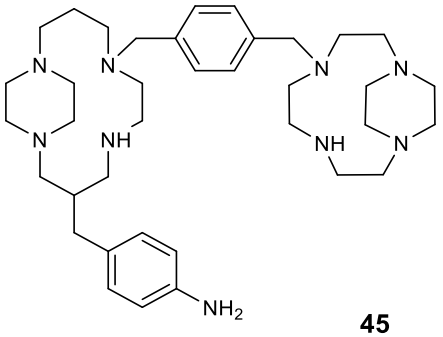
Method 2

BH_3.THF (1 M, 14 ml, 14 mmol) was added slowly via septum to (**40**) (0.25 g, 0.36 mmol) under atmosphere nitrogen. The mixture was heated to reflux for 1 week. After that time dry MeOH was added to quench the excess of BH_3 reagent after effervescence had ceased, HCl (1 M, 10 ml) was added and the mixture was concentrated the residue was taken to water (50 ml) and the pH was increased to 14 by addition on KOH pellets. The aqueous solution was then extracted with $CHCl_3$ (5 x 40 ml). The organic layer was combined and dried over ($MgSO_4$) then *vacuo* down to yield a light yellow solid (0.23 g, 96%). ^{1}H NMR ($CDCl_3$): δ 1.67 (m, 4H, CH_2-β-N), 1.75 (m, 4H, CH_2-α-N), 2.02 (m, 2H, CH_2-β-N), 2.47 (m, 13H, CH_2-α-N x 6, CH-β-N x 1), 2.65 (m, 10H, CH_2-α-N), 2.88 (m, 6H, CH_2-α-N), 3.10 (m, 4H, CH_2-α-N), 3.28 (m, 2H, CH_2-CAr), 3.45 (m, 4H, CH_2-α-N), 3.66 (m, 4H, CH_2-CAr), 6.61 (m, 2H, CH-Ar), 6.93 (m, 2H, CH-Ar), 7.16 (m, 4H, CH-Ar). MS (ESI): 660.6 $[(M+H)]^+$.

Method 3

$NaBH_4$ (0.18 g, 5 mmol) and S_8 (0.32 g, 10 mmol) were suspended in EtOH (10 ml) and stirred under nitrogen for 1 h. Compound (**40**) (0.05 g, 0.072 mmol) dissolved in EtOH (10 ml) was added and the reaction mixture was heated at reflux 1 week under nitrogen. The resulting solution was then cooled, filtered and washed the precipitate with EtOH (3 x 10 ml) to get brown solid (0.14 g) did not give ^{1}H NMR. The filtrate was concentrated then taken up to water (40 ml). The pH was increased to 14 by addition on KOH pellets and extracted with $CHCl_3$ (5 x 50 ml). The organic extracts were combined, dried over anhydrous ($MgSO_4$), filtered and concentrated *in vacuo* to yield a yellow solid (0.026 g). The desired product was not isolated using this synthetic procedure**.**

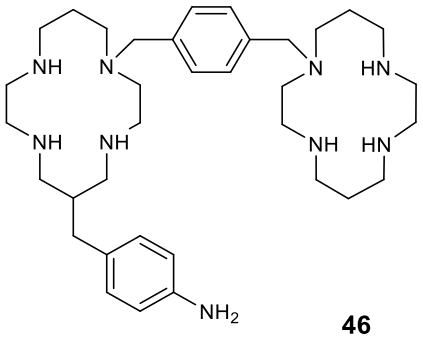
6.8.21. Synthesis of 4-((8-(4-((1,4,7,10-tetraazabicyclo[8.2.2]tetradecan-4-yl)methyl) benzyl)-1,5,8,12-tetraazabicyclo[10.2.2]hexadecan-3-yl)methyl)aniline (45)



45

$NaBH_4$ (56 mg, 1.5 mmol) and S_8 (96 mg, 3 mmol) were suspended in dry THF (10 ml) and stirred under argon for 1 h. 8-(4-((1,4,7,10-tetraazabicyclo[8.2.2]tetradecan-4-yl)methyl)benzyl)-3-(4-nitrobenzyl)-1,5,8,12-tetraazabicyclo[10.2.2]hexadecane (**41**) (50 mg, 0.075 mmol) in THF (30 ml) was added and the reaction was heated under reflux overnight then left to cool. The resulting solution was washed with 5% NaOH solution (5 x 20 ml), the organic layer was dried (Na_2SO_4), filtered and concentrated *in vacuo* to yield a brown solid (47 mg, 100%). ^{1}H NMR ($CDCl_3$): δ 0.68-1.18 (m, 6H, CH_2-β-N x 1, CH_2-N x 2), 1.51-1.72 (m, 2H, CH_2-N), 1.74-3.47 (m, 37H, CH_2-β-N x 1, CH_2-N x 18), 3.48-3.71 (m, 4H, CH_2-CAr), 6.52-6.65 (m, 2H, CH-Ar), 6.85-7.08 (m, 6H, CH-Ar); ^{13}C NMR ($CDCl_3$): δ 14.32 (CH_2-β-N), 14.52 (CH_2-N), 18.39 (CH-β-N), 21.23 (CH_2-N), 25.50 (CH_2-N), 25.70 (CH_2-N), 29.57 (CH_2-N), 29.77 (CH_2-N), 30.27 (CH_2-N), 30.38 (CH_2-N), 30.49 (CH_2-N), 32.82 (CH_2-N), 34.24 (CH_2-N), 34.44 (CH_2-N), 34.85 (CH_2-CAr), 45.01 (CH_2-N), 45.22 (CH_2-N), 53.35 (CH_2-N), 53.55 (CH_2-N), 67.98 (CH_2-N), 68.19 (CH_2-N), 87.64 (CH_2-N), 87.91 (CH_2-N), 89.73 (CH_2-CAr), 99.90 (CH_2-CAr), 125.59 (CH-Ar), 127.71 (CH-Ar), 128.38 (CH-Ar), 129.83 (CH-Ar), 135.86 (C-Ar), 137.76 (C-Ar), 143.90 (C-Ar), 151.60 (C-Ar). HRMS (ESI) calc. 316.7597 found; 316.7580 $[(M+2H)]^{2+}$.

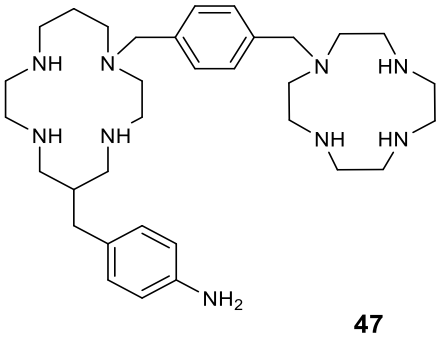
6.8.22. Synthesis of 4-((1-(4-((1,4,8,11-tetraazacyclotetradecan-1-yl)methyl)benzyl)-1,4,8,11-tetraazacyclotetradecan-6-yl)methyl)aniline (46)



46

1-(4-((1,4,8,11-Tetraazacyclotetradecan-1-yl)methyl)benzyl)-6-(4-nitrobenzyl)-1,4,8,11-tetraazacyclotetradecane (**37**) (0.094 g, 0.14 mmol) and 5% Pd/C (0.20 g) in glacial acetic acid (50 ml) were placed under an atmosphere of H_2 for 4 h. After this time the flask was flushed with N_2 and the solution filtered through diatomaceous earth and washed with HOAc (6 x 20 ml). The filtrate was *vacuo* down to yield a light yellow solid (0.085 g, 100%). ^{1}H NMR ($CDCl_3$): δ 0.85 (m, 1H, CH-β-N), 1.10-1.69 (m, 6H, CH_2-β-N), 1.90-2.22 (m, 14H, CH_2-α-N x 6, CH_2-CAr x 1), 2.23-2.43 (m, 6H, CH_2-α-N), 2.74 (m, 4H, CH_2-α-N), 2.93 (m, 8H, CH_2-α-N), 2.29-3.70 (m, 6H, CH_2-CAr x 2, CH_2-α-N x 1), 6.45-7.13 (m, 3H, CH-Ar), 7.26 (m, 3H, CH-Ar), 7.38-7.59 (m, 2H, CH-Ar). MS (ESI): 629.4 $[(M+Na)]^+$.

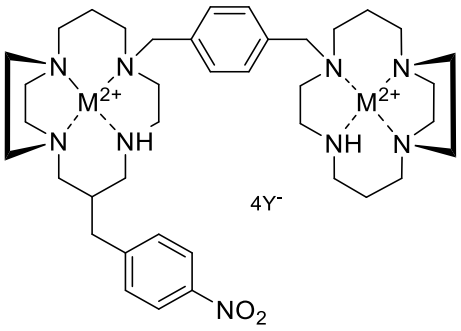
6.8.23. Synthesis of 4-((1-(4-((1,4,7,10-tetraazacyclododecan-1-yl)methyl)benzyl)-1,4,8,11-tetraazacyclotetradecan-6-yl)methyl)aniline (47)



47

1-(4-((1,4,7,10-Tetraazacyclododecan-1-yl)methyl)benzyl)-6-(4-nitrobenzyl)-1,4,8,11-tetraaza cyclotetradecane (**38**) (0.030 g, 0.049 mmol) and 5% Pd/C (0.20 g) in glacial acetic acid (50 ml) were placed under an atmosphere of H_2 for 4 h. After this time the flask was flushed with N_2 and the solution filtered through diatomaceous earth and washed with HOAc (6 x 20 ml). The filtrate was *vacuo* down to yield a light yellow solid (0.028 g, 100%). ^{1}H NMR ($CDCl_3$): δ 0.89 (m, 2H, CH_2-β-N), 1.19-1.38 (m, 4H, CH_2-N), 2.24-2.55 (m, 5H, CH_2-N x 2, CH-β-N x 1), 2.58-3.15 (m, 16H, CH_2-N x 7, CH_2-CAr x 1), 3.40-4.00 (m, 12H, CH_2-N x 5, CH_2-CAr x 1), 4.46-4.68 (m, 2H, CH_2-CAr), 7.03-7.28 (m, 3H, CH-Ar), 7.29-7.72 (m, 5H, CH-Ar). MS (ESI): 636.1 $[(M+CH_3COO^- -2H)]^+$.

6.8.24. Synthesis of metal complexes of ligand 8-(4-((1,5,8,12-tetraazabicyclo[10.2.2] hexadecane-5-yl)methyl)benzyl) -3-(4-nitrobenzyl)-1,5,8,12 tetraazabicyclo[10.2.2] hexadecane (40)



$[M_2\mathbf{40}]Y_4$

$[Cu_2\mathbf{40}]Y_4$ $M^{2+} = Cu^{2+}$, $Y^- = ClO_4^-$
$[Zn_2\mathbf{40}]Y_4$ $M^{2+} = Zn^{2+}$, $Y^- = CH_3CO_2^-$
$[Ni_2\mathbf{40}]Y_4$ $M^{2+} = Ni^{2+}$, $Y^- = CH_3CO_2^-$
$[Ni_2\mathbf{40}]Y_4$ $M^{2+} = Ni^{2+}$, $Y^- = NO_3^-$

General method D

The appropriate ligand (~ 200 mg) was dissolved in MeOH (10 ml) and a solution of metal salt (1.1 molar equivalents per macrocycle) in methanol (10 ml) was added dropwise. The mixtures were stirred at RT for 3 h and then at reflux for 2 h. The complexes either remained in solution or precipitated out. The solutions were concentrated *in vacuo*, redissolved in a minimum volume of MeOH and purified by size exclusion chromatography (Sephadex LH20). Alternatively, the precipitates were collected by filtration, washed with MeOH (5 x 20 ml) followed by Et_2O (2 x 20 ml) and dried *in vacuo*.

8-(4-((1,5,8,12-tetraazabicyclo[10.2.2] hexadecane-5-yl)methyl)benzyl) -3-(4-nitrobenzyl) -1,5,8,12-tetraazabicyclo[10.2.2]hexadecane copper(II) perchlorate $[Cu_2\,40](ClO_4)_4$

Ligand (**40**) (50 mg, 0.072 mmol), copper(II) perchlorate hexahydrate (56 mg, 0.15 mmol). A blue solution was observed immediately which developed into a purple precipitate after a few minutes. The purple product was collected by filtration (52 mg, 60%). Elemental analysis: (%) calc. for $C_{39}H_{63}N_9O_{18}Cl_4Cu_2$: (M-2$ClO_4^-$+2$H_2O$): C 44.57; H 6.33; N 11.99. Found: C 44.57; H 5.90; N 10.73. HRMS calc. for $C_{39}H_{62}Cl_2Cu_2N_9O_{10}$: 506.1289; found 506.1348 $[(M\text{-}H\text{-}(ClO_4)_2)]^{2+}$ and 466.1937; found 466.1998 $[(M\text{-}H\text{-}(ClO_4^-)_4 + (CH_3COO^-)_2]^{2+}$.

8-(4-((1,5,8,12-tetraazabicyclo[10.2.2] hexadecane-5-yl)methyl)benzyl) -3-(4-nitrobenzyl) -1,5,8,12-tetraazabicyclo[10.2.2]hexadecane zinc(II) acetate $[Zn_2\mathbf{40}](OAc)_4$

Ligand (**40**) (61 mg, 0.088 mmol), zinc(II) acetate (34 mg, 0.184 mmol). The crude product was purified by size exclusion chromatography (Sephadex LH20) to yield a light yellow solid (77 mg, 77%). Elemental analysis: (%) calc. for $C_{47}H_{75}N_9O_{10}Zn_2$: C 53.41; H 7.15; N 11.93. Found: C 53.81; H 7.56; N 11.61. HRMS: calc. For $C_{43}H_{67}N_9O_6Zn_2$: 468.6961; found 468.6964 $[(M-(CH_3COO^-)_2)]^{2+}$.

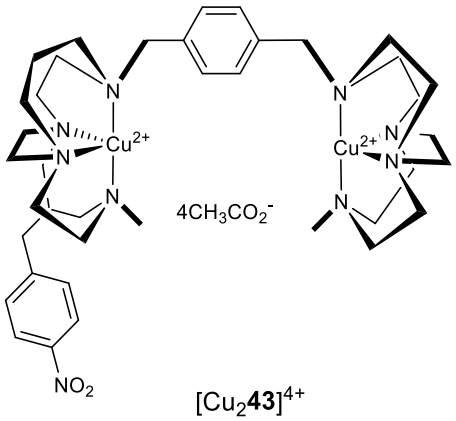
8-(4-((1,5,8,12-tetraazabicyclo[10.2.2] hexadecane-5-yl)methyl)benzyl) -3-(4-nitrobenzyl) -1,5,8,12-tetraazabicyclo[10.2.2]hexadecane nickel(II) acetate $[Ni_2\mathbf{40}](OAc)_4$

Ligand (**40**) (80 mg, 0.116 mmol), nickel(II) acetate (60 mg, 0.243 mmol). The crude product was purified by size exclusion chromatography (Sephadex LH20) to yield a light green solid (110 mg, 90%). Elemental analysis: (%) Calc. for $C_{47}H_{75}N_9O_{10}Ni_2$ ($M+3.9CH_3OH+3.1H_2O$) ($M+4CH_3OH+3H_2O$: C 50.01; H 7.82; N 10.31. Found: C 50.03; H 7.45; N 9.95. HRMS: calc. for $C_{47}H_{75}N_9O_{10}Ni_2$: 431.6928; found 431.6926 $[(M-4H-(OAc)_4+2CH_3O^-)]^{2+}$.

8-(4-((1,5,8,12-tetraazabicyclo[10.2.2] hexadecane-5-yl)methyl)benzyl) -3-(4-nitrobenzyl) -1,5,8,12-tetraazabicyclo[10.2.2]hexadecane nickel(II) nitrate $[Ni_2\mathbf{40}](NO_3)_4$

Ligand (**40**) (70 mg, 0.10 mmol), nickel(II) nitrate (61 mg, 0.21 mmol). The crude product was purified by size exclusion chromatography (Sephadex LH20) to yield a light orange solid (50 mg, 50%). MS (ESI): 993.4 $[(M-(NO_3^-)+2H)]^+$.

6.8.25. Copper(II) complex of ligand 4-methyl-11-(4-((10-methyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradecan-4-yl)methyl)benzyl)-6-(4-nitrobenzyl)-1,4,8,11 tetraazabicyclo[6.6.2] hexadecane (43)



$[Cu_2\mathbf{43}]^{4+}$

General method C

4-methyl-11-(4-((10-methyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradecan-4-yl)methyl) benzyl)-6-(4-nitrobenzyl)-1,4,8,11-tetraazabicyclo[6.6.2] hexadecane copper(II) acetate $[Cu_2\mathbf{43}](CH_3COO)_4$

Amounts: 4-methyl-11-(4-((10-methyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradecan-4-yl)methyl) benzyl)-6-(4-nitrobenzyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (**43**) (30 mg, 0.043 mmol); copper(II) acetate monohydrate (188 mg, 0.094 mmol). The crude product was purified by size exclusion chromatography (Sephadex LH20) to yield a blue solid (40 mg, 89%). MS (ESI): 452.6 $[(M\text{-}4OAc^- + 2COO^-)]^{2+}$ and 466.7 $[(M\text{-}2OAc^-)]^{2+}$.

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