THE UNIVERSITY OF HULL

Development of optimised MRI contrast agents for copper(I) click reactions

Being a thesis submitted for the degree of

MSc in the University of Hull

By

Farah K.M.Aloraibi BSc

Abstract

This research has been focused on synthesising three gadolinium(III) 1,4,7-triacetic acid-1,4,7,10-tetraazacyclododecane (DO3A) macrocyclic complexes (Gd**6**, Gd**7**, Gd**8**) as potential T_1 MRI contrast agents. Alkyne derivativised chelators were synthesised with three different alkyne pendent arms (propargyl, propargyl acetamide and butyne) which are suitable for Cu(I) catalysed cycloaddition 'click' chemistry with azide derivatives to form 1,2,3-triazole rings.

Triazole formation with benzyl azide was used as "proof of concept" to form chelators and gadolinium complexes (Gd**6**, Gd**7**, Gd**8**) that could be assessed for their properties as MRI contrast agents. One of the complexes was then selected for conjugation with an azide derivative of a thymidine DNA base (AZT, a clinically approved drug molecule) to link via the triazole group and demonstrate the widespread utility of this approach.

Relaxivity measurements were carried out on the synthesised gadolinium(III) complexes to evaluate their potential as MRI contrast agents with Gd**8** showing the highest T_1relaxivity result. Therefore, Gd**8** (relaxivity, 5.74 mM^{-1} s^{-1}) was conjugated with the DNA base derivatives (3'-Azido-3'-deoxythymidine, AZT) to give the desired product in an 89% yield with a relaxivity of 4.33 mM^{-1} s^{-1} indicating the potential of these compounds for future *in vivo* applications in MRI studies.

Risk Assessment

All experiment 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 (F.A1-F.A9), signed by the undertaking student, supervisor (Professor S.J. Archibald) and the departmental safety officer (Dr T. McCreedy) before any practical work started.

Acknowledgements

First and foremost, praise and thanks to God, the Almighty, for His showers of blessings throughout my research work and for giving me the opportunity to complete this thesis successfully. Without Him, I can do nothing. And may peace and blessings be on the most noble of prophets and messengers, our Prophet Mohammed, and on his family.

I wish to give my heartfelt thanks to my husband (Dr Mustafa Alyassiry) whose unconditional love, patience, and continual support of my academic endeavours over the past several years enabled me to complete this thesis.

I would like to express my deep and sincere gratitude to my research Supervisor Professor Steve Archibald for giving me the opportunity to do research and providing invaluable guidance throughout this research. His dynamism, vision, sincerity and motivation have deeply inspired me. I am extremely grateful for what he has offered me.

I would like to say thanks to my friends and research colleagues for their constant encouragement and genuine support throughout this research work and everyone who helped me in the lab, in no particular order: Alicja, Beckie, Hayley, Rhiannon, Zainab, Juozas, Ben and Boon-Uma.

I also dedicate this MSc thesis to my four lovely children, Ahmad, Alhorr, Kaseem, and Mishkat who are the pride and joy of my life. I love you more than anything in my life and I appreciate all your patience and support during mummy's MSc studies.

I owe a special thanks to my family, my mum, dad, my brothers and my sister who supported me and helped me throughout my life and during this study.

Abbreviations

1/τr	Tumbling rate
Bo	Magnetic field
CAs	Contrast agents
CT	X-ray computed tomography
CuAAC	Copper(I) catalysed alkyne-azide cycloaddition
DCM	Dichloromethane
DMF	Dimethylformamide
DOE	Design of Experiment
DOTA	1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid
EG	Ethylene glycol
FDA	Food and Drug Administration
FID	Free induction decay
FTIR	Fourier transform infrared
HRTEM	High Resolution Transmission Emission Microscopy
ICP	Inductively coupled plasma
IR	Infrared
LCST	Low critical solution temperature
Mo	Magnetisation vector
MRI	Magnetic resonance imaging
MS	Mass spectrometry
NMR	Nuclear magnetic resonance
NSF	Nephrogenic systemic fibrosis
NTA	Nanoparticle tracking analysis
q	Number of inner sphere water molecules
PET	Position emission tomography
r1	T_1 relaxation rate
r2	T_2 relaxation rate
rf	Radiofrequency
T_1	Longitudinal or spin-lattice relaxation

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Chapter 1

Introduction

1.1. Molecular imaging

Molecular imaging can provide characterisation and measurement of the key biological processes in the body at the molecular level. The field of molecular imaging is considered a growing research field in which a range of imaging modalities can be applied,[1] see Figure 1, including single-photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), positron emission tomography (PET) and some optical techniques.

At present, clinical work is more focused on structural imaging techniques such as MRI (which can be used without a molecular contrast agent) and CT to provide anatomical rather than functional images of the organs, this can be of major benefit to people with cancer, for example, but there is the potential to gather more information using molecular imaging.[2,3]

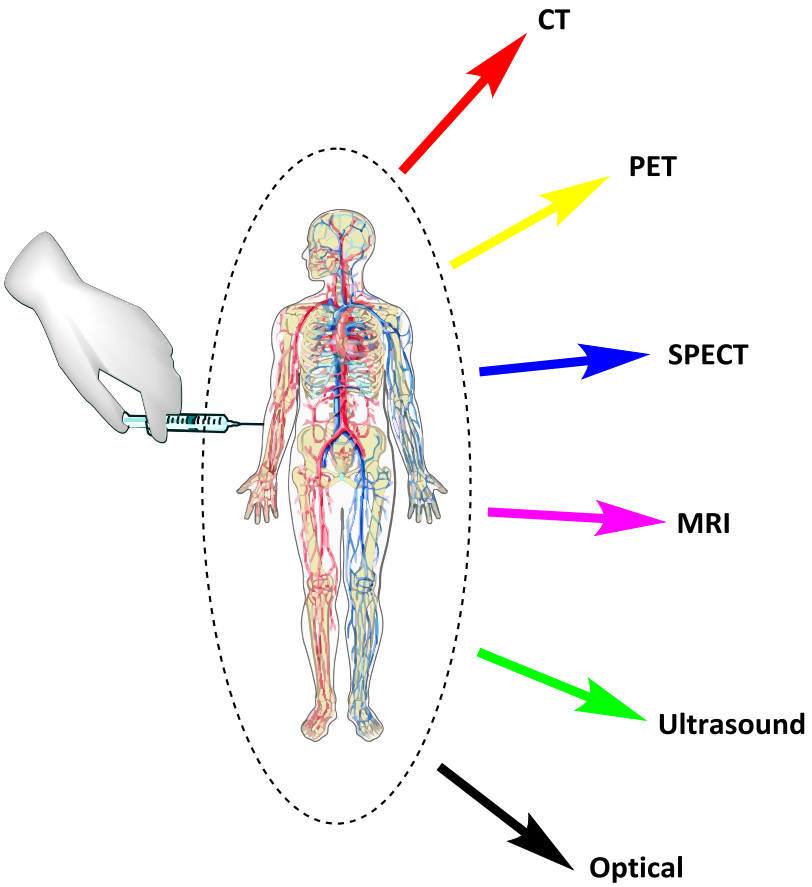


Figure 1 - Imaging modalities that are used for clinical and preclinical non-invasice imaging..

1.1.1. Imaging techniques

The field of medical imaging has expanded with molecular imaging, as these techniques can detect the molecular level biochemical process changes in tissues and tumours. They have also been applied in the drug delivery field to determine optimal methods and track delivery and release processes. In oncology, visualisation of the molecular processes during the growth of the tumour are important to selecting the best available treatment.[4]

The basis of these imaging techniques depends on the localisation of the agent or the interaction with biological targets. Each of these imaging modalities can be discussed in terms of the advantages and disadvantages associated with its clinical applications,[5,6] see Figure 2.

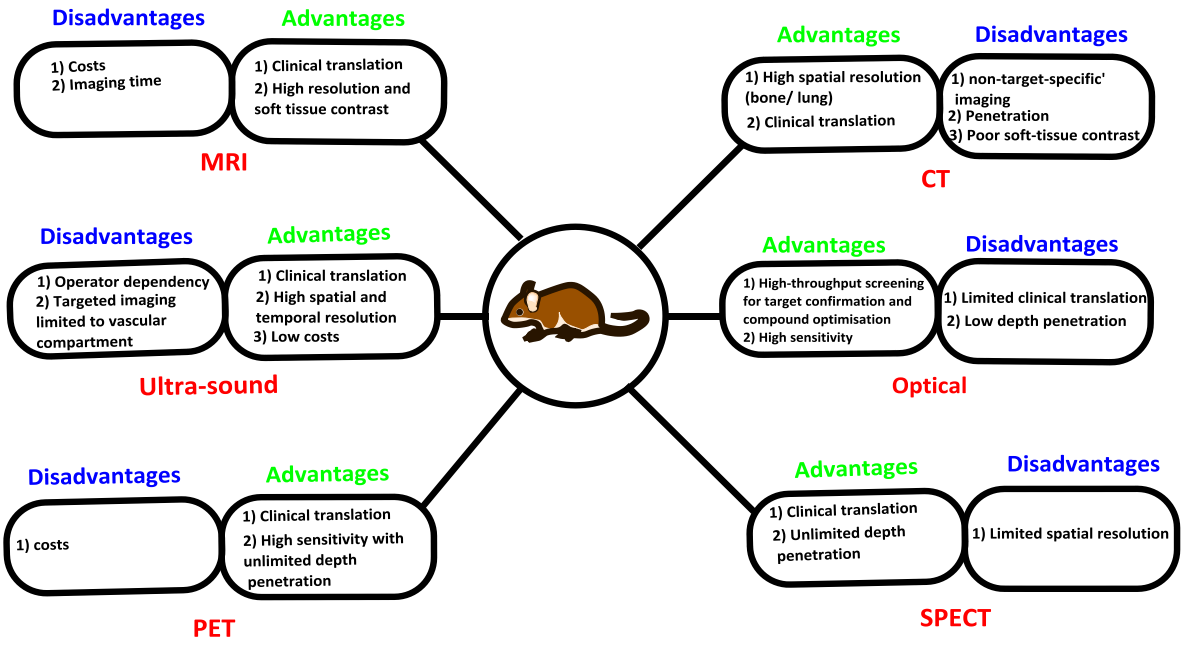


Figure 2 - Characteristics of the medical imaging modalities.

1.1.1.1. Magnetic resonance imaging (MRI)

Magnetic resonance imaging (MRI) is a highly effective and common diagnostic imaging technique for clinical applications due to its capability to provide anatomical and functional images with high resolution.[7]

This imaging modality has some advantages compared to other medical imaging techniques (PET, CT, X-ray), such as that the fact that it does not require harmful ionising radiation and that it can directly detect soft tissues, it also has high spatial resolution and provides good contrast between tissue types.

An MRI scan of the human body utilises the net magnetisation that is produced under the application of an external magnetic field where the protons in the tissues will align in the direction of the magnetic field (B_o),[8,9] see Figure 3.

Figure 3 - An example of an MRI scanner.[9]

The basic principle that MRI relies on is proton nuclear magnetic resonance by applying low intensity radio frequency (RF) waves along with a gradient magnetic field. Then, by alteration of the frequency pulse sequences in the magnetic field gradient, contrasts between various

tissues in the body are observed that are dependent on the concentration of water protons. This gives variation in the relaxation times of the water molecules protons due to their different environments. Hence, MRI provides information on the distribution of the water protons within a biological system which can be used to form images with good contrast between tissue types.[9,10]

1.1.1.2. General principles

MRI utilises one of the most abundant isotopes in nature, ^{1}H, see Table 1, and is dependent on the magnetic response produced by interaction with an external magnetic field. Both water and fat contain high percentages of hydrogen atoms (^{1}H) which have nuclear spin of I = ½.[9]

Nuclide	Spin	Magnetic Moment	Gyromagnetic Ratio
^{1}H	½	2.79285	26.752
^{2}H	1	0.85745	4.1067
^{13}C	½	0.7023	6.7272
^{14}N	1	0.40356	1.9328
^{31}P	½	1.1317	10.84

Table 1 - Magnetic properties of nuclei commonly observed using NMR spectroscopy.

Protons are positively charged particles with spin around their internal axis. They rotate constantly, unless influenced by external factors such a magnetic field. The behaviour can be related to a bar magnet and represented by a vector, see Figure 4. This property occurs when nuclei have an odd number of neutrons or protons.[10,11]

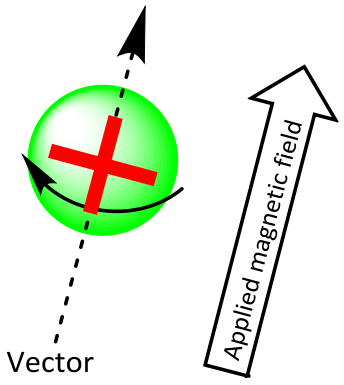


Figure 4 - A positively charged nucleus and themagnetic vector that is present when the nucleus has spin.

The interaction between nuclei, which possess spin, and an externally applied magnetic field, produce magnetic resonance. ^{1}H is the most common isotope with nuclear spin and has a high response to the applied magnetic field because it has a nucleus consisting of one proton with a half-integer spin, ½, and has approx. a 100 % abundancy in nature. The abundancy of protons in water and fat of the human body reaches approximately 63%; therefore, ^{1}H has the key role in formation of clinical MRI images.[12,13]

At equilibrium, the spin vectors of ^{1}H in the body have a random direction when no magnetic field is applied (B_0), see Figure 5, while in the presence of a magnetic field the alignment of most of the spin vectors will occur in the same direction as the B_0 due to their weak magnetic behaviour.

In other words, when the human body is placed in the magnetic field (B_0), the nuclei will align to the magnetic field in either a parallel or antiparallel direction. The most favourable alignment is the parallel alignment, as it represents the lowest energy state.[14]

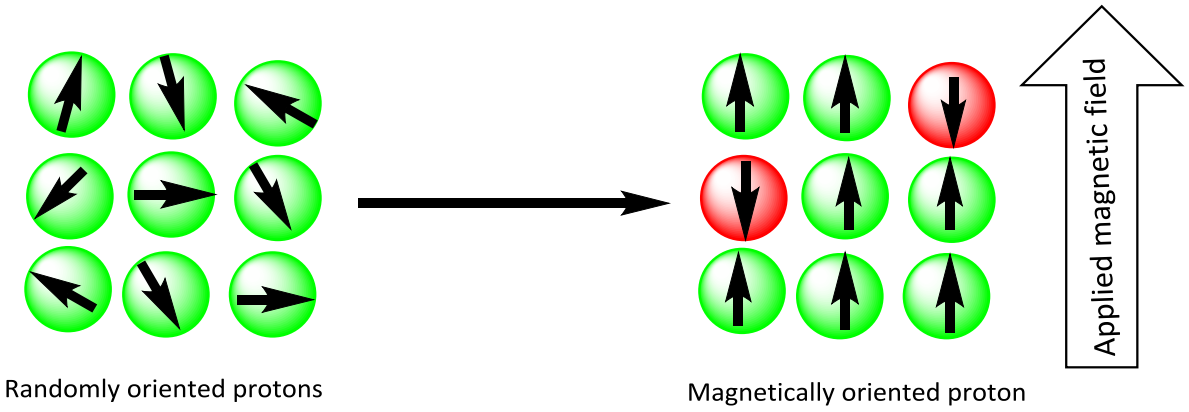


Figure 5 - Diagram to show the effect of an applied magnetic field (B_0) on the set of individual protons in fluid or tissue.

Some nuclei will cancel each other out if they are in opposite directions, while the rest will produce what is called net magnetisation (M). The speed of precession of the protons around B_0 is called the precessional frequency (ω_0) and can be used to determine the energy of radio frequency through Larmor's equation ($\omega_0 = B_0\gamma$).[16,17] There are two types of relaxation times (T_1, T_2). Fat tissues usually show short T_1 relaxation times and appear bright in the T_1-weighted imaging, while other tissues such as tumours or water will show long T_1 relaxation times and will appear dark in T_1 imaging and the opposite effects are observed with T_2 weighted imaging.

The most common T_1 relaxation contrast agents that can be administered to a patient to influence these properties are gadolinium(III) complexes.[20]

1.1.1.3. Contrast agents for MRI

Contrast agents (CAs) are required for MRI when there is only a small variation in relaxation times between the diseased and healthy tissues (poor contrast). The aim is to enhance the image contrast between the tissues, organs or to observe biological processes. Hence, one way that this can be achieved is by positive contrast by shortening T_1 time, this class of compounds are called T_1 agents, while the CAs that provide negative contrast by shortening the T_2 relaxation time are called T_2 agents. They can also be used to show physiological function or blood flow. Generally,T_1 CAs are more commonly used in clinical applications for a few reasons such as less background noise and higher resolution images.[23]

Use of contrast agents with MR imaging has been greatly developed in the past two decades and has become an integral part of the process, with around 45-50% of clinical scans using contrast agents. Developments with selective contrast agents will improve visibility, consequently the usability of contrast agents.[24]

$$R_1\,(1/T_1) = r_1[CA]+R_1\,\text{tissue}$$

$$R_2\,(1/T_2) = r_2[CA]+R_2\,\text{tissue}$$

In general, the application of a particular CA is dependent on its magnetic behaviour, such as paramagnetic or superparamagnetic. As mentioned, this results in shortening the relaxation time (either T_1 or T_2). The CA efficiency at shortening the relaxation time of water protons is known as the relaxivity rate (r), where the T_1/T_2 are relaxation times measured in s^{-1},[25] and the r values for any particular tissue depends on the temperature and the strength of the applied magnetic field (B_o). The units of r_1 and r_2 are $mM^{-1}s^{-1}$.

T_1 or T_2 properties of a particular CA are indicated by the ratio between r_2 and r_1,for T_1 agents the ratio is between 1 and 2, while for T_2 agents the ratio has a higher value, approximately 10 or more (e.g. with iron oxide nanoparticles).[26] Presently, research work is ongoing to improve the properties of contrast agents to reduce the risk of toxicity, increase their ability for tissue or tumour targeting and to optimise the relaxivity properties. There are many published studies which present a variety of potential molecules for use as MRI contrast agents with gadolinium(III) macrocyclic complexes and iron oxide nanoparticles dominating the research area.[26,27]

1.1.1.4. Gadolinium-based T_1 contrast agents

Gadolinium(III) based contrast agents are the most common T_1 CAs. Their location in the body results in an increase in the observed signal, leading to brighter areas in the image. Gd(III) has a number of magnetic properties that make it an ideal candidate for use in contrast agents. For example, the Gd(III) ion has seven unpaired *f*-electrons making it a highly paramagnetic metal centre and it has a symmetrical S-state in the electron arrangement which offers ideal properties for the local magnetic field. It also has a long electron spin relaxation time.[28]

A key concern associated with the preparation of any Gd(III) based contrast agent is the stability of the metal ion complex. This is due to the high toxicity of free Gd(III) ions in the human body, which is related to the similarity of their ionic radii with calcium(II).Gd(III) has a higher charge and hence higher affinity for the biological molecules that bind to or interact with Ca(II), which interferes with the calcium signalling processes in biological systems. Therefore, the Gd(III) metal ion always needs to be chelated by polydentate ligands.[29]

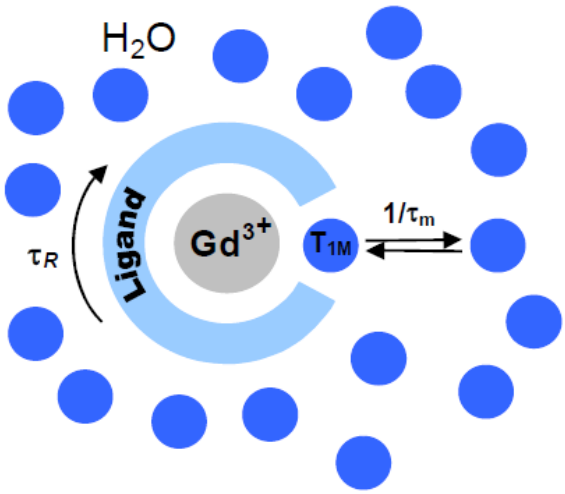


Figure 6 - Schematic representation of a contrast agent.[18]

Another important design feature for the Gd(III) complexes is that they should have at least one water molecule coordinated (q), leading to a rapid relaxation and water exchange ($1/\tau_m$) process to optimise relaxivity, see Figure 6. This allows water molecules from the outer sphere to exchange and attach to the Gd(III) ion and be influenced by the paramagnetic properties. There are also other aspects that affect relaxivity properties, including the residency time (τ_m) and the tumbling correlation time (τ_R).[30] Overall, the design of gadolinium(III) compounds as CAs needs consideration of these key properties to optimise

contrast properties, with particular focus on the ability to accommodate a high number of bound water molecules q whilst retaining stability, short water residence time in the inner sphere and a slow tumbling rate (τ_R).

Safety requirements restrict the increase in q number to get higher relaxivity for contrast agents administered to the human body, as it will affect the thermodynamic stability of the complex. If the Gd(III) ion becomes labile and is released from the chelator, consequently the patient could suffer severe complications including nephrogenic fibrosis (NSF). Therefore, health regulatory authorities are carefully examining stability data before approval of new constrast agents and revisiting the profiles of existing clinically approved compounds. [31]

The most common classes of organic ligands which are approved for effective encapsulation of metal ions such as gadolinium(III) are either diethylenetriamine derivatives (such as DTPA) or tetraaza macrocyclic compound (based on the cyclen macrocycle), which have been designed to chelate the central metal ion to leave only one or two sites for water molecule coordination. Examples of approved contrast agents that are gadolinium(III) complexes and have been licensed by the US Food and Drug Administration (FDA)[32,33] are shown in Figure 7.

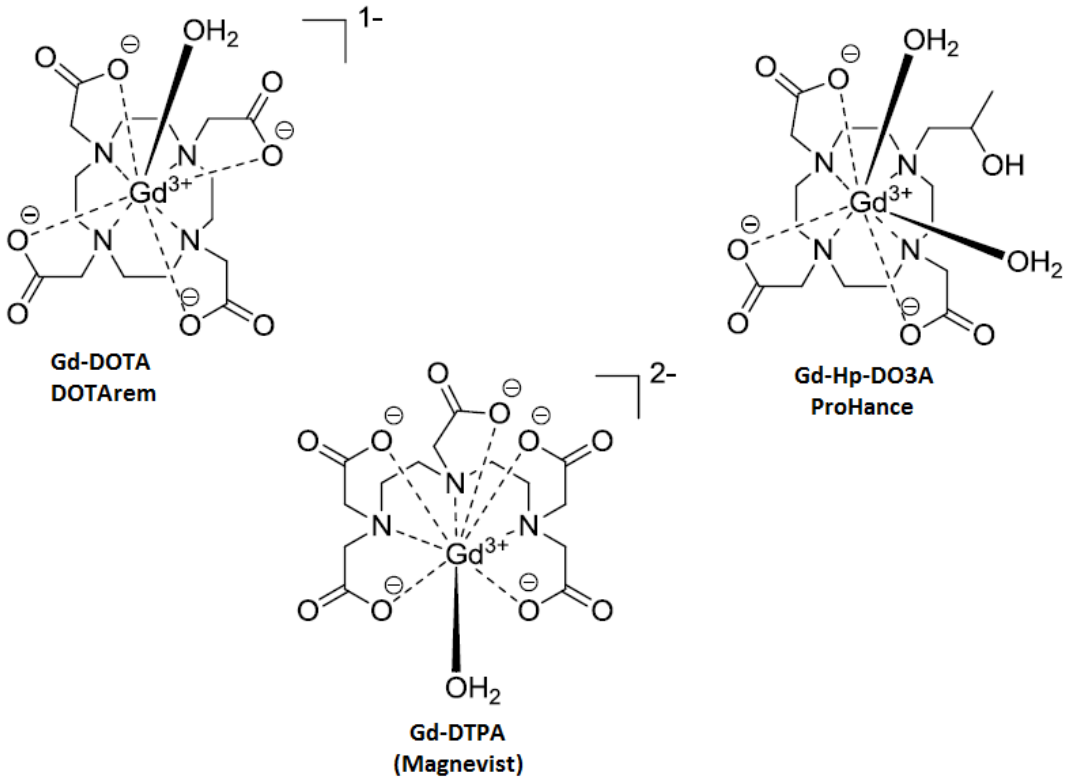


Figure 7 - Chemical structures of gadolinium-based compounds approved for use as T1 contrast agents clinically.

Gd-DOTA (DOTArem) as a macrocyclic gadolinium(III) complex and is one of the most commonly used MRI contrast agents. Another similar clinically approved CA is Gd-DTPA (Magnevist), in this case using an acyclic chelator. Both complexes are ionic in nature (overall negative charge) and both have eight coordination bonds formed with the chelator and one site available for a water molecule to bind. The third example shown in Figure 7 is Gd-PH-DO3A, which is a neutral complex with seven bonds to the chelator and two sites for water molecule coordination. With regard to stability, the macrocyclic complexes showed higher stability when compared with the cyclic complexes (DTPA) and further limit the chances of releasing free toxic Gd(III) ions.[33,34]

All four carboxylate groups in DOTA are not required for stable chelation, therefore, one acid group can be used to conjugate the chelator to a biological molecule of interest or it could be replaced by another group allowing a more convenient conjugation strategy. In this work the Cu(I) catalysed "click" reaction (explained in detail section 1.2) was selected as the conjugation method. This requires preparation of alkyne functionalised aza-macrocyclic chelators, such as propargyl-DO3A,[50] and propargylacetamide-DO3A, as precursors.[45]

Propargyl-DO3A has been synthesised by Faulkner *et al.*[50] and "clicked" with xylene diazide forming two triazole rings participating in the metal binding coordination sphere, see Figure 8.

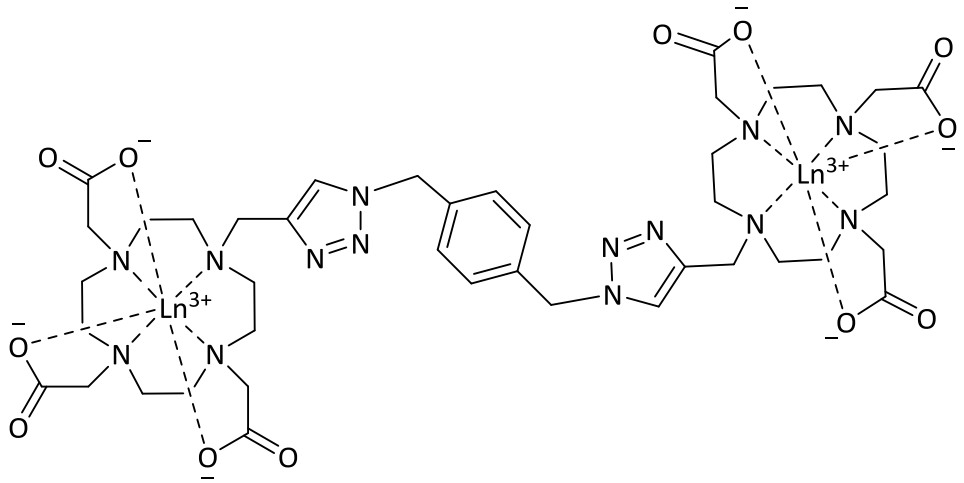


Figure 8 - An example of a triazole functionalised DO3A-Ln complex prepared using a bis-macrocyclic DO3A chelator.[50]

Propargylacetamide-DO3A has been synthesised by Hulme *et al*.[47], and coordinated to europium cation (Eu^{3+}) then "clicked" to an azide functionalised dansyl sulphonamide to produce a luminescent probe, see Figure 9. They showed that the acetamide linker can act as an energy transfer bridge between the europium complex and dansyl ring system increasing the luminescence of the europium ion tenfold.[45,56]

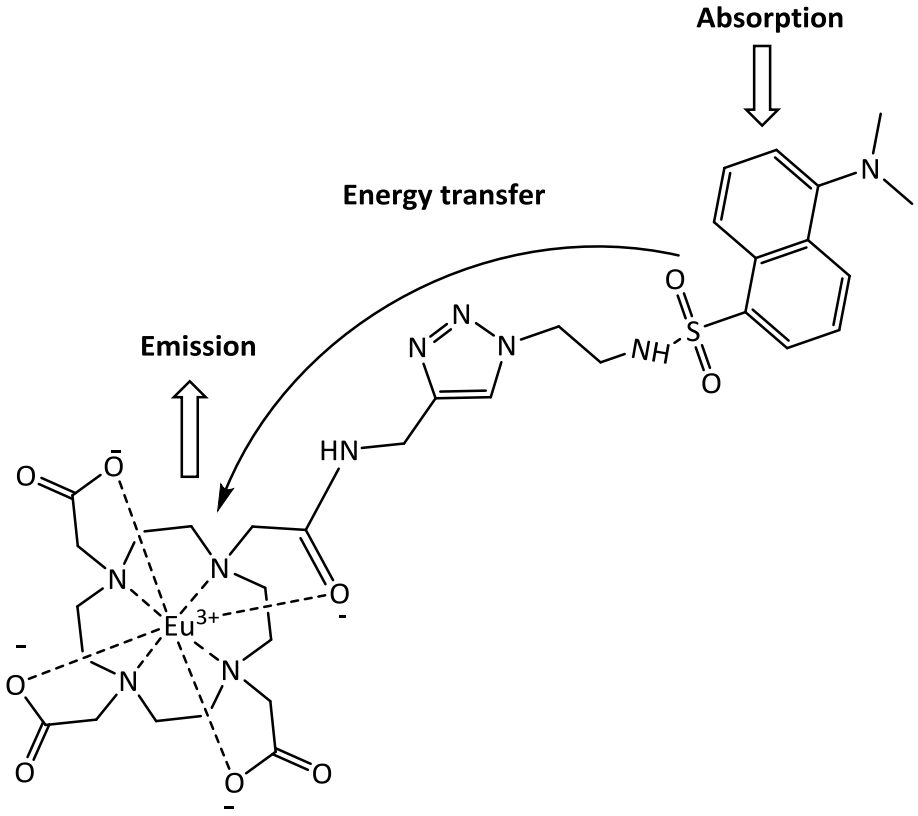


Figure 9 - An example of a sensitised Eu(III) chelate coupled with a dansyl sulphonamide group.[45]

1.2. Click chemistry

Click chemistry is a term presented for the first time by the Sharpless group in 2001 to describe the highly effective reaction of organic components that has significant advantages compared with the other slower and lower yielding methods. The compounds generated in this research project are made by the Cu(I) catalysed azide-alkyne cycloaddition (CuAAc) method.

The Cu(I) catalysed click chemistry is a Huisgen 1,3 dipolar cycloaddition which leads to 1,4 disubstituted 1,2,3-triazole and is formed from a reaction between an azide and terminal alkyne in the presence of copper.[35] This connection is rapidly formed, selective, and produces high yields with no or few by products. Thus, this type of chemistry is ideal for bioconjugation and peptide ligation. The formed triazole linker has a planar geometry and can be considered as a peptide bond mimic.

Initially, Husigen 1, 3-dipolar cycloaddition reactions had to be carried at high temperatures and suffered from long reaction time and lack of isomeric purity,[36]see Figure 10.

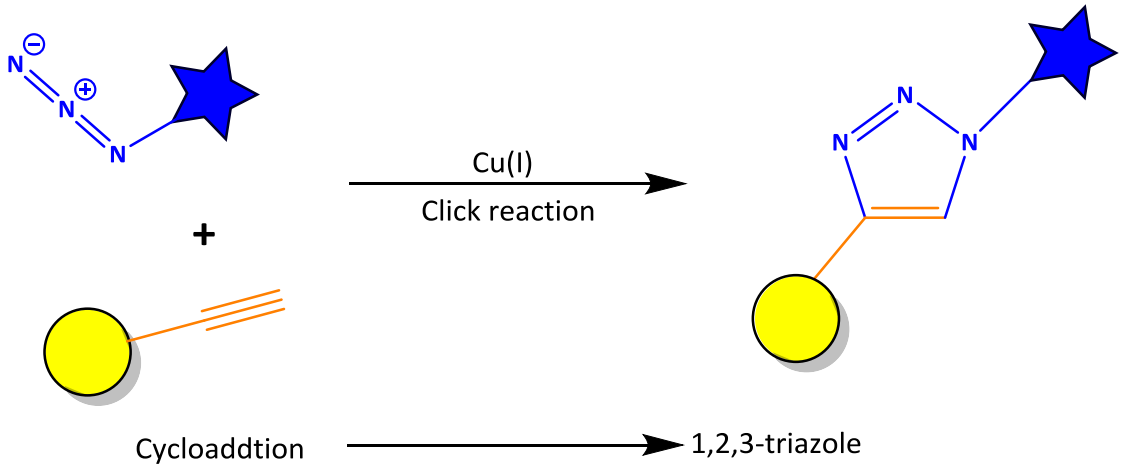


Figure 10 - Azide-alkyne Huisgen cycloaddition (click reaction) method to give a 1,2,3-triazole.

The use of copper(I) catalysis results in a multistep process with the copper involved in the intermediate steps, in the first step the acetylide forms, see Figure 11, via copper through the coordination with the alkyne. The second step consists of formation of a copper(III) metallacycle via binding between the azide and the copper, which then leads to the ring contraction that gives the desired 1,2,3-triazole product.[36]

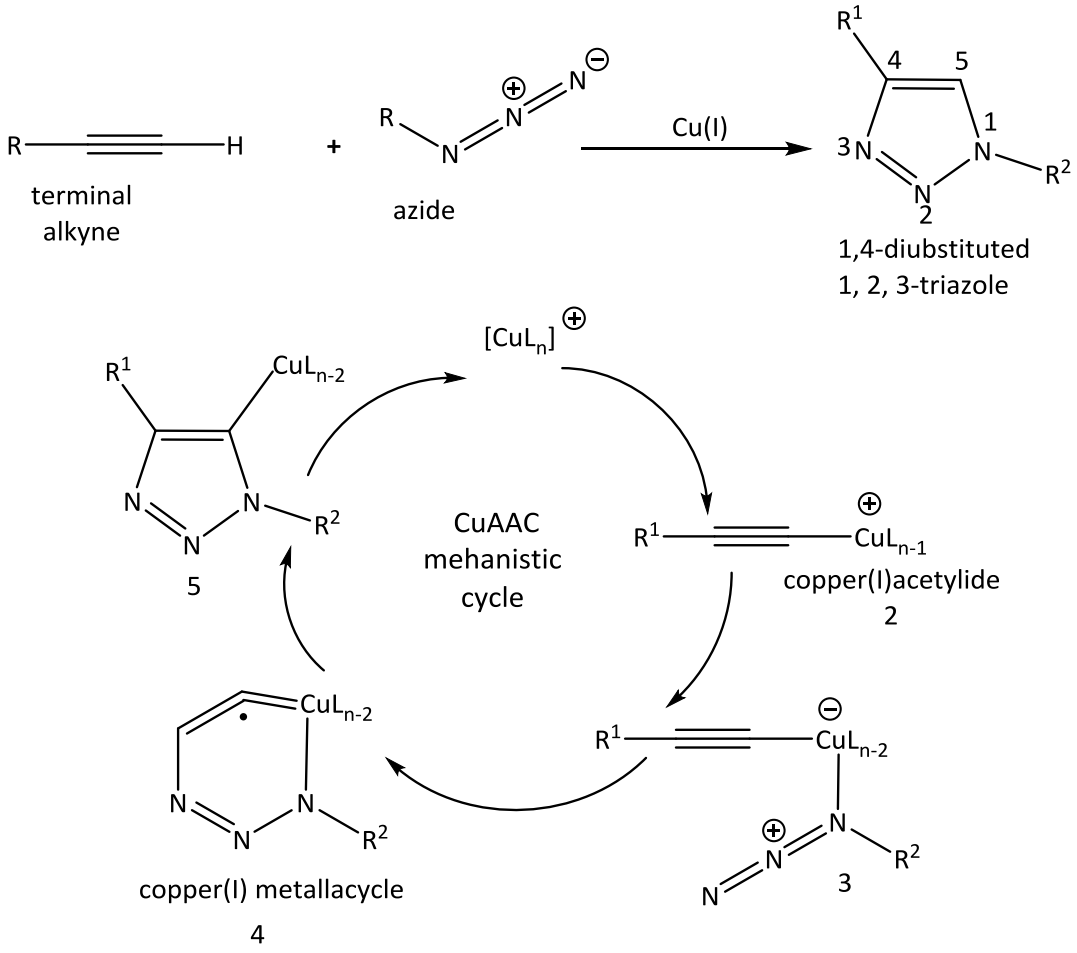
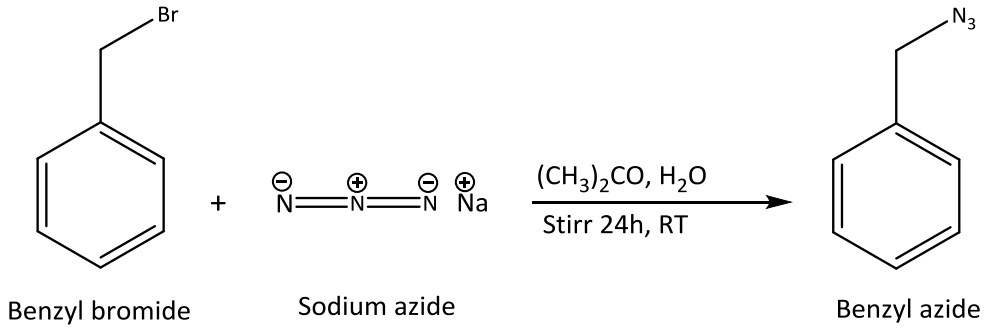


Figure 11 - Proposed mechanism of the copper-catalysed alkyne-azide cycloaddition (CuAAc). Reactions are faster when R^1 & R^2 are electron-withdrawing groups.[36]

The copper ion has been employed as the most effective catalyst either starting from a metallic form or as a salt to promote the 1,3-dipolar addition click chemistry reaction, Copper sulphate is frequently used with sodium ascorbate in a process developed by the Sharpless group,[35] where they performed an optimised click reaction catalysed by $CuSO_4$ in the presence of sodium ascorbate dissolved in a mixture of water: *tert*-butanol (2:1 ratio) at room temperature. Sodium ascorbate is a very important reagent for the production of the reactive catalytic Cu(I) species due to the rapid oxidation of Cu(I) back to Cu(II) by ambient oxygen.

Organic azides are potentially explosive, therefore, special care should be applied in their preparation and handling. The general approach is to use a mineral azide to form stabilised organic azides which can be isolated, or to generate them *in situ*,[38] see Scheme 1.



Scheme 1 - Generation of azides from azide salts and brominated precursors.

An advantage of click chemistry is that it can be used to generate compounds of biological interest, where the structure can effectively mimic the amide bond of the peptide. Compounds have been produced with inhibitory activity for various diseases, such as HIV or as potential antifungals.[39,40] However, there is another application of click chemistry in the formation of targeted imaging agents for use in techniques such as PET, SPECT, as luminescent probes and as MR contrast agents. This can be achieved via 1,2,3-triazole linking of imaging agents with relevant biological molecules such as DNA bases, peptides or glucose.

For use with peptides, click chemistry can provide a number of routes to structural modification.

It can be used to functionalise or derivatise peptides, or in peptidomimetics which form the key methods of producing new agents in this research area,[41,42] see Figure 12.

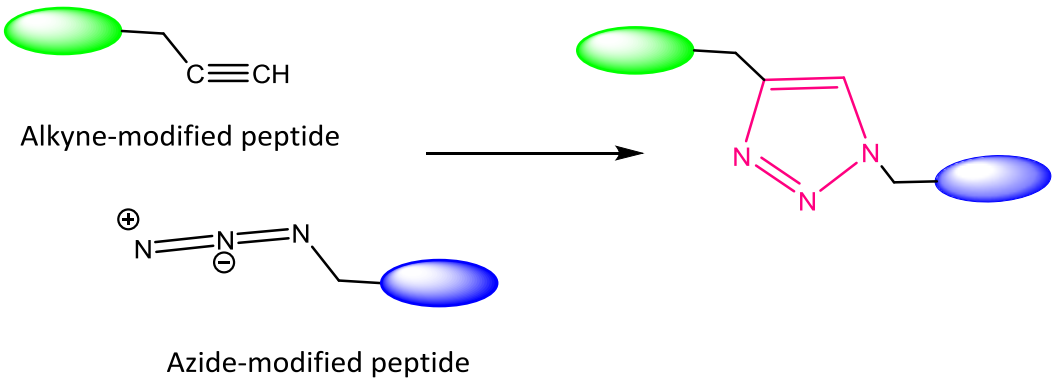


Figure 12 – Click reaction of an alkyne-functionalised peptide with an azide-modified substrate in the presence of Cu(I) to form a triazole-linked conjugate.[4]

1.2.1. Cu(I) catalysed "click" reactions between gadolinium(III) complexes with an alkyne group and an organic azide

The Cu(I) catalysed "click" reaction has been used previously to produce contrast agents for imaging based on lanthanide metal chelates. A lot of work has gone into development of alkyne or azide functionalised metal chelators which can be conjugated to biological molecules, such as peptides or sugars, however the conditions for these processes are not consistent and further optimisation is required.[43] As mentioned previously, the paramagnetic properties of Gd(III) enable its use as an MRI contrast agent. The choice of chelating moiety affects the kinetic and thermodynamic stability and the selection of an appropriate biologically active molecule can be used to enable targeted delivery of the agent.[43]

Figure 13, presents a literature example of an alkyne functionalised Gd(III)-DOTA chelator conjugated to an azide functionalised icosahedral closo-B_{12} cage generating an MRI contrast agent with improved retention time and excellent contrast enhancement.[41]

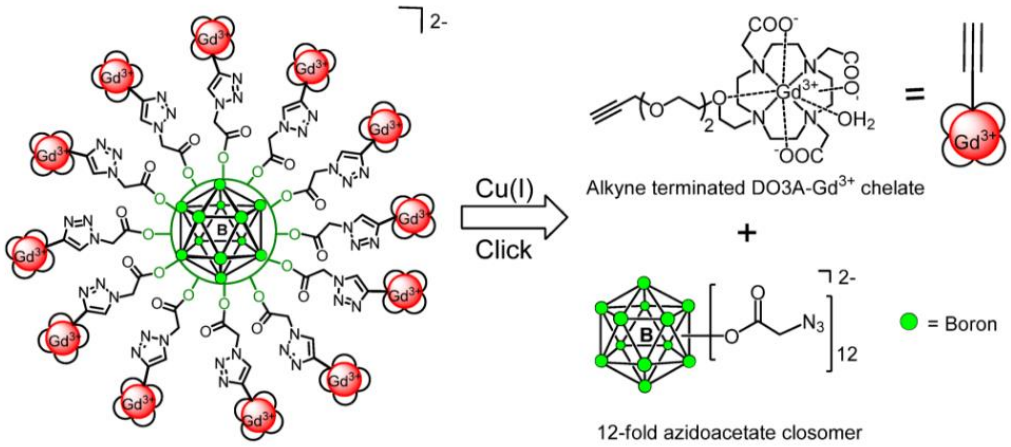


Figure 13 - Schematic representation of click-assisted synthesis of a [closo-B12]$^{2-}$ scaffold supporting twelve Gd^{3+}-DOTA chelates.[43]

Figure 14, shows another example published by the Parac-Vogt group,[44] in which a functionalised a Gd(III) DOTA complex is conjugated with boron-dipyrromethene derivatives (BODIPY) to give a bimodal contrast agent for MRI and optical imaging. The evaluation of relaxometric and luminescent properties of this complex suggested good potential for effective application as a bimodal contrast agent.[44,45]

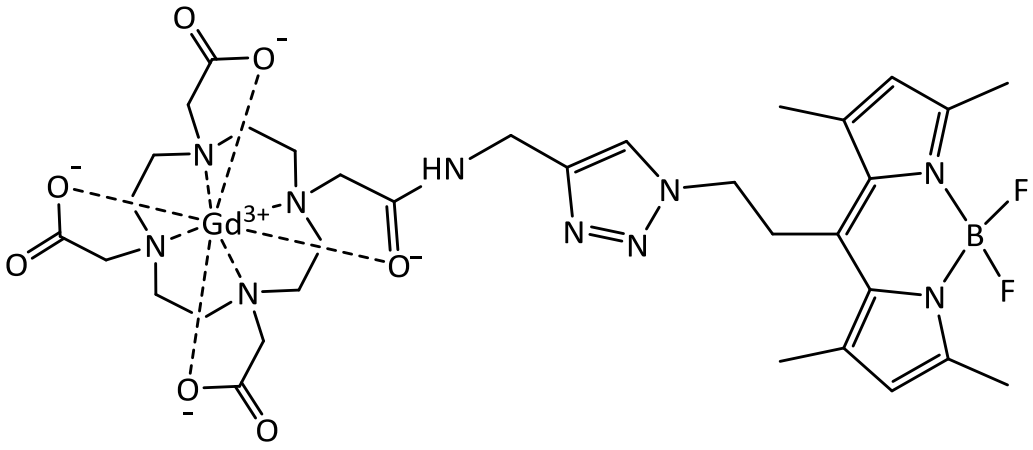


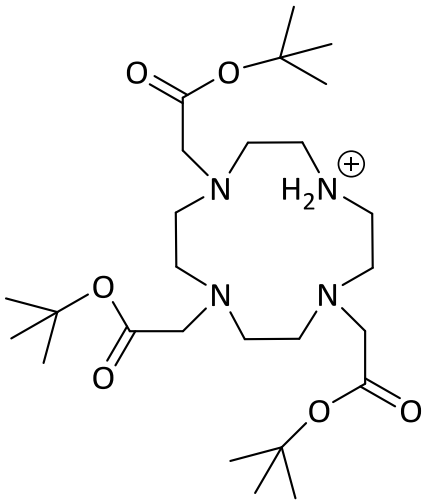
Figure 14 - A Gd-DOTA-BODIPY derivative produced by Parac-Vogt and co-workers.[44]

1.3. Aim of the MSc project

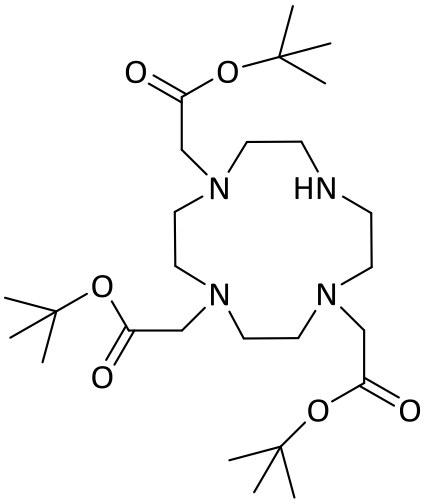
The overall aim of this research project is to use a Cu(I) catalysed cycloaddition click reaction, to develop validated and optimised methods to conjugate an MRI contrast agent containing Gd(III) with bioactive molecules.

The work has been carried out in four steps:

1- The synthesis of macrocyclic ligands based on cyclen backbone following the literature methods, including DO3A macrocyclic ligands that can be functionalised with different alkyne containing groups.

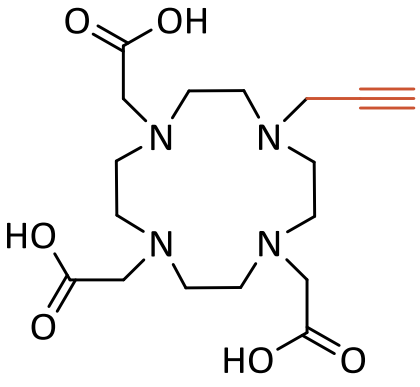


DO3A hydrobromide salt

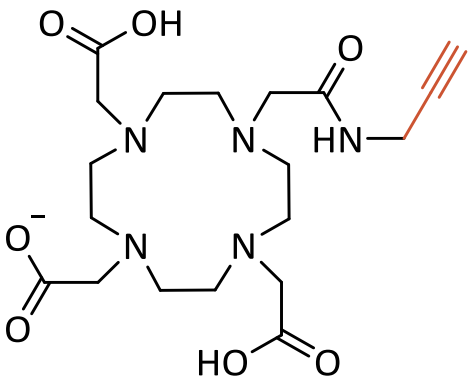


DO3A

2 –Synthesise two known functionalised chelators based on DO3A macrocycle with alkyne groups (propargyl, propargyl acetamide) via modification of reported literature methods.

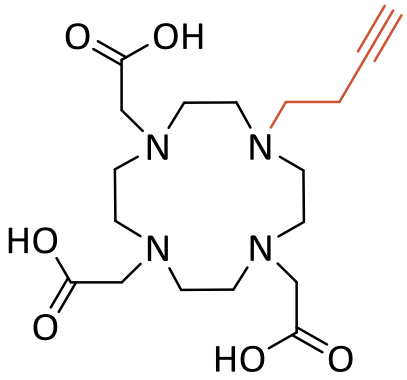


DO3A-Propargyl arm



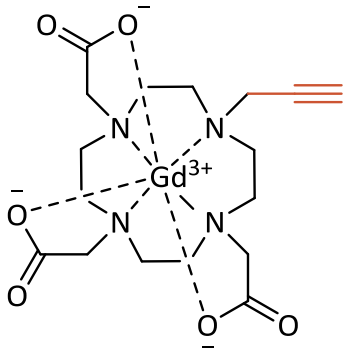
DO3A-Acetamide arm

3- Synthesis of a novel DO3A macrocyclic chelator functionalised with butyne arm using analogous methods to the previous chelators.

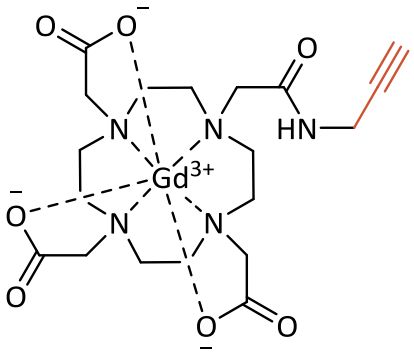


DO3A +Butyne arm

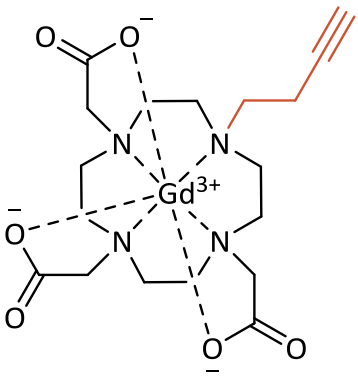
4- Synthesising of gadolinium(III) complexes of the three synthesised chelators (propargyl, acetamide, butyne).



Gd(III) +propargyl arm

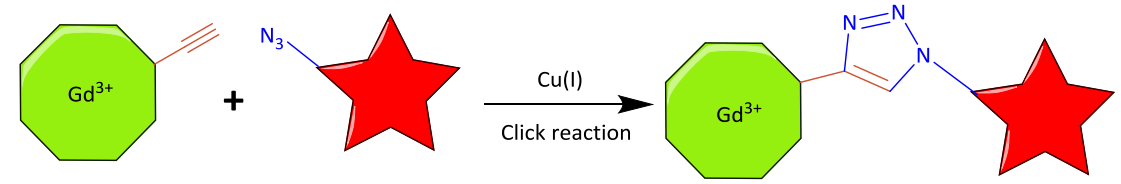


Gd(III) + acetamide arm



Gd(III) + butyne arm

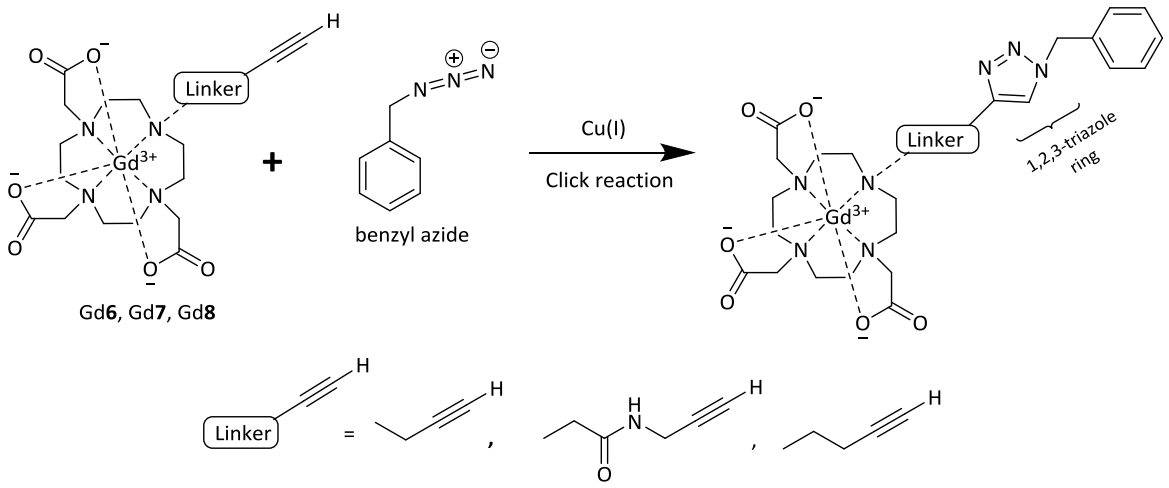
5- Cu(I) catalysed “click” chemistry reactions between the prepared Gd(III) complexes with simple azide (benzyl azide) to determine the optimised reaction conditions followed by assessment of their relaxivity properties.



Gd(III) complexes

Benzyl azide
(commercially available)

Conjugation via triazole linker



Scheme 2 - General synthetic route Cu(I) catalysed "click" chemistry reactions between the prepared Gd(III) complexes with benzyl azide.

5- Synthesis of a novel conjugate between the highest relaxivity complex of the three Gd(III) complexes produced with azide functionalised deoxythymidine (commercially available) to demonstrate the potential for bioconjugation.

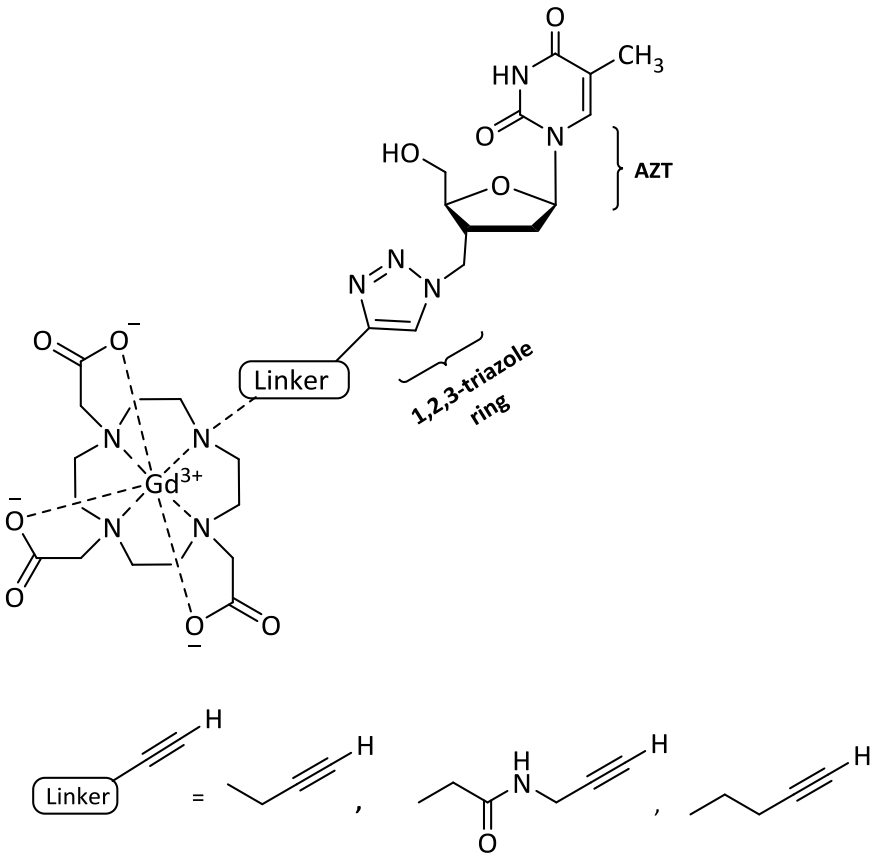


Figure 15 - General overview of the target molecules in the research work.

Chapter 2

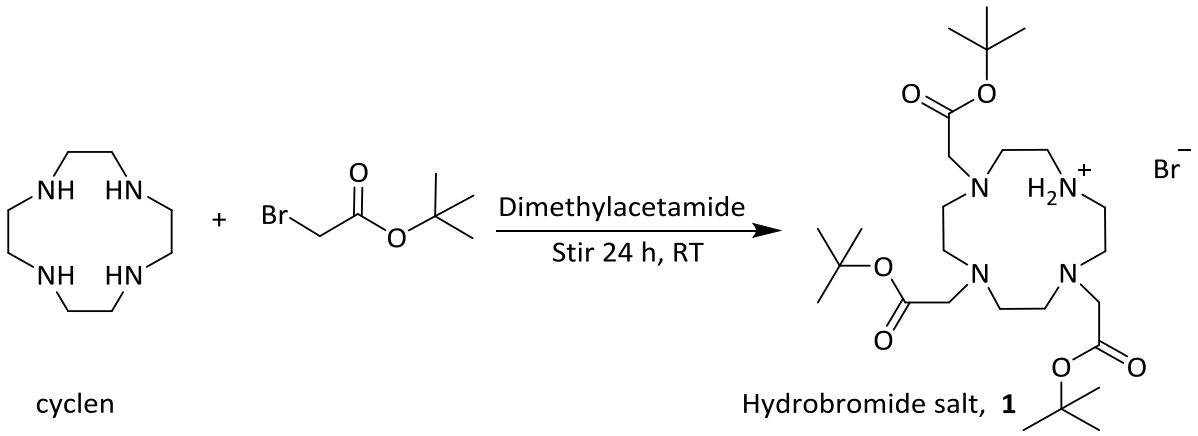
Synthesis of functionalised macrocyclic chelators

2.1 Synthesis of macrocyclic ligands based on DO3A

The initial aim of this research work was to synthesise a range of DO3A derivatives by functionalisation with different alkyne groups for subsequent conjugation with azide functionalised molecules. DO3A is a cyclen derivative with three carboxylate pendent arms, which has been extensively utilised as a metal chelator in biomedical applications. DO3A can be prepared from cyclen by a method reported by Jagadish *et al*.[47,48] This method was selected due to the short reaction time, high selectivity and the purity of the isolated three arm substituted product which does not require chromatographic purification.

The Jagadish preparation method can be divided into two steps: preparation of ^{t}Bu-DO3A as the hydrobromide salt (**1**), see Scheme 3, followed by neutralisation to yield the free base *t*-Bu-DO3A (**2**). [47]

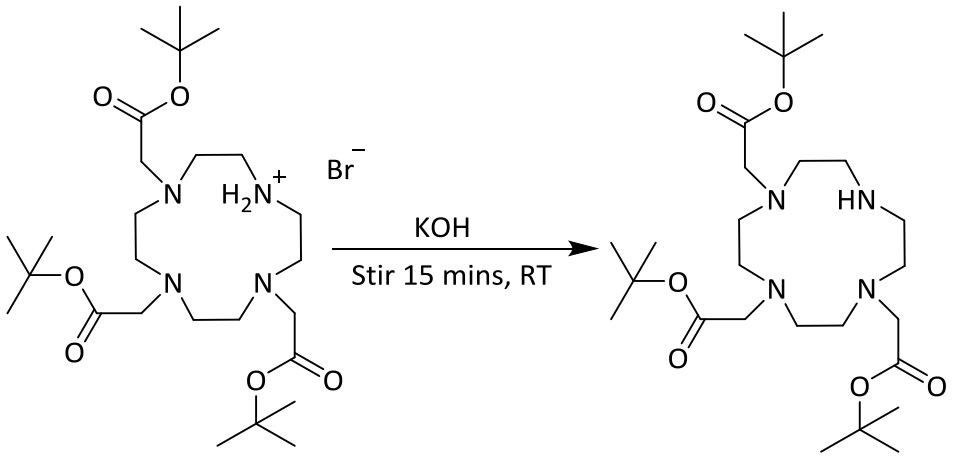
Cyclen was mixed with *t*-butyl bromoacetate at low temperature (−20 °C) to avoid tetraalkylation and left stirring for 24 h at room temperature. The tri-substituted product precipitates as the hydrobromide salt and can be recovered by filtration in a high yield of 73%. The product (**1**) was fully characterised by MS, ^{1}H NMR, ^{13}C NMR and CHN analysis.



*Scheme 3 -Preparation of the DO3A hydrobromide salt **1**.*

The neutralisation step was carried out as follows: the HBr salt (**1**) was dissolved in water at 70 °C and a 10% aqueous solution of potassium hydroxide was added, causing the free base DO3A **2** to precipitate in high purity with a yield of 92%, see Scheme 4. The identity of the product **2** was confirmed by MS, ^{1}H NMR and CHN analysis. ^{1}H NMR showed two signals at 1.42-1.48 ppm integrating to 9 and 18 protons, corresponding to the two environments for the *tert*-butyl ester groups (either attached to the nitrogen atom opposite the unsubstituted

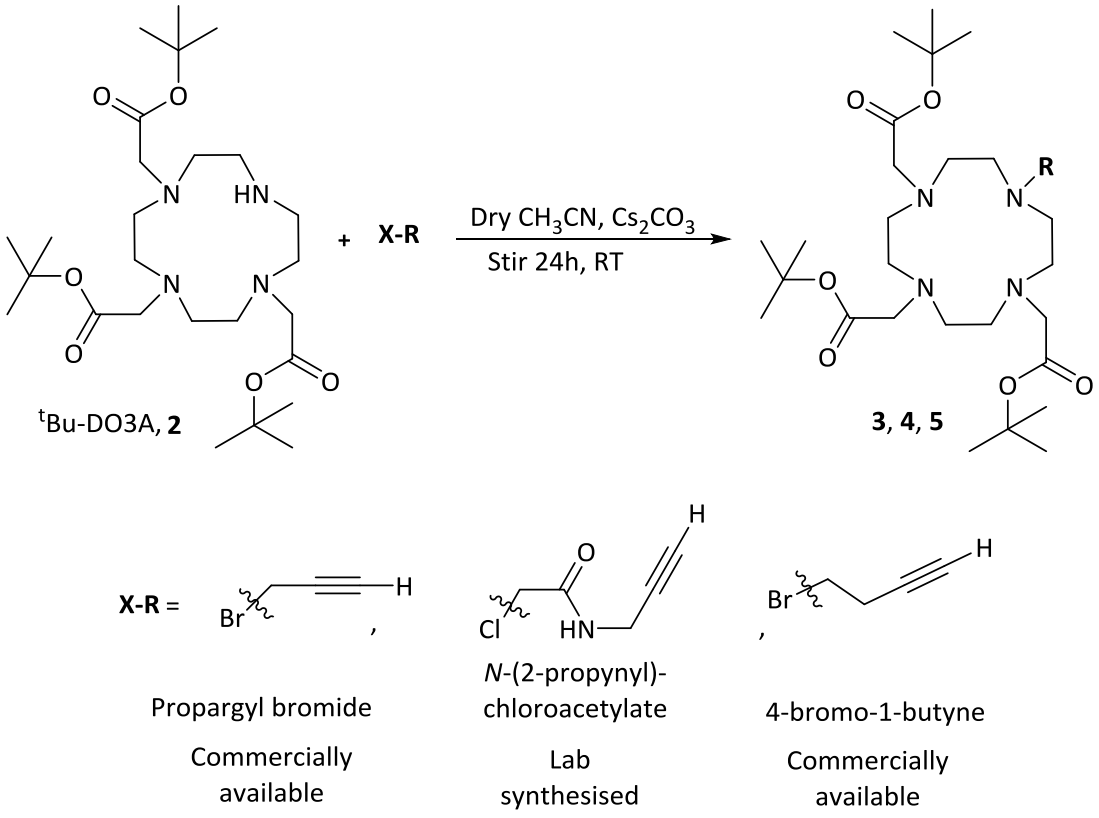
secondary amine or on one of the nitrogen atoms flanking the secondary amine group, respectively).



*Scheme 4 - Preparation of free base tBu-DO3A, **2**.*

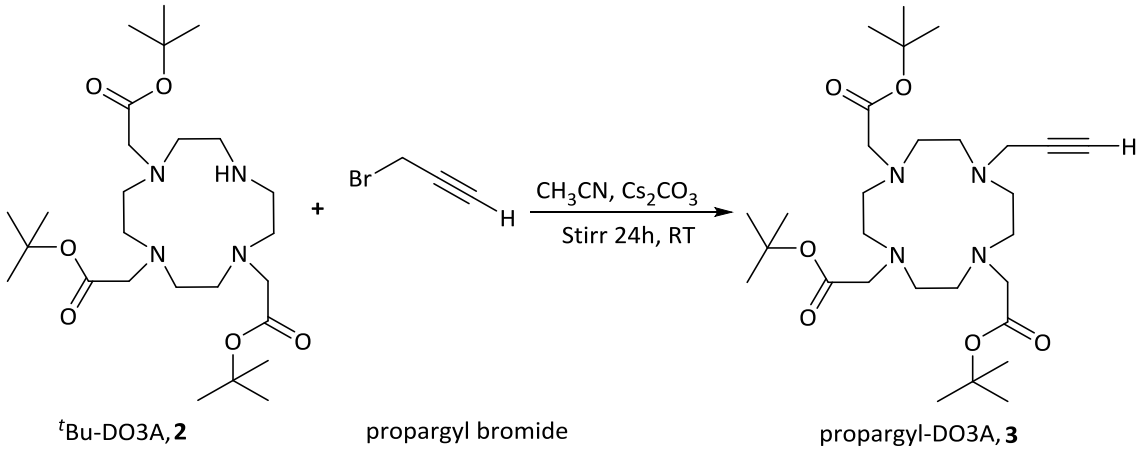
2.2. Synthesis of alkyne functionalised DO3A derivatives

Three different alkyne arms were selected for DO3A functionalisation: propargyl, butynyl (both of which have commercially available precursors) and as the third pendent arm, propargyl acetamide, which could be introduced using a precursor synthesised by modifying the published syntheses of related molecules,[50] see Scheme 5. Briefly, previously prepared ^{t}Bu-DO3A (**2**), alkynyl bromide and caesium carbonate in 1:1.5:2.4 molar ratio were stirred under inert atmosphere of nitrogen in dry acetonitrile at room temperature for 24 h. The Cs_2CO_3 base was preferred over sodium or potassium carbonate due to its higher solubility in acetonitrile and the larger size of the caesium cation preventing its complexation by DO3A.[50] The undissolved base was removed by filtration and products purified by liquid chromatography on silica gel.



*Scheme 5 -General synthetic route to form the functionalised alkynyl derivatives with tBu-DO3A (**2**) and produce **3**, **4**, **5** respectively.*

The propargyl group was the first functional group used in this work to attach on to the DO3A macrocyclic chelator as it had been previously validated for the conjugation with azide groups using Cu(I) catalysed click chemistry. It can be prepared by reacting ^{t}Bu-DO3A with propargyl bromide by following a modified version of the literature method reported by Jauregui e*t al.*[45] The Jauregui preparation method of **3** reacted ^{t}Bu-DO3A (**2**) with propargyl bromide in the presence of CS_2CO_3 using dry acetonitrile as solvent, see Scheme 6.



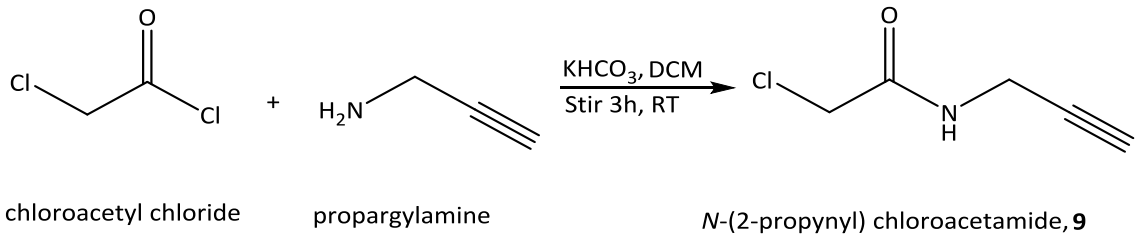
Scheme 6 - Synthetic route of propargyl-functionalised tBu-DO3A derivatives.

Base (CS_2CO_3) was used in the reaction to ensure removal of the NH proton, the reaction mixture was stirred under nitrogen for 24 h at room temperature, and the crude product was purified using silica chromatography with DCM: MeOH eluent to give the expected product **3** as a pale oil in a 78% yield.

The identity of product **2** was confirmed by MS, ^{1}H NMR and CHN analysis, where the MS analysis contained a peak at m/z 553 $[M+H]^{+}$ that was assigned to the molecular ion. ^{1}H NMR also confirmed that the S_N2 nucleophilic substitution occurred with the presence of the alkyne proton as a broad singlet at 2.1 ppm with integration value of 1. In addition, the peak at 1.47 is assigned to the t-butyl group, and the doublet peak at 3.5 corresponds to the CH_2 of the arm, whilst the multiplet peak attributed to the protons of the cyclen ring appeared at 2.75-2.83 ppm.

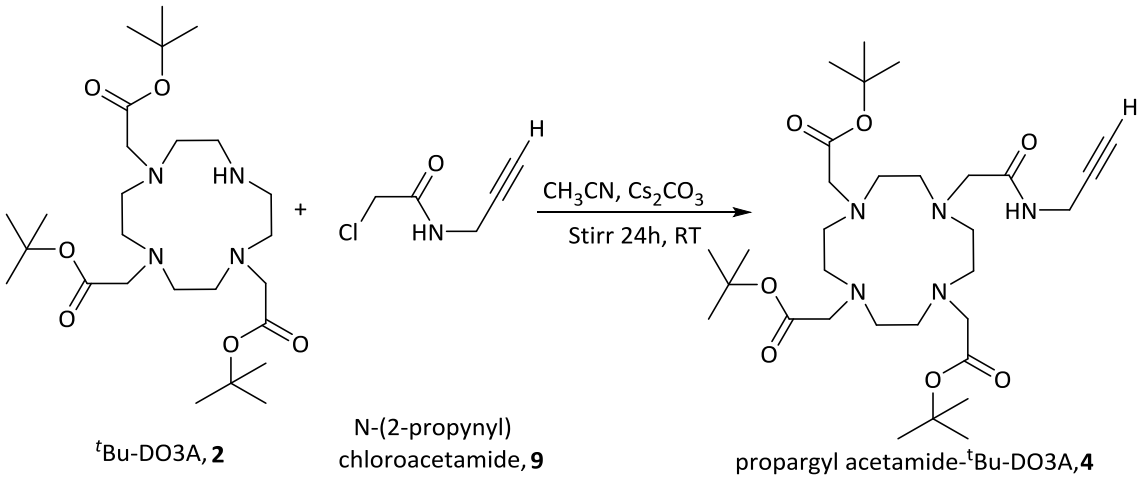
The second alkyne group attached to the DO3A was *N*-(2-propynyl)-chloroacetamide, as the longer arm could potentially enhance the relaxivity rate in MRI application, this precursor was successfully synthesised by following the published method reported by Jauregui *et al.*[45,52]

Compound **9** was prepared by reacting chloroacetyl chloride with propargyl amine in dry DCM in the presence of $KHCO_3$ at RT for 3h, see Scheme 7. The insoluble precipitate was filtered off and filtrate was washed with 5% $NaHCO_3$, dried over magnesium sulphate and concentrated by rotary evaporation to give the crude product of **9** as a brown solid. The crude compound was then purified by liquid chromatography on silica gel with 25% ethyl acetate/hexane mixture as eluent to give the pure product **9** in 81% yield. Structure and purity of **9** was confirmed by ^1H NMR, MS and CHN analyses.



*Scheme 7 - Synthetic route of acetamide arm (**9**) in situ.*

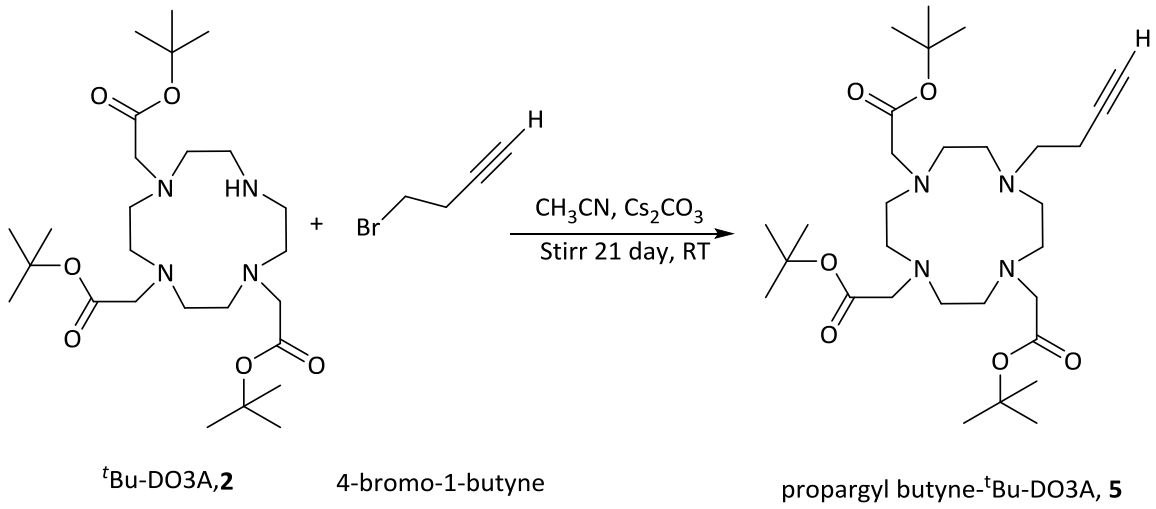
The acetamide arm precursor **9** was reacted with ^{t}Bu-DO3A using the same reaction conditions as described for preparation of propargyl ^{t}Bu-DO3A (**3**), see Scheme 8.



*Scheme 8 - Synthetic route to form N-functionalised tBu-DO3A derivatives with the acetamide arm (**4**).*

After 24h, the reaction was complete and the desired product **4** had formed as a yellowish oil in a 76% yield, see scheme 8. Identity and purity was confirmed by ^1H NMR, MS and CHN analyses of **4**. MS showed a peak for the molecular ion of the desired product at m/z 611 $[M+H]^+$ and with a sodium ion at m/z 633 $[M+Na]^+$. No unreacted DO3A starting material was observed (the peak appears at m/z 515) or any other impurities.

The third arm selected from the possible alkyne derivatives was 4-bromo-1-butyne, which is commercially available and the bromine is good leaving group for the substitution reaction. This reaction was carried out under the same conditions as described above in section 2.1.2, to form the desired product **5**, see Scheme 9.



^{t}Bu-DO3A,**2** 4-bromo-1-butyne propargyl butyne-^{t}Bu-DO3A, **5**

*Scheme 9 - Synthetic route to form N-functionalised tBu-DO3A with propargyl butyne arm (**5**).*

The reaction was left to stir for 21 days with regular monitoring by silica TLC and mass spectrometry, see Figure 16, which showed the formation of product at m/z 567 $[M+H]^+$, The product still showed the presence of unreacted starting materials (^{t}Bu-DO3A at m/z 515) after this reaction time.[53]

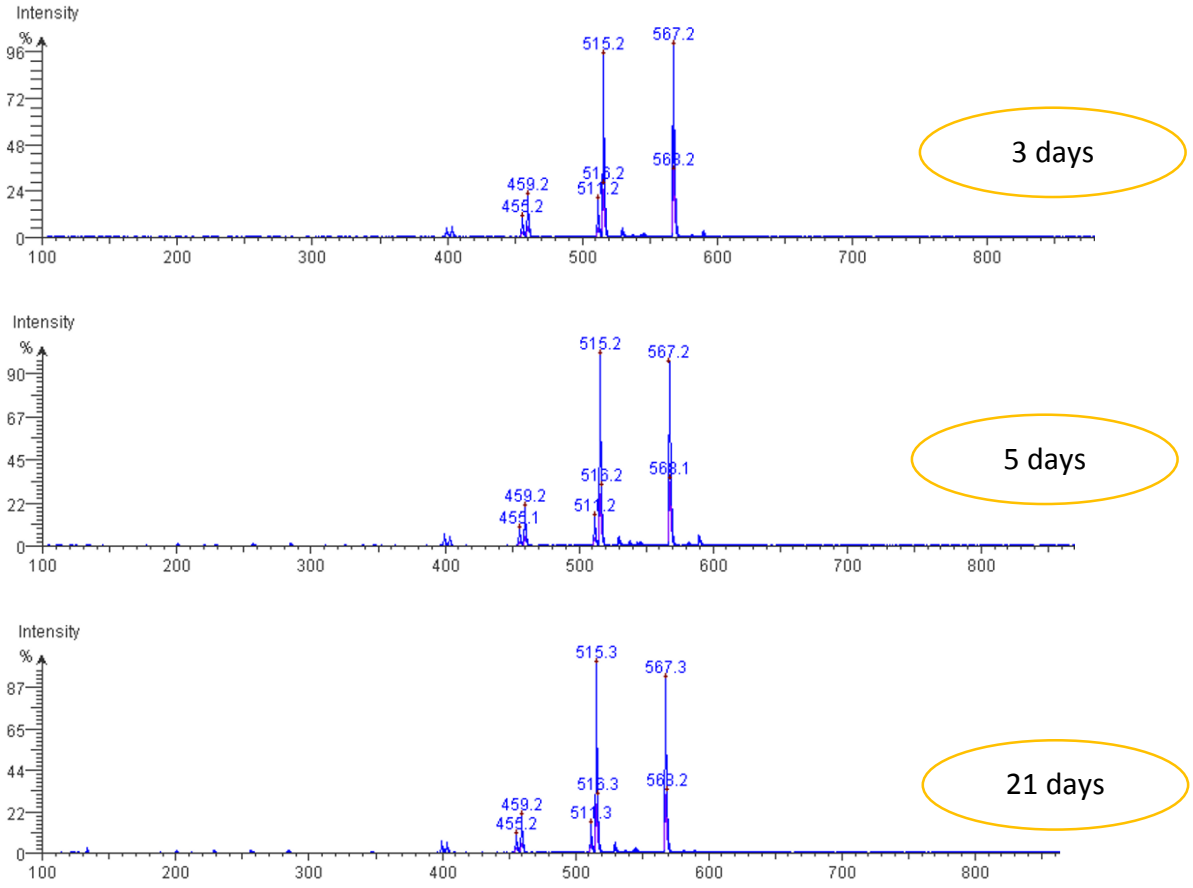


*Figure 16 - Mass spectrum of **8** contained starting materials DO3A at m/z 515 after 3, 5 and 21 days.*

The reaction was monitored by TLC and MS at three different times (3, 5, and 21 days), and shows no significant change by continuing the stirring for a longer time period.

At this stage, the reaction was terminated and the synthesised ligand **5** purified by silica gel chromatography with DCM (97%): MeOH (3%) as eluents. TLC and MS analysis results showed that the desired product was present after column chromatography but still had impurities of starting materials present (product at m/z 567 $[M+H]^+$, (DO3A) at m/z 515), see Figure 17.

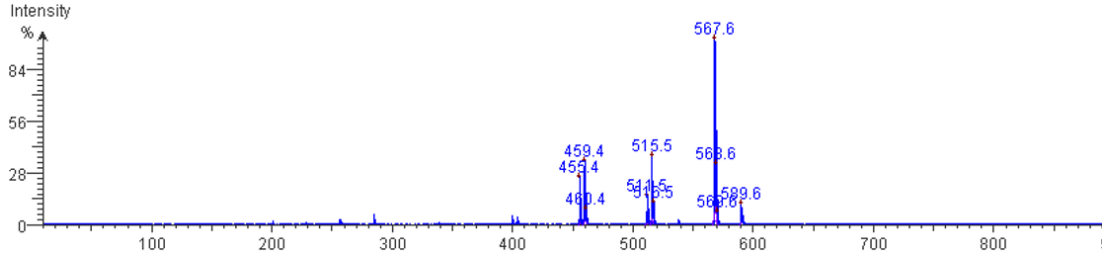
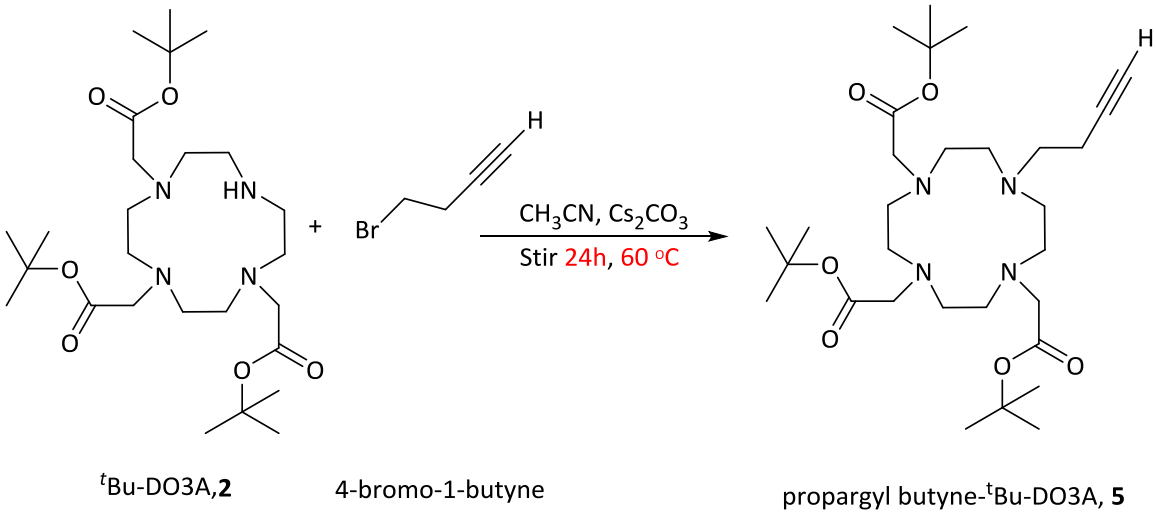


*Figure 17 - Mass spectrum of purified **5** still contained starting materials DO3A at m/z 515.*

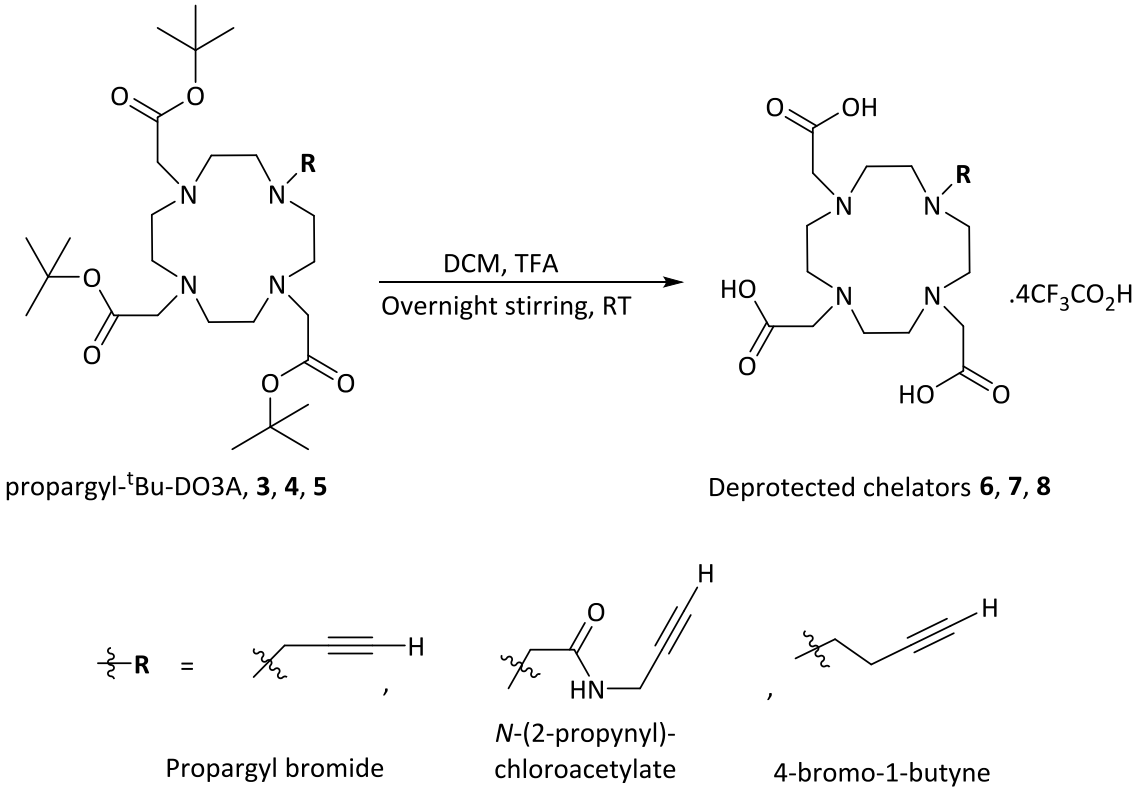
Further attempts were made to resynthesise **5** ligand with the same molar ratio but using different solvents (DMF, THF, and dry MeCN) and changing the base used (DIPEA, caesium carbonate). All of these reactions were carried out under nitrogen, stirring at RT for three weeks with daily monitoring by TLC and. The best results were observed with dry acetonitrile in the presence of caesium carbonate.[54] This reaction was then optimised by adjusting the temperature (60 °C), stirring for a shorter time period (24 h) and changing the eluent for the silica gel chromatography after developing new conditions on silica TLC (DCM:acetone: MeOH/ 6:3.5:0.5). The pure compound was then isolated as a novel compound from this research. The identity and purity of compound **5** was confirmed by ^{1}H NMR, HRMS and CHN analyses, see Scheme 10.



*Scheme 10 - Successful synthetic route to form the novel compound **5**.*

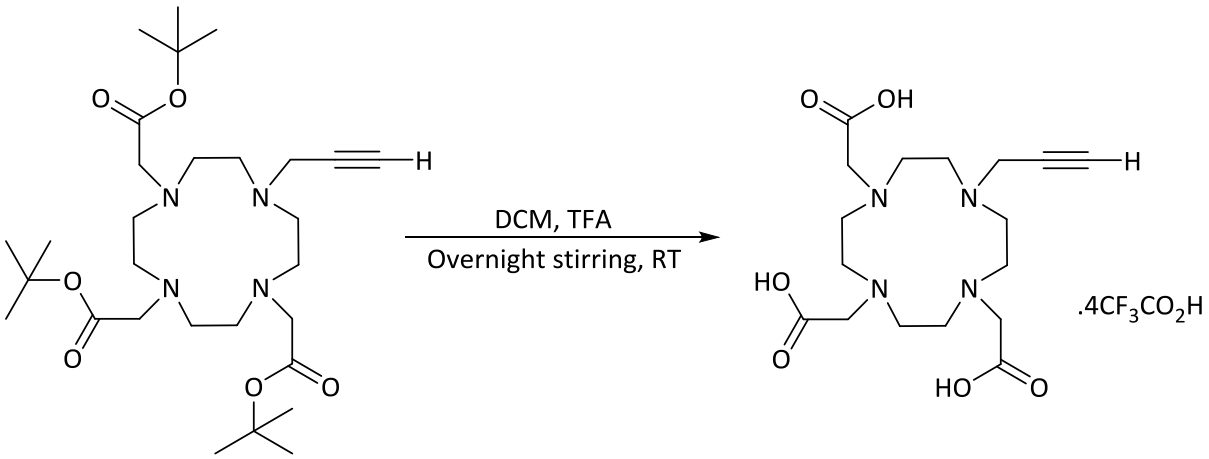
2.3 Deprotection of synthesised DO3A chelators with alkyne functionalised groups (3, 4, 5).

The deprotection reactions were carried out on the synthesised ligands (**3**, **4**, and **5**) by dissolving them in DCM, then adding TFA dropwise followed by stirring overnight at room temperature, see Scheme 11.The solvent was removed with a dry ice cooled rotary evaporator. The product was then dissolved in the minimum amount of methanol and added dropwise to 50ml diethyl ether which caused precipitation. The white fluffy solid was collect via centrifugation, was redissolved in water, washed and then freeze dried to give the products **6**, **7**, and **8**.[51] These compounds (**6**, **7**, and **8**) were characterised by MS, ^{1}H NMR, and CHN analysis.



*Scheme 11 - Deprotection Synthetic route of synthesised ligands (**3**, **5**, and **6**) leading to deprotected chelators (**6**, **7**, and **8**).*

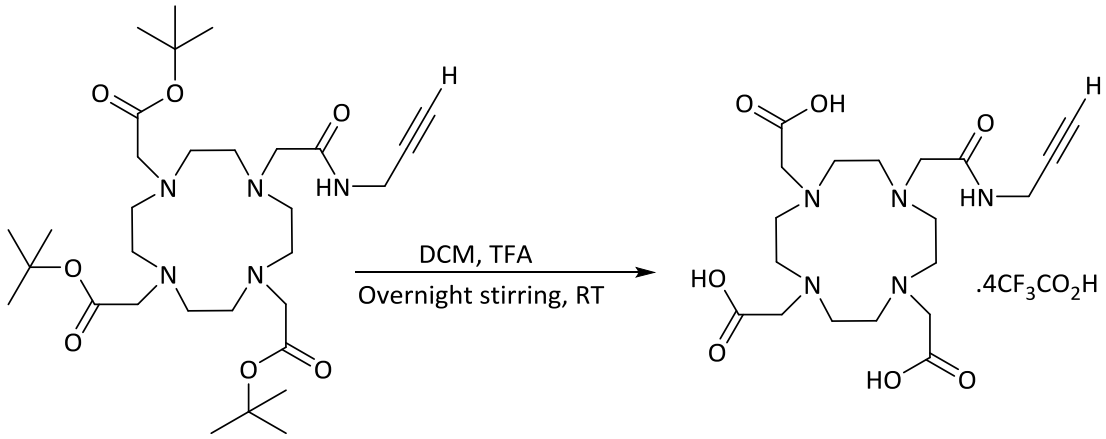
To give a drawn out example, the deprotection reaction was performed as described above for product **3** to give product as a white solid in 86% yield, see Scheme 12.



Propargyl-^{t}Bu-DO3A, **3** Deprotected chelator, **6**

*Scheme 12 - Deprotection of precursor propargyl tBu-DO3A (**6**).*

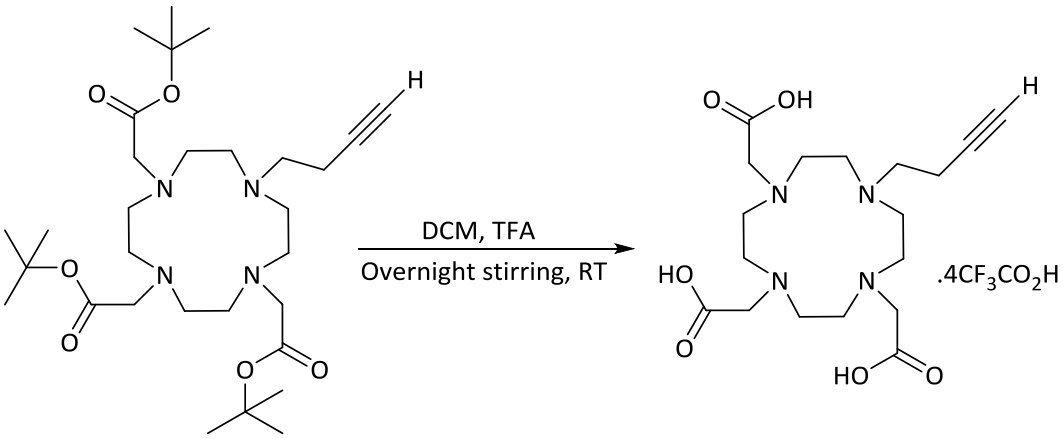
The same conditions were used for product **4** to give product **7;** formed as a pale yellow fluffy powder in 79% yield, see Scheme 13.



Propargylacetamide-^{t}Bu-DO3A, **4** Deprotected chelator, **7**

*Scheme 13 - Deprotection reaction to form chelator **7**.*

5 was then deprotected forming the product **8**, as fluffy white powder with 86% yield, see Scheme 14. Identity and purity of **8** was confirmed by ^{1}H NMR, MS and CHN analyses.



Butynyl-^{t}Bu-DO3A, **5**

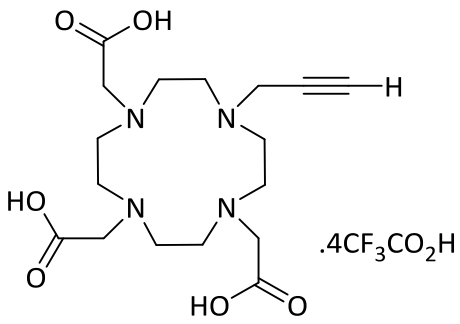
Deprotected chelator, **8**

*Scheme 14 - Deprotection reaction to form chelator **8**.*

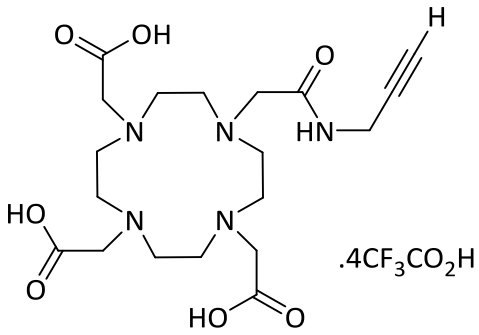
2.4. Summary of chelator synthesis

Three chelating ligands (**6**, **7**, and **8**), see Figure 18, were synthesised and functionalised with alkyne arms (propargyl, acetamide propargyl and butyne). Their preparation from DO3A and halogenated alkynyl precursors was optimised for reaction temperature and time.

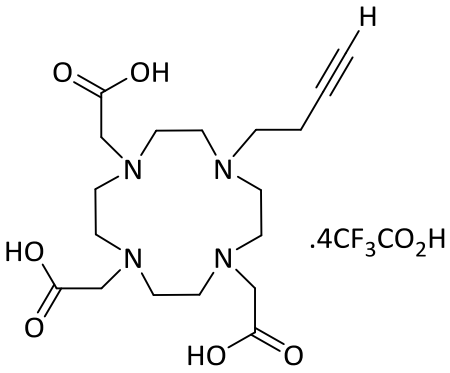
These three ligands, two of them (**6**, **7**) were known and published in the literature and the third synthesised ligand (**8**) is novel and synthesised in this research work. Identity and purity of these products (**6**, **7**, and **8**) was confirmed by ^{1}H NMR, MS and CHN analyses.



Deprotected propargyl-DO3A, **6**



Deprotected propargyl acetamide-DO3A, **7**



Deprotected propargyl butyne-DO3A, **8**

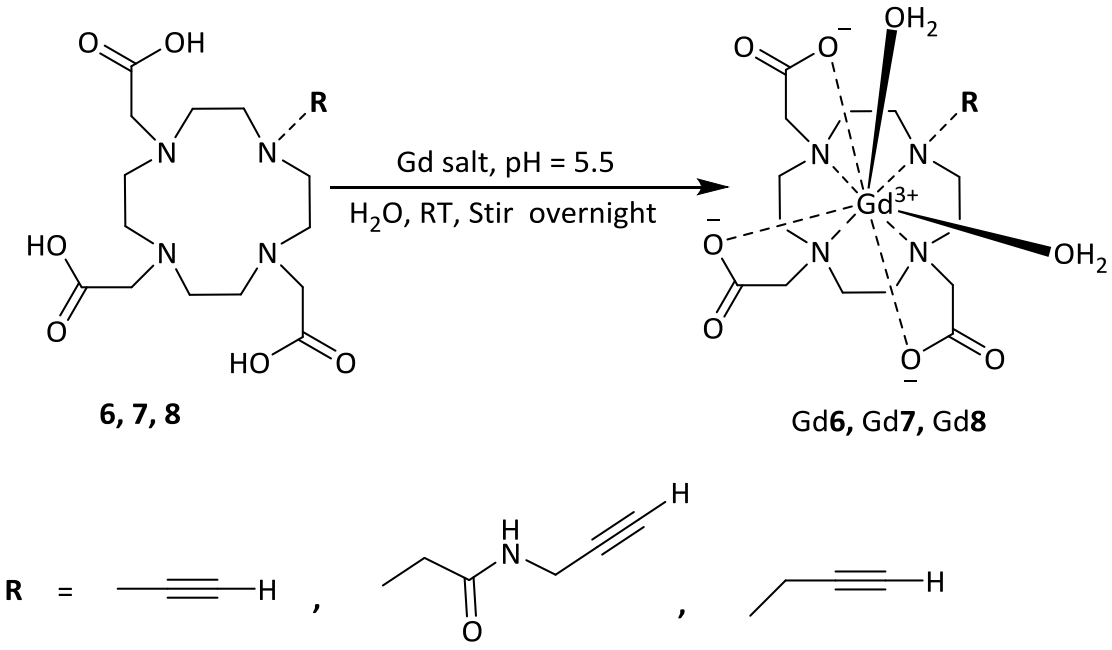
*Figure 18 - The three synthesised chelators (**6**, **7**, and **8**) with three different alkyne arms (propargyl, acetamide, and butyne).*

Chapter 3

Gadolinium(III) complex formation

3.1. Gadolinium(III) complex formation with alkyne-functionalised DO3A.

The second stage of this project consists of preparation of gadolinium(III) complexes with the previously prepared alkyne functionalised DO3A. As the synthesised ligands (**6**, **7**, and **8**) all contain the DO3A component, they can form up to seven coordination bonds and chelate di- or trivalent metal cations of suitable size. Gadolinium(III) is paramagnetic and has a symmetric S electronic state that is ideal for use as an MRI contrast agent, thus, it was chosen as the preferred lanthanide metal to coordinate with the chelators to form Gd**6**, Gd**7**, Gd**8**, see Scheme 15.



*Scheme 15 - Overall Gd(III) complexation reactions with synthesised ligands **6**, **7**, and **8**.*

The aim in formation of the Gd^{3+}complexes prior to "clicking" the DO3A macrocycle with an azide functionalised derivative is to prevent the Cu^{2+} ion (catalyst) from complexing with these macrocyclic ligands, complicating purification and compromising the Gd(III) chelation necessary for MRI CA activity.[45]

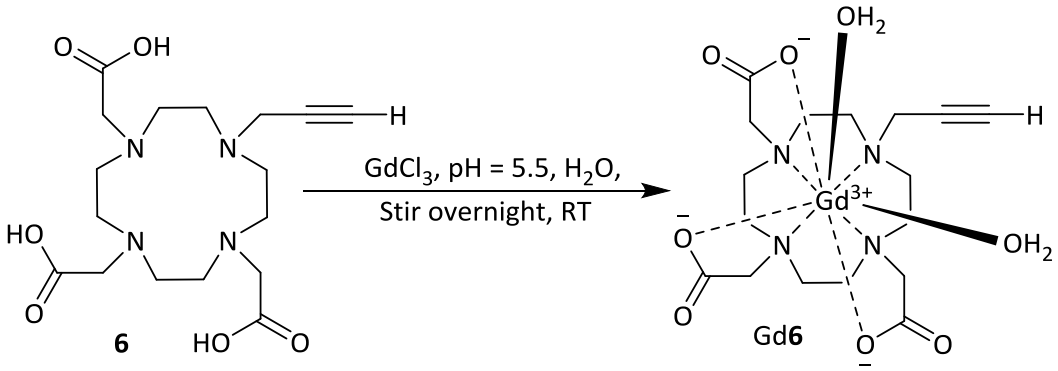
Complexation reactions were carried following a modified literature method.[45] Briefly, free carboxylate ligands (**6**, **7**, and **8**) and gadolinium chloride were dissolved in water, pH was adjusted to 5.5 using a dilute solution of sodium hydroxide and the reaction was stirred overnight at room temperature. Adjustment of pH is important because at lower pH nitrogen atoms in DO3A will protonate compromising their coordination potential and higher pH would

transform $GdCl_3$ into insoluble $Gd(OH)_3$ that would precipitate and not be available for reaction.[45,50]

Any excess of free unreacted Gd^{3+} was precipitated in $Ga(OH)_3$ form by increasing the pH of the solution to 9-10 and removed by centrifugation. The pH of supernatant containing products (Gd**6**, Gd**7**, and Gd**8**) was adjusted to 7 by addition of dilute HCl solution and tested for free Gd ions using the xylenol orange indicator.[51,55] The change of the colour to purple indicates the presence of free Gd. If this was the case, the pH was increased again and the removal procedure to precipitate $Gd(OH)_3$ repeated. When no free Gd(III) was detected, the supernatant was lyophilised to give the Gd^{3+}-DO3A complexes as solids.

3.1.1 Preparation of gadolinium(III) and propargyl functionalised DO3A complex (Gd6).

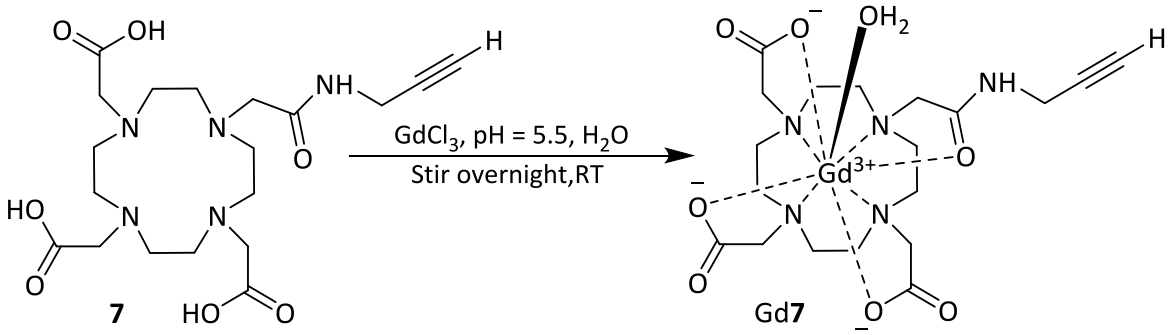
The gadolinium complex Gd**6** was prepared and purified following the procedure described above in 3.1, see Scheme 16. It was obtained as a white solid in a 74% yield. The identity of Gd**6** was confirmed by mass spectrometry and CHN analysis.



*Scheme 16 - Gd^{3+} complex formed with **6**.*

3.1.2. Preparation of gadolinium and acetamide linker containing alkyne functionalised DO3A complex (Gd7).

The gadolinium complex Gd**7** containing the propargylacetamide arm was prepared and purified following the procedure described in 2.3.1. It was obtained as a white solid in an 82% yield, see Scheme 17. The identity of Gd**7** was confirmed by mass spectrometry and CHN analysis.

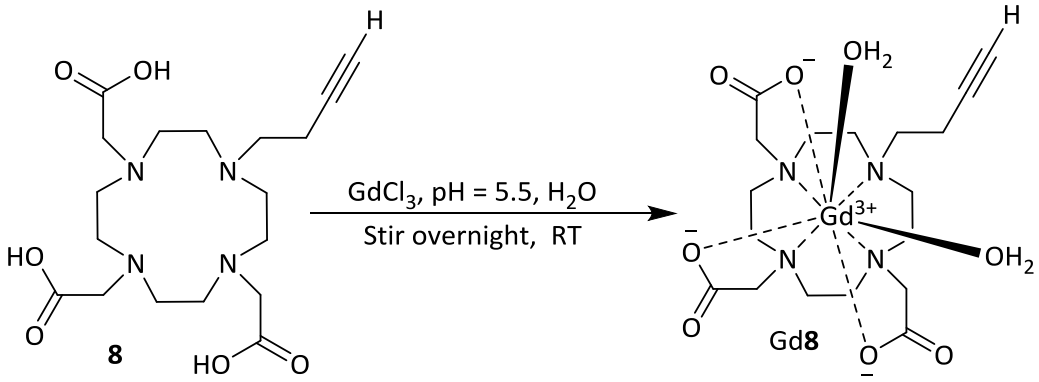


*Scheme 17 - Gd^{3+} complex formed with **7**.*

The chelator **7** differs from compounds **6** and **8** with the presence of acetamide group, which rigidifies the structure and may contribute (albeit more weakly than the carboxylate donors) to the coordination of the metal centre.

3.1.3. Preparation of gadolinium and butynyl functionalised DO3A complex (Gd8).

A novel gadolinium(III) complex Gd**8** based on butynyl functionalised DO3A was prepared and purified following the procedure described in 3.1. It was obtained as a light brown solid in 87% yield. Successful complex formation of Gd**8** was confirmed by mass spectrometry and CHN analysis, see Scheme 18.



Scheme 18 - Gd^{3+} complex formed with 8.

Borbas *et al*.[56] reported an alternate method for DO3A functionalised with ethane azide, which, once "clicked" with alkyne derivatised hydroporphyrine, yielded a similar triazole containing conjugate to those targeted in this work. Their compound is potentially useful in fluorescence imaging or for targeted photodynamic therapy, see Figure 19.

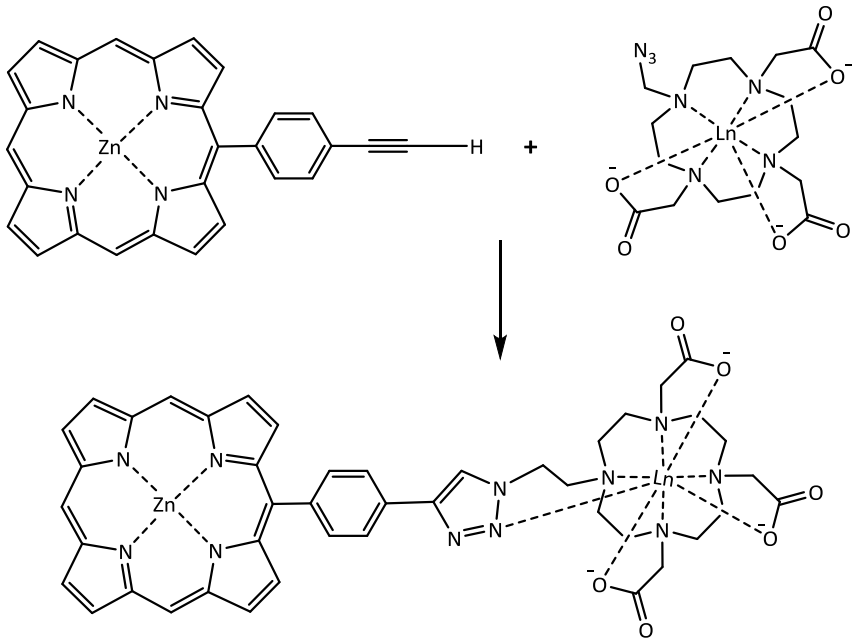


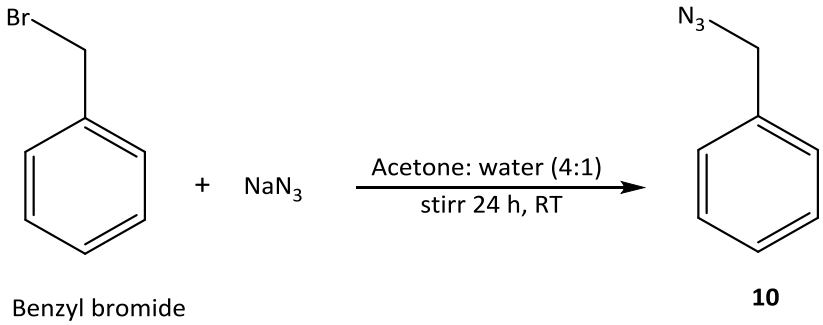
Figure 19 - An example of a Ln(III) chelate coupled with alkyne hydroporphyrine.[56]

Chapter 4

Click reactions with alkyne chelator complexes

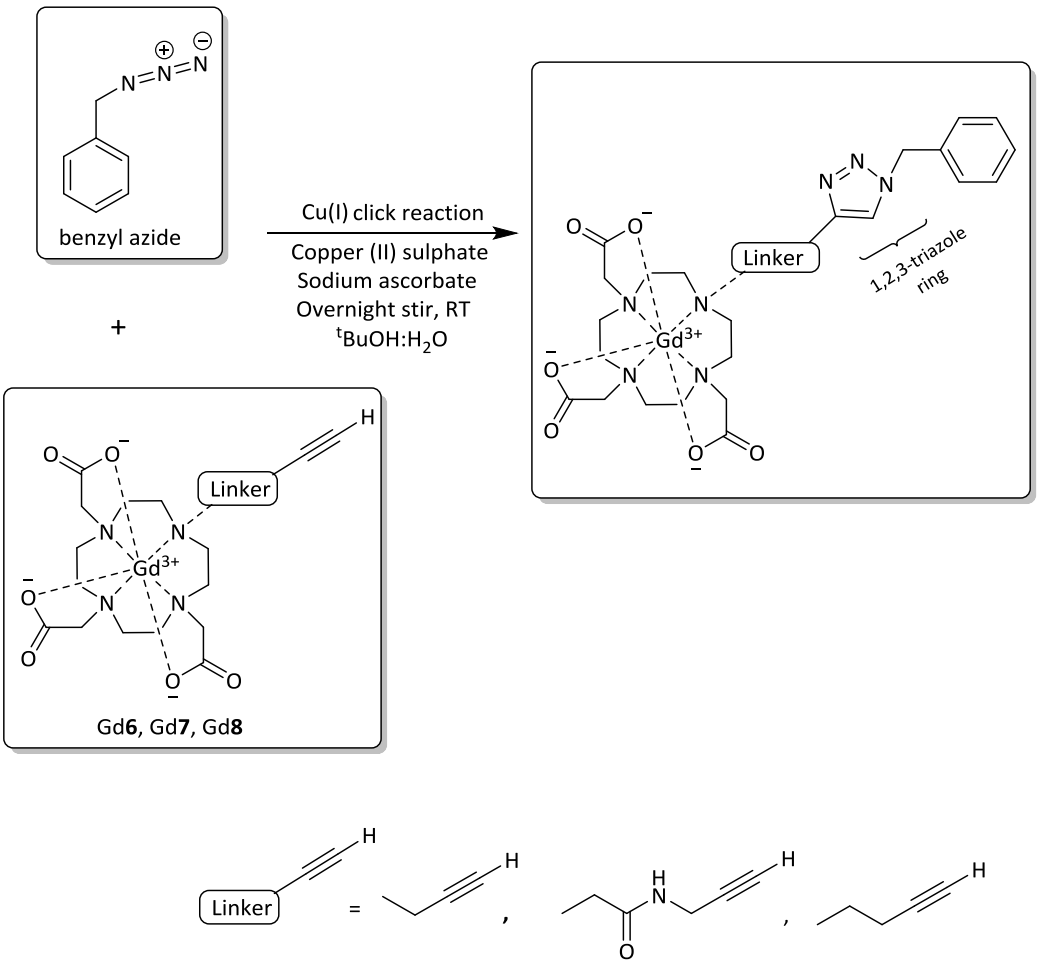
4.1. Model click reaction between alkyne-functionalised DO3A and alkyl azide.

Once alkyne functionalised chelates Gd**6**, Gd**7**, Gd**8** were prepared, a simple organic azide was required in order to investigate the chelate reactivity in the Cu(I) catalysed "click" reaction. Due to its stability and the ease of preparation from benzyl bromide, benzyl azide was selected.[58] It was prepared from benzyl bromide and sodium azide, as described in the literature, see Scheme 19. The formed benzyl azide was extracted into dichloromethane (DCM), the combined organic phases were dried over magnesium sulphate and evaporated at reduced pressure to give product **10** in 83% yield. The identity of the product was confirmed by ^{1}H NMR and MS analyses.



*Scheme 19 - Synthesis of benzyl azide **10**.*

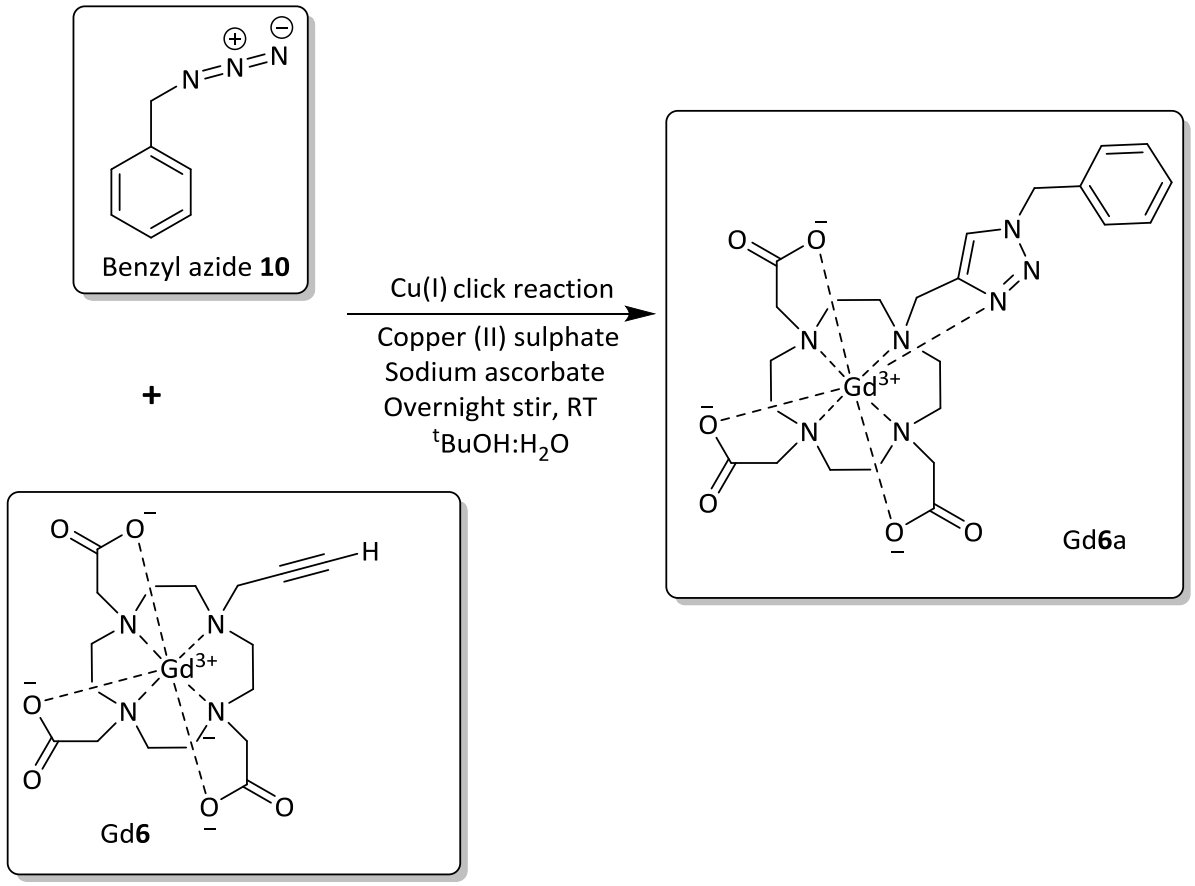
The model "click" reaction between benzyl azide and the prepared chelates Gd**6**, Gd**7**, Gd**8** was carried out following a similar protocol to that described by Sharpless *et al*.[70] Alkyne and azide precursors were stirred at room temperature overnight in *t*-BuOH and water mixture (1:1) in the presence of *in situ* generated Cu(I) catalyst. $CuSO_4$ (0.1 eq) was used as copper source and sodium ascorbate (1 eq, 10 fold excess to Cu) as a reducing agent, see Scheme 20.



*Scheme 20 - Cu(I) catalysed cycloaddition "click" reaction between benzyl azide and Gd**6**, Gd**7**, Gd**8** complexes.*

4.1.1. "Click" reaction between propargyl functionalised DO3A and benzyl azide.

Conjugate Gd**6**a was prepared using a standard protocol described above as light yellow powder in a 74% yield (see section 4.1), see Scheme 21.

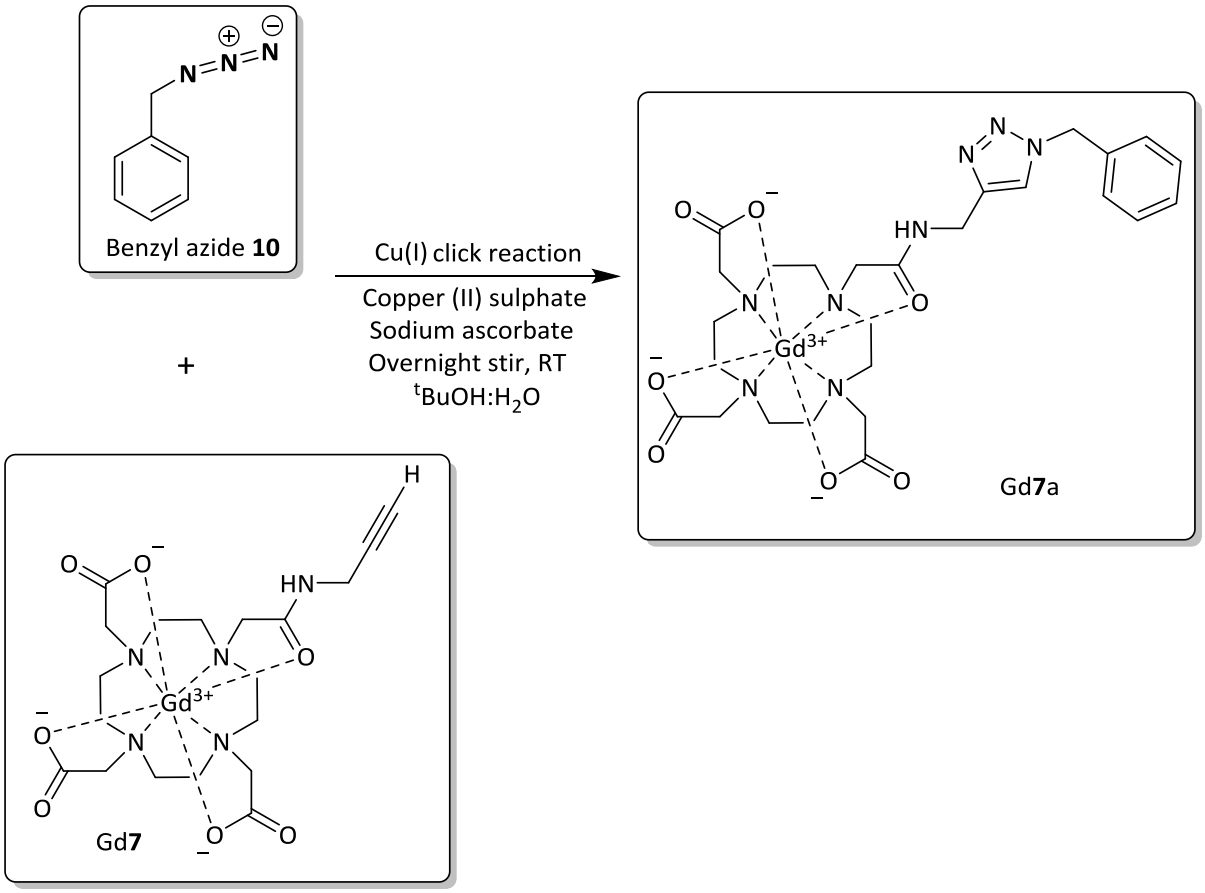


*Scheme 21 - Cycloaddition "click" reaction between Gd**6** and benzyl azide.*

The reaction progress was monitored by mass spectrometry showing reaction completion after 18h (overnight reaction). The copper catalyst was removed from the reaction mixture by passing it through a cation exchange resin. The identity of conjugate Gd**6**a was confirmed by MS, ICP and CHN analyses.

4.1.2. Click reaction between acetamide-functionalised DO3A and benzyl azide.

Conjugate Gd**7**a was prepared using the protocol described above (see section 4.1) as brown solid with 81% yield, see Scheme 22.

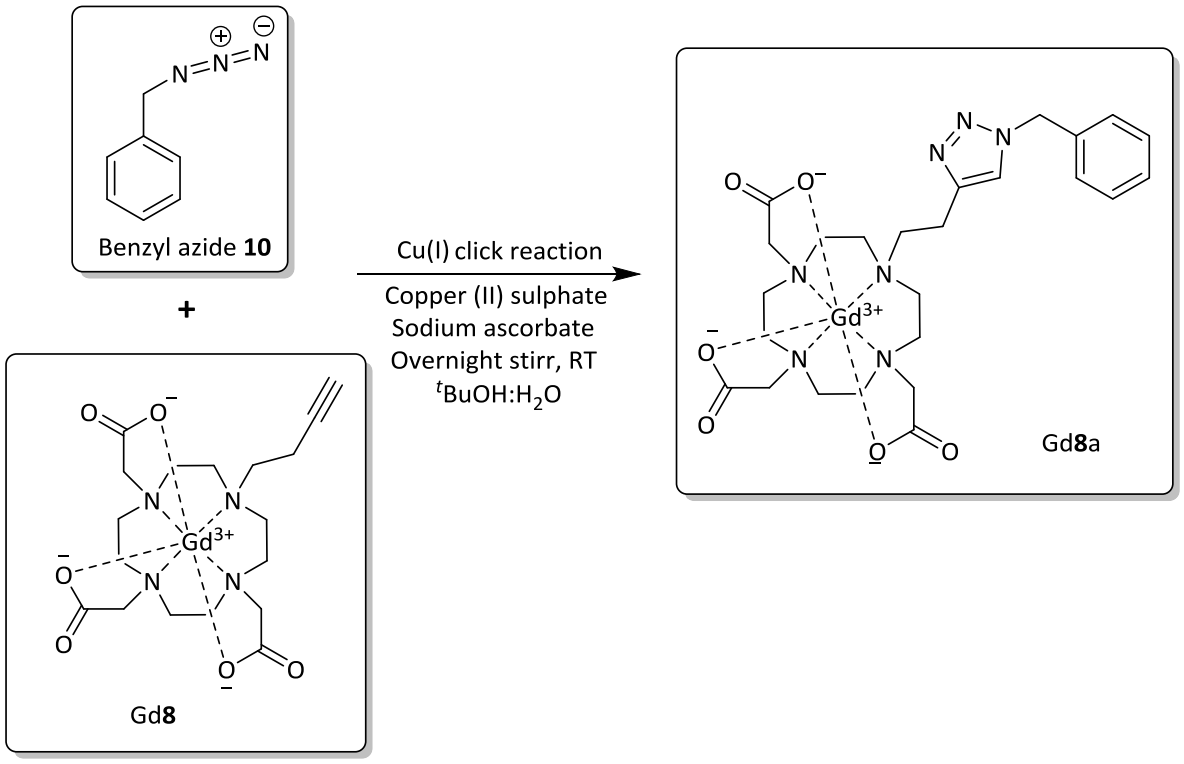


*Scheme 22 - Cycloaddition click reaction between benzyl azide and Gd**7** forming Gd.**7**b.*

The reaction progress was monitored by mass spectrometry showing the completion after 18h (overnight reaction). Copper catalyst was removed from the reaction mixture by passing it through cation exchange resin. The structure of conjugate Gd**7**a was confirmed by MS, ICP and CHN analyses.

4.1.3. Click reaction between butyne-functionalised DO3A and benzyl azide

Conjugate Gd**8**a was obtained using the standard protocol described above (see section 4.1) as a white powder with 85% yield, see Scheme 23.



*Scheme 23 -Cycloaddition click reaction between benzyl azide and Gd**9** forming 1,2,3-triazole.*

The reaction progress was monitored by mass spectrometry showing completion after 18h (overnight reaction). Copper catalyst was removed from the reaction mixture by passing it through a cation exchange resin. The identity of conjugate Gd**8**a was confirmed by MS, ICP and CHN analyses.

4.1.4. Summary of the synthesis of gadolinium complexes and their conjugation via model "click" reaction.

The main aim of this research is to synthesise alkyne-functionalised DO3A chelators suitable for click chemistry with biologically relevant azide functionalised molecule.

The reactivity of three Gd(III) chelates (Gd**6**, Gd**7**, and Gd**8**) in "click" reaction conditions was confirmed by reacting them with benzyl azide, see Figure 20. The identity of the resulting conjugates was determined by MS, ICP and CHN analyses.

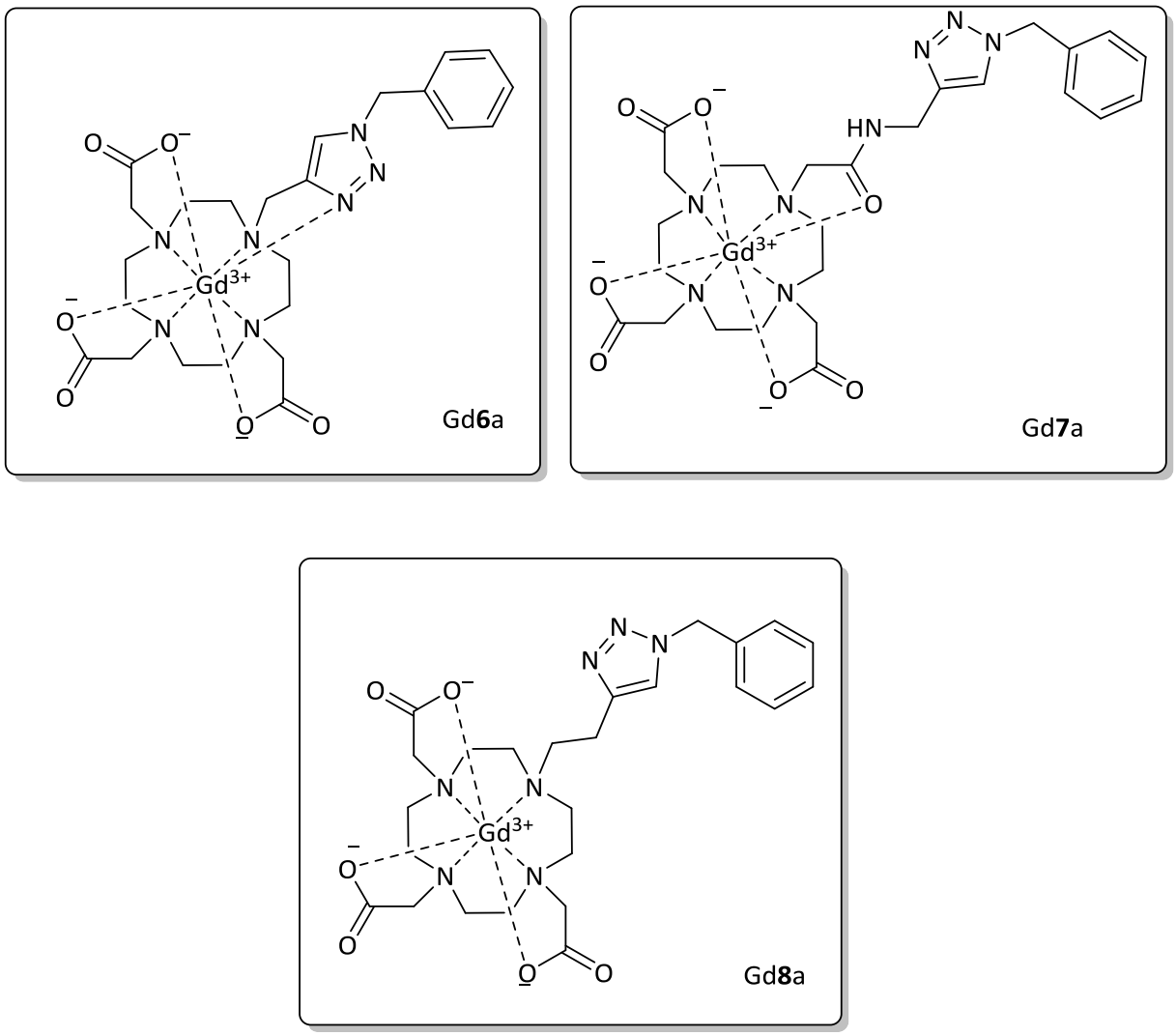


*Figure 20 - The synthesised a model compounds (Gd**6**a, Gd**7**a, and Gd**8**a).*

Chapter 5

Relaxivity measurements and click conjugation with a biomolecule

5.1 T_1 relaxation studies for Gd(III) complexes.

Relaxivity rates (R) of MRI contrast agents (CA) are very important as they determine the contrast of MR images.

R can be described as the ability of a selected CA on shortening the relaxation times of water protons, where a large R value can be linked to the magnetic properties of the contrast agents, the value of R can be determined via equations below with unit of $mM^{-1}\ s^{-1}$:

$$(1/T_1)_{obsd} = (1/T_1)_d + R_1 \times [CA]$$

where $(1/T_1)_{obsd}$ and $(1/T_1)_d$ characterise the measured relaxation rate of the solvent with or without paramagnetic species respectively, [CA] present the concentration of CA. T_1 relaxivity and subsequently R_1 values were measured for the three synthesised gadolinium complexes (Gd**6**, Gd**7**, and Gd**8**), in order to demonstrate the efficiency of these complexes as T_1 MRI contrast agents. T_1 relaxation times were measured in aqueous solutions over different gadolinium(III) concentrations (0.2, 0.5, 1.0, 1.5 and 2.0 mM) for each complex after dilution with distilled water at a field strength of 1.5 T.

5.1.1 T_1 relaxation studies for Gd6, Gd7, Gd8

Relaxivity rates were determined for Gd**6**, Gd**7**, Gd**8** across range of concentration values (0.5, 1, 1.5, 2 and 2.0 mM) at pH=7, which means that all the N and O donors are expected to be deprotonated and coordinated to the gadolinium(III) metal centre.

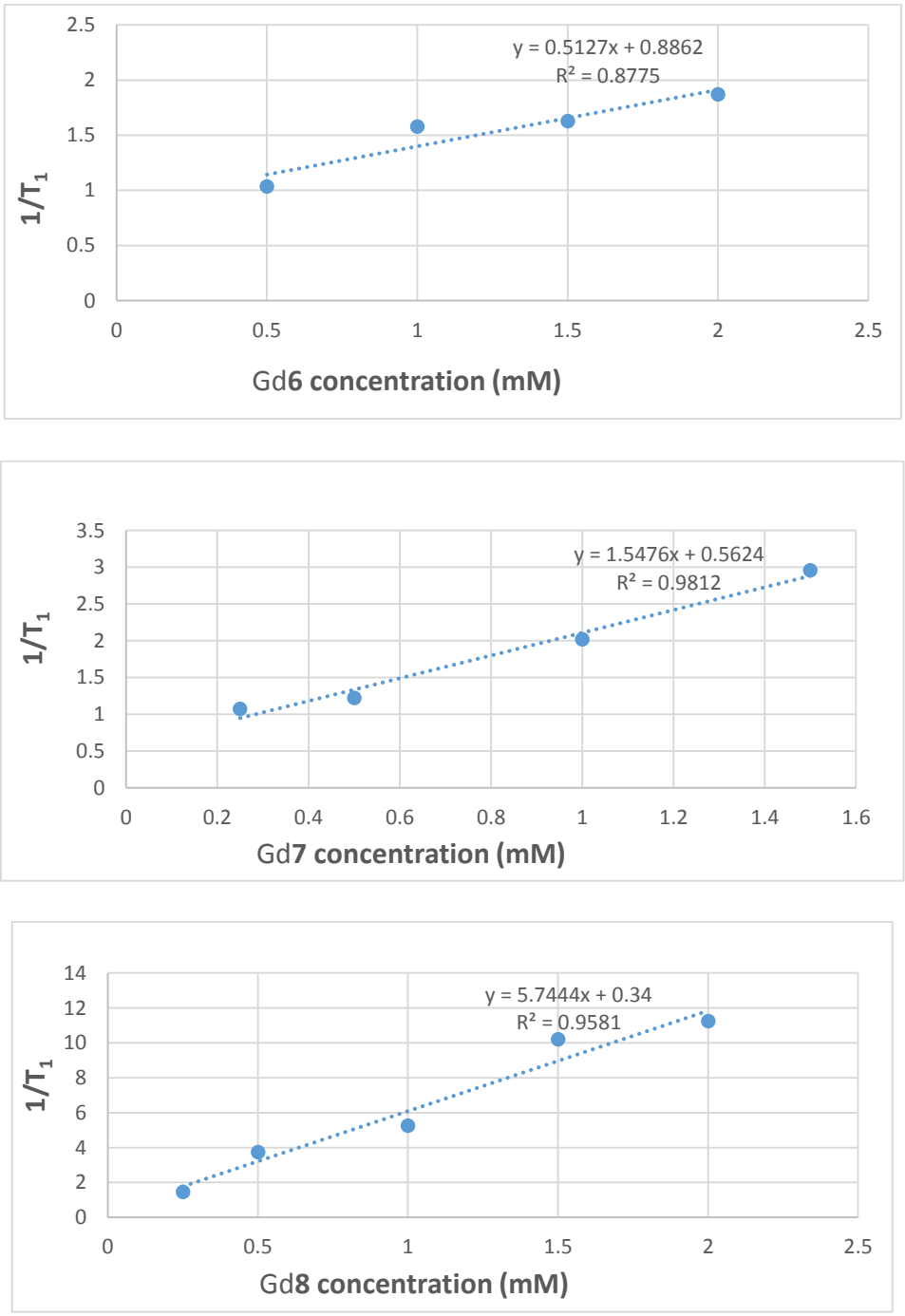


*Figure 21 - Graphs to show concentration vs. 1/T for Gd**4**, Gd**7**, and Gd**9**.*

Figure 21, show graphs of the MRI relaxivity measurements for the synthesised Gd**6**, Gd**7**, Gd**8** indicating the linear relationship between the relaxation rates $1/T_1$ (r_1) vs various

concentrations of these complexes to give T_1 relaxivity values of0.512 mM^{-1} s^{-1} , 1.512 mM^{-1} s^{-1} and 5.744 mM^{-1} s^{-1} , with correlation coefficienents of 0.877, 0.981 and 0.9581 respectively. This indicates their potential use as T_1 weighted contrast agents in MRI applications.

Comparison between the relaxivity rates for the prepared complexes Gd**6**, Gd**7**, Gd**8** shows higher relaxivity for Gd**8** for T_1 as 5.744 mM^{-1} s^{-1} in comparison to the other complexes Gd**7** and Gd**8** (0.512 mM^{-1} s^{-1} and 1.512 mM^{-1} s^{-1} respectively). The reason behind the higher relaxivity of Gd**8** is not immediately obvious but may be related to water exchange rates, which indicates promising utility of Gd**8** in future MRI studies.

5.2. Click chemistry

5.2.1 Click chemistry with DNA bases

There has been significant interest in click chemistry reactions with DNA and DNA bases, The copper catalysed alkyne-azide cycloaddition (CuAAc) can be used to label single bases or oligonucleotides with various groups such as sugars, peptides, and fluorescent dyes. These processes can also be used to synthesise DNA catenanes, to cyclise DNA, to join oligonucleotides to PNA and to produce analogues of DNA with other modified molecules and backbones.[71]

In the literature there are many examples of click reactions with AZT to attach different molecules for a variety of applications.[71] Keduo *et al.* designed a reaction to link AZT with betulin/betulinic acid (BA) by click chemistry via a triazole linkage. This potentially offers a new direction for the modification of anti-HIV triterpenes where one of them can be a large natural product,[72] see Figure 22.

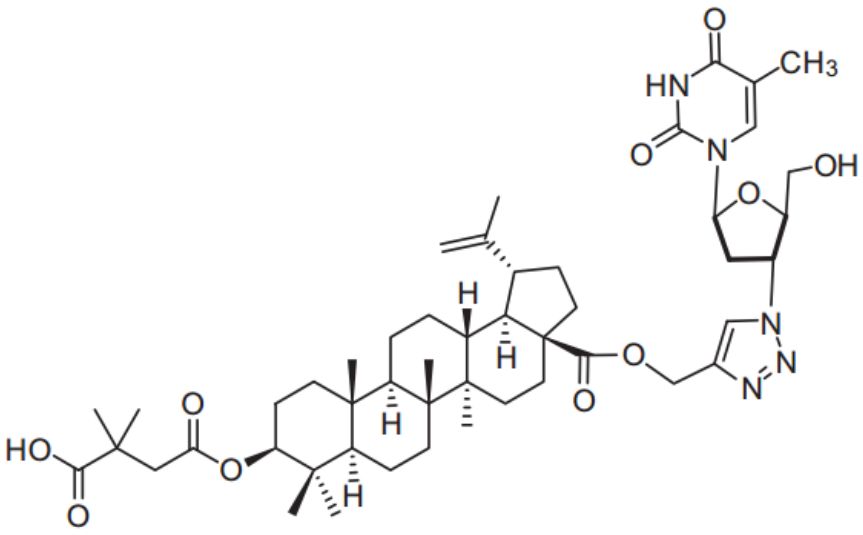


Figure 22 - AZT conjugation with betulin via click chemistry.[73]

The Wang group reported a further example as they successfully prepared an AZT 1,2,3 triazole derivative with submicromolar potency against HIV-1 and observed antiviral activity from the cytopathic effect that suggested a mechanism of translocation of the triazoles to the P-site of HIV reverse transcriptase,[73] see Figure 23.

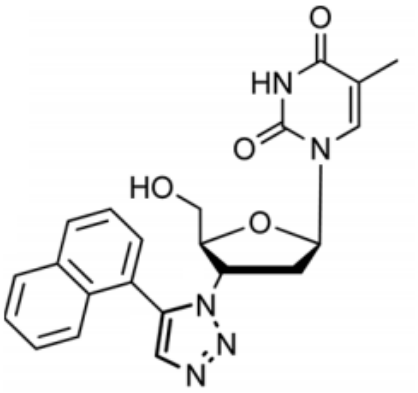


Figure 23 - Design of a 1,2,3-triazole HIV inhibitor.[73]

A third example of AZT conjugation was prepared using SiO_2 nanoparticles as a platform for delivery of 3'-triazole analogues of AZT-triphosphate into cells using the 'click'-reaction between AZTTP and modified nanoparticles containing the alkyne groups. The conjugate was tested and shown to be incorporated into the growing DNA chain with the similarity of the SiO_2 compound to AZTTP as the nanocomposites can penetrate into cells to inhibit the reproduction of POX and Herpes viruses at non-toxic concentrations.[74]

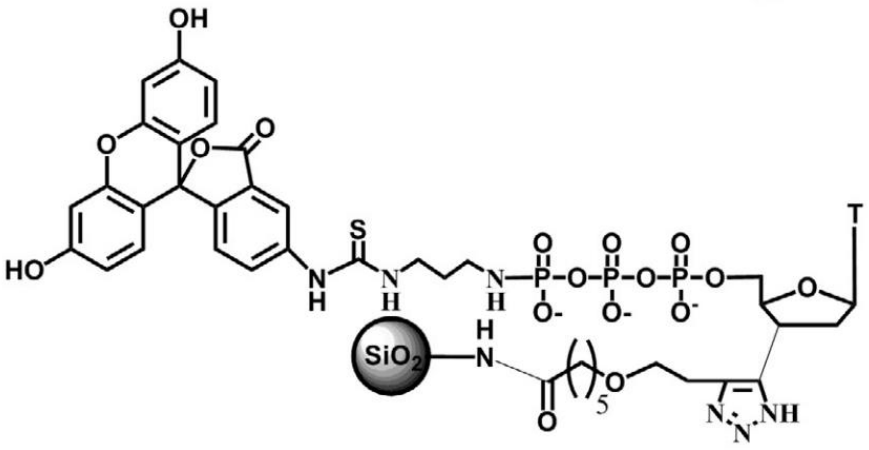


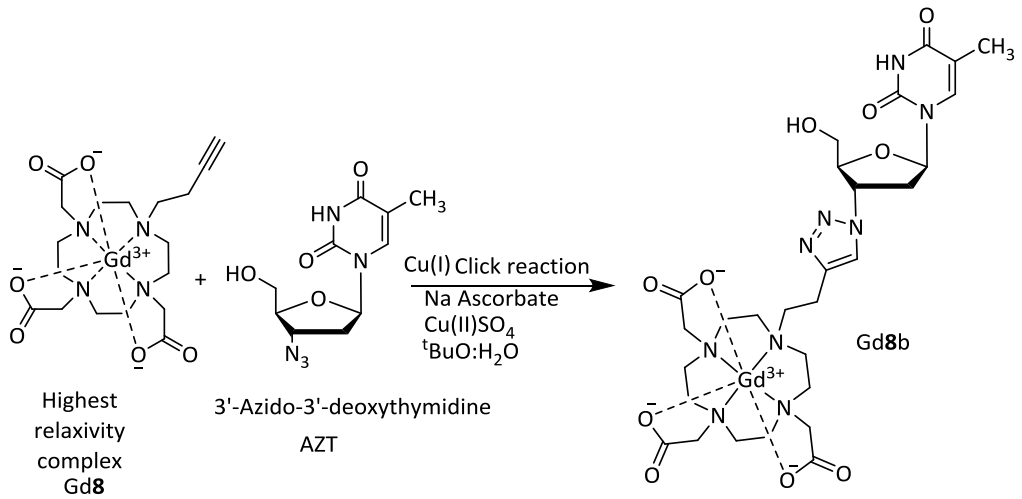
Figure 24 - Preparation of the SiO_2-dNTP nanocomposites.[74]

for particular work AZT which is DNA base was used as antiviral drug, where can be used in the click reaction between an azide on the drug molecule and the alkyne derivative MRI contrast agents that been developed herein.

5.2.2 Conjugation of Gd8 with azide functionalised deoxythymidine (AZT).

AZT has been used previously in click reactions but there are no reports of it being use for click reactions with imaging agents as proposed in this work. Linking an organic azide with a substituted alkyne using Cu(I) catalysed cycloaddition reaction offers a facile technique in order to append CA chelates to biological molecules. The key deliverable is the conjugation of an imaging component (Gd^{3+} complex functionalised with an alkyne arm) to a DNA base derivatives (3'-azido-3'-deoxythymidine, AZT) via the cycloaddition reaction click reaction. The conjugate can be analysed for its proton relaxation (relaxivity) efficiency to see if it generates significant contrast enhancement in MR imaging. Due to the high relaxivity properties, Gd**8** was selected for conjugation with AZT.[64]

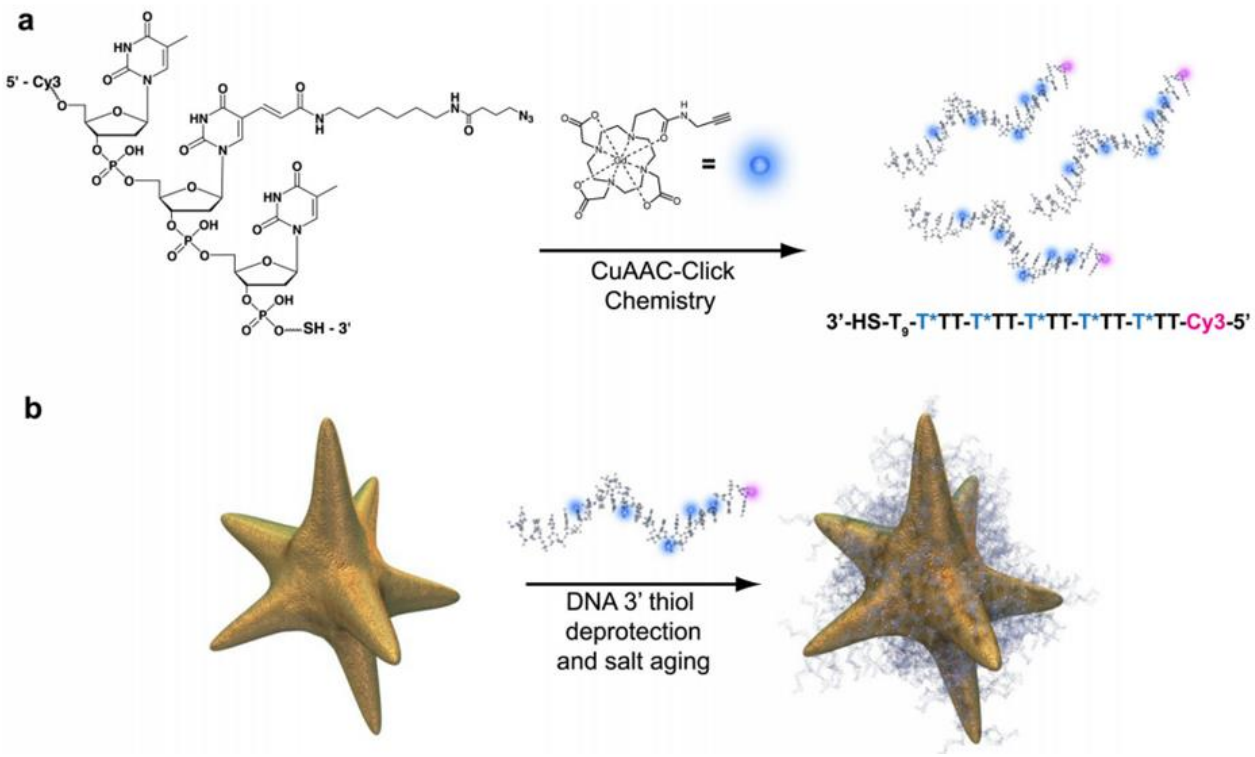
Click cycloaddition reaction between Gd**8** and a selected AZT was performed using the standard protocol described by Sharpless et al.[70] Alkyne and azide precursors were stirred at room temperature overnight in *t*-BuOH and water mixture (1:1) in presence of *in situ* generated Cu(I) catalyst, see Scheme 24. $CuSO_4$ (0.1 eq) was used as copper source and sodium ascorbate (1 eq, 10-fold excess to Cu) as a reducing agent to produce the desired compound as pale oily product in an 89% yield.



*Scheme 24 - Synthesised assembly by cycloaddition between Gd**8** and AZT.*

Rotz *et al.*[68] have recently developed a gold–based dual modality agent with high relaxivity. They investigated the effects on the proton relaxation (relaxivity) by generating a nanoconjugate contrast agent via a covalent attachment between a Gd(III) complex with an alkynyl acetamide arm to react with an azide group on thiolated DNA (Gd-DNA), see Scheme

25(a). This was then followed by attachment to the surface of gold nanostars (DNA-Gd@stars), leading to efficient delivery of Gd(III) and biocompatibility in vitro to produce significant contrast enhancement, see Scheme 25(b).[68]



Scheme 25 - Preparation of DNA-Gd@stars. (a) Cy3-labeled 24-mer poly-dT oligonucleotide is modified via the covalent attachment of Gd(III) to each of five azide-bearing dT bases per strand. (b) Functionalized oligonucleotides are deprotected, revealing the 3' thiol, and are conjugated to nanostars through a series of increases in salt concentration called salt aging.[68]

5.2.1 T1 relaxation studies for Gd8b

The T_1 relaxation time was measured for the synthesised conjugated Gd**8**b using a 1.5 T MRI scanner. The sample was diluted with distilled water and serious of solutions were prepared with different concentrations (0.25, 0.5, 1, 1.5, and 2 mM)

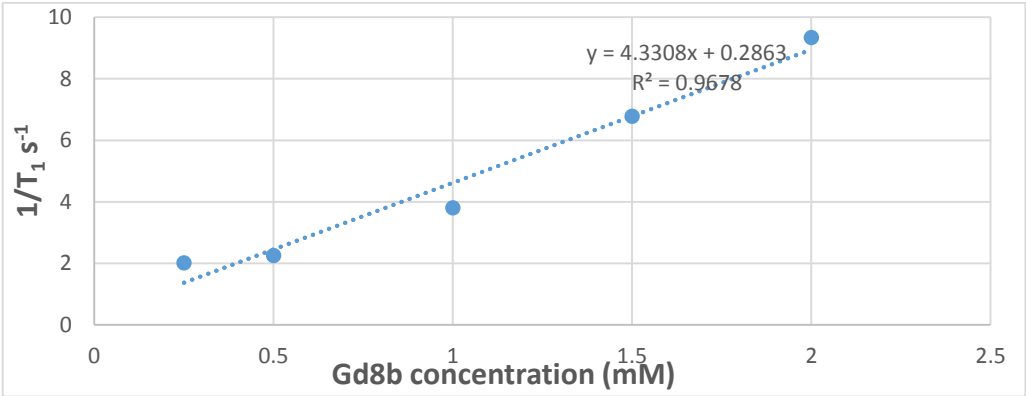


Figure 25 - Graph to show concentration vs. 1/T for Gd8b.

The relaxivity plot , see Figure 23, shows a linear relationship for Gd**8**b to give a relaxivity value of 4.33 mM^{-1} s^{-1} with acorrelation coefficient 0.9678. This shows that the high relaxivity properties of this compound have not been disrupted by the conjugation reaction suggesting that this approach can be used for a wide range of DNA conjugates.

Chapter 6

Conclusions

6.1 Conclusions

Novel chelator **8** was successfully synthesised as an alkyne functionalised DO3A for click chemistry reactions. A further two chelators functionalised with different alkyne derivatives (propargyl **6** and propargyl acetamide **7**) were also resynthesised successfully by using modified literature protocol and complexation of gadolinium(III) (a lanthanide metal) to give complexes Gd**6**, Gd**7** and Gd**8** was achieved, see Figure 25, and the MR relaxivity properties of these complexes investigated.

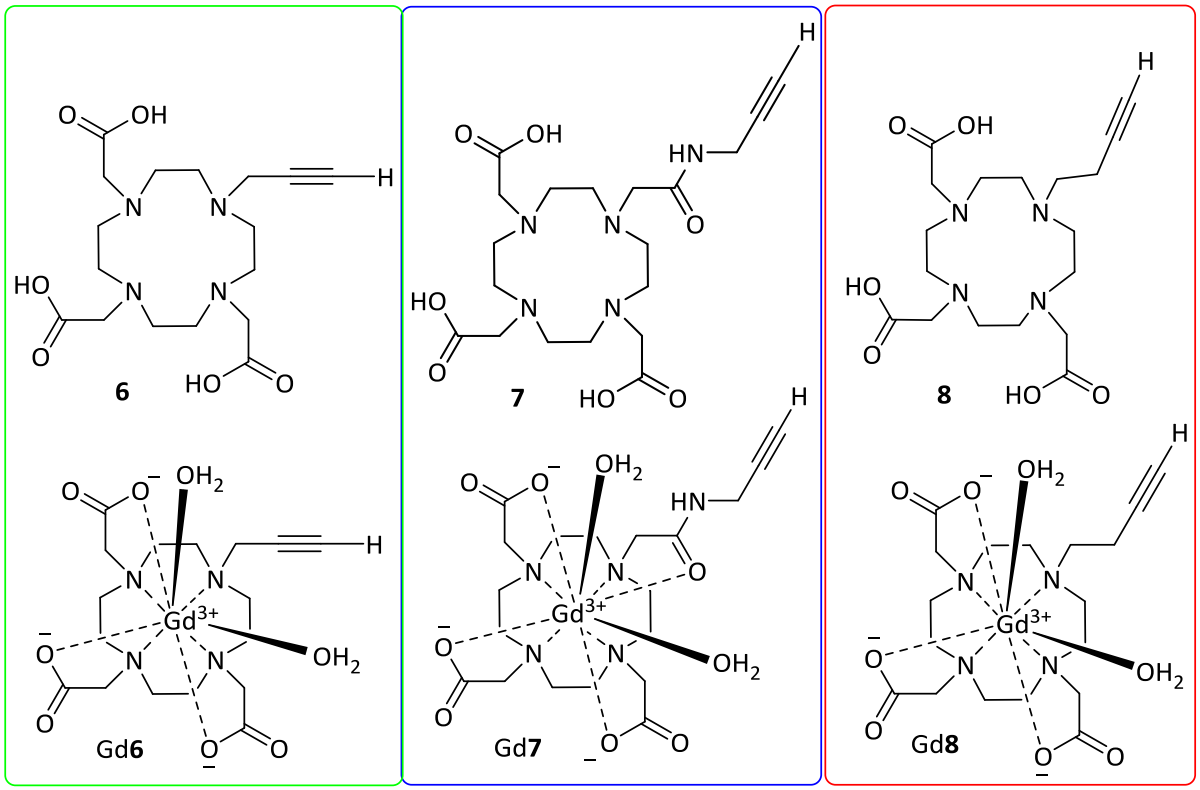


*Figure 26 - Three synthesised ligands (**6**, **7**, **8**) with their Gd(III) complexes (Gd**6**, Gd**7**, Gd**8**).*

The investigation of complex reactivity in Cu(I) catalysed "click" reaction with a simple organic azide (benzyl azide) was also carried out to form the conjugates Gd**6**a, Gd**7**a, Gd**8**a, see Figure 26, which were prepared in 74%, 81% and 85% yields, respectively, and their identity confirmed by MS, ICP and elemental analyses.

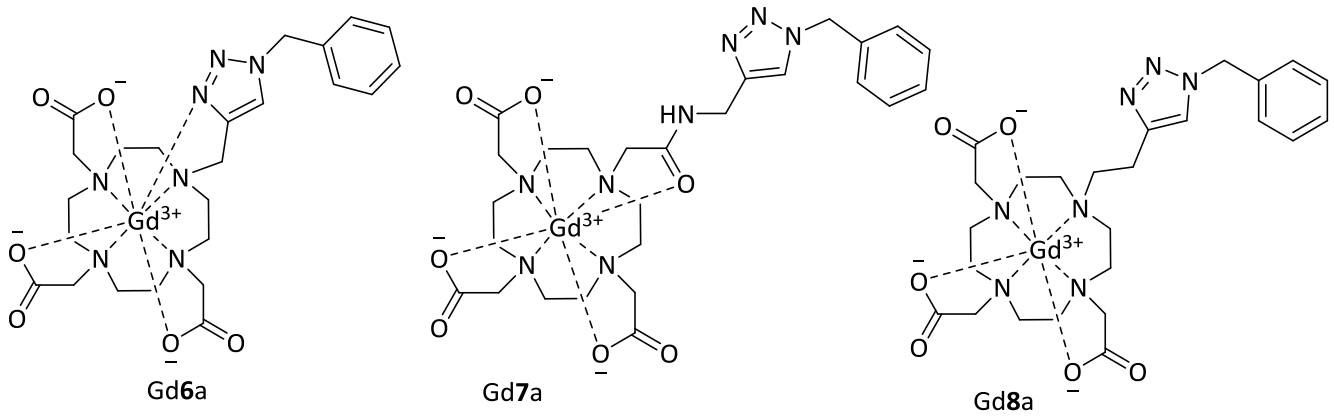


*Figure 27 – Structure of conjugates Gd**6**a, Gd**7**a, Gd**8**a prepared by model benzyl azide – Gd-chelate "click" reaction.*

Relaxivity measurements were made on the synthesised Gd(III) complexess (Gd**6**, Gd**7** and Gd**8**) showing the highest relaxivity for Gd**8** (5.74 mM^{-1} s^{-}) in comparison to the other two chelators (Gd**7** and Gd**8**) with values of 0.512 mM^{-1} s^{-1} and 1.512 mM^{-1} s^{-1} respectively.

The preparation of a novel MRI contrast agent containing Gd(III) (synthesised from Gd**8**) utilising click chemistry to conjugate with 3-azide-3-deoxythymidine (AZT), to produce the product Gd**8**b as a light brown solid in an 89% yield, see Figure 27.

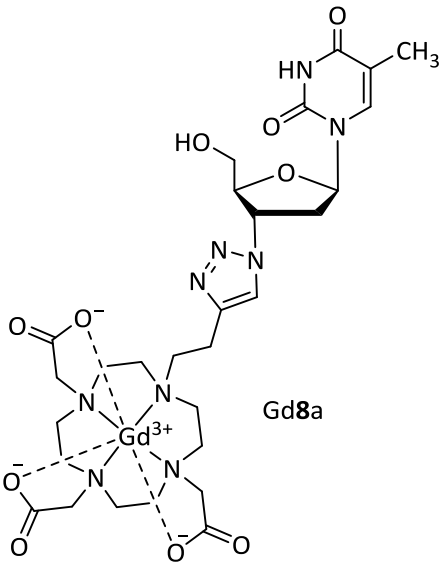


*Figure 28 - Synthesised conjugate Gd**8**b by cycloaddition between Gd**8** and AZT.*

Chapter 7

Experimental

7.1. General methods for synthetic experiments:

^{1}H NMR and ^{13}C NMR were obtained using a Jeol JNM-LA400 spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C in the solvents indicated, referenced against standard internal TMS or residual non deuterated solvent signal. Chemical shifts (δ) are quoted in parts per million (ppm). Splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), m (multiple) and br (broad). Deuterated solvents were purchased from either Goss chemicals Ltd or Cambridge Isotopes Ltd.

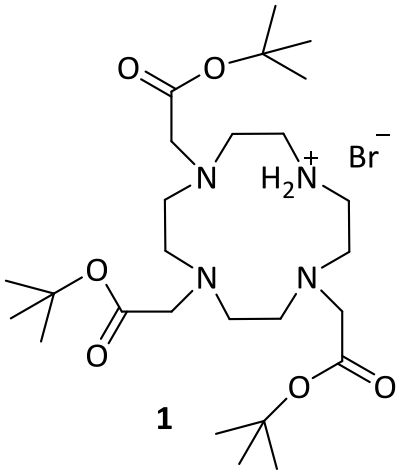
Electrospray ionisation (ESI) mass spectra were recorded on low resolution Varian 500-MS LC-ion trap system. The compound to be analysed was diluted in methanol and approximately 0.1 µL was added and run in 80% MeOH:20% H2O. Accurate mass spectrometry measurements (HRMS) were recorded at the EPSRC National Mass Spectrometry Service Centre at the University of Swansea using a LQT Orbitrap XL. Elemental analysis were performed by combustion using a CHN analyser EA1108 (Carlo Erba).
Inductively Coupled Optical Emission Spectroscopy (ICP-OES) analysis was carried out using a Perkin Elmer Optima 5300 DV. All the samples were in solid state, digested with aqua regia in glass sample vials on a hotplate. Calibration standards were prepared at 1 and 10 ppm from 1000 ppm concentrates of gadolinium which was purchased from Romil UK.

7.2. Materials

All solvents and reagents were purchased, primarily, from Fisher Scientific and Sigma Aldrich among other commercial suppliers and were used without further purification unless otherwise stated. When dry solvents were required, the solvent was either dried using 3 Å molecular sieves in the lab or taken from a sure-seal bottle to ensure the absence of water (acetonitrile (MeCN) and dichloromethane (DCM) were dried following Williams et.al method[69]). TLC was used to monitor the reaction progress with F254 silica plates and visualisation by UV light and stains. Silica gel (60A, 35-70 micron) was used to perform column chromatography.

7.3. Synthesis details

6.3.1 Synthesis of 1, 4, 7-tris (tert-butoxycarbonylmethyl)-1, 4, 7, 10-tetraazacylododecane hydrobromide.[47]

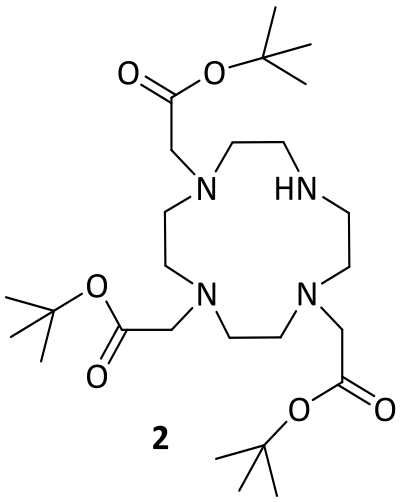


1

Following the literature method,[47] to a suspension of cyclen (5.00 g, 29 mmol) and sodium acetate (7.86 g, 96 mmol) in *N*,*N*-dimethylacetamide (DMA, 60 mL) at -20 °C was added a solution of *tert*-butyl bromoacetate (18.70 g, 14.1 mL, 96 mmol) in DMA (20 mL) dropwise over a period of half an hour. The temperature was maintained at -20 °C during the addition, after which the reaction mixture was allowed to come to room temperature. After 24 h of vigorous stirring, the reaction mixture was poured into water (300 ml) to give a clear solution. Solid $KHCO_3$ (15.00 g, 150 mmol) was added portion wise, and intermediate compound precipitated as a white solid. The precipitate was collected by filtration and dissolved in $CHCl_3$ (250 ml). The solution was washed with water (100 ml), dried ($MgSO_4$), filtered, and concentrated to about 20-30 ml. Ether (250 ml) was added, **1** was crystallised as a white fluffy solid. (13.6 g, 73%).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.47 (s, 9H, C($\underline{C}H_3$)$_3$), 1.48 (s, 18H, C($\underline{C}H_3$)$_2$), 2.61-2.94 (m, 12H, N-CH_2), 3.29 (m, 4H, N-CH_2), 3.42 (s, 2H, N-CH_2-C=O), 3.65 (s, 4H, N-CH_2-C=O), 10.31 (s, 1H, NH), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 28.43 C($\underline{C}H_3$)$_3$, 28.76(C($\underline{C}H_3$)$_3$), 47.23(C($\underline{C}H_3$)$_3$), 49.23 (N-CH_2), 51.33 (N-CH_2), 51.21 (N-CH_2), 58.24 (N-CH_2), 76.84 ($\underline{C}$(CH_3)), 77.1 ($\underline{C}$(CH_3)), 169.61 (C=O), 170.46 (C=O). Elemental analysis for $C_{26}H_{51}BrN_4O_6$. calcd. C, 52.43; H, 8.63; N, 9.41, found: C, 52.30; H, 8.75; N, 9.18. ESMS$^+$ m/z [M-Br]$^+$594.

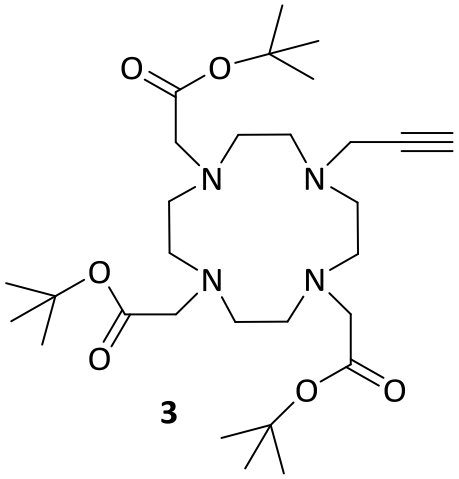
7.3.2 Synthesis of 1, 4, 7-tris (tert-butoxycarbonylmethyl)-1, 4, 7, 10-tetraazacyclododecane (tBu-DO3A).



2

Following the literature method,[47] hydrobromide salt (**1**) (5.00 g, 8.40 mmol) was dissolved in water (250 ml) at 70 °C. The solution was allowed to cool to 40 °C, after which 10% aqueous KOH solution (9.4 ml, 16.8 mmol) was added the reaction mixture was stirred for 15 min then filtered to yield a white solid (**2**) in a 94% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.46 (m, 27H, $C(CH_3)_3$), 2.33-2.71 (m, 4H, N-CH_2), 2.82-2.91 (m, 12H, N-CH_2), 3.21 (s, 6H, N-CH_2-CO). ^{13}C NMR (400, MHz, $CDCl_3$) δ: 28.11 (C($\underline{C}H_3)_3$), 28.29 (C($\underline{C}H_3)_3$), 47.51 (C($\underline{C}H_3)_3$), 51.11 (N-CH_2), 52.41 (N-CH_2), 52.33 (N-CH_2), 53.98 (N-CH_2), 58.01 (N-CH_2), 79.94 (N-$\underline{C}H_2$-CO), 80.76 (N-$\underline{C}H_2$-CO), 171.44 (C=O), 171.87 (C=O). Elemental analysis for $C_{26}H_{50}N_4O_6$ calcd. C, 57.26, H, 9.87, N, 10.27; found: C, 57.61; H, 9.57, N, 10.30. ESMS$^+$ m/z $[M+H]^+$ 515.

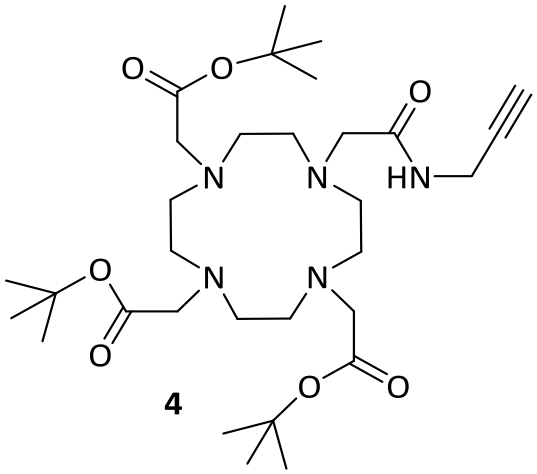
7.3.3 Synthesis of 1, 4, 7, 10-tetraazacyclododecane (tri-tert-butyl 2,2',2''-(10-(prop-2-yn-1-yl)-1,4,7-triyl) triacetate (propargyl functionalised DO3A).[50]



3

A modified method based on the reported procedure was used to synthesis **3**, 4,7-tris-(tert-butylacetate)-1,4,7,10-tetraazacyclododecane (**2**) (100 mg, 0.194 mmol) was dissolved in dry acetonitrile and the base caesium carbonate (157 mg, 0.48 mmol) was added. The mixture was stirred for 20 minutes at room temperature. After that, propargyl bromide (27 mg, 0.23 mmol) was added to a solution then the reaction mixture was stirred at room temperature. The solids from the reaction mixture were filtered off and the solvent was removed. The product was purified using silica gel chromatography with DCM/MeOH (9:10) as eluent, to give (**3**) as a brownish oil (48 mg) in a 44 % yield. ^{1}H NMR ($CDCl_3$): δ 1.46 (s, 27H, CC$\underline{H}_3$), 2.15 (s, 1H, CC$\underline{H}$), 2.69 (s, 4H, ring C$\underline{H}_2$), 2.83 (s, 12H, ring C$\underline{H}_2$), 3.19 (s, 6H, C$\underline{H}_2$-CO), 3.44 (s, 2H, C$\underline{H}_2$-CCH); ^{13}C NMR ($CDCl_3$) δ 28.23, 43.06, 51.57, 51.76, 52.08, 56.78, 72.53, 80.73, 171.62. ESMS^{+} m/z $[M+H]^{+}$ 553.5.

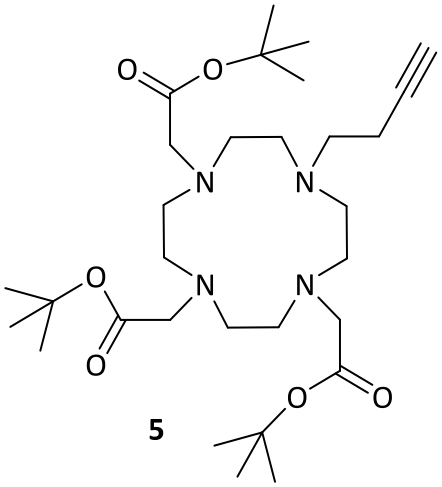
7.3.4 4,10-Bis-tert-butoxycarbonylmethyl-7-[(2-propynylcarbamoyl)-methyl]-1,4,7,10-tetraaza-cyclododec-1yl}-acetic acid tert-butyl ester (4).[45]



4

A modified method based on the reported procedure was used to synthesis **4**, A solution of 1, 4, 7, 10-tetraazacyclododecane (tri-tert-butyl 2,2',2''-(10-(prop-2-yn-1-yl)-1,4,7-triyl)triacetate (**2**) (100 mg, 0.19 mmol), *N*-(2-propynyl)chloroacetamide (**9**)(30 mg, 0.23 mmol) and Cs_2CO_3 (157 mg, 0.48 mmol) in dry MeCN (10 mL) was stirred under nitrogen at room temperature for 24 h. The solids from the reaction mixture were filtered off and the solvent was removed. Product was purified on silica gel chromatography using DCM/MeOH (9:10) as eluent, to give **4** as light brown solid (51 mg) in a 44% yield. ^{1}H-NMR ($CDCl_3$, 400 MHz). δ 1.46-1.48 (s, 27H, 9 x C$\underline{H}_3$), 2.50 (b, 4H, ring C$\underline{H}_2$), 2.69 (m, 4H, ring C$\underline{H}_2$), 2.83 (s, 16H, ring C$\underline{H}_2$), 3.11 (t, 1.6 Hz, 1H, CC$\underline{H}$), 3.26 (s, 4H, C$\underline{H}_2$CO), 3.37 (s, 2H, C$\underline{H}_2$CO), 3.61 (s, 8H, CH_2), 4.02 (d, 2.7 Hz, 2H, C$\underline{H}_2$CCH), 9.27 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$, 400 MHz). 27.33, 27.52, 51.22, 53.42, 55.14, 67.11, 80.22, 80.93, 172.45, 172.80. ESMS$^+$ m/z $[M+H]^+$611.0.

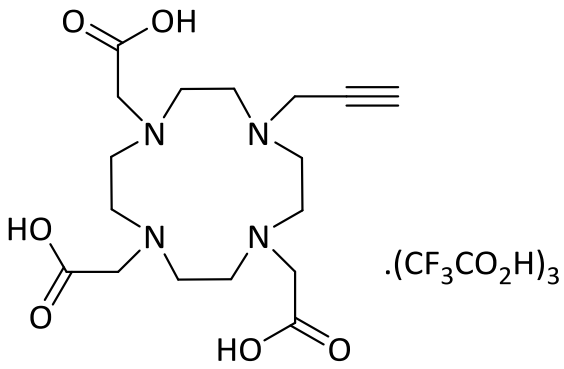
7.3.5 Synthesis of 1, 4, 7, 10-tetraazacyclododecane- tri-tert-butyl 2, 2', 2''-(10-(but-3-yn-1-yl)-1, 4, 7-triyl)triacetate.[45]



5

A modified method based on the reported procedure was used to synthesis **5**, A solution of 4,7-tris-(tert-butylacetate)-1,4,7,10-tetraazacyclododecane (**2**) (100 mg, 0.194 mmol), 1-bromo2-butyne (31 mg, 0.23 mmol) and Cs_2CO_3 (157 mg, 0.48 mmol) in dry MeCN (10 mL) was stirred under nitrogen at room temperature for 24 h. The solids from the reaction mixture were filtered off and the solvent was removed. Product was purified on silica gel chromatography using DCM: acetone: MeOH: (6:3.5:0.5) as eluent, to give (**5**) as light brownish oil (38 mg) in a 34% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43 (s, 9H, 3 x C$\underline{H}_3$), 1.48 (s, 18H, 6 x C$\underline{H}_3$), 2.25 (dt, 2H, C$\underline{H}_2$CCH), 2.62 (s, 4H, ring C$\underline{H}_2$), 2.78 (s, 12H, ring C$\underline{H}_2$), 2.81 – 2.99 (m, 8H, ring C$\underline{H}_2$), 3.11–3.25 (m, 2H, C$\underline{H}_2$CH$_2$CCH), 3.52 (m, 1H, CC$\underline{H}$), 3.91 (s, 2H, C$\underline{H}_2$CO), 4.26 (s, 4H, C$\underline{H}_2$CO). ^{13}C NMR ($CDCl_3$) δ 28.21, 29.13, 29.25, 53.25, 54.01, 55.22, 56.71, 58.18, 68.73, 70.26, 171.54, 171.91; HRMS$^+$ for $C_{30}H_{54}N_4O_6$ 567.4116 m/z $[M+H]^+$ found 567.4102 m/z $[M+H]^+$.

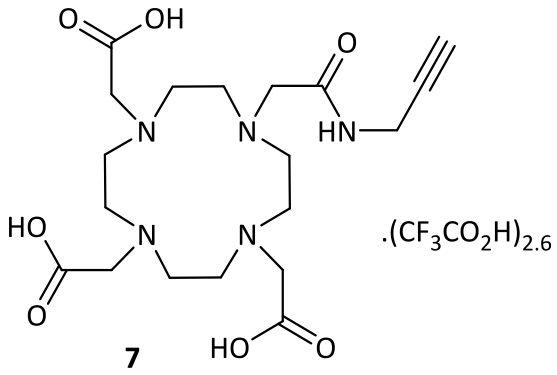
7.3.6 Synthesis of 1, 4, 7, 10-tetraazacyclododecane-2, 2', 2''-(10-(prop-2-yn-1-yl)-1,4,7-triyl) triacetic acid.[50]



6

A modified method based on the reported procedure was used to synthesis **6**, 1, 4, 7, 10-tetraazacyclododecane (tri-tert-butyl 2, 2', 2''-(10-(prop-2-yn-1-yl)-1, 4, 7-triyl)triacetate (**3**) (100 mg, 0.18 mmol) was dissolved in a mixture of DCM (6 mL) and TFA (6 mL). The solution was stirred overnight at room temperature. The volatiles were removed by rotary evaporation, and the residue dissolved in the minimum amount of methanol. Addition of diethyl ether yielded the desired compound as a white fluffy solid (42 mg) in a 62% yield. The volatiles are removed with a dry ice rotary evaporator and the product was dissolved in a minimum amount of methanol and add dropwise to ether, which triggered precipitation. The white fluffy solid was collect via centrifuge and dissolved in H_2O and freeze-dried. ^{1}H NMR (400 MHz, D_2O): δ 2.82 (m, 1H, CC<u>H</u>), 3.02 (m, 8H, ring C<u>H</u>$_2$), 3.21 (m, 8H, ring C<u>H</u>$_2$), 3.52 (s, 2H, C<u>H</u>$_2$-CCH), 3.73 (m, 6H, C<u>H</u>$_2$-CO). Elemental analysis calculated for $C_{17}H_{28}N_4O_6.(CF_3CO_2H)_3(H_2O)_2$:C 36.23, H 4.63, N 7.35; found C 36.52, H 4.91, N 7.67. ESMS$^+$ m/z $[M+H]^+$ 385.1.

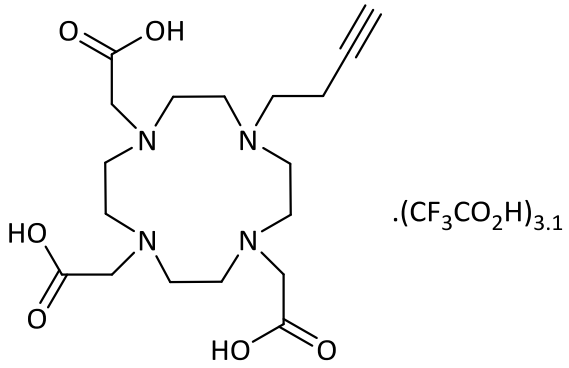
7.3.7 4, 10-Bis-carboxymethyl-7-[(2-propynylcarbamoyl)-methyl]-1,4,7,10-tetraaza-cyclododec-acetic acid (7).[45]



7

A modified method based on the reported procedure was used to synthesis **7**,[45] *t*-butyl propargylacetamide compound **4** (100 mg, 0.16 mmol) was dissolved in a mixture of DCM (6 mL) and TFA (6 mL). The solution was stirred overnight at room temperature. The volatiles were removed by rotary evaporation, and the residue dissolved in the minimum amount of methanol. Addition of diethyl ether yielded the desired compound as a white solid (50 mg) in a 71% yield. The volatiles are removed with a dry ice rotary evaporator and the product was dissolved in the minimum amount of methanol and added dropwise to ether, which caused precipitation. The white fluffy solid was collect via centrifuge, dissolved in H_2O, and freeze-dried. ^{1}H NMR (400 MHz, D_2O), 2.61 (t, J = 2.5 Hz, 1H, C≡C<u>H</u>); 3.12 (m, 3H, CH_2 ring), 3.18 (b, 3H, ring C<u>H</u>$_2$), 3.35-3.39 (m, 6H, ring C<u>H</u>$_2$), 3.43 (s, 2H, ring C<u>H</u>$_2$), 3.55 (s, 2H, ring C<u>H</u>$_2$), 3.74 (s, 4H, CH_2, C<u>H</u>$_2$CO), 3.81 (s, 2H, C<u>H</u>$_2$CO), 3.92 (d, 2H, J = 2.5 Hz, C<u>H</u>$_2$C≡CH). ^{13}C NMR (400 MHz, D_2O), 28.38, 49.11, 51.25, 54.44, 54.12, 56.12, 56.10, 73.42, 82.27. Elemental analysis calculated for $C_{19}H_{31}N_5O_7.(CF_3CO_2H)_{2.6}(H_2O)_{0.5}$:C 39.39, H 4.59, N 9.49. Found: C 39.55, H 4.77, N 9.83. ESMS$^+$ m/z $[M+Na]^+$464.1.

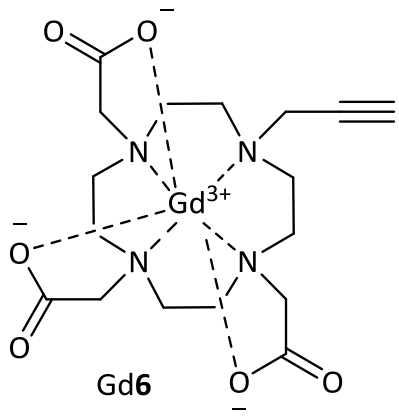
7.3.8 Synthesis of 1,4,7,10-tetraazacyclododecane-2,2',2''-(10-(but-3-yn-1-yl)-1,4,7-triyl)triacetic acid.[50]



8

A modified method based on the reported procedure was used to synthesis **7**, 1, 4, 7, 10-tetraazacyclododecane-tri-tert-butyl 2, 2', 2''-(10-(but-3-yn-1-yl)-1,4,7-triyl) compound **4** (100 mg, 0.17 mmol) triacetate was dissolved in a mixture of DCM (6 mL) and TFA (6 mL). The solution was stirred overnight at room temperature. The volatiles were removed by rotary evaporation, and the residue dissolved in the minimum amount of methanol. Addition of diethyl ether yielded the desired compound as a light yellow solid (47 mg) in a 67 % yield. The volatiles are removed with a dry ice rotary evaporator and the product was dissolved in a minimum amount of methanol and add dropwise to ether, which caused precipitation. The white fluffy solid was collect via centrifuge and dissolved in H_2O and freeze-dried.^{1}H NMR (400 MHz, D_2O), 2.32 (s, 1H, C$\underline{H}_2$CCH); 2.62-4.41(m, 24H, C$\underline{H}_2$), 4.61 (t, 1H, C≡C$\underline{H}$).^{13}C NMR (400 MHz, D_2O), 31.28, 35.26, 42.37, 45.11, 45.89, 47.88, 50.60, 51.39, 52.56, 72.79, 74.18, 173.90, 174,84). Elemental analysis calculated for $C_{18}H_{30}N_4O_5.(CF_3CO_2H)_{3.1}(H_2O)$: C 37.75, H 4.60, N 7.28. Found: C 38.21, H 4.87, N 7.55. ESMS$^+$ m/z $[M+H]^+$399.4.

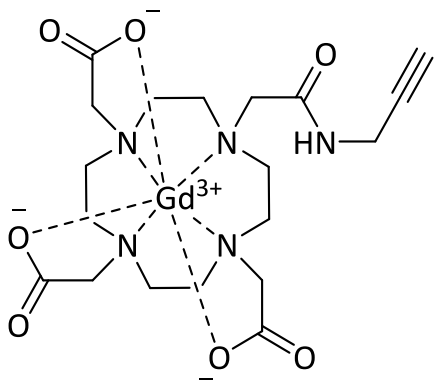
7.3.9 Gadolinium (III) 1,4,7-tris(carbonylmethyl)-10-(prop-2-ynl,)-1,4,7,10 tetraazacyclododecane.(Gd6).[45]



Gd**6**

A modified method based on the reported procedure was used to synthesis Gd**6**, (4,7-biscarboxymethyl-10-prop-2ynyl-1,4,7,10-tetraazacyclododec-1-yl)-acetic acid (**6**) (100 mg, 0.13 mmol) was dissolved in a mixture of 10 mL of methanol and triethylamine (0.15 mL). $GdCl_3.6H_2O$ (53 mg, 0.14 mmol) was added to the solution. The solution was stirred for two days at 50 ^oC. The residue was redissolved in methanol and triturated with diethyl ether to give the desired product as white solid (31 mg) in a 44% yield. Elemental analysis calculated for $C_{17}H_{25}GdN_4O_6.(CH_3OH)_2$.: C 37.86, H 5.52, N 9.30. Found C 38.21, H 5.87, N 9.63. $ESMS^+$ m/z $[M+H]^+$ 540.1.

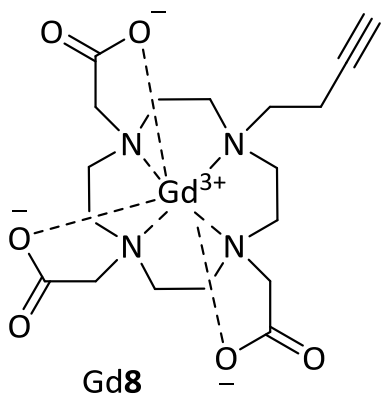
7.3.10 Gadolinium(III) 4,10-Bis-carboxymethyl-7-[(2-propynylcarbamoyl)-methyl]-1,4,7,10-tetraaza-cyclododec-1-yl-acetate. (Gd7).[45]



Gd**7**

A modified method based on the reported procedure was used to synthesis Gd**7**, 1,4,7,10-tetraazacyclododecane-2,2',2''-(10-(2-oxo-2-(prop-2-yn-1-ylamino)ethyl)-1,4,7-triyl)triacetic acid (**7**) (100 mg, 0.13 mmol) was dissolved in a mixture of 10 mL of methanol and triethylamine (0.10 mL). $GdCl_3.6H_2O$ (55 mg, 0.15 mmol) was added to the solution. The solution was stirred for two days at 50 ^oC. The residue was re-dissolved in methanol and triturated with diethyl ether to give pale yellow solid in a 82% yield. Elemental analysis calculated for $C_{19}H_{28}N_5O_7Gd.(CH_3OH)_{1.3}$: C 38.25, H 5.25, N 10.99. Found: C 38.57, H 4.10, N 11.11. $ESMS^+$ m/z $[M+H]^+$ 597.1.

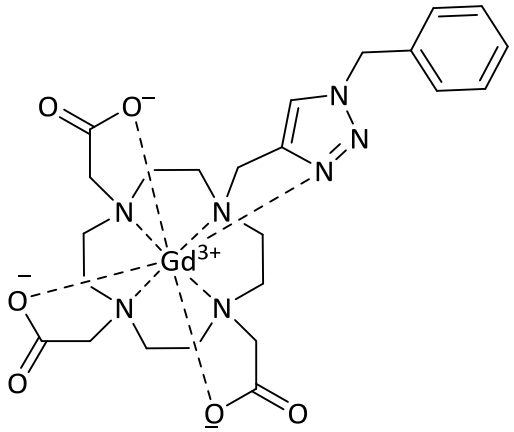
7.3.11 Gadolinium(III) complex of 1, 4, 7, 10-tetraazacyclododecane-2, 2', 2''-(10-(but-3-yn-1-yl)-1, 4, 7-triyl)triacetic acid. (Gd8).[45]



Gd**8**

A modified method based on the reported procedure was used to synthesis Gd**8**, 1,4,7,10-tetraazacyclododecane-2,2',2''-(10-(but-3-yn-1-yl)-1,4,7-triyl)triacetic acid **8** (100 mg, 0.13 mmol) was dissolved in a mixture of 10 mL of methanol and triethylamine (0.15 mL), $GdCl_3.6H_2O$ (55 mg, 0.15 mmol) was added to the solution. The solution was stirred for two days at 50^oC, the residue was redissolved in methanol and triturated with diethyl ether to give white powder (35 mg) in a 48% yield. Elemental analysis calculated for $C_{18}H_{27}GdN_4O_6.(CH_3OH)(H_2O)_{1.5}$: C 39.09, H 5.34, N 9.58. Found: C 39.45, H 5.64, N 9.78. ESMS$^+$ $[M+H]^+$ 540.1.

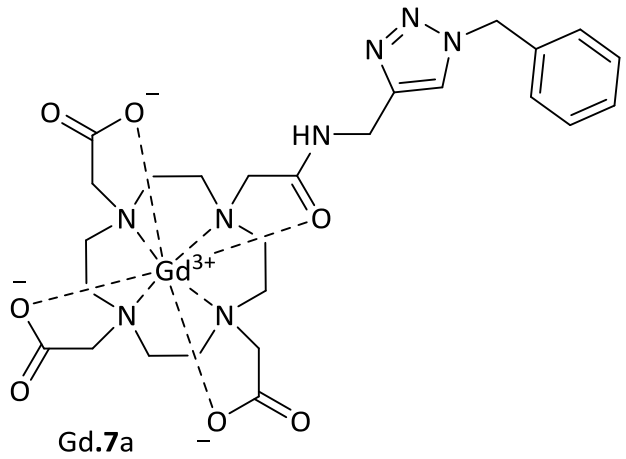
7.3.12 Gadolinium(III) 1,4,7-tris(carbonylmethyl)-10-(1-benzyl-1,2,3-triazol-4-ylmethyl)-1,4,7,10-tetraazacyclododecane (Gd6a).[70]



Gd.6a

A modified method based on the reported procedure was used to synthesis Gd**.6a**, gadolinium(III) 1,4,7-tris(carbonylmethyl)-10-(prop-2-ynl,)-1,4,7,10tetraazacyclododecane Gd**6** (0.100 g, 0.18 mmol) and benzyl azide (15 mg, 0.22mmol) were dissolved in water: *tert*-butanol (1:1). $CuSO_4.5H_2O$ (2.5 mg, 0.009 mmol) was added to reaction mixture. sodium ascorbate (3.6 mg, 0.018 mmol) were dissolved in a separate flask and was introduced to the reaction mixture by syringe in dropwise manner, the solvent was removed under reduced pressure when reaction was complete, forming a white powder (79 mg) in a 74% yield. $ESMS^-$ $[M-H]^-$ 673.1, ICP: Gd:23.41 found 19.663.

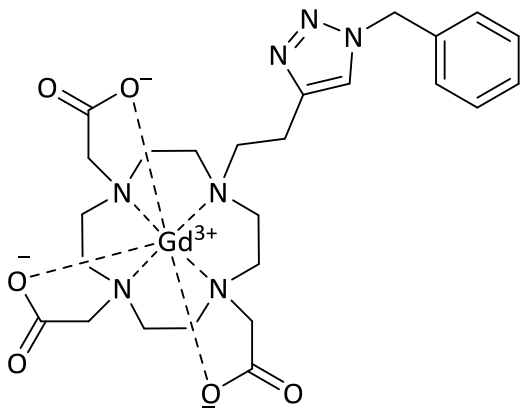
7.3.13 Gadolinium(III) 1,4,7,10-tetraazacyclododecane-2,2',2''-(10-(3-benzyl-4H-1,2,3-triazol-5-yl)methyl)propionamide)-1,4,7-triyl)triacetate complex.(Gd.7a).[70]



Gd.**7**a

A modified method based on the reported procedure was used to synthesis Gd**.7a**, gadolinium(III) 4,10-Bis-carboxymethyl-7-[(2-propynylcarbamoyl)-methyl]-1,4,7,10-tetraaza-cyclododec-1-yl-acetate (Gd**7**) (100 mg, 0.16 mmol) and benzyl azide (27 mg, 0.20 mmol) were dissolved in water: *tert*-butanol (1:1), then $CuSO_4.5H_2O$ (2 mg, 0.008 mmol) was added to reaction mixture. sodium ascorbate (31 mg, 0.016 mmol) were dissolved in a separate flask and was introduced to the reaction mixture by syringe in dropwise manner, the solvent was removed under reduced pressure when the reaction reached completion, forming a white solid (78 mg) in a 67 % yield. $ESMS^+$ $[M+H]^+$ 730.1, ICP: Gd:21.57 found 17.541.

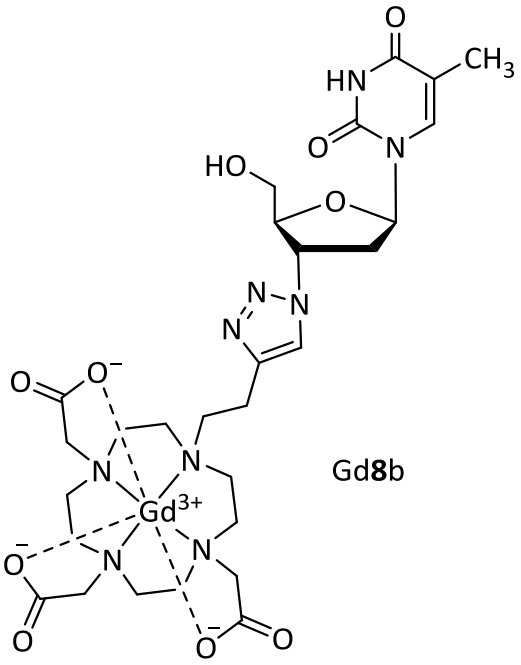
7.3.14 Gadolinium(III) 1,4,7,10-tetraazacyclododecane-(3-benzyl-5-propyl-1,2,3 triazole1)-1,4,7-triyl) triacetic acid).(Gd.8a).[70]



Gd.**8**a

A modified method based on the reported procedure was used to synthesis Gd.**8a**, gadolinium(III) 1,4,7,10-tetraazacyclododecane-(3-benzyl-5-propyl-1,2,3 triazole1)-1,4,7-triyl) triacetic acid)(Gd.**8**a) (100 mg, 0.18 mmol) and benzyl azide (30 mg, 0.22 mmol) were dissolved in water: *tert*-butanol (1:1), then $CuSO_4.5H_2O$ (2.3 mg, 0.009 mmol) was added to reaction mixture. Sodium ascorbate (3.5 mg, 0.018 mmol) were dissolved in a separate flask and was introduced to the reaction mixture by syringe in dropwise manner, the solvent was removed under reduced pressure after the reaction was complete, forming a white powder (72 mg) in a 59% yield. $ESMS^+$ $[M+H]^+$687.1, ICP: Gd:22.93 found 18.623.

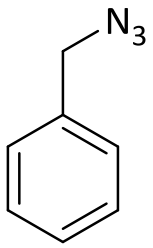
7.3.15 Gadolinium(III)1,4,7-tris(carbonylmethyl)-10-(4-(2,3-dideoxyuridine-1,2,3-triazol-4-yl)-1-methyl-benzene)-1,4,7,10-tetraazacyclododecane.[70]



Gd**8**b

A modified method based on the reported procedure was used to synthesis Gd**.8b**, gadolinium(III) complex of 1, 4, 7, 10-tetraazacyclododecane-2, 2', 2''-(10-(but-3-yn-1-yl)-1, 4, 7-triyl)triacetic acid. (**Gd8**) (30 mg, 0.055 mmol) and AZT (18 mg, 0.066 mmol) were dissolved in water: *tert*-butanol (1:1), then $CuSO_4.5H_2O$ (0.7 mg, 0.0028 mmol) was added to reaction mixture. Sodium ascorbate (1mg, 0.0055 mmol) were dissolved in a separate flask and was introduced to the reaction mixture by syringe in dropwise manner. When reaction reach competition, the solvent was removed under reduced pressure to give a pale yellow product (33 mg) in a 73 % yield. $ESMS^+$ $[M+H]^+$821.1, ICP: Gd:19.18 found 17.402.

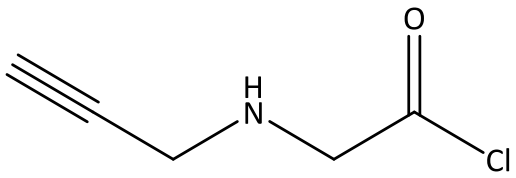
7.3.16 Synthesis of benzyl azide.[70]



9

A modified method based on the reported procedure was used to synthesis **9**, to a stirred solution of benzyl bromide (2.57 g, 15 mmol) in a mixture of acetone/water (4:1, 50 mL), sodium azide (1.47 g, 23 mmol) was added. The resulting suspension was stirred for 24 h at room temperature. When the reaction was complete, DCM was added (3x30) and the product was extracted into the organic phase. Th oirganic layer was separated, dried over magnesium sulphate and isolated by evaporation under reduced pressure, producing a yellow oil in a 77% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25 –7.69 (m, 2H), 4.31 (s, 1H). $ESMS^+$ $[M+H]^+$ 134.1.

7.3.17 Synthesis of N-(2-propynyl) chloroacetamide and N-(2-propynyl) bromoacetamide (10).[45]



10

A modified method based on the reported procedure was used to synthesis **10**, to chloroacetyl chloride (4.257 g, 38 mmol) was added drop wise to a stirred solution of propargylamine (1.38 g, 23 mmol) and $NaHCO_3$ (6.27 g, 75 mmol) in 50 mL of dry DCM. The solution was stirred for 3 h and then filtered and washed with 5% $NaHCO_3$. The collected organic layer was dried over magnesium sulphate and solvent removed under reduced pressure to give a brown solid crude material. The product was purified using silica gel chromatography with 25% ethyl acetate/hexane as eluent, to give (**9**) as a white solid in a yield of 81%). ^1H NMR ($CDCl_3$, 400 MHz): δ 2.11 (s, 1H), 3.93 (s, 2H), 4.02 (d, 2H), 6.31 (m, 1H). $ESMS^+$ m/z $[M+H]^+$ 132.1.

8. References

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