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Review

Biomedical applications of macrocyclic ligand complexes

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Contents

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ABSTRACT

Macrocyclic chelators can form highly stable complexes with transition metals and lanthanides. In this review, the recent advances towards biomedical applications of macrocyclic complexes are outlined. The use of such complexes in imaging as MRI contrast agents, radiopharmaceuticals and luminescent probes is discussed. The considerable scope for future development of novel metal based therapeutics based on protein binding, targeting of radioisotopes or dual function agents is also highlighted.

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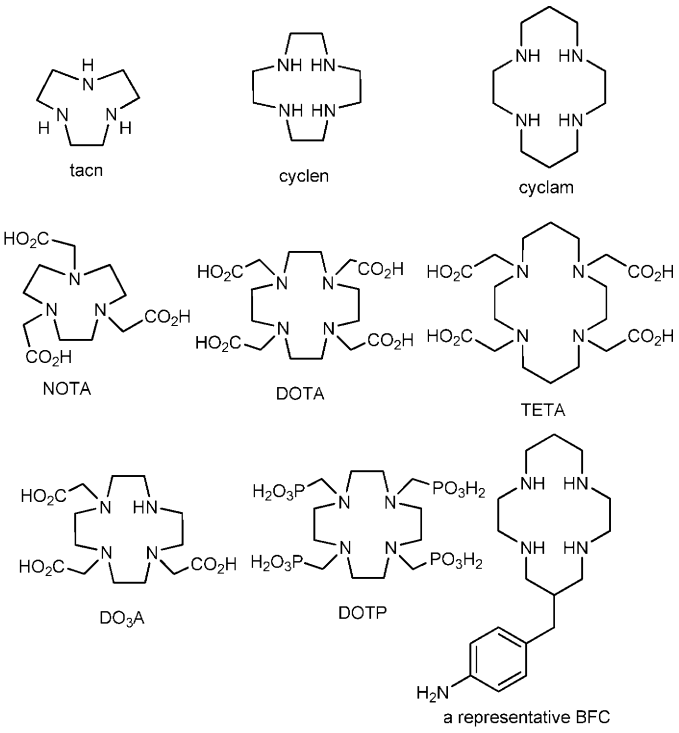
1. Introduction

Research in medicinal inorganic chemistry has expanded in recent years by exploiting a variety of chelating ligands to modify and control the properties of metal ions in biological systems [1–3]. Macrocyclic chelators offer the benefit of high stability complex formation and, through functionalization, the opportunity to fine tune the coordination environment. The pharmaceutical industry has yet to appreciate the impact coordination chemistry can have on the design of new medicines [4]. This may change in the future as skilled multi-disciplinary practitioners develop their research using a strategic approach to complex design.

This article offers an overview of the research reported in the last 5 years (2005–2009) into biomedical applications of macrocycles, although some earlier articles have been cited to provide relevant background information. Porphyrin compounds have not been included in this review, although they have a number of important developing biomedical applications e.g. as phototherapeutic agents, X-ray radiation enhancers and boron neutron capture agents [5–8]. The focus of this survey is on saturated macrocyclic ligand design and tuning the properties by chelator modification. The opportunity to add pendant arms to unsaturated nitrogen donor atoms (e.g. in aza macrocycles) has driven this expanding area of research.



Metal ions have key properties that can be applied to image or interact with biological systems. Five main topics were selected for this review to reflect the volume of research published. Biological imaging applications dominate, with magnetic resonance imaging (MRI), single photon emission computed tomography (SPECT), positron emission tomography (PET) and optical imaging the main techniques featured [9,10]. On the therapeutic side, with the exception of the use of α- and β-emitting radioisotopes, the applications of macrocyclic metal complexes are more limited. The range of chelators used in therapeutic and diagnostic radiopharmaceuticals are similar and so are grouped together for discussion in Section 4.

Research into biomedical applications of macrocycles developed during the 1980s and 1990s and has been particularly vibrant in the 21st century. It is built on the foundations of macrocyclic research carried out in the 1960s and 1970s by Busch, Curtis, Pedersen and many others [11,12]. Review articles on metals in medicine were collected together in a special issue of *Chemical Reviews* in 1999, which provides a comprehensive introduction to the field, including data on both cyclic and acyclic chelators [13–15]. More recently (2009) a special issue of *Accounts of Chemical Research* was published containing useful reviews on the chemistry of molecular imaging [16–19]. Refinement in the approach to chelator design has come with a more subtle understanding of binding kinetics, catalytic mechanisms and donor interactions [20–23].

The majority of the work discussed in this review article focuses on the azacrown macrocycles. The most commonly used of these are tacn (1,4,7-triazacyclononane), cyclen (1,4,7,10-tetraazacyclododecane) and cyclam (1,4,8,11-tetraazacyclotetradecane). This is due to their cavity size, the wide range of developed synthetic procedures for macrocycle synthesis and a variety of methods for functionalisation via the incorporation of pendant arms. The inclusion of acetate pendant arms provides strong binding donor groups that are compatible with both transition metal and lanthanide ions. The NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid), DO3A (1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid), DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) and TETA (1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid) chelators form the basis of many of the bifunctional chelators (BFCs) that are designed for specific targeted binding of metal complexes to proteins or other biological macromolecules. It is important to consider that coordination modes of these aza acid chelators can be pH/protonation dependent as they will ultimately be used in aqueous systems [24].

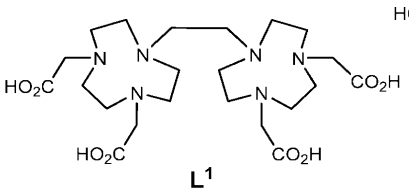
There have been some reviews specifically focussing on the medical applications of aza macrocycles [25,26]. Stability is of course a key issue for any complex in a biological system, as to retain its desired functionality the metal ion must remain bound, and in some cases the complex must be excreted intact. The factors influencing stability of macrocyclic complexes have been studied for many years, and more recent advances include the use of chelators exploiting rigidity to influence the kinetics of decomplexation and enhance stability [27–30].

2. MRI contrast agents

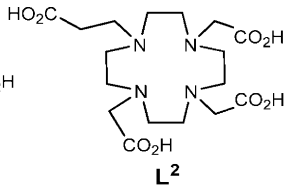
Magnetic resonance imaging contrast agents have been produced as highly stable complexes that can be administered to a patient at relatively high doses (concentration ca. 1 mmol in blood pool) to enhance the contrast between diseased tissue or show metabolic changes [31]. This has been a key clinical success in the application of macrocyclic chemistry to medicine. Research is ongoing to produce responsive molecular imaging agents [32].

Contrast agents in MRI accelerate the relaxation of water protons in proximity to the compound. For gadolinium(III) containing compounds their efficacy can be measured in terms of longitudinal proton relaxivity. This relaxivity parameter quantifies the extent to which the compound catalyses the shortening of the T_1 relaxation time of the protons in bulk water. Coordination interactions are important in transmitting the effect of the paramagnetic ion to the bulk solvent. This inner sphere contribution is generally the major contribution in new higher efficiency contrast agents that are being investigated. The rotational correlation time of the molecule (sometimes referred as the 'tumbling rate') is also a key parameter influencing relaxivity which can be varied by increasing size and reducing flexibility in the molecule [33]. It can also be affected

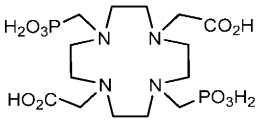
by the presence of other species in the solution such as proteins or salts.



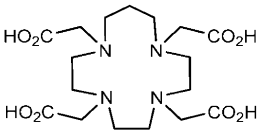
L^1



L^2



L^3



L^4

2.1. Metal ion selection

Gadolinium(III) complexes dominate the research work into macrocyclic contrast agents (CAs) and their clinical application in magnetic resonance imaging [31,32,34]. Gadolinium(III) has two key properties that make it an ideal choice, it is highly paramagnetic due to its seven unpaired electrons and also has relatively slow electronic relaxation due to its symmetric S-state. These paramagnetic CAs shorten the longitudinal relaxation times of bulk water protons. The chelators are generally designed to encapsulate the metal ion leaving only one or two sites available for a water molecule to bind [35,36]. There have been recent concerns regarding the stability of the non-macrocyclic (DTPA based) chelators causing the disease nephrogenic systemic fibrosis (NSF) by release of the gadolinium(III) ion in vivo, and so the high stability of macrocyclic derivatives is particularly desirable [37,38].

There is still interest, but considerably fewer publications, concerning high spin manganese(II) contrast agents. One example is the design of a tacn macrocyclic chelator $\mathbf{L^1}$ that has been synthesised to form dinuclear manganese(II) complexes for use as MRI CAs [58]. This mirrors the development of multinuclear gadolinium(III) complexes to offer greater relaxivity per molecule. However, the stability of the dinuclear complex was lower than that of the comparable NOTA complex.

The thermodynamics and kinetics of dissociation, particularly in the presence of competing cations, and water exchange are very important in the design of such compounds, see Table 1 for selected equilibrium (stability) constant data. A comparison of the kinetics of formation and dissociation of the lanthanide complexes of the DOTA type chelator $\mathbf{L^2}$, where one acetate arm has been replaced by a propionoate, was carried out [50]. These chelators have the advantage of more rapid water exchange improving the relaxivity properties of the gadolinium(III) chelators relative to the clinical DOTA agents. The resulting complex is less stable than $[GdDOTA]^-$ but considerably more inert than $[GdDTPA]^{2-}$.

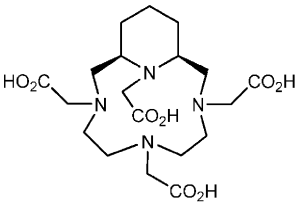
A further alternative for stable coordination to gadolinium(III) is to replace the acetate arms with phosphonate binding groups. A bisphosphonate version of the DOTA chelator was produced, $\mathbf{L^3}$, where two of the acetate arms have been replaced by methyl phosphonate arms [52]. The properties of the lanthanide complexes were investigated using potentiometric titration and NMR, and shown to fall between those observed for DOTA and the tetra(methyl phosphonate) cyclen derivative. Of additional interest was the rigidity, which followed the same trend, demonstrating that it can be tuned by sequential addition of methyl phosphonate arms.

To probe the coordination environment in CAs, the gadolinium(III) ion is frequently replaced with europium(III), and the europium(III) complex can be studied using NMR or luminescence techniques, see Section 3.2 [59]. Structural studies can also be carried out using EPR, direct observation of the geometries of the gadolinium(III) macrocyclic complexes is possible and the properties of two DOTA type gadolinium(III) complexes have been investigated [60]. In this case, the combination of chiral arms and a C-nitrobenzyl substituent fixes the complexes into either square anti-prismatic or twisted square anti-prismatic geometries, allowing the relationship between electronic relaxation and coordination environment to be probed. This study showed that electronic spin relaxation, which is a key parameter influencing the relaxivity, is largely environment independent.

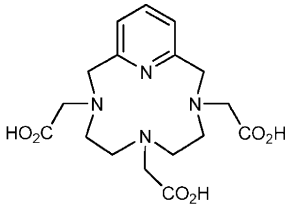
2.2. Chelator design

The gadolinium(III) complexes discussed so far are all based around the 12-membered cyclen macrocycle functionalised with pendant arms. There have been some recent efforts to expand the array of frameworks that are used in CA design.

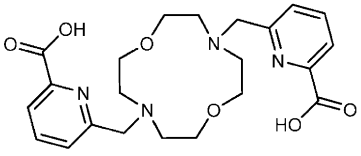
One area for chelator variation is the ring size, although it is known that the 14 membered ring cyclam ligand TETA forms much less stable complexes with lanthanides. Studies were made of the intermediate tetraaza tetraacetate macrocycle TRITA, $\mathbf{L^4}$ [61]. It was found that while the relative stability of the lanthanide complex was lower than DOTA it was again higher than some acyclic chelators that are currently used in vivo. Care should still be exercised as the issues with NSF show that maximising stability may be the best approach.



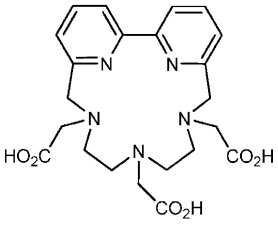
L^5



L^6



L^7



L^8

Alternatives have been investigated that retain the 12-membered tetraaza ring but increase its rigidity. An example links one of the backbone nitrogens into a piperidine ring while maintaining the four acetate pendant arms [62]. The properties of a series of related compounds as MRI contrast agents were investigated using in vivo studies and the gadolinium(III) complex of $\mathbf{L^5}$ was identified as a lead candidate with reduced kidney retention and improved liver uptake in comparison to clinically used DOTA complexes.

A further, even more rigid, alternative is to use the pyridine equivalent $\mathbf{L^6}$, although this can now only incorporate three pendant arms at the nitrogen positions. The equilibrium constants and complexation/decomplexation kinetics were studied

Table 1
Selected stability constants for the ligands discussed and relevant pre-2005 data[a].

Macrocyclic chelator and metal ion	Equilibrium constant ($\log K_{ML}$)	Ref.	Macrocyclic chelator and metal ion	Equilibrium constant ($\log K_{ML}$)	Ref.
NOTA (included for comparison)			**L[27]** (R = $CH_2CH_2CO_2H$)		[41]
Magnesium(II)	9.69	[39]	Europium(III)	25.53	
Calcium(II)	8.92	[39]	Gadolinium(III)	25.04	
Copper(II)	21.63	[39]	Lutetium(III)	25.5	
Zinc(II)	18.3	[40]			
DOTA (included for comparison)			**L[27]** (R = CH_2-*p*-$C_6H_4NH_2$)		[41]
Calcium(II)	17.2	[42]	Europium(III)	25.0	
Copper(II)	22.2	[43]	Gadolinium(III)	24.04	
Zinc(II)	21.1	[43]	Lutetium(III)	24.0	
Lanthanum(III)	22.9	[44]			
Europium(III)	24.7	[44]			
Gadolinium(III)	24.7	[44]			
Lutetium(III)	25.4	[44]			
DOTP (included for comparison)			**L[50]**		[48]
Calcium(II)	10.3	[45]	Calcium(II)	3.07	
Copper(II)	25.4	[46]	Copper(II)	27.34	
Zinc(II)	24.8	[46]	Zinc(II)	21.03	
Lanthanum(III)	27.6	[47]	Cadmium(II)	15.91	
Europium(III)	28.1	[47]			
Gadolinium(III)	28.8	[47]			
Lutetium(III)	29.6	[47]			
TETA (included for comparison)		[46]	**L[51]**		[49]
Calcium(II)	8.42		Calcium(II)	3.45	
Nickel(II)	19.91		Nickel(II)	21.92	
Copper(II)	21.74		Copper(II)	27.21	
Zinc(II)	16.62		Zinc(II)	20.16	
Cadmium(II)	18.25		Cadmium(II)	17.03	
Lead(II)	14.3		Lead(II)	12.85	
L[2]		[50]	**L[52]**		[51]
Magnesium(II)	7.62		Manganese(II)	11.64	
Calcium(II)	12.09		Cobalt(II)	16.42	
Manganese(II)	16.74		Nickel(II)	20.04	
Copper(II)	17.91		Copper(II)	21.58	
Zinc(II)	19.64		Zinc(II)	18.16	
			Cadmium(II)	17.9	
			Lead(II)	13.58	
L[3]		[52]	**L[53]**		[53]
Calcium(II)	15.1		Copper(II)	14.71	
Copper (II)	24.9		Zinc(II)	11.53	
Zinc(II)	22.5				
Yttrium(III)	26.6				
Lanthanum(III)	23.3				
Europium(III)	25.6				
Gadolinium(III)	25.7				
Lutetium(III)	26.4				
L[6]		[54]	**L[57]**		[55]
Magnesium(II)	12.35		Copper(II) $[Cu(HL)]^+$	16.3	
Calcium(II)	12.72		Copper(II) [CuL]	13.9	
Copper(II)	18.79				
Zinc(II)	20.48				
Yttrium(III)	20.63				
Europium(III)	20.26				
Gadolinium(III)	20.39				
Ytterbium(III)	20.28				
Gd**L[26]** ($\log K_{GdLM}$ for ternary complexes)		[56]	*monoalkylphosphonate esters of DOTP* (gadolinium(III))		[57]
Calcium(II)				16.09	
Copper(II)	1.87		Ester group = CH_2OH	16.50	
Zinc(II)	5.39		Ester group = CH_2CH_3	14.4	
	5.28		Ester group = OCH_2CH_3	12.19	
			Ester group = O-*n*-C_4H_9		

[a] General conditions: T = 298 K, I = 0.1 or 1.0 M (KCl, KNO_3 or NMe_4NO_3).

for lanthanide ion complexes of **L[6]** [54]. Both the stability constants and the mechanism of complex formation were shown to be similar to lanthanide-DOTA complexes, however, complex formation was at least ten times faster suggesting that this chelator may have greater potential in radiopharmaceutical applications where rapid complex formation is the key factor, see Section 4.

As an alternative to the tetraaza 12-membered ring a diazadioxa chelator was synthesised, **L[7]**. Despite only having two nitrogen positions for attachment of additional chelating groups, the

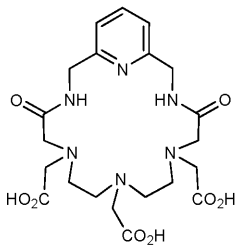
Table 2
Longitudinal relaxivity (T_1) parameters for selected gadolinium(III) complexes.

Compound	Relaxivity, r^a ($mM^{-1}\ s^{-1}$)	Temperature (K)	pH	Field strength (MHz)	Comments	Ref.
$[GdDOTA]^-$	3.96	310	H_2O No buffer	60	–	[65]
GdDO3A	6.95 3.20 (+lactate)	298	6.5	25	Relaxivity recorded in absence and presence of lactate.	[66]
Gd(1-methylphosphonic acid DO3A)	4.7	310	7	20	–	[67]
Gd(tris-3,2-hydroxy-pyridonate)tacn	13.1	298	7.0	20	Complex has three inner sphere water molecules.	[64]
$[GdDOTP]^{5-}$ Monoalkylphosphonate esters of DOTP	3.5	310	10	20	All recorded at pH 7 apart from DOTP (pH 10 fully deprotonated). The relaxivity contribution for these compounds is entirely outer/second sphere (no H_2O bound).	[57]
Ester group = CH_2OH	2.2		7.0			
Ester group = OCH_2CH_3	2.1		7.0			
Ester group = O-*n*-C_4H_9	2.3		7.0			
Ester group = CH_2CH_3	2.8		7.0			
GdDO3A(phosphonate)					Steric factors give a reduction in relaxivity.	[68]
Bisethyl phosphonate ester	3.44	310	7.0	20		
Monoethyl phosphonate ester	3.49					
No phosphonate ester group	4.57					
Gd**L**^{3}	3.6	298	7.4	20	Relaxivity is constant above pH 7.The pH dependence is due to phosphate protonation.	[52]
$[Gd\mathbf{L}^{5}]^-$	3.0	296	7.0	20	-	[62]
Gd**L**^{16}	5.3	298	7.0	20	-	[69]
Gd**L**^{17}	9.5	298	7.0	20	The faster exchange rate improves relaxivity	[70]
Gd**L**^{21}	Relaxivity profile	298	7.0	Variable	The relaxivity profile of the water protons were interpreted using a new theoretical framework	[71]
$[Gd\mathbf{L}^{24}]^-$	6.7	298	7.0	10	-	[72]
Gd**L**^{25}	7.2	294	7.0	300	Relaxivity is constant over pH 5–7.	[73]
$[Gd\mathbf{L}^{28}]_2$	1.9	298	7.0	20	–	[74]
$[Gd\mathbf{L}^{30}]^{3+}$	1.79	298	H_2O No buffer	20	–	[75]
$[Gd\mathbf{L}^{48}]^-$	4.77	310	H_2O No buffer	60	–	[65]

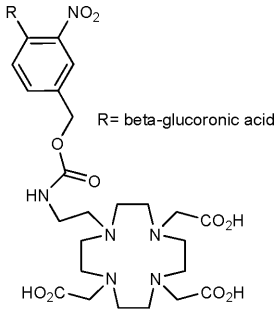
macrocycle is octadentate with pyridyl carboxylate pendant arms [63]. The europium(III) and terbium(III) complexes were studied and showed that an equilibrium exists between nine- and ten-coordinate species in solution with one or two bound waters respectively.

Raymond and co-workers have been investigating tripodal pyridonate constructs as lanthanide chelating agents for many years. In some recent work they used tacn to arrange three hydroxypyridonate groups in a suitable motif to form highly stable gadolinium(III) complexes, although the macrocycle in this case is a structural rather than a coordinating unit. These complexes have an increased hydration number of three compared to one or two bound water molecules for the DOTA/DO3A type complexes and hence have improved relaxivity properties, see Table 2 [64].

Some larger ring macrocyclic chelators and their complexes have also been studied as potential MRI CAs. The fifteen membered ring bipyridine analogue, **L**^{8}, has been synthesised and complexation of lanthanide ions investigated [76]. A pyridyl 18-membered hexaaza macrocycle with carboxymethyl pendant arms, **L**^{9}, was synthesised and lanthanide complexes characterised [77]. Cellular labelling studies were carried out using MRI (gadolinium(III) complex) and fluorescence microscopy techniques (terbium(III) complex).



L^{9}

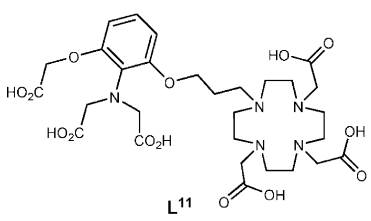


L^{10}

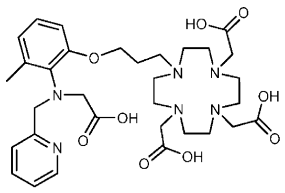
2.3. Responsive CAs

There has been an increased effort to expand the utility of contrast agents by modifying them to report on their biochemical environment as an alternative to passive concentration based image enhancement [18,78]. The general approach has been to incorporate binding units that will either open up water coordination positions at the gadolinium centre or slow the rotational correlation time, both offering enhanced relaxivity. For example, a physiologically responsive MRI contrast agent was produced

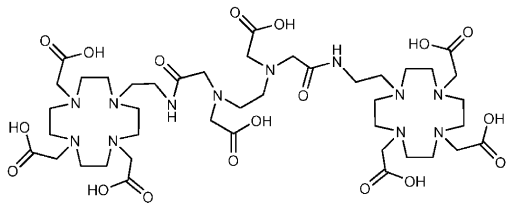
by modifying the cyclen framework to incorporate a pendant β-glucoronic acid, **L[10]**, that could be cleaved in vivo unmasking a coordination site on the gadolinium ion and hence increasing the relaxivity [79].



L[11]



L[13]



L[12]

In recent years the research groups led by Meade and by Logothetis and Toth have made significant advances in the area of MRI contrast agents sensors [80,81]. Having originally synthesised the first MRI responsive compounds to sense calcium(II) binding, Meade and co-workers more recently produced compounds that can sense zinc(II) binding by causing an increase in relaxivity due to a change in hydration number at the gadolinium(III) centre, see Table 3 [82]. **L[13]** has a DO3A chelator and a zinc(II) binding unit [80] and the gadolinium(III) complex shows over 100% increase in relaxivity in the presence of zinc(II). It was shown that an acetate pendant is required on the zinc(II) chelating unit to ensure the gadolinium centre is coordinatively saturated in the absence of zinc(II).

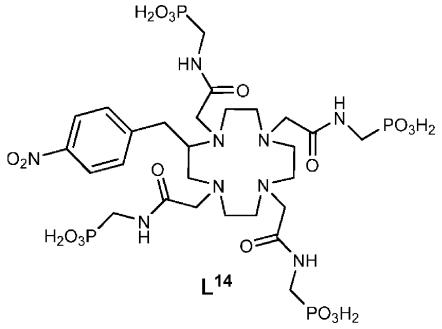
Similarly Logothetis, Toth and co-workers produced a new generation of calcium(II) binding compounds building on previous work in this area and showing a combination of rigidity and increased hydration number to improve relaxivity. Bis-macrocyclic complexes linked by calcium(II) binding groups, **L[12]**, were used to form ion sensitive contrast agents [81]. An increase in the relaxivity offered by the two gadolinium(III) centres located in the macrocyclic units is observed on binding to calcium(II) but not to magnesium(II). This increase is mainly due to the change in hydration at the gadolinium(III) centres with a smaller component relating to the reduced rotational correlation time due to increased rigidity.

Other groups have also been investigating the synthesis of improved calcium(II) activated CAs. Some of the previous issues with such systems include the concentration detection range and the sensitivity. Dhingra and co-workers have designed a system, **L[11]**, that combines a lower affinity calcium binding group to allow rapid changes in calcium concentration to be detected with a ca. 100% increase in relaxivity [83]. Further modifications may be required to improve the physiological response, which can be strongly influenced by anion binding.

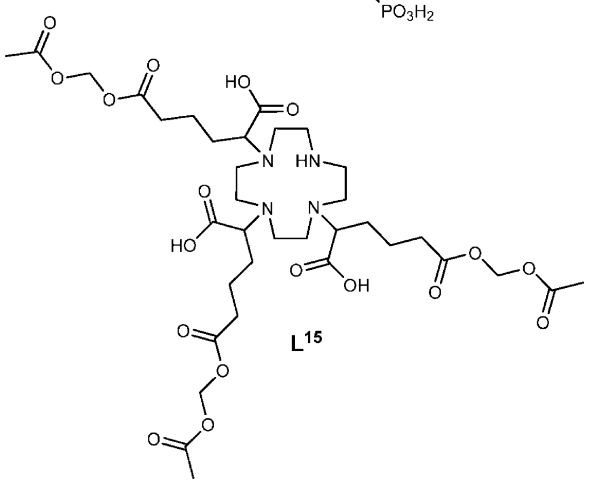
Table 3
Longitudinal relaxivity parameters for targeted and responsive MRI agents.

Compound	Relaxivity, r^a ($mM^{-1}\,s^{-1}$)	Temperature (K)	pH	Field strength (MHz)	Comments	Ref.
Gd**L[10]**	2.85 3.68 (after hydrolysis)	310	7.4	60	Glucoronic acid hydrolysis results in a 20% increase in relaxivity.	[79]
Gd**L[11]**	3.5 6.9 (Ca^{2+})	300	7.4	400	97% relaxivity increase upon addition of 1 equiv. of Ca^{2+}	[83]
Gd_2**L[12]**	5.4	298	7.0	500	A 32% increase in relaxivity is observed on binding Ca^{2+}	[81]
Gd**L[13]**	2.3 5.1 (Zn^{2+})	310	7.2	60	Zinc(II) gives 121% increase in relaxivity.	[80]
Gd**L[15]**	9.9 (no enzyme) 5.7 (no enzyme + carbonate) 10.5 (enzyme + carbonate)	298	7.4	20	Enzyme (porcine liver esterase)	[84]
Gd**L[26]**	3.4	298	9.5	20	Variable pH profile: relaxivity is higher at lower pH when protonated	[56]
Gd**L[63]**	11.7/8.6	298/310	H_2O	60	Targeted: Bombesin peptide analogue (Lys^3-Bombesin) at two temperatures.	[85]
Gd(monopeptide **L[63]**)	19.2/14.7		No buffer			
GdDO3A(monophosphinate bisphosphonate)	7.4	298	7.5	20	The bisphosphonate group allows for anchoring to bone tissue to give a selective agent.	[86]
GdDO3Aprogesterone	3.77	310	H_2O No buffer	60	This compound has no spacer group between DO3A and progesterone attached to the macrocyclic N.	[87]
Gd(DO3A-Pr-ATP)$(H_2O)_2$	6.51	308	H_2O No buffer (5.4)	24	ATP appended DO3A linked with a propyl group.	[88]

In an effort to create pH responsive MRI contrast agents, functionalized macrocycles such as **L[14]** were designed which could be incorporated into the larger structure of a PAMAM dendrimer to give 96 gadolinium-containing macrocyclic units per dendrimer [89]. The phosphonate groups are gradually protonated as the pH drops and transfer of the relaxation effects to the surrounding solvent becomes more efficient, with the relaxivity rising from 10.8 $mM^{-1}\,s^{-1}$ at pH 9 to 24.0 $mM^{-1}\,s^{-1}$ at pH 6.



L[14]

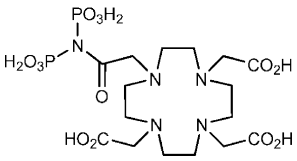


L[15]

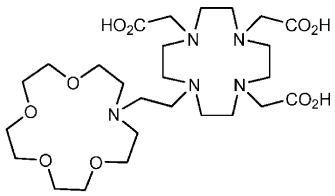
Another approach is to sense enzyme activity by providing a CA that also acts as an enzyme substrate. A cyclen chelator, **L[15]**, was designed to form a responsive MRI agent with gadolinium(III) [84]. The compound contains acetoxymethyl esters which can be cleaved by porcine liver esterase to give a ca. 85% increase in relaxivity due to suppression of interactions with carbonate anions that should be sufficient for detection in vivo. Clearly the interaction of coordinating anions can have significant impact on sensing capabilities, as Meade and co-workers also determined when they further elucidated the mechanism of their β-galactosidase activated CA [90]. The mechanism for water exclusion by the galactopyranose sugar prior to cleavage is dependent on the CA substitution pattern and isomeric form. Importantly, the binding of carbonate anions to the gadolinium centre is involved in the water exclusion process in some cases.

2.4. Tissue targeting of CAs

The simplest way to target a contrast agent to a particular part of the body or tissue type, for enhanced imaging of that specific location, is to use the natural transport/binding processes in vivo. For example, the addition of phosphate type groups could result in bone uptake. A novel cyclen based macrocycle for applications in bone imaging and therapy was produced, **L[16]** [69]. The gadolinium complex was investigated using ^{1}H and ^{17}O relaxometric studies. Bone uptake was modelled using hydroxyapatite demonstrating strong but reversible binding that occurred rapidly under physiological conditions.



L[16]

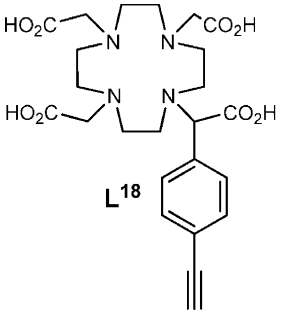


L[17]

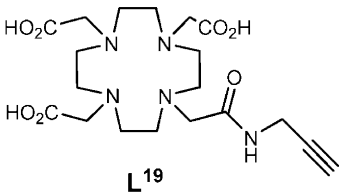
Another important aim is blood pool imaging, which requires the agent to remain in the blood stream for longer periods. A DO3A framework functionalised with an aza-15-crown-5, **L[17]**, was used to form a complex with gadolinium(III) that was investigated in vivo [70]. The agent showed improved retention times in the vasculature, low cytotoxicity and an appropriate water exchange rate.

It is challenging to produce compounds that permeate cell membranes in sufficient quantities for detection. The current clinical agents are only useful for mapping the extracellular and vascular regions of the body. Meade and co-workers have addressed this by conjugating GdDOTA to polyarginine oligomers and stilbene derivatives [91]. Cellular uptake was determined using X-ray fluorescence (synchrotron) and ICP-MS. Imaging agents can alternatively be modified to target cellular receptors which may be over expressed in some disease states. This could give important diagnostic information. Meade and co-workers attached progesterone derivatives to a GdDO3A backbone and investigated receptor binding properties [87]. The progesterone receptor expression profile can be a prognostic marker in breast cancer.

Common functional groups used for the attachment of bifunctional chelators to targeting groups such as antibodies and peptides include carboxylic acids, amines, isothiocyanates and aldehydes/ketones. More recently maleimides, alkynes and vinyl sulfones have become increasingly popular. Rapid and efficient reactions are required and so it was an obvious choice to investigate other 'click' chemistry type reactions, such as those between azides and alkynes. DOTA derivatives have been synthesised with alkyne functional groups for use in such reactions [92]. Biomolecules such as the somatostatin peptide analogue octreotate have been labelled using the Huisgen [3 + 2] cycloaddition with macrocyclic derivatives such as **L[18]**. Meade and co-workers produced an alternative DO3A chelator functionalised with an alkynyl group to exploit this reaction in the assembly of multimeric arrays of MRI contrast agents, **L[19]** [93]. Benzyl groups or a cyclodextrin were used as central units for attachment of the gadolinium(III) bound chelators to give complexes with increased relaxivity due to an increased number of metal centres and reduced flexibility, see Table 4.



L[18]



L[19]

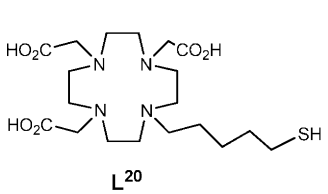
A thiol functional group can be used to attach the gadolinium(III) complex to the surface of a gold nanoparticle forming a high relaxivity array, **L[20]** [94]. In this case, the nanoparticle was also coated with sugars to provide water solubility and tissue targeting. In vivo imaging of a brain tumour (mouse) showed that the sugar configuration influenced tissue uptake.

Table 4
Longitutidinal relaxivity parameters for MRI contrast agents with multiple gadolinium(III) metal ions.

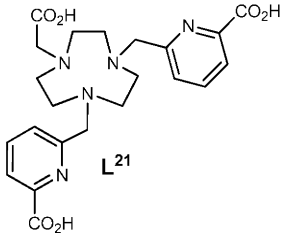
Compound	Relaxivity per mM Gd^{3+}, r^a ($mM^{-1}\ s^{-1}$)	Temperature (K)	pH	Field strength (MHz)	Comments	Ref.
Gd **L**^{14} dendrimer	10.8 24.0	298	9.5 6.0	20	Recorded at two pH values to show the effects of dendrimer protonation.	[89]
Molecules produced on reaction of Gd**L**^{19}	3.21 (Gd**L**^{19}) 5.90 (3 Gd**L** units) 10.97 (6 Gd**L** units) 12.20 (7 Gd**L** units)	310	H_2O No buffer	60	Molecules possessing 3, 6 or 7 Gd**L** units.	[93]
GdDO3A sugar conjugates	1.4–25.2	310	7.2	60	Galactose, lactose or glucose attached to gold nanoparticles with different chain lengths. The Gd^{3+} concentration was determined using ICP techniques.	[94]
Amide coupled calixarene with GdDOTA	18.3	310	H_2O No buffer	20	Four GdDOTA units are attached to a calix[4]arene via amide bonds on the upper rim.	[95]
Gd**L**^{23}	6.19 (no Fe^{2+}) 11.0 per Gd (1:3 ratio Fe:Gd**L**^{23})	298	5.0	20	Rigid structure is formed around the Fe^{2+} centre	[96]
PAMAM dendrimer conjugates of Gd**L**^{24}	8mer 10.1 16mer 14.1 59mer 18.6	310	7.5	20	–	[97]
Gd**L**^{100}	17.3	298	7	20	Micellar compound. Relaxivity is consistent for slow moving supramolecular adducts with the neutral Gd(III)-DOTA monoamide moiety.	[98]

2.5. Optimizing relaxivity

A number of strategies have been adopted to produce contrast agents with enhanced properties such as lower toxicity and increased relaxivity [99,100]. It is likely that higher stability macrocyclic chelators will remain more desirable than acyclic complexes.



L^{20}



L^{21}

Again the majority of the work in this area has focused on modification of the cyclen macrocyclic framework. A new, potentially octadentate, ligand based on the tacn framework has been synthesised incoporating picolinate pendant arms, **L**^{21} [71]. The gadolinium(III) complex shows improved stability compared to NOTA and relaxivity values were recorded giving results similar to commercial MRI agents. However it is still questionable whether the properties are ideal for in vivo application.

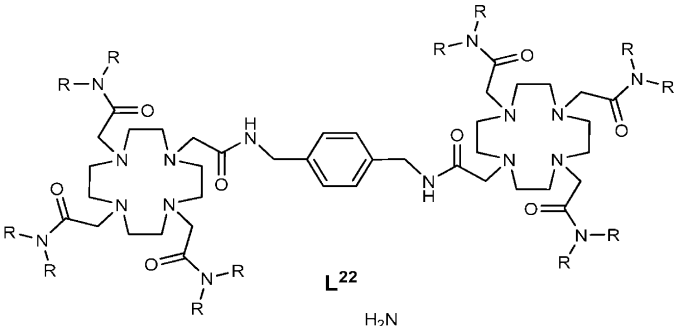
Agents can be designed that have enhanced properties in vivo by blocking any interactions with biologically relevant molecules which reduce relaxivity, in particular binding to coordinating anions. The effects of the coordination of L-lactate to gadolinium DO3A were investigated [66]. The resulting complex was characterised by multinuclear relaxometric measurements which showed that the coordination sphere has a heptadentate macrocylic ligand and a bidentate lactate which blocks inner sphere water coordination.

Another strategy to increase relaxivity is to increase the number of metal centres, see Table 4, a multimeric compound based on a BFC was discussed in Section 2.4. Linked cyclen macrocylic ligands have been synthesized with either *p*-xylyl, **L**^{22}, or flexible aliphatic linkers [101]. They were initially studied for activity as mRNA cleavage catalysts, see Section 5, but also have potential application as MRI contrast agents. Peters and co-workers have functionalised a calixarene to give four gadolinium(III) DOTA units attached on the upper rim [95]. The relaxivity is limited by the residence time of the inner sphere water molecule, however the rigidity is close to optimum.

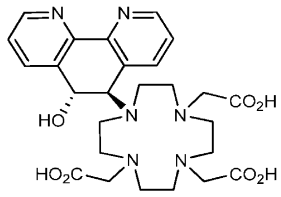
An alternative approach is to attach a coordinating group to the CA that does not interact with the gadolinium(III) centre and could interact with an external metal centre allowing a multimetal complex to be assembled. A cyclen derivative was synthesised to produce such heteromultimetallic arrays incorporating highly paramagnetic metal ions resulting in a compound with high relaxivity [96]. **L**^{23} was initially complexed to gadolinium(III) and then titrated with iron(II) or nickel(II) to form tris complexes coordinating to the phenanthroline unit.

The use of phosphorous oxo groups in the pendant arms of cyclen complexes has been shown to increase the rate of water exchange at the metal centres and a series of complexes of this type have been characterized. The main advantage of these complexes is that the small structural changes should not have a major impact on stability, see Table 1. Efforts have been made to understand the influence of phosphonate and phosphinate groups on complex geometry. The yttrium(III) complex of a monophosphinate derivative of the DOTA ligand, octadentate chelating agent, 1,4,7,10-tetraazacyclododecane-4,7,10-triacetic-1-methyl[(4-amino-phenyl)methyl]phosphinic acid **L**^{24}, showed unusual behaviour in the solid state [102]. It crystallised with three different coordination arrangements in one unit cell, two twisted square anti-prismatic configurations and one square pris-

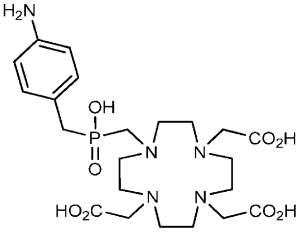
matic configuration. This has implications for water exchange rates. **L[24]**, can be converted to an isothiocyanate derivative for use as a bifunctional chelator to attach gadolinium(III) macrocyclic units to polyamidoamine dendrimers via thiourea linkages [97]. These compounds can be used to form effective macromolecular MRI contrast agents [72]. Dendrimers were synthesised with eight, sixteen and fifty nine Gd-macrocycle units and relaxivity increases were observed due to slowed molecular tumbling reducing the rotational correlation time. However a key parameter is the internal flexibility which requires significant improvement if the full potential for relaxivity is to be achieved. The equivalent non-alkylated phosphonic acid chelator was extensively studied to determine how protonation of the phosphonate affected the isomer ratio and the water exchange rate [67]. A further series of lanthanide complexes of the phosphinic acid DOTA ligands were characterised crystallographically with the structures showing ocatadentate binding of the ligand in all structures with a coordinated water molecule only present for neodymium(III) and terbium(III) [103].



L[22]



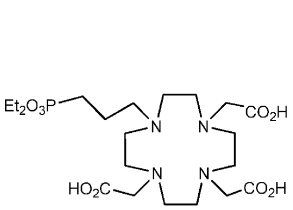
L[23]



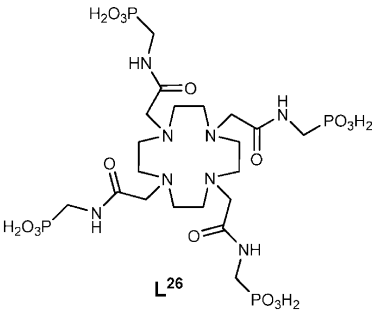
L[24]

The steric requirement of the phosphonate group is thought to be the key factor in influencing water exchange rates and a series of monophosphonic acid DO3A derivatives were studied incorporating a varying number of ethyl groups [68]. The water exchange rate was shown to be dependent on the ratio of the two isomers formed. Mayer and co-workers studied the lanthanide complexes of a series of DO3A phosphonate ligands, including **L[25]**, where the phosphonate group is at a greater distance from the metal centre [73]. For phosphonate ester arms, variation was observed in the number of coordinated water molecules and the deprotected phosphonates behaved differently with pH dependent relaxivity observed for the gadolinium(III) complexes.

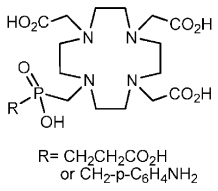
The gadolinium(III) complex with the amide phosphonate derivative of DOTA **L[26]** has previously been used to map tissue pH by MRI. In a more recent study Sherry and co-workers demonstrated that the agent must be prepared carefully as different complexes can be formed dependent on the pH at complexation [56]. This work also further elucidates the pH mapping behaviour showing the role of the phosphonates in the enhanced relaxivity. To determine the influence of phosphinate species on complex formation and dissociation, DO3A chelators with one phosphinate arm were studied [41]. It was found that the (2-carboxyethyl) phosphinic acid derivative **L[27]** formed complexes faster than DOTA. The yttrium(III) complexes were both highly thermodynamically stable and kinetically inert suggesting that gadolinium(III) analogues would be suitable for in vivo applications.



L[25]

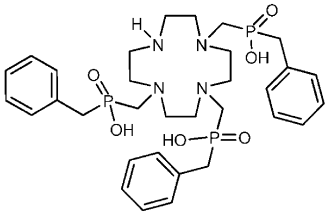


L[26]



R= $CH_2CH_2CO_2H$ or CH_2-p-$C_6H_4NH_2$

L[27]



L[28]

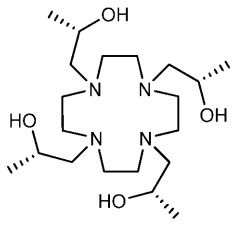
A tris N-substituted cyclen phosphinate ligand, **L[28]**, was synthesised, and the lanthanide complexes investigated [74]. The gadolinium(III) and terbium(III) complexes were found to be dimeric eight coordinate compounds both in the solid state and in solution. The relaxivity/luminescence properties observed are consistent with complete exclusion of water from the inner sphere.

Studies of the second sphere hydration have been carried out on a series of tetraphosphonate ester/phosphinate DOTA analogues which again have no water molecule bound to the metal centre [57]. The relaxivity did not show significant variation between the different derivatives and so the water molecules are probably located next to the phosphorous-oxygen atoms. A monophosphinate/bisphosphonate DOTA analogue was prepared which has increased relaxivity due to a slowed tumbling rate in the presence of magnesium(II) or calcium(II) ions [86]. This is thought to be due to the formation of coordination polymers, and attachment to hydroxyapatite as a bone model also shows a similar increase in relaxivity.

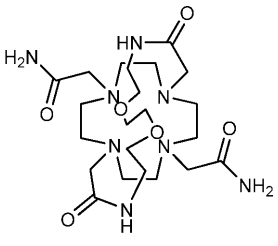
In an effort to attach a CA to a naturally occurring phosphate derivative, the gadolinium(III) complex of an ATP linked DOTA derivative was synthesised. The researchers were targeting a reduction in toxicity and increase in relaxivity for the new CA [88].

2.6. PARACEST, MR spectroscopy and other applications

An alternative to the gadolinium(III) CAs which shorten the longitudinal relaxation time of bulk water protons are PARACEST agents [104]. These compounds alter the image contrast by chemical exchange saturation transfer (CEST), where selectively saturated spins are transferred from one chemical pool to another. This can be further enhanced by the use of exchangeable protons on a chelating ligand bound to a paramagnetic centre.



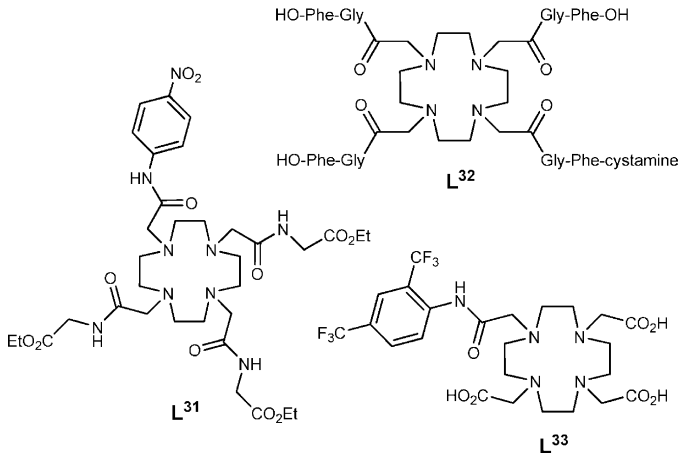
L[29]



L[30]

Europium(III) complexes have been a common choice as PARACEST agents with exchangeable protons located on hydroxyl or amide groups on the pendant arms in close proximity to the metal centre. A europium(III) complex of a cyclen ligand with pendant hydroxyethyl arms, **L[29]**, was investigated, showing that the rapid exchange of the hydroxyl protons limited the application as a CEST agent [105]. Different isomers can show markedly different PARACEST properties. Isomer selectivity in amide analogues of lanthanide-DOTA complexes was achieved by linking two of the pendant arms to form a macrobicyclic chelator **L[30]** [75]. However, only a twisted square anti-prismatic geometry was observed which will limit their application as PARACEST contrast agents.

The PARACEST work discussed so far has been carried out by Sherry and co-workers, one of the leading groups in this field. They have also recently shown that the electronics of the ligand system, such as **L[31]**, can have a major influence on the contrast properties of the complex [106]. An increased CEST contrast effect was observed in the MRI images obtained with the compound containing a nitro electron withdrawing substituent instead of the reduced electron donating (amino) form.



Modification of PARACEST agents was carried out to allow conjugation of the europium(III) compounds without disruption of the PARACEST properties [107]. This was achieved by unsymmetric functionalisation of a cyclen unit with one pendant arm terminating in a cystamine, **L[32]**, that offers potential for attachment to biologically active molecules via disulfide bond formation with the thiol.

Another potential application of lanthanide compounds in magnetic resonance techniques is to MR spectroscopy (MRS) in biological systems. Lanthanide complexes of heptadentate ligands based on the cyclen framework have been developed as magnetic resonance spectroscopy shift agents for detection of lactate in a clinical setting using ^{1}H NMR [108]. The effects on anion binding of the different lanthanide ions and the influence of steric interactions from the ligand were investigated.

Another technique that exploits the properties of lanthanides to enhance in vivo spectroscopy involves compounds with CF_3 reporter groups placed in close proximity to a paramagnetic lanthanide centre using a macrocyclic chelator such as **L[33]** [109]. The lanthanide improves the longitudinal relaxation rate of the ^{19}F nucleus to give suitable properties for use of these compounds as MRS agents.

There is an increasing interest in the potential use of hyperpolarised organic molecules in the clincial setting to track metabolic processes. The application of hyperpolarised metal ions as contrast agents in magnetic resonance imaging has been investigated by Sherry and co-workers in their study of the ^{89}Y complex of DOTA [110]. It was demonstrated that the hyperpolarisation could be successfully carried out and that the long T_1 times observed for the complex would be appropriate for in vivo metabolic imaging.

3. Fluorescent and luminescent macrocyclic complexes

Macrocyclic complexes incorporating optical imaging elements fall into two categories: macrocycles that have been tagged with fluorescent dyes or complexes where the metal ion can luminesce. The development of luminescent lanthanide probes has been an area of major activity in the last 5 years.

3.1. Fluorophore tagged macrocycles

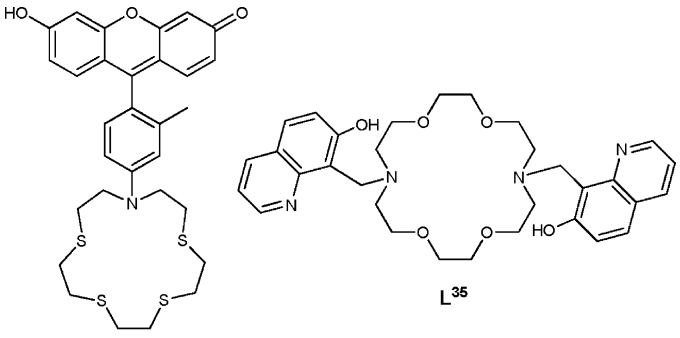
Macrocycles can be combined with fluorescent organic units to form fluorescent sensors, this is particularly valuable when specific binding is required to selectively sense metal ions. Often the coordination of the metal ion inhibits a quenching electron transfer process, for example by binding to an amino nitrogen, and the fluorescence response can then be observed. This section is limited to discussion of examples where a metal ion binds to the fluorescent macrocycle presenting representative examples of the research in this area.

A mercury sensor based on a NS_4 crown ether conjugated to an optical dye was produced, **L[34]**. It has a high quantum yield (Φ = 2% for free ligand and 72% for the Hg bound form in aqueous solution) and can be used to either detect mercury in cells or determine if toxic levels are present in fish [111]. Another mercury sensor was developed using a diaza-18-crown-6 ligand with appended hydroxyquinolines, **L[35]** [112]. It binds to mercury(II) ions in aqueous solution to give a fluorescent response, forming an unusual dimercury complex. Selective fluorescence enhancement for other diamagnetic metal ions has been observed, for example zinc selectivity over cadmium, mercury and lead is observed for a pyridyl octa-aza macrocycle functionalized with naphthyl methyl groups [113]. Lippolis and co-workers have synthesized related anthracenyl derivatives with 15 membered trioxa-diaza macrocycles [114]. In this case fluorescent enhancements with zinc(II) or cadmium(II) were only observed at certain pH values. However it is not just the variation of the binding unit that should be considered when developing a metal sensing system, the fluorophore itself can influence the selectivity, particularly when it has donor capability to interact with the metal ion [115].

A further example of fluorophore coordination is observed for the pH sensitive fluorescence of zinc(II) complex with benzimidazolyl functionalized cyclen [116]. In this case the coordination of the benzimidazole group lowers the pK_a of the N–H and an increased quantum yield is observed on deprotonation at higher pH. Tuning of the response in different pH windows can also be carried out by modifying the pendant arms in the chelate and hence influencing the interaction of the metal centre with the coordinating fluorophore, this has been demonstrated using an aminomethylanthracene functionalized cyclen system [117]. Another way to influence selectivity is to constrain the macrocycle into a more rigid configuration, an oxa-aza macrocycle with a pendant anthracenyl group shows improved selectivity for zinc(II) and mercury(II) when a rigid bridging unit is added [118]. The enhancement or disruption of excimer formation via stacking of two phenanthroline units in a macrocyclic ligand was investigated and shown to be dependent on the metal ion, with zinc(II) encouraging and copper(II) inhibiting excimer formation [119]. This could be used to discriminate binding of these metal centres.

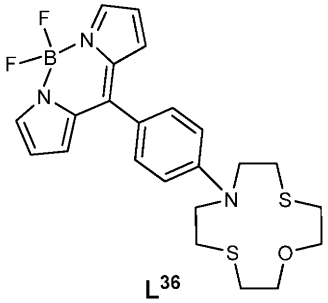
The BODIPY fluorophore is particularly popular in cellular assays due to the ideal wavelengths of excitation and emission and its high quantum yield. An oxa-aza cryptand has been attached to a boron dipyrromethene unit to give a potassium ion binding group for use

in biological systems [120]. The linked units are then attached to dextran and the system can be used as a sensor for extracellular potassium concentration with a sufficiently bright response to be detected by commercial plate readers. In an alternative system, a mixed donor macrocycle has been covalently attached to a BODIPY to give a fluorescent sensor for iron(III), **L[36]** [121]. Competition studies were carried out and it was demonstrated that detection is possible down to micromolar levels in aqueous solution. Oxadiazole fluorophores are well known for their amine activated fluorescence and can be attached to the cyclen backbone to produce sensors for copper(II) and zinc(II) that are compatible with biological systems [122].

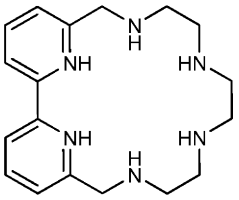


L[34]

L[35]



L[36]



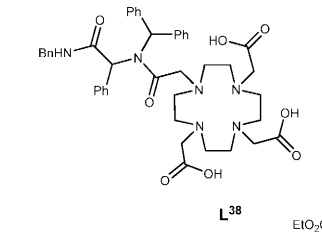
L[37]

The dizinc(II) complex of an hexaazamacrocyclic ligand **L[37]** acts as a fluorescent sensor for uridine and uridine containing ribonucleotides with the formation of a π-stacked exciplex between the bipyridyl unit in the ligand and a zinc(II) bound uridine giving an emission at 440 nm [123]. Spiccia and co-worker took a similar approach to the sensing of thymidine nucleotides using a zinc(II) cyclen core with two pyrene groups appended [124], in this case binding of the guest enhances the pyrene excimer emission via orientation of hydrogen bond interactions with the linking groups to the pyrene moieties.

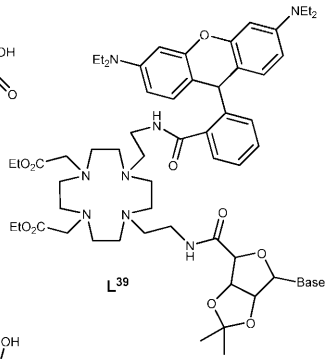
Watkinson and co-workers took an elegant approach to generating a selective zinc sensor by using a 'click' approach (Huisgen [3+2] cycloaddition) to attach a fluorescent group to a cyclam macrocycle [125]. The fluorescence is activated by coordination of the metal centre to both the macrocycle and the fluorophore linking triazole heterocycle, and selectivity is observed for zinc(II) over cadmium(II).

3.2. Luminescent lanthanide probes

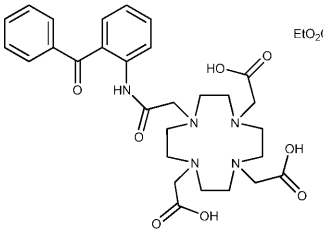
A significant body of work has been generated exploiting the luminescence properties of lanthanides. Lanthanide ions have sharp emission peaks but are poor chromophores. They are generally excited by the use of a sensitising chromophore which transfers energy to the metal centre [126,127]. They are also rapidly quenched by OH or NH oscillators and so water coordination is undesirable, see Table 5 for luminescence parameters of complexes.



L[38]



L[39]



L[40]

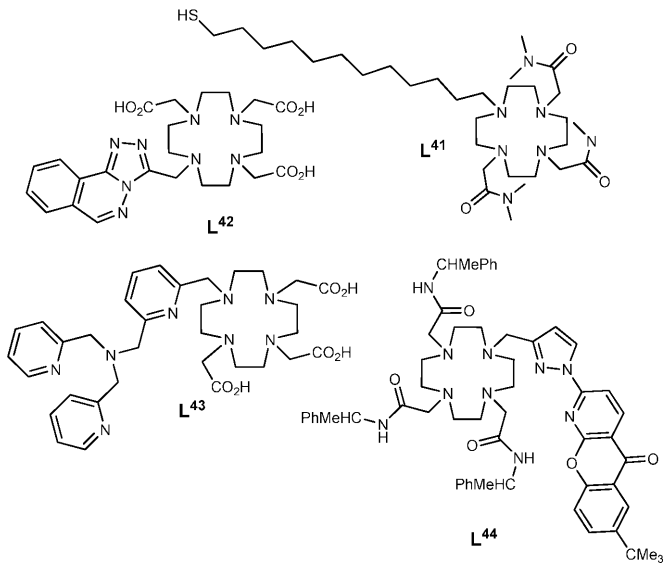
3.2.1. Chelator design: pendant arms and chromophores

Macrocyclic chelators are ideal for wrapping up the metal centre (as in MRI) and aza donors can be functionalised to incorporate sensitising chromophores. There have been significant developments in synthetic methodology to produce these chelators. For example, Faulkner and co-workers have used the Ugi four component reaction to prepare novel DOTA derivatives, such as **L[38]** [128]. The Ugi reaction is particularly useful in the rapid formation of large libraries of compounds to test their biological activity. The next stage is to take a toolkit approach incorporating peptide vectors for targeting biological systems. Synthetic methodology was also developed to produce asymmetrically N-functionalised cyclen chelators with three different pendant arms, such as **L[39]**, to offer both a sensitiser and a quencher to a complexed lanthanide ion [129]. The quencher unit is a nucleotide that can recognize substrates via base pairing, which then reduces the quenching effect on the energy transfer between the antenna and the metal centre.

The selection and attachment strategy for the chromophore can be of key importance, For example, the influence of the spacing and orientation of the benzophenone sensitising unit on a europium(III) complex of a DO3A macrocycle, **L[40]**, was investigated [130]. It was demonstrated that a longer linker could offer equally efficient optical properties due to an increased radiative rate constant.

Another desirable feature that is increasingly being investigated is the ability to attach the probes to a surface or nanoparticle. Towards this goal, cyclen derivatives have been produced with thiol groups attached, **L[41]**, for immobilization of the chelating unit on the surface of gold nanoparticles (see also **L[20]** in Section 2.4) [131]. The water solubilized nanoparticles can be functionalized with antennae groups to allow sensitisation of the lanthanide luminescence and give a sensor molecule in which the luminescence can be turned off by coordination of quenching anions, such as phosphate.

Chromophore selection is dependent on appropriately matching the energy levels of the lanthanide. For example, a DO3A macrocycle was appended with a hydralazine derived chromophore, **L[42]**, which was shown to successfully sensitise europium(III), neodymium(III), ytterbium(III) and erbium(III) [133]. Luminescence lifetime measurements established that the ligand is octadentate in these complexes.



An alternative approach is to move away from the 'standard' cyclen macrocycle and incorporate the sensitising group into the chelator backbone. New 18-membered hexaaza macrocycles have been produced that have a terpyridine unit as part of the macrocycle backbone as well as three carboxymethyl arms [143]. The photophysical properties of the europium(III) complex were examined, and direct sensitisation of the lanthanide luminescence was observed with quantum yields of 13–18%. The fifteen membered ring bipyridine analogue was also synthesised with the europium(III) and terbium(III) solutions showing similar luminescence quantum yields in the 10–20% range [76].

Nonadentate tacn chelators with three hydroxyquinolate arms were produced and shown to act as efficient sensitisers of near-IR luminescence of ytterbium(III) and neodymium(III) [140]. These are highly stable complexes in aqueous solution and show one of the highest reported quantum yields for ytterbium(III) luminescence (see Table 5).

For a biological probe, it is clearly important to test the luminescence properties in aqueous systems and make sure that quenching is minimized. A nonadentate chelator can be used to make sure that there is no free site for water molecule coordination. For example, a series of nonadentate macrocycles based on the cyclen framework with a tetraazatriphenylene pendant arm were synthesized, see Table 5 [147]. The terbium and europium complexes, which are cationic, neutral or anionic, have emission quantum yields in the range 15–40% in aqueous solution. Preliminary in vitro cellular experiments show that these compounds could be highly effective as luminescent probes in biological systems.

3.2.2. Applications in sensor technology

Lanthanide luminescent units can be used as part of metal ion sensors which can be turned on and off by controlling the luminescence quenching by water molecules. The europium(III) complex of **L^{43}** was synthesised as a responsive probe for zinc(II), where binding to zinc(II) modulates the inner sphere hydration of the europium affecting both emission intensity and luminescence lifetime [134].

Parker leads one of the most successful research groups in this area, they have developed understanding of key processes in the potential use of macrocyclic lanthanide complexes as cellular sensors, see Table 5 [148,149]. Much of their recent work in developing luminescent lanthanide based probes for biological systems has used aza-xanthone sensitising chromophores appended on to a cyclen backbone, such as **L^{44}** with a pyrazoyl-1-aza-xanthone group [135]. The terbium(III) complex of **L^{44}** has appropriate properties for sensing applications with cellular uptake and low sensitivity to excited state quenching. Europium(III) complexes of azathiaxanthone derivatives show similar potential utility, and with a sulfonamide arm incorporated these complexes can be used as single component ratiometric sensors for pH measurements between pH 6 and 8 [146]. The mechanisms of quenching of the excited state in terbium(III) and europium(III) DO3A type macrocyclic complexes were also studied with a variety of sensitising groups [150]. Urate, ascorbate and some catechols quenched by a mechanism that involved transient formation of an exciplex between the complex and the reductant. A series of complexes were screened to identify those that resisted quenching revealing that the sterically hindered complexes which inhibited urate binding and those that formed non-covalent interactions with proteins were the best candidates [151].

Cellular localization of terbium(III) complexes of this type (conjugated to octa-peptides or a tetra-guanidinium cation) was examined [152]. In this case, the cellular uptake could be followed by two-photon microscopy detecting the emission from the terbium(III) complex and different localization profiles that were dependent on the conjugated group. In a related study, this group also looked at how the variation of substituents on the aza-xanthone unit influences cellular uptake and localization properties of the europium(III) and terbium(III) complexes [153]. A significant influence was demonstrated on the speed of uptake into the cells, showing ester derivatives to be taken up much faster than acid or amide functionalized compounds. The response of the aza-xanthone and azathiaxanthone complexes to their environment was investigated, particularly focussing on their application as cellular sensors for anions [145]. A selective response was observed for the europium(III) complexes in binding to citrate or bicarbonate anions, suggesting good potential for further study. The group moved on to target the analysis of citrate in biological fluids, particularly aiming to elucidate the relationship between citrate levels in clinical prostate fluid samples and the onset or progression of prostate adenocarcinoma [154]. These are much more rapid methods than enzymatic assays.

Parker and co-workers developed a sensor for urate ions with both the europium(III) and terbium(III) complexes required to accurately determine the concentration of the analyte in diluted urine samples using a ratiometric assay [155]. The luminescence of the lanthanide is quenched by electron transfer from the bound urate ion. A similar ratiometric sensing system for intracellular pH was also developed with an alkylsulfonamide group incorporated as a pH sensitive binding moiety [156]. The intensity ratio vs. pH was plotted and intracellular pH could be measured in the range pH 6–8 using a suitable microscope. The mechanisms of cellular uptake were investigated in a later study which showed that this series of compounds were taken up into cells by macropinocytosis rather than other endocytosis mechanisms [157]. This is useful as it is easier for the complexes to escape from the macropinosomes once inside the cell for transfer to other organelles.

Gunnlaugsson and co-workers designed cyclen complexes that bind anions to a terbium(III)/macrocyclic unit using a combination of H-bonding and coordination interactions [158]. It was observed that H-bonding with some anions quenched the luminescence and others such as phosphate anions showed a concentration dependent response. Gunnlaugsson and co-workers also designed a cyclen chelator with a pendant phenanthroline unit to bind to a d-block transition metal ion forming an array of three lanthanide complexes around an octahedral metal centre [159]. Formation of the assemblies could be monitored by the luminescence as the lanthanide centre is sensitised by the phenanthroline unit. They went on to develop these complexes as DNA probes by using a DNA

Table 5
Selected luminescence data for lanthanide complexes.

Compound	Luminescence lifetimes in H_2O and D_2O (ms)		Number of bound water molecules (q value)	Quantum yield of luminescence (Φ_{H_2O})	Ref
Eu**L**^{7}	0.52	1.56	1.25	–	[63]
Tb**L**^{7}	1.48	2.32	0.92		
Eu**L**^{8}	0.56	1.84	1.19	10%	[76]
Tb**L**^{8}	1.28	2.06	1.18	21%	
Sm**L**^{8}	0.0015	0.034	1.32	0.06%	
Dy**L**^{8}	–	–	–	0.06%	
Eu**L**^{10}	–	–	1.2	–	[79]
Eu**L**^{11}	–	–	0.17	–	[132]
(+Ca^{2+})			0.88		
Eu_2**L**^{12}	0.54	1.84	1.3	–	[81]
(+5 equiv Ca^{2+})	0.47	1.62	1.5		
Tb**L**^{13}	1.97	2.71	0.3	–	[80]
(+zinc)	1.46	2.65	1.0		
Eu**L**^{15}	0.307	0.813	2.1	–	[84]
Eu**L**^{21}	0.54	1.67	1.2	5%	[71]
Tb**L**^{21}	1.49	2.46	1.0	43%	
Eu_2**L**^{22}, R = H	0.507	2.265	0.9	–	[101]
Eu_2**L**^{22}, R = CH_3	0.553	1.851	1.1		
Eu**L**^{25}	0.41	1.54	1.84	–	[73]
(pH7)					
[Eu**L**^{28}]$_2$	1.10	0.60	0	0.2%	[74]
[Tb**L**^{28}]$_2$	3.14	2.47	0	11%	
Eu**L**^{30}	0.787	1.433	0.15	–	[75]
Tb**L**^{38}	1.75	2.48	0.5	–	[128]
Eu**L**^{38}	0.55	2.07	1.3		
Eu**L**^{40}	0.59	2.4	1.08	0.4%	[130]
Eu **L**^{42}	0.620	1.670	0.9	–	[133]
Yb **L**^{42}	1.87×10^{-3}	7.88×10^{-3}	0.3		
Nd **L**^{42}	0.09×10^{-3}	0.33×10^{-3}	–		
Er **L**^{42}	$<0.02 \times 10^{-3}$	1.3×10^{-3}	–		
Eu**L**^{43}	0.99	1.59	0.2	–	[134]
(+zinc)	0.40	1.72	2		
[Tb**L**^{44}]$^{3+}$	0.50	0.42	0	18%	[135]
Eu**L**^{45}	0.42	2.36	2.04	–	[136]
Eu**L**^{46}	1.03 (41%) 0.60 (22%) 0.04 (37%)		Multiple species formed, q value not calculated (lifetimes H2O only)	–	[137]
Tb**L**^{46}	1.93 (81%) 0.72 (19%)				
[Eu_2**L**^{73}]$^{6+}$	0.31	1.7	1.8 per Eu	–	[138]
Yb**L**^{99}	20×10^{-3}	5.34×10^{-3}	0.5	–	[139]
Eu**L**^{99}	0.404	0.904	1.3		
[Yb**L**^{99}Re(Bpy)(CO)$_3$]$^{+}$	1.47×10^{-3}	5.30×10^{-3}	0.4		
Yb tris((hydroxyquinolinyl)methyl)tacn	2.05×10^{-3}	8.63×10^{-3}	–	0.14%	[140]
Nd tris((hydroxyquinolinyl)methyl)tacn	0.16×10^{-3}	0.41×10^{-3}		0.016%	
Eu tris(carbamoylmethyl) cyclen	0.256	1.56	2.0	–	[141]
Eu tris[(N-ethyl)carbamoylmethyl]cyclen	0.252	1.38	2.2		
Eu tris((N,N-diethyl)carbamoylmethyl) cyclen	0.231	0.68	2.0		
Eu tris(hydroxypyridonate)tacn	–	–	2.9	–	[64]
[Tb(DO2A)(H_2O)$_3$]$^{+}$	1.1	2.6	2.4	–	[142]
[Tb(DO2A)(dipicolinic acid)]$^{-}$	1.9	2.2	0.3	10%	
Monoalkylphosphonate esters of DOTP					
EuL (ester group = CH_2OH)	0.88	1.86	0.41	–	[57]
EuL (ester group = OCH_2CH_3)	1.13	1.93	0.14		
EuL (terpy analogue of **L**^{8})	1.70	1.06	0.05	17.5%	[143]

Selected complexes studied by Parker and co-workers

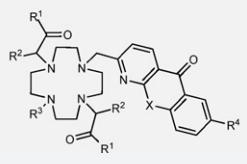
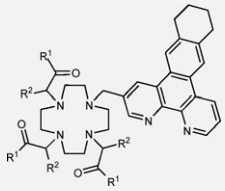
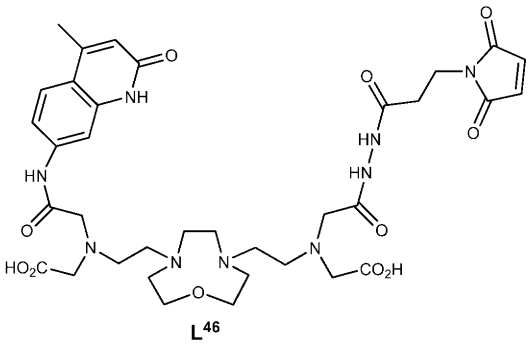


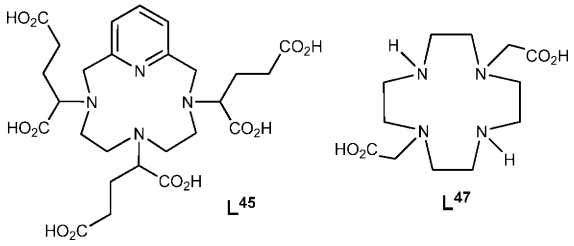
Table 5 (*Continued*)

Compound	Luminescence lifetimes in H_2O and D_2O (ms)		Number of bound water molecules (q value)	Quantum yield of luminescence (Φ_{H_2O})	Ref
EuL (R^1 = OH, R^2 = R^4 = H, R^3 = CH_2CO_2H, X = O)	0.57	2.02	1.2	6.9%	[144]
TbL (R^1 = OH, R^2 = R^4 = H, R^3 = CH_2CO_2H, X = O)	1.82	2.73	0.6	24%	
EuL (R^1 = OH, R^2 = H, R^3 = CH_2CO_2H, R^4 = CO_2H, X = O)	0.6	2.08	1.1	8%	
TbL (R^1 = OH, R^2 = H, R^3 = CH_2CO_2H, R^4 = CO_2H, X = O)	1.89	2.88	0.64	12%	
EuL (R^1 = OH, R^2 = H, R^3 = CH_2CO_2H, R^4 = CO_2Me, X = S)	0.51	1.62	1.3	2.2%	
TbL (R^1 = OH, R^2 = H, R^3 = CH_2CO_2H, R^4 = CO_2Me, X = S)	0.49	0.60	1.25	2.1%	
EuL (R^1 = NHCH(CO_2Et)CH_2Ph, R^2 = R^3 = R^4 = H, X = S)	0.32	0.49	1.1	8.9%	
TbL (R^1 = NHCH(CO_2Et)CH_2Ph, R^2 = R^3 = R^4 = H, X = S)	0.059	0.06	n/a	–	[145]
EuL (R^1 = NHCHPhMe, R^2 = H, R^3 = H, R^4 = CO_2Me, X = O)	0.32	0.62	1.33	–	
EuL (R^1 = OH, R^2 = R^4 = H, R^3 = $CH_2CH_2NHSO_2Me$, X = S)	0.48	0.76 (pH 4.5)	0.6	1.0%	[146]
	0.41	0.48 (pH 8.0)	0.1	0.9%	
EuL (R^1 = OH, R^2 = $CH_2CH_2CO_2H$, R^3 = $CH_2CH_2NHSO_2Me$, R^4 = H, X = S)	0.59	1.09 (pH 3.0)	0.6	1.8%	
	0.71	1.00 (pH 5.5)	0.2	2.0%	
	0.47	0.53 (pH 8.0)	0	1.7%	
EuL (R^1 = OH, R^2 = $CH_2CH_2CH_2CO_2H$, R^3 = $CH_2CH_2NHSO_2Me$, R^4 = H, X = S)	0.74	0.83 (pH 4.5)	0	6.1%	
	0.42	0.47 (pH 8.0)	0	5.4%	
					
EuL (R^1 = NHCHPhMe, R^2 = H)	0.96	0.63	0	16%	[147]
TbL (R^1 = NHCHPhMe, R^2 = H)	0.64	0.58	0	40%	

binding ruthenium complex in the phenanthroline binding position [160]. Use of neodymium(III) or ytterbium(III) as the lanthanides gave a dual near infra red and optical probe. This complements the work carried out by Faulkner and co-workers to produce hybrid d-f block arrays and study the sensitisation mechanism [161].



L^{46}



L^{45} L^{47}

Other approaches to sensing technologies have also been investigated. For example, europium(III) complexes of cyclen based ligands are of interest as DNA hydrolysis catalysts, see Section 6, and have been studied by direct excitation luminescence spectroscopy [141]. The complex of 1,4,7-tris(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane shows strong binding of the europium(III) ion to a dianionic phosphate ligand consistent with the catalytic role of stabilising negative charge on the phosphorane transition state.

The complexation process was studied for a europium(III) ion and a 12-membered azamacrocycle **L^{45}**, which has a rigidifying pyridyl group and N-glutaryl pendant arms, using luminescence spectroscopy [136]. A complexation mechanism is proposed that is a mixture of those proposed for DOTA and the analogous N-glutaryl derivative.

A mixed donor tridentate macrocycle, **L^{46}**, was used as the basis of a multifunctional construct for the sensitisation of lanthanide luminescence and attachment to biological macromolecules [137]. The macrocycle based design was shown to provide better protection from bound solvent quenching of the lanthanide luminescence than related linear chelating units, and the incorporated thiol reactive group was successfully used to attach proteins.

The terbium complex formed with a DO2A chelator, **L^{47}**, has been used to develop a detection system for bacterial spores using the sensitised luminescence due to the interaction of terbium(III) with dipicolinate, which is a major constituent of the spores, see Table 5 [142].

4. Radiopharmaceuticals

The use of macrocycles as chelators in radiopharmaceuticals and the formation of bifunctional chelators became more widespread in the 1980s and 1990s [162]. An expanding selection of radioisotopes is being evaluated with opportunities for new molecular imaging agents and targeted therapeutics [163].

4.1. Isotopes and macrocycle selection

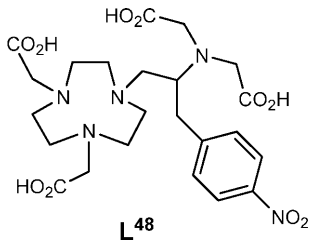
The DOTA/DO3A chelators again dominate the selection of macrocycles for radioisotope complex use in vivo [164]. Due

to the wide variety of isotopes available and the requirement for rapid complex formation there are also a number of both larger and smaller ring macrocycles that have been investigated [165]. Isotopes for imaging are gamma and positron emitters with alpha and beta particle emitters of interest for targeted radiotherapy applications. Clinically used metal isotopes include ^{99m}Tc, ^{111}In, ^{68}Ga and ^{90}Y, with many more under investigation.

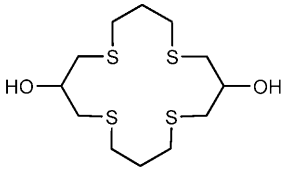
The metastable isotope ^{99m}Tc has been the mainstay of targeted radiopharmaceutical imaging but is usually bound to either monodentate or acyclic ligands. A series of technetium complexes of the tacn macrocycle were synthesized and characterized [166]. Few compounds are known with technetium in the oxidation states III, IV and VII. The high stability of the technetium(VII) trioxo complex is particularly unusual. Alberto worked with the researchers that had produced the high valent technetium oxo tacn compounds to come up with a novel labelling strategy whereby a molecule can be attached to this core via a [3+2] cycloaddition with an alkene to form a glycolato complex with a technetium(V) centre [167]. Hence, biological molecules could be tagged with an alkene for subsequent reaction with this complex.

^{111}In is used as an imaging substitute for therapeutic radiolanthanides as it binds effectively to the conjugated DO3A chelator. Giblin and co-workers used this isotope in a complex conjugated to plasminogen activator receptors in an attempt to image the metastatic potential of breast cancer tumours [168]. In an investigation of other isotopes, the tacn based NOTA chelator was used to label the bombesin peptide with either ^{68}Ga or ^{64}Cu for targeted positron emission tomography imaging of tumours [169].

A study was carried out to determine the coordination properties of octadentate tacn based ligands with lanthanides. The aim is to use these compounds in radioimmunotherapy, where the therapeutic isotope is bound to a BFC which is conjugated to an antibody [170]. **L^{48}** was radiolabelled with ^{177}Lu and displayed excellent stability both in serum (stable for 14 days with no measurable loss of radioactivity) and in vivo [65].



L^{48}



L^{49}

Labelling of a DOTA conjugate with the somatostatin peptide was carried out using cobalt isotopes, where the ^{57}Co isotope was used to determine the potential for labelling this system with the positron emitting ^{55}Co isotope [171]. In a comparison of the cobalt(II) complexes with gallium(III), it was demonstrated that varying the metal ion significantly influences key properties such as receptor affinity, biodistribution and pharmacokinetics. This is a key observation for the future design of such systems.

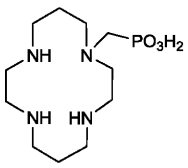
Macrocycles incorporating donors other than nitrogen have been investigated to give higher affinity in selected cases. An unusual example is the use of a metal complex to bind an inorganic anion. Rhodium(III) and iridium(III) complexes of a [16]aneS$_4$ diol, **L^{49}**, were produced for labelling with a radioactive astatide ion [172]. The astatine isotope (^{211}At) is an alpha particle emitter that can be produced using a medical cyclotron. It has appropriate properties for applications in targeted radiotherapy but methods to deliver it to target cells require development. These compounds were easily synthesised and preliminary stability investigations were promising.

Mixed donor macrocycles provide a further opportunity and new N_2S_2 chelators have been developed with backbone carbon attached carboxylates [173]. Different sized rings were produced (12, 13 and 14 membered) and ^{64}Cu radiolabelling carried out but further work is required to develop these chelators for in vivo use.

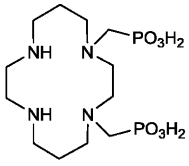
4.2. Selection of pendant arms

As in previous examples, aza macrocycles offer the opportunity for incorporation of different functional groups on the pendant arms. Key factors for radiopharmaceuticals are the rate of complex formation, complex stability (see Table 1) and the tissue localisation in vivo.

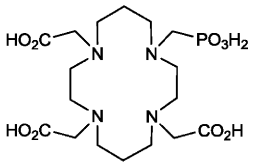
In the synthesis of novel chelators for MRI, the incorporation of phosphonate type chelating side arms was used to influence the water exchange kinetics, but in the case of radiopharmaceutical chelators the complex stability is the key parameter. A cyclam derivative with a methyl phosphonic acid pendant arm, **L^{50}**, was synthesised and shown to bind copper(II) with a high preference over other metal ions [48]. X-ray structural data show the copper(II) ion is encapsulated by the N_4O donor set of the macrocycle in a square based pyramidal geometry.



L^{50}

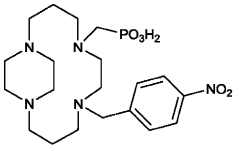


L^{51}

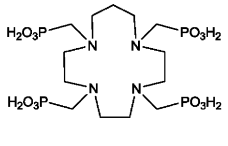


L^{52}

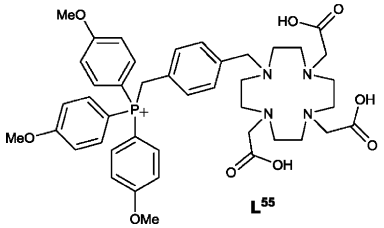
A cyclam ligand was also produced with methylphosphonate groups appended in the 1,4-positions, **L^{51}**, and complexes formed with copper(II), zinc(II) and nickel(II) [49]. All complexes showed significant acid stability and the nickel(II) complex could be isolated at different pHs with varying protonation patterns on the phosphate groups. A related novel ligand derivative with one methylphosphonate arm and three acetate arms, **L^{52}**, was synthesised and thermodynamic stability of divalent transition metal complexes studied [51]. In general the complexes were shown to be as stable as those formed with TETA.



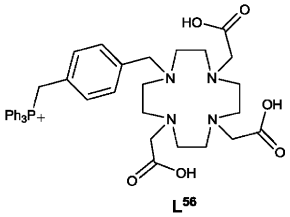
L^{53}



L^{54}



L^{55}



L^{56}

Kotek and co-workers produced a methylphosphonate functionalised cyclam, **L^{53}**, which has an ethyl bridge between two adjacent nitrogens in the cyclam ring [53]. This compound may form high stabilty radioisotope complexes for biomolecule conjugation (rigidified chelating systems are discussed in Section 4.3). The synthetic procedures for functionalisation of a piperazino containing cyclam macrocycle are complicated by the reduced

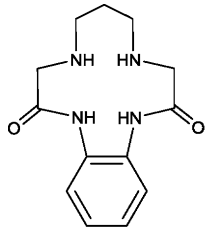
reactivity of the secondary amine position due to hydrogen bonding interactions across the cavity.

The smaller ring 13-membered tetraazamacrocycle with pendant methylphosphonate arms **L54** was investigated alongside the tetraacetate derivative (TRITA) for use as a therapeutic agent when coordinated to ^{153}Sm or ^{166}Ho [174]. **L54** complexes were shown to increase both stability and bone uptake, which are comparable to agents currently under clinical evaluation. The radiometal complexes were shown to be stable for up to 5 days in human serum at 37 °C.

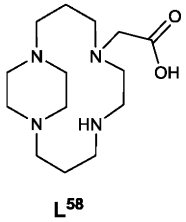
The pendant arm groups can be used to direct tissue uptake and influence pharmacokinetics rather than metal ion coordination. ^{64}Cu labelling experiments were carried out using DO3A ligands with a tetraphenylphosphonium group attached to the macrocyclic backbone, **L55** [175]. The biodistributions of the compounds were investigated showing significant substitution patterns and linking group effects in tumour bearing mice, with the introduction of methoxy groups improving the clearance from the liver and lungs. The complexation properties of a related DO3A derivative with a pendant triphenylphosphonium arm, **L56**, were investigated with diagnostically important metal ions [176]. In this case the aim was to target the mitochondrial membrane and probe for any alterations in membrane potential. A further phosphonium DO3A ^{64}Cu complex has been prepared by Liu and co-workers for comparison with a standard technetium drug in monitoring of multi-drug resistance transport processes in tumours [177]. However, these compounds were more effective for monitoring the cell efflux effects that reduce the radiotracer uptake.

4.3. Rigid macrocycles and cryptands

One method to achieve increased stability of the metal complexes is to rigidify the chelator thus slowing down the kinetics of dissociation. This work has mainly been aimed at copper(II) complexes which are often thermodynamically stable but kinetically labile [178,179].



L57

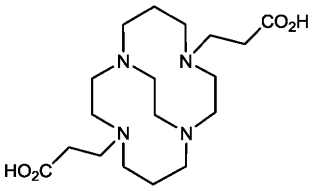


L58

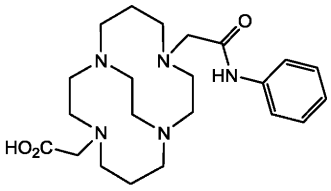
A simple way to approach this is to incorporate amide groups in the backbone, however this changes the nature of the donor atoms with deprotonated amide nitrogens coordinating to the metal centre. Tetraazamacrocycles incorporating both amide groups and a backbone benzene ring were synthesised as chelators for copper radionuclides. When deprotonated, the 13-membered macrocycle **L57** forms a highly stable copper(II) complex and the ^{67}Cu labelled compound was stable in serum [55].

Ethylene bridges between either adjacent or non-adjacent nitrogens of cyclam macrocycles can be used to increase stability via increased rigidity. Cyclam chelators with adjacent nitrogens bridged (sometimes referred to as 'side bridged') can be functionalised to provide novel ligands with an ester or acetate pendant arm [180]. The X-ray structure of the copper(II) complex of **L58** shows the cyclam ring in the expected trans-II configuration with the pendant arm coordinating in the protonated form as a carboxylic acid. The phosphonate cyclam **L53** previously discussed is a further example of this chelator type. Brechbiel and co-workers have produced a hybrid of these two chelators where the side bridged macrocycle has one acetate and one phosphonate arm [181]. This compound was labelled with ^{64}Cu and showed favourable in vivo stability.

The kinetic inertness of copper(II) complexes of a series of non-adjacent N bridged (sometimes referred to as 'cross bridged') carboxymethyl functionalised cyclams has been related to their electrochemical properties [182]. This study shows the importance of macrocycle rigidity to kinetic stability. A cross bridged cyclam macrocyclic ligand with pendant propionoate arms, **L59**, has also been developed as a chelator for ^{64}Cu and has similar high stability to decomplexation under acidic conditions to the acetate analogue previously studied [183]. However, this copper(II) complex is significantly easier to reduce (by a margin of +400 mV) and shows poorer bio-clearance properties, suggesting the reduction potential is particularly important to the in vivo profile. A detailed study was carried out by the same group to probe the in vivo properties of ^{64}Cu labelled cross bridged cyclam compounds with one amide and one acetate arm **L60** [184]. These compounds are models of peptide conjugates and show sufficient stability for investigation as targeted PET imaging agents for clinical use.



L59

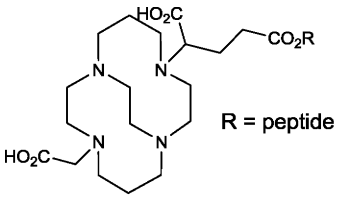


L60

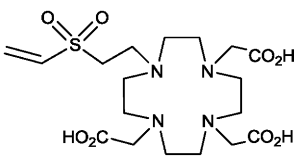
Another approach to high stability chelators for copper(II) is the use of cryptand ligands. Hexaaza cryptand cages have been conjugated to antibodies for use in PET imaging on complex formation with ^{64}Cu [132]. The chelator was attached to antibodies, reacted with the radioisotope quantitatively, and showed no disruption of antibody binding, suggesting these compounds could be of use in clinical diagnostic imaging.

4.4. Bifunctional chelators (BFCs)

The concept of a bifunctional chelator (BFC) is extremely important in the targeting of radiopharmaceuticals to different tissue types or a tumour [185]. Attaching macrocycles to biomolecules can be achieved by functionalising the framework in a number of different ways. An unsymmetric N-functionalised bifunctional chelator version has been produced of the cross bridged cyclam chelators, **L61**, as discussed in Section 4.2 [186]. This follows on from the report of a C-functionalised BFC by Archibald and co-workers [187,188]. The latest compound is versatile and has been used to label a tumour targeting peptide, providing an important advance towards clinical applications of copper isotopes in therapy and imaging.



L61

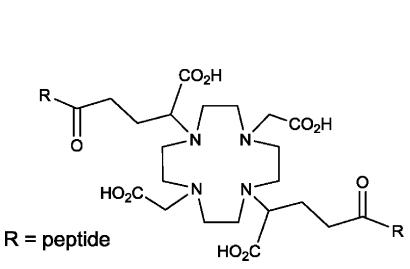


L62

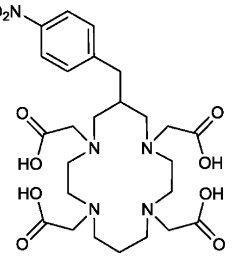
A new bifunctional chelator based on DO3A, **L62**, was developed for labelling antibodies with ^{64}Cu or ^{111}In [189]. It offers a useful approach to labelling via reaction of a vinyl sulfone linker which can be coupled to either surface lysines on the antibody or reduced disulfide bridges (cysteine thiols). A related method, this time targeting the formation of disulfide bonds, was investigated

with ethane thiol functionalised DO3A, which could be labelled with radioactive lanthanides to give complexes with high in vivo stability (serum stability of up to 5 days for the ^{153}Sm complex and 2 days for the ^{166}Ho complex) [190].

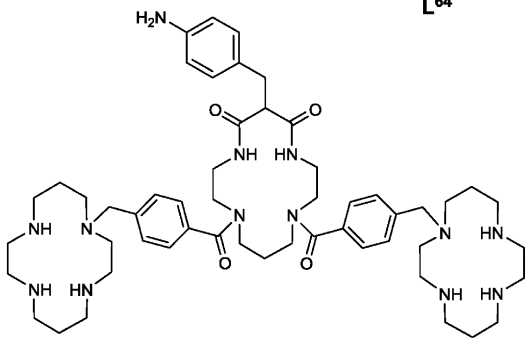
Synthetic work with the sarcophagine type hexaza cryptands has produced a range of bifunctional chelators including a benzyl acetic acid functionalised version which was conjugated to the RGD peptide for integrin receptor imaging [191]. An alternative to this is another acid functionalised sarcophagine produced by Donnelly and co-workers which shows highly effective conjugation and complex formation [192].



L^{63}



L^{64}



L^{65}

Maecke and co-workers have investigated divalent functionalisation of the cyclen macrocyclic framework to provide metal complexes for targeted tumour imaging and therapy [85]. The chelator, **L^{63}**, has two non-adjacent nitrogens functionalised with pendant arms that are attached to peptide vectors, such as bombesin, to offer improved tumour targeting. The compound was labelled with ^{177}Lu and in vitro biological studies used to evaluate the selective binding potential. One concern with BFC-natural peptide (such as bombesin, which binds to gastrin releasing peptide receptor) conjugates is that metabolites may be partially responsible for tumour uptake. Linder and co-workers studied the metabolites of the DO3A mono-bombesin system, that is in clinical trials, coordinated to ^{177}Lu [193]. In this case, they determined that tumour uptake in vivo is due to uptake of the parent compound and not the metabolites.

Meares and co-workers used the nitrobenzyl functionalised TETA ligand **L^{64}**, which was first reported in 1985, to label liposomes with ^{64}Cu [194]. The chelator was reduced and then attached via a polyethylene glycol linker to a lipid and then used to tag the liposomes. Preliminary in vivo data showed that the liposomes could be tracked using PET imaging and this technique could potentially be used to monitor drug delivery. In a different approach to increasing the payload for delivery, new multimacrocyclic chelators, such as **L^{65}**, have been developed for attachment of two or three copper centres to an antibody [195]. Conjugation to the B72.3 monoclonal antibody and radiolabelling with ^{64}Cu gave radiopharmaceuticals with higher tumour specificity than monomacrocyclic equivalents.

It is also worth noting that more subtle modification of known compounds can give improvements to in vivo properties. For example, the pharmacokinetics and tumour uptake of ^{64}Cu DOTA labelled peptides can be enhanced by the incorporation of polyethylene glycol chains or tri-glycine linkers [196].

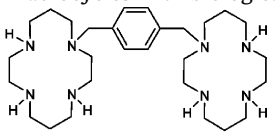
5. Protein binding macrocyclic complexes

Metal complexes which directly bind to proteins have become increasingly of interest as diagnostic probes and therapeutic drugs. They can bind via coordination interactions or weaker interactions between the ligands and the protein [197]. For example, Caravan and co-workers have produced a gadolinium(III) complex, MS-325 that binds to human serum albumin to provide blood pool contrast in MRI imaging [17].

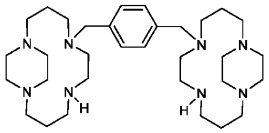
This section details research into macrocyclic complexes that bind to proteins via coordinate bonds and focuses on one particular example. The spatial arrangement of the coordination sites for protein binding is determined by the donor orientation of the macrocycle and the geometric preferences of the metal centre, and hence can be tuned by ligand design and metal ion selection. There is the potential for a new set of rules for designing protein targeting pharmacophores involving both coordination and secondary interactions.

A significant body of work has been reported by Sadler and co-workers and by Archibald and co-workers in studies of molecules that bind to CXCR4, a cell surface receptor protein. Sadler has studied the metal complexes of the known drug molecule Plerixafor, **L^{66}**, and Archibald has investigated complexes formed with new cross- and side bridged macrocyclic chelators. CXCR4 is a chemokine receptor involved in the HIV infection process and implicated in the metastatic spread of some cancers [198,199]

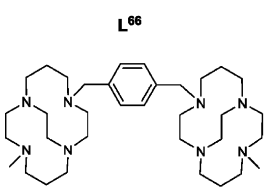
The configuration of the cyclam ring has been shown to be of particular importance in metal complexes which bind to proteins. Sadler and co-workers have continued their research into the metal complexes of the well known biologically active para-xylyl bridged cyclam **L^{66}**, in this study examining the configuration of the nickel(II) complex and its interaction with a protein [200]. The macrocycle is in the cis-V configuration both in the solid state and in solution, which is likely to be the most biologically active form. Protein binding interactions of the metal complexes of **L^{66}** were studied using X-ray crystallography to determine the structure of lysozyme protein with the complex bound [201], representing an important advance in the study of interactions of cyclam type macrocycles with biological macromolecules.



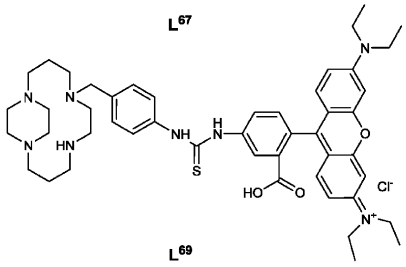
L^{66}



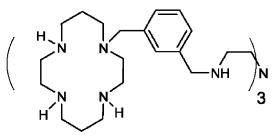
L^{67}



L^{68}



L^{69}



L^{70}

Side bridged cyclam macrocycles (with an ethyl bridge between two adjacent nitrogens) were used to restrict the configuration of the zinc(II) complex with bis-macrocyclic ligand **L[67]**. This complex specifically binds to the CXCR4 chemokine receptor and inhibits HIV infection. It was demonstrated that the configurational restriction generated an optimised compound for receptor binding considerably improving the anti-HIV properties relative to Plerixafor and its metal complexes [202]. Archibald and co-workers have also investigated the copper(II) complexes of **L[67]** [203]. This again increases the activity of the drug compound against the HIV virus, in comparison to the copper(II) complex of **L[66]** due to improved binding to the CXCR4 chemokine receptor protein. Related monoring trans-II fixed copper complexes can be tagged with a fluorescent dye **L[69]** and shown to bind to CXCR4 [204]. However, further work is required to develop these small molecule optical probes as some non-specific uptake is observed that is attributed to the rhodamine dye component. The most recent report shows the extremely potent anti-HIV activity of a cross bridged bis-macrocyclic copper(II) complex, **L[68]**, which may be linked to its increased residence time at the receptor [205]. The stronger binding and slower kinetics of dissociation from the protein may explain the improved activity. Related constrained 'side' bridged and 'cross' bridged bis-cyclen ligands have been synthesised and monometallic copper(II) complexes studied as intermediates in the formation of bimetallic species but no investigation of protein binding or biological activity have yet been carried out [206].

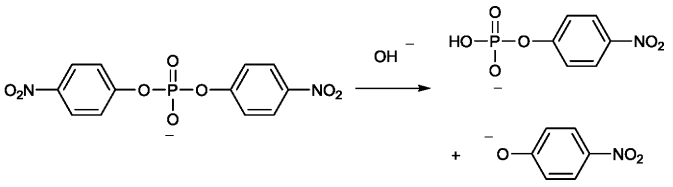
A next step in this research work could be to expand the number of rings and determine the protein binding properties. Such complexes have been reported in the literature but no protein binding studies have been carried out. Complex formation of copper(II) with tris-macrocyclic compounds based on cyclams linked with tris(2-aminoethyl)amine, **L[70]**, was investigated showing stepwise translocation processes of the copper(II), after initial binding, to each of the macrocyclic ring cavities [207]. Tris-macrocyclic cyclam systems with a central benzyl unit have also been synthesised and the palladium(II) complex isolated [208]. Configurationally restricted analogues will be of future interest.

6. Hydrolysis of biological molecules

Zinc(II), copper(II) and the lanthanide ions are commonly used in small molecule mimics of hydrolytic enzymes such as nucleases and proteases [209–211]. The Lewis acidity of the metal ion is the key property for activation of the substrate towards hydrolysis. There is an additional complication with copper(II) systems as there is the potential for oxidative cleavage involving the copper(I)/(II) couple and oxygen species. The examples in this section focus mainly on hydrolysis, with some examples where the role of oxygen is investigated.

The properties of the chelator (donor type and orientation) will have a key influence on the catalytic process. Morrow and co-workers conducted a study of several monometallic macrocyclic complexes [212]. They concluded that an optimised RNA cleavage catalyst should not have exceptionally strong donor groups from the macrocycle and should potentially incorporate functional groups that encourage selective phosphate ester anion binding while destabilising the M–OH interaction.

A frequently exploited model system for determining phosphate diester cleavage rates is bis(*p*-nitrophenyl) phosphate diester, BNPP, as it has a convenient spectroscopic handle for monitoring hydrolysis, see below [213,214]. Selected BNPP cleavage rate constants for the compounds discussed are collected in Table 6.



6.1. Lanthanide(III) complexes

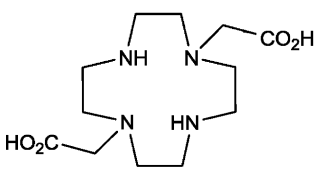
As Lewis acidity is of key importance the higher positive charge of the lanthanide ions is frequently left unmasked by using donor atoms from amide or alcohol pendant groups rather than carboxylates.

In an effort to produce artificial nucleases, the lanthanide complexes of two macrocycles, **L[71]** and **L[72]**, were studied for their ability to hydrolyze BNPP [215]. They are both bis-acid chelators and so the compounds have an overall positive charge. The europium(III) **L[71]** (DO2A) complex showed faster hydrolysis than europium(III) **L[72]** and the preliminary rate constant was fitted to a dimer-monomer model. A further study of the cleavage activity of europium(III) complexes of the dicarboxylate ligand **L[72]** towards a phosphodiester RNA analogue was carried out by Morrow and co-workers [216]. The results showed formation of a hydroxy bridged species at increased pH but both monomeric and dimeric complexes showed low activity in the cleavage reaction, potentially due to the inclusion of the two carboxylate donors per ligand.

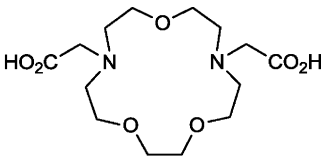
Dinuclear europium(III) complexes for the cleavage of RNA have been formed with ligand **L[73]** [138]. The most important feature of these complexes is that at neutral pH they coordinate water molecules rather than hydroxides, as is observed for dinuclear copper(II) or zinc(II) complexes, which are more easily displaced to allow binding of the phosphate diester species for cleavage. These compounds are amongst the most active synthetic catalysts known but there is still only weak cooperativity between the two metal centres.

6.2. Zinc(II) and copper(II) complexes with triaza macrocycles

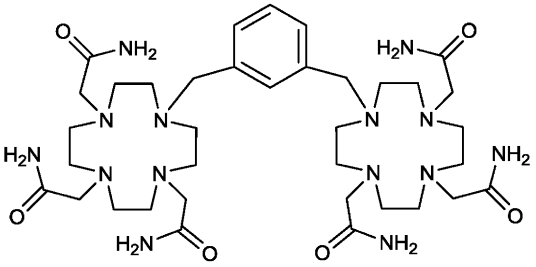
There is considerable interest in the use of triaza macrocycles with first row transition metals (mainly zinc(II) and copper(II)) to form catalysts for carboxy and phosphate ester hydrolysis, with particular relevance to RNA as a target [214,222], see Table 7. The triaza macrocycles are a good match with first row transition metals as they leave two or three free sites on the metal centres that can coordinate water/hydroxide. The simplest compounds studied are monomacrocyclic compounds. The kinetics and mechanism of phosphate diester hydrolysis by the copper(II) complex of 1,4,7-trimethyl-1,4,7-triazacyclonane/Me_3tacn was investigated in depth [214]. BNPP was used as a model compound and the complex was shown to be one of the most efficient divalent metal complexes for cleavage, but the rate constant is still several orders of magnitude below enzymatic systems. Spiccia and co-workers have collected together rate constants for the cleavage of BNPP and NPP of copper(II) tacn derivatives to give a useful set of reference data [214].



L71



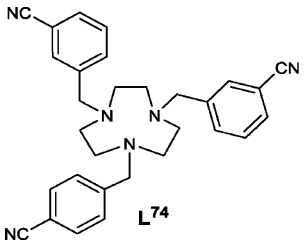
L72



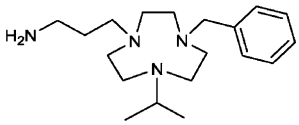
L73

New N-functionalised tacn complexes were used to prepare unusual ferromagnetically coupled hydroxy bridged copper(II) dimers [221]. The phosphate ester hydrolysis properties were investigated and the copper(II) complex of **L74** was shown to have one of the highest rates of BNPP cleavage reported so far for a mononuclear copper(II) tacn complex.

Spiccia and co-workers also produced a novel series of non-symmetrically N-functionalised tacn chelators **L75** [223]. These routes allowed synthesis of ligand systems which have a mixture of pendant arms and the resulting copper(II) complexes were tested for their ability to hydolyse BNPP. The rate constants were rationalized using the structural information on the complexes from X-ray crystallography.



L74



L75

The zinc(II) complexes of 1-oxa-4,7,10-triazacyclododecane, 1,5,9-triazacyclododecane, and 1-hydroxyethyl-1,4,7-triazacyclononane were investigated for their ability to cleave the RNA analogue 2-hydroxypropyl-4-nitrophenyl phosphate(HpNP) [228], see Table 7. All compounds showed cleavage inhibition in the presence of uridine suggesting that the uridine sequences in RNA will not be cleaved by these complexes.

Improvements in the catalytic rates and turnovers within synthetic systems could potentially be achieved by the use of dinuclear or trinuclear systems where the metal ions can catalyse the hydroytic process in a cooperative fashion. The dinuclear zinc(II)

Table 6
Selected rate constants for hydrolysis of bis(p-nitrophenyl)phosphate (BNPP) or p-nitrophenylphosphate (NPP).

Compound	Substrate	Rate, k (either pseudo first order, s^{-1}, or second-order rate constant, $M^{-1}\,s^{-1}$)	Temperature (K)	pH	Comments	Ref.
$[Eu\mathbf{L^{72}}(OH_2)_2]^+$	BNPP	1.01×10^{-7} 1.45×10^{-8}	298	8.50 7.01	[complex] = 1.0 mM and [BNPP] = 0.10 mM I = 0.10 $(CH_3)_4NCl$.	[215]
$[Zn_2\mathbf{L^{84}}]^{4+}$	BNPP	1.28×10^{-3} 1.35×10^{-3}	298	8 9	[complex] = 0.3 – 8 mM, [buffer] = 50 mM, I = 0.1 M (NaCl)	[217]
[Zn (monoring analogue of $\mathbf{L^{87}}$)(OH)]$^+$ $[Zn_2\mathbf{L^{87}}(OH)]^{3+}$	BNPP	19.5×10^{-5} 123×10^{-5}	308	–	Buffer used to adjust pH to form hydroxide species, pK_a = 8.0.	[218]
$[Zn_2\mathbf{L^{90}}(OH)_2]^{2+}$	BNPP	9.6×10^{-4}	308	–	Only the dihydroxide species is active (pK_a values of 7.60 and 8.82)	[219]
Silica bound copper(II) tacn complexes	BNPP		295	7.8	The surface of the silica was functionalized with either ethyl or propanol groups to influence hydrophobicity.	[220]
Four carbon link to surface (hydrophobic)		0.97×10^{-10}				
Eight carbon link to surface (hydrophobic)		1.3×10^{-10}				
Eight carbon link to surface (less hydrophobic)		0.89×10^{-10}				
$[Cu(Me_3tacn)(OH_2)_2]^{2+}$	BNPP	0.66×10^{-4}	323	7.4	[complex] = 2 mM	[214]
$[Cu(Me_3tacn)(OH_2)(OH)]^+$	BNPP	650×10^{-4}	323	–	[BNPP] = 15 μM, [complex] = 1.0 – 7.5 mM, [buffer] = 50 mM, pH range 6.3 – 9.1, I = 0.15 M (taken from initial rate measurements)	[214]
Copper(II) dimers	BNPP		323	7.4	[BNPP] = 15 μM, [complex] = 1.0 mM, I = 0.15 M ($NaClO_4$) Solvent mixture 1:3 $MeCN/H_2O$.	[221]
Me_3tacn		11.2×10^{-5}				
1,4-Dimethyl-tacn		1.24×10^{-5}				
1,1-Benzyl-4,7-dimethyl-1,4,7-tacn		7.01×10^{-5}				
1,4,7-Tris(3-cyanobenzyl)-1,4,7-tacn ($\mathbf{L^{74}}$)		30.2×10^{-5}				
$[Cu(Me_3tacn)(OH_2)_2]^{2+}$	NPP	1.93×10^{-6}	323	7.4	[complex] = 3 mM, [substrate] = 15 μM	[214]

Table 7
Rate constants for cleavage of RNA, RNA analogue and other phosphate ester substrates.

Compound	Substrate[a]	Rate constant k[b]	Temp./K	pH	Comments	Ref.
$[Eu\mathbf{L^{72}}(OH_2)_2]^+$	HpPNP	1.7×10^{-5} s^{-1}	298	8.0	[complex] = 1 mM	[216]
$[Eu_2\mathbf{L^{73}}]^{6+}$	HpNP 5′UpU3′	3.5 M^{-1} s^{-1} 0.021 M^{-1} s^{-1}	298	7.6	–	[138]
[Eu(tris(carbamoylmethyl)cyclen)]$^{3+}$ [Eu(tris((N-ethyl)carbamoylmethyl)cyclen)]$^{3+}$ [Eu(tris((N,N-diethyl)carbamoylmethyl)cyclen)]$^{3+}$	HpNP	0.042 M^{-1} s^{-1} 0.12 M^{-1} s^{-1} 0.054 M^{-1} s^{-1}	298	7.6	–	[141]
$[Zn(cyclen)(OH_2)]^{2+}$ $[Zn(12[ane]N_3O)(OH_2)]^{2+}$ $[Zn(12[ane]N_3)(OH_2)]^{2+}$ $[Zn(N\text{-hydroxyethyltacn})(OH_2)]^{2+}$	HpNP	1.5×10^{-3} M^{-1} s^{-1} 2.8×10^{-2} M^{-1} s^{-1} 1.8×10^{-2} M^{-1} s^{-1} 7.2×10^{-2} M^{-1} s^{-1}	298	pH/rate profiles recorded	The limiting second-order rate constants were determined at high pH.	[212]
$[Zn_2bis(12[ane]N_3)]^{4+}$ $[Zn_3tris(12[ane]N_3)]^{6+}$ (linked by oxymethyl benzene group)	5′CAACAC3′	0.24×10^{-6} s^{-1} 0.44×10^{-3} s^{-1}	308	7.50	–	[224]
$[Zn_2\mathbf{L^{76}}(OCH_3)]^{3+}$	Methyl (2-chloro-4-nitrophenyl) phosphate diester	1400 M^{-1} s^{-1}	298	9.8	Methanolysis of methyl aryl phosphate diesters (rate enhancement 1.2×10^{12})	[225]
$[Zn\mathbf{L^{89}}]^{2+}$ (protonated form)	ATP	0.07 M^{-1} s^{-1}	298	5.4	Requires 2nd equivalent of zinc for cleavage.	[226]
$[Cu_2\mathbf{L^{77}}]^{4+}$	5′pCpA bond in 5′AACAUC3′	1.3×10^{-5} s^{-1}	323	7.4	[complex] = 50 μM [RNA] = <0.2 nM	[227]

[a] HpNP = 2-hydroxypropyl-4-nitrophenyl phosphate.
[b] Either pseudo first order or second-order rate constant.

methoxide complex formed with the bis-macrocyclic propyl bridged 1,5,9-triazacyclododecane ligand $\mathbf{L^{76}}$ (bis[12]aneN$_3$) showed a remarkably high catalytic activity for the cleavage of model RNA systems in methanol, particularly in comparison to the activity under aqueous conditions [225,229]. A synergistic effect of the medium was proposed with, amongst other factors, enhancement of the catalytic activity via desolvation and stabilisation of a charge dispersed transition state.

Calixarenes functionalised with copper(II) complexes of [12]aneN$_3$ macrocycles at the upper rim, such as $\mathbf{L^{77}}$, have been used for the cleavage of single strand ribonucleotides [227]. Di- and trimetallic complexes were investigated. A cooperativity enhancement due to multiple metal centres was observed for the dicopper compounds but there was no evidence for the involvement of the third copper(II) centre in the trimetallic species.

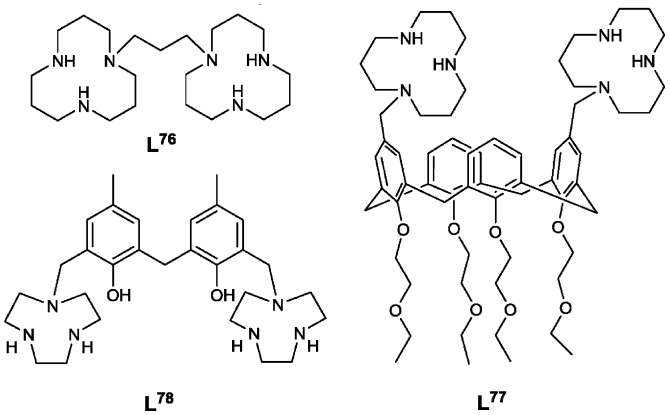
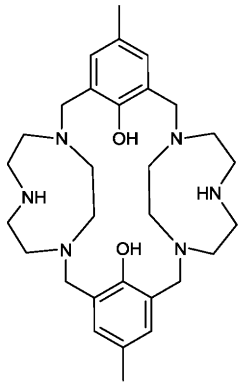


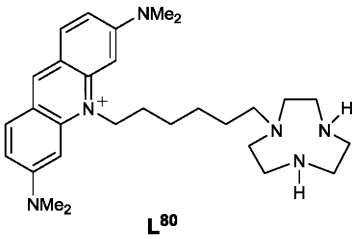
Table 8
Catalytic cleavage of DNA using macrocyclic complexes.

Compound	Supercoiled plasmid DNA substrate	Rate (k either pseudo first order or indication of timescale)	Temperature (K)	pH	Comments	Ref.
1,7-Dimethylcyclen	pBR322	6×10^{-4} s^{-1}	310	7.2	No metal ions present, [cyclen] = 0.2 mM, converted to linear form	[236]
$[Cu_2\mathbf{L^{79}}]^{2+}$	pBR322	Linear DNA appears after 0 h. DNA completely degraded after 6 h (in presence of hydrogen peroxide).	310	7.2	Oxidative mechanism for the copper(II) species.	[231]
$[Zn_2\mathbf{L^{79}}]^{2+}$		ca. 70% cleaved to linear DNA after 40 min (in the presence of mercaptoethanol).				
$[Cu\mathbf{L^{80}}]^{3+}$	pBluescript II	ca. 8% cleavage after 12 h.	323	7.8	[complex] = 0.5 mM, no difference was observed for aerobic and anaerobic conditions	[232]
$[Cu\mathbf{L^{83}}]^{4+}$	pUC19	1.7×10^{-4} s^{-1}	310	7.5	Saturation kinetic profile (ca. 10^8-fold rate acceleration over uncatalyzed reaction)	[238]
$Zn_2\mathbf{L^{85}}$	pUC 19	Compared to $[Zncyclen]^{2+}$ control, an increase from 13.6% vs 7.8% of the nicked form was observed	310	7.8	Incubated with DNA for 12 h	[239]
Copper(II) peptide nucleic acid linked cyclen	pUC 19	ca. 55% plasmid relaxation after 4 h	310	7.0	[complex] = 0.143 mM	[235]

A new bis-macrocyclic chelator was synthesised by linking two tacn macrocycles through a bis-cresol, **L[78]**, to provide two tetradentate coordination sites [230]. However, at lower pH values the macrocycle remains protonated and only coordinates through two of the ring nitrogen donors. The X-ray structure of the copper(II) complex shows two independent five coordinate metal centres bound to the four available donors and a nitrate counter anion.



L[79]



L[80]

In the formation of a more rigidly linked structure, two tacn macrocycles were bridged by the two 4-methyl phenol units to form **L[79]**, providing two N_3O_2 cavities and forming five coordinate bimetallic complexes with copper(II) and zinc(II) [231]. The complexes bind to calf thymus DNA and show nuclease activity in the presence of H_2O_2, see Table 8.

An alternative approach to improve DNA cleavage by metal complexes is to attach an intercalating group bringing the metal ion in close proximity to the target. A copper(II) macrocyclic complex, **L[80]**, was synthesised by Burstyn and co-worker [232]. However the results were mixed, with cleavage experiments showing that the free complex with no intercalating group is more active at low concentrations, see Table 8. A potential future target is a cooperative system with multiple metal centres and an intercalating group.

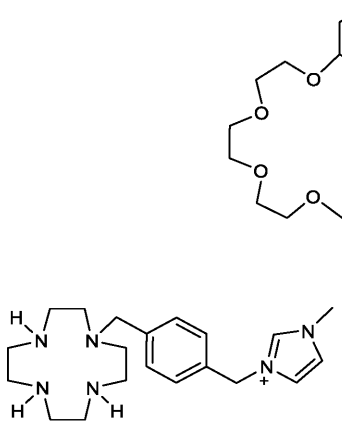
The next step up from a linked cooperative system containing two metal centres is a system containing three metal centres. Three [12]aneN_3 chelators were linked, the zinc(II) complex formed and a comparison of cleavage properties with short RNA strands made between di- and trinuclear compounds [224]. Selectivity was observed for different phosphodiester cleavage sites for each of the two compounds, however this was only applicable to short RNA strands as random background cleavage became an issue as the strand length increased.

Another approach Burstyn investigated was the immobilisation of copper(II) tacn units onto a silica surface [220]. Different spacers were used and modifications were made to the hydrophobicity of the surface, with the octyl spacer providing the optimum catalytic decomposition of BNPP. The main issue to be solved is the inefficient mass transfer, as the rate is slowed both by the access to the catalytic units and inhibition by the product remaining on the surface. A more porous support may improve the catalytic rates.

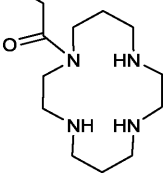
6.3. Zinc(II), cobalt(II) and copper(II) complexes with tetradentate and larger macrocycles

Larger ring macrocyclic complexes are also suitable for the formation of hydrolysis catalysts and in these examples activity is frequently increased by the addition of pendant arms. Once the number of donors in the macrocyclic rings has exceeded four there is an increased likelihood of dimeric complexes forming with a stoichiometry of M_2L.

Zinc(II), copper(II) and cobalt(II) complexes of a mono-imidazolium armed cyclen ligand, **L[81]**, were synthesised and analysed for their plasmid DNA cleavage properties [233]. The high activity observed, with the cobalt(II) complex the most active, was attributed to the presence of the imidazolium positive charge activating the phosphate ester. In an effort to mimic metalloenzyme type substrate binding, a crown ether receptor was combined with an azamacrocycle to form a hybrid receptor, **L[82]**, that was shown to bind both a zinc(II) ion and a series of phosphate derivatives [234]. The copper(II) complex was also synthesised and showed poor binding of phosphate.



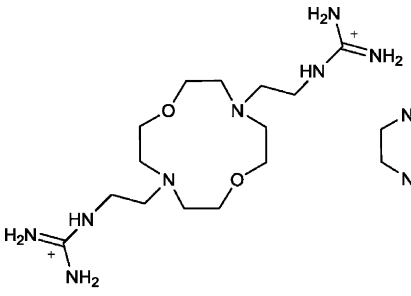
L[81]



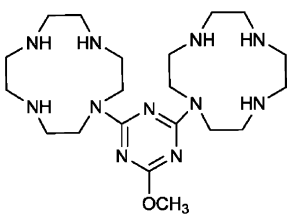
L[82]

Peptide nucleic acid linked cyclen complexes were synthesised as DNA cleavage agents and the cleavage of supercoiled DNA studied with zinc(II), cobalt(II) and copper(II) ions bound [235], see Table 8. It was suggested that the cleavage took place through a hydrolytic rather than an oxidative pathway, but the copper(II) complex was the most efficient catalyst, producing selectively nicked DNA in high yields. The abilities of cyclen type macrocycles to cleave DNA in the absence of metal ions has also been investigated with 1,7-dimethyl cyclen showing the ability to hydrolytically cleave DNA, it was noted that catalytic cleavage only occurred for N-alkylated cyclens [236]. Anthracene appended cyclens have also been studied for DNA cleavage potential as the free macrocycles [237]. It was noted for these compounds, that appending two anthracene intercalating groups promoted stronger binding and more efficient DNA cleavage.

The 14 membered oxa-aza macrocycle **L[83]** has been synthesised with bis-guanidinium pendant arms and the copper(II) complex binds to calf thymus DNA with the positively charged guanidinium groups improving the interaction [238]. The guanidinium groups also enhance the catalytic cleavage with cooperative effects increasing the overall activity of the copper(II) catalyst.



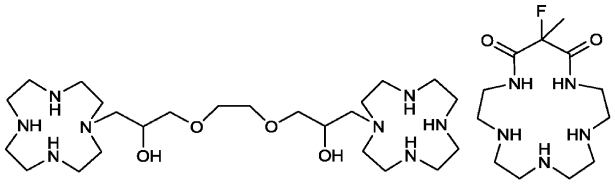
L[83]



L[84]

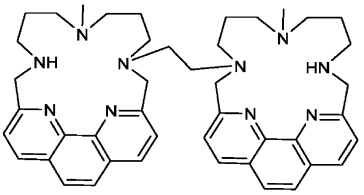
In an analogous fashion to the tacn ligand, cyclen can be used as a stable unit to form multinuclear catalysts. A series of new linked

cyclen ligands with different triazine and pyridine spacers were synthesized, such as **L[84]**, and the zinc(II) complexes shown to be active catalysts for the cleavage of a carboxyester model under physiological conditions [240]. In the dinuclear systems, the spacer length influences the degree of cooperativity between the zinc centres but does not have a major effect on the rate as the two metal centres can act independently. The zinc(II) **L[84]** complex is an active catalyst for the hydrolysis of BNPP and the influence of the spacer was used to determine the importance of cooperativity between the two metal centres [217]. Optimizing the cooperative process increased the catalytic rates and gave species that are active at pH < 9.

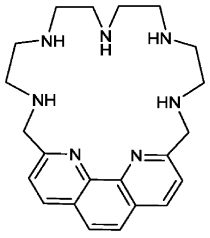


L[85]

L[86]



L[87]



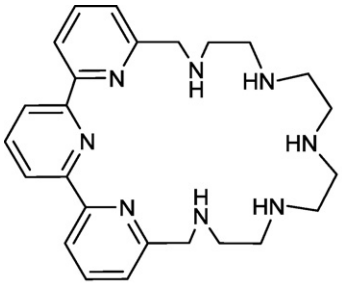
L[88]

A longer spacer in the form of a polyether chain was investigated with bis-cyclen complexes of zinc, **L[85]**, which were tested as DNA cleavage agents [239]. It was shown that the compounds catalyze the cleavage of supercoiled DNA to produce selectively nicked DNA. Related bis-macrocyclic zinc(II) cyclen compounds with both rigid and flexible spacers have also been synthesised and their interactions with plasmid DNA probed [241]. Increasing the rigidity of linker groups gave improved DNA cleavage properties for these examples.

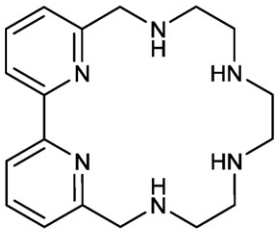
The pentadentate macrocycle 15-fluoro-15-methyl-1,4,7,10,13-pentaazacyclohexadecan-14,16-dione was synthesized, **L[86]** and copper(II) and zinc(II) complexes formed [242]. The X-ray structures showed that coordination was either via the three amine nitrogens as a tridentate ligand or on deprotonation through all five nitrogens as a pentadentate ligand. Their interaction with DNA was investigated and some copper(II) complexes induced a B-to Z-DNA transition but no cleavage was observed. Combining a pentadentate macrocycle with a cooperative strategy, a novel phenanthroline based bis-macrocyclic ligand, **L[87]**, was synthesised and the complexes formed with a series of transition metals [218]. The bis-zinc(II) complex efficiently hydrolyzed BNPP with cooperative interaction of the two metal centres and π-stacking with the substrate.

A macrocyclic ligand also incorporating a phenanthroline, but in this case a heptadentate 28 membered ring with five secondary amine donors, **L[88]**, was investigated for its ability to form mono- and bi-nuclear complexes with copper(II) and their binding interactions with DNA [243]. The larger terpyridine analogue, **L[89]**, which binds zinc(II) at one end of a cavity was shown to catalyse the hydrolysis of ATP in the presence of a second metal ion, which acts as a co-factor to transfer the phosphoryl group from the ATP to a macrocyclic amino group [226].

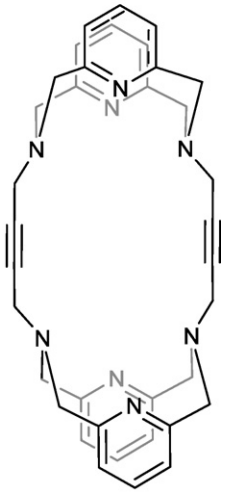
Bazzicalupi and co-workers also investigated the DNA cleavage properties of zinc(II) complexes with related bipyridine containing chelators, such as **L[90]** [219]. Many of the compounds synthesised had poorly nucleophilic Zn-OH units and were inactive in the cleavage of BNPP, however they were still active in the cleavage of DNA due to favourable positioning of the unit by intercalation demonstrating the enhancement from optimised location.



L[89]



L[90]



L[91]

The anion binding properties of the dicopper(II) complex of the 24 membered [18]aneN6 were investigated, showing significant selectivity for phosphate monoesters and it was suggested further improvements to selectivity could be achieved by the addition of C-substituted functionalised side chains, mirroring the strategy adopted with smaller ring macrocycles [244].

A large ring alkyne macrocycle with two pyridyl groups at each end of the cavity, **L[91]**, was synthesised and the dicopper(II) complex shown to be a phosphoryl transfer catalyst [245]. This complex promotes the transesterification of simple inert dialkyl phosphates and the mechanism was investigated using DFT calculations.

7. Other applications of macrocyclic complexes in biological systems

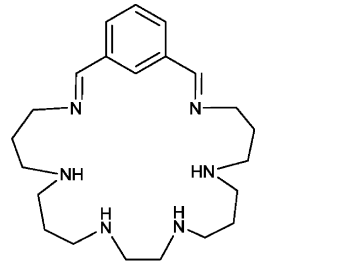
7.1. Detection and delivery

Examples of fluorescent groups that can report on reactions or for the detection of analytes are discussed in Section 3. Electrochemical detection of analytes is also possible using macrocyclic chelators, and is usually dependent on a binding interaction with the coordinated metal centre. For example, the adsorption of a copper(II) complex formed with hexaaza macrocycle, **L[92]**, onto a graphite electrode gives an electrochemical sensor which selectively detects L-glutamate in the presence of L-aspartate [246]. The two copper ions are thought to be coordinated at either end of the cavity with the amino acid binding in a bridging mode. The cyclen macrocycle has been used as part of a sensing unit by attachment of an electrochemical reporter which responds to binding events at the metal centre. Copper(II) and zinc(II) complexes of a cyclen appended with a pendant ferrocenyl, **L[93]**, have also been studied [223]. The copper(II/I) redox couple shows a strong solvent dependency related to the coordinating ability of the solvent and the ferrocenyl couple showing much weaker effects. The zinc(II) complex was studied for its ability to recognise thymine

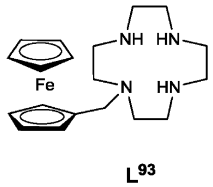
with the aim of electrochemical sensing in aqueous systems. This system needs further development as only minor changes in the ferrocene redox potential were observed under physiological conditions.

Another analytical method is to use the metal complex as a tag that can be detected in subsequent mass spectrometry analysis. This could be used as a system for proteomic analysis of the proteins using mass spectrometry. The isothiocyanate functionalised DOTA ligand, **L[94]**, was used to label proteins with lanthanide complexes by reaction with surface lysine residues forming thiourea linkages [247]. The advantage of using lanthanide labels is that due to their similar properties the lanthanide ion can be varied and the resulting complexes used for multi-element labelling that can be detected by ICP-MS.

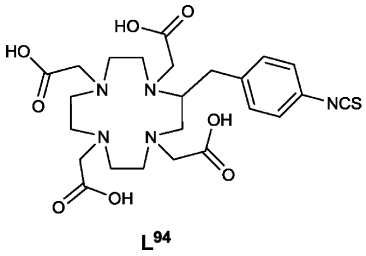
The detection of a biological binding event can be achieved by monitoring a change in the coordination environment of a metal centre using EPR. Watkinson and co-workers attached biotin to a cyclam ligand used the Huisgen [3+2] cycloaddition to form a triazole heterocycle linking the two units [248]. The triazole coordinates to the copper(II) centre until the biotin binds to its protein partner (avidin) and the interaction is disrupted. The modular synthesis lends itself to the facile production of a series of protein binding probes.



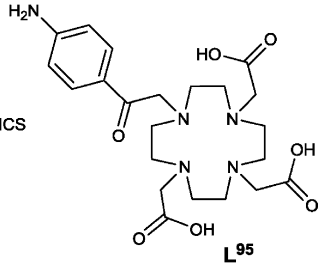
L[92]



L[93]



L[94]



L[95]

Amide functionalised DO3A lanthanide complexes have been developed as luminescent probes for protease activity [249]. **L[95]** is produced when an amide group attached to a peptide substrate is cleaved to unmask the primary amine, which is a significantly better antenna group for sensitisation of terbium(III) luminescence. This results in a time dependent increase in intensity that can be linked to enzyme activity.

A further potential application is the delivery of payloads to cells and tissues. Tacn ligands were functionalised with long chain alkanes and used to form CuL_2 sandwich complexes that assembled into metalloliposomes in aqueous solution for DNA delivery into mammalian cells [250]. Both the dissociation rates and redox potentials of the copper(II) ions were proposed to influence the efficiency of the transfection process.

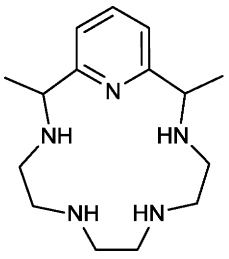
7.2. Therapeutic applications

Therapeutic drugs are of interest with potential compounds including cytotoxics to target diseases such as cancer and enzyme mimics to influence biochemical pathways (therapeutic radiopharmaceuticals are discussed in Section 4).

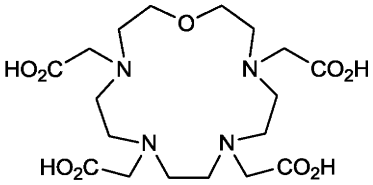
Ruthenium complexes with non-macrocyclic ligands have been of interest as a potential next generation of cytotoxic anti-cancer drugs [1]. A series of ruthenium [9]aneS_3 complexes was synthesised with chelating dicarboxylate ligands [251]. Both mononuclear and bridged dinuclear compounds were characterised and the mechanism of isomerization elucidated for one of the species. The control of coordination environment with such chelators may offer some advantages in controlling the biological properties.

Active enzyme mimics incorporating macrocyclic chelators have been tested in biological systems, with particular success in the generation of superoxide dismutase (SOD) mimics. Riley and co-workers played a key role in opening up this research area in the 1990s and researchers are continuing to investigate the potential of such compounds [252]. The use of pentaaza-macrocyclic manganese(II) compounds as SOD mimetics has been further investigated [253]. Chelators such as **L[96]** that forms the basis of Riley's compounds, give seven coordinate complexes. The rates of water release in the formation of six coordinate intermediates were thought to be of particular importance with the exchange rates shown to be related to the π-acceptor abilities of the ligands.

Alternatively, as in biology, other metals such as copper(II) can be used as the redox component in SOD mimics. Carboxylate functionalised macrocyclic copper(II) complexes were examined for their superoxide scavenging ability and their potential as in vivo therapeutic agents [254]. The most active complex was not stable at physiological pH but the best compound formed with ligand **L[97]** offered a complementary combination of stability and activity.



L[96]



L[97]

Another application of coordination chemistry to medicine is the use of strongly binding ligands in chelation therapy where the concentration of 'free' metal ions is reduced by complex formation. Carboxylate functionalised tacn chelators were used to deplete iron in cellular systems in an effort to cause toxicity to cancer cells, particularly targeting the transferrin receptor and ribonucleotide reductase [255]. Bifunctional chelator versions were also produced, such as **L[98]**, to allow conjugation of dyes and tracking of the chelator uptake by the cells [170]. Chong and co-workers then went on to attach bile acid to this chelator to target these chelators to colon cancer cells which overexpress the bile acid transporter [256]. The cytotoxicity was comparable to the free chelator and cellular uptake was observed by attachment of a fluorescent dye. These compounds have potential for use as targeted clinical agents with higher cytotoxicities observed than for current clinical iron chelating agents.

Metal ion chelation can also be used to prevent or interrupt essential disease development pathways. The role of copper (and potentially other metal ions) in prion related disorders has been under recent investigation. Although the exact role in disease progression is unclear, some copper porphyrin based compounds have been investigated for their ability to inhibit the formation of abnormal prion protein in cell lines and intriguingly their efficacy was linked to both copper chelating ability and SOD type activity [257].

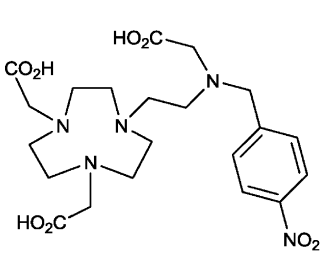
7.3. Dual modality and dual functional agents

More complex constructs that have multiple functions are being produced. Advances in medical imaging technology could be achieved by combining imaging and therapeutic compounds or the production of dual modality agents which can be used to image the target using two different imaging techniques (e.g. MRI and fluorescence) [258].

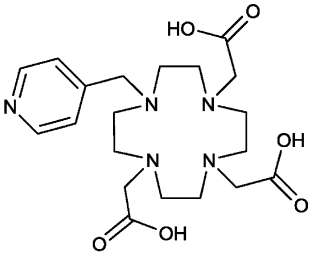
A heterobimetallic complex containing a rhenium(I) bipyridine chromophore linked to a DO3A gadolinium(III) complex via a pyridyl group on the ligand, **L^{99}**, was investigated as a dual modality MRI luminescence imaging agent [139]. The luminescence lifetime of the rhenium(I) unit is sufficient for gating out background fluorescence from cellular components. However, the concentration ranges for detection of each of the components are not an ideal match. Improvements need to be made to the MRI component to facilitate detection at nanomolar concentrations, which equates with the efforts to provide higher relaxivity agents that were discussed in Section 2. New synthetic routes to link fluorescent/luminescent groups and macrocycles are required [259].

An alternative attempt to combine fluorescence and MRI imaging agents focussed on the use of a larger fluorescent construct that could support multiple gadolinium(III) chelate units to increase relaxivity. DO3A type chelators were used to attach GdL units to a Qdot fluorescent nanoparticle giving a dual modality fluorescence/magnetic resonance contrast agent [260]. This combines near-IR fluorescence imaging, which has increased tissue penetration, with MRI contrast properties, improving the potential for translation to in vivo diagnostic applications. Another group focused on new constructs for cancer imaging, again attaching gadolinium(III) DOTA complexes to the surface of nanoparticles. Electrodeposition was used to build up layers on the surface of a hybrid silica nanoparticle with a ruthenium bipyridine luminescent core [261]. Non-covalent functionalisation with peptides modified the construct to target human colon cancer cells (HT-29).

An alternative use of the gadolinium(III) unit is to act as a quencher for the MRI signal of another nucleus. A dual modality imaging agent that is activated by the caspase-3 protease enzyme was designed by attaching a substrate peptide (DEVD) tagged with both a dye and ^{19}F group to a gadolinium(III) cyclen unit [262]. When the peptide is cleaved the dye fluorescence increases and the ^{19}F is no longer subject to the paramagnetic relaxation enhancement effect of the gadolinium(III) (the ^{19}F relaxation time increases from 0 to 0.38 s). This gives a dual response probe with a detectable response via both fluorescence and the ^{19}F MR signal.



L^{98}

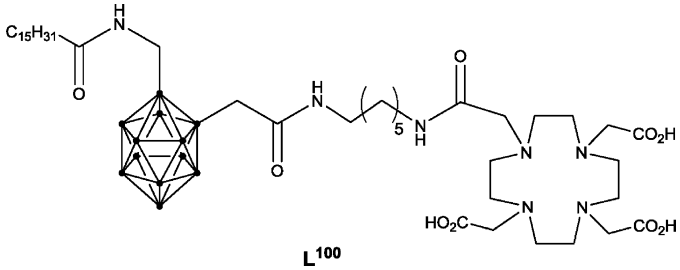


L^{99}

DOTA chelators can be used to label somatostatin peptides with PET emitters such as ^{64}Cu or ^{177}Lu to monitor their accumulation in the tumour tissue due to selective binding or uptake [263]. Dual imaging agents were then generated by combining this construct with a fluorescent cyanine dye, however in vivo studies showed that the conjugates did not show the desired receptor mediated targeting properties. This study illustrates the information that can be gained by the dual functional approach validating the concept. Further work will be required to ensure that target localisation is achieved with this system.

The isothiocyanate functionalised DOTA BFC was used to attach ^{64}Cu labelled macrocycles to magnetic nanoparticles for dual modality PET/MRI imaging probes [264]. A novel coupling method was developed with a diamine spacer incorporated to link to aldehyde groups generated from dextran sulfate coated onto the iron oxide nanoparticles.

A combined imaging/therapeutic agent allows the biodistribution to be tracked and potentially the concentration of the agent to be determined. If an external force is going to be applied to activate the therapy this is particularly valuable. A dual functional agent of this type has been synthesised to combine boron neutron capture therapy and imaging via MRI [98], in which a gadolinium(III) DO3A unit is linked to a decaborane and a long hydrophobic chain attached to allow targeting of the tumour cells via the overexpressed lipoprotein transporters, **L^{100}**. The gadolinium(III) centre also improves the neutron capture properties of the conjugate.



L^{100}

8. Conclusions

Macrocyclic complexes have key properties, such as high kinetic and thermodynamic stability, that match well with biomedical applications. Optimization of chelator design together with a developing understanding of how to transport and control the localisation of metal complexes in vivo will underpin future success in this area.

Molecular imaging will continue to offer the greatest opportunity for successful to clinical use. Goals include the imaging of the metastatic spread of cancer; an important aim for the future as this is the key factor in poor clinical outcome for cancer patients [265]. But there is also considerable scope for therapeutics with both cytotoxics and protein binding metal complexes offering potential for future medical impact.

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