# Design of targeting ligands in medicinal inorganic chemistry

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***Received 31st March 2006***
***First published as an Advance Article on the web 20th April 2006***
**DOI: 10.1039/b514859f**

This *tutorial review* will highlight recent advances in medicinal inorganic chemistry pertaining to the use of multifunctional ligands for enhanced effect. Ligands that adequately bind metal ions and also include specific targeting features are gaining in popularity due to their ability to enhance the efficacy of less complicated metal-based agents. Moving beyond the traditional view of ligands modifying reactivity, stabilizing specific oxidation states, and contributing to substitution inertness, we will discuss recent work involving metal complexes with multifunctional ligands that target specific tissues, membrane receptors, or endogenous molecules, including enzymes.

## 1 Introduction

Medicinal inorganic chemistry is a discipline of growing significance in both therapeutic and diagnostic medicine. The discovery and development of the antitumour compound cisplatin (*cis*-$[Pt(NH_3)_2Cl_2]$) played a profound role in establishing the field of medicinal inorganic chemistry. Cisplatin, and the second generation alternative carboplatin, are still the most widely used chemotherapeutic agents for cancer, greatly improving the survival rates of patients worldwide.

The history and basic concepts of medicinal inorganic chemistry have been recently reviewed.[1–3] The field now encompasses active metal complexes, metal ions, and even metal binding compounds as potential agents. Metal ions can be introduced into a biological system either for therapeutic effect or as diagnostic aids. Alternatively, metal ions can be removed from a biological system by judicious use of metal-binding molecules (termed ligands from the Latin word ligare, meaning that which binds).

Ligands are most often, but not limited to, organic compounds that bind metal ions, thus modifying the physical and chemical properties of the ion(s). Ligands can be introduced into a system to limit the adverse effects of metal ion overload, inhibit selected metalloenzymes, or facilitate metal ion re-distribution. Some of the aforementioned effects include modifying reactivity and lipophilicity, stabilizing specific oxidation states, and contributing to substitution inertness; however purposeful design today can go well beyond these effects. Tailored, multifunctional ligands for metal-based medicinal agents offer many exciting possibilities, and can play an integral role in muting the potential toxicity of a metallodrug to have a positive impact in areas of diagnosis and therapy.

In biological systems, metal ions exist as electron-deficient cations and are hence attracted to electron-rich biological molecules such as proteins and DNA. Biological systems themselves provide innumerable examples of 'designer ligands'

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that bind metal ions to perform important biological functions. Examples are the iron metalloprotein haemoglobin, which acts as a molecular shuttle for oxygen *via* reversible binding of $O_2$ to the iron centre, as well as manganese superoxide dimutase (MnSOD) and catalase, metalloenzymes that are both part of the body's antioxidant defence system. Building on the knowledge of the mode of action of MnSOD, synthetic mimics have been developed utilizing ligands that closely match the properties of the native enzyme system.[4] Our increased understanding of metalloenzyme function has also led to the development of artificial metalloenzymes, capable of numerous stereospecific transformations.[5]

This review will discuss how tailored, multifunctional ligands are contributing to exciting advances in medicinal inorganic chemistry. Topics covered include combination agents, targeted chemotherapeutics, lanthanide-based diagnostics, radiopharmaceuticals, and metal binding agents, ending with a section describing how the field of glycobiology has contributed to the development of improved metal-based drugs. Multifunctional ligands have found application at the forefront of all areas of medicinal inorganic chemistry research.

## 2 Combination agents

Chelation (characteristic of a chela, the pincer-like claw of a crustacean) is used here to describe the process of binding a ligand to a metal ion as opposed to the controversial medical procedure. Chelation offers an approach to fine-tune a metal's properties; it also offers a means to modify a metal-based drug by introducing ligands that are themselves active pharmaceutical agents. In this manner, a single chemical entity can be developed that is able to modulate multiple targets simultaneously, possibly delivering superior efficacy against complex diseases.[6] In the case of metal-containing combination agents, the pharmaceutically active ligand must either have an endogenous metal binding function, or a ligating moiety must be appended to form the complex. Cu(II) and Au(I) complexes of the pharmaceutically active compounds clotrimazole and ketoconazole are examples of combination agents exhibiting potent activity against the parasite *Trypanosoma cruzi*, the causative agent of Chagas disease.[7] Both clotrimazole and ketoconazole contain an imidazole moiety that binds to the metal ion, with the resulting compounds showing enhanced effects compared to the non-metal analogs.

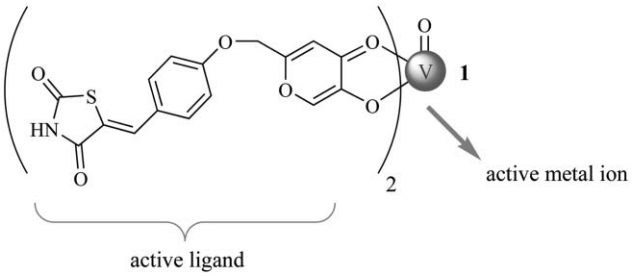


**Fig. 1** Vanadium–thiazolidinedione combination agents (**1**).[8]

Similarly, vanadium (as vanadyl ($VO^{2+}$) or vanadate ($VO_4^{3-}$)) is known to enhance the effects of insulin, whereas the thiazolidinediones act by indirectly improving peripheral insulin sensitivity. The combination of these two agents could therefore potentially modulate multiple targets, offering a new treatment option for diabetes therapy. We have recently reported the synthesis and biological evaluation of a series of vanadium–thiazolidinedione combination agents for diabetes therapy.[8] These vanadium–thiazolidinedione complexes **1** (Fig. 1) showed promising characteristics in an *in vivo* study.

## 3 Anticancer agents

The development of metal-based agents for the diagnosis (*vide infra*) and therapy of cancer has greatly increased survival rates of cancer patients worldwide. The platinum-based compound cisplatin, and the second generation alternative carboplatin, are still the most widely used chemotherapeutic agents for treating solid malignancies. Both compounds appear to act by forming adducts with DNA, thereby interfering with transcription and DNA replication to trigger apoptosis of the cell. Unfortunately, platinum-based anticancer agents are non-specific, resulting in significant toxicity. In addition, cancers of various types, including lung, colorectal, and ovarian are intrinsically resistant to platinum-based agents. The development of tissue-selective agents for cancer therapy, based on our increased understanding of the biochemical differences between normal and cancerous tissue, is now a realizable goal.

Recent advances in ligand design have resulted in potent anti-tumour compounds that are active in cisplatin resistant

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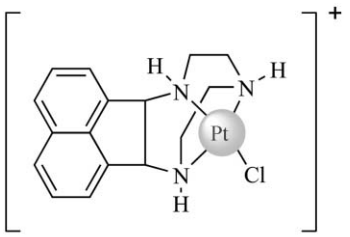
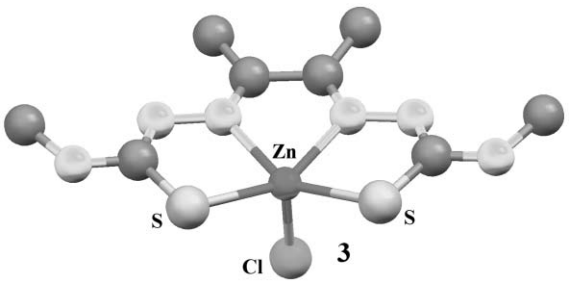
*Chris Orvig was born and raised in Montréal. He received his BSc in chemistry from McGill University in 1976 and subsequently pursued graduate studies (NSERC – of Canada scholar) in technetium chemistry at MIT with Prof. Alan Davison, receiving the PhD in 1981. He was then an NSERC postdoctoral fellow with Prof. Kenneth N. Raymond at the University of California, Berkeley in 1981–83. After one year with the late Prof. Colin J. L. Lock at McMaster University, he joined the faculty of the Department of Chemistry at the University of British Columbia in 1984, where he is now Professor of Chemistry and Pharmaceutical Sciences, and Director of the Medicinal Inorganic Chemistry Group, as well as graduate advisor. His scientific interests are firmly based in the areas of medicinal inorganic chemistry and coordination chemistry—he has been involved over the years with radiopharmaceutical chemistry, metal ion decorporation, and metal ion neurotoxicology, as well as chemotherapeutic metal complexes and ligands. Orvig is the inorganic chemistry editor of Can. J. Chem. and sits on numerous editorial boards and advisory panels. He has received various research and teaching awards, has published more than 150 research papers, and is a co-inventor on many issued patents; he is also a certified ski instructor.*




**2**

**Fig. 2** Pt(II)-intercalative compound **2**.[9]




**3**

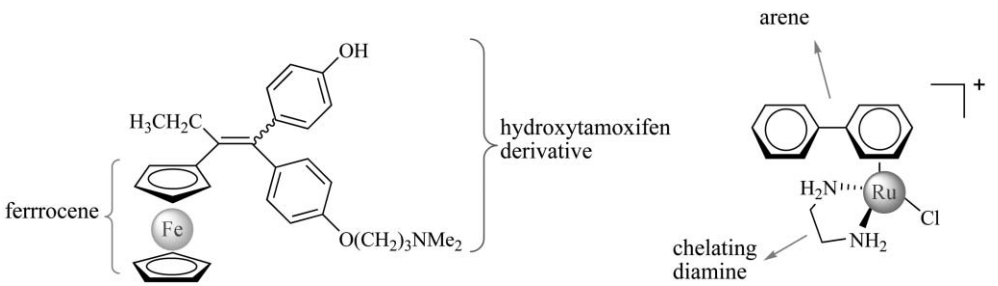
**Fig. 3** The crystal structure[10] of a Zn(II) bis(thiosemicarbazone) complex **3**.

cell lines, and also include additional features to allow for an increased understanding of the mechanism of action of the drug. As an example, a series of Pt(II) compounds with additional intercalative moieties have been developed utilizing a π-conjugated macrocyclic ligand (Fig. 2).[9] The resulting derivatives **2** were shown to have enhanced *in vitro* activity compared to cisplatin in all cell lines tested. The enhanced activity could be attributed to the intercalative moieties, because the macrocyclic derivative without the appended naphthalene did not show the same level of activity. These compounds were further appended with the luminescent probe rhodamine to investigate cell uptake.

Mechanistic details of cellular uptake and mode of action of chemotherapeutics are incomplete, hence new modalities that could offer important therapeutic insights are much sought after. While addition of an optical probe may allow for visualization of the intercalative Pt(II) compounds *in vitro*, the physicochemical properties of the resultant molecules are most likely changed with the addition of the probe. The ideal situation would be to have the optical probe built-in to the molecule. An example of chemotherapeutic agents satisfying this criterion is a series of Zn bis(thiosemicarbazone) complexes (Fig. 3) developed by Dilworth and co-workers.[10] Thiosemicarbazones and their metal complexes exhibit numerous therapeutic properties, including anti-tumour activity. While the $Zn^{2+}$ complexes **3** have been shown to be active as anti-tumour agents, only recently has the intrinsic fluorescence, interpreted in terms of intraligand excitation,[11] been utilized to display uptake and distribution of thiosemicarbazone complexes in a variety of cancer cell lines. Optical imaging allows for minor structural changes to be assessed at the cellular level, greatly aiding in the design of new and improved anti-tumour agents.

Organometallic compounds, metal complexes with at least one metal–carbon bond, have been studied extensively as anticancer agents. The metallocenes in particular have provided numerous examples of potential chemotherapeutics that act *via* mechanisms different from that of cisplatin. Ferrocene appended tamoxifen derivatives, termed ferrocifens and hydroxyferrocifens, have been recently reported by Jaouen and co-workers.[12] A phenyl ring of tamoxifen, a widely used anti-estrogen drug for therapy of hormone-dependent breast cancers, was replaced by a ferrocene moiety in an effort to increase cytotoxicity. One of the hydroxyferrocifens **4** (Fig. 4), displayed exceptional antiproliferative activity in both hormone-dependent and hormone-independent breast cancer cells *in vitro*. Tamoxifen is only active against tumours that are estrogen-receptor positive; thus it was proposed that the hydroxyferrocifens act *via* a combined anti-estrogenic and cytotoxic effect. The facile oxidation of the ferrocene moiety, leading to the production of reactive oxygen species (ROS) *via* Fenton chemistry, was highlighted as a potential mechanism for the observed increase in cytotoxicity.[12]

Organometallic ruthenium(II) arene compounds have also shown considerable promise as cancer therapeutics, with evidence suggesting that these compounds have an altered biological profile compared to other metal-based anticancer complexes.[13] The ligands were found to play a critical role in the activity of the $[(\eta^6\text{-arene})Ru(ethylenediamine)Cl]^+$ complexes **5** (Fig. 4) by enhancing DNA intercalation (arene) and limiting non-specific reactivity with cellular components (chelating bidentate ligand). These complexes display significant selectivity for binding guanine (N7) bases of DNA, facilitated by H-bonding between the ethylenediamine $NH_2$ groups and the exocyclic oxygens of guanine.[13] Very recently




**4** **5**

**Fig. 4** Examples of organometallic anticancer agents; a hydroxyferrocifen **4**,[12] and a ruthenium arene anticancer agent **5**.[13]

Sadler and co-workers[14] determined that under physiologically relevant conditions guanine (as guanosine 3′,5′-cyclic monophosphate cGMP) binding to **5** occurred even in the presence of a 250-fold excess of the sulfur-containing biomolecule glutathione (GSH). Cells contain high concentrations of GSH; this tripeptide is thought to play important roles in cellular detoxification and resistance mechanisms to platinum anticancer agents. Interestingly, the authors determined that oxidation of a ruthenium-coordinated GSH leads to facile ligand exchange for guanine (N7), a process that could play a significant role in the biological activity of this class of compounds.[14]

## 4 Lanthanide probes

### 4.1 Lanthanide complexes as MRI agents

Magnetic resonance imaging (MRI) is now a powerful tool in the field of diagnostic medicine, with demonstrated clinical ability as a non-invasive, non-radiative technique. MRI has historically focused on anatomical structure as the inherent contrast results from the physical properties and local environment of water molecules. The physical basis of image enhancement, and considerations for the improvement of image contrast *via* contrast agents have been recently reviewed in this journal.[15] The electronic properties of lanthanide ions, in particular $Gd^{3+}$, with its high magnetic moment ($\mu^2$ = 63 $\mu_B{}^2$), are very useful for enhancing MRI scans. In the absence of a suitable ligand however, nonphysiological metals such as the lanthanides (many of which are toxic, *e.g.* $Gd^{3+}$), hydrolyze, accumulate in tissues, or are excreted too rapidly to be of use in imaging. The ligand therefore plays an important role in minimizing toxicity while ensuring a long enough plasma half-life for the intended use.

Polyaminocarboxylate ligands such as diethylenetriaminepentaacetic acid (DTPA) and tetraazamacrocycles such as 1,4,7,10-tetrakis(carboxymethyl)-1,4,7,10-tetraazacyclododecane (DOTA) form lanthanide complexes with high thermodynamic stability, kinetic inertness, and minimal toxicity. In addition, these ligands leave at least one vacant coordination position open for a coordinated water molecule to ensure contrast enhancement. Recent developments in this area have concentrated on increasing the observed contrast in MR images. In this section we will highlight how ligand design has contributed to the development of contrast agents that respond to specific enzymes, pH, and changes in tissue physiology and metabolism.

An initial report in the area of functional MRI agents was by Meade and co-workers,[16] who developed a Gd-DOTA derivative **6** (Fig. 5) that was capped by a galactopyranose residue, blocking the close approach of water molecules and thereby limiting the contrast enhancement to outer-sphere relaxation. The exchange of coordinated water molecules with the bulk solvent is the primary mechanism by which these chelates affect water proton relaxation; modulating the number of water molecules ($q$) coordinated in the inner sphere modulates the relaxivity.

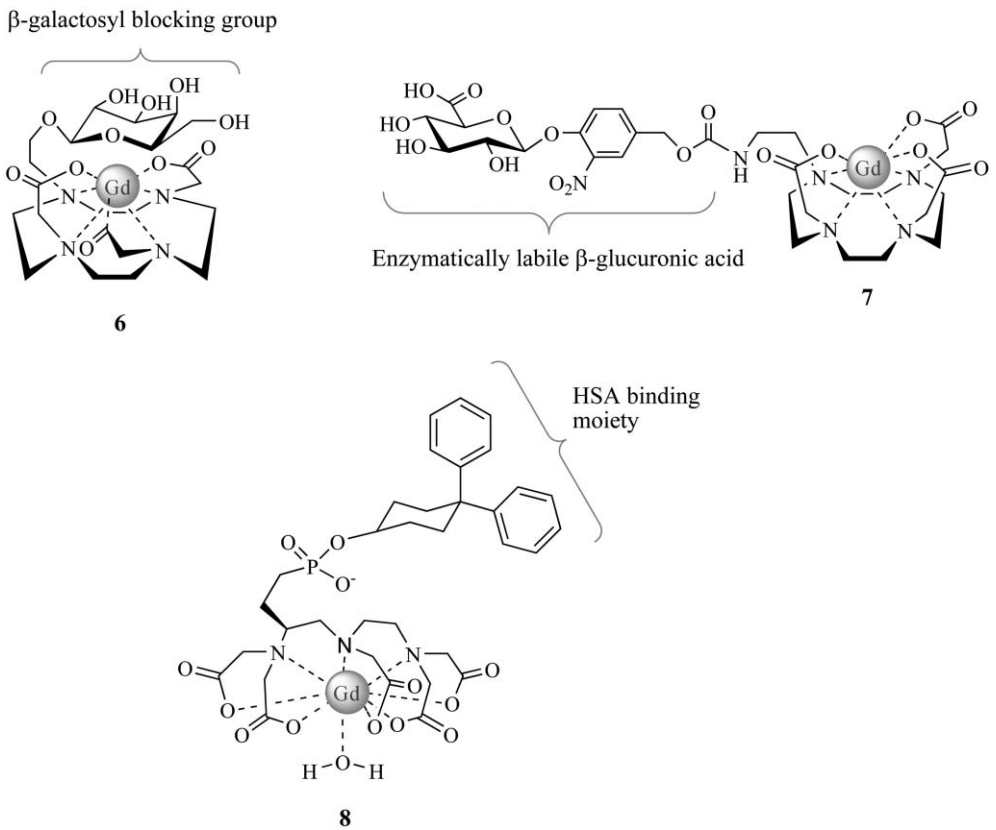



**Fig. 5** Three examples of functional MRI agents: (**6**) A β-galactosidase sensitive imaging agent;[16] (**7**) An imaging agent responsive to the oncologically relevant enzyme β-glucoronidase;[17] (**8**) A blood pool imaging agent that interacts with human serum albumin (HSA).[19]

Exposure of the molecule to the β-galactosidase enzyme irreversibly removes the blocking group causing a transition from a weak to a strong relaxivity state. This 'smart' MRI contrast agent, shown to be enzymatically responsive, demonstrated the feasibility of using such compounds to enhance images of physiological states *in vivo*. More recently, this same group developed a contrast agent **7** (Fig. 5) that was sensitive to the oncologically relevant enzyme β-glucoronidase.[17] Enzymatic hydrolysis of the β-glucoronic acid led to a change in water proton relaxation, demonstrating the potential of this approach for imaging cancer cell masses *via* changes in enzyme levels. The study also highlighted the effect of buffer composition on the relaxivity of the MR agent.

The efficacy of a metallopharmaceutical, as with any drug, depends on its fate once administered. The biological half-life, stability, interaction with endogenous molecules, and localization of a metal-based drug must be carefully considered to fit the intended application. The interaction of metallopharmaceuticals with the serum proteins transferrin and human serum albumin (HSA) (both proteins that have a strong affinity for metal ions) is an important aspect of metal-based drug design. Serum protein binding can lead to premature decomposition of a metal-based agent through ligand exchange, but can also be used to advantage by delaying systemic clearance as well as limiting metal redox conversion.[18] As an example, the presence of an HSA binding moiety greatly enhances the utility of a $Gd^{3+}$-containing blood pool imaging agent **8** (Fig. 5) *via* receptor-induced magnetization enhancement (RIME). Caravan and co-workers[19] developed a $Gd^{3+}$-DTPA analog with an attached lipophilic diphenyldicyclohexyl group that reversibly binds to site II on HSA and currently awaits approval in the United States. HSA binding prolongs plasma half-life, increasing the residence time of the agent in the blood pool. The high relaxivity of **8** when bound to HSA is primarily due to a substantial increase in the rotational correlation time ($\tau_R$) of the agent upon binding. This example highlights how ligand design can have a profound effect on the biolocalization of a metallopharmaceutical, and more specifically, how added targeting features can enhance the efficacy of metal-based agents.

Advances in ligand design have also led to the development of contrast agents that respond to changes in pH. A pH-responsive contrast agent was recently reported by Sherry and co-workers[20] using a DOTA ligand **9** (Fig. 6) with an appended *p*-nitrophenol moiety. The *p*-nitrophenol arm was found to be sensitive to changes in pH, dissociating from the metal complex as the pH was lowered. Acid-catalyzed dissociation of the *p*-nitrophenol arm ($pK_a = 7.36 \pm 0.04$) from the $Gd^{3+}$ complex of **9** was found to increase the hydration state from $q = 1$ to $q = 2$, with a corresponding 71% increase in relaxivity.[20]

One of the major difficulties in the development of better targetted MRI contrast agents is the need to correct for concentration effects, as signal enhancement depends on the absolute concentration of the agent. In order to overcome this limitation, **para**magnetic **c**hemical **e**xchange **s**aturation **t**ransfer (PARACEST) agents[21] have been developed, utilizing ligands with protons in slow exchange with bulk water on the

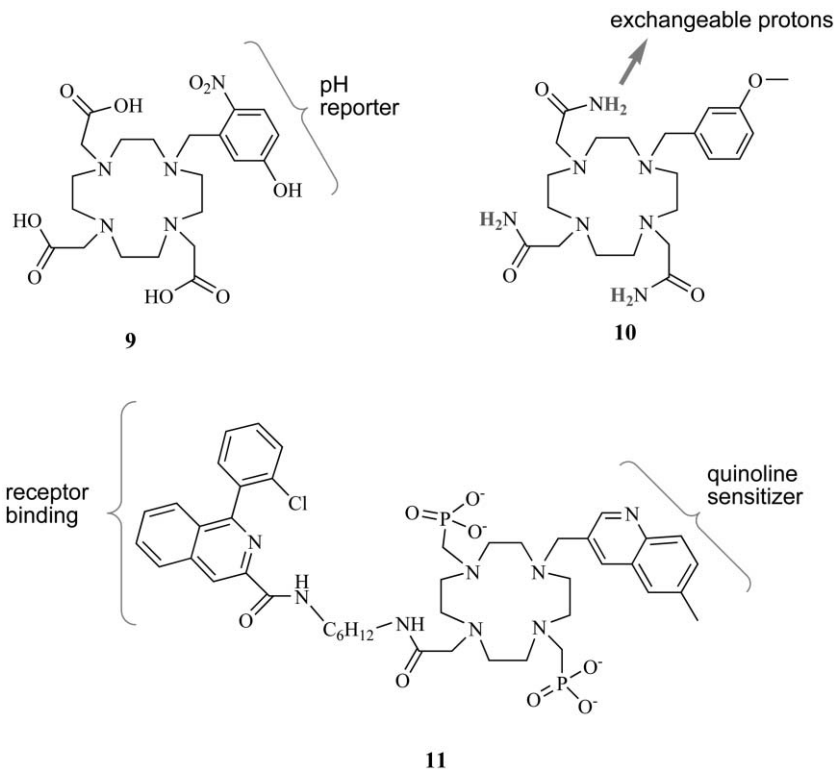



**Fig. 6** DOTA ligands for functional MRI agents; pH-responsive[20] (**9**); (**10**) with exchangeable protons for imaging using PARACEST[22]; (**11**) for use in bimodal (fluorescence and MRI) imaging.[27]

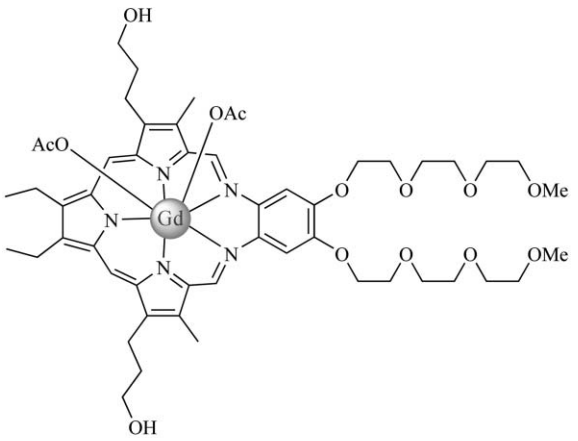
 

NMR timescale. Saturation transfer to the water resonance, *via* irradiation of the exchangeable proton pool, leads to a decrease in water signal intensity, with the difference used to produce the MR image. Exchangeable protons can be amides, hydroxyl groups, or even metal-bound water protons if exchange with bulk water is suitably slowed. Using this approach, Aime and co-workers have developed a PARACEST agent that is responsive to lactate concentration.[22] In this study, an $Yb^{3+}$ complex of a DOTA analog **10** (Fig. 6) was prepared, with six exchangeable amide protons. Lactate was found to bind to this complex, with the amide protons shifting downfield ($\sim$10 ppm) as a result of ternary complex formation. Selective irradiation of the two amide signals lead to a saturation transfer effect sensitive to the presence of the metabolite. PARACEST agents have recently been used for temperature monitoring[23] as well as the tuneable imaging of cells.[24]

The development of contrast agents that target specific receptors is an area of considerable interest, potentially playing an important role in signalling the early onset of disease. Receptor imaging using MR is only possible, however, if the receptor is expressed at a high density, and if the contrast agent is present in sufficient concentration. It has been estimated that at least $10^6$ receptor-bound contrast agents are required per cell to garner a 2 : 1 signal to noise enhancement in a conventional image.[25] Despite these limitations, receptor imaging *via* MR is being actively pursued.

One candidate receptor for MRI is the peripheral benzodiazepine receptor (PBR), overexpression of which has been observed in certain cancers.[26] Compound **11** (Fig. 6) is an example of a receptor targeting ligand based on the DOTA framework, with nanomolar affinity for PBR.[27] Metal complexes of this ligand were reported to be capable of either fluorescence ($Eu^{3+}$) or MR ($Gd^{3+}$) imaging of the PBR receptor, depending on the lanthanide ion used. Quinoline-sensitized fluorescence from the $Eu^{3+}$ complex of **11** was used to study uptake and localization in glioblastoma (brain cancer) cells. The isostructural $Gd^{3+}$ complex yielded a contrast enhancement (signal to background ratio) of 1.7 : 1 in the same cells, demonstrating that this complex does indeed have potential as a receptor-targeted MRI agent. Many suitable targets exist for receptor-based MRI agents, and no doubt further reports in this area will soon appear.

Sessler and co-workers[28] have developed an interesting class of dual-function MRI contrast agents based on the texaphyrins, pentaaza Schiff base macrocycles similar to the porphyrins. These macrocycles have a larger central core than the porphyrins and so are better able to form thermodynamically stable complexes with larger metal cations such as the trivalent lanthanides. The $Gd^{3+}$ complex of a texaphyrin **12** (Fig. 7) was shown to localize in tumours (as do the porphyrins) *via* MR and also enhance X-ray radiation therapy of certain tumours. Compound **12** is easily reduced ($E_{1/2} = -0.041$V *vs.* NHE) and is hypothesized to scavenge electrons produced as a result of the interaction of X-rays with water, augmenting the concentration of tissue damaging ROS during X-ray radiation therapy.[28] Subsequent studies have shown that **12**, without externally applied X-rays, induces oxidative stress, triggering cell death in a broad range of cancers.[29]




**12**

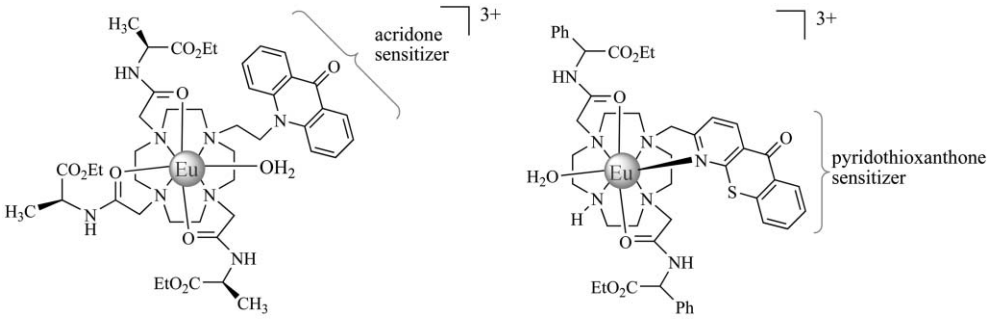
**Fig. 7** A dual-function $Gd^{3+}$-texaphyrin derivative **12** for cancer imaging and therapy.[28]

### 4.2 Lanthanide complexes as optical probes

The large Stokes shift and long radiative lifetimes commonly displayed by lanthanide ions make them ideally suited for use as optical probes. A recent review in this journal by Parker[30] highlighted the advances that have been made in understanding how complex structure and dynamics affect the spectral properties of lanthanide complexes. This knowledge has been applied to the development of lanthanide complexes with tailored ligands containing sensitizers for use as small molecule probes. The altered emission properties of these complexes upon interaction with small molecules have allowed for the detection of endogenous anions in the biological milieu.[31] In addition, imaging of cellular uptake and localization of specific small molecules could increase our understanding of the physiological processes they influence.

In a comprehensive report by Parker and co-workers,[31] a series of twelve $Eu^{3+}$ complexes based on the DOTA framework were synthesized and compared as ratiometric probes for bicarbonate ($HCO_3^-$). One of the cationic derivatives **13**, exhibiting three ester-protected alanine side-chains is shown in Fig. 8. The heptadentate ligands were affixed with an acridone chromophore for long-wavelength sensitization of the $Eu^{3+}$ emission; long-wavelength excitation limits biomolecule co-excitation. Bicarbonate binding was found to alter band intensity ratios as well as the radiative lifetime of the $Eu^{3+}$ emission, allowing for selective detection of $HCO_3^-$ in the presence of other anions such as lactate, citrate and phosphate prevalent in biological systems. The uptake and distribution of these small molecule probes *via* fluorescence microscopy was also reported, in NIH 3T3 mouse fibroblast cells.[31]

Using similar technology to that just described for bicarbonate detection, an $Eu^{3+}$ complex **14** (Fig. 8) has recently been reported as a chemoselective probe for citrate.[32] The probe was found to be specific for citrate and largely unaffected by changes in pH (between pH 4 and 8.2).




13 14

**Fig. 8** Lanthanide-based optical probes for bicarbonate[31] (**13**) and citrate[32] (**14**).

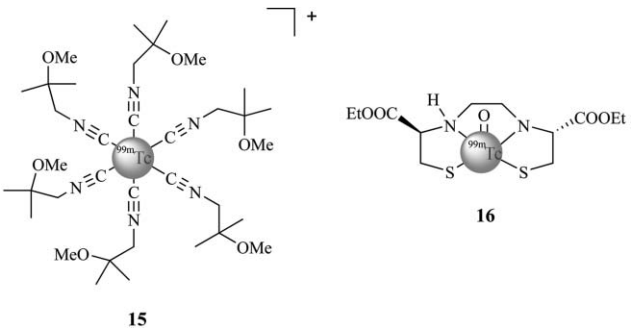
## 5 Radiopharmaceuticals in nuclear medicine

Nuclear medicine relies on the use of radiopharmaceuticals (compounds containing radionuclides) for the diagnosis, and more recently the treatment, of disease. Diagnostic radiopharmaceuticals provide a non-invasive means of assessing the physiology and morphology of organs and tissues and have become very important in the diagnosis of disease and assessing the progress of treatment strategies. The field has been recently reviewed by Liu in this journal,[33] and in this section we will cover how recent advances in multifunctional ligand design have contributed to the furthering of the field.

There are two general techniques used to produce diagnostic images, single photon emission computed tomography (SPECT) utilizing gamma-emitting isotopes, and positron emission tomography (PET) utilizing positron-emitting isotopes. Metallic radionuclides (such as $^{99m}$Tc, $^{186}$Re, $^{64}$Cu, $^{111}$In, $^{68}$Ga, $^{90}$Y) offer considerable advantages over their non-metal counterparts due to their wide range of nuclear properties (type of radiation, half-life, particle energy, coordination chemistry, ease of isolation) and thus can be chosen for the properties that best fit the intended application. $^{99m}$Tc is the most commonly used isotope in nuclear medicine (85% of all procedures) due to its ideal nuclear properties ($t_{1/2}$ = 6.01 h, $\gamma$ = 142.7 keV) and easy isolation as $Na^{99m}TcO_4$ from a $^{99}$Mo generator. Rhenium, the third row transition metal analog of technetium, exhibits similar chemistry to that of technetium, a fact which can be exploited in developmental work. In addition, Re itself has particle emitting radioisotopes ($^{186/188}$Re) with physical properties useful in therapeutic applications if suitable target specificity in disease tissue can be obtained. Ligand design must ensure rapid complexation and uptake by the target tissue, driven by the relatively short half-life of the radionuclide.

Initially the field of nuclear medicine depended on the development of small molecule radiopharmaceuticals such as $^{99m}$Tc-Sestamibi **15** for myocardial perfusion imaging, and $^{99m}$Tc-Bicisate **16** for imaging cerebral blood flow, where the biodistribution and targeting ability of the compounds depended on the lipophilicity, size, and charge of the complex (Fig. 9).[33] Many of these radiopharmaceuticals are still in use today.

More recently, advances in the design of small-molecule radiopharmaceuticals incorporate chemical properties that



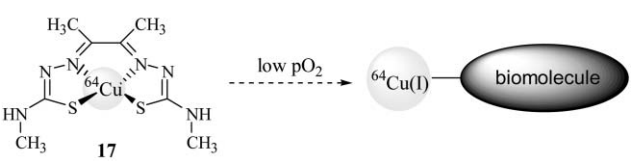

15 16

**Fig. 9** $^{99m}$Tc-based diagnostic radiopharmaceuticals $^{99m}$Tc-Sestamibi (**15**) and $^{99m}$Tc-Bicisate (**16**).

result in increased targeting *in vivo*. As an example, Welch and co-workers have reported a $^{64}$Cu-bis(thiosemicarbazone) complex **17** (Fig. 10) that selectively localizes in hypoxic tissue.[34] Certain tumours exhibit regions where blood flow is restricted and thus the concentration of oxygen is low, limiting the uptake of conventional chemotherapeutics. In this case, the use of the diacetyl-bis($N^4$-methylthiosemicarbazone) ligand imparts a low redox potential (−297 mV) on the $^{64}$Cu(II) complex leading to facile reduction to $^{64}$Cu(I) under low $pO_2$ conditions.

The bis(thiosemicarbazone) ligand is only a weak chelator for $^{64}$Cu(I) and so ligand exchange occurs with intracellular macromolecules, trapping the radionuclide in hypoxic tissue. The $^{64}$Cu radioisotope decays by a number of pathways, allowing for both diagnostic imaging *via* PET as well as therapy *via* β-particle emission. Indeed, **17** has shown efficacy for inhibiting solid tumour growth.[35]

The development of target-specific radiopharmaceuticals, through the conjugation of radionuclides to biomolecules, has enjoyed considerable success in nuclear medicine. The size of




17

**Fig. 10** $^{64}$Cu-bis(thiosemicarbazone) complex **17** localization under conditions of hypoxia.[34]

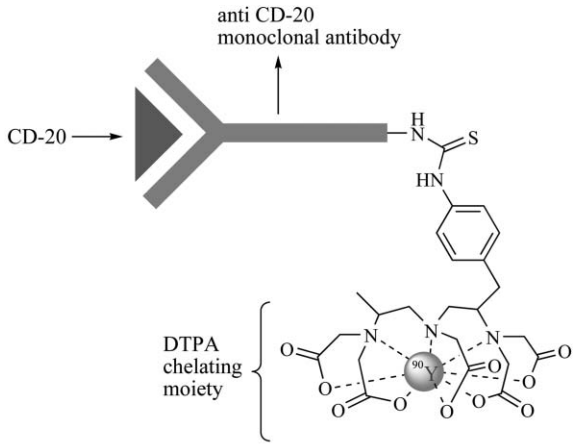



**Fig. 11** A cartoon depiction of the radiolabelled monoclonal antibody $^{90}$Y-ibritumomab tiuxetan **18**.

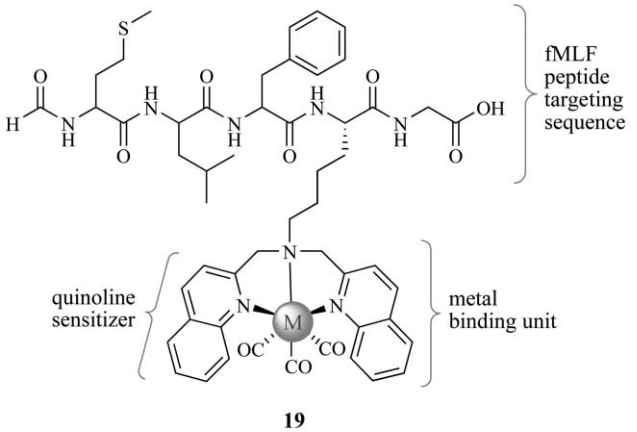



**Fig. 12** A formyl peptide receptor (FPR) binding sequence with attached metal chelating function **19** for use as a fluorescent (M = Re) and radioactive (M = $^{99m}$Tc) probe.[37]

the metal chelate and distance from the biomolecule, as well as the overall complex stability are important design considerations for target-specific radiopharmaceuticals. The goal is to interfere minimally with the targeting of the biomolecule while limiting decomposition of the agent through ligand exchange processes *in vivo*. The therapeutic potential of nuclear medicine lies in the possibility of designing target-specific radiopharmaceuticals to deliver therapeutic doses of ionizing radiation to disease sites. Specificity is very important so as not to introduce widespread radiation damage. The FDA approval of the radiolabelled monoclonal antibody $^{90}$Y-ibritumomab tiuxetan **18** (Fig. 11) for the treatment of non-Hodgkins lymphoma was a significant milestone in the area of target-specific radiopharmaceuticals.[36] A DTPA linker was attached to the monoclonal antibody to chelate $^{90}$Y, ensuring the tight binding of this tissue-damaging radionuclide. The antibody is directed to the antigen CD-20 which is expressed on the surface of B-cell lymphomas.[36] The β-particle, emitted during $^{90}$Y decay, has a mean tissue penetration distance of 5.3 mm; thus it is useful for killing tumour cells that are in close proximity to B-cell lymphomas yet do not express CD-20, or those that are poorly vascularized.

Valliant and co-workers have recently developed a general method for the incorporation of radionuclides into peptides for the preparation of target-specific radiopharmaceuticals.[37] In this interesting study a chelating function was attached by a linker to an amino acid that could be integrated into a peptide using a conventional automated synthesizer. To demonstrate this idea, the chelating moiety was incorporated into a targeting sequence for the formyl peptide receptor (FPR) **19** (Fig. 12). Metal complexes using the $\{M(CO)_3\}^+$ (M = $^{99m}$Tc/Re) core, developed by Jaouen[38] and Alberto[39] and co-workers, were synthesized to study the characteristics of the labelled peptide. The $\{M(CO)_3\}^+$ (M = $^{99m}$Tc/Re) core has found widespread application in nuclear medicine offering advantages in terms of stability, kinetic inertness, and size. The two quinoline arms of the chelating moiety were included in the ligand design to afford a Re complex with appropriate fluorescent properties to be used for *in vitro* microscopy studies. Fluorescence microscopy studies demonstrated that the peptide conjugate exhibited cell uptake in human leukocytes, and was internalized into the cytoplasm.[37] This work demonstrated how ligand design can be used to prepare complementary fluorescent and radioactive probes, potentially increasing our understanding of the mechanisms underlying the targeting of radiopharmaceuticals to specific molecular receptors.

## 6 Metal binding and sequestration

To this point we have discussed how target-specificity in ligand design for metal-based pharmaceuticals can have significant benefit. These same design principles can also be of great benefit when the goal is limiting the adverse effects of metal ion overload, inhibiting selected metalloenzymes, or facilitating metal ion re-distribution. Ligands can be used to treat metal ion overload diseases such as Wilson's disease (Cu) and hemochromatosis (Fe), by administration of the metal ion chelators penicillamine and desferrioxamine (DFO), respectively. Although metal chelating agents represent a much-needed therapeutic strategy, they are accompanied by undesirable side-effects. Long-term use of strong chelators that are not tissue-specific, such as DFO, can be expected to affect the homeostasis of numerous biometals and disturb normal physiological functions of essential metal-requiring biomolecules such as metalloenzymes. The ability to target tissues selectively, *via* the coupling of chelation agents with targeting moieties, may minimize the toxicity of metal binding agents and enhance their effectiveness. Target-specific metal-ion passivation holds great promise for the treatment of neurodegenerative disease; excess metals have been implicated in catalyzing peptide aggregation and oxidative stress in the brain, ultimately leading to cell death. Molecules designed to sequester excess metals in the brain and aid in the removal of such metals are attractive potential therapies for neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease (AD), Creutzfeldt-Jakob disease, and amyotrophic lateral sclerosis.[40] Using this approach, a DTPA analog **20** (Fig. 13) was developed to chelate excess metals (especially Cu,

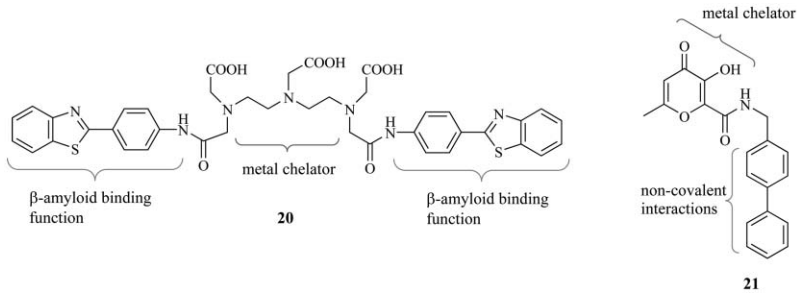



**Fig. 13** A targeted agent **20** for AD therapy,[41] and a pyrone matrix metalloproteinase inhibitor **21**.[42]

Zn, and Fe) and disrupt aberrant metal–peptide interactions in AD.[41] This bifunctional agent was appended with two benzothiazol-2-yl-phenyl functions that are known to target the amyloid-β (Aβ) peptide, a biomolecule closely linked to the etiology of AD. This compound was shown to reduce oxidative stress in an *in vitro* model, and attenuate cerebral Aβ pathology in a mouse model.[41]

Ligand design has also played an important role in the development of metalloenzyme inhibitors. In a recent study Cohen and co-workers[42] reported a series of pyrone-based inhibitors of $Zn^{2+}$-dependent matrix metalloproteinases. Inhibition of these enzymes is a potential treatment strategy for cancer and heart disease. Compounds were developed **21** (Fig. 13) that could chelate the catalytic $Zn^{2+}$ as well as further anchor the compound *via* noncovalent interactions within the active site. Computer modelling was used to design optimal fragments to link to the pyrone metal binding moiety. Biological testing validated the results of the computational study, with the pyrone-based inhibitors showing increased potency compared to hydroxamate-containing inhibitors.[42]

## 7 Glycobiology

The rapidly expanding field of glycobiology[43] has had a positive impact on medicinal inorganic chemistry. Appending a carbohydrate to a metal-binding ligand has the ability to reduce toxicity, and improve solubility and molecular targeting to a wide variety of metal-based drugs. Direct metal ion–carbohydrate interactions are, however, difficult to study due to the multifunctionality, complicated stereochemistry, and weak coordinating ability typical of carbohydrates. Carbohydrate ligands with chelating functions for metal ions have thus been developed to generate a well-defined binding environment as well as increase the stability of the resultant metal complexes. A potential benefit of this approach is that the carbohydrate can remain pendant, thereby being freely available to interact with carbohydrate transport and metabolic pathways in the body. One of the first examples of this approach was a carbohydrate-linked cisplatin analog **22** (Fig. 14) for chemotherapy.[44] More recently, a series of platinum terpyridine complexes containing a glycosylated acetylide unit were reported (Fig. 14).[45] One derivative **23** was found to be significantly more cytotoxic than cisplatin (up to ~100-times higher in potency) and it was concluded that the glycosylated arylacetylide unit not only enhanced complex solubility but was a key structural motif in governing the observed cytotoxicity.

Radiolabelled carbohydrates have found widespread utility in the field of nuclear medicine. Currently, 2-[$^{18}$F]-fluoro-2-deoxy-D-glucose (FDG) **24** (Fig. 15) is the most widely used carbohydrate-based diagnostic imaging agent. FDG, imaged by positron emission tomography (PET), has proven very useful for the detection of tumours and metastatic tissue and also for the assessment of tissue viability in cardiac patients.[46]

The high cost of PET and the relatively short half-lives of PET emitters have, however, limited their utility and hence led to the search for alternatives to FDG. A series of carbohydrate-appended $^{99m}$Tc complexes have thus been developed specifically for tumour imaging (Fig. 15).[47,48] Taking advantage of a commonly used $N_2S_2$ donor set for the $\{^{99m}Tc{=}O\}^{3+}$ core, $^{99m}$Tc-ethylenedicysteinedeoxyglucose (ECDG) **25** was developed and found to have similar *in vivo* tumour imaging capabilities when compared with FDG.[47] However, the compound displayed limited brain uptake possibly as a result of the large size of the complex.

Our work in this area has centered on the development of carbohydrate-appended $^{99m}$Tc complexes utilizing the $\{^{99m}Tc(CO)_3\}^{+}$ core.[48] A glucosamine-coupled derivative **26**

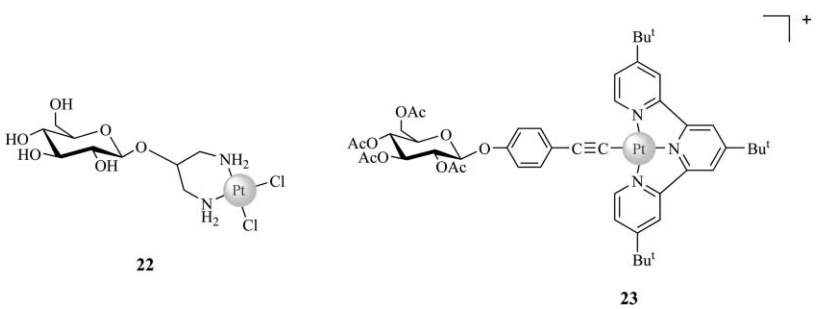



**Fig. 14** A carbohydrate cisplatin analog **22**[44] and a platinum terpyridine complex containing a glycosylated acetylide unit **23**.[45]

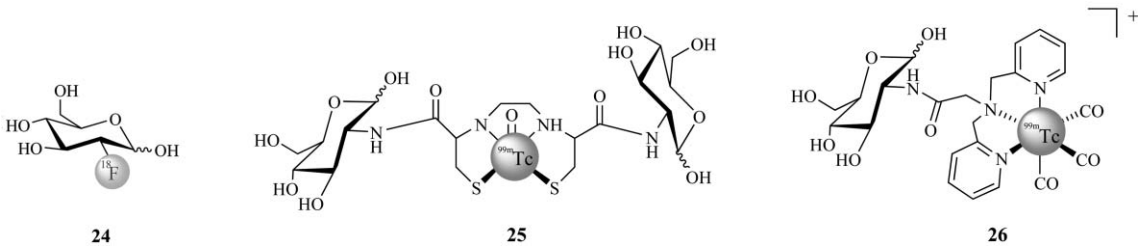


**Fig. 15** Radiolabelled carbohydrates 2-[$^{18}$F]-fluoro-2-deoxy-D-glucose (FDG) **24**; $^{99m}$Tc-ethylenedicysteinedeoxyglucose (ECDG) **25**;[47] and the $^{99m}$Tc-glucosamine conjugate **26**.[48]

(Fig. 15) with a 2,2′-dipicolylamine binding unit has shown exceptional *in vitro* stability and imaging studies are on-going. Due to the overall positive charge on this molecule, neutral forms are being prepared in order to test the effects of charge on the localization of these carbohydrate-based imaging agents.

Carbohydrate-binding proteins play an important role in many biological processes. For example, the lectins, a large group of carbohydrate binding proteins, mediate immune function, infectious cycles, and metastasis. The development of lectin-mediated MRI agents was recently reported using lanthanide complexes of DOTA monoamide-linked glycoconjugates **27** (Fig. 16).[49]

Preliminary biodistribution studies using $^{153}$Sm-labelled β-galactosyl glycoconjugates indicated specific uptake in hepatocyte cells known to express lectins that recognize terminal β-galactosyl residues. Research has shown that glycoconjugate–lectin interactions depend on matching the relative orientation and spacing of the carbohydrate residues of the glycoconjugate with the Carbohydrate Recognition Domains (CRDs) on the lectins.[50] Significant opportunities exist for the development of improved metal-based agents for interacting with carbohydrate binding proteins. In addition, changes in glycosylation patterns on the cell surface are often associated with cancer and chronic inflammation,[51] and thus it may be possible to selectively detect and treat these diseases with suitably tagged carbohydrates.

## 8 Outlook

The field of medicinal inorganic chemistry has benefited greatly from advances in ligand design, leading to the development of improved diagnostic and therapeutic agents. As we move beyond our traditional view of ligands as just metal binders, ligands are increasingly being used to enhance the applicability of metal-based agents. Numerous opportunities exist to design ligands that better target disease tissue or specific endogenous molecules. Indeed, in this review we have highlighted numerous cases where tailored ligands have made a positive impact on the field of medicinal inorganic chemistry. As our understanding of biological processes and disease physiology improves, opportunities for the design of new metal-based and metal-binding agents will arise.

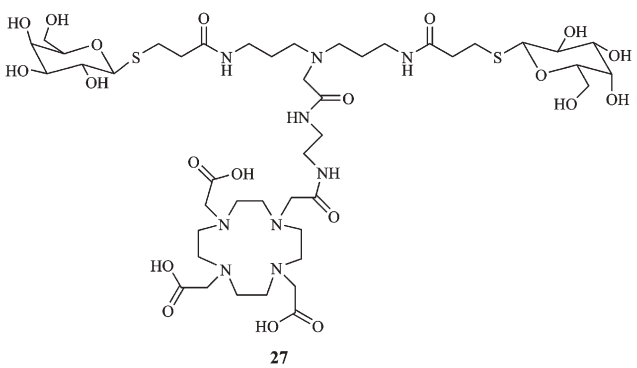


**Fig. 16** A DOTA monoamide-linked glycoconjugate **27** for lectin imaging.[49]

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