Technetium and Gallium Derived Radiopharmaceuticals: Comparing and Contrasting the Chemistry of Two Important Radiometals for the Molecular Imaging Era

Mark D. Bartholomä,[†,§] Anika S. Louie,[‡] John F. Valliant,[‡,*] and Jon Zubieta[†,*]

Department of Chemistry, Syracuse University, Syracuse, New York 13244, and Department of Chemistry, McMaster University, Hamilton, Canada ON L8S 4M1

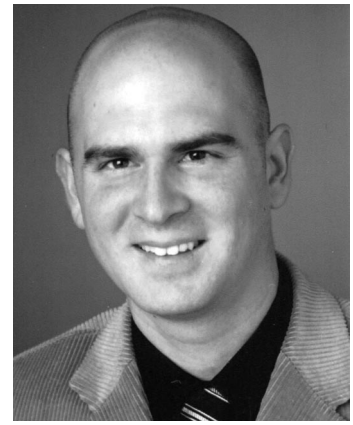
Received March 3, 2010

Contents

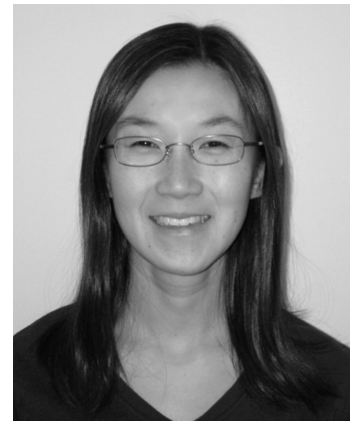
1. Introduction

The objective of medical imaging using molecular probes and perfusion radiotracers is to provide rapid, noninvasive evaluation of physiology, pathology, and/or organ function. The benefits of using molecular imaging is that it enables the early detection of disease, facilitates expedient deployment of therapies, allows for selection of the most potent interventions, and is a way to assess early on during therapy

* Corresponding authors: (J.F.V.) E-mail: valliant@mcmaster.ca. Fax: 905-522-7776. Tel.: 905-525-9140 ext. 22840. (J.Z.) E-mail: jazubiet@syr.edu. Fax: 315-443-4070. Tel.: 315-443-2547.
† Syracuse University.
‡ McMaster University.
§ Current address: Children's Hospital Boston, Division of Nuclear Medicine, Department of Radiology, 300 Longwood Avenue, Boston, MA 02115.



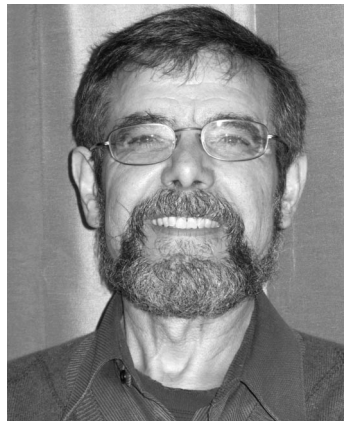
Mark Daniel Bartholomä was born in Neunkirchen (Germany) and received his diploma (2002) and his doctoral degree (2007) from the Saarland University in Saarbrücken (Germany) under the supervision of Prof. Kaspar Hegetschweiler on the synthesis and complex formation of multidentate derivatives of 1,3,5-triamino-1,3,5-trideoxy-*cis*-inositol. After a postdoctoral appointment in Prof. Jon Zubieta's research group at Syracuse University working on Re/Tc conjugated nucleoside analogues for noninvasive imaging from 2007 to 2009, he joined Prof. Alan Packard's group in 2010 at Harvard Medical School to study ^{18}F radiolabeled perfusion tracers. Educated as a coordination chemist, his research interests focus on the development of novel ligand systems and investigation of their corresponding complex formation as well as their biochemical background and medical applications including the development of radiopharmaceuticals.



Anika Louie received her B.Sc. in chemistry from the University of Victoria (Victoria, B.C.). She is currently a Ph.D. candidate at McMaster University in Professor John Valliant's group where she is focused on the development of targeted Re(I)− and Tc(I)−metallacarborane complexes for imaging neuroreceptors.

10.1021/cr1000755
Published on Web 04/23/2010

John Valliant is an Associate Professor of Chemistry and The Scientific Director and CEO of the Centre for Probe Development and Commercialization (CPDC) at McMaster University. Dr. Valliant's research program is focused on the development of new radiolabeling methods and compound discovery strategies to create novel molecular imaging probes and therapeutic radiopharmaceuticals and the bioinorganic chemistry of carboranes. Dr. Valliant was the recipient of the 2007 Journal of Labelled Compounds and Radiopharmaceuticals Award for Young Scientists. Dr. Valliant is also the founder of the CPDC, a center that is designed to foster the advancement of probes from discovery through to clinical use and commercialization. Dr. Valliant received his Ph.D. from McMaster University in 1997 and completed a postdoctoral fellowship with Drs. Alun Jones (Harvard Medical School) and Alan Davison (M.I.T.) prior to joining McMaster in 1999 as an Assistant Professor.



Jon Zubieta is a native of Brooklyn, New York, where he received his early education in the exceptional parochial school system before attending Fordham University for his B.S. degree. He received his doctoral degree from Columbia University under the supervision of Professor Stephen J. Lippard and subsequently did postdoctoral work with Professors Ron Mason, KCB, FRS, and John Postgate, FRS at the University of Sussex. He began his academic career at SUNY Albany in 1973, where he rose to the rank of Professor. In 1990, Zubieta moved to Syracuse University where he became a Distinguished Professor in 2004 and served as chairman of the Department from 2005 to 2009. In 2009, he was admitted as a Fellow of the Royal Society of Chemistry. Zubieta's research interests include the structures and properties of metal oxide hybrid materials, the electrochemistry of inorganic materials, and the design of novel radiopharmaceuticals.

the efficacy of a particular course. These unique capabilities in turn improve patient outcomes and patient safety and they can reduce hospital stays, thereby helping to mitigate the soaring cost of modern healthcare and greater economy. Radioactive probes offer considerable advantages over existing and less sensitive diagnostic methods with respect to imaging-specific biological targets, particularly those present at low concentrations.

Nuclear medicine relies on two main imaging modalities: single photon emission computed tomography (SPECT) and positron emission tomography (PET). PET offers higher resolution and sensitivity, while SPECT offers the advantages of more readily available, longer-lived radioisotopes that have lower direct costs. It should be pointed out here that there have been a number of developments around new SPECT and PET systems including disease-specific imaging cameras that are changing paradigms around traditionally held beliefs about PET and SPECT. Readers are directed to reviews on detector and instrument developments that are beyond the scope of the current review.[1−8]

SPECT radiotracers are generally small molecules (generally MW < 2000) labeled with a gamma-emitting isotope for diagnosis, such as ^{123}I, ^{111}In, ^{67}Ga, and ^{99m}Tc. New methods to incorporate clinically useful radionuclides (medical isotopes) into targeting vector molecules to impart specificity and selectivity are at the heart of modern radiopharmaceutical research and development. Advances are generally achieved through a greater understanding of the coordination chemistry of the medical radionuclides and by developing creative labeling and bioconjugation strategies.

Of the isotopes currently in use, ^{99m}Tc has become the workhorse of diagnostic nuclear medicine and is used in some chemical form in the majority of diagnostic scans conducted each year in hospitals worldwide.[9−35] This preferred use of ^{99m}Tc radiopharmaceuticals reflects the ideal nuclear properties of the isotope and, until recently, the convenient availability from commercial generators. ^{99m}Tc emits a 140 keV γ-ray with 89% abundance, which is nearly optimal for imaging with commercial gamma cameras. The absence of corpuscular radiation allows the injection of activities of more than 1.11 GBq (30 mCi) with low radiation exposure to the patient. The 6 h half-life allows for centralized preparation of radiopharmaceuticals in radiopharmacies, distribution to hospitals, administration, time for accumulation in the target tissue, and collection of the image while still ensuring minimal radiation dose to the patient.

The importance of ^{99m}Tc for diagnostic radioimaging applications is evident from the nearly 19×10^6 radiopharmaceutical injections in the United States in 2007 for cardiac, bone, lung, kidney, liver, and gall bladder scans. This represents about 85% of all radiopharmaceutical injections in that period. The importance of ^{99m}Tc is further illustrated by cardiac imaging procedures. Approximately 9×10^6 SPECT perfusion stress tests are administered annually in the United States using ^{99m}Tc radiopharmaceuticals. The alternatives to stress testing with ^{99m}Tc are generally inferior. For example, imaging with ^{201}Tl results in lower resolution and higher radiation dose to the patient, and the use of ^{201}Tl has declined steadily since the introduction of ^{99m}Tc reagents. Similarly, stress echocardiography is less sensitive and not useful for patients with previous heart attacks. Few physicians have the required expertise for stress testing with PET scans and MRI, and the techniques have very limited availability compared to ^{99m}Tc radiopharmaceuticals. Angiograms are expensive and invasive with ca. 1 in 1000 patients dying from the procedure.[36]

While ^{99m}Tc has become the medical isotope of choice for nuclear imaging, a key challenge is the continuing global shortage of the isotope because two aging nuclear reactors that provide a large fraction of the world's supply have been shut down for repairs and/or routine maintenance. The National Research Universal (NRU) Reactor in Chalk River, Canada, which provides 45% of the world supply of ^{99}Mo, the parent nuclide for ^{99m}Tc, was built in 1957 and is

approaching the end of its lifetime, and operations are likely to cease sometime between 2010 and 2020. Similarly, the High Flux Reactor (HFR) in Petten, The Netherlands, which supplies 30% of the world supply of ^{99}Mo and was built in 1961, is currently not operating as it undergoes six months of prescribed maintenance.

A number of alternatives have been proposed. One involves using existing smaller reactors, including ones at the University of Missouri, Columbia, and McMaster University to produce ^{99}Mo. This approach, while feasible, requires capital and operating investment around both production and processing. Other plans include the construction of new isotope reactors, the development of technology to manufacture ^{99}Mo using low-enriched uranium (Babcock & Wilcox Co.), the use of linear accelerators and cyclotrons, and the conversion of nuclear power reactors that have accessible cores. There are also proposals to address acute shortages through increased production from reactors in Belgium, France, Argentina, and South Africa. A most hopeful development is the recognition of the urgency of the situation by the United States Congress, which recently passed legislation RR3276, *American Medical Isotope Production Act of 2009*,[37] promoting the safe and reliable domestic production of ^{99}Mo/^{99m}Tc. A report for long-term options from an expert panel created by the Canadian government has also reviewed the situation and is available online.[38] None of the proposals are optimal, and all will take time to implement.

When problems are this complex, the most successful solution rarely involves investing in the status quo. A more prudent approach to shoring up what is a tenuous supply chain is to develop surrogate agents derived from other relevant medical isotopes produced from sources other than the reactors that manufacture ^{99}Mo. By diversifying supply and investment in isotope production, individual countries should be able to provide their patient populations with a more reliable imaging service and at the same time spur on economic growth in a new industry.

One obvious alternative to ^{99m}Tc are isotopes of gallium including ^{67}Ga for SPECT and ^{68}Ga for PET. The prevailing question is whether the chemistry of gallium is sufficient enough that complexes can be developed that offer equivalent or superior in vivo stability, ease of radioconjugate formation, and range of biodistributions that are characteristic of ^{99m}Tc agents. This review seeks to address these questions by comparing and contrasting the chemistry of the two radiometals. This includes reviews of the production of the radioisotopes, their availability and ease of use in the nuclear medicine clinics, the coordination chemistries of Ga compared to Tc, and what can be learned from the relevant radiopharmaceutical chemistry of these elements for the future development of alternative imaging agents. Our hypothesis in this critical, rather than comprehensive, review is that it should be possible to use the knowledge gained from the attempted development of targeted Tc radiopharmaceuticals over the past 20 years to guide the creation of effective Ga-based probes. This will include both surrogates for existing Tc agents and creation of a new generation of molecular imaging probes.

2. Technetium-99m

2.1. ^{99m}Tc: Properties and Production

^{99m}Tc is available from a commercial generator technology (Figure 1) where a parent isotope (^{99}Mo) is loaded onto an alumina column and the desired daughter is isolated by passing a saline solution through the system. This technology was developed in the 1960s at BNL and revolutionized radiopharmaceutical chemistry.[39] The parent isotope is produced by bombardment of ^{98}Mo with thermal neutrons to provide the radioactive, 66 h half-life ^{99}Mo, which more recently has been separated as a fission product. ^{99}Mo is processed as molybdate, $^{99}MoO_4^{2-}$, loaded onto an alumina column that is encased in a shielded, portable container. Through a β-decay process, ^{99m}Tc is produced as $^{99m}TcO_4^-$, pertechnetate, which is eluted from the column with a 0.15 M saline solution. At this ionic strength, the singly charged $^{99m}TcO_4^-$ species elutes while the $^{99}MoO_4^{2-}$ remains adsorbed on the column. The ^{99m}Tc is obtained in high specific activity (so long as the generator is eluted regularly) containing only minute quantities of contaminants.[40]

2.2. Coordination Chemistry of Technetium and General Categories of Technetium Radiopharmaceuticals[41−43]

Technetium is a transition metal that presents a major challenge with respect to designing radiopharmaceuticals that have suitable in vivo properties. For instance, ^{99m}Tc cannot

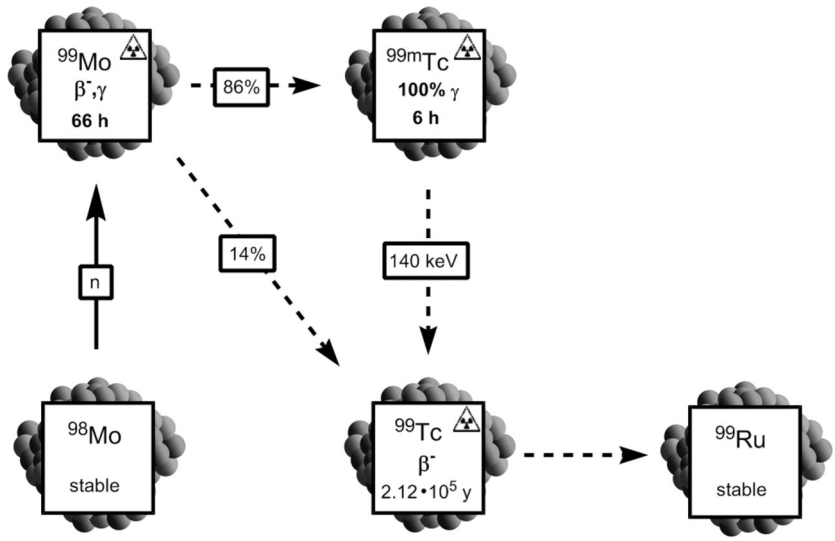


Figure 1. Generation and decay of ^{99m}Tc using the ^{99}Mo−^{99m}Tc generator.

be substituted for a hydrogen atom in a targeting molecule, unlike radiohalogens such as ^{18}F. As noted previously, the radioisotope is eluted from the generator as a dilute $^{99m}TcO_4^-$ solution. Consequently, in order to link the radionuclide to a targeting vector, pertechnetate, a Tc(VII) species, must be reduced to a suitable oxidation in the presence of appropriate ligands or directly complexed with suitably hard ligands. The development of technetium imaging agents requires both a familiarity with the rather complex coordination chemistry of the Group VII metals and an appreciation for the design of suitable ligands that provide robust molecular imaging probes.

Technetium radiopharmaceuticals have traditionally been classified in broad terms based on the role of technetium on the ultimate fate of the complex. The two categories of radiopharmaceuticals are commonly referred to as technetium-essential and technetium-tagged agents. For a technetium-essential compound, technetium incorporation is key in determining the structure and the overall physicochemical character and hence localization or biological fate of the molecule.[44,45] The best known example of this class of reagent is ^{99m}Tc−sestamibi,[46] $[^{99m}Tc(CNR)_6]^+$ (R = $CH_2C(CH_3)_2OCH_3$), which is sold under the trade names Cardiolite and Miraluma with respect to its applications in myocardial perfusion and breast tumor imaging, respectively (Figure 2). Other examples of this type of radiopharmaceutical include the heart-seeking complexes ^{99m}Tc−teboroxime (Cardiotec)[47] and ^{99m}Tc−tetroformin (Myoview),[48] the cerebral perfusion agent ^{99m}Tc−bicisate (Neurolite),[49] the renal-imaging reagents ^{99m}Tc−gluceptate (Glucoscan)[50] and ^{99m}Tc−mertiatide (Technescan MAG_3),[51] and the bone-imaging agent ^{99m}Tc−oxidronate (Osteoscan HDP)[52] (Figure 2). The reagents of this class are generally low molecular weight complexes whose biological distribution is determined by perfusion and the physicochemical characteristics, such as size, shape, charge, and lipophilicity, of the coordination complexes. Uptake is generally geared toward target high-capacity systems and processes, including phagocytosis, hepatocyte clearance, glomerular filtration, and bone absorption.

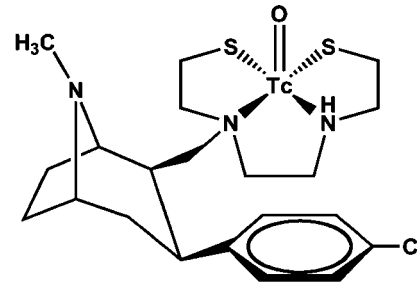


Figure 3. $[^{99m}TcO(TRODAT)]$ radiopharmaceutical for the imaging of the neuronal dopamine transporter.

The second type of technetium reagent is referred to as technetium-tagged or technetium-inessential. In this case, the biodistribution of the technetium-containing reagent is determined by some receptor site chemistry or enzymatic process associated with a substrate carrier molecule. In the ideal case, the affinity for the biological target should be independent of the presence of the technetium. This seldom occurs with probes of MW < 1000. One of the limited numbers of examples from this class of compounds are tropane analogues that bind to the dopamine transporter (DAT). TRODAT is perhaps the best studied and has been investigated as a probe for the diagnosis of Parkinson's disease (Figure 3).[53−59] In this example, a phenyltropane

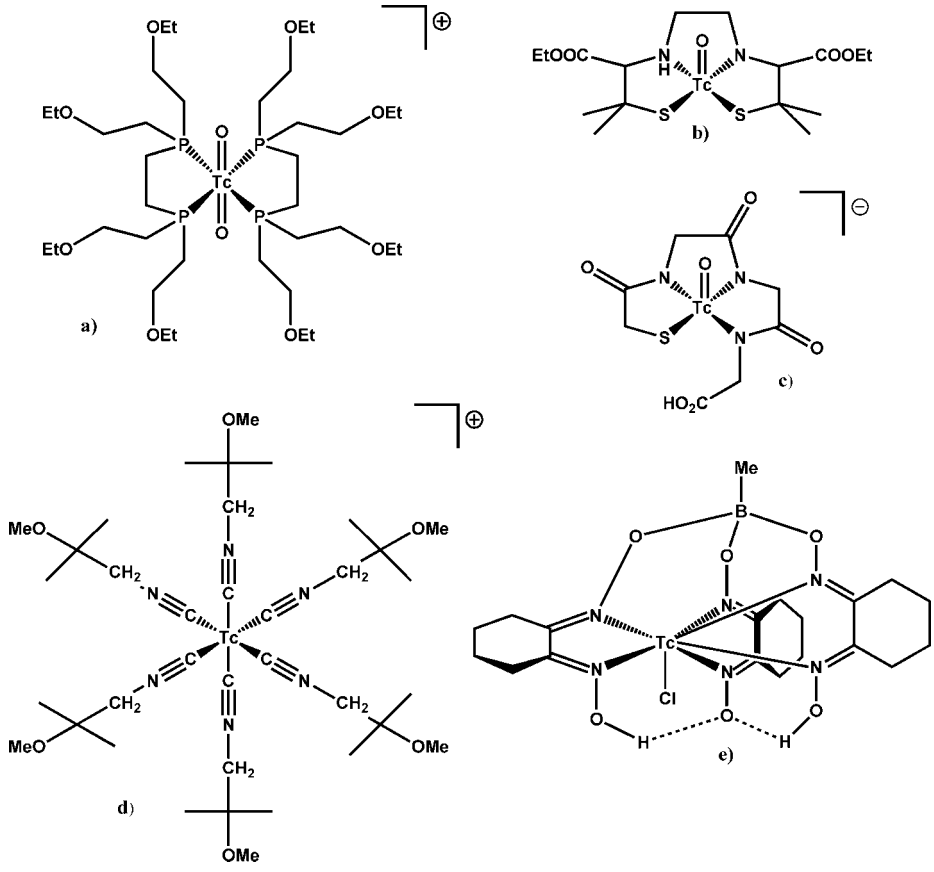


Figure 2. Structures of selected simple coordination complex radiopharmaceuticals: (a) ^{99m}Tc−tetroformin, (b) ^{99m}Tc−bicisate, (c) ^{99m}Tc−MAG_3, (d) ^{99m}Tc−sestamibi, and (e) ^{99m}Tc−teboroxime.

pharmacophore is used as a targeting vector for specific monoamine transporter proteins, which is a low-capacity system.

Two general methods have been developed to coordinate ^{99m}Tc to receptor-specific molecules. In one approach, chelation of the metal is designed in such a manner that the resulting complex mimics the geometry of known and high-affinity receptor ligands. An interesting application of this approach was used in attempts to develop imaging agents for the estrogen receptor, which is overexpressed on certain tumor types[60−66] (Figure 4). For compounds that were derived from bidentate ligands, the products exhibited low in vivo stabilities. The more stable compounds with tetradentate chelates unfortunately exhibited only modest affinity. While the integrated approach is appealing, it may be more suitable for high molecular weight species where the impact of the size of the metal complex can be minimized compared to that in small molecules.

The second strategy for designing receptor/site-specific ^{99m}Tc compounds is the conjugate or pendant method, in which a ^{99m}Tc−chelate moiety is attached to a molecule with high binding affinity. Bifunctional chelates provide an effective strategy for binding the radioactive metal cation to the biologically active molecule.[34,67−72] The strategy involves a three-component system of a biologically active molecule, a bifunctional chelate/spacer group, and the radioactive metal (Figure 5). The bifunctional chelate is designed to form a linkage bond to the metal radionuclide in vivo, preventing leakage while also providing a second functional group at the other terminus that is used to form a strong covalent bond to the targeting molecule. Traditional concerns include the stability/inertness of the metal chelate, the level of purity of the labeled product, and the rapid, preferably single-step synthesis of the labeled complex. More recently there has been a focus on the impact of the hydrophobic nature of the chelate and its metal complex on pharmacokinetics and the development of labeling methods that produce compounds in high effective specific activity.[73,74]

Whatever approach is adopted, the design strategy depends on an understanding of the fundamental coordination chemistry of technetium. Factors such as coordination preferences, stable oxidation states, robust core structures, and ligand selection are crucial in the design of effective radiopharmaceuticals. Creation of new strategies in turn depends upon innovations and advances in our understanding of the basic coordination chemistry of technetium.[9,41]

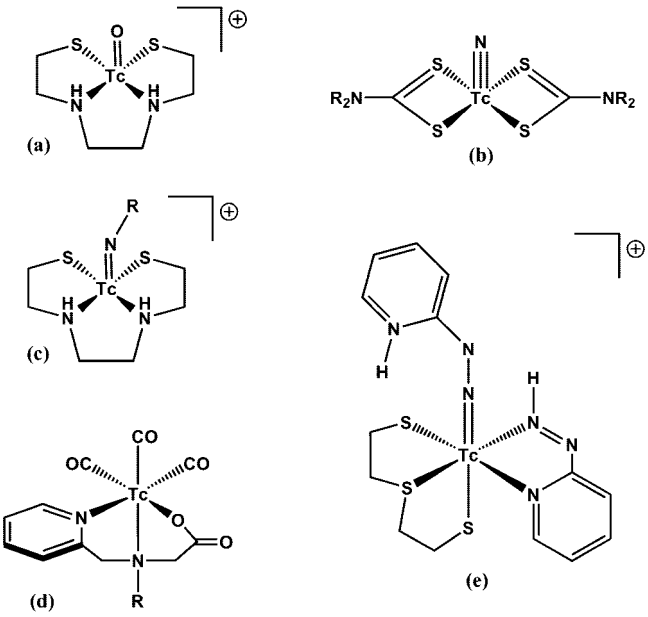


Figure 6. Common core structures for technetium and rhenium complexes: (a) Tc(V)−oxo; (b) Tc(V)−nitrido; (c) Tc(V)−imido; (d) Tc(V)−hydrazido; (e) Tc(I)−tricarbonyl.

Characterized compounds of technetium exist in oxidation states between −1 and +7.[75] On the one hand, this characteristic of the chemistry suggests considerable structural diversity to be exploited in the design of the radiopharmaceuticals. On the other hand, design consideration must deal with the potential for complex redox chemistry leading to complex product mixtures and the potential for labile complexes. Fortunately, the stable and readily accessible oxidation states are characterized by chemically robust core structures that can be exploited as platforms for radiopharmaceutical design. Since the coordination chemistry of technetium is the subject of several excellent reviews, we will highlight the chemistry that is most relevant to our discussion.

Common core structures for technetium are illustrated in Figure 6. The most extensively studied core is the $\{Tc(V)O\}^{3+}$ moiety, although the intensity of this area of research has decreased recently with the discovery of a convenient means to make Tc(I) complexes (vide infra).[76−107] Complexes of the Tc(V) core generally adopt square-pyramidal geometry with the π-bonding oxo-group in the apical position. Six coordination through ligation *trans* to

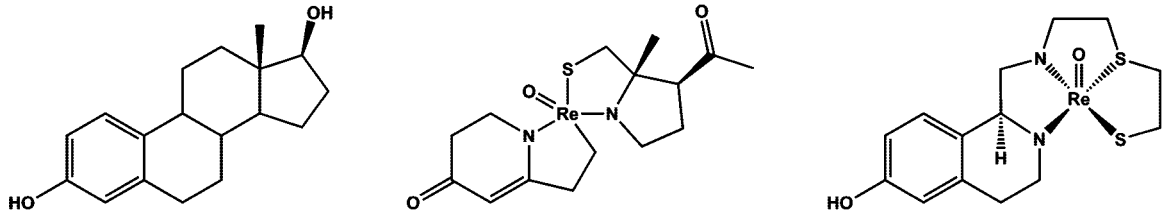


Figure 4. Estradiol and a rhenium mimic.

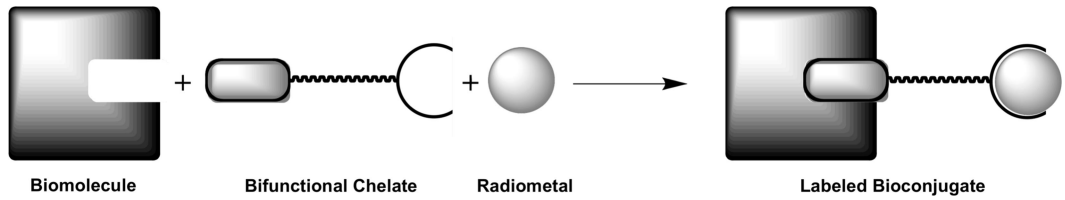


Figure 5. Schematic representation of the bifunctional chelate strategy.

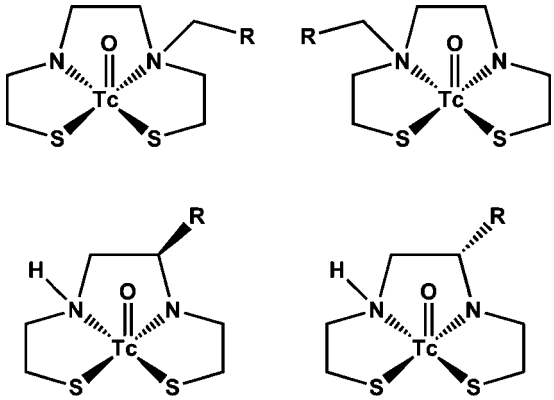


Figure 7. Tc(V)−oxo complex isomers: (a) optical isomers of a $\{Tc(V)O\}^{3+}$ core N_2S_2 complex; (b) *syn*- and *anti*-isomers of the $[TcO(N_xS_{4-x})]$ class of compounds.

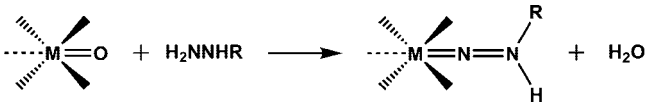


Figure 8. Preparation of a metal−hydrazido complex from the metal−oxo precursor by a simple condensation reaction.

the oxo-group is relatively uncommon due to the strong *trans* labilizing influence of the oxo-group, the steric crowding, and the displacement of the technetium out of the basal plane in the direction of the oxo-group. The core is stabilized by σ- and π-donating groups where amino, amido, and thiolate ligands, as well as tetradentate ligands of the $N_{4-x}S_x$ class, have been extensively investigated.[71−101] A representative example is provided by the peptide-based chelator mercapto acetylglycylglycylglycine (H_5MAG_3) (Figure 2).[101] Several other donor types, such as N_xP_y and S_xP_y types, have been developed to avoid the problem of *syn*- and *anti*-isomers of the $[TcO(N_{4-x}S_x)]$ class, which exhibit different pharmacokinetic properties (Figure 7). Other approaches have exploited (3 + 1) or (3 + 2) mixed ligand complexes that, while effective, have somewhat challenging properties with respect to translation to routine clinical use.[105−107]

The technetium−organoimido core $\{Tc(NR)\}^{3+}$ is isolobal with the $\{TcO\}^{3+}$ core, but is hydrolytically unstable under physiological conditions.[108−110] The structurally similar nitrido core $\{TcN\}^{2+}$ provides a building block with a different oxidation state for evaluating the influence of overall complex charge on biodistribution profiles.[111−116] However, additional steps are required to convert the parent pertechnetate to $\{Tc(V)N\}^{2+}$, rendering this approach problematic for development of imaging probes using traditional instant kit technologies.

An alternative approach to the design of compounds with stable and substitution inert metal−ligand bonds is metal−organohydrazine chemistry, first introduced in 1991.[117,118] The chemically robust metal−organohydrazino unit is readily accessible from the metal−oxo core by a simple condensation reaction (Figure 8). Furthermore, the effectiveness of the metal−organohydrazine interaction can be improved by exploiting the chelate effect in compounds devised for example from 6-hydrazinonicotinic acid (HYNIC).[119] An attractive feature of the HYNIC system is its simple bioconjugate chemistry, which can be used to derivatize a wide range of different targeting vectors.[41]

However, while the technetium− and rhenium−organohydrazino cores display the advantages of facile preparation and chemical robustness, the intimate details of the chemistry are complex, dependent upon reaction conditions and the presence of coligands.[120−126] Since the HYNIC ligand acts as a bidentate chelate, it occupies only two coordination sites on the metal, requiring a variety of coligands to satisfy the metal coordination requirements. A variety of coligands have been explored, including triphenylphosphine sulfinates,[127−131] nicotinic acids,[127,129,132] peptides,[133] ethylenediaminediacetic acid (EDDA),[134−137] glucarate,[138] glucamine,[138] tricine,[129,131,132,139] glucoheptonate,[140] mannitol,[140] and thiolates.[141,142] While many efforts have been made to establish the identities of the complexes on the tracer scale, the chemistry remains controversial. Several binary and ternary systems have been developed, including phosphine- and nicotinyl-containing HYNIC chelators (Figure 9).[140,143−145] The continuing difficulties with the HYNIC technology reflect the dependence of the coordination chemistry on reaction conditions and the low stabilities of complexes incorporating hydrophilic ligands. With the increasing requirements of regulators to have fully characterized products, translation of the HYNIC system to general clinical use will be problematic and will require in-depth characterization of all compounds at both the macroscopic and tracer levels.

While the interest in the metal−oxo and metal−organohydrazino cores remains unabated, other core geometries allow the introduction of novel chelators and targeting strategies. The organometallic nature of the $\{Tc(CO)_3\}^{+1}$ core

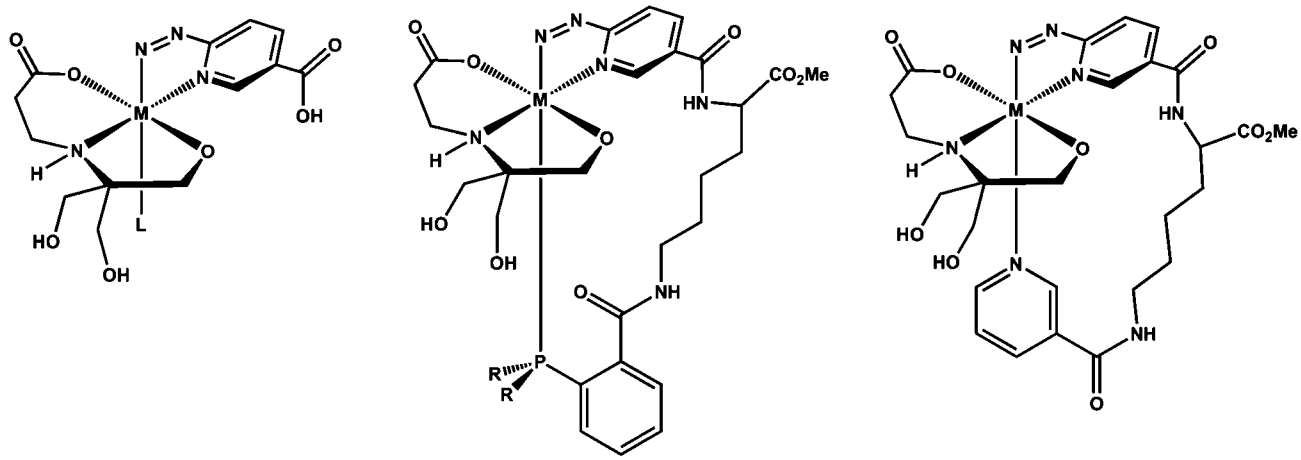


Figure 9. Example of technetium complexes with HYNIC and tricine and coligand. The phosphine and nicotinyl containing chelators obviate the need for a second coligand.

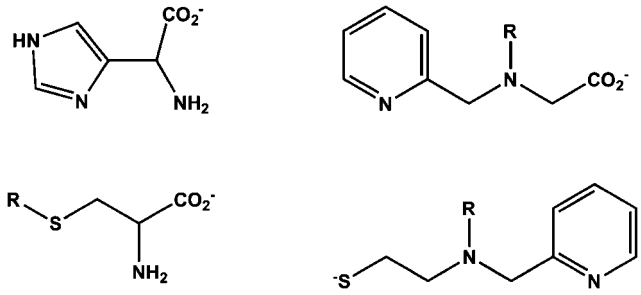


Figure 10. Representative tridentate ligands for chelation to the $\{M(CO)_3\}^+$ core.

has generated renewed interest in the design of ^{99m}Tc radiopharmaceuticals.[146−156] The core offers a number of attractive features: (i) The synthetic precursor $\{Tc(CO)_3(H_2O)_3\}^{+1}$ can be readily prepared from the pertechnetate salt under reducing conditions developed by Alberto et al. (ii) $\{^{99m}Tc(CO)_3(H_2O)_3\}^+$ is water-soluble and the aqua ligands readily undergo ligand exchange. (iii) The $\{Tc(CO)_3\}^+$ core is chemically robust and maintains its integrity under the most forcing conditions (in addition, since the electronic configuration of the metal in the $\{Tc(CO)_3\}^+$ core is d^6 low spin, the complexes are typically inert). (iv) The core is organometallic in nature, making the chelation more covalent in character. (v) The core is lipophilic.

While the robustness of complexes is purely kinetic in nature, essentially all types of donor atoms have been used (Figure 10). Studies by Schibli et al. on the influence of denticity on the fate of bidentate and tridentate $\{Tc(CO)_3\}^+$ core complexes indicate that complexes of the bidentate chelators are more likely to be retained in the liver and kidneys than the corresponding tridentate complexes.[157] The differences may be related to the susceptibility of the third, aqua ligand to exchange with proteins in the blood.[158,159] It is also noteworthy that the tridentate chelator can be readily modified to provide complexes with cationic, neutral, or anionic overall charge. Because the pharmacokinetic profiles of the complexes can also be influenced by introduction of additional functional groups (Figure 11), Tc(I) systems can be readily adapted to achieve the desired distribution when incorporated into targeting vectors.

3. Gallium Radioisotopes

3.1. Gallium Radioisotopes: Properties and Preparation

Currently, 30 different gallium isotopes are known including the two stable, nonradioactive isotopes ^{69}Ga and ^{70}Ga with natural abundancies of 60.11% and 39.89%, respectively. Radioactive gallium isotopes cannot be found in nature. Out of the existing radioactive isotopes, only ^{66}Ga, ^{67}Ga, and ^{68}Ga are radionuclides possessing decay properties and availabilities appropriate for use in clinical PET and SPECT studies.[160,161]

3.2. Gallium-67

Gallium-67 is cyclotron produced by the ^{68}Zn (p, 2n)−^{67}Ga reaction, as illustrated in Figure 12, in which a thin layer of enriched ^{68}Zn is electrochemically plated on an appropriate metal target (typically zinc or copper). After irradiation, gallium is dissolved off the target with an appropriate acid (e.g., HCl) and separation along with concentration is achieved by solvent/solvent extraction, ion-exchange chromatography, or extraction chromatography.[162−166] In most cases, the radionuclide is obtained in hydrochloric acid solutions of various concentrations. ^{67}Ga is readily available at reasonable cost (ca. $19/mCi), and its half-life is sufficiently long ($t_{1/2}$ = 78.3 h) to allow shipment of the radioisotope over long distances and central radiopharmacy preparation of radiopharmaceuticals.

For clinical applications, citric acid is often added as a solubilizer followed by neutralization and sterilization of the aqueous solution.[167] Gallium-67 is a pure γ-ray source and decays by electron capture to stable ^{67}Zn (Table 1). Several gamma photons of different energy are emitted at 93 keV (36%), 185 keV (20%), 300 keV (16%), and 394 keV (5%). The most widespread application for ^{67}Ga is the use in inflammation and tumor imaging where 2−5 mCi of carrier-free gallium citrate are administered intravenously ("gallium-scan", ^{67}Ga scintigraphy).[168] Despite the extensive use of ^{67}Ga salts in routine clinical applications for over 30 years, the

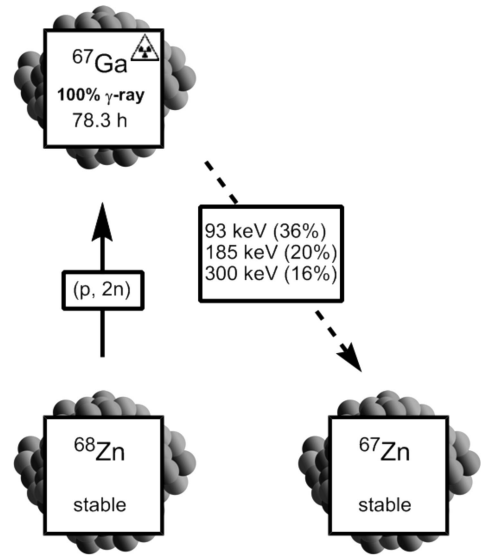


Figure 12. Production and decay of ^{67}Ga.

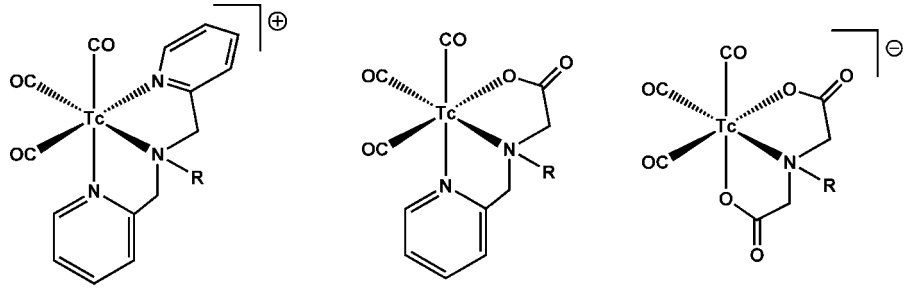


Figure 11. Examples of cationic, neutral, and anionic $\{^{99m}Tc(CO)_3\}^+$ core complexes.

Table 1. Gallium Radioisotopes

	Ga-67	Ga-68	
imaging modality	SPECT	PET	
decay mode	e^--capture	e^+-emission	
	0.091 MeV (2.9%)	β^+ Energies	Emitted γ
	0.093 MeV (35.7%)	**1899 keV (88%)**	**0.51 MeV (176%)**
	0.185 MeV (19.7%)	822 keV (1%)	0.80 MeV (0.4%)
	0.209 MeV (2.2%)		1.08 MeV (3.5%)
	0.300 MeV (16.0%)		1.24 MeV (0.14%)
	0.394 MeV (4.5%)		1.87 MeV (0.15%)
	0.888 MeV (0.1%)		
physical half-life	78.26 h = 3.26 d	67.71 min = 1.13 h	
specific activity	5.97×10^4 Ci/g; 2 210 TBq/g	4.10×10^7 Ci/g; 1.51×10^{18} Bq/g	
preparation	^{68}Zn (p,2n)−Ga^{67} cyclotron	Ge^{68}/Ga^{68} generator	

amount of work on bioconjugate-derived radiopharmaceuticals based on ^{67}Ga is comparatively small.

3.3. Gallium-68

Gallium-68 decays by positron emission (89%) and electron capture (11%) with a half-life of 67.71 min. The average positron energy per disintegration is 1899 keV (Table 1). The positron emission energy is higher than that for fluorine-18, the most widely used PET isotope, which potentially can lead to lower spatial resolution. The physical half-life is sufficiently long to allow preparation and purification of molecular probes and for imaging so long as the pharmacokinetics of the agent are sufficiently rapid. The half-life, however, does not allow for widespread shipping of the isotope, a problem which has been addressed through the development of a generator system.

The major advantage of this particular radionuclide lies in its accessibility from a ^{68}Ge/^{68}Ga generator system, which provides a non-cyclotron based and cost-effective source of the isotope (Figure 13). For gallium-68, the parent isotope is germanium-68 with a half-life of 270.8 days, allowing the manufacturing of long-lived generator systems (theoretically useful for 1−2 years) suitable for radiopharmaceutical applications. In addition, the chemical properties of Ge(IV) and Ga(III) are sufficiently different such that several methods for efficient separation have been proposed.[169−173] One of the challenges with a ^{68}Ge/^{68}Ga generator is the lack of efficient production methods for ^{68}Ge, which is generated using high-energy accelerators via the (p, 2n) reaction on gallium targets. Targets typically accumulate 33 000−45 000 μA·h of beams during an irradiation period of 4 weeks, to produce 14.8−18.5 GBq (400−500 mCi) of ^{68}Ge. The thick target yield (μCi/(μA·h)) is 91.4% if an energy of 50 MeV is used.[174]

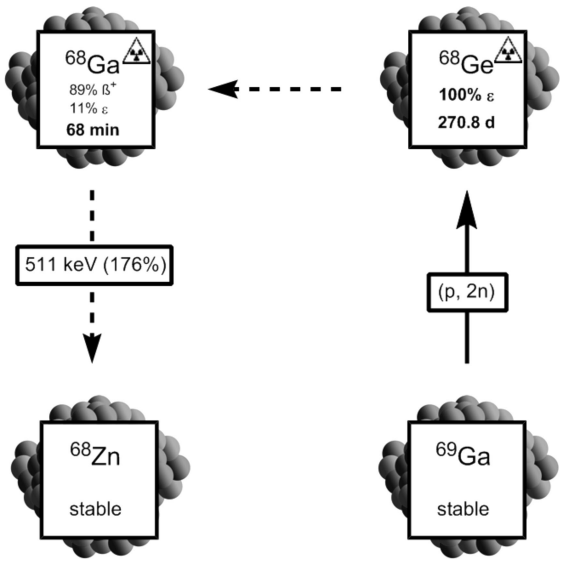


Figure 13. Production and decay of ^{68}Ge and ^{68}Ga.

3.4. ^{68}Ge/^{68}Ga Generators

A number of different generators with different stationary phases of inorganic and polymer-based composition for the ^{68}Ge/^{68}Ga pair has been developed and recently reviewed in detail by Maecke et al.[175] In early attempts, inorganic oxides such as Al_2O_3 or ZrO_2 were used as immobilized packing materials.[176] In these generators, carrier-free ^{68}Ge is neutralized, complexed with EDTA, and absorbed onto the column; ^{68}Ga can then be eluted with a 5 mM EDTA solution (elution yields ≈ 70−80%). An important factor for the preparation of radiopharmaceuticals is a straightforward synthesis of the radionuclide without necessity of further processing. Since these early systems provided the radionuclide in complexed form, destruction of the EDTA complex was necessary, which rendered the preparation of the radiopharmaceutical tedious, time-consuming, and with a reduced overall yield. Thus, further development focused on systems employing ^{68}Ga(III) in its hydrated form. Attempts using Al_2O_3 or $Fe(OH)_3$ stationary phases with HCl as the eluent, ZrO_2 or SiO_2 with HNO_3, as well as SnO_2 with HCl (∼80%) have been reported, but their clinical use failed because of oxides present in the eluate.[177,178] However, moderate yields (∼56%) and very low breakthrough of the column packing material and the parent isotope were reported for a CeO_2/0.02 M HCl generator.[179]

A different approach for purification of ^{68}Ga is the use of organic, polymer-based stationary phases with high-affinity functionalities. A macroporous styrene−divinylbenzene copolymer with *N*-methylglucamine groups has been described recently.[172,179] ^{68}Ga is eluted with the low affinity chelator sodium citrate in good yields (∼80%), and the ^{68}Ge leakage is reported to be less than 0.0004%. Even though the styrene−divinylbenzene matrix remains intact if exposed to 3.9×10^6 Gy of radiation, loss of the polyol functionalites required for the binding of Ge(IV) is observed. Further investigations on the leaching of the organic matrices have to be carried out before clinical applications are undertaken. Another complementary approach is a pyrogallol−formaldehyde resin with high affinity for Ge(IV), where ^{68}Ga is obtained as $^{68}GaCl_4^-$ using 5.5 M HCl as eluent.[180] The $[^{68}GaCl_4]^-$ complex is then adsorbed on a small anion-exchange column to remove low levels of Ge(IV) breakthrough (<1 ppm). Elution with small volume of water results

in the decomposition of the chloro complexes, and concentrated solutions of ^{68}Ga(III) in 0.5 M HCl are finally obtained (80%).

A commercially available generator based on titanium dioxide (Cyclotron Co., Ltd., Obninsk, Russia) is currently used worldwide and has helped to push forward activities around the development of ^{68}Ga based radiopharmaceuticals. The advantages of this particular generator include the use of a nontoxic packing material and, most importantly, the elution of free, cationic ^{68}Ga with low acid concentration of 0.1 M HCl, allowing universal application for radiopharmaceutical preparations. According to the manufacturer, the life span of the generator is 2 years and the ^{68}Ga yield is ~60% in 3−5 mL of eluate for a new generator and no less than 25% after 1 year of operation or 200 elutions. The ^{68}Ge breakthrough is reported to be between 0.001 and 0.005%.

3.5. Eluate Concentration and Purification

A general disadvantage related to all gallium generators is the large primary ^{68}Ga eluate volume leading to a low ^{68}Ga concentration. Thus, it is often the case that additional concentration of the radioactive solution is necessary in order to obtain high yields of radiolabeled compounds, which is a serious disadvantage, particularly compared to technetium generators where Curies of activity can be eluted in less than 100 mL and used directly. There have been a number of strategies to address the concentration issue (vide infra). An alternative approach, which to our knowledge has not been studied, is to use a rapid evaporator system like the V10 system from Biotage, which we have found particularly useful for drying aqueous systems containing ^{99m}Tc complexes.[181]

Beyond concentration issues, a general disadvantage found in most generator systems is contamination of the eluate with the long-lived parent nuclide ^{68}Ge(IV) and other cationic metal ions such as Ti(II) (from the column material), Zn(II) (^{68}Ga decay), and Fe(III). The impurities reduce ^{68}Ga labeling yields and specific activities, especially if low concentrations of the labeling precursor are usual.

Several post elution methods have been described to obtain ^{68}Ga(III) in sufficient concentrations with low amounts of cation impurities. One strategy exploits the fact that Ga(III) forms strong anionic complexes $[GaCl_n]^{(n-3)-}$ ($n = 4$ or 6) in hydrochloric acid solutions (>4.5 M). These complexes can then be separated effectively from metal cations and organic impurities by strong anion-exchange chromatography. The elution with water leads to the decomposition of the gallium chloro complexes, producing hydrated ^{68}Ga(III) in small volumes.[171,182] It also has been found that ~80% of radioactivity is present in the first 1 mL of eluate and that fractionation results in lower breakthrough and lower concentration of other metal cation contaminants.[183] Cation-exchange chromatography with an acetone/HCl mixture as the mobile phase was also investigated for the purification and concentration of the generator eluate. With an optimized ratio of 80% acetone in 0.15 M HCl, the majority of impurities were eluted and purified ^{68}Ga was obtained.[170] Some groups have more recently developed automated systems for purification, concentration of the generator eluates, and labeling of a suitable ligand.[184,185]

In the case of the ^{68}Ge/^{68}Ga system, both fundamental requirements for a generator strategy, a long-lived parent nuclide, and an efficient separation of ^{68}Ga are thus fulfilled, making this system ideal for the development of PET radiopharmaceuticals. Today, several ^{68}Ge/^{68}Ga generator systems are commercially available from distributors in Russia, Europe, and the United States. With the availability and reliability of commercial generator systems, ^{68}Ga has the potential to become as useful for PET as ^{99m}Tc is for SPECT imaging.

3.6. Gallium: Properties and Coordination Chemistry

Gallium is a nonphysiological metal of group 13 of the Periodic Table. Because of its low redox potential, the solution chemistry in aqueous media is exclusively represented by the stable oxidation state +III. The low-valent oxidation state +I is not of significance under aqueous conditions and has consequently no relevance in the design of radiopharmaceuticals. In aqueous solution, the free hydrated Ga(III) cation is only stable under acidic conditions. In the pH range of 3−7, hydrolysis to insoluble $Ga(OH)_3$ occurs in the absence of stabilizing ligands. At high pH > 7, gallium hydroxide redissolves as $[Ga(OH)_4]^-$. In contrast to its group 13 congener indium hydroxide, gallium hydroxide is amphoteric, dissolving in acidic as well as alkaline media. The kinetic of multidentate ligands used for radiolabeling is slow, and rapid hydrolysis of Ga(III) to insoluble $Ga(OH)_3$ can take place in the labeling process (for Tc(V), reaction with poor ligands in water leads to the insoluble and unreactive TcO_2 hydrate). Hydrolysis and formation of insoluble gallium hydroxide in the preparation of radiopharmaceuticals remains a problem that can be avoided by ligand-exchange reaction in the presence of weak, stabilizing ligands such as citrate, acetate, or oxalate.

It was shown that, at physiological pH, a $Ga(OH)_4^-$ concentration up to 2.5×10^{-6} M can be obtained without the formation of insoluble $Ga(OH)_3$.[155] At concentration levels used for radiopharmaceuticals, precipitation of $Ga(OH)_3$ does not occur at physiological pH because of the almost exclusive formation of soluble $[Ga(OH)_4]^-$. Additionally, it should be noted that, in nonaqueous hydrochloric media (HCl > 3 M), Ga(III) forms hexa- and tetracoordinate chloro complexes, a fact which is exploited in the purification of radioactive gallium isotopes.[168,169]

The Ga(III) cation can be classified as hard Lewis acid because of its high charge density and small ionic radius (0.62 Å). As a result, its chelate chemistry is dominated by strong bonding to highly ionic, nonpolarizable hard Lewis bases such as nitrogen and oxygen donor atoms. Thus, ligands with carboxylate, phosphonate, hydroxamate, and amine functionalities form thermodynamically stable complexes with Ga(III), but also softer donor atoms such as phenolate and thiol groups were found to be effective.[186−191] Because of its small cationic radius, Ga(III) is often six-coordinate in a distorted octahedral fashion.[192] Especially in physiological media, gallium complexes with vacant coordination sites (five- and four-coordinate) are more sensitive to hydrolysis for steric and electronic reasons. Coordination chemistry and biological properties of Ga(III) are very similar to high-spin Fe(III) because of their comparable ionic radii (0.65 Å for Fe(III)), charge, and electronic configuration (no ligand field stabilization energy).

3.7. Gallium Chelators

The optimal chelator for radiopharmaceutical purposes should form complexes of high thermodynamic stability and/or kinetic inertness to avoid any premature ligand-exchange reactions or hydrolysis in vivo. Another important feature is rapid and efficient chelation of the radiometal at a pH that will not degrade biovectors. A related concern is the similarity of the coordination chemistry of trivalent gallium and iron, which must be taken into account when selecting or designing gallium chelates and imaging agents. Ligand exchange is performed with transferrin, an abundant plasma protein that has two iron binding sites with high affinity to Ga(III). At bicarbonate concentrations typical for blood serum, the formation constants are log $\beta_1 = 20.3$ and log $\beta_2 = 39.6$ for Ga(III) and log $\beta_1 = 22.8$ and log $\beta_2 = 44.3$ for Fe(III).[168,193]

Despite the original belief that, in targeted radiopharmaceuticals, the chelate played no role in targeting, there is an abundance of evidence in the literature to the contrary. Several publications and conference presentations have reported that the nature of a bifunctional chelate (geometry, lipophilicity, overall charge) plays a crucial role in determining the biodistribution of targeted radiopharmaceuticals. In recent years, various chelating agents of different structural types have been proposed for in vivo use with high selectivity and stability for binding of Ga(III). In this review, a brief summary of common gallium chelating ligands that fulfill the key requirements for radiopharmaceutical applications is presented. Additionally, the focus is on agents suitable for use as bifunctional chelates and the production of targeted imaging probes. For a more comprehensive review, we direct readers to a number of excellent articles covering a broader range of established and emerging gallium ligands.[34,168,175,192,194,195]

Table 2. Formation Constants of Ga(III) with Various Ligands

	K_1
oxalic acid	6.45
citric acid	10.02
transferrin	20.3
DOTA	21.33
EDTA	21.7
DTPA	23.32
NOTA	30.98
6SS	41.0

Even though it has been shown that four- and five-coordinate gallium complexes are of sufficient stability for use in vivo, saturation of the coordination sphere of gallium is desirable because coordinatively unsaturated complexes are generally more prone to ligand exchange or hydrolysis. Consequently, polydentate ligands with hard donor groups remain first choice for gallium labeled biomolecules. The most prominent multidentate representatives are the acyclic ligands 6SS (=*N,N′*-bis(2,2-dimethyl-2-mercaptoethyl)ethylenediamine-*N,N′*-diacetic acid), TAME Hex (=tris(aminomethyl)ethane-*N,N,N′,N′,N″,N″*-hexaacetic acid), DTPA (=diethylenetriaminepentaacetic acid), and deferoxamine as well as the macrocyclic NOTA (=1,4,7-triazacyclononane-1,4,7-triacetic acid) and DOTA (=1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) chelators shown in Figure 14.

The ligand 6SS provides a $N_2O_2S_2$ donor set of two amine-N, two carboxylate-O, and two thiol-S atoms. Even though thiols are not considered to be hard bases, they still are excellent donors for gallium reflected in the high complex stability (Table 2). Under in vitro and in vivo challenge conditions (blood serum), the corresponding Ga(6SS) complex is stable for at least 1 h. After rapid blood clearance, the complex is metabolized through the liver, showing rapid washout as well. Molecular mechanics calculations indicated

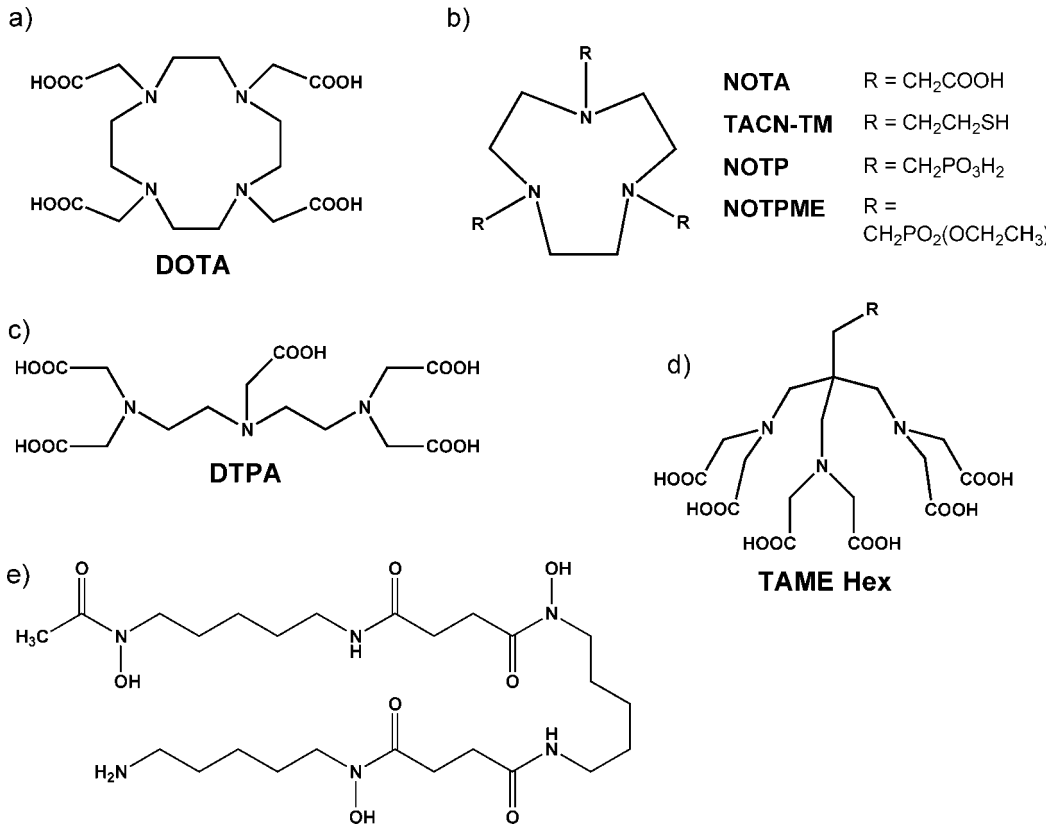


Figure 14. Schematic representations of the ligands DOTA, NOTA and derivatives, DTPA, TAME Hex, and deferoxamine.

a six-coordinate complex geometry for the Ga(6SS) complex. Moreover, the two *gem*-dimethyl groups increase the stability almost by a factor of 2 compared to its unmodified version EDDASS (=*N*,*N*′-bis(2-mercaptoethyl)ethylenediamine-*N*,*N*′-diacetic acid). Additionally, the labeling efficiency was >90% (30 min, room temperature) which is suitable for some, but not all, radiopharmaceutical applications. A bifunctional version B6SS (=1-(4-carboxymethoxybenzyl)-*N*,*N*′-bis[(2-mercapto-2,2-dimethyl)ethyl]-1,2-ethylenediamine-*N*,*N*′-diacetic acid), carrying a methoxybenzyl tether equipped with a carboxylic acid function for covalent coupling to biomolecules, has also been reported.[190,196]

The ligand TAME (TAME = 1,1,1-tris(aminomethyl)-ethane) is often used as a building block for more complex ligands. For example, the salicylaldimine derivative of TAME, H_3[(5-MeOsal)$_3$TAME], in combination with ^{68}Ga can be used to assess myocardial blood flow.[197] More recently, the synthesis of a bifunctional, multidentate TAME derivative, namely, TAME Hex, was described[198] where the ligand possesses nine potential donor atoms for metal binding. The radiolabeling yield of TAME Hex with ^{67}Ga was >99% (10 min, 100 °C), and the gallium complex was stable for 10 days in a ligand challenge experiment using a 1000-fold excess of DTPA. The bifunctional ligand carries a *p*-aminobenzyloxy moiety introduced at the 2-position of TAME, which can be easily transformed into its isothiocyanate for conjugation.

The ligand DTPA is one of the most commonly used acyclic ligands in radiochemistry and has been linked to biomolecules through reaction of the dianhydride. DTPA is not only used as gallium complexing agent, it is also successfully applied as chelator for many hard Lewis acids such as In(III) and rare earth metal cations.[199−206] This potentially octadentate ligand is able to form gallium chelate complexes of high stability that can be obtained in high radiochemical yield. Trivalent gallium is usually six-coordinate in its complexes, and DTPA is thus able to saturate the coordination sphere while allowing the covalent coupling to small biomolecules via a noncoordinated carboxylic acid function. The commercial availability of DTPA and its dianhydride facilitates its widespread use as a bifunctional chelator. Compared to macrocyclic ligand NOTA, however, Ga(DTPA) is of lower thermodynamic stability. Surprisingly and to the best of our knowledge, neither a single-crystal structure analysis nor solution conformation studies have been performed to date to elucidate the coordination geometry of the Ga(DTPA) complex in detail.

Ligands based on polyaza-macrocycles, e.g., 1,4,7-triazacyclononane (TACN, [9]aneN3) and 1,4,7,10-tretraazacyclododecane (Cyclen, [12]aneN4), have also proven to be effective chelating agents for trivalent gallium. Several multidentate derivatives with additional thiol-S, carboxylate-O, and phosphate-O donors have been described in the literature.[207−213] The most popular representatives in this category are the ligands NOTA and DOTA, carrying one additional acetic acid group at each nitrogen atom of the macrocycle (Figure 14). The thermodynamic stability of Ga(III) complexes of both ligands is sufficiently high enough for use in clinical practice (see Table 2). However, the Ga(NOTA) complex is of much higher stability than its corresponding DOTA counterpart, which is a consequence of the good fit of the small Ga(III) cation in the cavity of the nine-membered triaza-macrocycle of NOTA.[214] Switching to a 12-membered ring in the case of DOTA results in an unfavorable large coordination geometry and, consequently, in steric strain in the ligand backbone, which causes a decrease in stability.

In the crystal structures of Ga(DOTA-D-PheNH_2) and Ga(DOTA), Ga(III) is coordinated by a N_4O_2 donor set in which the cation is encapsulated in a *cis*-pseudooctahedral fashion by the DOTA subunit while one carboxylic acid group is deprotonated and does not coordinate to the metal. The remaining carboxylic acid function is either conjugated to the amino acid via the amide in Ga(DOTA-D-PheNH_2) or does not bind to the metal.[215,216] NOTA, on the other hand, binds Ga(III) with a low-strain pseudooctahedral *fac*-N_3O_3 donor set as illustrated by the crystal structure of the NOTA surrogate NODASA (=1,4,7-triazacyclononane-*N*-succinic acid-*N*′,*N*″-diacetic acid). A bifunctional NOTA derivative with a pendant carboxylic acid function for coupling is also available.[217,218] Another bifunctional NOTA analogue is NODAGA (=1,4,7-triazacyclononane-*N*-glutamic acid-*N*′,*N*″-diacetic acid).[219,220] Both ligands are of particular interest because of their higher labeling efficiencies compared to corresponding DOTA derivatives and the superior stability of their Ga(III) complexes. Other TACN-based ligands are TACN−TM (=1,4,7-tris(2-mercaptoethyl)-1,4,7-triazacyclonane), a derivative with three additional ethylmercapto arms,[194] and the two phosphonate-containing counterparts NOTP (=1,4,7-triazacyclononane-*N*,*N*′,*N*″-tris(methylenephosphonic)acid),[213,221] NOTPME (=1,4,7-triazacyclononane-*N*,*N*′,*N*″-tris(methylenephosphonate−monoethylester)),[222,223] and NOKA (=6,6′,6″,-(1,4,7-triazonane-1,4,7-triyl)tris-(methylene)tris(5-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one) carrying koijic acid functionalities.[224] This class of macrocyclic triaza ligands displays a distinctive tendency toward the small Ga(III) cation originating mostly from the size-selectivity effect. These ligands are able to encapsulate Ga(III) with high specificity, and uncharged complexes of this nature are less sensitive toward proton-catalyzed dissociation than anionic complexes. An illustrative example on the importance of the overall charge is the neutral complex Ga(NOTA), which decomposes only at low pH.

4. Bifunctional Strategies

The design of most targeted molecular imaging agents consists of a ligand system that binds to a radiometal and contains a functional group suitable for linking the complex to a targeting biomolecule (Figure 15). Examples of reactive functionalities used to link chelates to vectors include aromatic isothiocyanates, isocyanates, carboxylic acid as active esters, iodo-acetamides, and mixed anhydrides among other derivatives that can react with nucleophilic sites (−NH_2, −SH, or −OH) on or attached as a prosthetic group to the targeting vector.[225] Recently, click chemistry involving azides and alkynes, and epoxides are becoming increasingly popular bioconjugate chemistry strategies. The optimal reaction conditions for conjugation include mild aqueous conditions close to physiological pH, short reaction times, and minimal purification.[226] Some chelate systems already contain reactive conjugation sites, while others require modifications to introduce the linker site, as is usually the case when using click chemistry.

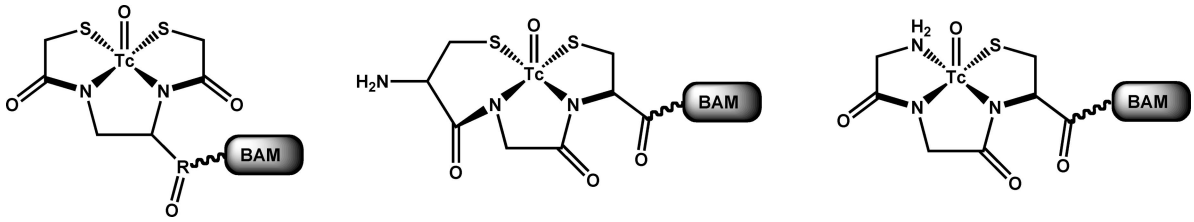


Figure 15. Schematic representations of the conjugation of $\{Tc(V)O\}^{3+}$ core complexes to biomolecules.

4.1. Conjugation Strategies for ^{99m}Tc

Conjugation strategies for linking technetium-99m complexes to targeting vectors have been extensively investigated and involve a range of different oxidation states,[34,227] with the most common being +1, +3, and +5. Here we focus on the most common ligand systems and the modes used to link them to the targeting vectors over the last 10 years and compare the systems for technetium-99m and gallium.

The technetium(V)−oxo and technetium(V)−organohydrazino cores are the most extensively studied. The $\{TcO\}^{+3}$ unit is generally coordinated to a tetradentate ligand that utilizes a carboxylic acid side-group to link to biomolecules. The technetium oxo-bifunctional chelate, MAG_3H_5 (Figure 2), for instance, can be readily derivatized as the S-acetyl MAG_3−ethyl ester or S-acetyl MAG_3−hydroxysuccinimidyl ester for conjugation to vectors including monoclonal antibodies. A similar technetium−oxo system that has been linked to biomolecules is derived from the N_xS_{4-x}-class of ligands (Figure 6). These tetradentate ligands have been conjugated via carboxylic acids (COOH), amino (NH_2), and thiocyanate (NCS) groups to biomolecules, including peptides.[228−236]

As mentioned previously, the HYNIC ligand has facile bioconjugate chemistry. HYNIC-peptide conjugates are formed via amide linkage formed through reaction of the active ester derivative of the ligand (Figure 16). This approach has been used for radiolabeling antibodies, human serum albumin, a variety of medium to high molecular weight proteins, oligopeptides, and small biomolecules.[237−280] The issue of the poorly characterized coordination chemistry of the associated bioconjugates, as mentioned previously, detracts from the attractiveness of this ligand system.[281]

The accessibility of the +1 oxidation state for technetium has brought about the development of new bifunctional

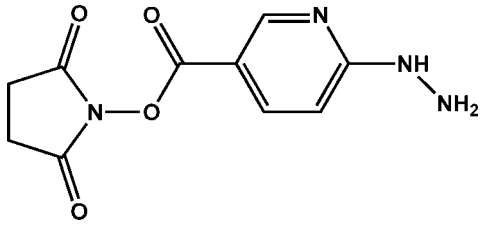


Figure 16. *N*-Hydroxysuccinimidyl hydrazinonicotinate.

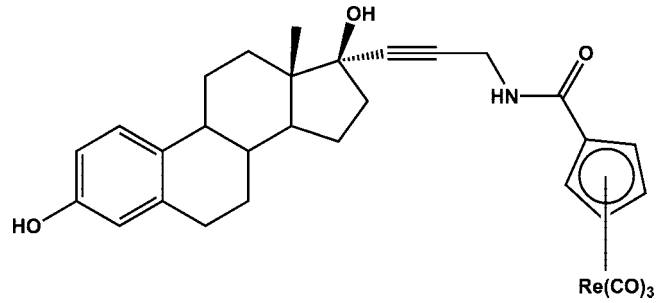


Figure 18. Coupling of $[Re(CO)_3(CpCO_2H)]$ to estrynamide.

ligand systems. An example of a bifunctional chelate, based on lysine, that uses the technetium(I) oxidation state is the single amino acid chelate system (SAAC) (Figure 17). The ligand can be readily incorporated into any peptide using standard solid- or solution-phase coupling methodologies as if it were a natural amino acid using traditional automated peptide synthesis.

Tc(I) can also form bifunctional organometallic complexes that are sufficiently robust to be used to prepare bioconjugates.[282] Similar to bifunctional chelate ligand systems, modifications to organometallic ligands include the introduction of carboxylic acid groups as a site of conjugation. An example of this is the preparation of estrynamide with $CpRe(CO)_3CO_2H$ via an amide linkage (Figure 18).[282] Further modification to the cyclopentadiene ligand has been explored with the development of a diazocyclopentadiene derivative. The rapid coupling of the diazocyclopentadiene with boronic acid derivatives with no addition of catalyst demonstrates nearly ideal conditions to generate stable organometallic complexes; however, the high level of instability of diazocyclopentadiene may limit future use.[283]

The multifunctional nature of both a targeting biomolecule and a suitable imaging radiometal chelate unit can complicate the preparation of the conjugate due to potential side reactions or the lack of specificity of the coupling step. The use of multistep syntheses, including protective groups, to address this problem can significantly impact the overall efficiency of the preparations. This problem has been addressed recently by the introduction of "click" chemistry to the preparative arsenal of bifunctional chelates.[283,284] "Click" chemistry generally refers to the copper(I) catalyzed [3 + 2]-cycloaddition of terminal alkynes and azides. The

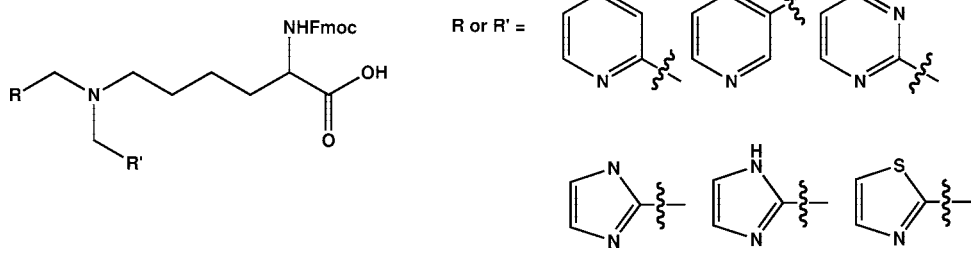


Figure 17. Examples of lysine-based bifunctional chelators for the $\{^{99m}Tc(CO)_3\}^+$ core.

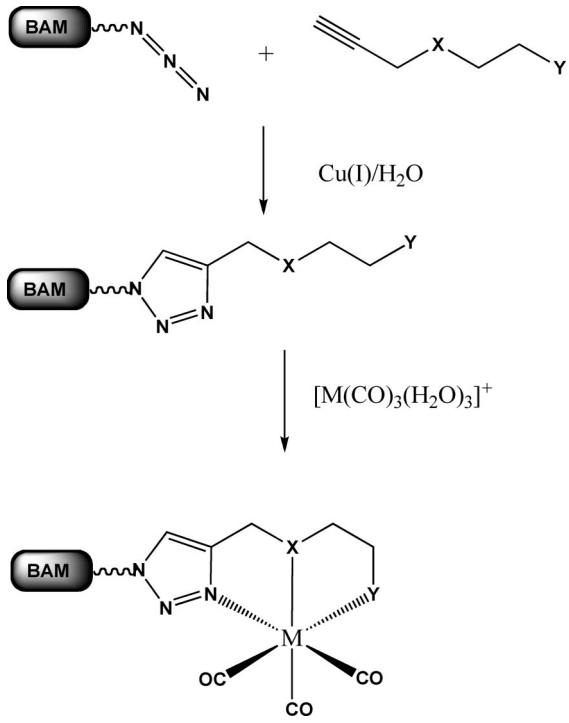


Figure 19. Schematic of the modular "click-to-chelate" approach.

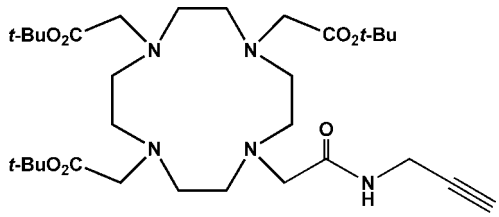


Figure 20. Alkyl derivative of DOTA−tris(*tert*-butyl ester).

many advantages of click chemistry include efficiency, selectivity, avoidance of side reactions, and mild conditions. As shown in Figure 19, the approach allows the facile synthesis of bifunctional ligands in which 1,4-disubstituted triazoles form an integral part of the metal chelating system. In addition to the tridentate ligands of Figure 19, the alkyne derivative of DOTA−tris-*tert*-butyl ester, shown in Figure 20, has been coupled to folate-γ-(4-azido)butane amide and shown to efficiently radiolabel with ^{67}Ga.[284]

4.2. Conjugation Strategies for ^{68}Ga

The development of gallium-based agents utilizes similar conjugation strategies as technetium-99m. DOTA forms a stable metal−chelate complex and has been linked to various biomolecules (Table 3) via either an amide or thiourea linkage. Modification to one of the carboxylic acid groups on DOTA to generate a NHS-ester was used to link the chelate to a range of different vectors including biotin and small peptides.[225,285] *p*-Isothiocyanatobenzyl DOTA is the most widely used DOTA derivative and has been used with both proteins and antibodies.[211] DOTA−tris(*tert*-butyl ester) (Figure 20) is a popular ligand for attaching DOTA to a peptide on solid support with analogy to the SAAC system, except that it can only be used to derivatize the N-terminus of the peptide chain attached to the solid support or to a lysine side chain.[225] One disadvantage of this ligand system is the long deprotection times required to cleave the *tert*-butyl esters. Alternative protecting groups, including tris(allyl ester), tris(methyl ester), and tris(benzyl ester), have been explored to address this issue.

Table 3. Summary of Conjugation Strategies and Chelate Systems for Targeted Gallium Radiopharmaceuticals

conjugation strategy	chelate	biomolecule	reference
amide formation	DOTA	biotin	285
		peptide	225
		MMP-2	288
		octreotate	289
		human epidermal growth factor (h EGF)	286
		bombesin	287
		somatostatin	295
thiourea formation	DOTA	protein and antibody	225
amide formation	NOTA	peptides (TATE) [Tyr^3]−octreotide	289−291
thiourea formation	NOTA	cRGDyK	292
		RGD peptide [Tyr^3]−octreotide	289
amide formation	CHX-A	Re(Arg^{11})−CCMSH	294
	DFO	antibody	293

DOTA-derived human epidermal growth factor (hEGF) was prepared by coupling of *N*-sulfosuccinimide ester of DOTA to hEGF. Binding data for this complex showed high affinity (~ 2 nM) in U343 glioma cells and A431 cervical carcinoma cells. Biodistribution studies showed accumulation of radioactivity in xenografts and in EGFR-expression organs, indicating its potential for imaging EGFR overexpressed in tumors.[286] Similar strategies have been used to prepare a bombesin Ga derivative, which showed high and specific internalization in animal models. However, the replacement of gallium with ^{177}Lu resulted in slightly reduced (~20%) tumor uptake and residence time.[287] For diagnostic imaging of somatostatin receptor-positive tumors, [^{68}Ga-DOTA,Tyr^3]octreotide was developed. The gallium complex showed distinctly better preclinical, pharmacological performance than the ^{111}In-labeled analogue.[288] Following promising in vivo and in vitro studies, the Ga derivative showed approximately 2.5 times greater tumor uptake in a mouse and lower kidney uptake than the ^{111}In or ^{90}Y DOTA−octreotide derivatives.[289] Further modifications to the labeling strategy of DOTA with gallium have been investigated by Cantorias and co-workers, who used microwave-assisted heating to label DOTA-Re(Arg^{11})CCMSH with ^{68}Ga in less than 1 min. With the reduction of reaction time, the use of high-performance liquid chromatography (HPLC) purification could be used to generate a high specific activity conjugate in a reasonable time frame (less than 30 min for synthesis and purification).[290]

Recently, labeling of NOTA octreotide (TATE) with ^{68}Ga was achieved in nearly quantitative yield within 10 min at room temperature. The complex was incubated and found to be stable in the reaction mixture of phosphate buffer and human plasma.[291] The NOTA-based chelator has also been used for coupling with RGD peptides via thiourea formation, leading to quantitative labeling with ^{68}Ga at room temperature.[289] NOTA-cRGDyK, conjugated through a isothiocyanatobenzyl linkage, was labeled in high yields and showed high affinity to $a_v\beta_3$ integrin and showed specific uptake to angiogenic muscle in vivo.[292]

An alternative to cyclic chelator NOTA, deferoxamine (DFO), has been linked to antibodies by two methods: (i) antibody−lysine conjugation and (ii) mild reduction of antibody to generate thiols. Labeling with ^{67}Ga was efficient; however, the complex was poorly retained within cells after

antibody internalization and catabolism.[293] Other bifunctional chelators for gallium, including CHX-A″, which was linked to the melanoma-targeting peptide (Arg^{11})CCHMSH, could be labeled efficiently with ^{68}Ga.[294] The commercial availability of these different bifunctional chelates as bulk chemicals and products suitable for manufacturing under GMP guidelines, particularly the NOTA and DOTA derivatives, will unquestionably help support the development of new gallium agents.

5. Comparing and Contrasting Gallium and Technetium

The development of targeted metal-based radiopharmaceuticals is a nontrivial task which is evident by the fact that, after a herculean effort by technetium researchers in academia and in industry, there have been few truly effective agents that are in widespread clinical use. This is due to a combination of technical and economic factors. However, with molecular imaging moving to the forefront of modern medicine, particularly as we enter the era of personalized medicine, there is an increasing demand for new agents and a need for innovative radiopharmaceutical discovery and design paradigms. There is also a change being driven by the shortage of technetium, whose abundance and highly subsidized costs influenced much of the radiopharmaceutical development work that has taken place since the 1970s.

Going forward, it is difficult to predict what the clinical use of various isotopes will be. However, it is clear that the growth of the field will continue to rely upon advances in our understanding of the coordination and bioconjugate chemistry of radiometals in concert with the development of new imaging cameras including high-resolution disease-specific systems and hybrid imaging tools such as PET−MRI and SPECT−CT. We can also learn a number of valuable lessons from the years spent investigating the radiopharmaceutical chemistry of technetium to help expedite work with other promising radiometals like ^{68}Ga. These include the following:

5.1. Keep the Chelate Chemistry Simple

One of the lessons learned from the development of radiopharmaceuticals derived from the Tc(V) core is that, the more complex the coordination chemistry, the more difficult it is to develop a viable agent. Researchers making new chelates need to avoid creating systems that form multiple stereoisomers (or avoid reactions that are not enantio- and diastereoselective). Products of this nature generally have different biodistribution and metabolism profiles and will therefore have to be fully characterized or separated prior to clinical use, which increases translational costs and complexity. On a related note, the chemistry used to prepare the chelate should involve as few steps as possible, involve a minimum number of orthogonal protecting groups, and be scalable to the preparation of multigram quantities of product.

5.2. Versatile Bioconjugate Chemistry is Key

Finding the appropriate biovector, site of conjugation, linker group, and tether length to produce a viable molecular imaging probe is a complex and lengthy process. Ligands that have limited flexibility with respect to how they are linked to targeting vectors will prevent downstream optimization (usually around pharmacokinetic optimization), which in almost all cases is the critical step to successful translation. The flexibility is paramount to preventing premature catabolism, optimizing the site of derivatization, and adjusting the polarity of the overall conjugate as a means of fine-tuning the route of clearance and accessing the target of interest.

5.3. Engineer Quality Early in your Radiochemistry

The ability to label compounds at room temperature in solvents that are biocompatible increases the general utility of a ligand system. Of equal importance is the development of analytical methods that adequately demonstrate the purity of new complexes and can be used to measure the effective specific activity. For technetium, much of the early quality assurance and quality control (QA/QC) work was done using iTLC (instant thin-layer chromatography) which is suitable for Tc-essential compounds but not for targeted agents. For the latter, the free ligand or impurities where the metal is bound to sites on the vector other than the chelate are not detectable by iTLC and can, in many cases, compete with the compound of interest for the target. Impurities can lead to erroneous conclusions about an agent's ability to reach a target of interest. An additional benefit to developing good analytical procedures early is that it will facilitate later translation and regulatory approvals. Most countries require, or are shortly moving toward requiring, GMP manufacturing for all new agents, so well-developed analytical methods are essential.

5.4. Do not Ignore Pharmacokinetics Considerations in Early Compound Design

Many of the early Tc-bifunctional ligands were as lipophilic as steroids, such that, when they were linked to targeting vectors, they significantly increased nonspecific binding. As mentioned in point (1), new Ga and Tc chelates should engineer in the ability to adjust the log P of their chelates and be able to change the charge of the overall complex as a means to optimize the route of clearance.

5.5. Look to Other Fields for Innovation and Inspiration

Some of the most exciting innovations in the field have been inspired from outside the traditional realm of Tc coordination chemistry. The quintessential example is the use of organometallic chemistry to develop Tc compounds pioneered by Jones and Davison and driven to a new level with the discovery of the $\{Tc(CO)_3\}^+$ core. With rapid developments in microfluidic chemistry, high-content drug screening, dendrimer sciences, and dedicated imaging camera development, there are many opportunities to push the boundaries of the field and overcome current issues.

5.6. Match the Isotope with the Appropriate Targeting Vector

One mistake that groups made in the past was to become attached to a particular medical isotope. Whether or not the properties of the radionuclide matched the intended application, attempts were made to develop a viable agent. The field is shifting and researchers are now selecting the isotope and/or chelate that best matches with the pharmacokinetics of

the targeting vector while also considering the procedures and schedules that are followed in clinical nuclear medicine facilities. This trend must continue because the field of radiometal chemistry needs to identify viable new probes to meet the expectations of clinicians and patients.

6. References

(1) Rhodes, D. J.; O'Connor, M. K.; Phillips, S. W.; Smith, R. L.; Collins, D. A. *Mayo Clin. Proc.* **2005**, *80*, 24.
(2) O'Connor, M. K.; Phillips, S. W.; Hruska, C. B.; Rhodes, D. J.; Collins, D. A. *Breast J.* **2007**, *13*, 3.
(3) Hruska, C. B.; Phillips, S. W.; Whaley, D. H.; Rhodes, D. J.; O'Connor, M. K. *Am. J. Roentgenol.* **2008**, *191*, 1805.
(4) Moses, W. W. *Nucl. Instrum. Methods Phys. Res., Sect. A* **2004**, *525*, 249.
(5) Berman, C. G. *Cancer Control* **2007**, *14*, 338.
(6) Garcia, E. V.; Faber, T. L. *Cardiol. Clin.* **2009**, *27*, 227.
(7) Slomka, P. J.; Patton, J. A.; Berman, D. S.; Germano, G. *J. Nucl. Cardiol.* **2009**, *16*, 255.
(8) Garcia, E. V.; Faber, T. L. *Curr. Cardiovasc. Imaging Rep.* **2009**, *2*, 230.
(9) Abram, U.; Alberto, R. *J. Braz. Chem. Soc.* **2006**, *17*, 1486.
(10) Mindt, T.; Struthers, H.; Garcia-Garayoa, E.; Desbouis, D.; Schibli, R. *Chimia* **2007**, *61*, 725.
(11) Alberto, R. *Comp. Coord. Chem. II* **2004**, *5*, 127.
(12) Jurisson, S. S.; Lydon, J. D. *Chem. Rev.* **1999**, *99*, 2205.
(13) Liu, S.; Edwards, D. S. *Chem. Rev.* **1999**, *99*, 2235.
(14) Liu, S.; Edwards, D. S.; Barrett, J. A. *Bioconjugate Chem.* **1997**, *8*, 621.
(15) Dilworth, J. R.; Parrott, S. J. *Chem. Soc. Rev.* **1998**, *27*, 43.
(16) Hom, R. K.; Katzenellenbogen, J. A. *Nucl. Med. Biol.* **1997**, *24*, 485.
(17) Eckelman, W. C. *Eur. J. Nucl. Med.* **1995**, *22*, 249. [and references therein].
(18) Schwochau, K. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2258.
(19) Jurisson, S.; Berning, D.; Jia, W.; Ma, D. *Chem. Rev.* **1993**, *93*, 1137.
(20) Nicolini, M., Mazzi, U., Eds. *Technetium, rhenium and other metals in chemistry and nuclear medicine*; Servizi Grafici Ed.iali pnc: Padova, Italy, 2002; Vol. 6.
(21) Nicolini, M., Bandoli, G., Mazzi, U., Eds. *Technetium, rhenium and other metals in chemistry and nuclear medicine*; SGE Ed.iali: Padova, Italy, 1999; Vol. 5.
(22) Nicolini, M., Bandoli, G., Mazzi, U., Eds. *Technetium and rhenium in chemistry and nuclear medicine*; Raven Press: New York, 1990.
(23) Steigman, J.; Eckelman, W. *The chemistry of technetium in medicine*; National Academy Press: Washington, DC, 1992.
(24) Shiue, C. Y.; Eck, S. *Handbook of Radiopharmaceuticals*; Welch, M. J., Radvanly, C. S., Eds.; John Wiley & Sons: New York, 2003; pp 467−486.
(25) Herschman, H. R. *Science* **2003**, *302*, 605.
(26) Xavier, C.; Pak, J.-K.; Santos, I.; Alberto, R. *J. Organomet. Chem.* **2007**, *692*, 1332.
(27) Pinkerton, T. C.; Desilets, C. P.; Hoch, D. J.; Mikelsons, M. V.; Wilson, G. M. *J. Chem. Educ.* **1985**, *62*, 965.
(28) Deutsch, E.; Libson, K.; Jurisson, S.; Lindoy, L. F. *Prog. Inorg. Chem.* **1983**, *30*, 75.
(29) Int. J. Appl. Radiat. Isot. 1983, *33*, 793 [special issue on Tc].
(30) Deutsch, E.; Bushong, W.; Glavan, K. A.; Elder, R. C.; Sodd, V. J.; Scholz, K. L.; Fortman, D. L.; Lukes, S. J. *Science* **1981**, *214*, 85.
(31) Deutsch, E.; Libson, K. *Comments Inorg. Chem.* **1984**, *3*, 83.
(32) Deutsch, E., Nicolini, J., Wagner, H. N., Eds. *Technetium in chemistry and nuclear medicine*; Cortina International: Verona, Italy, 1983.
(33) Clark, M. J.; Podlielski, L. *Coord. Chem. Rev.* **1987**, *78*, 253.
(34) Liu, S. *Adv. Drug Delivery Rev.* **2008**, *60*, 1347.
(35) Garcia-Garayoa, E.; Schibli, R.; Schubiger, P. A. *Nucl. Sci. Tech.* **2007**, *18*, 88.
(36) Forrester, J. S., Jr.; Liebson, P. R.; Parrillo, J. E.; Klein, L. W. *Prog. Cardiovasc. Dis.* **2002**, *45*, 49.
(37) Open Congress for the 111th United States Congress. http://www.opencongress.org/bill/111-h3276/show, March 12, 2009.
(38) National Resources Canada News Room. http://www.nrcan.gc.ca/media/newcom/2009/200951-1-eng.php, May 5, 2009.
(39) Homepage of Brookhaven National Laboratory. www.bnl.gov/bnlweb/history/tc-99m.asp, March 12, 2009.
(40) Eckelman, W. C.; Volker, W. A.; Bonardi, M. *Nucl. Med. Biol.* **2008**, *35*, 523.
(41) Liu, S. *Chem. Soc. Rev.* **2004**, *33*, 445.
(42) Banerjee, S. R.; Maresca, K. P.; Francesconi, L.; Valliant, J.; Babich, J.; Zubieta, J. *Nucl. Med. Biol.* **2005**, *32*, 1.
(43) Bartholoma, M.; Valliant, J.; Maresca, K. P.; Babich, J.; Zubieta, J. *Chem. Commun.* **2009**, 493.
(44) Banerjee, S.; Pillai, M. R. A.; Ramamoorthy, N. *Semin. Nucl. Med.* **2001**, *31*, 260.
(45) Jain, D. *Semin. Nucl. Med.* **1999**, *29*, 211.
(46) Prats, E.; Aisa, F.; Abos, M. D.; Villavieja, L.; Garcia-Lopez, F.; Asenjo, M. J.; Razola, P.; Banzo, J. *J. Nucl. Med.* **1999**, *40*, 296. [and references therein].
(47) Johnson, L. L.; Seldin, D. W. *Am. J. Cardiol.* **1990**, *66*, 63E.
(48) Woolley, G. T.; Kitson, S. L.; Reid, R. G. *J. Labelled Compd. Radiopharm.* **2007**, *50*, 468.
(49) Kocmur, M.; Milcinski, M.; Budihna, N. V. *Eur. J. Nucl. Med.* **1998**, *25*, 1412.
(50) Brooks, K. E.; Rawal, N.; Henderson, A. R. *Clin. Chem.* **1986**, *32*, 2195.
(51) Folks, R. D.; Garcia, E. V.; Taylor, A. T. *J. Nucl. Med. Technol.* **2007**, *35*, 27.
(52) Majewski, W.; Zimmer, A. M.; Spies, S. M. *J. Nucl. Med. Technol.* **1983**, *11*, 23.
(53) Kung, H. F.; Kung, M.-P.; Wey, S.-P.; Lin, K.-J.; Yen, T.-C. *Nucl. Med. Biol.* **2007**, *34*, 787.
(54) Acton, P. D.; Meyer, P. T.; Mozley, P. D.; Plossl, K.; Kung, H. F. *Eur. J. Nucl. Med.* **2000**, *27*, 1714.
(55) Mozley, P. D.; Schneider, J. S.; Acton, P. D.; Plossl, K.; Stern, M. B.; Siderowf, A.; Leopold, N. A.; Li, P. Y.; Alavi, A.; Kung, H. F. *J. Nucl. Med.* **2000**, *41*, 584.
(56) Zhuang, Z.-P.; Plossl, K.; Kung, M.-P.; Mu, M.; Kung, H. F. *Nucl. Med. Biol.* **1999**, *26*, 217.
(57) Dresel, S. H. J.; Kung, M.-P. T.; Huang, X.-F.; Plossl, K.; Hou, C.; Shiue, C. Y.; Karp, J.; Kung, H. F. *Eur. J. Nucl. Med.* **1999**, *26*, 342.
(58) Meegalla, S. K.; Plossl, K.; Kung, M.-P.; Stevenson, D. A.; Mu, M.; Kushner, S.; Liable-Sands, L. M.; Rheingold, A. L.; Kung, H. F. *J. Med. Chem.* **1998**, *41*, 428.
(59) Kung, H. F.; Kim, H.-J.; Kung, M. P.; Meegalla, S. K.; Plössl, K.; Lee, H.-K. *Eur. J. Nucl. Med.* **1996**, *23*, 1527.
(60) Hom, K. R.; Chi, D. Y.; Katzenellenbogen, J. A. *J. Org. Chem.* **1996**, *61*, 2624.
(61) Skaddan, M. B.; Wüst, F. R.; Jonson, S.; Syhre, R.; Welch, M. J.; Spies, H.; Katzenellenbogen, J. A. *Nucl. Med. Biol.* **2000**, *27*, 269. [and references therein].
(62) Katzenellenbogen, B. S.; Katzenellenbogen, J. A. *Breast Cancer Res.* **2000**, *2*, 335.
(63) Welch, M. J.; Downer, J. B.; Katzenellenbogen, J. A. *Dev. Nucl. Med., Curr. Dir. Radiopharm. Res. Dev.* **1996**, *30*, 137.
(64) Chi, D. Y.; O'Neil, J. P.; Anderson, C. J.; Welch, M. J.; Katzenellenbogen, J. A. *J. Med. Chem.* **1994**, *37*, 928.
(65) O'Neil, J. P.; Wilson, S. R.; Katzenellenbogen, J. A. *Inorg. Chem.* **1994**, *33*, 319.
(66) Chi, D. Y.; Katzenellenbogen, J. A. *J. Am. Chem. Soc.* **1993**, *115*, 7045.
(67) Yeh, W. M.; Sherman, D. G.; Meares, C. F. *Anal. Biochem.* **1979**, *100*, 152.
(68) Meares, C. F.; Wensel, T. G. *Acc. Chem. Res.* **1984**, *17*, 202.
(69) Meares, C. F. In *Protein Tailoring for Food and Medical Uses*; American Chemical Society Symposium; Feeney, R. E., Whitaker, J. R., Eds.; Marcel Dekker: New York, 1986; p 339.
(70) Lewis, J. S.; Anderson, C. J. *Methods Mol. Biol.* **2007**, *386*, 227.
(71) Brechbiel, M. W.; Gansow, O. A.; Atcher, R. W.; Schlom, J.; Esteban, J.; Simpson, D.; Colcher, D. *Inorg. Chem.* **1986**, *25*, 2772.
(72) Hnatowich, D. J.; Mardirossian, G.; Rusckowski, M.; Fogarasi, M.; Virzi, F.; Winnard, P. *J. Nucl. Med.* **1993**, *34*, 109.
(73) Maresca, K. P.; Hillier, S. M.; Femia, F. J.; Zimmerman, C. N.; Levadala, M. K.; Banerjee, S. R.; Hicks, J.; Sundararajan, C.; Valliant, J.; Zubieta, J.; Eckelman, W. C.; Joyal, J. L.; Babich, J. W. *Bioconjugate Chem.* **2009**, *20*, 1625.
(74) He, H.; Lipowska, M.; Chirstoforou, A. M.; Marzilli, L. G.; Taylor, A. T. *Nucl. Med. Biol.* **2007**, *34*, 709.
(75) Blower, P. J.; Prakash, S. *Perspect. Bioinorg. Chem.* **1999**, *4*, 91.
(76) Papadopoulos, M.; Nock, B.; Maina, T.; Pirmettis, I.; Raptopoulou, C.; Tasiopoulos, A.; Troganis, A.; Kabanos, T.; Terzis, A.; Chiotellis, E. *J. Biol. Inorg. Chem.* **2001**, *6*, 159.
(77) Vanbilloen, H. P.; Bormans, G. M.; DeRoo, M. J.; Verbruggen, A. M. *Nucl. Med. Biol.* **1995**, *22*, 325.
(78) Rao, T. N.; Adhikesavalu, D.; Camerman, A.; Fritzberg, A. R. *J. Am. Chem. Soc.* **1990**, *112*, 5798.
(79) Meegalla, S. K.; Plössl, K.; King, M.-P.; Chumpradit, S.; Stevenson, D. A.; Kushner, S. A.; McElgin, W. T.; Mozley, P. D.; Kung, H. F. *J. Med. Chem.* **1997**, *40*, 9. [and references therein].
(80) Rajagopalan, R.; Grummon, G. D.; Bugaj, J.; Hallemann, L. S.; Webb, E. G.; Marmion, M. E.; Vanderheyden, J. L.; Srinivasan, A. *Bioconjugate Chem.* **1997**, *8*, 407.
(81) Pollak, A.; Roe, D. G.; Pollock, C. M.; Lu, L. F. L.; Thornback, J. R. *J. Am. Chem. Soc.* **1999**, *121*, 11593.

(82) Wong, E.; Fauconnier, T.; Bennett, S.; Valliant, J.; Nguyen, T.; Lau, F.; Lu, L. F. L.; Pollak, A.; Bell, R. A.; Thornback, J. R. *Inorg. Chem.* **1997**, *36*, 5799.
(83) Liu, S.; Edwards, D. S.; Looby, R. J.; Poirier, M. J.; Rajopadhye, M.; Bourque, J. P.; Carroll, T. R. *Bioconjugate Chem.* **1996**, *7*, 196.
(84) Kasina, S.; Sanderson, J. A.; Fitzner, J. N.; Srinivasan, A.; Rao, T. N.; Hobson, L. J.; Reno, J. M.; Axworthy, D. B.; Beaumier, P. L.; Fritzberg, A. R. *Bioconjugate Chem.* **1998**, *9*, 108.
(85) Zhu, Z.; Wang, Y.; Zhang, Y.; Liu, G.; Liu, N.; Rusckowski, M.; Hnatowich, D. J. *J. Nucl. Med. Biol.* **2001**, *28*, 703. [and references therein].
(86) Van Domselaar, G. H.; Okarvi, S. M.; Fanta, M.; Suresh, M. R.; Wishart, D. S. *J. Labelled Compd. Radiopharm.* **2000**, *43*, 1193.
(87) Goodbody, A.; Pollak, A. Peptide-chelator conjugates for diagnostic imaging. PCT Int. Appl. W09603427, Feb. 8, 1996.
(88) Pollak, A.; Goodbody, A. E.; Ballinger, J. R.; Duncan, G. S.; Tran, L. L.; Dunn-Dufault, R.; Meghji, K.; Lau, F.; Andrey, T. W. *Nucl. Med. Commun.* **1996**, *17*, 132.
(89) Lister-James, J.; Knight, L. C.; Mauer, A. H.; Bush, L. R.; Moyer, B. R.; Dean, R. T. *J. Nucl. Med.* **1996**, *37*, 775.
(90) Pearson, D. A.; Lister-James, J.; McBride, W. J.; Wilson, D. M.; Martel, L. J.; Civitello, E. R.; Taylor, J. E.; Moyer, B. R.; Dean, R. T. *J. Med. Chem.* **1996**, *39*, 1361.
(91) Wishart, D. S. *Biotechnology*, 2nd ed.; Ratledge, C., Kristiansen, B., Eds; Wiley-VCH Verlag GmbH: Weinheim, Germany, 2001; p 325.
(92) Ben-Haim, S.; Kahn, D.; Weiner, G. J.; Madsen, M. T.; Waxman, A. D.; Williams, C. M.; Clarke-Peason, D.; Coleman, R. E.; Maguire, R. T. *Nucl. Med. Biol.* **1994**, *21*, 131.
(93) Luyt, L. G.; Hunter, D. H. 217th ACS National Meeting, Anaheim, CA, March 21−25; *Book of Abstracts* 1999, NUCL-184.
(94) Oya, S.; Kung, M.-P.; Frederick, D.; Kung, H. F. *Nucl. Med. Biol.* **1995**, *22*, 749.
(95) Hansen, L.; Hirota, S.; Xu, X.; Taylor, A. T.; Marzilli, L. G. *Inorg. Chem.* **2000**, *39*, 5731.
(96) Tisato, F.; Refosco, F.; Ossola, F.; Bolzati, C.; Bandoli, G. *Transition Met. Chem.* **1997**, *22*, 606.
(97) Ossola, F.; Tisato, F.; Refosco, F. *Inorg. Chim. Acta* **2002**, *330*, 17.
(98) Bolzati, C.; Tisato, F.; Refosco, F.; Bandoli, G.; Domella, A. *Inorg. Chem.* **1996**, *35*, 6221.
(99) Kurti, L.; Papagiannopoulou, D.; Papadopoulos, M.; Pirmettis, I.; Raptopoulou, C. P.; Terzis, A.; Chiotellis, E.; Harmata, M.; Kuntz, R. R.; Pandurangi, R. S. *Inorg. Chem.* **2003**, *42*, 2960.
(100) Karra, S. R.; Schibli, R.; Gali, H.; Katti, K. V.; Hoffman, T. J.; Higginbotham, C.; Sieckman, G. L.; Volkert, W. A. *Bioconjugate Chem.* **1999**, *10*, 254.
(101) Lei, K.; Rusckowski, M.; Chang, F.; Qu, T.; Mardirossian, G.; Hnatowich, D. J. *Nucl. Med. Biol.* **1996**, *23*, 917.
(102) Papadopoulos, M.; Pirmettis, I.; Tsoukalas, C.; Nock, B.; Maina, T.; Raptopoulou, C. P.; Pietzsch, H.-J.; Friebe, M.; Spies, H.; Johannsen, B.; Chiotellis, E. *Inorg. Chim. Acta* **1999**, *295*, 1.
(103) Seifert, S.; Gupta, A.; Syhre, R.; Spies, H.; Johannsen, B. *Appl. Radiat. Isot.* **2001**, *54*, 637.
(104) Syhre, R.; Seifert, S.; Spies, H.; Gupta, A.; Johannsen, B. *Eur. J. Nucl. Med.* **1998**, *25*, 793.
(105) Nock, B.; Maina, T.; Tisato, F.; Papadopoulos, M.; Raptopoulou, C. P.; Terzis, A.; Chiotellis, E. *Inorg. Chem.* **2000**, *39*, 2178.
(106) Femia, F. J.; Chen, X.; Babich, J. W.; Zubieta, J. *Inorg. Chim. Acta* **2001**, *316*, 145.
(107) Papagiannopoulou, D.; Primettis, I.; Pelecanou, M.; Komiotis, D.; Sagnou, M.; Benaki, D.; Raptopoulou, C.; Terzis, A.; Papadopoulos, M. S. *Inorg. Chim. Acta* **2007**, *360*, 3597.
(108) Bryan, J. C.; Burrell, A. K.; Miller, M. M.; Smith, W. H.; Burns, C. J.; Sattelberger, A. P. *Polyhedron* **1993**, *12*, 1769.
(109) Nicholson, T.; Davison, A.; Zubieta, J. A.; Chen, Q.; Jones, A. G. *Inorg. Chim. Acta* **1995**, *230*, 205.
(110) Arterburn, J. B.; Rao, K. V.; Goreham, D. M.; Valenzuela, M. V.; Holguin, M. S.; Hall, K. A.; Ott, K. C.; Bryan, J. C. *Organometallics* **2000**, *19*, 1789.
(111) Mallia, M. B.; Mathur, A.; Subramanian, S.; Banerjee, S.; Kothari, K.; Koiry, S. P.; Sarma, H. D.; Venkatesh, M. *Appl. Radiat. Isot.* **2006**, *64*, 361.
(112) Chu, T.; Li, R.; Hu, S.; Wang, Y.; Liu, X.; Wang, X. *J. Radioanal. Nucl. Chem.* **2004**, *261*, 199.
(113) Bolzati, C.; Refosco, F.; Cagnolini, A.; Tisato, F.; Boschi, A.; Duatti, A.; Uccelli, L.; Dolmella, A.; Marotta, E.; Tubaro, M. *Eur. J. Inorg. Chem.* **2004**, (9), 1902.
(114) Boschi, A.; Duatti, A.; Uccelli, L. *Top. Curr. Chem.* **2005**, *252*, 85.
(115) Baldas, J. *Top. Curr. Chem.* **1996**, *176*, 37.
(116) Bolzati, C.; Mahmood, A.; Malago, E.; Uccelli, L.; Boschi, A.; Jones, A. G.; Refosco, F.; Tisato, F. *Bioconjugate Chem.* **2003**, *14*, 1231.
(117) Schwartz, D. A.; Abrams, M. J.; Hauser, M. M.; Gaul, F. E.; Larsen, S. K.; Rauh, D.; Zubieta, J. *Bioconjugate Chem.* **1991**, *2*, 333.
(118) Abrams, M. J.; Juweid, M.; TenKate, C. I.; Schwartz, D. A.; Hauser, M. M.; Gaul, F. E.; Fuccello, A. J.; Rubin, R. H.; Strauss, H. W.; Fischman, A. J. *J. Nucl. Med.* **1990**, *31*, 2022.
(119) Babich, J. W.; Solomon, H.; Pike, M. C.; Kroon, D.; Graham, W.; Abrams, M. J.; Tompkins, R. G.; Rubin, R. H.; Fishman, A. J. *J. Nucl. Med.* **1993**, *34*, 164.
(120) Nicholsen, T.; Zubieta, J. *Polyhedron* **1988**, *7*, 171.
(121) Cowley, A. R.; Dilworth, J. R.; Donnelly, P. S. *Inorg. Chem.* **2003**, *42*, 929.
(122) Nicholson, T.; Lombardi, P.; Zubieta, J. *Polyhedron* **1987**, *6*, 1577.
(123) Dilworth, J. R.; Harrison, S. A.; Walton, D. R. M.; Schweda, E. *Inorg. Chem.* **1985**, *24*, 2594.
(124) Nicholson, T.; Cook, J.; Davison, A.; Rose, D. J.; Maresca, K. P.; Zubieta, J. A.; Jones, A. G. *Inorg. Chim. Acta* **1996**, *252*, 427.
(125) Hirsch-Kuchma, M.; Nicholson, T.; Davison, A.; Jones, A. G. *J. Chem. Soc., Dalton Trans.* **1997**, *318*, 9.
(126) Rose, D. J.; Maresca, K. P.; Nicholson, T.; Davison, A.; Jones, A. G.; Babich, J.; Fischman, A.; Graham, W.; DeBord, J. R. D.; Zubieta, J. *Inorg. Chem.* **1998**, *37*, 2701. [and references therein].
(127) Liu, S.; Hsieh, W. Y.; Kim, Y. S.; Mohammed, S. I. *Bioconjugate Chem.* **2005**, *16*, 1580.
(128) Decristoforo, C.; Mather, S. J. *Eur. J. Nucl. Med.* **1999**, *26*, 869.
(129) Decristoforo, C.; Faintuch-Linkowski, B.; Rey, A.; von Guggenberg, E.; Rupprich, M.; Hernandez-Gonzales, I.; Rodrigo, T.; Haubner, R. *Nucl. Med. Biol.* **2006**, *33*, 945.
(130) Dijkgraaf, I.; Kruijtzer, J. A. W.; Liu, S.; Soede, A. C.; Oyen, W. J. G.; Corstens, F. H. M.; Liskamp, R. M. J.; Boerman, O. C. *Eur. J. Nucl. Med. Mol. Imag.* **2007**, *34*, 267.
(131) Liu, S.; Hsieh, W. Y.; Jiang, Y.; Kim, Y. S.; Sreerama, S. G.; Chen, X. Y.; Jia, B.; Wang, F. *Bioconjugate Chem.* **2007**, *18*, 438.
(132) Rennen, H.; van Eerd, J. E.; Oyen, W. J. G.; Corstens, F. H. M.; Edwards, D. S.; Boerman, O. C. *Bioconjugate Chem.* **2002**, *13*, 370.
(133) Surfraz, M.B.-U.; King, R.; Mather, S. J.; Biagini, S.; Blower, P. J. *J. Bioinorg. Chem.* **2009**, *103*, 971.
(134) Santos-Cuevas, C. L.; Ferro-Flores, G.; Arteaga de Murphy, C.; Pichardo-Romero, P. A. *Nucl. Med. Commun.* **2008**, *29*, 741.
(135) Ferro-Flores, G.; Arteaga de Murphy, C.; Rodriguez-Cortes, J.; Pedraza-Lopez, M.; Ramirez-Inglesias, M. *Nucl. Med. Commun.* **2006**, *27*, 371.
(136) Liu, S.; Edwards, D. S.; Looby, R. J.; Harris, A. R.; Poirier, M. J.; Barrett, J. A.; Hemingway, S. J.; Carroll, T. R. *Bioconjugate Chem.* **1996**, *7*, 63.
(137) Storch, D.; Behe, M.; Walter, M. A.; Chen, J. H.; Powell, P.; Mikolajczak, R.; Macke, H. R. *J. Nucl. Med.* **2005**, *46*, 1561.
(138) Babich, J. W.; Fischman, A. J. *Nucl. Med. Biol.* **1995**, *22*, 25.
(139) Edwards, D. S.; Liu, S.; Harris, A. R.; Poirier, M. J.; Ewels, B. A. *Bioconjugate Chem.* **1999**, *10*, 803.
(140) Edwards, D. S.; Liu, S.; Ziegler, M. C.; Harris, A. R.; Crocker, A. C.; Hemingway, S. J.; Barrett, J. A.; Bridger, G. J.; Abrams, M. J.; Higgins, J. D., III *Bioconjugate Chem.* **1999**, *10*, 884.
(141) Banerjee, S. R.; Maresca, K. P.; Stephenson, K. A.; Valliant, J. F.; Babich, J. W.; Graham, W. A.; Barzana, M.; Dong, Q.; Fischman, A. J.; Zubieta, J. *Bioconjugate Chem.* **2005**, *16*, 885.
(142) Babich, J. W.; Graham, W.; Femia, F. J.; Dong, Q.; Barzana, M.; Ferrill, K.; Fischman, A. J.; Zubieta, J. *Inorg. Chim. Acta* **2001**, *323*, 23.
(143) Liu, S.; Harris, A. R.; Williams, N. E.; Edwards, D. S. *Bioconjugate Chem.* **2002**, *13*, 881.
(144) Purohit, A.; Liu, S.; Casebier, D.; Edwards, D. S. *Bioconjugate Chem.* **2003**, *14*, 720.
(145) Purohit, A.; Liu, S.; Ellars, C. E.; Casebier, D.; Haber, S. B.; Edwards, D. S. *Bioconjugate Chem.* **2004**, *15*, 728.
(146) Alberto, R. *J. Organomet. Chem.* **2007**, *692*, 1179.
(147) Alberto, R. *Bioorganometallics*; Jaouen, G., Ed.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2006; p 97.
(148) Alberto, R. *J. Nucl. Radiochem. Sci.* **2005**, *6*, 173.
(149) Alberto, R.; Schibli, R.; Angst, D.; Schubiger, P. A.; Abram, U.; Abram, S.; Kaden, T. A. *Transit. Met. Chem.* **1997**, *22*, 597.
(150) Alberto, R.; Schlibi, R.; Egli, A.; Schubiger, P. A.; Abram, U.; Kaden, T. A. *J. Am. Chem. Soc.* **1998**, *120*, 7987.
(151) Waibel, R.; Alberto, R.; Willuda, J.; Finnern, R.; Schibli, R.; Stichelberger, A.; Egli, A.; Abram, U.; Mach, J.-P.; Pluckthun, A.; Schubiger, P. A. *Nat. Biotechnol.* **1999**, *17*, 897.
(152) Egli, A.; Alberto, R.; Tannahill, L.; Schibli, R.; Abram, U.; Schaffland, A.; Waibel, R.; Tourwe, D.; Jeannin, L.; Iterbeke, K.; Schubiger, P. A. *J. Nucl. Med.* **1999**, *40*, 1913.
(153) Alberto, R.; Schlibi, R.; Schubiger, A. P.; Abram, U.; Pietzsch, H.-J.; Johannsen, B. *J. Am. Chem. Soc.* **1999**, *121*, 6076.
(154) Amann, A.; Descritoforo, C.; Ott, I.; Wenger, M.; Bader, D.; Alberto, R.; Putz, G. *Nucl. Med. Biol.* **2001**, *28*, 243.
(155) Schibli, R.; Schwartzbach, R.; Alberto, R.; Ortner, K.; Schmalle, H.; Dumas, C.; Egli, A.; Schubiger, P. A. *Bioconjugate Chem.* **2002**, *13*, 750.

(156) Alberto, R.; Ortner, K.; Wheatley, N.; Schibli, R.; Schubiger, A. P. *J. Am. Chem. Soc.* **2001**, *123*, 3135.
(157) Schibli, R.; LaBella, R.; Alberto, R.; Garcia-Garayoa, E.; Ortner, K.; Abram, U.; Schubiger, P. A. *Bioconjugate Chem.* **2000**, *11*, 345.
(158) Schibli, R.; Schubiger, P. A. *Eur. J. Nucl. Med. Mol. Imaging* **2002**, *29*, 1529.
(159) Liu, Y.; Spingler, B.; Schmutz, P.; Alberto, R. *J. Am. Chem. Soc.* **2008**, *130*, 1554.
(160) Audi, G.; Bersillon, O.; Blachot, J. A.; Wapstra, A. H. *Nucl. Phys. A* **2003**, *A729*, 3.
(161) *Health Physics & Radiological Health Handbook*, 3rd ed.; Williams & Wilkins: Baltimore, MD, 1998; pp 6−53.
(162) Helus, F.; Maier-Borst, W. *Radiopham. Labelled Compd., Proc. Symp.* **1973**, *1*, 317.
(163) van der Walt, T. N.; Strelow, F.W. E. *Anal. Chem.* **1983**, *55*, 212.
(164) Hupf, H. B.; Beaver, J. E. *Int. J. Appl. Radiat. Isot.* **1970**, *21*, 75.
(165) El-Azony, K. M.; Ferieg, K. H.; Saleh, Z. A. *Appl. Radiat. Isot.* **2003**, *59*, 329.
(166) van der Meulen, N. P.; van der Walt, T. N. *Z. Naturforsch.* **2007**, *62b*, 483.
(167) Ruth, T. J.; Pate, B. D.; Robertson, R.; Porter, J. K. *Nucl. Med. Biol.* **1989**, *16*, 323.
(168) Green, M. A.; Welch, M. J. *Nucl. Med. Biol.* **1989**, *16*, 435.
(169) Asti, M.; De Pietri, G.; Fraternali, A.; Grassi, E.; Sghedoni, R.; Fioroni, F.; Roesch, F.; Versari, A.; Salvo, D. *Nucl. Med. Biol.* **2008**, *35*, 721.
(170) Zhernosekov, K. P.; Filosofov, D. V.; Baum, R. P.; Aschoff, P.; Bihl, H.; Razbash, A. A.; Jahn, M.; Jennewein, M.; Rösch, F. *J. Nucl. Med.* **2007**, *48*, 1741.
(171) Velikyan, I.; Beyer, G. J.; Langström, B. *Bioconjugate Chem.* **2004**, *15*, 554.
(172) Andronov, V. G.; Bruskin, A. B.; Sevast'yanova, A. S.; Kodina, G. E.; Ochkin, A. V.; Myasoedova, G. V. *Radiochemistry* **2008**, *50*, 535.
(173) Nakayama, M.; Haratake, M.; Ono, M.; Koiso, T.; Harada, K.; Nakayama, H.; Yahara, S.; Ohmomo, Y.; Arano, Y. *Appl. Radiat. Isot.* **2003**, *58*, 9.
(174) Cyclotron Produced Radionuclides: Physical Characteristics and Production Methods. Technical Report Series No. 468; International Atomic Energy Agency: Vienna, Austria, 2009.
(175) Fani, M.; Andre, J. P.; Maecke, H. R. *Contrast Media Mol. Imaging* **2008**, *3*, 53.
(176) Greene, M.; Tucker, W. *Int. J. Appl. Radiat. Isot.* **1961**, *12*, 62.
(177) Kopecky, P.; Mudrova, B. *Int. J. Appl. Radiat. Isot.* **1974**, *25*, 469.
(178) Neirinckx, R. D.; Davis, M. A. *J. Nucl. Med.* **1979**, *20*, 1075.
(179) Nakayama, M.; Haratake, M.; Koiso, T.; Ishibashi, O.; Harada, K.; Nakayama, H.; Sugii, A.; Yahara, S.; Arano, Y. *Anal. Chim. Acta* **2002**, *453*, 135.
(180) Schuhmacher, J.; Maier-Borst, W. *Int. J. Appl. Radiat. Isot.* **1981**, *32*, 31.
(181) Causey, P. W.; Besanger, T. R.; Schaffer, P.; Valliant, J. F. *Inorg. Chem.* **2008**, *47*, 8213.
(182) Meyer, G. J.; Maecke, H.; Schuhmacher, J.; Knapp, W. H. *Eur. J. Nucl. Med. Mol. Imaging* **2004**, *31*, 1097.
(183) Breeman, W. A. P.; de Jong, M.; Blois, E.; Bernard, B. F.; Konijnenberg, M.; Krenning, E. P. *Eur. J. Nucl. Med. Mol. Imaging* **2005**, *32*, 478.
(184) Azhdarinia, A.; Yang, D. J.; Chao, C.; Mourtada, F. *Nucl. Med. Biol.* **2007**, *34*, 121.
(185) Decristoforo, C.; Knopp, R.; von Guggenberg, E.; Rupprich, M.; Dreger, T.; Hess, A.; Virgolini, I.; Haubner, R. *Nucl. Med. Commun.* **2007**, *28*, 870.
(186) Wong, E.; Caravan, P.; Liu, S.; Rettig, S. J.; Orvig, C. *Inorg. Chem.* **1996**, *35*, 715.
(187) Cox, J. P. L.; Craig, A. S.; Helps, I. M.; Jankowski, K. J.; Parker, D.; Eaton, M. A. W.; Millican, A. T.; Millar, K.; Beeley, N. R. A.; Bsoyce, B. A. *J. Chem. Soc., Perkin Trans.* **1990**, (9), 2567.
(188) Eisenwiener, K. P.; Prata, M. I.; Buschmann, I.; Zhang, H. W.; Santos, A. C.; Wenger, S.; Reubi, J. C.; Maecke, H. R. *Bioconjugate Chem.* **2002**, *13*, 530.
(189) Govindan, S. V.; Michel, R. B.; Griffiths, G. L.; Goldenberg, D. M.; Mattes, M. J. *Nucl. Med. Biol.* **2005**, *32*, 513.
(190) Sun, Y.; Anderson, C. J.; Pajeau, T. S.; Reichert, D. E.; Hancock, R. D.; Motekaitis, R. J.; Martell, A. E.; Welch, M. J. *J. Med. Chem.* **1996**, *39*, 2434.
(191) Caraco, C.; Aloj, L.; Eckelman, W. C. *Appl. Radiat. Isot.* **1998**, *49*, 1477.
(192) Bandoli, G.; Dolmella, A.; Tisato, F.; Porchia, M.; Refosco, F. *Coord. Chem. Rev.* **2009**, *253*, 56.
(193) Harris, W. R.; Pecoraro, V. L. *Biochemistry* **1983**, *22*, 292.
(194) Yang, D. J.; Azhdarinia, A.; Kim, E. E. *Curr. Med. Imaging Rev.* **2005**, *1*, 25.
(195) Anderson, C. J.; Welch, M. J. *Chem. Rev.* **1999**, *99*, 2219.
(196) Sun, Y.; Martell, A. E.; Motekaitis, R. J.; Welch, M. J. *Tetrahedron* **1998**, *54*, 4203.
(197) Green, M. A.; Welch, M. J.; Huffman, J. C. *J. Am. Chem. Soc.* **1984**, *106*, 3689.
(198) Arslantas, E.; Smith-Jones, P. M.; Ritter, G.; Schmidt, R. R. *Eur. J. Org. Chem.* **2004**, *19*, 3979.
(199) Liu, S.; Cheung, E.; Rajopadhye, M.; Williams, N. E.; Overoye, K. L.; Edwards, D. S. *Bioconjugate Chem.* **2001**, *12*, 84.
(200) Maecke, H. R.; Breeman, W. A. P. *Pept. Sci.* **2006**, *43*, 273.
(201) Kaltsas, G. A.; Papadogias, D.; Makras, P.; Grossman, A. B. *Endocr.-Relat. Cancer* **2005**, *12*, 683.
(202) Weiner, R. E.; Thakur, M. L. *BioDrugs* **2005**, *19*, 145.
(203) Weiner, R. E.; Thakur, M. L. *Appl. Radiat. Isot.* **2002**, *57*, 749.
(204) Goldsmith, S. J. *Future Oncology* **2009**, *5*, 75.
(205) Liu, S.; Edwards, D. S. *Bioconjugate Chem.* **2001**, *12*, 630.
(206) Jia, B.; Liu, Z.; Liu, Z. F.; Yu, Z. L.; Yang, Z.; Zhao, H. Y.; He, Z.; Liu, S.; Wang, F. *Bioconjugate Chem.* **2008**, *19*, 201.
(207) Clarke, E. T.; Martell, A. E. *Inorg. Chim. Acta* **1992**, *190*, 37.
(208) Ma, R.; Welch, M. J.; Reibenspies, J.; Martell, A. E. *Inorg. Chim. Acta* **1995**, *236*, 75.
(209) Craig, A. S.; Parker, D.; Adams, H.; Bailey, N. *J. Chem. Soc., Chem. Commun.* **1989**, (23), 1793.
(210) Broan, C. J.; Cox, J. P.; Craig, A. S.; Kataky, R.; Parker, D.; Harrison, A.; Randall, A. M.; Ferguson, G. *J. Chem. Soc., Perkin Trans.* **1991**, (1), 87.
(211) Kruper, W. J.; Rudolf, P. R.; Langhoff, C. A. *J. Org. Chem.* **1993**, *58*, 3869.
(212) Chappell, L. L.; Rogers, B. E.; Khazaeli, M. B.; Mayo, M. S.; Buchsbaum, D. J.; Brechbiel, M. W. *Bioorg. Med. Chem.* **1999**, *7*, 2313.
(213) Prata, M. I.; Santos, A. C.; Geraldes, C. F. G. C.; de Lima, J. J. *Nucl. Med. Biol.* **1999**, *26*, 707.
(214) Delgado, R.; Frausto da Silva, J. J. R. *Talanta* **1982**, *29*, 815.
(215) Heppeler, A.; Froidevaux, S.; Maecke, H. R.; Jermann, E.; Behe, M.; Powell, P.; Hennig, M. *Chem.−Eur. J.* **1999**, *5*, 1974.
(216) Viola, N. A.; Rarig, R. S., Jr.; Ouellette, W.; Doyle, R. P. *Polyhedron* **2006**, *25*, 3457.
(217) Andre, J. P.; Maecke, H. R.; Andre, J. P.; Zehnder, M.; Macko, L.; Akyel, K. G. *Chem. Commun.* **1998**, (12), 1301.
(218) Andre, J. P.; Maecke, H. R.; Kaspar, A.; Kunnecke, B.; Zehnder, M.; Macko, L. *J. Inorg. Biochem.* **2002**, *88*, 1.
(219) Velikyan, I.; Beyer, G. J.; Bergström-Pettermann, E.; Johansen, P.; Bergström, M.; Laangström, B. *Nucl. Med. Biol.* **2008**, *35*, 529.
(220) Velikyan, I.; Langstrom, B. 68Ga-labelling of a free and macromolecule conjugated chelator at ambient temperature. PCT Int. Appl. WO2008026051, March 6, 2008.
(221) Prata, M. I. M.; Santos, A. C.; Geraldes, C. F. G. C.; De Lima, J. J. P. *J. Inorg. Biochem.* **2000**, *79*, 359.
(222) Ramasamy, R.; Lazar, I.; Brucher, E.; Sherry, A. D.; Malloy, C. R. *FEBS Lett.* **1991**, *280*, 121.
(223) Lazar, I.; Ramasamy, R.; Brucher, E.; Geraldes, C. F. G. C.; Sherry, A. D. *Inorg. Chim. Acta* **1992**, *195*, 89.
(224) Yang, C. T.; Sreerama, S. G.; Hsieh, W. Y.; Liu, S. *Inorg. Chem.* **2008**, *47*, 2719.
(225) De Leon-Rodriguez, L. M.; Kovacs, Z. *Bioconjugate Chem.* **2008**, *19*, 391.
(226) Struthers, H.; Spingler, B.; Mindt, T. L.; Schibli, R. *Chem.−Eur. J.* **2008**, *14*, 6173.
(227) Wei, L.; Babich, J.; Zubieta, J. *Inorg. Chim. Acta* **2005**, *358*, 2413.
(228) Kasina, S.; Rao, T. N.; Srinivasan, A.; Sanderson, J. A.; Fitzner, J. N.; Reno, J. M.; Beaumier, P. L.; Fritzberg, A. R. *J. Nucl. Med.* **1991**, *32*, 1445.
(229) Liu, S.; Edwards, D. S.; Looby, R. J.; Poirier, M. J.; Rajopadhye, M.; Bourque, J. P.; Carroll, T. R. *Bioconjugate Chem.* **1996**, *7*, 196.
(230) Rajopadhye, M.; Edwards, D. S.; Bourque, J. P.; Carroll, T. R. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1737.
(231) DiZio, J. P.; Fiaschi, R.; Davison, A.; Jones, A. G.; Katzenellenbogen, J. A. *Bioconjugate Chem.* **1991**, *2*, 353.
(232) O'Neil, J. P.; Carlson, K. E.; Anderson, C. J.; Welch, M. J.; Katzenellenbogen, J. A. *Bioconjugate Chem.* **1994**, *5*, 182.
(233) Meegalla, S.; Plüssl, K.; Kung, M. P.; Chumpradit, S.; Stevenson, D. A.; Frederick, D.; Kunly, H. F. *Bioconjugate Chem.* **1996**, *7*, 421.
(234) Barrett, J. A.; Damphousse, D. J.; Hemingway, S. J.; Liu, S.; Edwards, D. S.; Looby, R. J.; Carroll, T. R. *Bioconjugate Chem.* **1996**, *7*, 203.
(235) Rajagopalan, R.; Grummon, G. D.; Bugaj, J.; Hallemann, L. S.; Webb, E. G.; Marmion, M. E.; Vanderheyden, J. L.; Srinivasan, A. *Bioconjugate Chem.* **1997**, *8*, 407.
(236) Tisato, F.; Porchia, M.; Bolzati, C.; Refosco, F.; Vittadini, A. *Coord. Chem. Rev.* **2006**, *250*, 2034.
(237) Greupink, R.; Sio, C. F.; Ederveen, A.; Orsel, J. *Pharm. Res.* **2009**, *26*, 2647.
(238) Salouti, M.; Rajabi, H.; Babaei, M. H.; Rasaee, M. J. *Nucl. Med. Biol.* **2008**, *35*, 763.

(239) Verbeke, K.; Hjelstuen, O.; Debrock, E.; Cleynhens, B.; DeRoo, M.; Verbruggen, A. *Nucl. Med. Commun.* **1995**, *16*, 942.
(240) Fonge, H.; de Saint Hubert, M.; Vunckx, K.; Rattat, D.; Nuyts, J.; Bormans, G.; Ni, Y. C.; Reutelingsperger, C.; Verbruggen, A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3794.
(241) Wong, E.; Kumar, V.; Howman-Giles, R. B.; Vanderheyden, J. L. *Cancer Biother. Radiopharm.* **2008**, *23*, 715.
(242) Biechlin, M. L.; Bonmartin, A.; Gilly, F. N.; Fraysse, M.; du Moulinet d'Hardemare, A. *Nucl. Med. Biol.* **2008**, *35*, 679.
(243) Blankenberg, F. G.; Vanderheyden, J. L.; Strauss, H. W.; Tait, J. F. *Nat. Protoc.* **2006**, *1*, 108.
(244) King, R. C.; Surfraz, M. B. U.; Biagini, S. C. G.; Blower, P. J.; Mather, S. J. *Dalton Trans.* **2007**, (43), 4998.
(245) Surfraz, M. B.; Biagini, S. C. G.; Blower, P. J. *Dalton Trans.* **2008**, (22), 2920.
(246) Babich, J. W.; Solomon, H.; Pike, M. C.; Kroon, D.; Graham, W.; Abrams, M. J.; Tompkins, R. G.; Rubin, R. H.; Fischman, A. J. *J. Nucl. Med.* **1993**, *34*, 1964.
(247) Greenland, W. E. P.; Howland, K.; Hardy, J.; Fogelman, I.; Blower, P. J. *J. Med. Chem.* **2003**, *46*, 1751.
(248) King, R.; Surfraz, M. B.; Finucane, C.; Biagini, S. C. G.; Blower, P. J.; Mather, S. J. *J. Nucl. Med.* **2009**, *50*, 591.
(249) Surfraz, M. B. U.; King, R.; Mather, S. J.; Biagini, S. C. G.; Blower, P. J. *J. Med. Chem.* **2007**, *50*, 1418.
(250) Liu, S.; Ziegler, M. C.; Edwards, D. S. *Bioconjugate Chem.* **2000**, *11*, 113.
(251) Liu, S.; Edwards, D. S.; Harris, A. R.; Hemingway, S. J.; Barrett, J. A. *Inorg. Chem.* **1999**, *38*, 1326.
(252) Liu, S.; Edwards, D. S.; Harris, A. R. *Bioconjugate Chem.* **1998**, *9*, 583.
(253) Surfraz, M. B. U.; King, R. C.; Mather, S. J.; Biagini, S. C. G.; Blower, P. J. *J. Inorg. Biochem.* **2009**, *103*, 971.
(254) von Guggenberg, E.; Sallegger, W.; Helbok, A.; Ocak, M.; King, R.; Mather, S. J.; Decristoforo, C. *J. Med. Chem.* **2009**, *52*, 4786.
(255) Fan, W. W.; Lin, Y.; Xian, Z.; Pang, Y.; Ma, C.; Tang, Z. G.; Zhang, J. B.; Wang, X. B. *Sci. China, Ser. B: Chem.* **2009**, *52*, 590.
(256) Harris, T. D.; Cheeseman, E.; Harris, A. R.; Sachleben, R.; Edwards, D. S.; Liu, S.; Bartis, J.; Ellars, C.; Onthank, D.; Yalamanchili, P.; Hemingway, S.; Silva, P.; Robinson, S.; Lazewatsky, J.; Rajopadhe, M.; Barrett, J. *Bioconjugate Chem.* **2007**, *18*, 1266.
(257) Liu, S.; Hsieh, W. Y.; Kim, Y. S.; Mohammed, S. I. *Bioconjugate Chem.* **2005**, *16*, 1580.
(258) Edwards, D. S.; Liu, S.; Barrett, J. A.; Harris, A. R.; Looby, R. J.; Ziegler, M. C.; Heminway, S. J.; Carroll, T. R. *Bioconjugate Chem.* **1997**, *8*, 146.
(259) Barrett, J. A.; Damphousse, D. J.; Hemingway, S. J.; Liu, S.; Edwards, D. S.; Looby, R. J.; Carroll, T. R. *Bioconjugate Chem.* **1996**, *7*, 203.
(260) Rajopadhye, M.; Harris, T. D.; Yu, K.; Glowacka, D.; Damphousse, R. P.; Barrett, J. A.; Hemingway, J.; Edwards, D. S.; Carroll, T. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 955.
(261) Rennen, H. J. J. M.; van Eerd, J. E.; Oyen, W. J. G.; Corstens, F. H. M.; Edwards, D. S.; Boerman, O. C. *Bioconjugate Chem.* **2002**, *13*, 370.
(262) Decristoforo, C.; Mather, S. J. *Eur. J. Nucl. Med.* **1999**, *26*, 869.
(263) Behe, M.; Kluge, G.; Becker, W.; Gotthardt, M.; Behr, T. M. *J. Nucl. Med.* **2005**, *46*, 1012.
(264) Branco de Barros, A. L.; Cardoso, V. N.; Mota, L.; das, G.; Leite, E. A.; de Oliveira, M. C.; Alves, R. J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2497.
(265) Wang, Y.; Liu, X.; Hnatowich, D. J. *Nat. Protoc.* **2007**, *2*, 972.
(266) Liu, G.; Mang'era, K.; Liu, N.; Gupta, S.; Rusckowski, M.; Hnatowich, D. J. *J. Nucl. Med.* **2002**, *43*, 384.
(267) Wang, Y.; Liu, G.; Hnatowich, D. J. *Nat. Protoc.* **2006**, *1*, 1477.
(268) Okarvi, S. M.; Jammaz, I. A. *Cancer Biother. Radiopharm.* **2006**, *21*, 49.
(269) Reyes, L.; Navarrete, M. *J. Radioanal. Nucl. Chem.* **2002**, *251*, 245.
(270) Van Gog, F. B.; Visser, G. W. M.; Gowrising, R. W. A.; Snow, G. B.; Van Dongen, G. A. M. S. *Nucl. Med. Biol.* **1998**, *25*, 611.
(271) Knieß, T.; Noll, S.; Noll, B.; Spies, H. *Forschungszentrum Rossendorf e.V., [Bericht] FZR* **1997**, *(FZR-200)*, 95.
(272) Le Gal, J.; Benoist, E. *Eur. J. Org. Chem.* **2006**, *6*, 1483.
(273) Le Gal, J.; Michaud, S.; Gressier, M.; Coulais, Y.; Benoist, E. *Bioorg. Med. Chem.* **2006**, *14*, 2904.
(274) Pillai, M. R. A.; Kothari, K.; Banerjee, S.; Samuel, G.; Suresh, M.; Sarma, H. D.; Jurisson, S. *Nucl. Med. Biol.* **1999**, *26*, 555.
(275) Luyt, L. G.; Jenkins, H. A.; Hunter, D. H. *Bioconjugate Chem.* **1999**, *10*, 470.
(276) Verbruggen, A. M. *Dev. Nucl. Med.* **1996**, *30*, 31.
(277) Eisenhut, M.; Lehmann, W. D.; Becker, W.; Behr, T.; Elser, H.; Strittmatter, W.; Steinstraesser, S.; Baum, R. P.; Valerius, T.; Repp, R.; Deo, Y. *J. Nucl. Med.* **1996**, *37*, 362.
(278) Delmon-Moingeon, L.; Moretti, J. L.; Mahmood, A.; Jones, A. G. *J. Med. Nucl. Biophys.* **1991**, *15*, 401.
(279) Shiba, K.; Mori, H.; Matsuda, H.; Tsuji, S.; Kinuya, K.; Hisada, K. *Appl. Radiat. Isot.* **1991**, *42*, 1159.
(280) Baidoo, K. E.; Scheffel, U.; Lever, S. Z. *Cancer Res.* **1990**, *50*, 799s.
(281) Banerjee, S. R.; Maresca, K. P.; Francesconi, L.; Valliant, J.; Babich, J. W.; Zubieta, J. *Nucl. Med. Biol.* **2005**, *32*, 1.
(282) Jaouen, G.; Top, S.; Vessieres, A.; Alberto, R. *J. Organomet. Chem.* **2000**, *600*, 23.
(283) Struthers, H.; Mindt, T. L.; Schibli, R. *Dalton Trans.* **2010**, *39*, 675.
(284) Mindt, T. L.; Muller, C.; Stuker, F.; Salazar, J.-F.; Hohn, A.; Mueggler, T.; Rudin, M.; Schibli, R. *Bioconjugate Chem.* **2009**, *20*, 1940.
(285) Blom, E.; Langström, B.; Velikyan, I. *Bioconjugate Chem.* **2009**, *20*, 1146.
(286) Velikyan, I.; Sundberg, A. L.; Lindhe, O.; Hoeglund, A. U.; Eriksson, O. W.; Werner, E.; Carlsson, J.; Bergström, M.; Laangström, B.; Tolmachev, V. *J. Nucl. Med.* **2005**, *46*, 1881.
(287) Schuhmacher, J.; Zhang, H.; Doll, J.; Maecke, H. R.; Matys, R.; Hauser, H.; Henze, M.; Haberkorn, U.; Eisenhut, M. *J. Nucl. Med.* **2005**, *46*, 691.
(288) Antunes, P.; Ginj, M.; Zhang, H.; Waser, B.; Baum, R. P.; Reubi, J. C.; Maecke, H. *Eur. J. Nucl. Med. Mol. Imaging* **2007**, *34*, 982.
(289) Fani, M.; Andre, J. P.; Maecke, H. R. *Contrast Media Mol. Imaging* **2008**, *3*, 67.
(290) Cantorias, M. V.; Figueroa, S. D.; Quinn, T. P.; Lever, J. R.; Hoffman, T. J.; Watkinson, L. D.; Carmack, T. L.; Cutler, C. S. *Nucl. Med. Biol.* **2009**, *36*, 505.
(291) Velikyan, I.; Maecke, H.; Langstrom, B. *Bioconjugate Chem.* **2008**, *19*, 569.
(292) Jeong, J. M.; Hong, M. K.; Chang, Y. S.; Kim, Y. J.; Lee, D. S.; Chung, J.-K.; Lee, M. C. *J. Labelled Compd. Radiopharm.* **2007**, *50*, S47.
(293) Govindan, S. V.; Michel, R. B.; Griffiths, G. L.; Goldenberg, D. M.; Mattes, M. J. *Nucl. Med. Biol.* **2005**, *32*, 513.
(294) Wei, L.; Zhang, X.; Gallazzi, F.; Miao, Y.; Jin, X.; Brechbiel, M. W.; Xu, H.; Clifford, T.; Welch, M. J.; Lewis, J. S.; Quinn, T. P. *Nucl. Med. Biol.* **2009**, *36*, 345.
(295) Meyer, G.-J.; Macke, H.; Schuhmacher, J.; Knapp, W. H.; Hofmann, M. *Eur. J. Nucl. Med. Mol. Imaging* **2004**, *31*, 1097.

CR1000755