

Matching chelators to radiometals for radiopharmaceuticals

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Radiometals comprise many useful radioactive isotopes of various metallic elements. When properly harnessed, these have valuable emission properties that can be used for diagnostic imaging techniques, such as single photon emission computed tomography (SPECT, e.g. ^{67}Ga , $^{99\text{m}}\text{Tc}$, ^{111}In , ^{177}Lu) and positron emission tomography (PET, e.g. ^{68}Ga , ^{64}Cu , ^{44}Sc , ^{86}Y , ^{89}Zr), as well as therapeutic applications (e.g. ^{47}Sc , $^{114\text{m}}\text{In}$, ^{177}Lu , ^{90}Y , $^{212/213}\text{Bi}$, ^{212}Pb , ^{225}Ac , $^{186/188}\text{Re}$). A fundamental critical component of a radiometal-based radiopharmaceutical is the chelator, the ligand system that binds the radiometal ion in a tight stable coordination complex so that it can be properly directed to a desirable molecular target *in vivo*. This article is a guide for selecting the optimal match between chelator and radiometal for use in these systems. The article briefly introduces a selection of relevant and high impact radiometals, and their potential utility to the fields of radiochemistry, nuclear medicine, and molecular imaging. A description of radiometal-based radiopharmaceuticals is provided, and several key design considerations are discussed. The experimental methods by which chelators are assessed for their suitability with a variety of radiometal ions is explained, and a large selection of the most common and most promising chelators are evaluated and discussed for their potential use with a variety of radiometals. Comprehensive tables have been assembled to provide a convenient and accessible overview of the field of radiometal chelating agents.

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1. Introduction

Radiometals are radioactive isotopes that can be harnessed for applications in medical diagnosis, as well as for cancer therapy. In order to apply these isotopes to specific biological applications, the “free” radiometal ions must be sequestered from aqueous solution using chelators (ligands) to obviate transchelation and hydrolysis. Chelators used for this application are typically covalently linked to a biologically active targeting molecule, making an active radiopharmaceutical agent. The chelator is used to tightly bind a radiometal ion so that when injected into a patient, the targeting molecule can deliver the isotope without any radiometal loss from the radiopharmaceutical, effectively supplying a site-specific radioactive source *in vivo* for imaging or therapy. A rapidly expanding number of radiometals are routinely produced, with a broad variety of half-lives, emission types, energies, and branching ratios (Table 1).^{1–4} The availability of a wide range of radiometal ions makes it possible to carefully pick the specific nuclear properties that are needed for a vast number of different applications. Some examples of radiometals that can be used for positron emission tomography (PET) imaging are

^{68}Ga , ^{64}Cu , ^{86}Y , ^{89}Zr , and ^{44}Sc , with PET imaging providing sensitive, quantitative, and non-invasive images of a variety of molecular processes and targets. Single photon emission computed tomography (SPECT) is an older and more ubiquitous imaging modality than PET, and, since its inception in the 1960s, $^{99\text{m}}\text{Tc}$ has been the workhorse isotope of SPECT. More recently, the radiometals ^{67}Ga , ^{111}In , and ^{177}Lu have been increasingly used for SPECT imaging in chelator-based radiopharmaceuticals. For therapy applications, particle emitters such as ^{111}In (Auger electron emitter), ^{90}Y and ^{177}Lu (β^-), and ^{225}Ac , ^{212}Pb , and ^{213}Bi (α), are being heavily investigated, typically in conjunction with antibody vectors (immunoconjugates) or peptides. Each radiometal ion has unique aqueous coordination chemistry properties; these must be properly attended to if these isotopes are to be safely harnessed for medical applications and use *in vivo*.

The major difference between radioactive (“hot”) and non-radioactive (“cold”) metal ion chemistry is that radiochemistry is typically performed under extremely dilute conditions, with radiometal ions typically being utilized at nM to pM concentrations.⁵ It is also important to note that several of the elements being discussed have multiple radioactive isotopes that are useful for diagnostic or therapeutic purposes (e.g. $^{86/90}\text{Y}$, $^{67/68}\text{Ga}$, $^{44/47}\text{Sc}$, $^{60/61/62/64}\text{Cu}$), and all isotopes of a given element have identical chemistry.^{6–11} This means that a single radiopharmaceutical agent can be radiolabeled with different isotopes

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of the same element (e.g. $^{86/90}\text{Y}$), and provide the same charge and physical properties, and therefore the same biological behavior and distribution *in vivo*.^{6–11} This class of radiopharmaceutical that utilizes two isotopes of the same element, such as ^{86}Y for PET imaging and ^{90}Y for therapy, has been referred to as a theranostic agent.^{12,13} An interesting note about ^{90}Y is that as a β^- emitter it is possible to perform biodistribution, imaging, and dosimetry studies with its bremsstrahlung X-rays, but because the spatial resolution and image quality obtained is very poor an imaging surrogate is typically used (e.g. ^{111}In or ^{86}Y).¹⁴ Alternatively, ^{90}Y is unique and in some circumstances can be used directly for PET imaging because it emits a very low abundance of positrons (0.003%), which can be used to collect imaging data superior to that obtained by ^{90}Y bremsstrahlung imaging.¹⁴

2. Radiometal-based radiopharmaceutical design

Ligands that are typically used to construct radiometal-based radiopharmaceuticals (not always with $^{99\text{m}}\text{Tc}$) are bifunctional chelators (BFCs), which are simply chelators with reactive functional groups that can be covalently coupled (conjugated) to targeting vectors (e.g. peptides, nucleotides, antibodies, nanoparticles). Common bioconjugation techniques utilize functional groups such as carboxylic acids or activated esters (e.g. *N*-hydroxy-succinimide NHS-ester, tetrafluorophenyl TFP-ester) for amide couplings, isothiocyanates for thiourea couplings, and maleimides for thiol couplings (Fig. 1).^{17,18} Click chemistry is gaining popularity in bioconjugate chemistry, with both the traditional copper(I) catalyzed azide-alkyne Huisgen 1,3-dipolar cycloaddition “click”

reaction (forming a 1,2,3-triazole-ring linkage), or newer copper-free reactions like strain-promoted azide-alkyne cycloadditions (e.g. dibenzocyclooctyne/azide reaction) and Diels-Alder click reactions (e.g. transcyclooctene/1,2,4,5-tetrazine) (Fig. 1).¹⁹ It is interesting to note that the transcyclooctene/1,2,4,5-tetrazine copper-free click coupling displays remarkably fast reaction kinetics, allowing for novel applications like *in vivo* pre-targeting, where the click reaction can occur *in vivo* at very dilute concentrations.^{20–24} The modular design of BFC systems allows for a theoretically limitless number of different vectors to be conjugated, providing molecular targeting to a constantly increasing number of biological targets.

The structure and physical properties of the radiometal-chelate complex have a large impact on the overall pharmacokinetic properties of a radiopharmaceutical, with many radiometal complexes being very hydrophilic and subsequently leading to rapid renal excretion when attached to small vectors like peptides and nucleotides (less prominent with large ~ 150 kDa antibodies).^{6–11,179} It has been observed in peptide-conjugates that keeping the radiometal ion and peptide constant, and changing only the chelator can have drastic effects on biodistributions.²⁵ Radiometal-based radiopharmaceuticals contain many synthetically exchangeable components, which can be separated into different modules: the radiometal, which changes the radioactive emission properties and half-life (γ for SPECT, β^+ for PET, and β^- or α particles, or Auger electrons, for therapy); the chelator, which must be carefully matched with the radiometal for optimal stability; the BFC-vector conjugation method, for different types of bioconjugation reactions and linkages; and the vector/targeting moiety, which allows for the selection of any known molecular target for site-specific delivery of the radioactive “payload” (Fig. 2).



Eric W. Price and Chris Orvig

Eric Price received his undergraduate education at the University of Victoria in Victoria, British Columbia, Canada, where he completed his honours BSc (2009) in Chemistry as a part of the CO-OP program, performing research both in industry and academia, including work at TRIUMF and an NSERC USRA research term with Dr Matthew Moffitt. In 2009 he began his PhD studies as an NSERC scholar in a joint project between Dr Chris Orvig at the University of British Columbia and Dr Michael J. Adam at TRIUMF, partially in collaboration with Nordion, researching new radiometal-based radiopharmaceuticals of $^{67/68}\text{Ga}$, ^{64}Cu , ^{111}In , ^{177}Lu , ^{89}Zr , and ^{225}Ac . He initiated, and has been pivotal in, an international research collaboration with Dr Jason S. Lewis and Dr Brian M. Zeglis, traveling to Memorial Sloan-Kettering Cancer Center in New York, USA, and has also collaborated with the BC

Cancer Agency in Vancouver, as part of his PhD work. In 2013 he won the Berson-Yalow award from the Society of Nuclear Medicine and Molecular Imaging.

Chris Orvig earned his Hons. BSc from McGill and his PhD as an NSERC of Canada scholar at MIT with Alan Davison, FRS. After postdoctoral fellowships with Kenneth N. Raymond at the University of California, Berkeley, and the late Colin J. L. Lock at McMaster University, he joined the University of British Columbia in 1984, where he is now Professor of Chemistry and Pharmaceutical Sciences. Orvig, a Fellow of the Royal Society of Canada, has received various teaching, research and service awards, and published more than 200 research papers. He is a co-inventor on many issued patents, and a certified ski instructor.

Table 1 Properties of some popular radiometal isotopes, EC = electron capture; some low abundance emissions have been omitted for brevity^{1–4,15,16}

Isotope	$t_{1/2}$ (h)	Decay mode	E (keV)	Production method
⁶⁰ Cu	0.4	β^+ (93%) EC (7%)	β^+ , 3920, 3000, 2000	Cyclotron, ⁶⁰ Ni(p,n) ⁶⁰ Cu
⁶¹ Cu	3.3	β^+ (62%) EC (38%)	β^+ , 1220, 1150, 940, 560	Cyclotron, ⁶¹ Ni(p,n) ⁶¹ Cu
⁶² Cu	0.16	β^+ (98%) EC (2%)	β^+ , 2910	⁶² Zn/ ⁶² Cu generator
⁶⁴ Cu	12.7	β^+ (19%) EC (41%) β^- (40%)	β^+ , 656	Cyclotron, ⁶⁴ Ni(p,n) ⁶⁴ Cu
⁶⁶ Ga	9.5	β^+ (56%) EC (44%)	β^+ , 4150, 935	Cyclotron, ⁶³ Cu(α ,n γ) ⁶⁶ Ga
⁶⁷ Ga	78.2	EC (100%)	γ , 93, 184, 300	Cyclotron, ⁶⁸ Zn(p,2n) ⁶⁷ Ga
⁶⁸ Ga	1.1	β^+ (90%) EC (10%)	β^+ , 1880	⁶⁸ Ge/ ⁶⁸ Ga generator
⁴⁴ Sc	3.9	β^+ (94%) EC (6%)	γ , 1157 β^+ , 1474	⁴⁴ Ti/ ⁴⁴ Sc generator
⁴⁷ Sc	80.2	β^- (100%)	γ , 159 β^- , 441, 600	⁴⁷ Ti(n,p) ⁴⁷ Sc
¹¹¹ In	67.2	EC (100%)	γ , 245, 172	Cyclotron, ¹¹¹ Cd(p,n) ^{111m,g} In
^{114m} In	49.5 d	EC (100%)	γ , 190	Cyclotron, ¹¹⁴ Cd(p,n) ^{114m} In or ¹¹⁶ Cd(p,3n) ^{114m} In
¹¹⁴ In (daughter)	73 s	β^- (100%)	β^- , 1989	
¹⁷⁷ Lu	159.4	β^- (100%)	γ , 112, 208 β^- , 177, 385, 498	¹⁷⁶ Lu(n, γ) ¹⁷⁷ Lu
⁸⁶ Y	14.7	β^+ (33%) EC (66%)	β^+ , 1221	Cyclotron, ⁸⁶ Sr(p,n) ⁸⁶ Y
⁹⁰ Y	64.1	β^- (100%)	β^- , 2280	⁹⁰ Zr(n,p) ⁹⁰ Y
⁸⁹ Zr	78.5	β^+ (23%) EC (77%)	β^+ , 897	Cyclotron, ⁸⁹ Y(p,n) ⁸⁹ Zr
²¹² Bi	1.1	α (36%) β^- (64%)	α , 6050 β^- , 6089	²²⁸ Pb/ ²¹² Pb generator
²¹³ Bi	0.76	α (2.2%) β^- (97.8%)	α , 5549 β^- , 5869	²²⁸ Th/ ²¹² Pb generator
²¹² Pb (daughter is ²¹² Bi)	10.6	β^- (100%)	α , 570	²²⁴ Ra/ ²¹² Pb generator
²²⁵ Ac	240	α (100%)	α , 5600–5830 (6)	²²⁶ Ra(p,2n) ²²⁵ Ac n-Capture of ²³² Th $\rightarrow$ ²³³ U $\rightarrow$ ²²⁵ Ac

Each radiometal ion has different chemical demands, including ligand donor atom preferences (e.g. N, O, S, hard/soft), coordination number, and coordination geometry; however, there are many key design considerations that can be applied universally.²⁶ Ligand synthesis should be relatively simple and avoid stereoisomers and non-enantio/diastereospecific reactions, modularity should be incorporated in BFC synthesis as much as possible to allow for incorporation of different bioconjugation handles, and modular synthesis also should allow for tuning of denticity and physical properties by changing the polarity and charge of the chelate (the degree of polarity can

be assessed by octanol–water partition coefficients (log *P*)), so that biodistribution properties can be adjusted.

The intention of this article is to act as a guide for researchers to find the optimal match of chelator with radiometal for radio-pharmaceutical applications. The methods by which chelators are evaluated with different radiometals will be discussed, and the current “gold standard” chelators for each relevant radio-metal ion will be identified. A large number of review articles with broader scopes have been written that discuss the application of radiometals in radiopharmaceuticals, covering topics ranging from chelators, coordination chemistry, synthetic

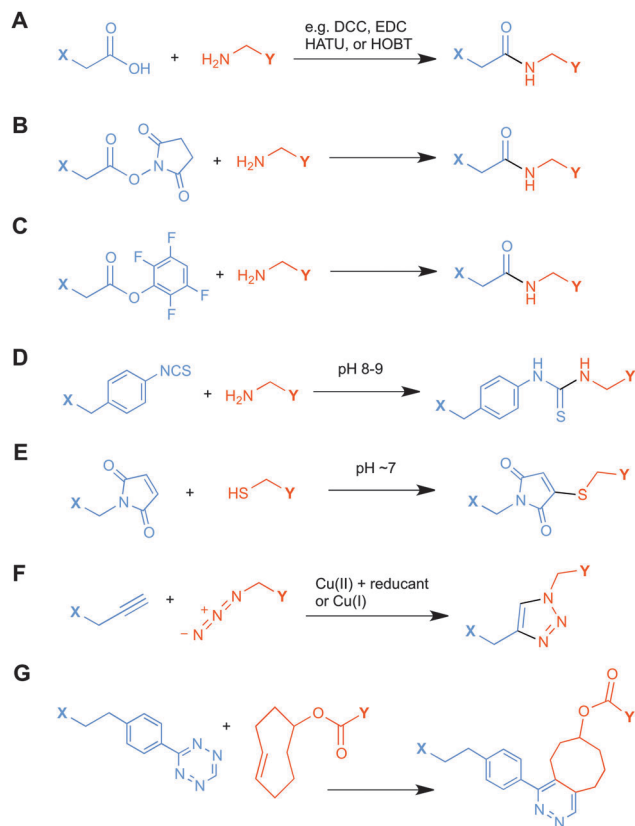


Fig. 1 Examples of bioconjugation reactions: (A) standard peptide coupling reaction between a carboxylic acid and a primary amine with a coupling reagent; (B and C) peptide coupling reactions between activated esters of tetrafluorophenyl (TFP) or *N*-hydroxysuccinimide (NHS) and a primary amine; (D) thiourea bond formation between an isothiocyanate and a primary amine; (E) thioether bond formation between a maleimide and thiol; (F) standard Cu(I) catalyzed Huisgen 1,3-dipolar cycloaddition ("click" reaction) between an azide and an alkyne; and (G) strain-promoted Diels-Alder "click" reaction between a tetrazine and transcyclooctene.

methodologies, radiometal production, radiochemistry, bioconjugation strategies, automation, molecular targeting groups (vectors), imaging modalities, and therapeutics.^{3,13,15,17,18,26–51} Technetium chemistry is not covered here, because the

chemistry of technetium, and the types of ligands used with it, differ drastically from those for the radiometals discussed here.^{26,35,52}

2.1 Macrocytic versus acyclic chelators

When designing new chelators, a historical glance at previous work reveals that macrocycles are generally more kinetically inert than acyclic chelators, even if their thermodynamic stabilities have been determined to be very similar.^{53–57} Macrocytic chelators require minimal physical manipulation during metal ion coordination, as they possess inherently constrained geometries and partially pre-organized metal ion binding sites, thereby decreasing the entropic loss experienced upon metal ion coordination.⁵⁸ To contrast this, acyclic chelators must undergo a more drastic change in physical orientation and geometry in solution in order to arrange donor atoms to coordinate with a metal ion, and subsequently they suffer a more significant decrease in entropy than do macrocycles (thermodynamically unfavorable). The thermodynamic driving force towards complex formation is therefore greater for macrocycles in general, a phenomenon referred to as the macrocycle effect.⁵⁸ A crucial property where most acyclic chelators excel and most macrocycles suffer is in the coordination kinetics and radiolabeling efficiency. The ability to quantitatively radiolabel/coordinate a radiometal in less than 15 minutes at room temperature is a common property of acyclic chelators, whereas macrocycles often require heating to 60–95 °C for extended times (30–90 minutes).^{59–61} Fast room temperature radiolabeling becomes a crucial property when working with BFC-conjugates of heat sensitive molecules such as antibodies and their derivatives, or when working with short half-life isotopes such as ⁶⁸Ga, ^{212/213}Bi, ⁴⁴Sc, and ⁶²Cu.

2.2 Matching chelators with radiometals – how are chelators evaluated?

When a new chelator is synthesized for the purpose of radio-metal ion sequestration, or an old chelator is repurposed for use with a new radiometal ion, initial screening experiments are usually done by simple radiolabeling to determine a number of factors: whether the chelator can bind the radiometal ion and effectively radiolabel in high yields (quantitative is best), what temperature is required (ambient temperature is best), and what reaction time is required (faster is better). Short half-life isotopes

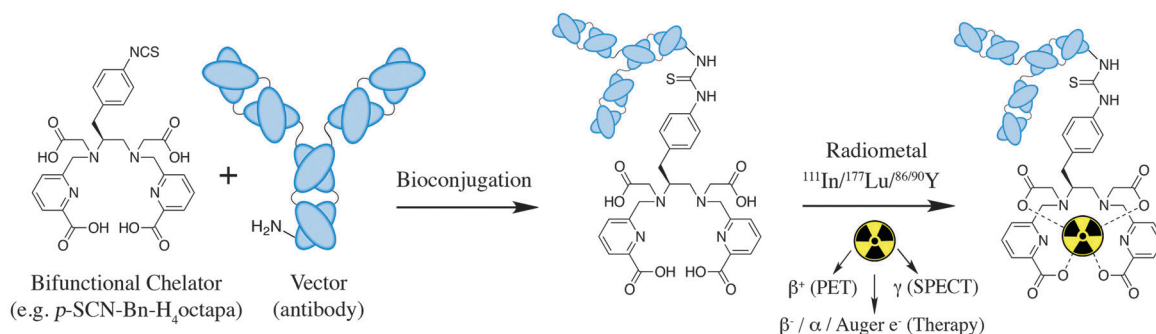


Fig. 2 Illustration of an archetypal radiometal-based radiopharmaceutical agent containing a bifunctional chelator (BFC) conjugated to a targeting vector (e.g. antibody, peptide, nanoparticle) using a variety of conjugation methods (e.g. isothiocyanate-amine coupling, peptide coupling, maleimide-thiol coupling, activated ester amide coupling, click-coupling) and then radiolabeled with a radiometal ion (e.g. ¹¹¹In³⁺/¹⁷⁷Lu³⁺/^{86/90}Y³⁺).

like ^{68}Ga are ideally matched with chelators that can radiolabel rapidly (fast radiolabeling kinetics). Longer half-life isotopes such as ^{111}In and ^{177}Lu allow for extended reaction times, but even if the half-life allows for long reaction times, completing the radiolabeling portion of radiopharmaceutical preparation is most convenient if finished in less than 10 or 15 minutes. As previously mentioned, room temperature radiolabeling is crucial for sensitive antibody vectors, which are degraded at elevated temperatures. DOTA inconveniently requires elevated temperatures for radiolabeling with essentially all radiometals (e.g. ^{44}Sc , ^{111}In , ^{177}Lu , $^{86/90}\text{Y}$, ^{225}Ac) but its abundant application in radiochemistry for decades, its exceptional *in vivo* stability, and the commercial availability of many different bifunctional DOTA derivatives and vector conjugates means that it is likely the most commonly used chelator to this day. Moving forward to the design and testing of new chelators, fast room temperature radiolabeling kinetics should be a priority; however, fast kinetics of radiometal incorporation (on-rate) and consequently low energetic barriers to radiometal–chelate complexation can also mean fast radiometal decorporation (off-rates) and low energetic barriers to radiometal release (Fig. 3). An arduous balancing act is required to obtain the best set of chelate properties for each application and radiometal ion, requiring the study and availability of a broad selection of different chelators with a variety of properties from which to choose.

When a chelator is identified through early screening to possess radiolabeling properties that are suitable for use with a particular radiometal ion, it must then be experimentally determined to be highly stable and inert. Further experiments are performed with the specific radiometal–chelate complex under conditions relevant to *in vivo* translation to judge its potential as the core component of a radiometal-based radiopharmaceutical. The result of radiometal loss from a radiopharmaceutical *in vivo* is the non-targeted distribution of the “free” radiometal ion in the body, and its exact fate and distribution in the body depends on the properties and biological behavior of the specific radiometal ion in question (Fig. 3). For example, ^{89}Zr and ^{68}Ga are known to accumulate in the bone when released from a BFC, where ^{64}Cu is known to accumulate in the liver. The fate of these radiometal ions can be tracked using PET/SPECT imaging in the living animal, and/or biodistribution experiments where animals are euthanized at predetermined time points, their organs harvested, and the distribution of radioactivity measured and calculated for the percentage of injected dose of radioactivity per gram of tissue (%ID/g). Each metal ion has its own unique properties that must be considered when constructing a radiometal-based imaging/therapeutic agent, such as its aqueous hydrolysis chemistry, redox chemistry, and affinity for native biological chelators.

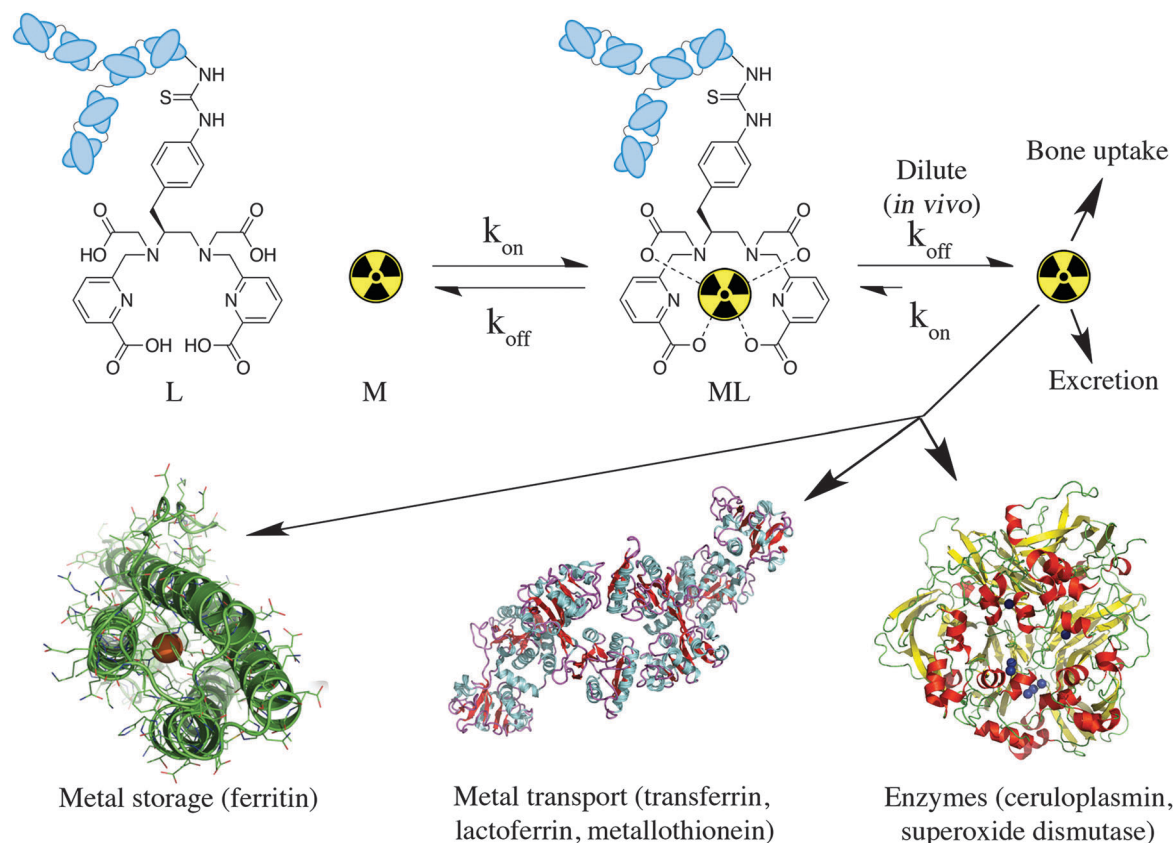


Fig. 3 Cartoon depiction of metal ion coordination kinetics, enhanced off-rate kinetics *in vivo* (extremely dilute conditions), and possible routes of radiometal ion loss *in vivo* (solid-state structures of ferritin H-chain homopolymer PDB file 1FHA, ceruloplasmin PDB file 2J5W, and apo-transferrin PDB file 2HAV shown).

2.3 Thermodynamic stability

When evaluating and selecting a chelator to match with a specific radiometal ion for use in a radiopharmaceutical, kinetic inertness *in vivo* is ultimately the most crucial consideration, even beyond that of the absolute thermodynamic stability of the metal–chelate complex. Thermodynamic stability/formation constants ($K_{ML} = [ML]/[M][L]$) are usually experimentally determined by potentiometric and/or spectrophotometric titrations, but evaluating kinetic inertness under conditions relevant to *in vivo* applications can be much more problematic. Thermodynamic stability constants can be a useful metric for preliminary comparisons of various chelators with a particular metal ion, but they do not predict *in vivo* stability with any level of competence.^{62,63} A thermodynamic parameter that provides more biologically relevant information than K_{ML} values is the pM value ($-\log[M]_{Free}$).^{64–67} The pM value is the negative log of the concentration of free metal ion uncomplexed by a given chelator under specific conditions. The pM value is a condition-dependent value that is calculated from the standard thermodynamic stability constant ($\log K_{ML}$), accounting for variables and conditions such as ligand basicity, metal ion hydrolysis, (physiological) pH, and ligand:metal ratio.

Stability constants and pM values provide a number for the direction and magnitude of the equilibrium in a metal–chelate coordination reaction under specific conditions, but give no kinetic information (*e.g.* off-rates for dissociation).^{68,69} This is a very important factor because the rate of dissociation *in vivo* is what governs the kinetic inertness of a radiometal complex, regardless of thermodynamic stability, and these off-rates are greatly influenced by the high dilution encountered *in vivo* when a tiny quantity of radiopharmaceutical (micro- or nanograms) is diluted into the blood pool (Fig. 3). Even more complicating is the abundant presence in the body of many strong native biological chelators and competing ions that can transchelate radiometals from BFC-conjugates. These are often present in higher concentrations than is the radiopharmaceutical, and include transport proteins such as transferrin, ceruloplasmin, and metallothionein, storage proteins like ferritin, and metal containing enzymes such as superoxide dismutase (Fig. 3). This wide range of complicating factors means that a single *in vitro* assay is typically not accurate in predicting *in vivo* stability.

2.4 Kinetics – acid dissociation and competitive radiolabeling

Acid dissociation experiments can be used to measure and assess the relative kinetic inertness of a metal complex to acidic conditions. Most complexes are found to de-ligate fairly quickly below pH 2.0,⁷⁰ and experiments evaluating the rate of dissociation at a constant pH (*e.g.* 2.0) have been performed to compare and evaluate chelators with a particular metal/radiometal ion.^{55,70,71} In these acid dissociation experiments, decomplexation can be observed over time with techniques such as high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), and nuclear magnetic resonance (NMR) for diamagnetic metal complexes. With the exception of copper chelates,^{68,72} acid dissociation experiments are not

commonly performed as they do not provide an accurate prediction of *in vivo* kinetic inertness, because low pH is not encountered in the blood or most organs (except the stomach) and radiometal dissociation typically occurs *via* transchelation to serum proteins and enzymes, and is not acid-mediated.^{69,73–77}

Competitive radiolabeling experiments can be performed by adding to a radiolabeling mixture an excess of non-radioactive ions, such as Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Cu^{2+} , Zn^{2+} , or Fe^{3+} , followed by addition of a chelator to evaluate the impact of these competing ions on radiolabeling yields.^{55,78–81} These experiments can reveal the radiolabeling selectivity of a chelator for a specific radiometal ion in the presence of other biologically relevant competing ions. Alternatively, if the radiometal complex is preformed under standard metal-free radiolabeling conditions, and is then added to a mixture containing these competing ions, stability to transchelation can be assessed. Because chelate-based radiopharmaceuticals are typically radiolabeled in strictly metal-free conditions (deionized ultrapure water, often passed through a metal-scavenging chelex resin), these experiments may not appear completely relevant for predicting *in vivo* stability and utility. Depending on the method of radiometal production and purification, the specific activity of radiometal ions can vary greatly, as can the concentrations of impurity metals ions.⁵ Radiometals are used in very small quantities and under extremely dilute conditions; therefore, any impurity metal ions present (even if only a few nanomoles) may actually be in excess of the radiometal ion concentration, and competitive binding could be problematic.^{5,82} The presence and quantity of metal ion impurities in radiometal mixtures depends on the source of the radiometal ion, method of production, and purification.^{5,82}

2.5 *In vitro* and *in vivo* stability

Experiments that are more pertinent to *in vivo* translation are metal-exchange competitions with biologically relevant mixtures, including blood serum, *apo*-transferrin, superoxide dismutase, and hydroxyapatite (bone).^{18,83–88} By incubating radiometallated chelators with these different competition mixtures, the quantity of radiometal that is transchelated from chelator to serum proteins/enzymes can be evaluated over time using size exclusion HPLC, iTLC, or disposable PD10 size exclusion columns.^{18,83–88} These experiments provide a directly relevant measure of stability and kinetic inertness by competition with the most likely transchelation culprits *in vivo*; additionally, in these *in vitro* assays the concentrations of these biological reagents can often be elevated above normal physiological levels to provide a more stringent challenge.

Ultimately the most relevant and practical test of radiopharmaceutical stability is *in vivo*. Biodistribution studies in healthy mice can be performed on “naked” radiometal–chelate complexes (no conjugated vectors) to assess clearance and uptake profiles and ensure no abnormal organ distribution or critical instability occurs. If a radiometal–chelate complex is very stable *in vivo*, the complex is typically cleared quickly from the animal through the kidneys/bladder/urine or digestive system/liver/feces depending on polarity and metabolism.⁸⁹

Unstable complexes often demonstrate persistent uptake in organs and tissues where the non-bound radiometal is known to associate (e.g. Zr^{4+} and lanthanides in the bone, Cu^{2+} in the liver).⁸⁹ The major drawback to this type of experiment with “naked” chelate complexes is that highly polar and charged radiometal–chelate complexes are typically cleared very quickly from the body, and therefore do not persist *in vivo* for long enough to encounter any significant challenge to their structural integrity.^{84,85} Conversely, highly lipophilic complexes tend to accumulate in the liver and digestive tract, regardless of stability.^{84,85}

To evaluate properly the stability and kinetic inertness of a chelate *in vivo*, a suitable vector must be attached (e.g. peptide, antibody, nanoparticle) so that the radiometal–chelate complex is made to persist in blood circulation for a substantial period of time, and can be monitored over several hours or days (depending on isotope half-life and the subsequent imaging/therapy window).⁹⁰ An additional concern with *in vivo* experiments is the significant normal variance between animals that introduces error; for example, 10 mice of the same sex and breed, procured from the same supplier, will often show significant differences in biodistribution of the same radiopharmaceutical. Additionally, the specific experimental techniques and methods (e.g. radiopharmaceutical preparation, injection, animal dissection, organ counting) used for these biodistribution experiments can cause large variability between data sets. Variables such as specific activity of the source radiometal, specific activity of the radiolabeled agent, radiolabeling temperature, purity of the final radiopharmaceutical preparation, injected mass of radiopharmaceutical, and injected volume can make large differences in biodistribution profiles. The same radiopharmaceutical used at different institutions may offer drastically different tumor uptake and organ distribution values, if the above variables are not tightly controlled, and even then differences between animals and between experimental techniques can introduce large variances. For these reasons it is crucial to include internal control experiments for every study; for example, evaluation of a new chelator *in vivo* should be done in parallel with an existing and established “gold standard” chelator so that a direct comparison can be made because comparison to previously performed studies (even in the exact same animal model and/or cell line) is often not reliable due to the above mentioned complications.^{79,83,85,91}

3. A selection of chelators and their most suitable radiometal companions

A survey of radiometal chelators has been undertaken, with the most relevant and promising examples being discussed. The most suitable chelate–radiometal matches have been identified, and detailed tables included, with the aim of providing a quick reference for those looking to find the optimal match between chelator and radiometal. A color-coding scheme has been used, and justification for the assignment of good (green), fair (orange), and poor (red) matches between chelators and radiometals can be found in the text and the provided

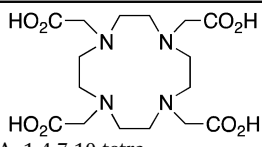
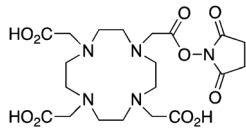
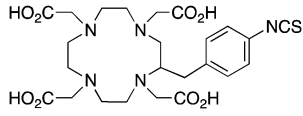
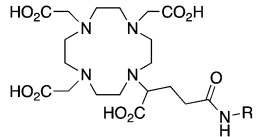
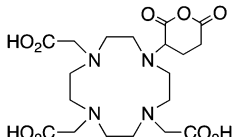
references. The color code indicates the authors' opinions on the general suitability of a chelator for use with a specific radiometal, accounting for factors such as radiolabeling conditions and *in vitro/in vivo* stability. An assignment of green may suggest that either a chelator is currently the “gold standard” for use with a given radiometal, or that early work with a new chelator looks very promising and *in vivo* studies have shown that it works comparably to the current “gold standards”. Assignment of orange to a chelator–radiometal pair may be made if the combination has been used *in vivo* successfully, but perhaps in recent years has been surpassed by a new and superior chelator and is no longer the best choice. Also, an assignment of orange could mean that a new chelator–radiometal ion pair looks very promising, but perhaps a bifunctional derivative has yet to be synthesized, or only preliminary *in vivo* work has been done and more study is required (e.g. no study of bioconjugates, or no direct comparisons to existing “gold standards” for internal reference). An assignment of red indicates that the highlighted chelator–radiometal ion pair is an unsuitable match, and generally should not be used due to severe instability or radiolabeling problems. These color “ratings” were made using the referenced studies and reflect the current trends in radiochemistry and nuclear medicine, with the goal of acting as a quick guide for those seeking to find an optimal chelate–radiometal ion pairing.

3.1 DOTA

DOTA is one of the primary workhorse chelators for radiometal chemistry, and is one of the current “gold standards” for a number of isotopes, including ^{111}In , ^{177}Lu , $^{86/90}\text{Y}$, ^{225}Ac , and $^{44/47}\text{Sc}$. DOTA has been extensively used with $^{67/68}\text{Ga}$, but is widely accepted to be less stable than its more petite macrocyclic counterpart NOTA (Table 2). ^{68}Ga -DOTATOC has been shown to exhibit superior *in vivo* properties to ^{111}In -DOTATOC (Octreoscan) despite non-optimal stability.⁹² Even though a chelator may be more stable and inert with a given radiometal (e.g. NOTA vs. DOTA for ^{68}Ga), it may not be the optimal choice for a certain application (e.g. a specific peptide vector). For example, NOTA is widely accepted to form a more stable complex with ^{68}Ga than does DOTA, but due to differences in charge and physical properties (e.g. neutral vs. charged complex), DOTA may provide superior *in vivo* properties with certain vectors.^{25,79} This example highlights the complex set of variables one must consider when constructing radiometal-based radiopharmaceuticals.

When designing new BFCs or modifying existing chelators to make BFC derivatives, care should be taken not to disrupt the original coordination sphere of the chelator. Some common bifunctional DOTA derivatives utilize one of the carboxylic acid arms for the site of vector conjugation, effectively blocking one of the coordinating carboxylate arms (amide carbonyl groups may still coordinate, albeit weakly) (Table 2).⁵⁹ DOTAGA, DOTASA, and various isothiocyanate derivatives of DOTA have been synthesized and solve this problem by conjugating to vectors through the carbon backbone and side-arm functionalization (Table 2).^{59,93–95} These bifunctional DOTA derivatives retain their maximum potential denticity (octadentate) as well

Table 2 DOTA and bifunctional derivatives, highlighting relevant radiometal ions, radiolabeling conditions, thermodynamic stability constants ($\log K_{ML}$), coordination geometry, and color-coded ranking

Chelator and common bifunctional derivatives	Radiometal ion	^a	Radiolabeling conditions	$\log K_{ML}$	Proposed geometry	Ref.
 DOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid, maximum CN = 8, donor set N ₄ O ₄	⁶⁴ Cu ²⁺	~	25–90 °C, 30–60 min, pH 5.5–6.5	22.2, 22.7	Distorted octahedron	84, 87, 90, 104–110
	^{67/68} Ga ³⁺	~	37–90 °C, 10–30 min, pH 4.0–5.5	21.3 (pM 15.2, 18.5)	Distorted octahedron	64, 92, 110–118
	^{44/47} Sc ³⁺	✓	95 °C, 20–30 min, pH 4.0	27.0 (pM 26.5)	Square antiprism	96–98
	¹¹¹ In ³⁺	✓	37–100 °C, 15–60 min, pH 4.0–6.0	23.9 (pM 17.8–18.8)	Square antiprism?	64, 85, 93, 114, 117, 119–124, 179
	¹⁷⁷ Lu ³⁺	✓	25–100 °C, 15–90 min, pH 4.0–6.0	23.5, 21.6 (pM 17.1)	Square antiprism	124–130
	^{86/90} Y ³⁺	✓	25–100 °C, 15–90 min, pH 4.0–6.0	24.3–24.9	Square antiprism	55, 70, 93, 124, 127, 130–132
	²¹³ Bi ³⁺	~	95–100 °C, ¹³³ 5 min, pH 6.0–8.7	—	Square antiprism	134, 135
	²¹² Pb ²⁺	~	25–75 °C, 30–60 min, pH 4.0–5.5	—	Square antiprism	127, 136–138
 DOTA-NHS-ester ¹³⁹	²²⁵ Ac ³⁺	✓	37–60 °C, 30–120 min, pH 6.0	—	Square antiprism?	100, 101
 p-SCN-Bn-DOTA (C-DOTA) ^{95,140,141}						
 DOTAGA, R = amide, NHS ester ¹⁴²						
 DOTAGA-anhydride ¹⁴³						

^a Green “✓” = good/best match, orange “~” = suitable match, or requires more evaluation but shows potential, red “✗” = poor/unstable match.

as the same thermodynamic stability and kinetic inertness as unadulterated DOTA.^{59,93,94}

Despite its slow radiolabeling kinetics and required heating, DOTA is currently the “gold standard” chelator for use with ¹¹¹In, ¹⁷⁷Lu, and ^{86/90}Y (Table 2). The coordination chemistry and properties of Y³⁺ and Lu³⁺ are very similar; they both preferentially form 8–9 coordinate complexes in square antiprismatic or monocapped square antiprismatic geometries, and are hard metal ions with a preference for hard ligand donors such as carboxylate-oxygens and amine-nitrogens. In³⁺ has similar properties to Y³⁺ and Lu³⁺, but being smaller forms 7–8 coordinate complexes, typically with square antiprismatic geometry. According to acid dissociation experiments performed at pH 2.0 with the ⁹⁰Y complexes of DOTA, DTPA (*vide infra*), and CHX-A''-DTPA, DOTA is the most kinetically inert to acid dissociation by a significant margin, with CHX-A''-DTPA showing moderate stability, and DTPA decomposing almost

immediately.⁵⁵ The validity of acid dissociation experiments is questionable, as conditions such as these are never encountered *in vivo* (except for the stomach), but they are a quantifiable metric for measuring off-rate kinetics and further substantiates the place of DOTA as the current “gold standard” chelator for ¹¹¹In, ¹⁷⁷Lu, and ^{86/90}Y.⁵⁵

Although very little investigation of this isotope has been performed, DOTA is also the current chelator of choice for ⁴⁴Sc, although the requisite high temperature (90 °C) radiolabeling conditions are not optimal.^{96–98} As ⁴⁴Sc has only recently become more popular in the literature, little *in vivo* work has been performed, and new chelators (preferably with ambient temperature radiolabeling properties) are of strong interest. Another isotope of recent interest for use with DOTA is the α -emitter ²²⁵Ac, which is a large actinide isotope that possesses no non-radioactive isotopologue, making study of its coordination chemistry difficult. The large macrocycles HEHA and PEPA

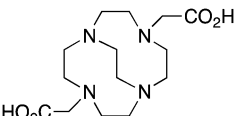
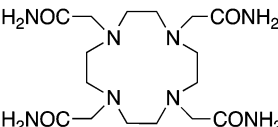
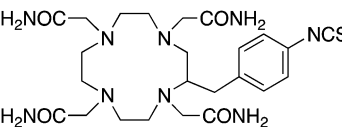
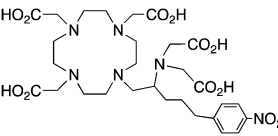
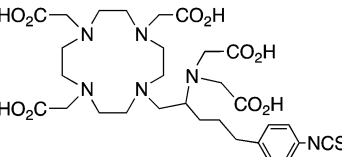
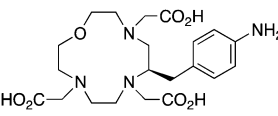
(*vide infra*) had previously been used most extensively with ^{225}Ac , but recently DOTA has been shown to have superior *in vivo* properties.⁹⁹ The long half-life of ^{225}Ac (10.0 days) lends well to radioimmunotherapy (RIT), and so the high temperatures required for ^{225}Ac radiolabeling with DOTA are very unfavorable for sensitive antibodies. The current method used for synthesizing ^{225}Ac -DOTA-antibody conjugates is to radiolabel the BFC *p*-SCN-Bn-DOTA at 60 °C with ^{225}Ac first, followed by antibody conjugation (thiourea coupling) and purification.^{100,101} It is important to realize that the isothiocyanate functionality in the BFC *p*-SCN-Bn-DOTA is very sensitive and degrades rapidly under these conditions. A study comparing the therapeutic effects of ^{225}Ac , ^{213}Bi , and ^{90}Y using an antibody vector demonstrated ^{225}Ac to be the most effective, with renal toxicity from the daughters ^{221}Fr and ^{213}Bi being the most significant concern.¹⁰² A recent clinical trial in humans with ^{225}Ac -DOTA-HuM195 (humanized anti-CD33

monoclonal antibody) has shown promise, with DOTA being the current “gold standard” for ^{225}Ac .^{100,103} A high denticity acyclic chelator (CN = 8–10) that could match the *in vivo* stability of DOTA and radiolabel at room temperature would be of great utility and would offer a more streamlined radio-pharmaceutical preparation than the one outlined above for *p*-SCN-Bn-DOTA.

3.2 DOTA derivatives: CB-DO2A, 3p-C-DEPA, TCMC, and Oxo-DO3A

CB-DO2A has only been investigated with the radiometal ^{64}Cu for radiochemical use (Table 3).^{68,144} An X-ray crystal structure of CB-DO2A with Ga^{3+} is available and shows a distorted octahedral coordination geometry, and acid stability experiments have been performed with Ga^{3+} , revealing impressive acid inertness.¹⁴⁵ Although very little work has been done with

Table 3 DOTA derivatives CB-DO2A, TCMC, 3p-C-DEPA, Oxo-DO3A, and bifunctional derivatives, highlighting relevant radiometal ions, radiolabeling conditions, thermodynamic stability constants ($\log K_{\text{ML}}$), coordination geometry, and color-coded ranking

Chelator and common bifunctional derivatives	Radiometal ion	^a	Radiolabeling conditions	$\log K_{\text{ML}}$	Proposed geometry	Ref.
 CB-DO2A, 4,10-bis(carboxymethyl)-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane, N_4O_2 , CN = 6	$^{64}\text{Cu}^{2+}$	~	80 °C, 30–60 min, pH 5–7	—	Distorted octahedron	68, 144
	$^{67/68}\text{Ga}^{3+}$	~	—	—	Distorted octahedron	145
 TCMC, 1,4,7,10-tetrakis(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane, N_4O_4 , CN = 8	$^{212}\text{Pb}^{2+}$	✓	37 °C, 30–60 min, pH 5–6.5	>19	Square antiprismatic	138, 147, 148, 150, 153, 154
			 <i>p</i> -SCN-Bn-TCMC ¹⁵⁰			
 3p-C-DEPA, N_5O_5 , CN = 10	$^{212/213}\text{Bi}$	✓	25 °C, 5–10 min, pH 5.5	—	Square antiprismatic	152
			 3p-C-DEPA-NCS ¹⁵²			
 <i>p</i> -NH ₂ -Bn-Oxo-DO3A, N_3O_4 , CN = 7	$^{64}\text{Cu}^{2+}$	~	25 °C, 10 min, pH 5.5	—	Distorted octahedron?	86, 87, 155, 156
	$^{67/68}\text{Ga}^{3+}$	~	25 °C, 10 min, pH 4–5	—	Distorted octahedron?	86, 157, 158

^a Green “✓” = good/best match, orange “~” = suitable match, or requires more evaluation but shows potential, red “✗” = poor/unstable match.

CB-DO2A, studies with the non-bifunctional $^{64}\text{Cu}(\text{CB-DO2A})$ have suggested that it had superior *in vivo* stability to DOTA and TETA, but inferior stability to CB-TE2A.¹³⁹ Further comparison of CB-DO2A to CB-TE2A, DOTA, and TETA revealed that CB-TE2A possessed superior acid and electrochemical inertness, which explains why DOTA and its cross-bridged derivative CB-DO2A have been replaced by CB-TE2A and are no longer a prominent subject of current research with ^{64}Cu .⁵⁶ Another interesting DOTA derivative is Oxo-DO3A, which is a heptadentate N_3O_4 macrocycle that has shown improved radiolabeling kinetics and stability with ^{64}Cu and $^{67/68}\text{Ga}$ compared to DOTA (Table 3). The $p\text{-NH}_2\text{-Bn-Oxo-DO3A}$ derivative is shown in Table 3, which can be directly coupled to a peptide *via* standard peptide coupling reactions, or transformed to a benzyl-isothiocyanate.

Although DOTA has been used successfully with ^{212}Pb , its slow radiolabeling kinetics and stability properties were not ideal.¹⁴⁶ Replacement of the carboxylic acid donor arms of DOTA with amide arms has produced the chelator TCMC (Table 3), which to date is the best chelator available for ^{212}Pb .^{147–149} Chappell *et al.* have shown TCMC to be superior to C-DOTA (Table 2) with ^{212}Pb due to improved radiolabeling kinetics and *in vivo* stability.^{150,151} Although not optimal for ^{212}Pb , 3p-C-DEPA (Table 3) has been shown to be an excellent chelator for $^{212/213}\text{Bi}$, with superior properties to C-DOTA.¹⁵² The DOTA derivative 3p-C-DEPA has demonstrated greatly improved radiolabeling kinetics with $^{212/213}\text{Bi}$ compared to DOTA, with comparable *in vivo* stability.¹⁵² 3p-C-DEPA can be considered to be one of the current “gold standards” for $^{212/213}\text{Bi}$; however, the NOTA analogue 3p-C-NETA (*vide infra*) also looks very promising and has not been directly compared with 3p-C-DEPA, and so the superior of the two has yet to be identified.

3.3 TETA

TETA is an octadentate N_4O_4 macrocyclic chelator that has only been investigated with ^{64}Cu for radiopharmaceutical applications (Table 4). TETA is generally not used anymore, and has been superseded by newer generation chelators such as NOTA, CB-TE2A, and CB-TE1A1P/CB-TE2P (Table 4) that are more stable and kinetically inert *in vivo*. Copper is a difficult metal ion to harness, as it is quite labile, has active redox chemistry between $\text{Cu}^{1+}/\text{Cu}^{2+}$ *in vivo*, and has a fast water exchange rate of $2 \times 10^8 \text{ s}^{-1}$.^{37,159} Copper(II) is a first row transition metal with a d^9 electron configuration, is best used with hexadentate chelators that saturate its coordination sphere, and has a preference for borderline soft ligand donor atoms such as amines, imines, and thiols.^{15,37} Despite being made obsolete in recent years, TETA has previously been used successfully for ^{64}Cu imaging with vectors like octreotide.^{160,161} TETA can radiolabel with ^{64}Cu at room temperature, but it lacks kinetic inertness and overall stability *in vivo*. Surprisingly, the TETA derivative TE2A (Table 4) shows enhanced kinetic inertness, despite the fact that TE2A is merely a hexadentate derivative of TETA that is missing two carboxylic acid arms.¹⁶² This effect is enhanced even further when the two secondary amines of TE2A are methylated or cross-bridged by an ethylene unit (Table 4).^{144,163} Unlike DOTA (cyclen-based), TETA (cyclam-based) has not been

heavily investigated with any radiometals other than ^{64}Cu ; however, some structural and physical data is available with other metal ions (Table 4).

3.4 TE2A, CB-TE2A, CB-TE1A1P, CB-TE2P, MM-TE2A, DM-TE2A

The cyclam-based macrocycle TETA has spawned a large number of successful derivatives (Table 4). Despite the requisite harsh radiolabeling conditions (95 °C), CB-TE2A has become one of the premier ^{64}Cu chelators, having superior *in vivo* stability to TETA, TE2A, DOTA, NOTA, and CB-DO2A.¹⁴⁴ The stability gains from adding an ethylene cross bridge to TETA/TE2A are significant, despite the removal of two coordinating carboxylic acid arms, and a subsequent decrease in denticity from 8 to 6. In order to surmount the harsh radiolabeling conditions required for CB-TE2A, several derivatives of CB-TE2A have been synthesized. Improved radiolabeling kinetics have been achieved by replacement of one (CB-TE1A1P) or both (CB-TE2P) carboxylic acid arms with methylenephosphonate groups, with *in vitro* and *in vivo* stability being retained or enhanced compared to the native CB-TE2A (Table 4).^{144,164–168} Most importantly, the methylenephosphonate derivatives CB-TE1A1P and CB-TE2P can be radiolabeled with ^{64}Cu at room temperature, although relatively slowly. Another noteworthy set of derivatives were made by methylating one (MM-TE2A) or two (DM-TE2A) nitrogen atoms of TETA/TE2A, instead of linking them together with an ethylene bridge (Table 4).¹⁶³ Surprisingly, these methylated TE2A derivatives possessed similar radiolabeling kinetics and stability to CB-TE2A.¹⁶³ This observation suggests that the high stability of CB-TE2A is not a result of steric protection by the cage-like structure created by the cross-bridged ethylene unit, but instead by changing the electronic properties of the nitrogen atoms (secondary to tertiary amines) and their donor properties. Although CB-TE2A has been the most heavily investigated ligand of the above mentioned TETA derivatives, the new methylenephosphonate derivatives CB-TE1A1P and CB-TE2P show the most promise, as they appear to retain the stability of CB-TE2A, while having greatly enhanced radiolabeling kinetics.

3.5 Diamsar and derivatives

The radiometal ion $^{64}\text{Cu}^{2+}$, similar to $^{68}\text{Ga}^{3+}$, has been extremely popular in recent years due to its favorable positron emission (β^+) properties for PET imaging and its intermediate half-life (12.7 hours), which is suitable for use with peptides, antibody fragments, and even whole antibodies. Unlike ^{68}Ga , ^{64}Cu also undergoes β^- emission, making it useful for therapy in addition to PET imaging. It follows, therefore, that there has been a strong interest in developing new and improved bifunctional chelators to optimize radiolabeling procedures and *in vivo* performance with isotopes of copper. In addition to the ubiquitous chelator NOTA (*vide infra*), new entrants such as CB-TE2A, CB-TE1A1P, CB-TE2P, MM-TE2A, and DM-TE2A have recently been determined to be excellent ^{64}Cu chelators (*vide supra*). The Sar family of chelators (hexamine sarcophagines) are hexaazamacrocyclic cage type ligands, and include the ligands SarAr, SarAr-NCS, diamSar, AmBaSar, and BaBaSar (Table 5).

Table 4 TETA, CB-TE2A and derivatives, and bifunctional derivatives, highlighting relevant radiometal ions, radiolabeling conditions, thermodynamic stability constants ($\log K_{ML}$), coordination geometry, and color-coded ranking

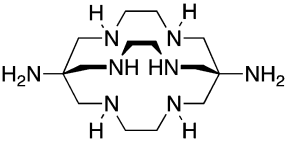
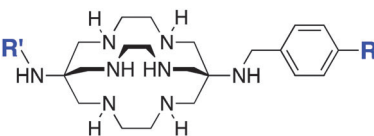
Chelator and common bifunctional derivatives	Radiometal ion	^a	Radiolabeling conditions	$\log K_{ML}$	Proposed geometry	Ref.
	$^{64}\text{Cu}^{2+}$	~	25 °C, 60 min, pH 5–7	21.9, 21.6	Distorted octahedron	160, 169
	$^{67/68}\text{Ga}^{3+}$	X	—	19.74 (pM 14.1)	Distorted octahedron	64
TETA, 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid, N_4O_4 CN = 8	$^{111}\text{In}^{3+}$	X	—	21.9 (pM 16.3)	Distorted octahedron?	64
	$^{177}\text{Lu}^{3+}$	X	—	15.3	Distorted dodecahedron?	170
	$^{86/90}\text{Y}^{3+}$	X	—	14.8	Distorted dodecahedron?	170
BAT (C-TETA derivative) ¹⁷¹						
$p\text{-NH}_2\text{-Bn-TE3A}^{172}$						
C-TETA ($p\text{-NO}_2\text{-Bn-TETA}$) ¹⁷³						
	$^{64}\text{Cu}^{2+}$	✓	95 °C, 60 min, pH 6–7	—	Distorted octahedron	68, 90, 106, 108, 164, 166–168, 174, 175
CB-TE2A, 4,11-bis-(carboxymethyl)-1,4,8,11-tetraazabicyclo[6.6.2]-hexadecane, N_4O_2 CN = 6						
CB-TE1A1P ^{164,177}						
CB-TE2P ^{164,177}						
MM-TE2A ¹⁶³						
DM-TE2A ¹⁶³						
TE2A ¹⁶²						

^a Green “✓” = good/best match, orange “~” = suitable match, or requires more evaluation but shows potential, red “X” = poor/unstable match.

One of the major benefits of these nitrogen rich chelators is that they can radiolabel with ^{64}Cu in only a few minutes (5–10 min) at room temperature, which is faster even than the recently devised ligands CB-TE1A1P and CB-TE2P (Table 4).^{37,178–180} Of all the chelators discussed for ^{64}Cu , Sar derivatives have the fastest room temperature radiolabeling kinetics. In addition to fast kinetics, they demonstrate excellent *in vivo* stability, and strong resistance to acid dissociation *in vitro*.^{178–181} One drawback to nitrogen rich N_6 chelators such as the Sar family is lipophilicity, as they

contain little or no acid functionality to add negative charge, and as a result form cationic or neutral complexes with ^{64}Cu (depending on the Sar derivative). Because ^{64}Cu pairs exceptionally well with peptides, a lipophilic radiometal–chelator complex can have deleterious affects on biodistribution and tumor targeting properties by directing activity to the liver and digestive tract (depending on hydrophilicity of the selected peptide). The Sar derivative BaBaSar adds two carboxylic acid functional groups to the Sar scaffold, thereby augmenting

Table 5 The copper chelators Diamsar, SarAr, AmBaSar, and BaBaSar, highlighting relevant radiometal ions, radiolabeling conditions, thermodynamic stability constants ($\log K_{ML}$), coordination geometry, and color-coded ranking

Chelator and common bifunctional derivatives	Radiometal ion	^a	Radiolabeling conditions	$\log K_{ML}$	Proposed geometry	Ref.
 Diamsar, N ₆ , CN = 6	⁶⁴ Cu ²⁺	✓	25 °C, 5–30 min, pH 5.5		Distorted octahedron or trigonal prism	178–181
 1- <i>N</i> -(4-Aminobenzyl)-3,6,10,13,16,19-hexaazabicyclo[6.6.6]-eicosane-1,8-diamine (SarAr) ^{178–182} SarAr R = NH ₂ , R' = H SarAr-NCS R = NCS, R' = H AmBaSar R = COOH, R' = H BaBaSar R = –COOH, R' = –CH ₂ –Ph–COOH						

^a Green “✓” = good/best match, orange “~” = suitable match, or requires more evaluation but shows potential, red “✗” = poor/unstable match.

hydrophilicity. Animal studies have been promising with the Sar family of chelators, but more work is required for further validation; furthermore, limited commercial availability and challenging synthesis may be a reason why widespread adoption has yet to occur.^{37,178–181}

3.6 NOTA, NETA, and TACN-TM

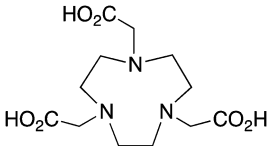
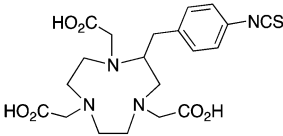
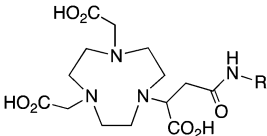
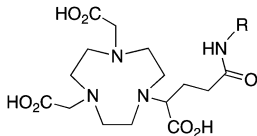
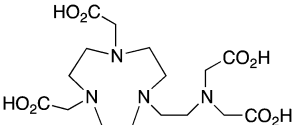
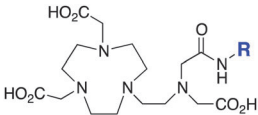
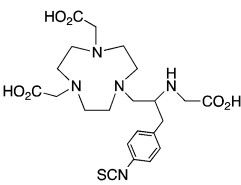
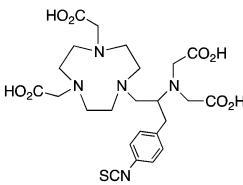
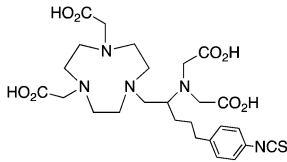
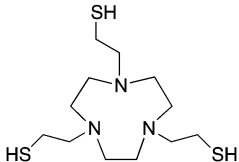
NOTA is a hexadentate N₃O₃ chelator, and is one of the oldest and most successful chelators for use with ^{67/68}Ga and ⁶⁴Cu (Table 6). NOTA is generally considered to be the “gold standard” for Ga³⁺ chelation, possessing favorable radiolabeling conditions (RT, 30–60 minutes) and excellent *in vivo* stability.^{110,117,183,184} Of all the isotopes discussed in this review, ⁶⁸Ga is probably the most popular in recent years. ⁶⁸Ga has a very favorable positron emission (1880 keV, 90%) for PET imaging, and a short half-life (68 min) suitably matched for imaging with peptide vectors. The recent development of several ⁶⁸Ge/⁶⁸Ga-generator systems with shelf-lives of ~9–12 months has propelled this radiometal into the spotlight of the international research scene.¹⁸⁵ ⁶⁷Ga is a longer-lived isotope of gallium that is suitable for SPECT imaging. Ga³⁺ is a hard acidic metal ion (pK_a 2.6) with an ionic radius of 62 pm (CN = 6), a preference for amine and oxygen donor atoms, and is ultimately a challenging metal ion to chelate because it has a strong affinity for hydroxide anions causing precipitation of gallium hydroxide (Ga(OH)₃) between pH 3–7.^{62,159,186}

Because of its potential clinical utility, many new gallium chelators have been published in the last ~5 years; however, most have not yet amassed the same amount of *in vivo* data as NOTA. NOTA has been shown to have superior radiolabeling properties and stability with ⁶⁴Cu when compared to common chelators such as DOTA, EDTA, DTPA, and TETA.^{155,187} NOTA can radiolabel with ⁶⁴Cu at room temperature in 30–60 minutes, making it compatible with heat sensitive antibody vectors.¹⁸⁸ Although many new chelators like TRAP, AAZTA (DATA), H₂dedpa, CP256, and PCTA (*vide infra*) show great promise for use with ^{67/68}Ga in early experiments, due to commercial availability of BFC derivatives and widespread acceptance, NOTA is still currently the “gold standard” for ^{67/68}Ga. A similar story can be told for ⁶⁴Cu, where a plethora of new chelators,

including the Sar family (*e.g.* SarAr, DiamSar), CB-TE2A, and various CB-TE2A derivatives (*e.g.* CB-TE1A1P, CB-TE2P, MM/DM-TE2A) appear to possess many superior properties, but NOTA is still the practical “gold standard” for ⁶⁴Cu.

The modified NOTA chelator NETA (Table 6) is an interesting derivative that shows promise with ^{86/90}Y, possessing fast radiolabeling kinetics similar to those of acyclic chelators (5–10 minutes, RT), as well as a high degree of stability and rigidity imparted by the macrocyclic framework.¹⁸⁹ A direct comparison between NETA and DOTA has demonstrated greatly enhanced radiolabeling kinetics for NETA with Y³⁺.¹⁸⁹ Biodistribution experiments performed with the ⁸⁶Y complexes of the non-bifunctional chelators NETA and DOTA have suggested that NETA had comparable clearance and stability properties to DOTA, even showing lower bone accumulation than DOTA.¹⁸⁹ NETA has been evaluated with other therapeutic isotopes for RIT, including ^{203/212}Pb, ^{212/213}Bi, and ¹⁷⁷Lu.^{149,190,191} NETA and bifunctional derivatives C-NETA and C-NE3TA (Table 6) were found to be unstable with ^{203/212}Pb isotopes, but looked very stable with ^{212/213}Bi, ⁹⁰Y, and ¹⁷⁷Lu during a period of 11 days in blood serum.^{191–193} Biodistribution studies of C-NETA complexes in healthy mice demonstrated excellent clearance and stability of the ^{212/213}Bi, ⁹⁰Y, and ¹⁷⁷Lu complexes, with the exception of high kidney uptake with the ^{212/213}Bi-C-NETA complex.¹⁹¹ Recent experiments with an isothiocyanate bearing derivative of C-NETA, 3p-C-NETA (Table 6) have demonstrated that ⁹⁰Y and ¹⁷⁷Lu radiolabeled immunoconjugates (trastuzumab) had very rapid room temperature radiolabeling kinetics, and excellent *in vivo* performance, with the potential to surpass DOTA and also DTPA analogues such as CHX-A''-DTPA.¹²⁷ 3p-C-NETA has been further evaluated with ^{212/213}Bi for RIT applications, and trastuzumab immunoconjugates have been shown to exhibit rapid radiolabeling kinetics and excellent *in vitro* and *in vivo* stability, suggesting improved stability over C-NETA, which showed high kidney accumulation of ^{212/213}Bi.^{191,194} It is interesting to note that these studies were actually performed with ^{205/206}Bi, as the longer half-lives (15.3/6.2 days, respectively) of these ^{212/213}Bi isotopologues are more amenable to extensive *in vitro* and *in vivo* study than are the short half-lives of ^{212/213}Bi.¹⁹⁴ Currently it is not clear if 3p-C-DEPA (Table 3) is

Table 6 NOTA, NETA, TACN-TM, and bifunctional derivatives, highlighting relevant radiometal ions, radiolabeling conditions, thermodynamic stability constants ($\log K_{ML}$), coordination geometry, and color-coded ranking

Chelator and common bifunctional derivatives	Radiometal ion	α	Radiolabeling conditions	$\log K_{ML}$	Proposed geometry	Ref.
 NOTA, 1,4,7-triazacyclononane-1,4,7-triacetic acid, CN = 6, N₃O₃	⁶⁴ Cu ²⁺	✓	25 °C, 30–60 min, pH 5.5–6.5	21.6	Distorted trigonal prism	187, 196
	^{67/68} Ga ³⁺	✓	25 °C, 30–60 min, pH 4.0–5.5	31.0 (pM 26.4, 27.9)	Distorted octahedron	110, 117, 118, 183, 184, 197, 198
	^{44/47} Sc ³⁺	~	95 °C, 20–30 min, pH 4.0	16.5 (pM 19.2)	Distorted octahedron	98
	¹¹¹ In ³⁺	~	60–95 °C, 20–30 min, pH 4.0–5.0	26.2 (pM 21.6)	Distorted octahedron	70, 117, 184, 199–201
 <i>p</i>-SCN-Bn-NOTA(C-NOTA)^{141,199}						
 NODASA, R = NHS ester, amide²⁰²						
 NODAGA, R = NHS ester, amide²⁰⁰						
 NETA, {4-[2-bis(carboxymethylamino)-ethyl]-7-carboxymethyl-[1,4,7]triazonan-1-yl}-acetic acid, N₄O₄, CN = 8	¹⁷⁷ Lu ³⁺	✓	25 °C, 5 min, pH 4.5	—	Square antiprism?	127, 149, 190, 191
	^{86/90} Y ³⁺	✓	25 °C, 5 min, pH 4.0	—	Square antiprism?	127, 189, 191
	^{212/213} Bi ³⁺	✓	25 °C, 5 min, pH 4.0	—	Square antiprism?	149, 190, 191, 194
	²¹² Pb ²⁺	✗	25 °C, 5 min, pH 4.0	—	Square antiprism?	191
 NETA-monoamide¹⁸⁹						
 C-NE3TA-NCS¹⁹¹						
 C-NETA-NCS¹⁹¹						
 3p-C-NETA^{127,193,194}						
 TACN-TM, <i>N,N',N''</i>, tris(2-mercaptoethyl)-1,4,7-triazacyclononane, N₃S₃ CN = 6	^{67/68} Ga ³⁺	✗	25 °C, 10 min, degassed ethanol	34.2	Distorted octahedron	110, 195, 203, 204
	¹¹¹ In ³⁺	✗	25 °C, 10 min, degassed ethanol	36.1	Distorted octahedron	99, 176, 177, 183

α Green "✓" = good/best match, orange "~" = suitable match, or requires more evaluation but shows potential, red "✗" = poor/unstable match.

superior to 3p-C-NETA (Table 6) for use with ^{212/213}Bi, and a direct comparison would be timely.

TACN-TM (Table 6) is an interesting thiol-based chelator, and shows extremely high thermodynamic stability constants

($\log K_{ML}$) with Ga³⁺ and In³⁺ of 34.2 and 36.1, respectively.^{99,176,177,183}

Another interesting thiol TACN derivative is TACN-HSB, which substitutes alkyl-thiols for thiophenol groups.¹⁹⁵ Despite the high thermodynamic stabilities, radiolabeling experiments required

the use of degassed ethanol due to instability of the thiol groups of TACN-TM/TACN-HSB to air, and ultimately these ligands could not be used for *in vivo* applications.¹⁹⁵ For these reasons, no bifunctional derivatives have been synthesized of these thiol-based TACN/NOTA derivatives, and further study has not been pursued.

3.7 DTPA, 1B4M-DTPA, and CHX-A''-DTPA

DTPA is one of the oldest and most pervasive acyclic chelators used in radiochemistry, and like most acyclic chelators it can be radiolabeled with many radiometal ions at room temperature in a matter of minutes (Table 7). As a first generation radiometal chelator, it suffers from stability issues *in vivo* with many radiometal ions and is universally not as stable as the macrocycles DOTA and NOTA, making its use obsolete in recent years.⁵⁶ DTPA has been successfully used as the BFC in the FDA-approved SPECT agent OctreoScan™ (¹¹¹In-DTPA-octreotide), a somatostatin-targeting peptide-conjugate used for imaging neuroendocrine tumors.^{205,206} DTPA has also been successfully used with radiometals such as ⁶⁴Cu, ¹¹¹In, ¹⁷⁷Lu, and ^{86/90}Y, but has been made redundant by more stable new chelators like DOTA, NOTA, and CHX-A''-DTPA (Table 7).^{56,161} The shortcomings of DTPA have been improved through design of novel derivatives, such as the DTPA derivatives 1B4M-DTPA (Tiuxetan) and CHX-A''-DTPA.^{56,207} 1B4M-DTPA is a bifunctional DTPA derivative that contains a single methyl group on one of its ethylene backbones, and has been successfully incorporated into the FDA-approved ⁹⁰Y therapeutic immunoconjugate Zevalin (Table 7).^{207–209,210}

Although DTPA and DOTA are the most commonly investigated chelate systems for Y³⁺, the chelator CHX-A''-DTPA (Table 7) shows significantly improved stability over DTPA; however, it is still generally considered to be less stable than DOTA.⁵⁶ The cyclohexyl backbone of CHX-A''-DTPA makes the chelator more rigid and imposes a degree of preorganization on the metal ion binding site, enhancing kinetic inertness, but retarding radiolabeling kinetics compared to those of DTPA.^{56,211} Due to the success of antibody vectors, developing acyclic chelators with fast radiometal ion coordination kinetics, as well as high stability and kinetic inertness comparable to that of macrocycles such as DOTA is an important goal. The work done towards developing CHX-A''-DTPA was particularly interesting because of the 4 possible isomers of this chelator. The CHX-B''-DTPA isomer was found to be much less stable than the CHX-A''-DTPA isomer *in vivo* with ⁹⁰Y, despite *in vitro* stability assays suggesting they were very similar.^{56,212} If CHX-DTPA were to be used as a racemic mixture it would result in significant decomposition of the less stable isomers and would lead to radiodemetalation *in vivo*, highlighting the importance of enantiopurity.^{56,212} Different isomers of a BFC–radiometal complex can have different stabilities and off-rates (kinetic inertness), potentially leading to differential biodistributions.^{42,59}

Although CHX-A''-DTPA has enhanced radiolabeling kinetics when compared to DOTA, both are still much slower than typical acyclic chelators such as DTPA and H₄octapa (Table 9), and often require mild heating (~37–60 °C) and

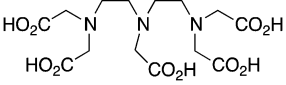
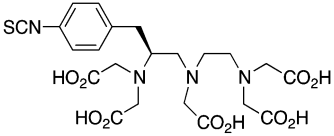
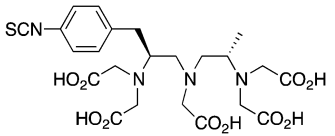
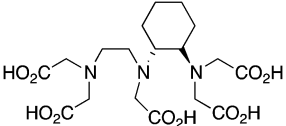
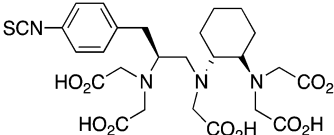
reaction times of 30–60 minutes to achieve reasonable yields. CHX-A''-DTPA has been heavily investigated for use with many radiometals, including ^{86/90}Y,^{6,7,55,56,212–220} ¹⁷⁷Lu,^{54,126,128} and ^{212/213}Bi,^{201,221–223} but work with ¹¹¹In has comparatively been limited.^{217,218,224–229} Studies that have been performed with ¹¹¹In(CHX-A''-DTPA) conjugates have shown good *in vivo* results, but to our knowledge little work has been done comparing it with the “gold standard” ¹¹¹In chelator DOTA.²²⁸ A study comparing the *in vivo* behavior of the affibody conjugate ^{114m}In-CHX-A''-DTPA-ABD-(Z_{HER2:342})₂ to ¹¹¹In-DOTA-ABD-(Z_{HER2:342})₂ suggests that both chelators have comparable stability and performance, with CHX-A''-DTPA exhibiting slightly higher tumor uptake, but also slightly higher non-target organ uptake (e.g. kidneys, liver, bone).²²⁸ ^{114m}In is a notable γ-emitting isotope because it has identical chemistry to ¹¹¹In, but a longer 49.5-day half-life and interesting decay scheme (Table 1).²²⁸ When ^{114m}In decays it emits a γ-ray for SPECT imaging, but the daughter nuclide ¹¹⁴In has a half-life of 72 seconds and emits high-energy (1989 keV) β[−] particles useful for therapy.²²⁸ When the parent ^{114m}In radiolabeled affibody internalizes into cells, it acts as an *in vivo* generator of ¹¹⁴In for intracellular delivery of therapeutic radioactivity (β[−] particles) to cancer cells.²²⁸

Investigations with ^{212/213}Bi have found DTPA to possess fast radiolabeling kinetics, but poor *in vivo* stability, ultimately rendering it unusable.^{119,230–232} CHX-A''-DTPA has been shown to possess enhanced stability with ^{212/213}Bi when compared to DTPA and 1B4M-DTPA, and even comparable stability to DOTA, while additionally possessing greatly enhanced radiolabeling kinetics (important for the short half-life of ^{212/213}Bi).^{201,221–223,231,233} The solid-state structure of the [Bi(CHX-A''-DTPA)]^{2−} complex suggests that the partially pre-organized binding site offered by the rigid cyclohexyl backbone may contribute to the enhanced stability of the complex (also suggested as the cause for enhanced stability with other isotopes such as ^{86/90}Y and ¹⁷⁷Lu).²²¹ Although comparable to DOTA, CHX-A''-DTPA is still considered to be inferior to 3p-C-NETA and 3p-C-DEPA for use with ^{212/213}Bi.

3.8 TRAP (PRP9) and NOPO

TRAP (PRP9) is a derivative of the macrocycle NOTA, wherein the traditional carboxylic acid arms have been replaced with phosphinic acid groups (Table 8).^{79–81,245–249} The most successful TRAP derivative, TRAP-Pr, contains an additional ethyl-carboxylate moiety extending distally from the phosphinic acid groups (Table 8).^{79–81,245–249} The most interesting property of the TRAP-Pr ligand is its improved apparent specificity for Ga³⁺ when compared to NOTA.^{78,81} This phenomenon is expressed by the difference in thermodynamic stability constants (log K_{ML}) of TRAP and NOTA for competing metal ions such as Mg²⁺, Ca²⁺, Cu²⁺, and Zn²⁺,⁸¹ and by competitive radiolabeling experiments done in the presence of an excess of these other ions.⁷⁸ As a general rule, radiolabeling experiments are performed in strictly metal-free water and so the selectivity of TRAP-Pr for Ga³⁺ may not appear to be totally relevant, but it does suggest that transchelation by these competing metal ions *in vivo* should occur to a lesser extent than with NOTA. Additionally, as discussed in the introduction, the enhanced

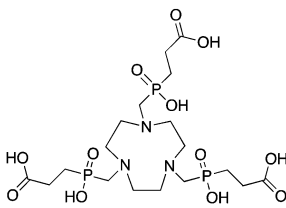
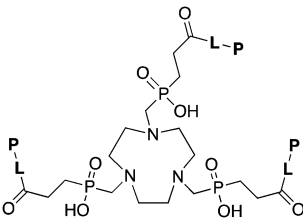
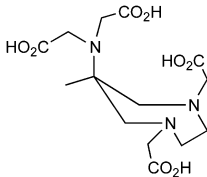
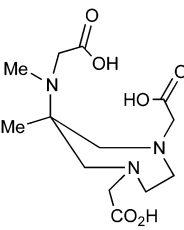
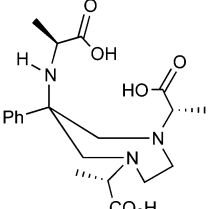
Table 7 DTPA, 1B4M, CHX-A''-DTPA, and bifunctional derivatives, highlighting relevant radiometal ions, radiolabeling conditions, thermodynamic stability constants ($\log K_{ML}$), coordination geometry, and color-coded ranking

Chelator and common bifunctional derivatives	Radiometal ion	^a	Radiolabeling conditions	$\log K_{ML}$	Proposed geometry	Ref.
 DTPA, diethylenetriaminepentaacetic acid, N ₃ O ₅ , CN = 8	⁶⁴ Cu ²⁺	✗	40 °C, 60 min, pH 6.5	21.4	Distorted octahedron?	119, 234
	^{67/68} Ga ³⁺	✗	25 °C, 30 min, pH 3.5	24.3, 25.5 (pM 20.2)	Distorted octahedron?	117, 119, 235
	^{44/47} Sc ³⁺	~	25 °C, 10 min, pH 6.0	—	Square antiprism?	16
	¹¹¹ In ³⁺	~	25 °C, 5–10 min, pH 4.5–5.5	29.0 (pM 24.9)	Pentagonal bipyramid or square antiprism	117, 119, 236–238
	¹⁷⁷ Lu ³⁺	~	25 °C, 10–20 min, pH 5.5	22.6	Square antiprism?	119, 131, 239
	^{86/90} Y ³⁺	~	25 °C, 10–20 min, pH 5.5	21.2, 22.0, 22.5	Monocapped square antiprism	55, 131, 132, 239
	⁸⁹ Zr ⁴⁺	✗	25 °C, 60 min, pH 7 (<0.1% yield)	35.8–36.9	Distorted dodecahedron	119, 240
	^{212/213} Bi ³⁺	✗	25 °C, 10–20 min, pH 5	35.6	Square antiprism	119, 230–232
 <i>p</i> -SCN-Bn-1B-DTPA ^{211,241}					 <i>p</i> -SCN-Bn-1B4M-DTPA ^{224, 242, 243}	
 CHX-A''-DTPA, 2-(<i>p</i> -isothiocyanatobenzyl)-cyclohexyldiethylenetriaminepentaacetic acid, N ₃ O ₅ , CN = 8	^{67/68} Ga ³⁺	✗	85 °C, 20 min, pH 5.5	—	Distorted octahedron?	217
	¹¹¹ In ³⁺	✓	25–60 °C, 30–60 min, pH 5.5	—	Square antiprism	217, 218, 224–229
	¹⁷⁷ Lu ³⁺	✓	37–75 °C, 30–60 min, pH 5–5.5	—	Square antiprism	54, 126, 128
	^{86/90} Y ³⁺	✓	37–75 °C, 30–60 min, pH 5–5.5	—	Square antiprism	6, 7, 55, 56, 212–220
	²¹³ Bi ³⁺	~	25 °C, 10–20 min, pH 5	—	Square antiprism	201, 221–223
 <i>p</i> -SCN-Bn-CHX-A''-DTPA ^{56,212,244}						

^a Green “✓” = good/best match, orange “~” = suitable match, or requires more evaluation but shows potential, red “✗” = poor/unstable match.

specificity of TRAP-Pr for ⁶⁸Ga over NOTA may be of benefit if trace-impurities are present in the stock radiometal solution (e.g. ⁶⁸Ge/⁶⁸Ga generator eluent, or commercially supplied ⁶⁴Cu). A similar derivative to TRAP is NOPO, which is a

Table 8 Promising $^{67/68}\text{Ga}$ chelators TRAP (PRP9, TRAP-Pr), AAZTA (DATA), and bifunctional derivatives, highlighting relevant radiometal ions, radiolabeling conditions, thermodynamic stability constants ($\log K_{\text{ML}}$), coordination geometry, and color-coded ranking

Chelator and common bifunctional derivatives	Radiometal ion ^a	Radiolabeling conditions	$\log K_{\text{ML}}$	Proposed geometry	Ref.
 TRAP (PRP9, TRAP-Pr), 1,4,7-triazacyclononane-1,4,7-tris[2-carboxyethyl]phosphonic acid], N_3O_3 CN = 6 (+ distal carboxylates CN = 9?)	$^{67/68}\text{Ga}^{3+}$	95 °C, 5 min, pH 3.2	26.24	Distorted octahedron	79–81, 91, 245–249
 NOPO ^{78,248,257}					
 AAZTA, and novel DATA derivatives, 1,4-bis(hydroxycarbonyl methyl)-6-[bis(hydroxycarbonyl methyl)] amino-6-methyl perhydro-1,4-diazepine, N_2O_4 or N_3O_3 , CN = 6	$^{67/68}\text{Ga}^{3+}$	25 °C, 1–5 min, pH 4–6.8	—	Distorted octahedron	253–256
 H_3L^1					
 H_3L^4					

^a Green “✓” = good/best match, orange “~” = suitable match, or requires more evaluation but shows potential, red “✗” = poor/unstable match.

triazacyclononane-triphosphate ligand that has a similar structure to TRAP-Pr, but with two terminal alcohol groups in place of carboxylic acids.^{78,248,250} Like TRAP, NOPO has shown promising properties for ^{64}Cu and ^{68}Ga radiolabeling.^{78,248,250}

The peptide conjugate TRAP(RGD)₃ was radiolabeled with ^{68}Ga in 5 minutes at 95 °C (HEPES, pH 3.2), and despite the high reaction temperature was able to radiolabel at lower ligand concentrations than the corresponding DOTA or NOTA conjugates.^{14,15} RGD (arginine–glycine–aspartic acid tripeptide) is a popular cyclic peptide vector that targets over-expression of integrin $\alpha_v\beta_3$ receptors on various types of cancer, and is commonly used as a proof-of-principle vector for studying new isotopes and new BFC.^{14,15,30,91,250,251} ^{68}Ga -TRAP(RGD)₃ demonstrated good *in vivo* tumor targeting properties, showing great promise for use in ^{68}Ga -based PET imaging agents,^{14,15} however, a recent comparative biodistribution study between ^{68}Ga -TRAP(RGD)₃ and ^{68}Ga -NODAGA-RGD revealed higher uptake in several non-target organs for the TRAP-based agent.^{79,91,246} Despite these apparent shortcomings, TRAP-Pr is a very promising ^{68}Ga chelator, which is a claim substantiated by the work of Dr Richard P. Baum at Zentralklinik Bad Berka in Germany, who has performed imaging experiments in human patients using ^{68}Ga -TRAP-peptide conjugates.²⁵²

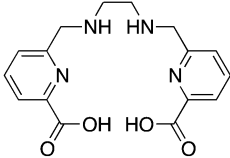
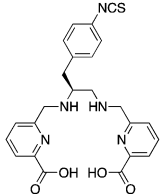
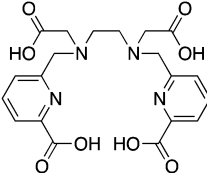
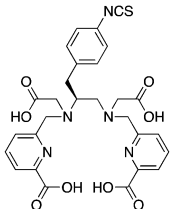
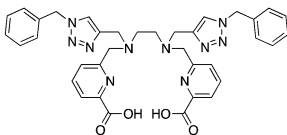
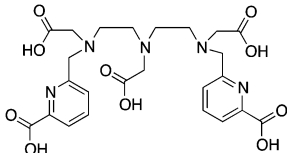
3.9 AAZTA and derivatives (DATA)

The novel AAZTA derivatives H_3L^1 and H_3L^4 are new $^{67/68}\text{Ga}$ -chelators that have shown improved radiolabeling and serum stability properties over the parent AAZTA chelator (Table 8). The parent AAZTA chelator was found to form multiple isomers with Ga^{3+} , and so the derivatives H_3L^1 and H_3L^4 were crafted such that one major isomer of the Ga^{3+} complex was kinetically trapped by attachment of bulky alkyl/aryl groups.^{253–256} These AAZTA derivatives (now called DATA) are promising new Ga^{3+} chelators with extremely fast radiolabeling kinetics (1–5 minutes at RT) and good preliminary *in vitro* and *in vivo* stability results (as non-bifunctional chelators). These chelators are very recent, as most of this work has just been published in 2013, and so more extensive *in vitro* and *in vivo* experimentation will likely follow in the coming years. Additionally, no bifunctional derivatives (*e.g.* peptide conjugates) have been published to date, which will be required for proper evaluation and comparison *in vivo* to existing “gold standards” like NOTA.

3.10 H_2dedpa , H_4octapa , H_2azapa , and H_5decapa

This family of ligands has only been published over the previous 3 years and has been referred to as the “pa family”,

Table 9 H₂dedpa, H₄octapa, H₂azapa, H₅decapa, and bifunctional derivatives, highlighting relevant radiometal ions, radiolabeling conditions, thermodynamic stability constants (log *K*_{ML}), coordination geometry, and color-coded ranking

Chelator and common bifunctional derivatives	Radiometal ion	<i>a</i>	Radiolabeling conditions	log <i>K</i> _{ML}	Proposed geometry	Ref.
 H₂dedpa, 1,2-[[6-(carboxy)pyridin-2-yl]methylamino]ethane, N₄O₂ CN = 6	⁶⁴ Cu ²⁺	~	25 °C, 5–10 min, pH 5.5	19.2 (pM = 18.5)	Distorted octahedron	269
	^{67/68} Ga ³⁺	✓	25 °C, 5–10 min, pH 4.5	28.1 (pM = 27.4)	Distorted octahedron	118, 268, 270
 <i>p</i>-SCN-Bn-H₂dedpa^{118,268}	¹¹¹ In ³⁺	✓	25 °C, 5–10 min, pH 4.5	26.8 (pM = 26.5)	Square antiprism	83, 85
	¹⁷⁷ Lu ³⁺	✓	25 °C, 5–10 min, pH 4.5	20.1 (pM = 19.8)	Square antiprism	83
 H₄octapa, <i>N,N'</i>-bis(6-carboxy-2-pyridylmethyl)ethylenediamine-<i>N,N'</i>-diacetic acid, N₄O₄ CN = 8	¹¹¹ In ³⁺	✓	25 °C, 5–10 min, pH 4.5	26.8 (pM = 26.5)	Square antiprism	83, 85
	¹⁷⁷ Lu ³⁺	✓	25 °C, 5–10 min, pH 4.5	20.1 (pM = 19.8)	Square antiprism	83
 <i>p</i>-SCN-Bn-H₄octapa⁸³	⁶⁴ Cu ²⁺	~	25 °C, 5–10 min, pH 5.5	—	Distorted octahedron?	84
	^{67/68} Ga ³⁺	✗	25 °C, 5–10 min, pH 4–4.5	—	Distorted octahedron?	84
 H₂azapa, <i>N,N'</i>-[1-benzyl-1,2,3-triazole-4-yl]methyl-<i>N,N'</i>-[6-(carboxy)pyridin-2-yl]-1,2-diaminoethane, N₆O₂ CN = 8 (benzyl groups as vector placeholder)	¹¹¹ In ³⁺	✗	25 °C, 5–10 min, pH 4.5	—	Square antiprism?	84
	¹⁷⁷ Lu ³⁺	✗	25 °C, 5–10 min, pH 4.5	—	Square antiprism?	84
 H₅decapa, <i>N,N'</i>-[[6-(carboxy)pyridin-2-yl]methyl]diethylenetriamine-<i>N,N',N''</i>-triacetic Acid, N₅O₅ CN = 10	¹¹¹ In ³⁺	✗	25 °C, 5–10 min, pH 4.5	27.56 (pM = 23.1)	Square antiprism	83, 85

^a Green “✓” = good/best match, orange “~” = suitable match, or requires more evaluation but shows potential, red “✗” = poor/unstable match.

as they are all crafted around central picolinic acid (“pa”) binding moieties (Table 9). This work originated in chelators made by Rodriguez-Blas and coworkers, originally purposed as

contrast agents for magnetic resonance imaging (MRI) and luminescence.^{258–267} H₂dedpa was the first chelator in this family to be investigated, with its acyclic hexadentate scaffold

being ideal for $^{67/68}\text{Ga}$, radiolabeling in less than 10 minutes at room temperature and forming a very symmetrical hexadentate coordination geometry (determined by solid-state X-ray structure).^{118,262,264,265,267} *In vitro* analysis of the $[\text{}^{67/68}\text{Ga}(\text{dedpa})]^+$ complex demonstrated excellent stability and resistance to transchelation by *apo*-transferrin and blood serum.¹¹⁸ The bifunctional derivative *p*-SCN-Bn- H_2dedpa has been synthesized and conjugated to the cyclic peptide RGD, radiolabeled with ^{68}Ga and ^{64}Cu , and has shown promising results for *in vivo* tumor targeting and PET imaging.^{268,269} The H_2dedpa chelator has also been functionalized with lipophilic moieties, and the resulting cationic ^{68}Ga complexes have been investigated for cardiac perfusion imaging.²⁷⁰ Although preliminary work looks very promising for H_2dedpa , especially towards the production of kit-formulations with its very fast room temperature radiolabeling kinetics, more *in vivo* validation is required. As is the case with all new bifunctional chelators, the largest barrier to widespread adoption is commercial availability, as the synthesis and purification of most nitrogen and oxygen rich chelators are typically tedious and challenging.

H_2azapa was built on a variation of the click-to-chelate approach, where the peptide vector is conjugated to the chelator by click-chemistry, and the resultant triazole-rings are uniquely positioned so that they are capable of coordinating to radiometal ions in the inner-coordination sphere.^{32,84,271} The triazole-containing $\text{H}_2\text{dedpa}/\text{H}_4\text{octapa}$ derivative, H_2azapa , has been synthesized and evaluated with ^{64}Cu , ^{68}Ga , ^{111}In , and ^{177}Lu (Table 9).⁸⁴ Preliminary results were promising with ^{64}Cu , but *in vitro* stability assays suggest that complexes with ^{68}Ga , ^{111}In , and ^{177}Lu were unstable.⁸⁴ Due to the very lipophilic character of the neutral $[\text{Cu}(\text{azapa})]$ complex, *in vivo* evaluation revealed high liver and digestive tract uptake as expected, therefore more hydrophilic conjugates (*e.g.* peptide conjugates) must be synthesized and evaluated in the future.⁸⁴ The large decadentate derivative of H_4octapa , H_5decapa , was evaluated with ^{111}In *in vitro* and *in vivo*, and was found to be significantly less stable than were H_4octapa and DOTA (Table 9).^{83,85}

H_4octapa is a derivative of H_2dedpa , with an additional two carboxylic acid arms increasing the maximum denticity from 6 to 8 (Table 9).^{83,85,262,267} H_4octapa has recently been found to exhibit ideal properties for ^{111}In and ^{177}Lu radiochemistry, radiolabeling in quantitative yields at room temperature in less than 10–15 minutes.^{83,85} Direct comparisons between H_4octapa and DOTA with both ^{111}In and ^{177}Lu have demonstrated similar *in vivo* properties and stability, with H_4octapa having greatly enhanced radiolabeling kinetics (significantly faster even than CHX-A''-DTPA), suggesting that it may be a suitable alternative and improvement to DOTA.^{83,85} Based on its excellent properties with ^{177}Lu , H_4octapa may also be a suitable match for the similar isotopes $^{86/90}\text{Y}$, although no results have been published towards this goal thus far. Further preclinical work must be performed to validate H_4octapa as a suitable alternative to DOTA, although a direct comparison has been made with ^{111}In and ^{177}Lu *in vitro* and *in vivo*.^{83,85}

It appears that CHX-A''-DTPA , $\text{C-NETA}/3\text{p-C-NETA}$, and H_4octapa are the best current alternatives to DOTA for fast

radiolabeling kinetics and excellent *in vivo* stability with ^{111}In for SPECT imaging and dosimetry, and ^{177}Lu and $^{86/90}\text{Y}$ for therapeutic applications.^{83,85,127} It is currently not clear how $\text{C-NETA}/3\text{p-C-NETA}$ perform with ^{111}In because, to our knowledge, no studies have been published, and work with ^{111}In and CHX-A''-DTPA is limited. If a SPECT imaging surrogate isotope such as ^{111}In is required for pre-therapy imaging and dosimetry, it appears that currently CHX-A''-DTPA and H_4octapa are the most promising alternatives to DOTA.^{83,85} A direct comparison between all of these chelators with ^{111}In , ^{177}Lu , and $^{86/90}\text{Y}$ in the same animal model at the same time would be of significant value.

3.11 HBED and SHBED

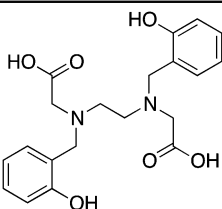
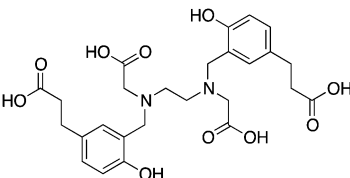
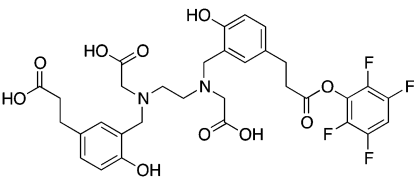
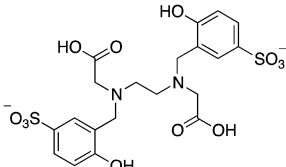
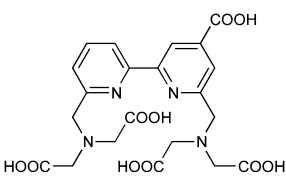
HBED and its derivative SHBED present an interesting example of chelator development, as the addition of an aromatic *para*-sulfonate group in SHBED to the phenol functionality of HBED serves to decrease the pK_a of the phenolic protons and alter their hard/soft metal-donor properties (Table 10). The original purpose of adding this aryl-sulfonate group to HBED was to increase its negative charge and hydrophilicity/solubility (fully deprotonated SHBED has a 6^- charge *vs.* 4^- for HBED), but the effect of changing the donor properties of the attached phenolic oxygen highlights an interesting option for tuning other chelators that contain aromatic donor groups.²⁷² Decreasing the pK_a of acidic functional groups in ligands can also help to improve radiolabeling efficiency: in this example by making the phenolic protons of SHBED more acidic and easier to deprotonate, and subsequently making SHBED a more effective ligand at lower pH. The bifunctional derivative HBED-CC has been conjugated to antibodies and was found to radiolabel much faster than NOTA, achieving a radiochemical yield (RCY) of 97% in under 5 minutes at room temperature, while also showing comparable *in vivo* performance to NOTA.^{273,274} Although HBED has been around for several decades, limited work with ^{68}Ga has been performed, and other isotopes such as ^{64}Cu and ^{111}In have not shown optimal properties.^{117,273–276}

HBED demonstrates that phenol and catechol donor groups are effective for Ga^{3+} , but interestingly recent Ga^{3+} chelators such as H_2dedpa , TRAP, PCTA, AAZTA (DATA), and CP256 have not utilized them. Although an effective gallium chelator, HBED has received limited clinical use and has been supplanted in recent years by a plethora of new ligands.

3.12 BPCA

BPCA is an interesting chelator that was published in 2010, which is based on a 2,2'-bipyridine backbone, and provides an octadentate N_4O_4 donor set (Table 10).^{277,278} BPCA was conjugated to a novel cholecystokinin C-terminal tetrapeptide (CCK4), which targets tumors expressing the cholecystokinin receptor subtype 2 (CCK2R), and radiolabeled with ^{111}In in high RCY after 1 hour at room temperature. The novel peptide-conjugate BPCA-(Ahx)₂-CCK4 was evaluated *in vitro* by serum stability transchelation assays, and *in vivo* by biodistribution and planar scintigraphy experiments. The same (Ahx)₂-CCK4 conjugate was made with CHX-A''-DTPA as an internal reference,

Table 10 HBED, SHBED, BPCA, and bifunctional derivatives, highlighting relevant radiometal ions, radiolabeling conditions, thermodynamic stability constants ($\log K_{ML}$), coordination geometry, and color-coded ranking

Chelator and common bifunctional derivatives	Radiometal ion	^a	Radiolabeling conditions	$\log K_{ML}$	Proposed geometry	Ref.
 HBED, <i>N,N'</i>-bis(2-hydroxybenzyl)-ethylenediamine-<i>N,N'</i>-diacetic acid, N_2O_4, CN = 6	^{67/68} Ga ³⁺	~	25 °C, 10–20 min, pH 4–4.5	38.5 (pM 28.6)	Distorted octahedron?	117, 273–276
	^{44/47} Sc ³⁺	✗	25 °C, 10 min, pH 6.0	—	Square antiprism?	16
	¹¹¹ In ³⁺	✗	25 °C, 10–20 min, pH 4–7	27.9 (pM 17.9)	Distorted octahedron?	117, 275, 276
 HBED-CC^{274,279}						
 (HBED-CC)TFP²⁷⁴						
 SHBED, <i>N,N'</i>-bis(2-hydroxy-5-sulfobenzyl)-ethylenediamine-<i>N,N'</i>-diacetic acid, N_2O_4, CN = 6	^{67/68} Ga ³⁺	~	25 °C, 10–20 min, pH 4–4.5	37.5 (pM 28.3)	Distorted octahedron?	117, 272, 276
	¹¹¹ In ³⁺	~	25 °C, 10–20 min, pH 4–7	29.4 (pM 20.6)	Distorted octahedron?	117, 272, 276
 BPCA, N_4O_4, CN = 8	¹¹¹ In ³⁺	~	25 °C, 60 min, pH 5	—	Square antiprism?	277, 278

^a Green “✔” = good/best match, orange “~” = suitable match, or requires more evaluation but shows potential, red “✗” = poor/unstable match.

and it was demonstrated that BPCA possessed superior stability to CHX-A''-DTPA in serum (89% vs. 45% stable after 2.5 hours, respectively). Biodistribution and planar scintigraphic imaging studies showed that BPCA-based conjugates had higher tumor uptake, lower background organ uptake, and higher tumor/muscle ratios than CHX-A''-DTPA, suggesting that BPCA is a very promising new chelator for ¹¹¹In. A study evaluating BPCA with ¹⁷⁷Lu and ^{86/90}Y would be of great interest.

3.13 CP256

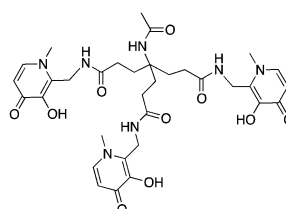
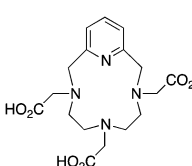
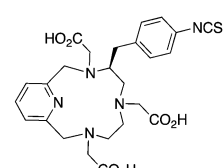
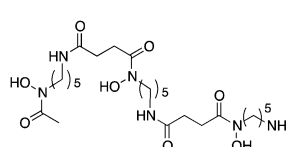
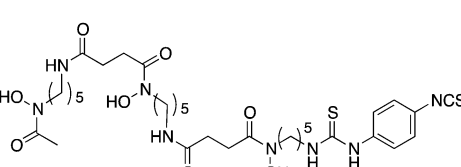
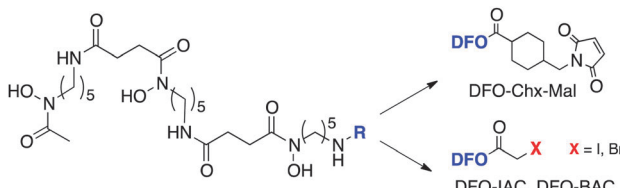
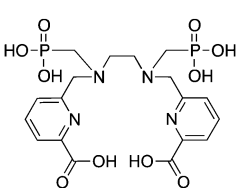
CP256 and its bifunctional derivative YM103 are acyclic tripodal tris(hydroxypyridinone) ligands that can rapidly radiolabel with ⁶⁸Ga in 5 minutes at room temperature (Table 11).²⁸⁰ The maleimide derivative YM103 was conjugated to cysteine residues of the protein C2Ac, and *in vivo* experiments in normal healthy mice (no tumor models) showed rapid clearance through the kidneys with no observable decomposition after

1.5 hours.²⁸⁰ This new chelator was published less than 2 years ago, and further studies in tumor bearing mice will help to establish its potential utility. CP256 has an interesting acyclic tripodal design, similar to L3 (hydroxamate-based, Fig. 3), and most interestingly both chelators are oxygen-rich with O₆ donor sets, suggesting a potential untapped application as ⁸⁹Zr chelators (yet to be investigated, to our knowledge).

3.14 Desferrioxamine (DFO)

Desferrioxamine B (DFO) is a bacterial siderophore that natively binds Fe³⁺, and has also been used extensively with isotopes of gallium and zirconium (Table 11). DFO is the only competent ⁸⁹Zr chelator available for radiolabeling and *in vivo* applications.²⁸¹ Zr⁴⁺ is a highly charged, very hard metal ion with a relatively small ionic radius (84 and 89 pm for CN = 8 and 9, respectively),¹⁸⁶ that is prone to forming insoluble polynuclear hydroxide species in aqueous solution under non-acidic conditions,

Table 11 The $^{67/68}\text{Ga}$ and ^{89}Zr chelators CP256, desferrioxamine (DFO), PCTA, H_6phospa , and bifunctional derivatives, highlighting relevant radiometal ions, radiolabeling conditions, thermodynamic stability constants ($\log K_{\text{ML}}$), coordination geometry, and color-coded ranking

Chelator and common bifunctional derivatives	Radiometal ion	<i>a</i>	Radiolabeling conditions	$\log K_{\text{ML}}$	Proposed geometry	Ref.
 CP256, O_6 CN = 6	$^{67/68}\text{Ga}^{3+}$	✓	25 °C, 5 min, pH 6.5	—	Distorted octahedron	280
CP256 = 4-acetylamino-4-[2-[(3-hydroxy-1,6-dimethyl-4-oxo-1,4-dihydro-pyridin-2-ylmethyl)-carbamoyl]-ethyl]-heptanedioic acid bis-[(3-hydroxy-1,6-dimethyl-4-oxo-1,4-dihydro-pyridin-2-ylmethyl)-amide]						
 PCTA, 3,6,9,15-tetraazabicyclo[9.3.1]-pentadeca-1(15),11,13-triene-3,6,9-triacetic acid, N_4O_3 CN = 7	$^{64}\text{Cu}^{2+}$	~	25 °C, 5 min, pH 5.5	19.1	Distorted octahedron	87, 90, 214, 290
Bifunctional derivative YM103 ²⁸⁰						
	$^{67/68}\text{Ga}^{3+}$	✓	25 °C, 5–10 min, pH 4–5	—	Distorted octahedron	86, 290–292
 <i>p</i> -SCN-Bn-PCTA ²¹⁴	$^{67/68}\text{Ga}^{3+}$	~	25 °C, 30 min, pH 3.5	28.6	Distorted octahedron?	235, 293
 DFO, desferrioxamine B, O_6 , CN = 6	$^{89}\text{Zr}^{4+}$	✓	25 °C, 60 min, pH 7–7.3	—	Distorted octahedron?	51, 130, 240, 281, 283–288, 294–300
 <i>p</i> -SCN-Bn-DFO ²⁹⁶	 Bifunctional DFO derivatives ^{297,301}					
 H_6phospa , N,N' -(methylenephosphonate)- N,N' -[6-(methoxycarbonyl)pyridin-2-yl]-methyl-1,2-diaminoethane, N_4O_4 CN = 8	$^{89}\text{Zr}^{4+}$	~	25 °C, 60 min, pH 7.4	—	Square antiprism?	289
Bifunctional derivative <i>p</i> -SCN-Bn- H_6phospa						

^a Green “✓” = good/best match, orange “~” = suitable match, or requires more evaluation but shows potential, red “✗” = poor/unstable match.

and subsequently is difficult to radiolabel effectively. DFO is thought to bind ^{89}Zr with its three hydroxamate groups in a hexadentate fashion, although no crystal structures have been obtained.²⁸² For many years the next best chelator for ^{89}Zr has been DTPA, which forms a thermodynamically stable complex with Zr^{4+} ($\log K_{\text{ML}} = 35.8\text{--}36.9$), but studies have shown that radiolabeling with ^{89}Zr is inefficient and *in vivo* stability is exceptionally poor.¹¹⁹ A DTPA-antibody conjugate of Zevalin was radiolabeled with ^{89}Zr , and radiochemical yields obtained were $<0.1\%$ after 1 hour at room temperature;²⁴⁰ in contrast, DFO can radiolabel with ^{89}Zr in quantitative yields ($>99\%$) after 1 hour. The new chelator H_6phospa has recently been studied with ^{89}Zr , and has shown greatly improved radiolabeling performance relative to DTPA, but still inferior to DFO (*vide infra*).

No FDA-approved radiopharmaceuticals currently utilize ^{89}Zr , although a number of ^{89}Zr -DFO-antibody conjugates are in clinical trials.²⁰⁸ ^{89}Zr has a long half life (78.5 hours), making it ideally paired with antibody vectors, as the biological half-life of an antibody is on the order of 2–3 weeks.³⁸ ^{89}Zr -DFO-Zevalin was the first ^{89}Zr antibody conjugate imaged in humans, and was shown to be a suitable PET surrogate for ^{90}Y -Zevalin dosimetry.²⁸³ A number of other studies have been performed using ^{89}Zr -DFO-antibody conjugates with success, such as ^{89}Zr -DFO-U36 (anti-CD446 chimeric mAb),^{208,284} ^{89}Zr -DFO-bevacizumab,^{208,285} ^{89}Zr -DFO-J591,²⁸¹ ^{89}Zr -DFO-TRC105,²⁸⁶ and ^{89}Zr -DFO-trastuzumab.^{208,287,288}

The high 4+ charge makes ^{89}Zr challenging to incorporate into BFC systems while still retaining bioequivalence with other common 3+ cationic metal ions (e.g. In^{3+} , Ga^{3+} , Y^{3+} , Lu^{3+}), because the chelate–radiometal charge and polarity is different. Evaluating the stability of a potential BFC for ^{89}Zr can be done *in vivo*, because ^{89}Zr that is lost from a BFC typically localizes in bone, as demonstrated by the highly unstable ^{89}Zr -DOTA- and ^{89}Zr -DTPA-based antibody conjugates with cetuximab that showed significant ^{89}Zr accretion in the thighbone 72 hours post injection.¹³⁰ ^{89}Zr -chloride and ^{89}Zr -oxalate have also been injected directly into mice, with ^{89}Zr -chloride forming colloids and accumulating in the liver, and the weakly chelated ^{89}Zr -oxalate demonstrating rapid bone uptake.²⁸¹ Although DFO is an excellent ^{89}Zr chelator, some decomposition can be observed over time *in vivo* as ^{89}Zr slowly accumulates in bone.^{27,51} The design of novel chelators for ^{89}Zr with improved solubility, *in vivo* stability, and chelation properties would be timely, considering there is currently only one option (DFO). Based on the success of DFO, it would appear that oxygen-rich donors are most suitable for ^{89}Zr chelation, with $\text{O}_6\text{--O}_8$ coordination being preferred, and donor groups including hydroxamates, carboxylates, carbonyls, catechols, and hydroxypyridinones being logical choices.

3.15 H_6phospa

Developing new chelating agents for ^{89}Zr is currently of great interest, as the only chelator available that can radiolabel ^{89}Zr with any level of proficiency is DFO. As discussed in Section 3.14, the best alternative chelator to DFO for ^{89}Zr radiolabeling is DTPA; however, with meager radiochemical yields of $<0.1\%$ after 1 hour at room temperature and poor *in vivo* stability, DTPA is not a viable alternative to DFO.²⁴⁰ DFO can radiolabel

with ^{89}Zr in quantitative yields ($>99\%$) after 1 hour at room temperature, and demonstrates imperfect but acceptable stability *in vivo*, and no acceptable alternatives have been published to date. The acyclic chelator H_6phospa (Table 11),^{264,265} and the bifunctional derivative *p*-SCN-Bn- H_6phospa are methylenephosphonate derivatives of H_4octa (Table 9) that have been recently studied with ^{89}Zr .²⁸⁹ The antibody conjugate H_6phospa -trastuzumab was able to achieve radiochemical yields of $\sim 8\%$ with ^{89}Zr after 1 hour at room temperature (PBS, pH 7.4).²⁸⁹ This work was compared to previous attempts to radiolabel H_4octa -trastuzumab with ^{89}Zr , which performed similarly to DTPA and exhibited essentially no radiolabeling after 1 hour.²⁸⁹ These results demonstrate that replacing the carboxylic acid arms of H_4octa with methylenephosphonate arms in H_6phospa improved radiolabeling kinetics with ^{89}Zr , suggesting that methylenephosphonate groups are more suitable than carboxylic acid groups for chelating ^{89}Zr .²⁸⁹ Although a RCY of $\sim 8\%$ is insufficient to supplant DFO as the “gold standard” chelator for radiolabeling with ^{89}Zr , it is a significant improvement over DTPA and H_4octa , and to our knowledge is the highest ^{89}Zr radiolabeling yield behind DFO to be published.

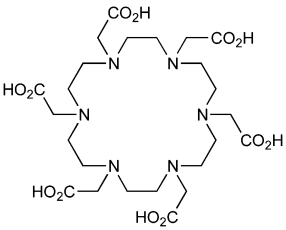
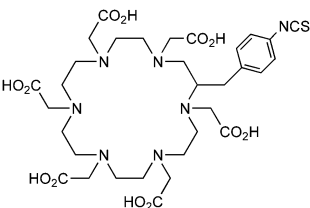
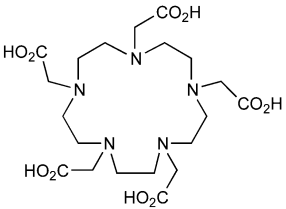
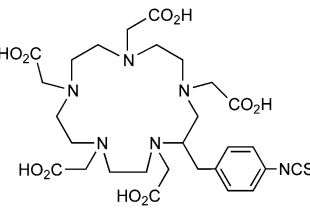
3.16 PCTA

The heptadentate macrocyclic chelator PCTA was originally synthesized by Sherry and coworkers as a potential MRI contrast agent (Table 11).²⁹⁰ PCTA has been recently re-purposed and evaluated with ^{68}Ga and ^{64}Cu , and has been shown to possess much faster radiolabeling kinetics than DOTA (5–10 min, RT).⁸⁶ The serum stability of the $^{67/68}\text{Ga}$ -PCTA complex was found to be superior to DOTA, and comparable to NOTA.⁸⁶ Additionally, the biodistribution profile of the non-bifunctional ^{68}Ga -PCTA revealed lower kidney retention than ^{68}Ga -NOTA, showing potential improvement over NOTA for use in peptide conjugates.⁸⁶ Comparisons of RGD conjugates of the bifunctional derivatives of both PCTA and NOTA have demonstrated excellent *in vivo* stability and comparable biodistribution profiles, with the PCTA-based agent having lower kidney uptake than its NOTA counterpart.²⁹¹ PCTA has also been investigated with ^{64}Cu , showing greater radiolabeling kinetics and yields to DOTA, and superior *in vitro* and *in vivo* stability.⁷⁵ PCTA has only recently been investigated, but further work may show it to be an excellent chelator for copper and gallium-based radiopharmaceuticals.

3.17 HEHA and PEPA

HEHA is a large 18-membered, 12 coordinate, macrocyclic chelator, and PEPA is a structurally similar 10 coordinate derivative (Table 12). Both HEHA and PEPA have primarily been investigated for ^{225}Ac complexation and RIT; preliminary radiolabeling and *in vitro/in vivo* stability experiments with ^{225}Ac had suggested that HEHA was superior to DOTA and PEPA.²²⁴ The antibody conjugate HEHA-MAB-201B was radiolabeled with ^{225}Ac and used to treat lung tumors in mice, and although effective at delivering large doses of ^{225}Ac to tumors, high radiotoxicity from decay daughter isotopes resulted in death.³⁰² More recently, using different *in vitro* experiments (e.g. cation exchange resin challenge) and different *in vivo* targets, DOTA has been shown to be the most suitable ^{225}Ac chelator.^{101,103,303} A recent clinical trial with ^{225}Ac -DOTA-HuM195

Table 12 The ^{225}Ac chelators HEHA, PEPA, and bifunctional derivatives, highlighting relevant radiometal ions, radiolabeling conditions, thermodynamic stability constants ($\log K_{\text{ML}}$), coordination geometry, and color-coded ranking

Chelator and common bifunctional derivatives	Radiometal ion ^a	Radiolabeling conditions	$\log K_{\text{ML}}$	Proposed geometry	Ref.
 HEHA, 1,4,7,10,13,16-hexaazacyclohexadecane-<i>N,N',N'',N''',N''',N'''</i>-hexaacetic acid, N_6O_6, $\text{CN} = 12$	$^{225}\text{Ac}^{3+}$	 37–40 °C, 30 min, pH 5.8–7	—	?	99, 101, 103, 303–305
 <i>p</i>-SCN-Bn-HEHA (C-HEHA)⁹⁹	$^{212/213}\text{Bi}^{3+}$	 25 °C, 15 min, pH 5–5.5	—	?	304
 PEPA, 1,4,7,10,13-pentaazacyclopentadecane-<i>N,N',N'',N''',N'''</i>-pentaacetic acid, N_5O_5, $\text{CN} = 10$	$^{225}\text{Ac}^{3+}$	 37–40 °C, 30–60 min, pH 5.8–7	—	?	99
 <i>p</i>-SCN-Bn-PEPA (C-PEPA)³⁰⁴					

^a Green “✓” = good/best match, orange “~” = suitable match, or requires more evaluation but shows potential, red “✗” = poor/unstable match.

(humanized anti-CD33 monoclonal antibody) in humans has shown promise.^{100,103} PEPA has also been investigated for use with $^{212/213}\text{Bi}$ for therapy applications, but was found to be unsuitable.³⁰⁴ α -emitting isotopes are currently of very high interest and potential impact, due to their effectiveness at killing cancer cells when they are tightly chelate-bound and safely delivered to their targets.^{3,134,149,151,210,303} α -particles travel a very short distance (μm range, compared to mm range for β^- particles) and have high linear energy transfer values (LET), meaning they deposit huge amounts of energy over a very short distance, making them very effective at treating various cancers when properly directed, but also potentially very toxic.^{3,134,149,151,210,303} Development of new chelators for use with α -emitting isotopes is currently of high impact in the field of BFC.

4. A brief look at less-developed chelators

A large number of chelators have been used transiently over the years for study and application with a variety of radiometals, but have not garnered very much interest from the radio-

chemistry community, have not been sufficiently developed to date, or have lacked a required level of proficiency (*e.g.* reaction kinetics, RCY, stability) for mainstream use. Additionally, a chelator may have shown great promise for use with a radiometal, but a suitable synthetic route to a bifunctional derivative may never have been published. Shown in Fig. 4 is a selection of chelators that have not been discussed here, but still may be of interest or of use to some researchers. These chelators may not have been found to be a suitable match to any of the current gamut of available radiometal ions discussed here, but production of more exotic radiometals in the future may bring them into relevance. Inspiration may also be drawn from the chemical structures, donor-atom types, connectivity, or geometries of these chelators, and potentially used towards new and novel chelators.

4.1 Conclusions

After examining the large number of highly competent radiometal chelators displayed in this review, some researchers may be daunted or even disheartened at the idea of creating new scaffolds with the existence of so many chelators that already work “well enough”. Radiometal chemistry teaches us that the intricate relationships amongst radiometal ion, chelator, linker,

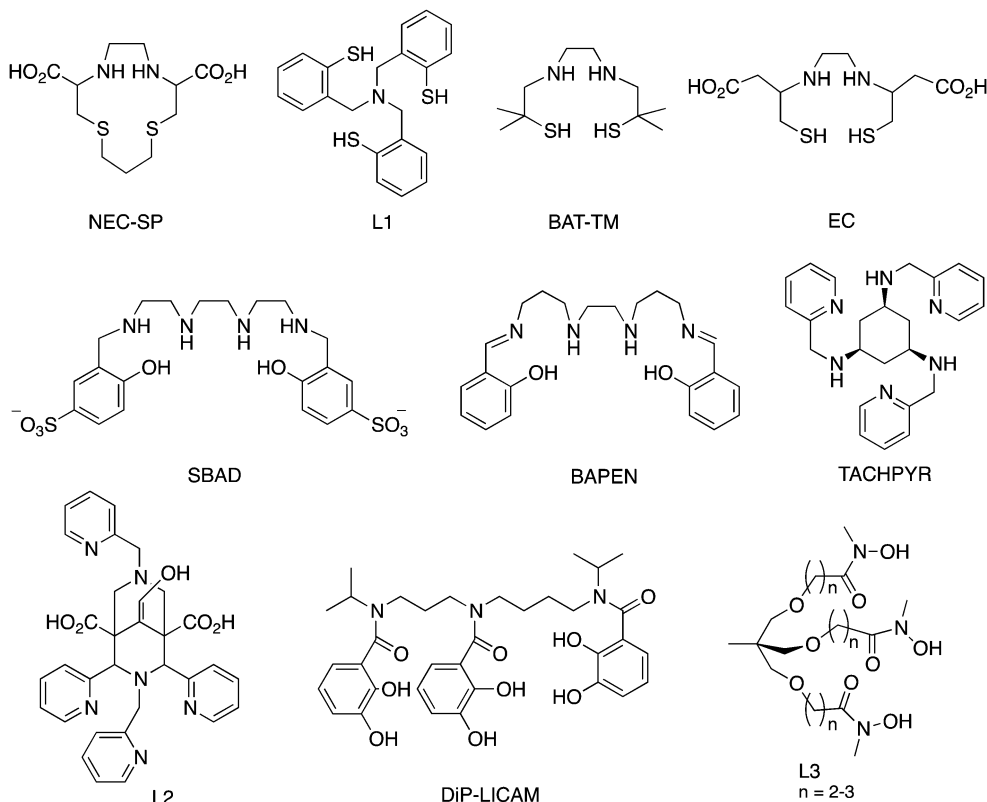


Fig. 4 A selection of less-discussed chelators with potential applications in radiometal chemistry, including NEC-SP,³⁰⁶ L1 (TACN-HSB, tris(2-mercaptobenzyl)amine),³⁰⁷ BAT-TM,^{308,309} EC,^{117,310} SBAD,³¹¹ BAPEN,^{312–314} TACHPYR,^{315–317} L2 (9-hydroxy-2,4-dipyridin-2-yl-3,7-bis(pyridin-2-ylmethyl)-3,7-diazabicyclo[3.3.1]nonane-1,5-dicarboxylic acid),³¹⁸ DiP-LICAM,^{319,320} and the hydroxamate-based L3.³²¹

biological vector (e.g. peptide, antibody, antibody fragment, nanoparticle), and animal/tumor models are surprisingly complicated and only superficially understood. In order to optimally tune the properties of radiometal-based radiopharmaceuticals, a large variety of different tools (e.g. chelators, linkers, vectors) are crucial to have available so that the physical properties of an agent can be easily modified. Factors such as the charge, polarity, denticity, donor atom type (e.g. N, O, S), and kinetics (e.g. macrocyclic vs. acyclic) must be modular in order to optimize *in vivo* behavior of a radiopharmaceutical, and this requires the availability of many different bifunctional chelators. The synthetic canon of radiopharmaceutical components will always have space for new innovation and curiosity so that radioactive imaging agents and therapeutics can be continually improved.

Current directions in radiochemistry and nuclear medicine highlight the importance of PET imaging, and subsequently the significance of optimal utilization of a growing number of β^+ emitting radiometal ions. Promising new PET isotopes such as ^{44}Sc and ^{89}Zr currently have poorly elaborated chelate chemistry, and moving forward the development of fast-radiolabeling and exceptionally stable chelators for these isotopes will be important. ^{68}Ga is perhaps the most promising of all isotopes discussed here, primarily due to its highly portable and long-lived generator system. To properly utilize ^{68}Ga and translate it to the clinic, the wide-range of excellent ^{68}Ga chelators that are currently under investigation must be transformed into viable imaging agents to provide useful diagnostic

tools to the medical community. α -emitting isotopes are also currently of strong interest, as they are extremely effective, when targeted, at site-specifically delivering therapeutic doses of radioactivity to a variety of cancers. The caveat of α -emitters is their potentially fatal toxicity, and therefore robust chelate chemistry is of utmost importance for the continued development of therapeutic agents based on isotopes such as ^{225}Ac , $^{212/213}\text{Bi}$, and ^{212}Pb .

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