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Bifunctional Chelates for Metal Nuclides

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Abstract

The use of “non-standard” metallic radionuclides continues to be an expanding field of investigation. Radiolabeling small molecules, peptides, proteins, and up to nano-particles are all areas of active investigation for both diagnostic and therapeutic applications. All require a common variable – the need for appropriate chelation chemistry for adequate sequestration of the metallic radionuclide that is equal to the intended application. A brief overview of the array of the chelation chemistry options available to researchers and the means for their selection is provided.

Introduction

Nature has provided a vast array of radionuclides with emission properties that that makes them valuable reagents for investigating basic problems in chemistry, biology, and medicine. These properties include γ-, β^+-, β^--, α-, and Auger emissions just to list some of those useful for medical diagnostic (γ–scintigraphy, SPECT, PET) and therapeutic applications. In addition to their radionuclidic properties, there is an even wider array of fundamental chemical properties that are available for researchers to exploit. However, the use of these same radionuclides is then constrained by limits of half-life, decay chain, production and availability, realistic chemical usage, and matching all of these properties appropriately to the intended biological application(s) which severely diminishes the number of choices to a select few (Table 1).[1] As much of these aspects have been well reviewed, the focus herein then is on those properties and the chemistry required for the use of those metallic radionuclides that have generally been accepted to be within those boundary conditions set forth above.

The utility of these metallic radionuclides has necessitated the development of metal chelating agents to effectively provide a handle over their behavior. These chelating agents have been termed “bifunctional chelating agents” since they have a metal binding moiety function and then also possess a chemically reactive functional group. The former then provides for the sequestration of the metallic radionuclide while the latter aspect provides the requisite chemistry for covalent attachment to a targeting vector of interest, such as a small molecules peptides (octreotide),[2] proteins (monoclonal antibody, Zevalin),[3] or nano-particles.[4]

There are a number of fundamental criteria that have to be met in the design of bifunctional chelating agents for such applications. Foremost seems based on the stability of the metal complex. Clearly, the consequences of loss or dissociation of the radionuclide are associated with toxicity in the case of therapeutics and poor image qualities for diagnostics. Fundamental coordination chemistry criteria such as: (1) charge; (2) matching cavity size of the chelating agent with the ionic radius of the radionuclide; (3) providing the appropriate chelate denticity

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or number of donor binding groups; and (4) providing donor binding groups of appropriate chemical character are all key elements. Two additional properties are also critical to consider: the rate at which the metal complex forms and the rate of dissociation. All of these criteria are interrelated. Cavity size must accommodate the ionic radius of the radionuclide such that all of required donor groups can be properly aligned for optimal binding to the metal ion in such a way to adequately encapsulate the ion thereby providing high stability and limiting dissociation. A listing of those metallic radionuclides that will be discussed here along with their selected properties of ionic radius, charge, and half-life is provided in Table 1. The suitable radiometals are diverse in their properties and coordination chemistry, so, unfortunately there is no bifunctional chelating agent suitable for all radionuclides.[5] Lastly, there are a number of copper radionuclides. These are not included here and are left for discussion in other papers in this issue.

Having then created a bifunctional chelating agent, validation of its suitability for biological applications still remains to be executed. There are a number of properties that can be used to validate acceptability of a novel bifunctional chelating agent, including: (1) thermodynamic stability constants; (2) transchelation studies; (3) acid catalyzed dissociation constants; and (4) serum stability studies. All of these properties do provide some information that can be used to suggest potential *in vivo* suitability. Serum stability can be a very useful tool and model that serves to predict and eliminate from contention those bifunctional chelating agents that are unsuitable for *in vivo* applications. None of these properties or models is predictive of actual *in vivo* stability of the metal complex. To assess real *in vivo* stability of the metal complex, evaluation in an appropriate animal model is necessary. The definition of appropriate animal model is variable, however clearly it should really reflect very closely the ultimate intended biological application. As yet, no *in vitro* model system replicates all of the ongoing processes and components of a living organism just as the therapeutic efficacy of a macromolecule can not be predicted from *in vitro* results.

Despite all those considerations, the development of bifunctional chelating agents has been rooted in making derivations from well established and defined inorganic chemistry chelating agents, *e.g.*, ethylenediamine tetraacetic acid (EDTA), diethylenetriamine pentaacetic acid (DTPA) (Figure 1), and 1,4,7,10-tetra-azacylcododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid (DOTA) (Figure 2), all of which as polyaminocarboxylate ligands vary coordination number from 6 to, and also cover acyclic and macrocyclic options to encapsulate the metal ion. Fundamental thermodynamic stability constants are known for these ligands with a variety of metal ions that have provided a starting point for their derivation into an array of bifunctional chelating agents (Table 2).[6] In addition to that data, actual biological data for some of these ligands complexing radionuclides of medical interest, both animal and human use, is available in the literature and an example of such is provided in Table 3.[7] Such data can be used to extrapolate to the creation of bifunctional chelating agents.

Historically, one of the earliest reports of a bifunctional chelating agent conjugated to an antibody made use of a natural product, desferrioxamine, for radiolabeling with ^{111}In.[8] Desferrioxamine and related compounds are well known chelators of Fe(III), and as such their derivation for use with In(III) and Ga(III) has precedence. Interestingly, more recently desferrioxamine has been investigated for sequestering ^{89}Zr through a somewhat complicated, yet elegant protocol that exploits that same Fe(III)/(II) chemistry for antibody labeling in support of immunoPET applications.[9,10] The Fe(III) complex was formed first with the desferrioxamine then activated for conjugation through extension of the terminal amine with succinic anhydride followed by conversion of the formed carboxylate into an active ester.[10] After conjugation, the Fe(III) was reduced and displaced with the ^{89}Zr.

Returning to polyaminocarboylate ligands, one can recognize that while bifunctional EDTA derivatives were initially reported for use with ^{111}In and ^{90}Y, their limited stability forced a move to develop bifunctional DTPA derivatives that would provide a more appropriate coordination number.[11,12] Concurrently, direct derivatives of DTPA, the cyclic anhydride (ca-DTPA) and the isobutylcarbonic anhydride (carb-DTPA) were also routinely in use (Figure 1).[13,14] Despite their products routinely being termed DTPA conjugates these conjugation products, in fact, are not DTPA chelators. This is due to the utilization of one carboxylate in the conjugation forming an amide that may or may not then bind to the metal effectively. Studies to determine the impact of this change in coordination number and donor character very clearly defined decreased *in vitro* and *in vivo* stability of these products.[12,15] Additionally, the cyclic anhydride with two reactive conjugation moieties suffered from potential cross-linking issues.

Full octadentate bifunctional DTPA derivatives addressed these deficiencies.[12,16-19] The numbers and variations on structure combined with variations in conjugation strategies that have been developed exceed the scope of this paper. However, a selection of representative structures is provided in Figure 1. The 1B4M-DTPA, also known as MX-DTPA or tiuxetan, has been developed as the chelating agent component of Zevalin for radiolabeling with either ^{111}In or ^{90}Y.[3] Further refinements in the pre-organization geometry of the DTPA donor elements ultimately led to the creation of the CHX-A" DTPA which has been reported to form stable complexes with ^{111}In, ^{177}Lu, ^{213}Bi, and to also be significantly more stable than the 1B4M-DTPA for sequestering ^{90}Y.[20-22] The CHX-A" DTPA is notable for being the chelating agent component in the first clinical antibody trial using an α-emitter, ^{213}Bi,[23] as well also being a commercially available product.

Despite the successes achieved with bifunctional DTPA derivatives, their overall stability complexing radionuclides such as ^{90}Y was noted as being less than perfect and could potentially contribute to toxicity.[24]

In response to that deficiency, full octadentate macrocyclic bifunctional DOTA derivatives have been developed for complexing ^{111}In, ^{86}Y, ^{90}Y, radio-lanthanides, ^{213}Bi, ^{212}Pb, and ^{225}Ac.[25-29] Again, the numbers and variations on structure combined with variations in conjugation themes that have been developed exceed the scope of this paper. However, a selection of representative structures is provided in Figure 2. Concurrently, a bifunctional DOTA that makes use of one carboxylate in an active ester form for protein conjugation has also been developed and is also a commercially available product (Figure 3).[30] As with analogous DTPA derivatives, the conjugation product really is not DOTA, but again a mono-amide product wherein the amide may or may not bind to the metal. While this DOTA mono-amide product provides convenience (another commercial product), the impact on the fundamental chemical characteristics by this change in donor number and character have not been well studied with the array of radionuclides that have been used with this agent. Considerable benefit on metal ion complex stability is no doubt conferred by the macrocyclic effect, yet actual stability constants remain to be reported for those metallic radionuclides with which this chemistry have been employed.

Limitations to the use of the DOTA derivatives is directly related to their exquisite stability; slower complex formation rates compromise radiolabeling yields, efficiency, and specific activity. The multi-step mechanism of complex formation severely limits the actual use of bifunctional DOTA agents and in fact may have contributed to some questionable results using this agent.[31] The slow formation rates can be in part traversed if the conjugate product is tolerant of being heated transiently.[2] Conversely, use of bifunctional DTPA ligands is not hampered by complex formation rates. Thus, one must very carefully choose which class of ligands is most appropriate for each specific application.

Bifunctional DOTA has also been used for ^{225}Ac, however, the reported radiolabeled conditions to force complexation are not acceptable for protein conjugates, hence formation of the complex has been performed first followed by conjugation via isothiocyanate chemistry with concomitant low efficiencies in both complexation and conjugation.[32]

Other macrocyclic ligands have been reported. There include a number of bifunctional NOTA agents (Figure 4).[33-36] NOTA is well established to form an exceedingly stable complex with Ga(III), and as such, one might think that PET agents would feature its use. Surprisingly, DOTA seems to be preferred despite there being no real substantiation as to the stability of the DOTA Ga(III) complex. One might speculate that use of DOTA in this specific instance may be directly linked to the commercial availability of the DOTA active ester derivative (Figure 3). One variant on DOTA that has seen significant use with ^{203}Pb and ^{212}Pb is the tetra-primary amide of DOTA termed TCMC (Figure 4).[37,38]

Bifunctional macrocyclic chelating agents with larger than the 12-membered ring DOTA, 14-membered, 15-membered, and 18-membered ring agents have also been developed (Figure 5). At least two different geometrically substituted 14-membered ring bifunctional TETA agents have been reported.[26,39] TETA had been promoted as being stable for copper radionuclides and that topic will be discussed in other papers in this issue. Oddly, TETA appears to have had no use with any other radionuclides. One 15-membered ring bifunctional PEPA has been investigated for complexing ^{213}Bi stably *in vivo* without success.[40] Lastly, one 18-membered ring bifunctional HEHA has been investigated for complexing ^{225}Ac and superior stability versus DOTA reported, yet still not adequate for in vivo use with this element.[41,42]

An area in the development of bifunctional chelating agents that has seen a surprising small level of investigation has been impact on radio-metal complex stability due to the stereochemical constraints of the chelating agent. Clearly, stereochemistry plays a serious role in the three dimensional geometry and arrangement of donor elements directed towards the metal ion and that optimization of these variables should equally lead to optimized chelation chemistry. The study that led to the development of the CHX-A" DTPA in fact investigated whether differences might even exist between radiolabeled enantiomeric forms of chelating agents post-conjugation.[21] Both serum stability and transchelation studies indicated that radiolabeled enantiomers behaved identically to their corresponding racemates, however *in vivo* studies that examined bone deposition as an indicator of ^{88}Y loss clearly demonstrated significant differences between enantiomeric conjugates. One might be tempted to attribute this result to stereochemical resolution due to complex formation except that the *in vitro* studies failed to, or were inadequate to detect this condition. This result does reflect two very critical aspects of bifunctional chelate development: stereochemistry can be important and should be studied, and that the importance of *in vivo* studies can not be discounted.

An array of reactive functional groups for conjugation of bifunctional chelating agents has also been reported in the literature of which a selection is depicted in Figure 6. Beyond just the simple amine or carboxylate for use in conjugation chemistry protocols, haloacetamide or maleimide have been reported for reaction with sulfhydryl moieties that are either extant of introduced,[43,44] isothiocyanates have been readily available for reaction with amine groups,[11,12] azides have been familiar as photoaffinity reagents,[11] a wide array of active ester chemistry for reacting with amines have been perhaps only partly explored,[30,45,46] and even an alkene derivative for use in Suzuki coupling chemistry has been reported.[18] The breadth of choices of conjugation reactive functional groups has explored all of these listed possibilites and more, however, the need for refinement of these choices and their actual usage remains an area where opportunities remain. Currently, active esters and isothiocyanate chemistry dominate the use of bifunctional chelating agents, primarily for peptides and proteins, respectively, perhaps more from convenience than actually having arrived at the terminus of

development. Clearly, protein conjugation remains inefficient regardless of conjugation chemistry employed, resulting in random product distributions, and with radiolabeling yields that achieve far lower than theoretical specific activities, critical to both imaging and therapeutic applications. All of these areas continue to call for improvement and refinement of more than the bifunctional chelate itself, but rather how they are actually employed.

On a related note, if one considers the numbers of both bifunctional DTPA and DOTA that have been reported in the literature, one must really begin to question the need for further permutations of the fundamental structure of either ligand. How many more structural variants of these as well as many of the others are really advancing the field of use of bifunctional chelating agents? There seems little chance that any further advances in stability with DTPA or DOTA will be forthcoming. This is particularly relevant to DOTA since no measurable advances in either stability or formation rate enhancement has been achieved. Needs remain to select new permutations in conjugation chemistry as noted above, however, the fundamental base bifunctional ligands for this purpose do seem to be well in hand for nearly every metallic radionuclide that may be required to ask and answer the vast majority of research and clinical questions relevant to their use. Exceptions to this are obvious and tend towards the more "exotic" metallic radionuclides such as ^{225}ac or ^{223}Ra. Clearly, unequivocally stable bifunctional chelating agents for these radionuclides remain to be developed that would permit their precise therapeutic benefits to be determined.

Lastly, the continued pursuit of exceptionally stable complexes has to be put into the context of actual use, i.e., just what is "good enough". One must balance the variable of radionuclide half-life with biological half-life versus actual biological application to assess just what is acceptable stability further balanced against potential toxicity. All of those parameters must yet again be weighed against actual feasibility of use of the chemistry. Clearly, we already have a great many of the requisite tools of bifunctional chelates with which to move forward to accomplished those refinements needed to develop both better and more effective imaging and therapeutic agents using metallic radionuclides.

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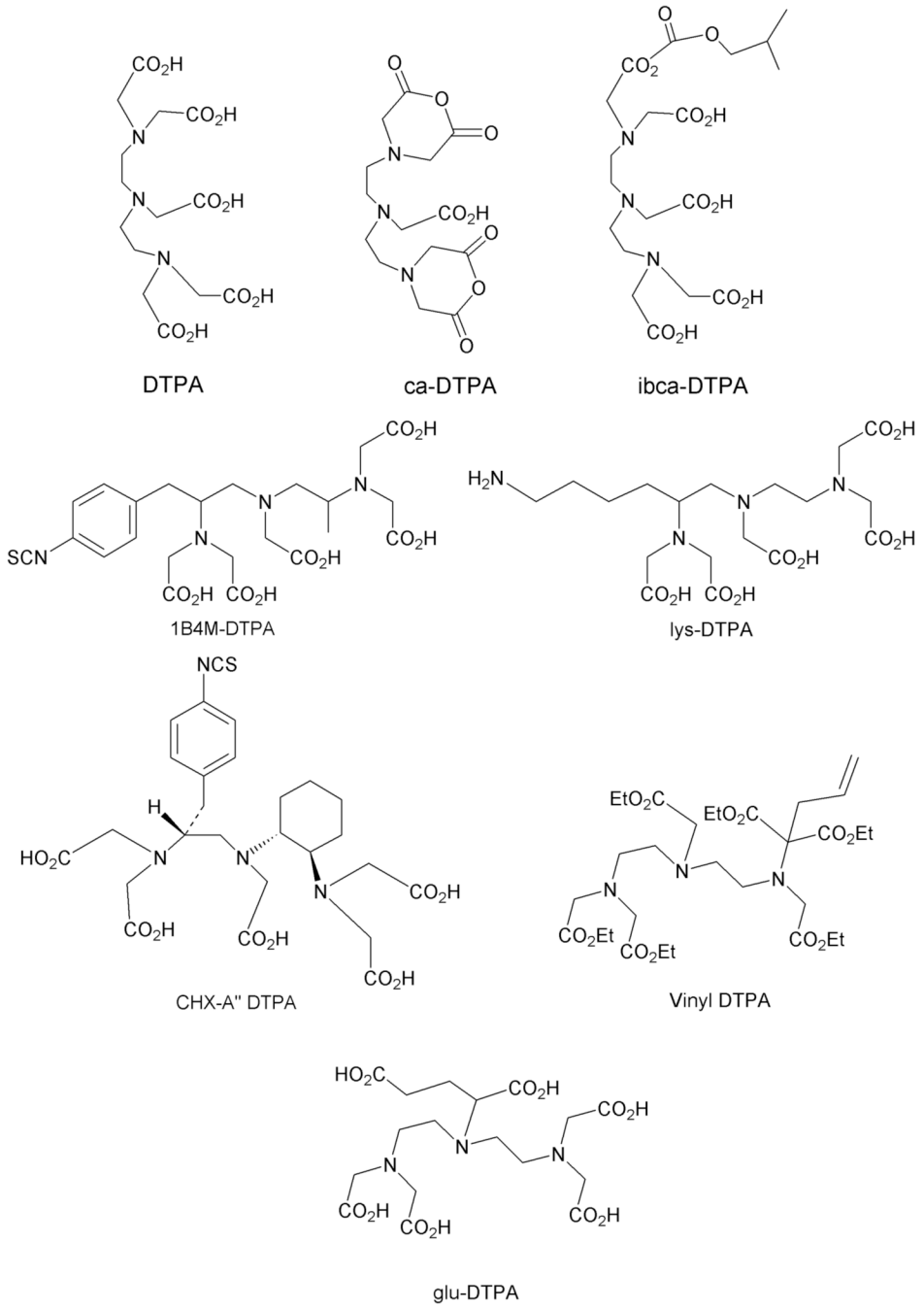


Figure 1.
Structures of DTPA, ca-DTPA, ibca-DTPA, 1B4M-DTPA, lys-DTPA, vinyl DTPA, glu-DTPA, and CHX-A” DTPA

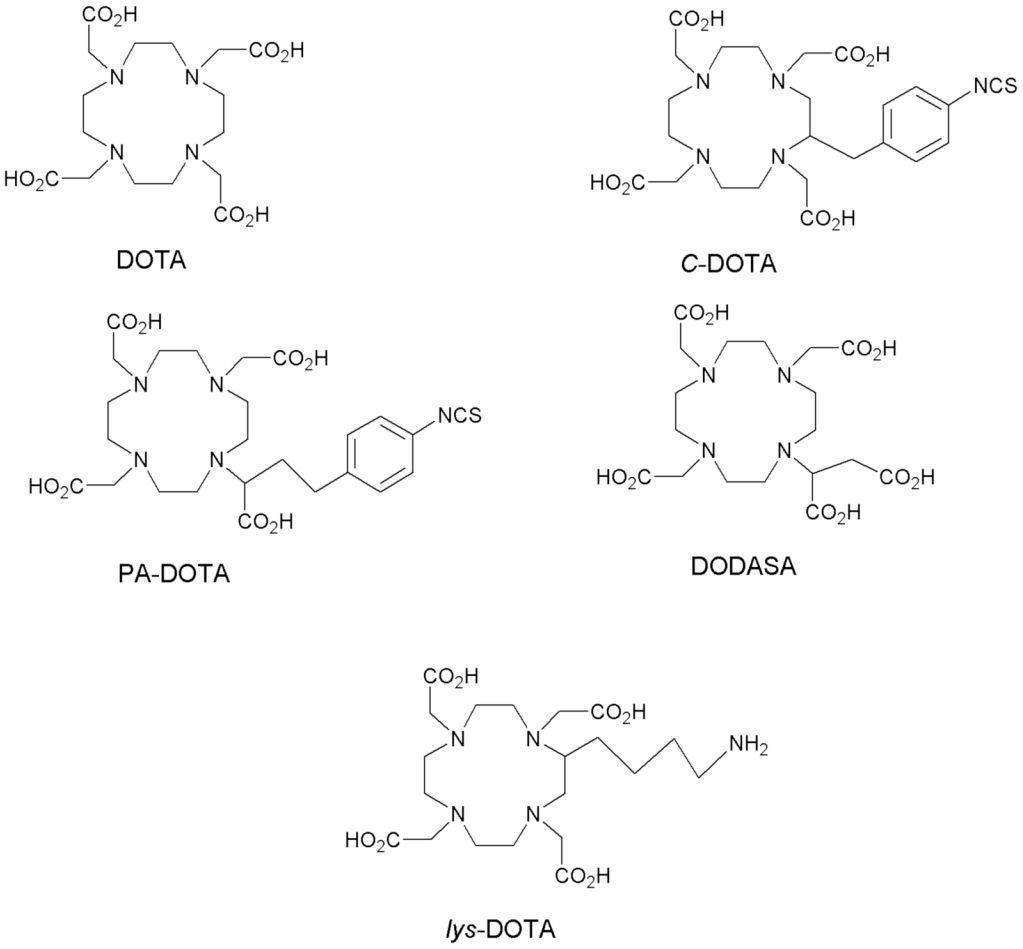


Figure 2.
Structures of DOTA, *C*-DOTA, PA-DOTA, DODASA, and *lys*-DOTA

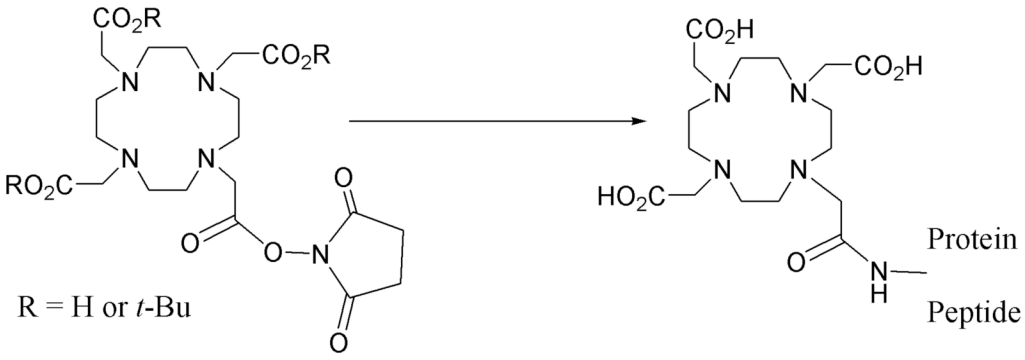


Figure 3.
Structures of DOTA hydroxysuccinimide active ester and its peptide or protein conjugation product.

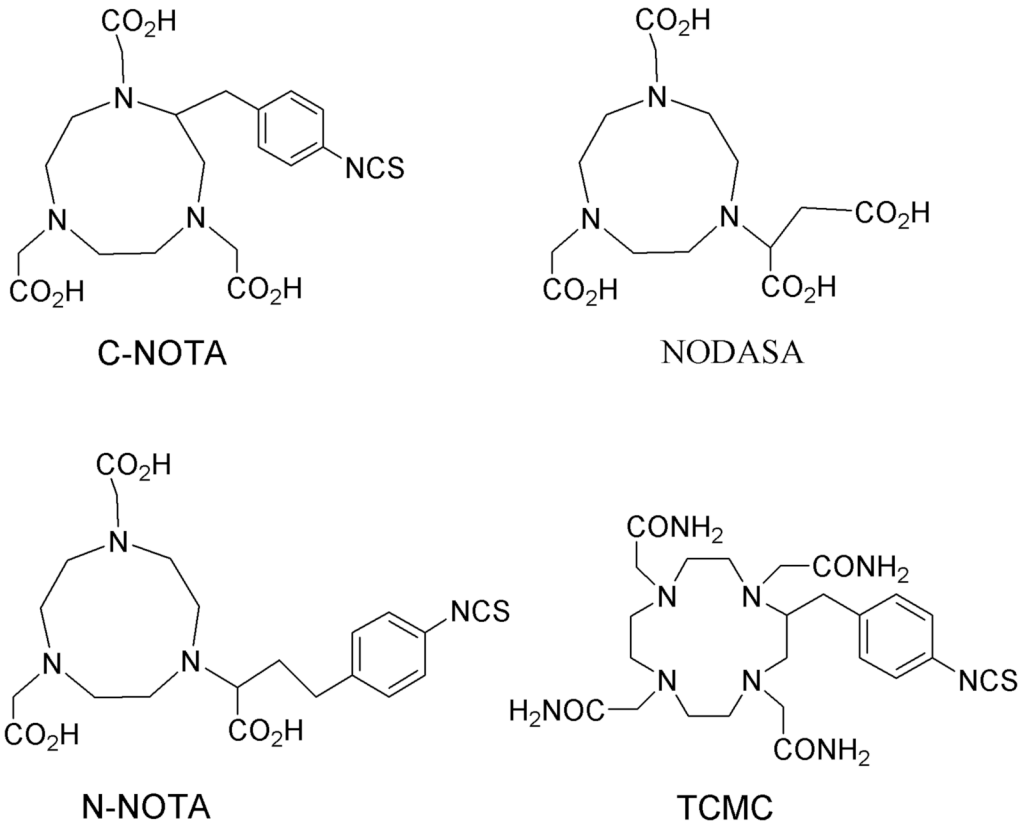


Figure 4.
Structures of C-NOTA, N-NOTA, NODASA DTPA, and TCMC

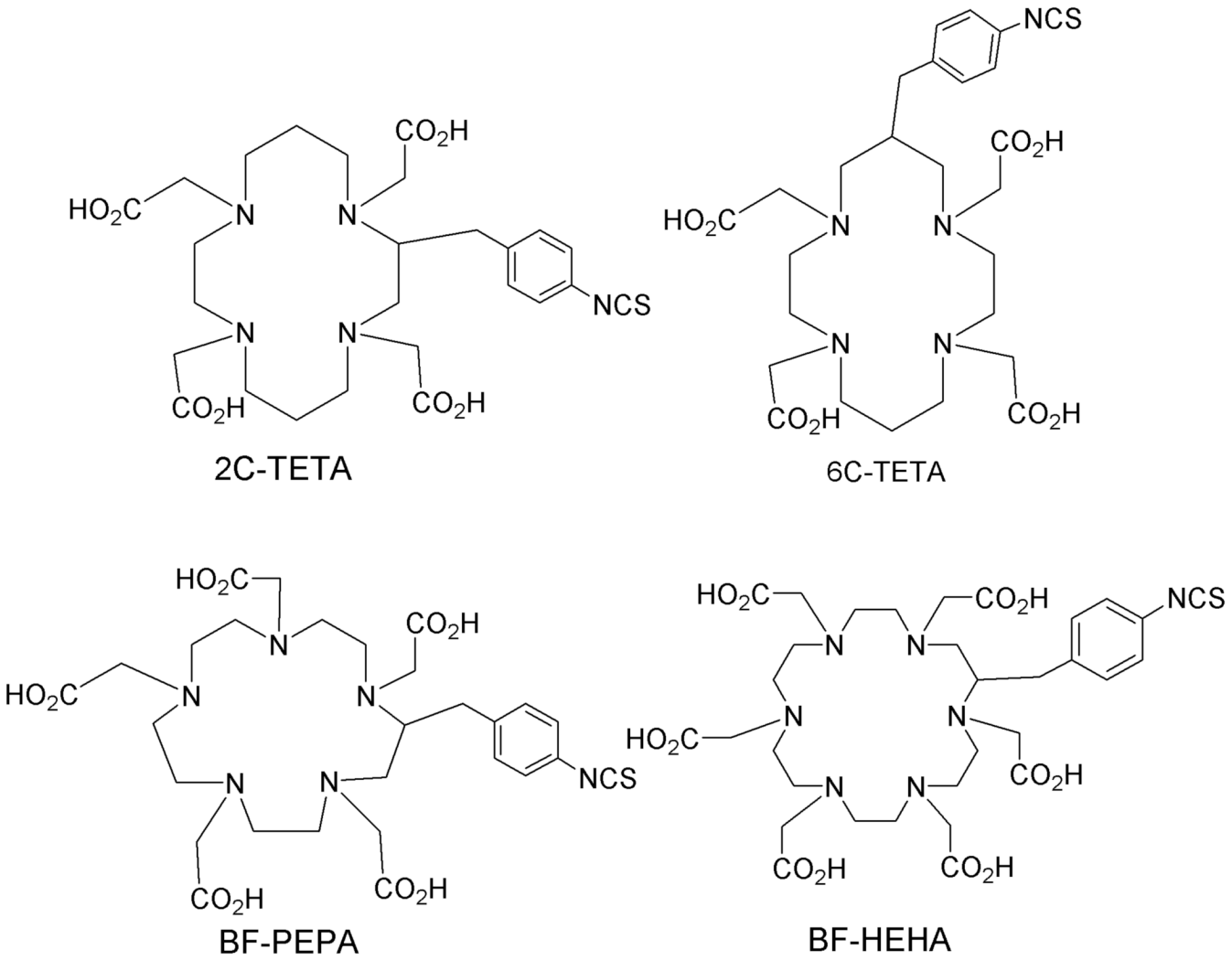


Figure 5.
Structures of 2C-TETA, 6C-TETA, BF-PEPA and BF-HEHA

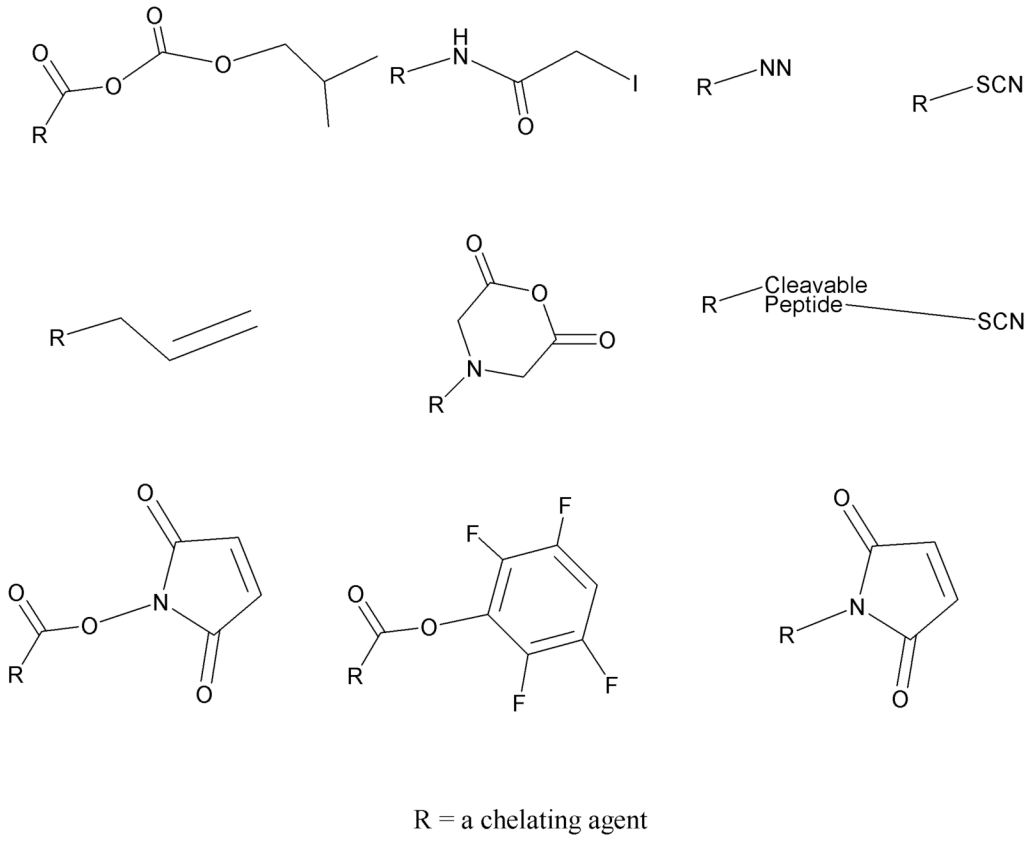


Figure 6.
Structure of some of the reactive functional groups that have been used for conjugation of bifunctional chelating agents to peptides and / or proteins

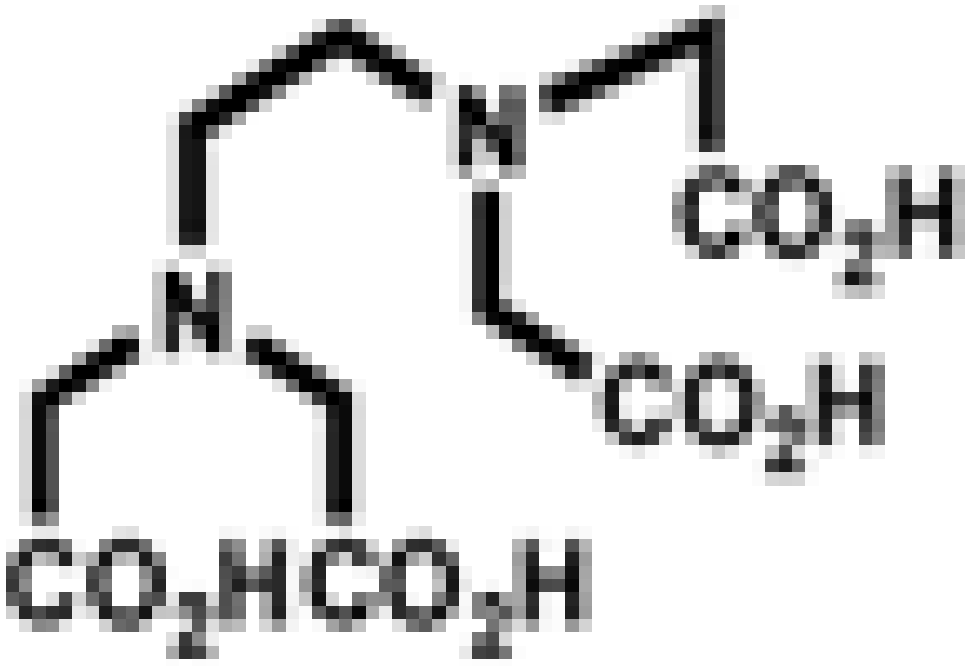
Table 1

Selected Properties of “Non-Standard” Radionuclides

Radionuclide	Ionic Radius*	Charge	$T_{½}$
$^{66}Ga/^{68}Ga$	62.0	+3	9.5 h/68 min
$^{86}Y/^{90}Y$	90.0	+3	14.7 h/2.67 d
^{111}In	80.0	+3	2.8 d
^{212}Pb	119.0	+2	10.64 h
$^{212}Bi/^{213}Bi$	103.0	+3	1.01 h/ 45.6 min
^{89}Zr	72.0	+4	3.27 d
^{177}Lu	86.1	+3	6.71 d
^{225}Ac	112.0	+3	10 d

Table 2

Selected Stability Constants for Acyclic Polyaminocarboxylate Chelates[6a]



	EDTA
Y(III)	18.09
In(III)	24.9
Bi(III)	27.8

Table 3

Plasma Level and Cumulative Urinary Excretion of Yttrium Chelates[7]

Ligand	4 hr		8 hr		24 hr	
	Plasma	Urine	Plasma	Urine	Plasma	Urine
EDTA	5.3	36.5	0.9	43.7	0.5	46.7
CDTA	5.7	68.4	2.3	87.1	0	97.6
DTPA	5.0	75.0	1.8	93.0	0	101.3