Bifunctional Chelators in the Design and Application of Radiopharmaceuticals for Oncological Diseases

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Abstract: Radiopharmaceuticals constitute diagnostic and therapeutic tools for both clinical and preclinical applications. They are a blend of a tracer moiety that mediates a site specific accumulation and an effector: a radioisotope whose decay enables either molecular imaging or exhibits cytotoxic effects. Radioactive halogens and lanthanides are the most commonly used isotopes for radiopharmaceuticals. Due to their ready availability and the facile labeling metallic radionuclides offer ideal characteristics for applications in nuclear medicine. A stable link between the radionuclide and the carrier molecule is the primary prerequisite for *in vivo* applications. The radionuclide is selected according to its physical and chemical properties i.e. half-life, the type of decay, the energy emitted and its availability. Bifunctional chelating agents are used to stably link the radiometal to the carrier moiety of the radiopharmaceutical. The design of the bifunctional chelator has to consider the impact of the radiometal chelate on the biological properties of the target-specific pharmaceutical. Here, with an emphasis on oncology, we review applications of radiopharmaceuticals that contain bifunctional chelators, while highlighting successes and identifying the key challenges that need to be addressed for the successful translation of target binding molecules into tracers for molecular imaging and endoradiotherapy.

Keywords: Bifunctional chelating agent (BFCA), DOTA, nuclear medicine, radiochemistry, radiometals, radiopharmaceuticals, targeting, theranostics.

INTRODUCTION

Radiopharmaceuticals are diagnostic and therapeutic tools for the early identification and treatment of pathological changes. The radioisotopes comprised in radiopharmaceuticals emit either gamma rays for diagnostic use or alpha or beta particles for therapeutic use [1]. The radiopharmaceuticals used in molecular imaging improve drug development since molecular and functional imaging enhance our understanding of disease and drug activity during preclinical and clinical trials [2, 3].

The term "molecular imaging" is broadly used in combination with imaging modalities that provide primarily morphological/anatomical as well as molecular information [4]. The definition of "molecular imaging" can be formulated as "the *in vivo* application of imaging probes for the non-invasive visualization, characterization, and quantification of physiological processes at the molecular level" [5, 6]. Molecular imaging provides the potential for earlier detection, characterization and "real time" monitoring of disease, evaluation of treatment as well as investigating the efficacy of drugs [7]. Nowadays, the most sensitive molecular imaging modalities are the radionuclide-based positron emission tomography (PET) and single photon emission computed tomography (SPECT) imaging techniques. They provide the sensitivity required to visualize and determine most interactions between physiological targets and specific biomolecules at the picomolar scale [8].

Imaging modalities can generally be divided into anatomical and molecular imaging techniques. In contrast to computed tomography (CT), magnetic resonance imaging (MRI) and ultrasound, the technologies used in anatomical imaging, which are characterized by a high spatial resolution, the molecular imaging modalities (optical imaging, PET and SPECT) offer the potential to identify changes due to diseases at molecular and cellular levels before tissue structural changes (e.g. metastasis) become visible. They rely on the injection of radiotracers at nanomolar blood concentrations.

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At higher doses radiopharmaceuticals exert cytotoxic effects. The strong cytotoxic effects obtained with beta- and alpha-emitting isotopes are used to treat tumors. This targeted radionuclide therapy is termed endoradiotherapy. It is an approach for the systemic treatment and represents an alternative to the chemotherapeutic treatment of malignant tumors.

The selection of the radionuclide depends on its physical and chemical parameters. In general, the element half-life, the type of decay, the type of emission(s) with the related energy, and of course the costs and availability should be considered before selecting a specific radionuclide [9]. A selection of radiometal nuclides suited for the incorporation into biomolecules is listed in Table **1**. In this review, we describe commonly used strategies for the radiolabeling of probes for imaging and therapy. Furthermore, an overview of the most useful radiometal-chelator systems for radiopharmaceutical application for molecular imaging is given with emphasis on oncological diagnosis and therapy.

THE COMPOSITION OF RADIOPHARMACEUTICALS

The majority of radiopharmaceuticals currently available for applications in nuclear medicine are based on radiometals. In general, a radiometal-based radiopharmaceutical can be divided into four parts: a targeting biomolecule, a pharmacokinetic modifying linker, the bifunctional chelating agent (BFCA), and the radiometal (e.g., ^{67}Cu, ^{90}Y, ^{99m}Tc, ^{111}In, ^{177}Lu, ^{186}Re, ^{188}Re, and ^{213}Bi) [10-12]. The targeting biomolecule serves as a "carrier" for the specific delivery of the radiometal isotope. The BFCA is a prerequisite for the radiolabeling of biomolecules with metallic radionuclides. In addition to the physical and chemical properties, the appropriate selection of the radiometal depends on factors such as the biodistribution of the radiometal used, the targeted tissue, and the clearance rate of the radiometal complex from both target and non-target tissues [13].

The use of radioactive metals has led to the development of several BFCAs that serve as a cross-linker between the carrier and the radiometal. Besides the coordinating sites that chelate the proper metallic radionuclide, the BFCA bears a functional group enabling the attachment to the carrier [12].

Different types of BFCAs with different donor atoms and chelator framework are required since the radiometals used in

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nuclear medicine vary significantly in their coordination chemistry depending on their nature and oxidation state. The electronegativity and oxidation state play a key role in the establishment of metal-ligand complexes. The complexes have to be formed in high yields (>99.5%) with high specific activities. The high stability and the preservation of the receptor-binding affinity of the labeled carrier are the crucial requirements for the *in vivo* application of radiometal-labeled biomolecules [14]. The labeling of radiopharmaceuticals with a radiometal can be difficult if the metal ion forms insoluble hydroxides at physiological pH. It is therefore necessary that the BFCAs complexes and stabilizes the radiometal. A high thermodynamic stability and kinetic inertness, under physiological conditions, are required to prevent the release and dissociation of the radiometal from its complex and thus avoid the accumulation in non-targeted organs. The radionuclides provide either the visualization source for molecular imaging or the source of the cytotoxic dose in radiotherapy.

The linker region can be varied in order to modify pharmacokinetic properties of the substance. This can, for example, lead to an improvement of the target to background/blood (T/B) ratios to reduce the accumulation in non-targeted organs, while maintaining the uptake rate at the site of interest. Several types of linkers were explored (cationic, anionic, neutral or metabolically cleavable) and reviewed comprehensively by Liu *et al*. [12, 15-17]. A selection of the most commonly used radioisotopes is shown in Table **1**.

Radiopharmaceuticals can be classified upon their application as diagnostic and therapeutic agents. Positron- and γ -emitting radioisotopes are utilized for diagnostic applications (PET or SPECT), while β-emitters are useful for therapeutic applications.

DIAGNOSTIC RADIOPHARMACEUTICALS

Generally, diagnostic radiopharmaceuticals are applied intravenously (i.v.). They can be detected in very low concentrations (low picomolar range) [8]. They can be labeled with a metallic (i.e. ^{99m}Tc, ^{68}Ga, and ^{111}In) or non-metallic (i.e. ^{18}F and ^{123}I) radionuclide. They rely on a gamma-emitter for SPECT or a positron-emitter for PET [5, 20-23]. Their ultimate goal is to enable an anatomical description of organs as well as to evaluate their physiological function. Additionally, they can be used to determine the efficiency of a therapeutic treatment. Besides ^{99m}Tc, which remains the most widely used SPECT isotope, ^{111}In is useful for gamma and SPECT imaging, and is often used as the imaging surrogate for ^{90}Y since ^{90}Y is a pure β-emitter. For PET imaging it is advantageous if the radionuclide does not emit radiation other than the 511-keV gamma photons from positron annihilation. This minimizes the impairment of the spatial resolution due to the high β^+ energy and reduces the radiation burden for the patient. The worldwide number of PET-based studies was assumed to reach 3.2 million in 2010 [22].

THERAPEUTIC RADIOPHARMACEUTICALS

Today disseminated disease is only accessible by chemotherapy. The chemotherapeutic agents used in systemic cancer therapy exert their effect mainly on proliferating cells. As a consequence normal tissue which is physiologically proliferating, such as bone marrow, intestinal or dermal epithelia is affected. Consequently, the systemic side effects of chemotherapeutic agents are one of the major problems of cancer chemotherapy [24].

This has led to the requirement of new therapeutic strategies. The aim of drug-targeting is the reduction of extra-target effects in order to reduce the therapeutic index. Selective delivery or the selective activation within the targeted tissue minimizes toxic side effects. The improvement of the pharmacokinetic profile should be accomplished by the drug targeting strategies. However, diverse drug-targeting concepts have been followed in order to fulfill those principles [24]. The majority of the active drug-targeting applications rely on receptor-based drug-targeting principles, where a previously modified delivery system with receptor-specific-ligands conveys the drug to its target (Fig. **1**).

External beam irradiation, implantable “seeds” or systemic administration are the three routes of site specific administration to convey therapeutic doses of ionizing radiation to specific disease sites [25]. Brachytherapy comprises the use of seeds, which are only beneficial for the treatment of accessible tumors [26, 27]. In contrast, the systemic administration of radiopharmaceuticals designed for tumor-specific localization makes it possible to treat disseminated tumors [9, 14, 28-33]. Radiolabeled molecules

Table 1. Commonly Used Radiometals and their Physical Characteristics

Element	Half-Life	Mode of Decay	E_β (KeV)	E_γ (KeV)	Availability/Production
^{99m}Tc	6.02 h	IT (100%)		141	^{99}Mo/^{99m}Tc Generator
^{186}Re	3.7 d	β^- (92%) EC (8%)	1071, 934	137	Reactor
^{188}Re	16.98 h	β^- (100%)	2116, 1965	155	^{188}W/^{188}Re Generator
^{64}Cu	12.7 h	β^+ (19%) EC (41%) β^- (40%)	656		Cyclotron
^{67}Cu	2.58 d	β^- (100%)	557, 484, 395	91, 93, 185	Accelerator
^{177}Lu	6.71 d	β^- (100%)			Reactor
^{86}Y	14.7 h	β^+ (33%) EC (66%)	2335, 2019, 1603, 1248, 1043		Cyclotron
^{90}Y	2.67 d	β^- (72%)	2288		^{90}Sr/^{90}Y Generator
^{66}Ga	9.5 h	β^+ (56%) EC (44%)	4150, 935		Cyclotron
^{67}Ga	3.26 d	EC (100%)		91, 93, 185, 296, 388	Cyclotron
^{68}Ga	68 min	β^+ (90%) EC (10%)	1880, 770		^{68}Ge/^{68}Ga Generator
^{111}In	2.8 d	EC (100%)		245, 172	Cyclotron
^{213}Bi	45.6 min	β^- (98%) α (2%)	8.38 MeV (from ^{213}Po)	440	^{225}Ac/^{213}Bi Generator

Data collected from Reichert *et al.* and Anderson *et al.* and the references cited therein [18, 19].

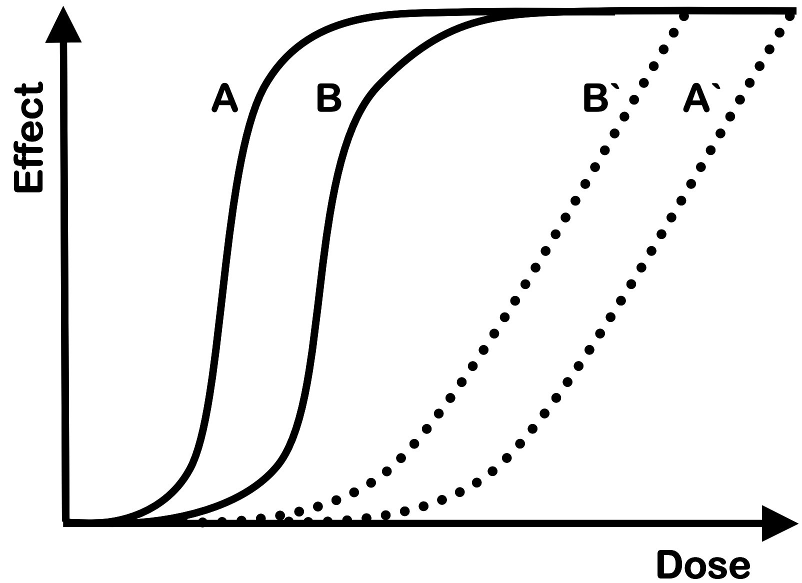


Fig. (1). Idealized scheme showing the dose effect relationship of targeted drugs and conventional drugs. Curve A illustrates the dose dependency of effects observed with drug-carrier conjugates: the therapeutic effect is achieved at lower doses than with conventional drugs (curve B). As a consequence, the corresponding side effects are minimized in the case of the targeted therapy (dotted line A') in comparison to the conventional therapy (dotted line B').

designed to deliver therapeutic doses of ionizing radiation are referred to as "therapeutic radiopharmaceuticals". Endoradiotherapy offers an additional advantage over the normal targeted therapy, since the ionizing radiation has the ability to penetrate all physiological barriers, cellular internalization is not required. High tumor accumulation ratios and a fast blood clearance, which minimizes radiation damage of non-targeted tissues, are the characteristics of an ideal therapeutic radiopharmaceutical. Therefore, complexes formed from radiometals and BFCAs should have a high thermodynamic stability and kinetic inertness (Fig. **2**).

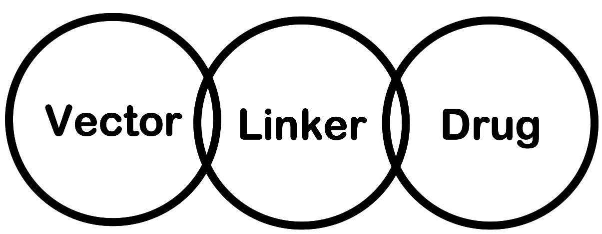


Fig. (2). The basic structure of targeted drugs. Whereas targeted prodrugs have to be designed to release the drug at the site of action, targeted radiopharmaceuticals require a stable linker that guarantees the retention at the site of action. This can be achieved by using bifunctional chelators.

The choice of the β-emitter depends on the size and the location of the tumor. While high-energy β-emitters such as ^{90}Y are used for the treatment of large tumors (long penetration range (~12 mm)), the medium or low-energy β-emitters such as ^{177}Lu are preferred for small tumors or metastases. Radiotherapy has been started five decades ago with the treatment of thyroid malignancy by radioiodine. The main prerequisite for radiotherapy is the specific localization of the therapeutic doses in the targeted tissues. The development of [^{111}In]DTPA-octreotide (OctreoScan) for the diagnosis of somatostatin receptor positive tumors has provoked the succession of new receptor-based target-specific therapeutic radiopharmaceuticals and motivated the introduction of new therapeutic radionuclides, as well as new bifunctional chelators (BFCAs) [14, 34-43].

THE CARRIER SYSTEMS

Peptide Radiopharmaceuticals

Due to their favorable pharmacokinetic profiles, small synthetic receptor-binding peptide-based radiopharmaceuticals have been established for diagnostic and therapeutic applications in oncology [28, 39]. Usually, the application of peptide carriers requires an over-expressed receptor and sufficient *in vivo* stability. Additionally, the conjugated chelator should not interfere with the binding affinity of the peptide.

The possibility to obtain these compounds by solid phase synthesis approaches, invented by the Nobel laureate Bruce Merrifield [44], has opened a new era in nuclear medicine [28, 29, 39, 45-48]. With automated solid phase peptide synthesis (SPPS), peptides can be produced in a straightforward manner for a variety of diagnostic and therapeutic applications [49, 50]. Due to their low molecular weight, fast clearance, rapid tissue or tumor accumulation and their low antigenicity, peptides are preferred over proteins, such as antibodies, for molecular imaging and tumor-targeting. Various receptors are over-expressed in particular tumor types and many of them have been targeted either by modifying natural peptides or by panning phage libraries [51, 52]. Endogenous peptides are usually associated with a short half-life and have their proper physiological effects, which lead to undesired adverse reactions. Several peptide modifications, like the conscious incorporation of *D*-amino acids, capping, amidation or cyclization, have been introduced to enhance the stability and to improve the receptor binding characteristics of peptides [53, 54]. Attachment of the BFCAs may alter the pharmacokinetics of the peptide carrier and should therefore be performed in a rational manner to avoid any interference with the targeting sequence [55, 56]. To avoid receptor saturation, which would result in an unfavorable pharmacokinetics, receptor-based radiopharmaceuticals are used in very low concentrations. The somatostatin receptor binding octreotide and its analogs are the most commonly applied peptide radiopharmaceuticals [57]. After labeling with ^{111}In, ^{90}Y, ^{64}Cu or ^{177}Lu, they have shown excellent results for the treatment of patients with neuroendocrine tumors. Examples of approved peptide radiopharmaceuticals are ^{111}In-DTPA-OctreoScan® (Fig. **3**) and the

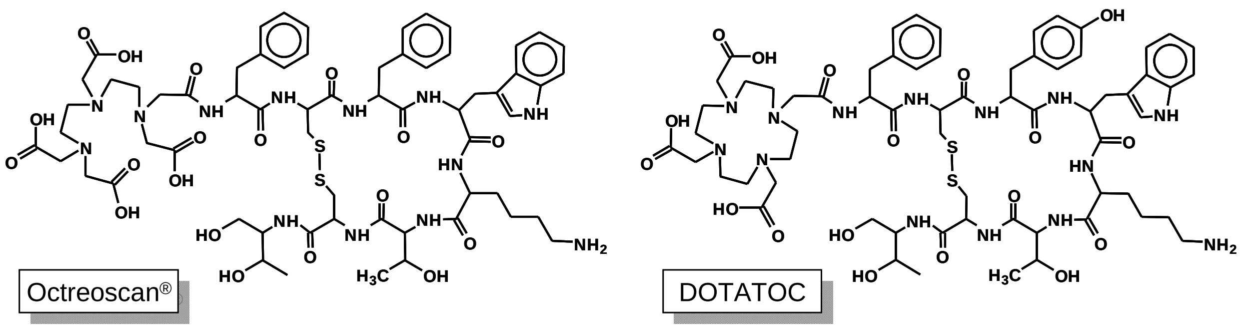


Fig. (3). The chemical structure of DTPA-OctreoScan as compared to DOTATOC. This kind of presentation illustrates the similarity of the acyclic chelator DTPA with the cyclic Chelator DOTA. The small change of the peptide (^{3}Tyr versus ^{3}Phe) and the physicochemical differences of the two different chelators cause significant changes of the pharmacokinetics of the two pharmaceuticals.

^{99m}Tc-labeled NeoTect, an analog which is used for the detection of lung cancer.

Due to its excellent performance the peptide derivative DOTATOC has become the gold standard of peptide radiopharmaceuticals. It has found wide application for large number of carcinoid tumors expressing somatostatin receptor subtypes SSTR2 and SSTR5. The binding to these receptors induces receptor mediated endocytosis of the tracer. DOTATOC labeled with ^{68}Ga allows the diagnosis by PET (as shown in Fig. **4**), ^{90}Y labeled DOTATOC has been proven to be effective for endoradiotherapy (Fig. **5**).

Monoclonal Antibodies

In 1993 the US Food and Drug Administration (FDA) approved the first murine monoclonal antibody (MAb) for the diagnosis of recurrent colorectal and ovarian cancer [39]. While the preclinical results were promising for MAbs, clinical studies often only showed limited accumulation in the tumor, accompanied by slow blood clearance, due to the high molecular weight, resulting in only modest tumor to blood/background (T/B) ratios. The poor penetrating properties due to the large molecular weight result in low tumor uptake and set hurdles to the use of short physical half-life radioisotopes such as ^{99m}Tc. Furthermore, the first generation of

Fig. (4). Positron emission tomography imaging of a 46 year old patient with a massive infiltration of the liver by metastases of neuroendocrine tumor (A). Status after intraaterial treatment with the beta emitting radiotherapeutics ^{90}Y-DOTATOC (B). ^{68}Ga-DOTATOC depicts the eradication of the liver metastases and the occurrence of multiple bone metastases.

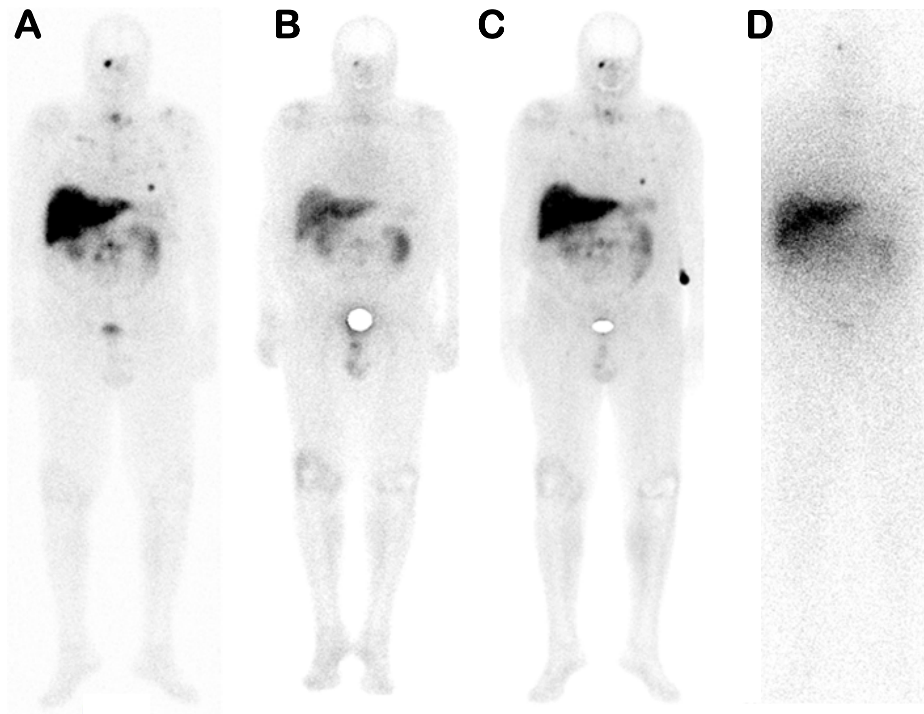


Fig. (5). Planar images of different revealing the influence of the coordination environment imposed by "chelators" together with the changes of image quality of different SSTR binding octreotide derived radiopharmaceuticals. (**A**) ^{177}Lu-DOTATOC (**B**) ^{111}In-octreoscan (**C**) ^{99m}Tc-HYNIC-[*D*-Phe^1,Tyr^3-octreotide] and (**D**) ^{90}Y-DOTATOC (recording of Bremsstrahlung).

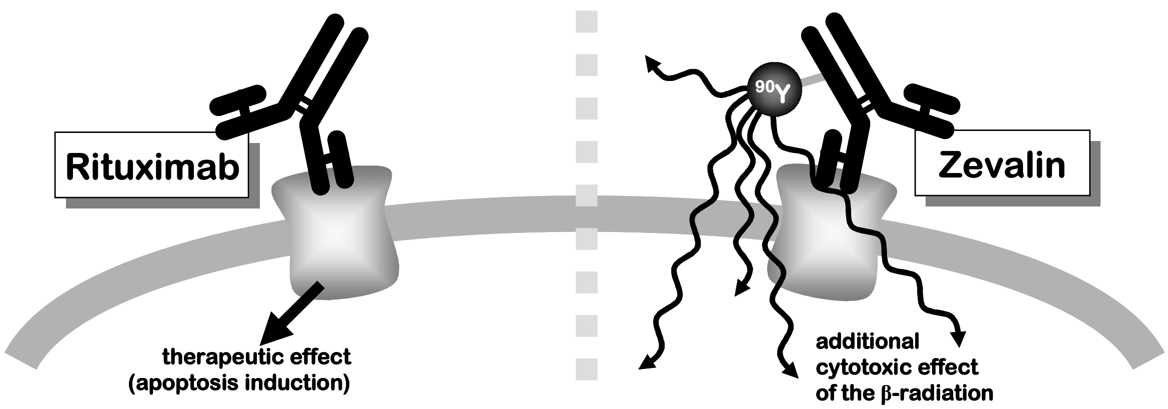


Fig. (6). Schematic representation of the effects of the anti-CD20 monoclonal antibody Rituximab and its ^{90}Y-labeled derivative Zevalin. The FDA-approved radiopharmaceutical Zevalin has an additional therapeutic effect caused by the ionizing radiation. This is exemplified by the high efficiency of Zevalin in the treatment of non-Hodgkin lymphomas [58].

MAbs bore the risk of immunogenic reactions due to murine origin of the protein. The approval of the ^{90}Y-labeled anti-CD20 monoclonal antibody (Zevalin®, Bayer Schering Pharma AG, Germany) was a considerable advance for radioimmunotherapy in oncology (Fig. **6**) [9, 32].

Other Carrier Systems

A wide variety of carrier systems have been tested for the systemic administration of diagnostic or therapeutic radiopharmaceuticals. The spectrum of different compounds covers small organic or inorganic molecules [59], peptides [36], peptidomimetics [60, 61], proteins [37], oligonucleotides [48, 62], or macromolecules, such as monoclonal antibodies [32], antibodies [31], microparticles [63], liposomes [64], nanoparticles [65], polymeric micelles [63], dendrimers [66], or hydrogels labeled with a radionuclide [65, 67].

Radiometals and the Bifunctional Chelating Agent (BFCA)

The stability, i.e. the release of the radiometal from a radiopharmaceutical in human serum is predominantly determined by the kinetic inertness of the chelator used. Generally, macrocyclic chelators such as DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) form more kinetically inert complexes than their acyclic analogs such as DTPA (diethylenetriamine-*N,N,N',N'',N''*-pentaacetic acid) (Fig. **7**). In contrast, acyclic chelators exhibit faster metal-binding kinetics compared with macrocyclic BFCAs. This is of great importance for radiometals with short half-lives. The chelators form coordinative bonds with central metal ions.

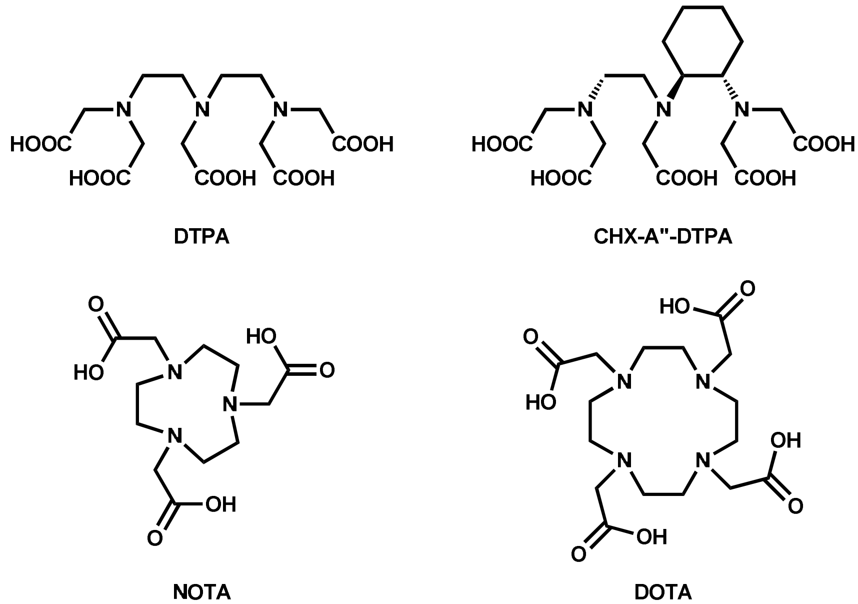


Fig. (7). The chemical structures of the typically used acyclic chelators DTPA and CHX-A''-DTPA, an isomer of DTPA with a cyclohexyl backbone substitution, as well as the macrocyclic chelators NOTA (1,4,7-triazacyclononane-1,4-7-triacetic acid) and DOTA.

Depending on the size, the charge, and the electron configuration of the radiometals, the coordination number differs from 2-8.

The design of radiometal-based pharmaceuticals relies on the organ or tissue to be targeted. The overall charge of the metal complex, as well as the nature of the radiometal influence the properties of the tracer. Usually, lipophilic complexes exhibit affinity to the liver, cationic complexes tend to accumulate in the heart, anionic complexes tend to clear through the kidneys, and non-charged complexes are required in order to achieve blood–brain barrier penetration.

The treatment of a broad variety of diseases such as heart diseases, brain disorders, infections, kidney and liver abnormalities as well as cancer have benefited from the progression made in the field of nuclear medicine [19]. Developments of radiopharmaceuticals benefit from the nuclear technology offered by nuclear reactors, accelerators, cyclotrons and generators. SPECT requires radiopharmaceuticals enclosing radionuclides with gamma radiation while PET necessitates radiopharmaceuticals labeled with positron-emitting radionuclides (Table **1**).

Metal-Based Radiopharmaceuticals

The labeling with a radiometal can be difficult since metal ions can form insoluble hydroxides at physiological pH. This leads to the necessity of BFCAs, as they can complex and stabilize the radiometal. Therefore, the conjugation to a BFCA which exhibits higher affinity to the radiometal than to the hydroxyl ions (OH^-) is a prerequisite. In order to achieve high specific activities, the BFCA conjugate has to be used in very low concentrations. The formation of insoluble hydroxides occurs faster than the complexation with the ligands offered by the BFCA. Therefore, the complexation is performed in the presence of weak coordinating ligands such as acetate or citrate, since they form weak coordination complexes with the labeling radiometal.

Despite the believe that the BFCAs are 'innocent' molecules, the nature of a BFCA (i.e. geometry, lipophilicity, overall charge) plays a key role in the pharmacokinetics of targeted radiopharmaceuticals [40]. This influence is decreased in larger biomolecules. Isomerism is an additional factor to be avoided or minimized since it can exert an impact on the physical and chemical properties of the radiopharmaceuticals, which in turn alters the pharmacokinetic properties [12]. Hydrophilic BFCAs have an additional advantage since they facilitate the blood clearance by renal tracer excretion.

The standard procedure for the measurement of the stability of radiometal-labeled biomolecules is the determination of its stability in human serum. The monitoring techniques are based on radio-TLC, radio-HPLC, and LC-MS [68-70].

Acyclic and macrocyclic chelators have been developed (Fig. **7**). The ultimate goal of the development of chelators is to obtain kinetically inert complexes and to reduce the rate of dissociation of the radiometal *in vivo*. For example, it has been shown that ^{90}Y−DTPA is not very stable *in vivo*, and uncomplexed ^{90}Y accumulates in the bone marrow. Consequently, the macrocyclic chelator DOTA, which forms more kinetically inert complexes, has replaced DTPA. Generally, acyclic chelators have faster metal-binding kinetics in comparison to their macrocyclic analogs. This behavior is of great importance for radiometals with short half-lives [17, 71, 72].

THE PRODUCTION OF THE RADIOISOTOPES

Production of the Radioisotope ^{99m}Tc

Radionuclide generators consist of a long-lived radionuclide (parent radionuclide) that decays to a short-lived isotope (daughter isotope). The design of the generator allows the separation of the radionuclide produced from the parent isotope by a single step elution from an ion exchange column. ^{99m}Tc is obtained from the parent radionuclide ^{99}Mo, which itself is available at low costs (Fig. **8**). Due to their large spectrum of applications, technetium generators are very economical and therefore a sustainable source for an isotope of medical interest.

The core of the generator is a solid column made up of activated (acidified) alumina. The molybdate anion (MoO_4^{2-}) polymerizes in acidic solution and forms a stable polymer that consists of $Al[Mo_6O_{24}]^{9-}$. This polymer is adsorbed on the column [12]. In contrary, the pertechnetate ion, which does not polymerize, does not bind to the column. Therefore, ^{99m}Tc can be eluted from the column as pertechnetate using physiological saline. The exclusive availability of $^{99m}TcO_4^-$ in saline explains why the chemistry has to be performed in aqueous solutions. Thirteen

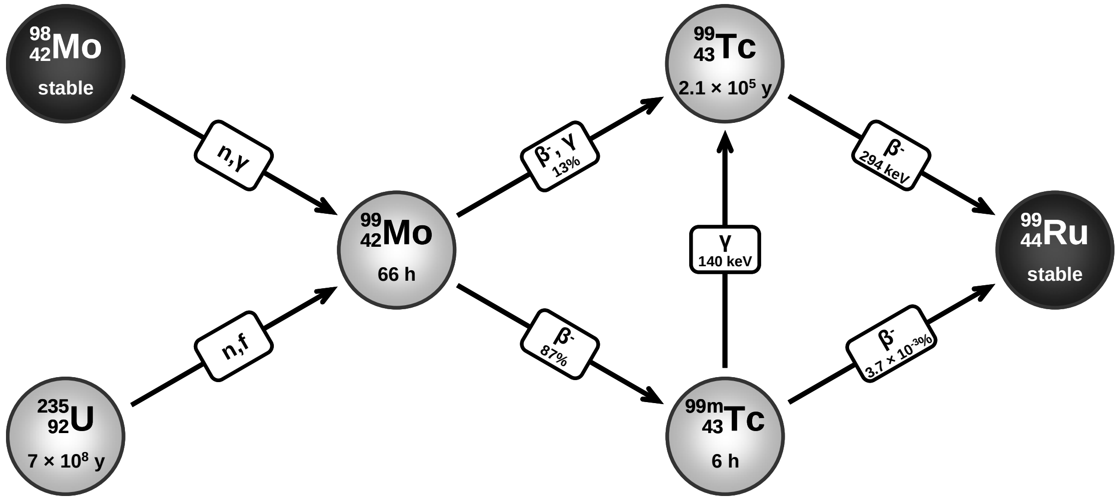


Fig. (8). Schematic presentation of the radioisotopes involved in the production and decay of the technetium-99m generator.

percent of ^{99}Mo decays directly to ^{99}Tc. Consequently, this isotope is a by-product, which is contained in the ^{99m}Tc eluate. This isotope is the only by-product found in the generator eluate. The yield of ^{99m}Tc depends on the age of the generator and the elution intervals. The ^{99m}Tc/^{99}Tc ratio (with a maximum of 87%) decreases with the regeneration time.

Production of Gallium Radioisotopes

Radioactive gallium isotopes cannot be found in nature. ^{68}Ga (half-life 68 min), ^{67}Ga (half-life 78.3 h (3.26 d)), and ^{66}Ga (half-life 9.5 h) are the three radioisotopes suited for the usage in nuclear medicine [73]. ^{68}Ga is the Ga-isotope most widely used in nuclear medicine. It serves as an efficient surrogate for ^{99m}Tc [73]. It is the daughter radioisotope of germanium-68 (^{68}Ge, half-life 270.8 days) (Fig. **9**).

While the short half-life of ^{68}Ga prohibits the shipment of the radioisotope over long distances, the long half-life of the parent radioisotope ^{68}Ge allows the production of generators (anticipated average life 1-2 years). This is the basis of a cost-effective production of ^{68}Ga. The use of different stationary and/or mobile phases resulted in the development of a diversity of ^{68}Ge/^{68}Ga generators, which were discussed in a recent review [74]. The high positron emission energy of ^{68}Ga (1899 keV) affects the spatial resolution. However, the half-life is excellent for preparation, purification, and imaging of tracers with rapid pharmacokinetics. The number of applications for ^{67}Ga and ^{66}Ga are relatively small in comparison with ^{68}Ga. ^{67}Ga was first produced for human use in 1953 [75]. ^{67}Ga decays by electron capture to form the stable isotope ^{67}Zn. It is a pure γ-emitter with multiple gamma photons of different energies. The half-life of 78.3 h makes it available from commercial sources. ^{67}Ga is most commonly used for inflammation and tumor imaging applications.

Production of ^{111}In and ^{113m}In

The most widely used radioisotope of indium is ^{111}In. It has a half-life of 2.83 days and is produced in cyclotrons by the ^{112}Cd(p,2n)/^{111}In nuclear reaction (Fig. **10**). It decays by electron capture with the emission of gamma photons of 173 and 247 keV (89% and 95% abundance, respectively).

Due to its appropriate gamma energy, half life and its excellent chemical properties, ^{111}In is widely used in gamma scintigraphy. It was first evaluated *in vivo* in 1969 [18]. The first FDA-approved peptide radiopharmaceutical for clinical use was the ^{111}In-labeled somatostatin analog OctreoScan®. The half-life of ^{111}In makes it ideal for applications in research where imaging is performed over longer intervals lasting up to several days [29].

^{113m}In (half-life = 1.7 h) is formed using the ^{113}Sn/^{113m}In generator where the parent isotope has a half-life of 115 days. The usage of ^{113m}In is limited by its high gamma energy of 392 keV.

Production of ^{86}Y and ^{90}Y

^{90}Y ($t_{1/2}$ = 64.06 h) and ^{86}Y ($t_{1/2}$ = 14.7 h) are the two radioisotopes of yttrium that have found applications as radiopharmaceuticals. ^{90}Y is a pure β-emitter. Because of its long penetration range (~12 mm) it is suited for endoradiotherapy of large tumors. ^{90}Y is usually produced from strontium-90 (half-life 27.8 year) as the parent nuclide (Fig. **11**) [76].

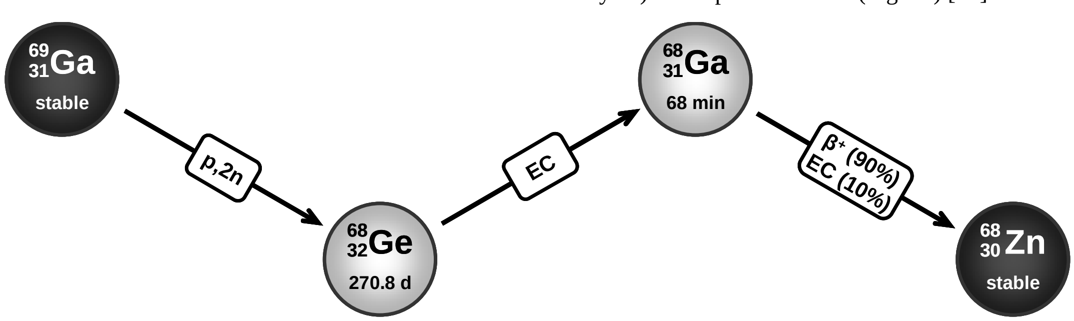


Fig. (9). Schematic presentation of the radioisotopes involved in the production and decay of the gallium-68 generator.

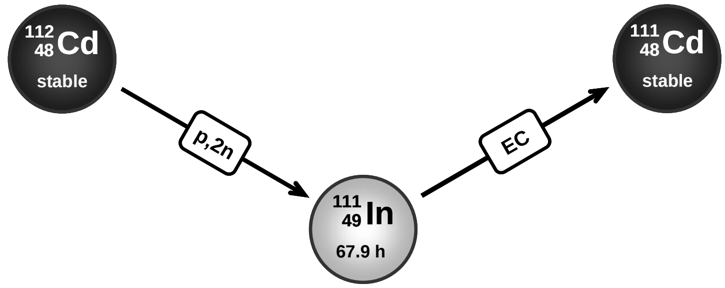


Fig. (10). Schematic presentation of the radioisotopes involved in the production and decay of indium-111.

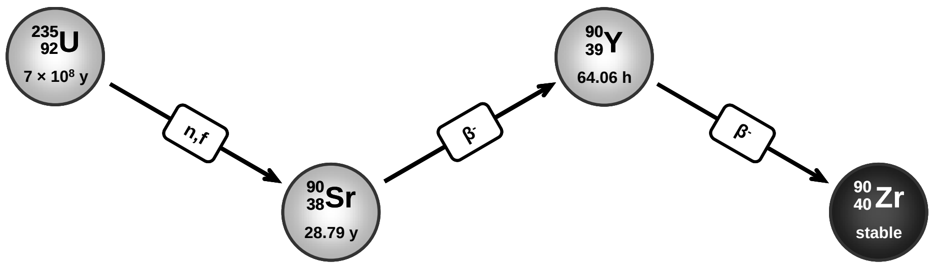


Fig. (11). Schematic presentation of the radioisotopes involved in the production and decay of the yttrium-90 generator.

The positron-emitting nuclide ^{86}Y has been used as a surrogate to ^{90}Y for PET imaging. It is produced by the ^{86}Sr(p,n)/^{86}Y nuclear reaction [77].

Production of ^{60}Cu, ^{61}Cu, ^{62}Cu, ^{64}Cu, and ^{67}Cu

A diversity of copper radioisotopes is available for theranostics. They are produced in cyclotrons or generators. ^{60}Cu, ^{61}Cu, ^{62}Cu, and ^{64}Cu are suited for diagnostic applications since their half-life range from 10 min to 12.7 h. The isotopes ^{64}Cu and ^{67}Cu are suited for therapeutic applications. The copper radioisotopes were reviewed comprehensively in another publication [78].

For published X-ray crystal structures of Cu, In, Ga, Y, and Zr coordination complexes the readers are referred to a very recent review by Wadas *et al*. [22].

Technetium Chemistry

^{99m}Tc has a half life of 6 h. It is the metastable nuclear isomer of the long-lived isotope ^{99}Tc. Upon decay to its ground state ^{99}Tc, a gamma energy of 140 keV is emitted. Due to its favorable characteristics ^{99m}Tc remains the dominant radioisotope for diagnostic applications. This preeminent position is due to the ease of production (^{99}Mo/^{99m}Tc generator), resulting in an availability at low costs and the versatile chemistry that has allowed the development of various tracers in a large number of technetium oxidation states. The 140 keV photons give excellent images regarding to sensitivity and resolution. The half-life of 6 h is long enough to perform the radiolabeling procedures but short enough to limit the radiation exposure of the patients. The oxidation states of ^{99m}Tc vary between -1 to +7, affording a wide range of coordination geometries [79]. The capability of ^{99m}Tc to accept a broad variety of donor ligands is an additional advantage. Usually, ^{99m}Tc is generated as pertechnetate ion (TcO_4^-), which is negatively charged. Since no effective chemistry can be applied to attach the negatively charged $^{99m}TcO_4^-$ to a biomolecule, it is necessary to reduce Tc(VII) to a lower oxidation state to produce a stable ^{99m}Tc-tracer conjugate.

Cardiolite (Tc-sestamibi, Fig. **12**), the first clinically applied radiometal-labeled pharmaceutical in nuclear medicine, is an example for a technetium essential radiopharmaceutical [40].

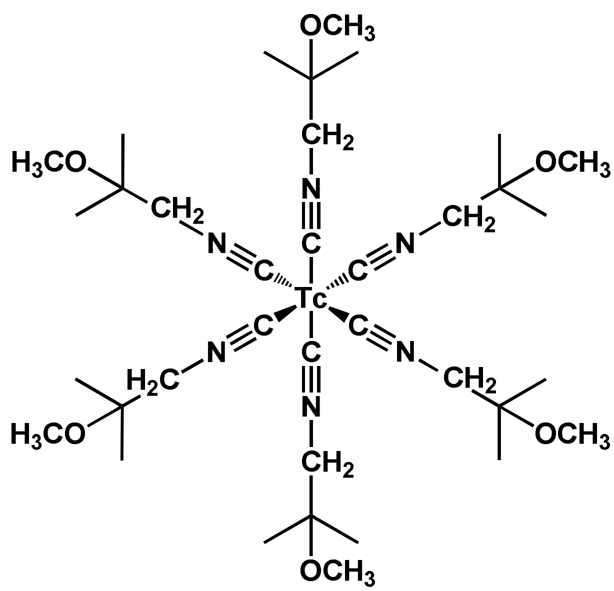


Fig. (12). The chemical structure of the cardiac tracer ^{99m}Tc-Cardiolite.

The targeting properties of the carrier molecule should be preserved after labeling with ^{99m}Tc [80]. In other words, the carrier molecule has to be adapted to the technetium core in order to dominate the pharmacokinetic properties of the conjugate. While peptides are a good choice for ^{99m}Tc-based radiopharmaceuticals, larger molecules have their limitations due to the poor match of their pharmacokinetic properties and the physical half-life of ^{99m}Tc.

Radiolabeling Approaches with ^{99m}Tc and $^{186/188}$Re

The labeling strategies for ^{99m}Tc/$^{186/188}$Re-labeled tracers can be established based on three different methodologies. The choice of ^{99m}Tc/$^{186/188}$Re-labeling approaches depends on the size of the tracer and the purpose of the study [12, 28, 41, 46, 47]. The first technique is referred to as the direct-labeling approach. It relies on the application of a reducing agent (such as $SnCl_2$) to convert a tracer containing a disulphide bridge to its corresponding precursor containing free thiols. These free thiol ligands serve as chelators for $^{99m}Tc/^{186/188}Re$. Despite the ease of this method, it is restricted to tracers containing disulphide-bonds and the reduction of disulphide-bonds may affect the biological activity, the pharmacokinetics and the stability *in vivo*. The second method is the pre-labeling approach where the complex of $^{99m}Tc/^{186/188}Re$ with the BFCA is formed and subsequently conjugated to the tracer moiety. The advantages are that the tracer does not have to be exposed to the labeling conditions and that a well-defined labeling chemistry can be used. However, this method is relatively complex and time consuming. The third method is the post-labeling approach or indirect labeling approach. In this approach a BFCA is first coupled to the tracer and then radiolabeled with ^{99m}Tc. The post-labeling approach is the most widely used method and a variety of BFCAs including mercaptoacetyltriglycine (MAG_3), 2-hydrazino-nicotinic acid (HYNIC), diaminodithiol (DADT) analogs, ethylenedicy-steine, tetraamines, and chelators of the N_3S type have been developed [12].

The Redox Chemistry of Technetium

The negatively charged pertechnetate anion ($^{99m}TcO_4^-$) eluted form the generator is the starting point of technetium chemistry. During the radiolabeling process a reducing agent is utilized to form the lower oxidation states of ^{99m}Tc. The resulting oxidation state of ^{99m}Tc depends on the reduction conditions, in particular the nature of the reducing agent and the ligands available [12]. The choice of the ligand system allows the manipulation of the pharmacokinetics (i.e. modulation of the overall charge and lipophilicity). The application of transient metals (i.e. Cu(I), or Fe(II)) as reducing agent, should be avoided since they compete with ^{99m}Tc for the ligands of the BFCAs. (Fig. **13**) summarizes various oxidation states and coordination geometries of technetium.

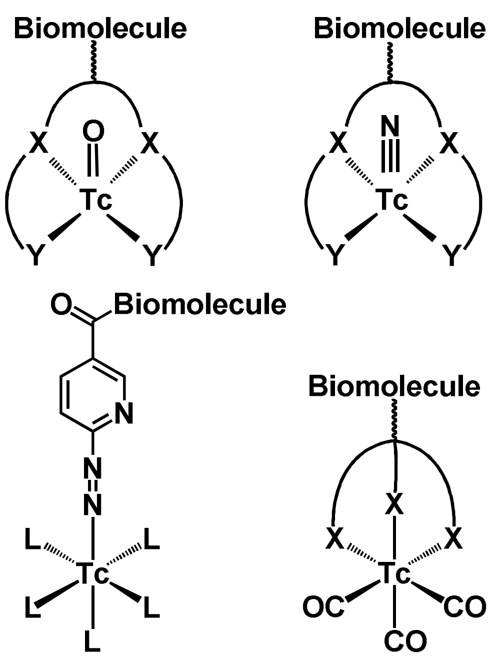


Fig. (13). Common core structures of technetium complexes, which illustrate the diversity of the chemistry of this metal.

Technetium Cores and BFCAs for Labeling with ^{99m}Tc

The formation of stable and kinetically inert ^{99m}Tc-complexes, in high yield at low concentration, are imperative prerequisites for the BFCA. Additionally, the BFCA has to preserve the oxidation state of ^{99m}Tc since a possible reduction or oxidation *in vivo* would result in transchelation to the chelating ligands present in biological systems. The use of isomeric chelators should be avoided or minimized as they show different pharmacokinetic properties [12, 40, 47, 73, 81]. The establishment of the shape of the BFCA framework as well as the selection of the donor atoms highly depend on the technetium cores. Among the diversity of ^{99m}Tc-cores, the technetium cores $[Tc{=}O]^{3+}$, $[Tc{\equiv}N]^{2+}$, $[Tc(CO)_3]^+$, and [Tc]HYNIC are preferred [38, 82-84].

The $[Tc{=}O]^{3+}$ Core

This core has gained acceptance in ^{99m}Tc-radiopharmaceuticals due to its stability in aqueous solutions in the presence of competitive chelators (Fig. **14**). Tc is in the oxidation state Tc(V) and forms quadratic pyramidal technetium oxo-complexes with tetradentate ligands of the general form N_xS_{4-x} (i.e. N_4, N_3S, or N_2S_2) [85, 86].

Generally, N can be amine or amide ligands, based on the combination of ligand systems; they can form cationic (dibasic ligands) or neutral (tribasic ligands) complexes. Modification or functionalization on the framework of these chelators results in the generation of isomers. In order to circumvent the isomerism, other donor types, i.e. N_4, N_xP_y and S_xP_y have been developed [87-89]. In an alternative strategy named peptidic chelating systems, similar frameworks were developed based on the tri- or tetra-peptide sequences, where the N_x moieties consist of Gly, Ala, Cys, Ser and/or their derivatives are used (i.e. GAGG, GSC, GGC, GAG). This strategy is of great benefit for peptide-based radiopharmaceuticals since the chelator sequence can be incorporated into the peptide during the SPPS. Two well known examples of this chelator system are the renal imaging tracers MAG_3 [90] and MAG_2 [91]. The thrombosis imaging agent ^{99m}Tc-P280 which is based on a peptidic chelating system was approved by the FDA [92]. It should be noted that the use of chelators containing protected thiols leads to a reduction of the efficiency of radiolabeling.

The Tc=O (3 + 1) system (SXS + RS) was investigated where X = S or NCH_3. In this system the tracer-bearing monodentate thiol ligand forms a stable complex with SXS ligands. Unfortunately, the stability of these complexes was found to be problematic *in vivo* and *in vitro* because of the transchelation of the RS^- group by glutathione [93]. In the case of X = NCH_3, the stability can be improved.

The $[Tc{\equiv}N]^{2+}$ Core

The nitrido ligand shows a high affinity for Tc(V) [94, 95]. It can be used to bind ^{99m}Tc to peptides and small molecules such as benzodiazepine receptor ligands [73]. Diphosphine ligands (PXP) are used as co-ligands to stabilize this core. The $[Tc{\equiv}N]^{2+}$ core has a higher redox stability compared to the corresponding metal-oxo analog. However, the requirement of additional preparative steps is considered as a limitation [96]. This core was reviewed elsewhere [97-100].

The $[Tc(CO)_3]^+$ Core

This Tc core was first described by Alberto *et al.*, who formed the complex $[^{99m}Tc(H_2O)_3(CO)_3]^+$ with the low oxidation state Tc(I) [40]. The labeling strategy was called Tc/Re-tricarbonyl technology, which was developed substantially by Alberto and co-workers and reviewed comprehensively [40]. This simple complex combines a "lower" organometallic half with three carbonyl ligands with an "upper" Werner-type half with three water molecules as ligands (Fig. **15**).

This core is very compact with a "closed" sphere and therefore favorable over the $[Tc{=}O]^{3+}$ core. The "open" pyramidal structure

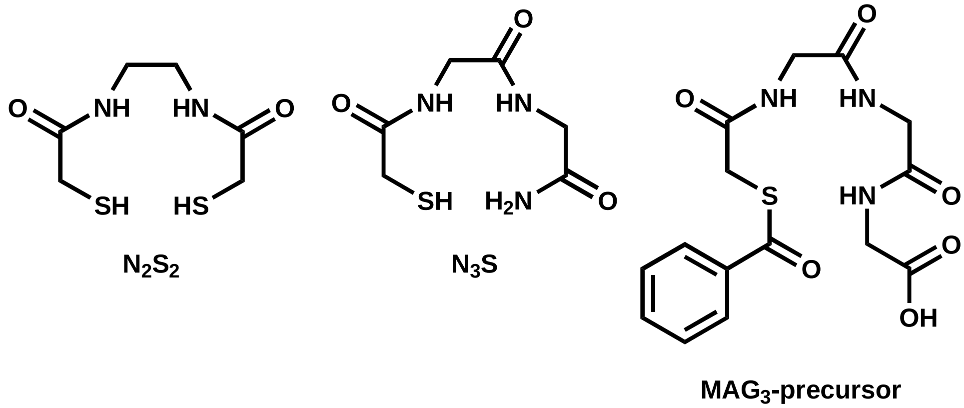


Fig. (14). The chemical structure of technetium chelators of the N_2S_2 and the N_3S type and the labeling precursor (*N*-[*N*-[*N*-[(benzoylthio)acetyl]glycyl]glycyl]glycine), which is used to prepare the kidney imaging tracer ^{99m}Tc-MAG_3.

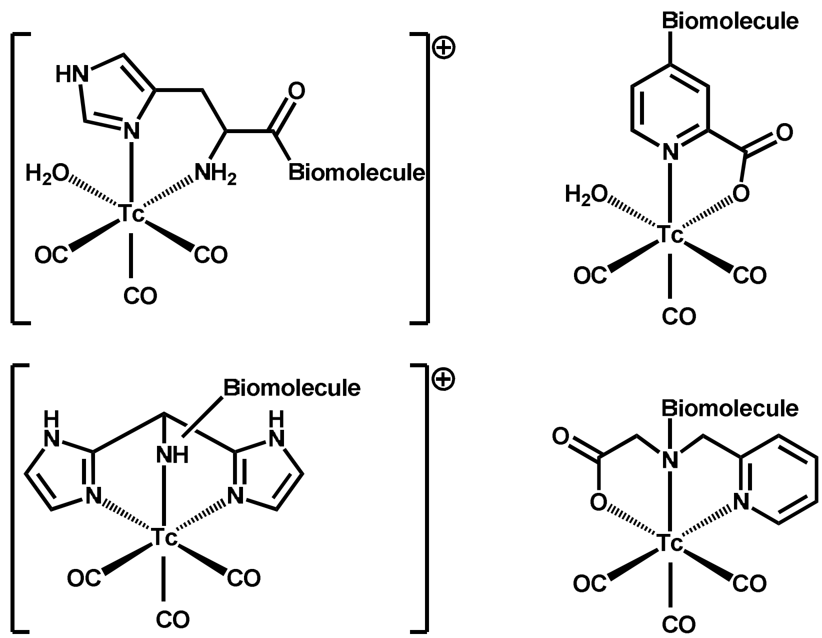


Fig. (15). The chemical structure of typical bidentate and tridentate technetium chelators of the tricarbonyl type.

of the tetradentate chelate makes Tc(V)-oxo-complexes prone to ligand attack. The compatibility with aqueous solutions (the starting material is soluble in aqueous solutions), the small size of the core and the labeling efficiency are considered as additional advantages. The reduction of pertechnetate Tc(VII) to Tc(I) using the stable and nontoxic potassium boranocarbonate, $K_2[H_3BCO_2]$, was developed by Schubiger's group [101]. The oxidation state Tc(I) allows the application of a broad variety of donor and acceptor atoms [102]. Imidazoles, pyridines, pyrazoles, amides, carboxylic acids, thioethers, thiols, phosphines and their combinations have been developed as mono-, bi- and tridentate ligand systems. The possibility of complexation with the imidazole ring in the side chain of histidine makes this strategy of special interest for the labeling of peptides, proteins and antibodies. Hexahistidine residues that are applied in the His-tag approach have been applied successfully for the labeling with ^{99m}Tc [40]. Tridentate chelators were found to be superior to the mono- and bidentate BFCAs regarding to the clearance and stability [103]. Recently Schibli and co-workers developed an excellent chelator system that relies on the preparation of histidine analog by means of the "Click Chemistry" [104, 105]. This strategy was referred to as "Click-to-Chelate" (Fig. **16**).

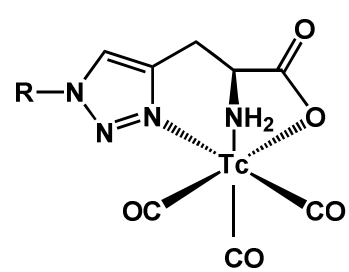


Fig. (16). The chemical structure of a technetium chelator obtained by the "Click-to-Chelate" strategy.

In another strategy, a BFCA was developed based on lysine derivatives for the coordination to the $[Tc(CO)_3]^+$ core. The type of chelators was named single amino acid chelates (SAACs). The chelators have been optimized to be compatible with peptide synthesis strategies (Fig. **17**) [73, 106, 107].

The [Tc]HYNIC core

The 6-hydrazinonicotinamide (HYNIC) core was firstly introduced by Abrams *et al.* for the labeling of human polyclonal

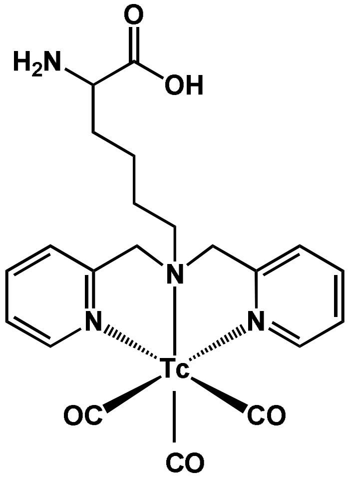


Fig. (17). The chemical structure of a technetium chelator of the single amino acid chelates type.

IgG with ^{99m}Tc [47]. The labeling efficiency of the HYNIC core is of great benefit for the ^{99m}Tc-labeling chemistry. Since HYNIC occupies only one or two coordination sites, co-ligands such as ethylenediaminediacetic acid (EDDA) or *N*-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)glycine (tricine) are required to stabilize either the quadratic pyramidal or the octahedral coordination sphere of technetium. The introduction of HYNIC can be considered as an additional breakthrough in Tc-coordination chemistry since it establishes new parameters that give rise to the formation of stable complexes with the radiometal that are superior to polydentate ligands.

The TcO_2^+[O=Tc=O]$^+$ Core

Linear or macrocyclic tetraamines such as cyclamen are able to form a stable octahedral complex with the TcO_2^+ core (Fig. **18**). The polydentate ligands can be amine, thioether, or phosphine donor atoms [108]. An example of a technetium radiopharmaceutical using the TcO_2^+ core is [$^{99m}TcO_2$(tetrofosmin)$_2$]$^+$ which was developed as a heart-imaging agent because of its cationic character [43]. The TcO_2^+ core has also been used in ^{99m}Tc labeling of the somatostatin analog ([DTPA]octreotide) [18, 109].

Rhenium

Rhenium is the group VII congener of technetium [18, 96, 110]. In contrast to the diagnostic ^{99m}Tc-radiopharmaceuticals, the $^{186/188}$Re-radiopharmaceuticals have found their application in nuclear medicine as therapeutics. The chemical behavior of rhenium is closely related to that of the homologous element technetium. The mixture of the naturally occurring stable rhenium isotopes ^{185}Re (37.4%) and ^{187}Re (62.6%) has been used as a substitute for technetium in the early research due to the similarity of their ionic radii, dipole moments, lipophilicity, and the resulting coordination chemistry [27, 83, 101, 111-113]. As a consequence, the BFCAs developed for the labeling with ^{99m}Tc can be applied for the $^{186/188}$Re-labeling of tracers. In general, the chelator systems used for ^{99m}Tc can be applied for $^{186/188}$Re.

The redox chemistry represents the main difference between rhenium and technetium: the Re-radiopharmaceuticals tend to be more prone to reoxidation than the Tc-analogs. This results in the formation of perrhenate (RhO_4^-), the starting material for the formation of the rhenium complexes. Due to the slow reduction rate of Re(VII) in $^{186/188}ReO_4^-$, a stronger reducing agent is required to reach the lower oxidation states. Therefore, the use of the HYNIC chelator system is limited to carrier molecules that can withstand harsh reduction conditions. Rhenium pharmaceuticals, including their applications, ligands, coordination, and chelator systems, have been comprehensively studied and reviewed [40, 79, 96, 104, 106, 109].

The Chemistry of the Radiometals

Due to the progress of PET imaging the replacement of ^{99m}Tc and its parent radioisotope ^{99}Mo will become a challenging task [73]. ^{67}Ga for SPECT and ^{68}Ga for PET are the candidates of choice to serve as ^{99m}Tc surrogates. The comparison of gallium and technetium has been reviewed [73] as well as the coordination and the radiopharmaceutical chemistry of gallium and indium have been reviewed [22, 114]. Gallium (III) and indium (III) species with filled d10 orbitals resemble ferrous ions, which have half-filled $3d^5$ orbitals. In consequence, the chemistry of gallium and indium is very similar to that of iron, even though the mentioned are no transition elements. The three metals show comparable ionization, ionic radii and coordination numbers as aquo cations. For gallium, indium and iron in the +3 oxidation state, the primary species in acidic aerated solutions, is the octahedrally coordinated M^{3+} cation.

All three metals are Lewis Acids, which hydrolyze as the solution's pH is increased and ultimately form their neutral trihydroxides with very low solubility products. Weak In(III) complexes hydrolyze easily, forming insoluble hydroxides at pH >3.4. In(III) (ionic radius 0.92 Å) is somewhat "softer" than Ga(III) and has a larger ionic radius than Ga(III) (ionic radius 0.62 Å) and Fe(III) (ionic radius 0.65 Å). Consequently, In(III) prefers higher

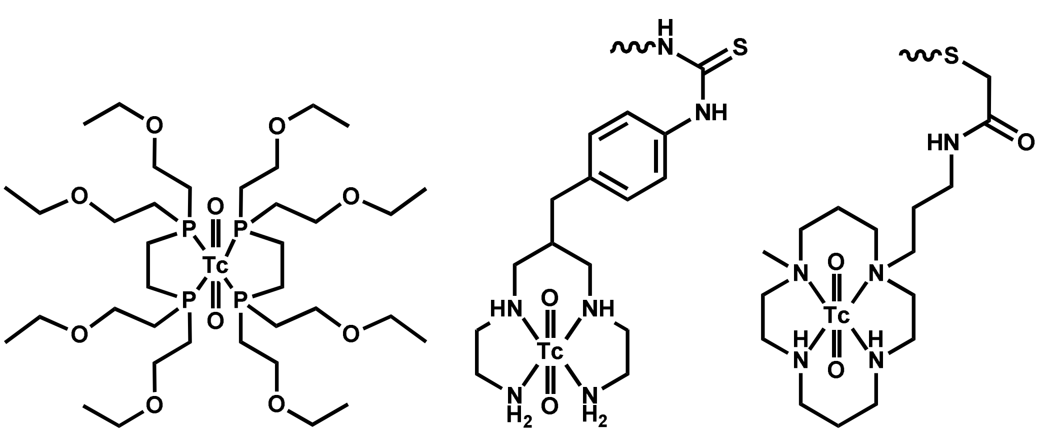


Fig. (18). The chemical structure of tetrofosmin and an acyclic tetraamine as compared to a cyclam derivative, three chelators suited for complexation of the TcO_2^+ core.

coordination numbers. Gallium and indium belong to the group IIIB metals of the periodic table of elements [115-117]. The most common oxidation state of gallium and indium in aqueous solution is +3. Due to their high charge density, they prefer hard bases, such as amines and carboxylates offered by polydentate chelators [118].

For the same reason, their hydrolysis at pH >4 remains a major problem during the labeling step. Consequently, the maximum pH to which a solution can be increased is limited.

This can be achieved with gallium complexes that are thermodynamically stable against hydrolysis or if the kinetics of ligand-to-hydroxide exchange is slow compared to the time. This is of practical importance since it narrows down the pH range within which the complex chemistry can be performed. If a gallium complex is used for pharmaceutical applications in aqueous solutions, the complex must be resistant to $Ga(OH)_3$ formation, to avoid the resulting precipitation of the radiocolloid.

Consequently, weak chelators (such as citrate, acetate or tartrate) are usually added to complex the metal and prevent precipitation. In the absence of stabilizing ligands and in pH ranges between pH 3 - 9.5, for instance, Ga(III) forms insoluble $Ga(OH)_3$. Above pH 9.6, Ga(III) is present as a soluble gallate ion ($Ga(OH)_4^-$). The differences of Ga(III) and In(III) in size and charge density are often reflected by their different coordination chemistry with DTPA, DOTA and NOTA. Depending on the ligand, the coordination number of Ga(III) in its complexes is 3, 4, 5 or 6. The coordination numbers of In(III) are 6 or 7 and only a few eight-coordinated In(III) complexes are known [22, 119]. Because of its small cationic radius, six-coordinated Ga(III) complexes with a distorted octahedral structure are most stable *in vivo* [114]. With respect to their coordination chemistry and biological properties, Ga(III) and In(III) display similarity with slight differences compared to the ferric ion Fe(III). This similarity to Fe(III) is important since Fe(III) competes *in vivo* especially for transferrin, which transports and stores Fe(III) [118]. Due to their strong transferrin binding capability free ^{68}Ga and ^{111}In tend to accumulate in the liver and the lungs [13].

The elements yttrium (z = 39) and lutetium (z = 71), are both members of the class of "heavy" rare earth elements. Despite its low atomic number, yttrium is classified along with the group of lanthanides and shows great similarity with this group. Significant cation-ligand attractions are thus largely electrostatic in character, and the complex species formed by yttrium and lutetium are rather comparable to those derived from calcium, strontium, and barium ions than to those derived from d-type transition metal ions. Y(III) and the trivalent lanthanides precipitate in the presence of hydroxide, phosphate or carbonate. Their extreme affinity to phosphate anions explains their high tendency to deposit in the bone [13].

Yttrium is the first member of the 4d transition series and shows remarkable similarities with the lanthanides and is often discussed in this context. The similarity is obvious, even with regard to the occurrence in nature, yttrium is almost always found together with the lanthanides in minerals.

The similarity yttrium with the lanthanides is a result of the fact that the atomic and ionic radii lie between holmium and erbium. For this reason, yttrium shows greater similarity with the heavier lanthanides, instead of the lighter lanthanides.

The complexes of Y mainly differ from those of the lanthanides in properties caused by the presence of the incompletely occupied 4f orbitals, i.e., the magnetic properties and absorption of light - emission phenomena. The coordination chemistry of lanthanides has found applications in the isolation and separation of the members of the series by fractional crystallization and precipitation. The trivalent lanthanide ions behave as hard acids and have small ionic radii, low and high oxidation states and polarizabilities. As hard acids are expected to strongly interact with hard bases lanthanide ions form stronger complexes with ligands with O and N donor atoms. The majority of the coordination compounds of oxygen-donor ligands have been prepared from anionic (chelate) ligands, but recently several lanthanide complexes of neutral oxygen and nitrogen donor ligands have been described.

In aqueous solution the ligands encounter a competition from water molecules for the coordination sites of the metal ion. The weakly basic N-donor coordination requires the displacement of the strongly bound water molecules. The ligand field stabilization effects result from the lack of extensive interactions, at least in the lanthanide complexes with the 4f orbitals. The lack of ligand field stabilization effects reduces the overall stability, but on the other hand, offers greater flexibility in geometry and coordination. The lanthanide ions show a tendency for increased coordination numbers compared to the d-transition metal ions.

The coordination numbers of lanthanides in complexes are generally higher than six, and vary from 6 to 12 (Fig. **19**). The coordination numbers in lanthanide complexes can be attributed to steric factors and electrostatic forces of attraction and repulsion, rather than the directional orientation of bonds by the deep-seated 4f orbitals of the metal ions. The large lanthanide ions have a tendency to accommodate more than six donor atoms in the coordination sphere [12]. The rate of formation of lanthanide complexes is slower than the corresponding complexes of transition metal ions.

The high coordination numbers that occur are a consequence of the prevailing electrostatic nature of the bonds between the metal and the ligand and depend on the size of the lanthanide ions. Since the 4f orbitals are adequately shielded by the $5s^25p^6$ octet they are mostly unavailable for bonding with the ligand orbitals, the structure of a lanthanide complex is therefore dictated by the following considerations: a) obtaining a spherical symmetry for the central metal ion b) minimizing the ligand-ligand and metal-metal repulsion, and (c) steric requirements of the ligand. Chelation invariably leads to greater stability with respect to dissociation of the component species than the complex formation does by the same donor atoms in non-chelated structures. As a consequence chelated species containing yttrium and the rare earth metal ions are more numerous than all other complex species.

Due to their potential applications as PET imaging agents, ^{68}Ga-labeled radiopharmaceuticals have received strong attention. The gamma emitter ^{111}In has been applied widely in gamma scintigraphy. ^{111}In-radiopharmaceuticals are used as imaging surrogates for the pure β-emitter ^{90}Y and lanthanides such as ^{177}Lu, which are mainly suited for therapeutic applications. They are used for biodistribution studies and for the determination of the dosimetry of their corresponding ^{90}Y analogs. Usually, NOTA and DOTA derivatives are used for ^{68}Ga and ^{111}In-labeled radiopharmaceuticals.

In(III) and Y(III) share a very similar coordination chemistry with DTPA and DOTA derivatives. The half-life of ^{111}In (2.8 days) is almost identical to that of ^{90}Y (2.7 days). However, ^{90}Y and ^{111}In labeled biomolecules do not share the same behavior *in vivo* since their pharmacokinetics are different [120, 121]. Therefore, several crucial factors should be addressed before applying ^{111}In-labeled biomolecules as imaging surrogates for ^{90}Y-labeled biomolecules.

The ionic radiuses present the major difference between indium and yttrium. This is reflected by the differences in the coordination chemistry with acyclic and macrocyclic chelators. For instance, ^{90}Y forms an octacoordinate DOTA-complex while ^{111}In prefers the coordination numbers six and seven and rarely forms eight-coordinated complexes [14, 68, 122, 123].

The different pharmacokinetic profiles can be illustrated best with the biodistribution data of ^{67}Ga, ^{90}Y and ^{111}In-labeled

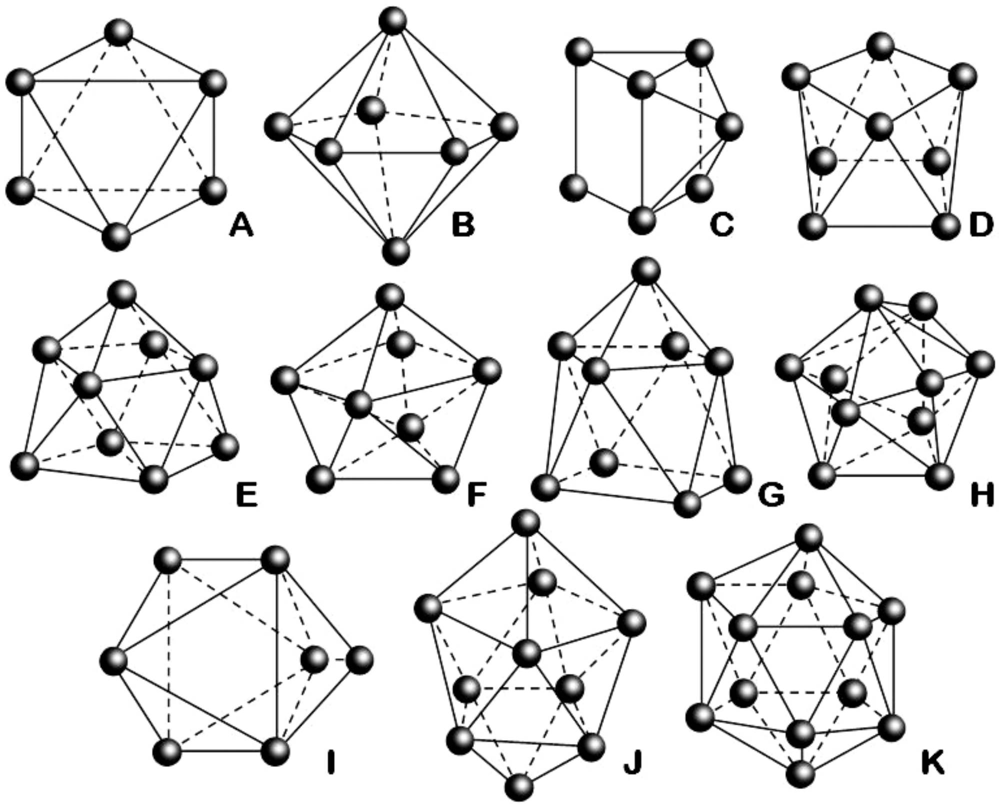


Fig. (19). Architectures of ideal polyhedra adopted by complexes at high coordination numbers. This selection illustrates the rich chemical and physical properties and functions giving access to well-defined and confined cavities, with high symmetry and stability. Key: **A** octahedron, **B** pentagonal bipyramid, **C** monocapped trigonal prism, **D** square antiprism, **E** tricapped trigonal prism, **F** triangular dodecahedron, **G** monocapped square antiprism, **H** bicapped dodecahedron, **I** monocapped octahedron, **J** bicapped square antiprism, **K** icosahedron.

DOTATOC (DOTA-Tyr^3-Octreotide, Fig. **3**) [14]. ^{67}Ga-DOTATOC has the highest tumor uptake rate followed by ^{90}Y-DOTATOC and ^{111}In-DOTATOC. However, ^{90}Y-DOTATOC and ^{111}In-DOTATOC show similar accumulation rates in normal tissues and ^{67}Ga-, ^{111}In- and ^{90}Y-DOTATOC show high stability in the serum. The kidney accumulation rate of ^{67}Ga-DOTATOC is drastically lower than that of ^{111}In- DOTATOC and ^{90}Y-DOTATOC. This improvement of the clearance profile has been ascribed to the free carboxylic group in ^{67}Ga-DOTATOC. The retention times of the ^{90}Y- and ^{111}In-labeled DOTA-derivative TA138, a small molecule receptor antagonist of integrin $\alpha_v\beta_3$, showed a clear shift of about ~4.5 min [124]. This difference in lipophilicity reflects the differences between ^{90}Y- and ^{111}In-labeled molecules. It should be mentioned that this difference depends on the molecular weight of the labeled biomolecule – the effect is negligible in the cases of ^{111}In and ^{90}Y-labeled antibodies while it is very clear in the cases of small biomolecules.

EDTA and DTPA were the first typical polyaminopolycarboxylate ligands tested as chelators for Ga(III) and In(III). The complexes formed with Ga(III) and In(III) have a six-coordinate octahedral configuration [18]. In order to improve the *in vivo* stability of Ga(III) and In(III) complexes, different acyclic EDTA-based frameworks such as hydroxyaromatic diamines and pyridoxylethylenediamine derivatives were investigated [18]. Unfortunately, their stability *in vivo* was insufficient. The breakthrough in this field was achieved by the introduction of macrocyclic chelators. The combinations of nitrogen and oxygen or nitrogen and sulfur donor atoms have been tested. NOTA, DOTA and TETA (Fig. **7** and Fig. **25**) represent the carboxylic acid derivatives of the macrocyclic chelators. Their crystal structures and stabilities have been determined, evaluated and reviewed [22]. Additionally, several triazacyclononane derivatives have been studied [125, 126]. The chelators containing amino and thiol donors which form stable complexes with ^{99m}Tc cores were also investigated to enhance the *in vivo* stability of In(III) and Ga(III) complexes [125, 127]. Further triazacyclononane derivatives have been synthesized and reviewed previously [18, 128].

Acyclic BFCAs

The design of acyclic BFCAs was based on the usage of EDTA for the complexation of trivalent metals. The low stability of their complexes *in vivo* set hurdles to their usage. This limitation was improved by the development of DTPA and its derivatives. They offer further coordinating ligands [129]. DTPA is used as a BFCA for gallium, indium and lanthanide cations. In general, its octadentate ligands are suited for the saturation of the coordination sphere of gallium (hexa-coordination) and indium (hepta- or octa-coordination). A large number of gallium tetradentate or hexadentate ligands have been developed (Fig. **20**).

The *O*-hydroxybenzyl derivatives of iminodiacetic acid were designed to provide an NO_3 donor set [130]. The tripodal NS_3 chelator tris(2-mercaptobenzyl)amine has been also found to form a stable tetradentate ligand with Ga(III). In the same study, it was found that this chelator forms penta-coordinated complexes with In(III) [131]. Another chelator has been designed based on the bis(aminothiolate) N_2S_2 chelator forming a square-pyramidal complex [132]. However, it has been found that the acyclic hexadentate chelator *N*,*N′*-ethylene-di-*L*-cysteine (($N_2O_2S_2$ donor) forms a very stable complex with Ga(III) [133].

DTPA and its cyclohexyl (CHX) derivative have been studied more recently as chelators for ^{68}Ga, ^{86}Y, and ^{111}In [134]. In another

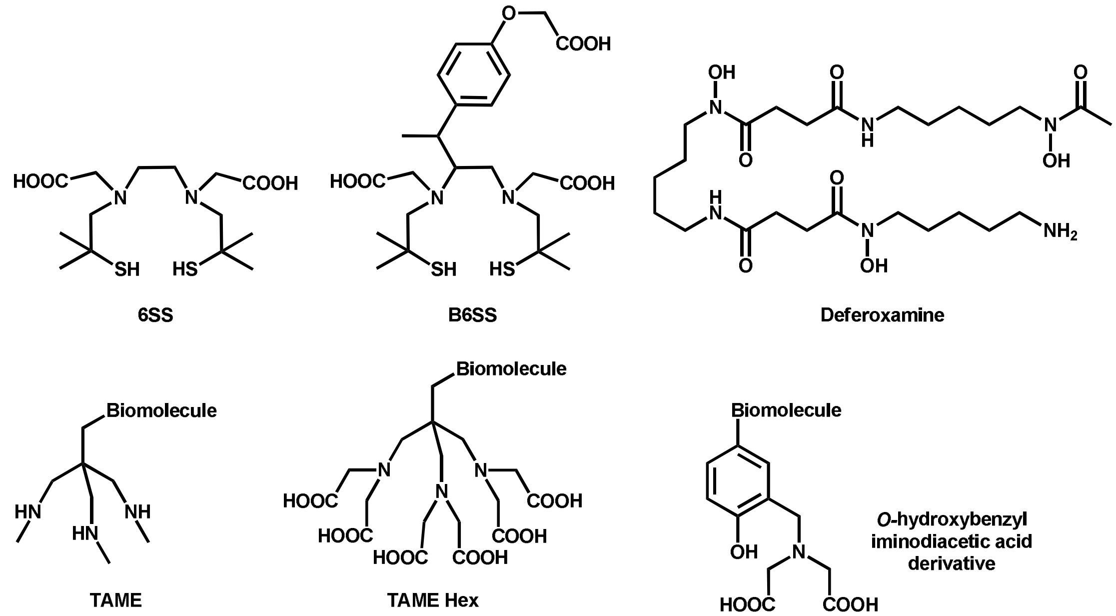


Fig. (20). The chemical structures of several chelators for gallium including *N*,*N′*-bis(2,2-dimethyl-2-mercaptoethyl)ethylenediamine-*N*,*N′*-diacetic acid (6SS), 1-(4-carboxymethoxybenzyl)-*N*-*N′*-bis[(2-mercapto-2,2-dimethyl)ethyl]-1,2-ethylenediamine-*N*,*N′*-diacetic acid (B6SS), deferoxamine, 1,1,1-tris(aminomethyl)ethane (TAME), tris(aminomethyl)ethane-*N*,*N*,*N′*,*N′*,*N″*,*N″*-hexaacetic acid (TAME Hex), and the *O*-hydroxybenzyl iminodiacetic acid derivative.

study, deferrioxamine-B (DFO) revealed a high thermodynamic stability of Ga(III) complexes [135]. This observation has provoked the development of radiopharmaceuticals based on DFO as a BFCA for Ga(III) [119, 136]. The radiolabeling of Zevalin with MX-DTPA was investigated to enable the labeling with either ^{111}In or ^{90}Y [137]. The introduction of backbone-substituted derivatives was an important advance in the development of acyclic chelators. CHX-DTPA forms stable complexes with ^{111}In, ^{177}Lu, ^{213}Bi, and ^{90}Y [138-140].

However, the *in vivo* stability of CHX-DTPA complexes was insufficient in the case of ^{90}Y, since it was found to be accompanied with bone cytotoxicity [121]. Compared to the common macrocyclic ligands, DTPA offers a lower kinetic inertness. Fig. (**21**) illustrates the structures of several DTPA derivatives.

In comparison to Ga(III), In(III) shows a higher affinity towards soft donor ligands. For instance, acyclic N_4O_2 aminophenols and transferrin bind Ga(III) more efficiently than In(III). On the other hand, the chelators NS_3, EDTA, DTPA, and DOTA form more stable complexes with In(III) than with Ga(III), resulting in different *in vivo* behavior [14].

As already mentioned, the hexadentate chelator ethylenedicysteine with the $N_2O_2S_2$ donors was found to form a highly stable

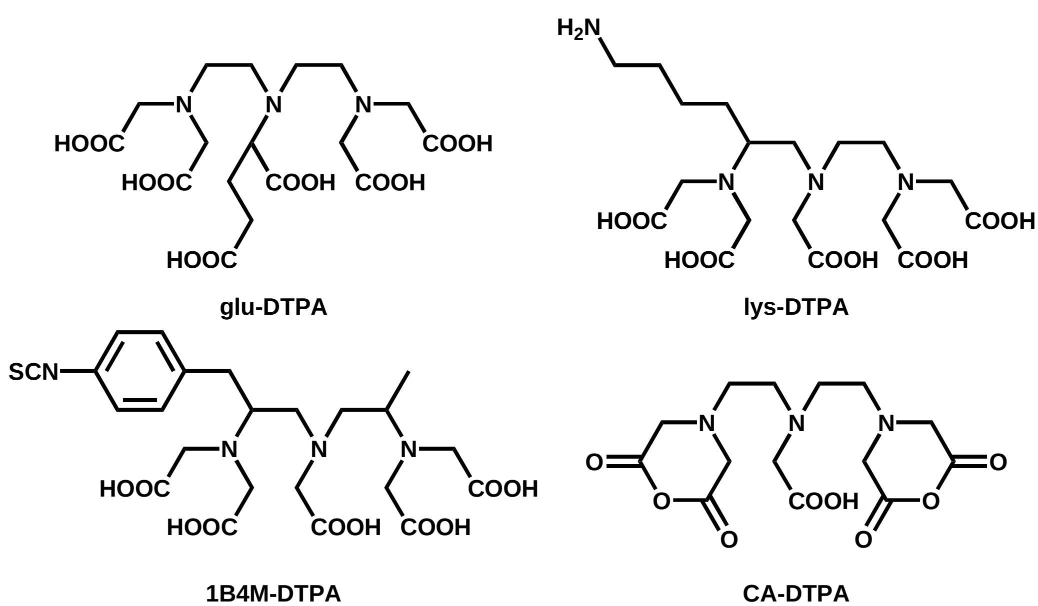


Fig. (21). The chemical structures of several DTPA derivatives including the two activated derivatives 2-(p-SCN-Bz)-6-methyl-DTPA and CA-DTPA, the cyclic anhydride of DTPA (CA-DTPA).

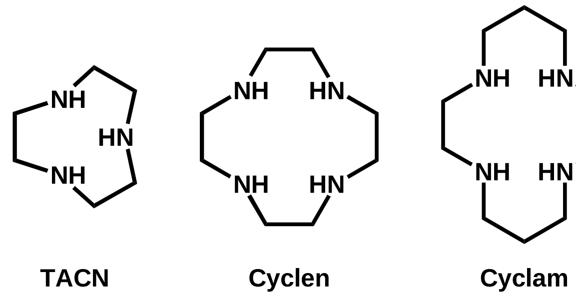


Fig. (22). Chemical structure of triazacyclononane, cyclen and cyclam, three polyazamacrocyles that constitute the basic structure of many macrocyclic BFCAs.

complex with ^{111}In [133]. The acyclic chelators EDTA and DTPA form very thermodynamically stable complexes with indium. The DTPA derivative, CHX-A″-DTPA, was found to efficiently complex the radiometals ^{68}Ga, ^{86}Y, and ^{111}In [134].

Due to its large ionic radius Y(III) prefers the coordination numbers 8 and 9. Y(III) has been considered as an acidic cation which is harder than Ga(III) or In(III) [22]. Similarly to Ga(III) and In(III), EDTA and DTPA have been shown to form stable complexes with Y(III) [141, 142]. However, the tris(carbamoyl-methyl) derivative of triazacyclononane has been also reported to form stable Y(III) complexes [143].

The 6SS ligand and its derivative B6SS (1-(4-carboxymethoxy-benzyl)-*N*,*N′*-bis[(2-mercapto-2,2-dimethyl)ethyl]-1,2-ethylenedia-mine-*N*,*N′*-diacetic acid) have also been described [115, 144, 145]. In general, they provide a $N_2O_2S_2$ donor set of two amine-N, two carboxylate-O, and two thiol-S atoms for Ga(III). Their stability in serum is sufficient in the case of ^{68}Ga (half-life 68 min), however, it is insufficient in the cases of longer-lived radiometals.

The chelator 1,1,1-tris(aminomethyl)ethane (TAME) has been used as a building block that offers nine donor atoms for the complexion of radiometals. Recently, the synthesis of TAME Hex as BFCA has been described [146]. It shows a high stability for gallium complexes. Deferoxamine is a chelator commonly used to remove excess iron from the body in acute or chronic iron poisoning [147]. Recently, the application of deferoxamine as a chelator for ^{67}Ga in the preparation of antibody conjugates has been evaluated [135].

Macrocyclic Chelators

Regarding the complex stability, macrocyclic chelators constitute the crème de la crème of the chelators. The general macrocyclic structure is based on polyazamacrocyles. NOTA, DOTA and their derivatives are the most widely used macrocyclic chelators. 1,4,7-triazacyclononane (TACN) is the nine-membered macrocyclic backbone of NOTA derivatives, while 1,4,7,10-tretraazacyclododecane (cyclen) is the twelve-membered macrocyclic backbone of DOTA derivatives (Fig. **22**).

Thiol, carboxylate, and phosphate donors are used as multidentate ligands for trivalent metals. The stability of the metal-chelator complexes of these derivatives is governed by the radiometal used. For instance, the ^{111}In-DOTA complexes are more stable than the ^{111}In-NOTA analogs. In contrast, the ^{68}Ga-NOTA complexes show a higher *in vivo* stability than the ^{68}Ga-DOTA derivatives.

NOTA is well-known to form an extraordinarily stable complex with Ga(III). The chemistry of the gallium and its BFCAs have been recently reviewed [73]. However, DOTA derivatives offer eight ligands atoms which make them appropriate for the labeling with ^{111}In, ^{90}Y and lanthanides (Ln). Derivatives of NOTA (Fig. **23**) and DOTA (Fig. **24**) are often used for the ^{68}Ga and ^{111}In-labeling of biomolecules.

Among them 1,4,7-triazacyclononane-1-succinic acid-4,7-diacetic acid (NODASA) and 1-(1-carboxy-3-carboxypropyl)-4,7-bis-(carboxymethyl)-1,4,7-triazacyclononane (NODAGA) are particularly useful for ^{68}Ga-labeling due to the high hydrophilicity and the stability of their ^{68}Ga chelates. In addition, a higher ^{68}Ga-labeling efficiency is achieved when compared to the corresponding DOTA analogs [148, 149]. The ^{68}Ga-NOTA complex is about 4

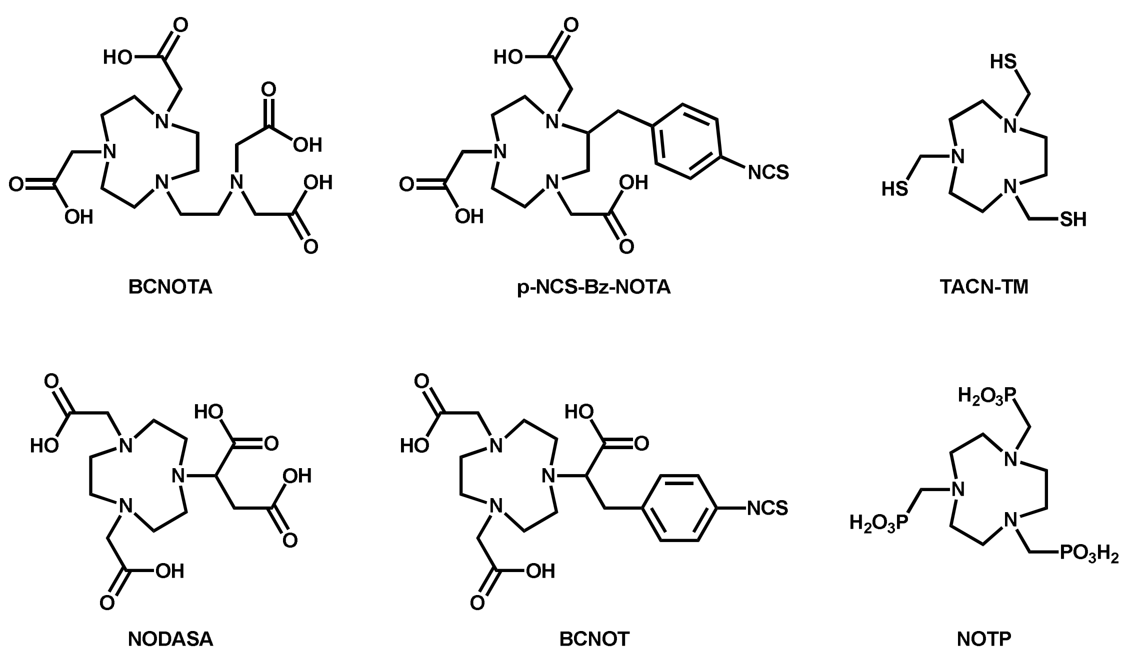


Fig. (23). The chemical structures of a selection of NOTA derivatives including 1,4,7-tris(2-mercaptoethyl)-1,4,7-triazacylclonane (triazacyclononane−TM), a derivative with three additional ethylmercapto arms, and the phosphonate derivative 1,4,7-triazacyclononane-*N*,*N′*,*N″*-tris(methylenephosphonic)acid (NOTP).

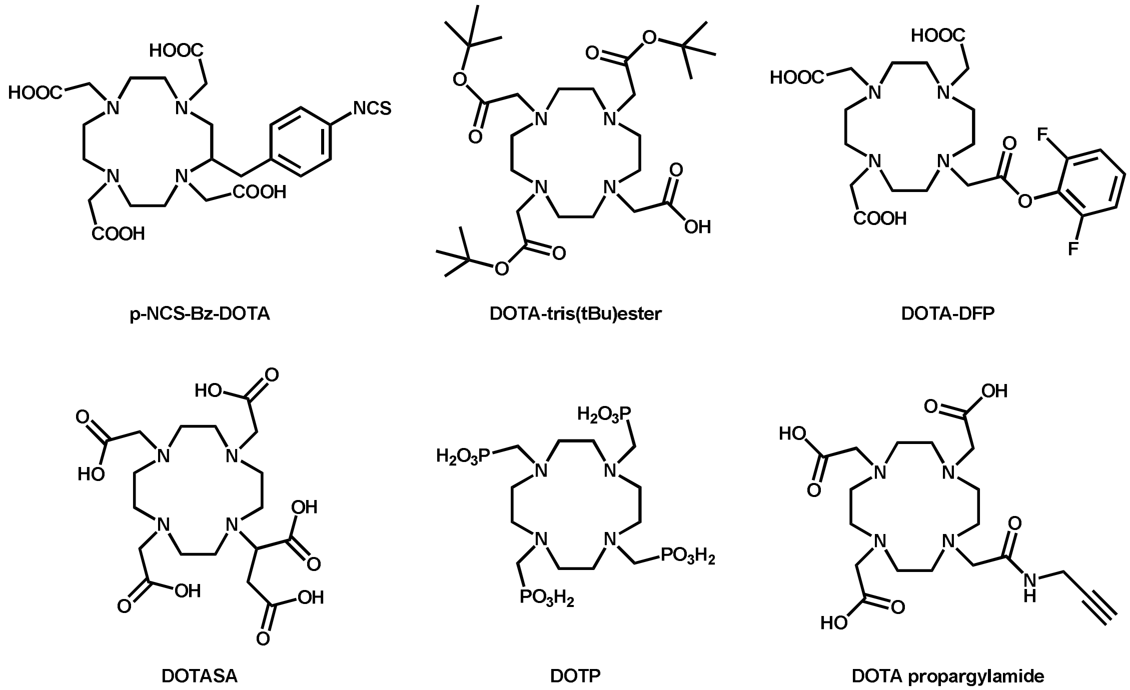


Fig. (24). The chemical structures of a selection of DOTA derivatives.

orders of magnitude more stable than the ^{111}In-NOTA complex, quite the reverse, the ^{111}In-DOTA complex is 2-3 orders of magnitude more stable than the ^{68}Ga-DOTA complex [150]. Owing to this high stability NOTA and its derivatives are valuable BFCAs for radiopharmaceutical applications [149, 151, 152]. DOTA has a larger cavity size than NOTA leading to lower stability of Ga-DOTA complexes, therefore, NOTA is considered superior to DOTA regarding the Ga(III) complexation [22]. Additionally, larger macrocyclic BFCAs based on 14-, 15-, and 18-membered rings were described. Fig. (**25**) shows the structures of the macrocyclic chelators 1, 4, 8, 11-tetraazacyclotetradecane-*N,N',N'',N'''*-tetraacetic acid (TETA), 1,4,7,10,13-pentaazacyclopentadecane-*N,N',N'',N''',N''''*-pentaacetic acid (PEPA) and 1,4,7,10, 13,16-hexaazacyclohexadecane-*N,N',N'',N''',N'''',N'''''*-hexaacetic acid (HEHA). TETA, a 14-membered ring BFCA has been used for copper radionuclides [153]. However, no other radionuclide can be potentially used with TETA. Attempts to use the 15-membered ring PEPA failed for the chelating of ^{213}Bi for *in vivo* applications [154]. The larger macrocyclic chelator TETA was tested as BFCA for Ga(III) complexation. Unfortunately, a relatively low stability was determined [155].

Numerous triazacyclononane derivatives have been used for chelating In(III). The NOTA complexes with In(III) were found to be less stable than the Ga(III)-NOTA complexes. The *tris*(phenylphosphinate) and the tris(mercaptoethyl) derivatives of triazacyclononane can both bind In(III) in a similar way [126, 156].

Several truncated derivatives of DOTA have been described (Fig. **26**). However, their major applications lie in the use as free chelates rather than biomolecule conjugated derivatives.

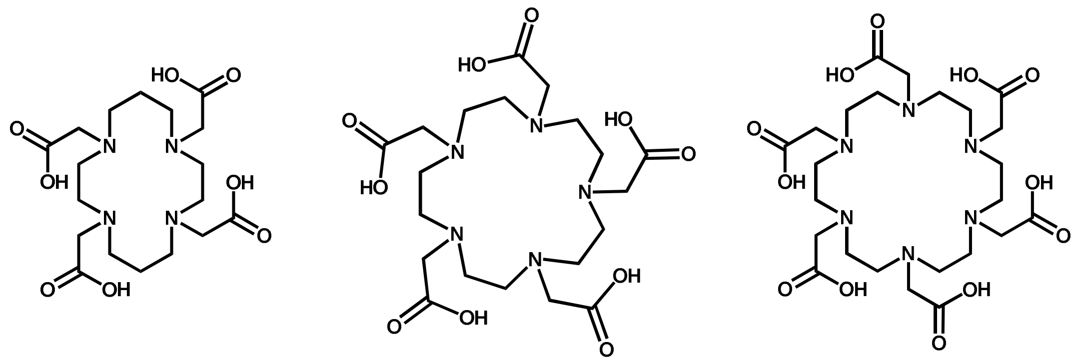
DO3A, a DOTA derivative shortened by one carboxylic arm, has been evaluated for In(III) complexation. It forms a seven-coordinate ligand complex with In(III) [117]. DOTA and its derivatives are the most efficient chelators for Y(III). As mentioned above, ^{90}Y-DOTATOC was found to be less stable than its ^{67}Ga analog. Recently, the pharmacokinetics of different ^{86}Y-DOTA derivatives were investigated to determine the influence of ^{86}Y-DOTA isomers on the biodistribution of oligonucleotides in mice [62]. Y(III)-TETA was found to be much less stable than the DOTA or DTPA analogs [157].

DOTA Derivatives for Trivalent Labeled Radiopharmaceuticals

Since DOTA accommodates a variety of di- and trivalent metal ions with high stability [158] its usage has been extended to chelate metals such as gadolinium, which are used as Magnetic Resonance Imaging (MRI) contrast agents [159]. The cyclic framework of DOTA is able to coordinate several radiometals with extremely high thermodynamic stability and kinetic inertness. The acetate chelating arms provide a high hydrophilic profile, which favors a rapid clearance from blood, liver, and kidneys. Furthermore, the low pKa values of the carboxylic groups minimize the acid-catalyzed dissociation of the radiometal *in vivo* [119].

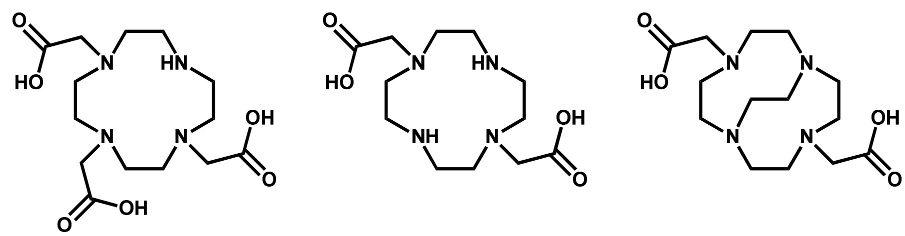
Due to its kinetic inertness, the chelator DOTA seems to be the best choice for trivalent-based radiopharmaceuticals, however, the labeling procedures are crucial and more dependent on labeling conditions. At room temperature, the complex formation rates of DOTA-labeled biomolecules are very slow [22, 119]. The essential prerequisites for an efficient radiolabeling of trivalent-DOTA based radiopharmaceuticals are a) the reaction temperature b) the reaction time c) the buffer salts and their concentration d) the pH e) the DOTA-containing tracer concentration and its purity and f) of course the absence of other metal ions such as Zn(II) and Fe(III) [72]. Whereas the elevated temperature required may not result in the degradation of small biomolecules, it remains a major obstacle for the radiolabeling of proteinaqueos radiopharmaceuticals such as antibodies. In such cases acyclic chelators provide an advantage since they show very high complex formation rates.

1,4,7,10-tetrakis(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane (TCMC), the tetraamide derivative of DOTA has been used extensively with ^{203}Pb and ^{212}Pb [160, 161]. Hopefully, substituted NOTA derivatives will help to overcome this problem for the ^{90}Y-labeled monoclonal antibodies [162]. It should be mentioned that the microwave technique has been successfully applied for the rapid formation of ^{68}Ga-DOTA complexes [163].



TETA PEPA HEHA

Fig. (25). The chemical structures of TETA, PEPA and HEHA, three large macrocyclic chelators.



DO3A DO2A CB-DO2A

Fig. (26). The chemical structures of three truncated DOTA derivatives.

The Attachment Position of Biomolecules

The attachment position of biomolecules is of vital importance for complex stability and the pharmacokinetic properties of the conjugate. DOTA will be used as an example to illustrate this, because numerous approaches were developed in order to covalently attach DOTA to carrier molecules. DOTA can be conjugated either via one of its carbon atoms presented by the acetate chelating arms, or via the bridges of the macrocycle. Conjugation of one of the four carboxylic acid arms to the biomolecule by an amide bond (Fig. **24**) [14, 164] leads to the loss of one strong ligand because the carbonyl-oxygen obtained after formation of an amide is a relatively weak donor when compared to the carboxylate-oxygen. This conjugation technique is considered as the standard method for solid phase synthesis since the amide bond formation is convergent with the peptide chemistry. The alternative conjugation via the bridge of the macrocyclus should show a lower influence on the kinetic inertness. Besides the *in situ* activation, [45, 165] protected or activated DOTA-derivatives were developed. The introduction of the prochelator tris-*t*Bu-DOTA is of importance for the synthesis of DOTA-derivatised peptides by solid phase synthesis. One disadvantage of this BFCA system is the long deprotection times required to cleave the *tert*-butyl esters [73]. This limitation has been subjected by the use of different protecting groups such as tris-allyl-DOTA, tris-methyl-DOTA, and tris-benzyl-DOTA esters [73]. The protected DOTA-derivatives require preactivation prior to coupling and harsh deprotection conditions to liberate the free chelator. Therefore, DOTA-active esters were found as an efficient tool to circumvent these problems [166]. Numerous DOTA-derivatives were used to conjugate DOTA to different biomolecules including peptides, biotin, antibodies, dendrimers etc. [167-169].

Conjugation Groups

Electrophiles, such as anhydrides, bromo- or iodoacetamides, isothiocyanates, *N*-hydroxysuccinimide (NHS) esters, carboxylic acid active esters (mostly phenolic active esters i.e. para-nitrophenol esters), and maleimides are typical groups for conjugation that have been developed to modify biomolecules with the appropriate BFCAs. Primary amines are reactive towards isothiocyanates and active esters, while maleimide is reactive towards thiols. Recently, “Click Chemistry” based on the [3 + 2]-azide-alkyne cycloaddition (catalyzed by copper(I)) has witnessed considerable development and become a key tool in bioconjugate chemistry [104]. Nucleophilic functionalities serve as "handle" between the biomolecule and the BFCA. They can be naturally-present or synthetically-introduced to the biomolecules. However, the azide or alkyne functions required for “Click Chemistry” have to be synthetically introduced to the biomolecules and the BFCAs.

DTPA dianhydride is a well-known example of an anhydride-based synthon. The reactivity of the anhydride groups in both aqueous and non-aqueous solution without the necessity to add an activating reagent is an advantage. However, the formation of dimeric conjugates of the biomolecule is an obvious disadvantage since it destroys the pharmacokinetics as well as the stability of the radiometal-DTPA complexes. Asymmetrically protected derivatives of DTPA have been developed. Unfortunately, the known syntheses are very laborious and can only be accomplished at low yields [170]. A bifunctional DOTA derivative that uses one carboxylate in an active ester form for protein conjugation has been developed and is also commercially available [171]. A series of DOTA-phenolic esters has been introduced by Mier *et al*. [171]. Their tunable reactivity towards amines makes them valuable active ester groups for the conjugation to macromolecules as well as small biomolecules. The increased stability makes them superior to the use of NHS-esters [165]. The stability depends on the phenolic component applied and allows to optimize protocols for the controlled conjugation to proteins, peptides, and other molecules of biological interest, without the necessity of the subsequent removal of the protecting groups [171].

A large variety of isothiocyanate analogs of DOTA and DTPA have been developed [129, 172]. Isothiocyanate-containing BFCAs also show a higher stability in aqueous solutions than their NHS-ester analogs. On the other hand they require a relatively alkaline pH which restricts their use in the case of alkaline-sensitive biomolecules. Another limitation to their usage is an Edman type degradation of the thiourea linkage. Therefore, they are not suited for the conjugation to the *N*-terminus of peptides [173]. Maleimide-based BFCAs have been developed as thiol reactive chelators that are stably linked via a thioether function [174, 175]. At neutral pH their selective reactivity towards thiols in the presence of amine groups is of great benefit.

Nowadays, "Click Chemistry" is one of the most studied methods used for the conjugation of BFCAs with the biomolecule to be labeled. "Click Chemistry" based on the formation of 1,2,3-triazole by the cycloaddition of alkynes and azides catalyzed by copper(I) [176]. This chemistry offers several advantages, such as efficiency, selectivity, and mild reaction conditions. The use of this chemistry in the formation of BFCAs has been referred to by Schibli and co-workers as "Click to Chelate" [105]. Recently, the application of this strategy to different BFCAs as well as with a diversity of biomolecules has been comprehensively studied and reviewed [177, 178]. Fig. (**27**) shows a selection of the most commonly applied conjugation reactions applied for the conjugation of BFCAs.

In single amino acid chelates (SAAC) the lysine-based BFCAs can be incorporated in the peptide sequence during the synthesis of the peptide [106]. This approach is limited to the labeling of peptide-based radiopharmaceuticals.

It should be mentioned that the conjugation of the BFCA to the biomolecule depends also on the biomolecule itself. For instance, the conjugation of the BFCAs to synthetic peptides is usually conducted during the synthesis steps of the peptide. This strategy allows the incorporation at a specific position and therefore the targeting properties can be preserved. Conversely, the conjugation of the BFCA to biologically synthesized biomolecules (i.e. antibodies) is performed after the synthesis of the biomolecule and, therefore, the conjugation is generally not site specific. This can result in alteration of the biological properties. Recently, an alternative method has been reported by Jeger *et al.* based on an enzymatic conjugation of different BFCAs or reagents to antibodies. The site-specific and stoichiometric modification of antibodies was done by bacterial transglutaminase forming an isopeptide bond between the γ-carboxamide group of glutamine with the primary ε-amine group of lysine [31].

CONCLUSIONS

Biochemistry, molecular biology and biotechnology have made major steps forward for the understanding of the mechanisms of diseases. However, this has not yet been satisfyingly translated into the development of new drugs. This is most obvious for tumor diseases – the vast majority of cancers remain incurable. The concept of "theranostics" has the potential to widen the spectrum of treatment options, by combining therapeutic agents with a corresponding diagnostic marker. Metallic radionuclides offer the best prospects for nuclear medicine applications. For example ^{99m}Tc, with its ideal properties is still the most widely used diagnostic radionuclide and more recently numerous radiopharmaceuticals labeled with ^{68}Ga and ^{64}Cu have been developed for PET imaging. Several β-emitting radiometals, such as ^{90}Y, ^{177}Lu, and ^{67}Cu, have been shown effective as therapeutic radiopharmaceuticals. The high *in vivo* stability of radiopharmaceuticals, that make use of BFCAs in combination with radiometal pairs, for both imaging and therapy (^{68}Ga/^{67}Ga, ^{62}Cu/^{67}Cu, ^{110}In/^{111}In, and ^{86}Y/^{90}Y), is the basis for the successful application of radiopharmaceuticals. The strategy of downsizing proteins shall allow to close the gap between drugs of a molecular weight below 500 Da – corresponding to the traditional understanding of druglikeness – and today's proteinaceous drug candidates. BFCAs provide ideal properties for the development of these novel carrier molecules into tracers for molecular imaging and radioimmunotherapy. Consequently targeted BFCAs provide the ideal prerequisites to pave the way for patient-specific therapies.

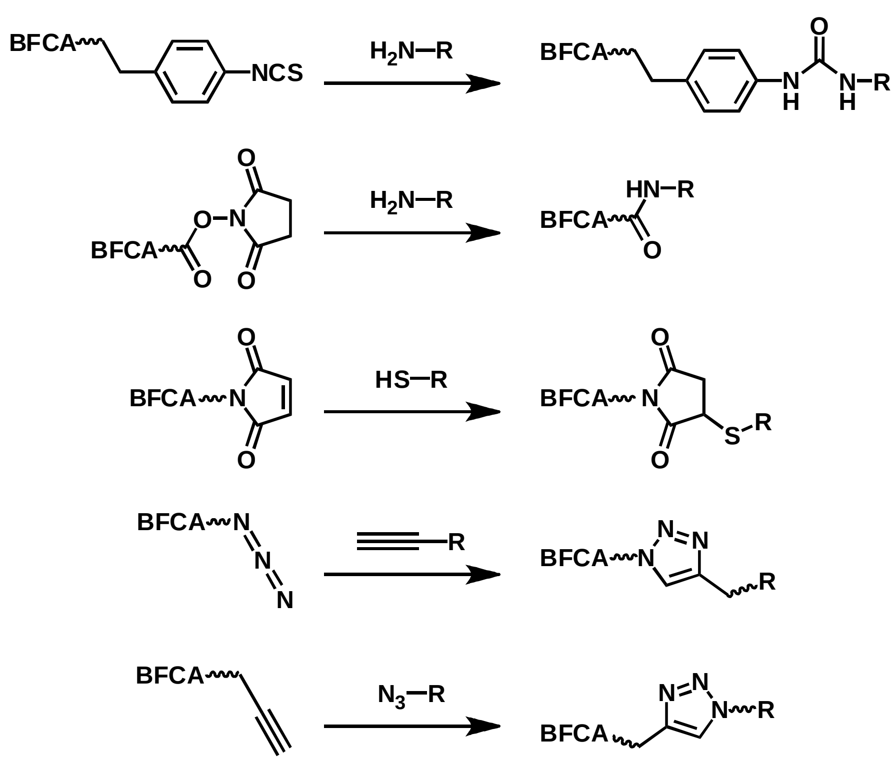


Fig. (27). The most commonly applied chemical reactions for the conjugation of chelators.

CONFLICT OF INTEREST

None declared.

ACKNOWLEDGEMENT

None declared.

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