Therapeutic Radiopharmaceuticals

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Received February 1, 1999 (Revised Manuscript Received June 14, 1999)

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I. Introduction

Therapeutic radiopharmaceuticals are radiolabeled molecules designed to deliver therapeutic doses of ionizing radiation to specific disease sites (most often cancerous tumors) with high specificity in the body. Unsealed source radiolabeled agents have been used for treatment of cancers for over five decades.[1−5] Therapies such as ^{131}I-sodium iodide for treatment of thyroid cancer and ^{89}Sr-strontium chloride and ^{32}P-sodium phosphate for the relief of bone pain associated with skeletal metastasis are well established.[1−8] Conventional or external beam radiotherapy plays a vital role in treatment of cancers; however, it is not effective for treatment of secondary or metastatic cancer sites outside of the treatment area. In contrast, systemic administration of radiopharmaceuticals that are designed for site-specific localization provides the opportunity for treatment of widely disseminated disease.[3,9−20] Ideally, therapeutic radiopharmaceuticals are designed to locate with high specificity at cancerous foci, even when their location in the body is unknown, while producing minimal or tolerable radiation damage to normal tissues.[3,12−15,17−24] Unfortunately, it is difficult to achieve this goal due to a variety of factors which are directly related to the chemical and physical characteristics of the radiopharmaceutical.[25−33]

Over the past two decades, interest in developing unsealed radiolabeled drugs for treatment of cancers has been increasing due to the emergence of a diversity of sophisticated molecular carriers (e.g., immuno-derived molecules, receptor-avid tracers, etc.).[15,18,27,34−50] Technological advances in the molecular biology, combinatorial chemistry, and peptide biochemistry arenas are providing novel molecular targeting vectors at an accelerating rate.[10,13,15,23,39,41,42,44,46,48,49,51−58] To fully realize the potential of these advances, it is critical that radiolabeling of these vectors be performed in a manner that will minimally alter or enhance their capacity for high specificity in vivo targeting of cancer cells.[12,13,27,38,50,59−61]

The design of each radiotherapeutic agent requires optimizing the balance between specific in vivo targeting of the disease site (i.e., cancerous tumor) and the clearance of radioactivity from nontarget radiosensitive tissues as well as the physical radioactive decay properties of the associated radionuclide.[12,29,50,61−70] Several difficulties are encountered in the design of highly selective radiolabeled drug carriers that must be addressed. These include problems related to efficient drug delivery, maximizing the residence time of radioactivity at target sites, in vivo catabolism and metabolism of the drug, and optimization of relative rates of the radiolabeled-drug or -metabolite clearance from nontarget sites to name a few.[71−74] Because of the multiple parameters that must be considered, developing effective radiotherapeutic drugs is a complex problem which is not simply accomplished by attaching a radionuclide, in any fashion, to a nonradiolabeled targeting vector.[12,24,27,38,48,63,66,75,76] The chemistry involved in the labeling process, therefore, is an integral and es-

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10.1021/cr9804386 CCC: $35.00
Published on Web 08/11/1999

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sential part of the drug design process. For example, if a radiometalated chelate is appended at some point to a biomolecular targeting entity, the structure and physicochemical properties of the chelate must be compatible with, and possibly even help promote, high specific uptake of the radiopharmaceutical at the diseased site.[41,63,66,77,78] At the very least, this radiometal chelate should not interfere with binding specificity and affinity to cancer cells. In addition, the radiolabeled moiety must be directly involved in optimizing the biochemical or cellular processes to residualize radioactivity at the tumor site and facilitate clearance of radioactivity from other nontarget parts of the body.[12,50,61,65,69,78,79] Clearly, the selection of the radionuclides and the chemical strategies used for radiolabeling of molecules are critical elements in the formulation of safe and effective therapeutic radiopharmaceuticals. The approach used in this review includes a section on the considerations involved in identifying the radionuclides that hold potential for the development of radioactive therapeutic drugs. Other sections include reviews of some of the synthetic and design strategies used for producing radiolabeled molecules and discussing the utility of specific radiopharmaceuticals that either are routinely used or hold potential for use in humans.

II. Therapeutic Radionuclides

An inherent determinant in developing any therapeutic radiopharmaceutical is the selection of appropriate radionuclides.[13,80−84] The choice of the radionuclide will depend on the nuclear emission properties, the physical half-life, the decay characteristics, the in vivo pharmacokinetics of the radiopharmaceutical, the cost, and availability.[83−86] Particle-emitting radionuclides are effective for delivering localized cytotoxic doses of ionizing radiation.[13,21,87−89] Radionuclides that decay by β-particle emission, α-particle emission, and Auger-electron emission have been used in nonsealed source radiotherapeutic agent development.[80,90] Each type of these particles has different effective range and linear energy-transfer (LET) properties. The type of particle emission that is applicable will depend on the size of the tumor, intratumor distribution (i.e., degree of heterogeneity of radiotracer deposition), pharmacokinetics of the tracer, and other factors.[13,80,87,88,91] γ-ray emission may or may not accompany the particle emission process and will contribute little to the therapeutic effectiveness but will augment irradiation to nontarget tissues. In cases where the γ-ray energy is in the diagnostically useful range, radionuclide imaging of the tracer biodistribution in patients is feasible.[13,83,85]

The physical half-life is a critical consideration in the design of therapeutic radiopharmaceuticals. The half-life of the radionuclide should be matched well with the in vivo biolocalization and clearance properties of the radiolabeled drug.[13,21,38,83,92] If the delivery of the radiotracer to tumors is slow and the residualization of radioactivity in the tumor and body residence time is prolonged, as is the case with radiolabeled monoclonal antibodies (MAbs), the half-life of the radionuclide should be long.[23,60,93] In contrast, if tumor uptake and clearance from the blood and extracellular fluid is rapid, radioisotopes with shorter half-lives are more appropriate.[13,14,68,83,85,92,94] From a practical standpoint, it is essential that the short-lived radionuclides be readily available in large quantities at affordable costs, otherwise they will not be able to satisfy potential patient treatment demands or achieve widespread medical applicability.

Specific activity (i.e., Ci/μg or GBq/μg) is also an important issue in designing many radiopharmaceuticals. Site-specific radiolabeled compounds should be prepared as high specific activity drugs since they target low-capacity systems (e.g., receptors or anti-

gens that are uniquely or overexpressed by cancer cells).[10,12,27,38,92] Routine availability of no-carrier-added (NCA) radioisotopes (e.g., ≥2−5 Ci/μg) is essential for widespread patient utilization and applicability of site-specific therapeutic radiopharmaceuticals.[10,83,85] Because of the constraints due to particle-emission properties, half-life, specific activity, cost, and availability, only a limited number of radionuclides have been used to design radiopharmaceuticals for therapeutic applications in medicine.

In some applications, radiolabeled molecules with maximal specific activities exhibit poorer in vivo selective tumor targeting than the corresponding radiopharmaceutical at reduced specific activities. For example, many radiolabeled MAbs administered at lower specific activities achieve higher tumor deposition and tumor to nontarget ratios.[38,86,95] It is postulated that unlabeled MAbs in the lower specific activity preparations will bind to circulating antigens and/or occupy antibody-avid binding sites present on normal tissues (e.g., liver) that are otherwise present at sufficient levels to bind with a large fraction of the high-specific activity radiolabeled MAb products.[42,96,97] Even under these circumstances, it is essential that the specific activity radionuclide reagent used to prepare these lower specific activity radiopharmaceuticals is high to provide a well-defined product that can be modified (i.e., carrier molecules added) to produce specific activities of the final radiopharmaceutical product that will provide optimal specificity for in vivo targeting of the cancers.

A. β-Particle Emitting Radionuclides

Radionuclides that decay by β-particle emission are used most extensively for radiotherapeutic applications in current clinical practice.[13,83,84,98] Utilization of β-particle emitters provides a mechanism to produce a highly homogeneous radiation dose even though their deposition is heterogeneously distributed in target tissues (e.g., tumors).[13,98,99] Particle ranges of high-energy electrons emitted from these radionuclides means that considerable cross-fire will occur with possible sterilization of untargeted neoplastic cells from radioisotopes deposited on neighboring tumor cells.[13,80] The β particles are high energy electrons emitted from the nucleus as a spectrum or continuum of energies (and ranges) up to a maximum value. The range of these high-energy electrons is much greater than α particles, and the low ionization density along their tracks accounts for their low LET.[98,100] Depending upon the β-particle energy, the tumor size for optimal curability will vary.[21,87] A simplified estimation of the radiation dose delivered to the tumor can be made, assuming complete (i.e., absorbed fraction = 1) and homogeneous absorption of all β-particle energies within the tumor by the following equation: D_β(Gy) = 19.9 × conc(MBq) × E_β(avg) (MeV) × T_{eff} (days). However, the microdosimetry is far more complex.[87,100,101] Smaller metastatic tumors targeted with higher energy β-particle emitters will receive a reduced radiation dose per emission, due to deposition of a larger fraction of the particle energy outside of the tumor volume.[21,87,99] In contrast, when the tumor is large in comparison to the range of ionizing particles, most of the energy is deposited within the tumor.

Table 1. Selected β-Particle Emitting Radionuclides with Therapeutic Potential

radionuclide	$t_{1/2}$ (days)	max E_β (MeV)	γ-ray energy (MeV)
^{32}P[a]	14.3	1.71	
^{47}Sc[b]	3.4	0.6	0.159 (68%)
^{64}Cu[b]	0.5	0.57	0.511 (38%)
^{67}Cu[b]	2.6	0.57	0.184 (48%)
			0.092 (23%)
^{89}Sr[a]	50.5	1.46	
^{90}Y[c]	2.7	2.27	
^{105}Rh[a]	1.5	0.57	0.319 (19%)
			0.306 (5%)
^{111}Ag[b]	7.5	1.05	0.342 (6%)
^{117m}Sn[a]	13.6	0.13	0.158 (87%)
^{131}I[a]	8.0	0.81	0.364 (81%)
^{149}Pm[a]	2.2	1.07	0.286 (3%)
^{153}Sm[a]	1.9	0.8	0.103 (29%)
^{166}Ho[a]	1.1	1.6	0.81 (6.33)
^{177}Lu[a]	6.7	0.50	0.113 (6.4%)
			0.208 (11%)
^{186}Re[a]	3.8	1.07	0.137 (9%)
^{188}Re[d]	0.7	2.11	0.155 (15%)

[a] Radionuclides produced in nuclear reactors. [b] Radionuclides produced in charged particle accelerators. [c] ^{90}Y is generated from a long-lived ^{90}Sr parent, which is reactor produced and supplied only as a NCA ^{90}Y product. [d] ^{188}Re is produced as a NCA reagent from a ^{188}W/^{188}Re generator system.

β-particle emitting radionuclides that hold potential for use as the cytotoxic compliment of therapeutic agents in human medicine have been identified in several review articles.[13,83,87,100] Table 1 provides a list of radionuclides that are used as FDA approved drugs as well as a limited selection of others with therapeutic potential.

These radioisotopes are produced in either nuclear reactors or particle accelerators. Most, but not all, are produced as high specific activity reagents. Some are available as NCA products from radionuclide generator systems (e.g., ^{90}Y, ^{166}Ho, and ^{188}Re).[83−85,102−104] The rationale for selection of one of these or other radionuclides for specific therapeutic applications including the relative advantages of one radionuclide compared to the other have been discussed elsewhere.[13,81−83,85]

B. α-Particle Emitting Radionuclides

Radionuclides that emit α particles are attractive in settings where it is more advantageous to use particulate radiation with a range of only a few cell diameters.[89,91,105,106] α particles are high-energy helium nuclei that produce high densities of ionization along their linear tracks and are classified as high LET radiation.[89,91,106,107] These monoenergetic emissions deposit their energy over short ranges (i.e., usually between 40 μm to 100 μm).[105,106,108] Because of their relatively short penetration in range and high LET, α particles have the capability for producing a high degree of tumoricidal activity while sparing the surrounding normal tissues.[89,99,108,109] Their high LET also makes α particles more effective in killing cancer cells that are in a hypoxic environment within the tumor.[21,91,108,109] In contrast to β-particle emitters, α-particle emitters are more compatible for use in treatment of tumors with small diameters and where

their localization within the tumor is more spatially homogeneous.

Although more than 100 radionuclides exist which decay by the emission of α particles, the vast majority have half-lives that are too long to be compatible with in vivo applications.[89,106,110] In addition, most α-particle emitters are difficult to produce in large quantities with acceptable radionuclidic purity. As a result, only two α emitters have received serious attention for radiotherapeutic applications: ^{211}At and ^{212}Bi. ^{211}At ($t_{1/2}$ = 7.2 h, E_α(avg) = 6.8 MeV) must be produced in particle accelerators, more frequently via the ^{209}Bi (α, 2n) reaction.[106,110,111] ^{212}Bi ($t_{1/2}$ = 1 h, E_α(avg) = 7.8 MeV) is obtained from the ^{224}Ra/^{212}Bi generator system.[106,107,112] Recently, an ^{225}Ac/^{213}Bi generator system was developed that could stimulate increased efforts to develop ^{213}Bi ($t_{1/2}$ = 0.76 h) radiopharmaceuticals.[113,114]

C. Low-Energy Electron Emitters

The majority of low-energy Auger electrons emitted during radioactive decay deposit their energy over subcellular dimensions, producing highly localized energy density in the immediate vicinity of the decay site.[115−117] In vivo and in vitro studies demonstrate that the toxicity of Auger-electron emitters approximates that for low LET radiation when the emitter is localized in the cytoplasm and that for high LET α particles when the Auger-electron emitter is covalently bound to DNA in the cellular nucleus.[118−120] Thus, to realize a high LET-like response, potential therapeutic radiopharmaceuticals labeled with Auger-electron emitters requires Auger election emitter uptake and residualization within the nuclei of cancer cells.[100,120,121] In addition, in order for complete eradication of cancer cells in a tumor, the Auger-emitting radiopharmaceuticals must be capable of localizing in all of the targeted cells. Thus, methods of selective guidance to target cells and subsequent introduction of these radionuclides intracellularly must be found. While efforts are being made in designing therapeutic radiopharmaceuticals with several promising Auger-electron emitters, the design of effective Auger-emitting targeting agents for in vivo targeting and treatment of cancers remains a challenge.

III. Therapeutic Radiopharmaceuticals for Routine Medical Use

A wide variety of radiolabeled agents have been applied clinically in the treatment of malignant and benign conditions over the past several decades. However, the number of therapeutic radiopharmaceuticals that received FDA approval for routine use in humans and available on a commercial basis has been comparatively small. Falling into this category are ^{131}I-sodium iodide for treatment of thyroid disorders, ^{32}P-phosphate for blood disorders, ^{32}P-sodium phosphate, ^{89}Sr−chloride, and ^{153}Sm−EDTMP for pain control in metastatic bone disease, ^{131}I-mIBG for neuroendocrine tumors, and ^{90}Y- or ^{32}P-colloids or -microspheres for intracavitary and intraarterial delivery to tumors.[1,2,4−6,6−8,122−128] Over the past several years, a great deal of effort has been made to identify and develop more sophisticated site-directed radiotherapeutic agents with a significant number currently undergoing further evaluation in human clinical trials.

A. ^{131}I-Sodium Iodide

The first therapeutic use of ^{131}I-radioiodide was reported in 1941.[129] ^{131}I-sodium iodide has and continues to be used extensively worldwide for treatment of hyperthyroidism and differentiated thyroid carcinoma.[1,6,130,131] ^{131}I-iodide is effective for treatment of these diseases due to the specificity of uptake by thyroid cells to provide selective irradiation (primarily from β-particle emissions) to the thyroid gland or thyroid cancer tumors.

There is a wide body of literature on the treatment of thyroid disease with ^{131}I-sodium iodide.[1,3,5,6,130,132] The doses administered to patients for effective treatment of thyroid disease usually ranges from 5 to 10 mCi (185−370 MBq) but can be somewhat higher.[3,130] Administration of this quantity of ^{131}I delivers a tyroid dose of approximately 100 Gy. The exact magnitude of the radiation dose to the gland depends on the percent iodide uptake and its size. The principal disadvantage of ^{131}I-radioiodine therapy is the high incidence of early or late hypothyroidism, making it necessary to monitor patients adequately after treatment.[3,6,133] Although there is a theoretical possibility of radiation-induced cancer or genetic defects, there is no experimental evidence to support this hypothesis based on several large follow-up studies conducted in humans over the past 30 years.[134,135]

^{131}I-iodide has been effective for eradicating differentiated thyroid carcinomas (follicular or papillary) that metastasized to distant sites (including bone and lung).[3,6,132] Following thyroidectomy, an ablative dose of ^{131}I-sodium iodide (usually in the range of 100−200 mCi (or 3700−7400 MBq) is administered.[6,132] The uptake of the functioning tumors is sufficiently great (about 0.1%/g average) to deliver a selective ablative tumor dose.[132] Response to therapy can be assessed by repeating radionuclide imaging with radioiodine and, if necessary, administering additional therapeutic doses until there is no longer evidence of tumor uptake.[6,133] The routine use of ^{131}I-sodium iodide for over 50 years to cure patients with metastatic thyroid carcinoma is well documented.[1,6,130,132] This simple radioactive anion ($^{131}I^-$) serves as the most prominent example demonstrating that widely disseminated tumors can be systemically targeted by a therapeutic radiopharmaceutical with sufficient specificity to totally eradicate the metastatic disease in humans.[136]

B. Intracavitary and Intraarterial Radiopharmaceuticals

Intracavitary Agents. ^{32}P-chromic phosphate colloid is an FDA-approved radiopharmaceutical used for treatment of intraperitoneal metastasis malignant effusions.[1,131,132] This drug replaced the formally used ^{198}Au-colloid because ^{198}Au emitted undesirable

high-energy γ radiation leading to unnecessary exposure of nontarget tissues and personnel.[132] It has been shown to be effective for treatment of ovarian cancer, where the entire peritoneal cavity is at risk from metastatic spread. It can also be used to reduce accumulative serosal cavities of the peritoneum pleura and pericardium, produced by dissemination of a variety of cancers (including ovarian, renal, breast, lung, and GI cancers).[132] A major problem with therapy ^{32}P-colloids (and other radiolabeled colloids) relates to nonspecificity of their localization and uptake in the cancer cells. These colloids are delivered with minimal or no discrimination to both normal and tumor tissues within the cavity.[131,132] Tumor-specific antibodies labeled with particle-emitting radionuclides (including ^{131}I, ^{90}Y, ^{177}Lu, etc.) are providing opportunities for specific targeting and delivery of the radionuclide to intracavitary tumor cells.[40]

Radiocolloids have been and are being evaluated for radionuclide synovectomy. Radionuclide synovectomy provides an alternative to surgical synovectomy (removal of the inflamed joint lining) in patients with chronic rheumatoid arthritis.[137−140] Several radionuclides have been used (including ^{90}Y, ^{188}Re, ^{169}Er, ^{32}P, ^{165}Dy, and ^{153}Sm) to prepare radiolabeled colloids, macroaggregates, and particulates.[139,140] In theory, it is possible to select the optimum radionuclide for specific joints and synovial thicknesses.[140] While studies have demonstrated the value and effectiveness of these radio-synovectomy agents in humans, much more effort will be required to develop a commercially available agent for routine use in patients.

Intraarterial Microspheres. Radiolabeled particles have been used for delivering a high localized radiation dose to tumors in a variety of organs following intraarterial administration for over 40 years.[141] Delivery of a highly selected distribution of radiation doses that are 20−30 times greater than that achievable by external beam therapy is possible by virtue of the preferential blood flow to the tumor relative to normal tissues.[142] The microspheres have sizes large enough (i.e., >10−15 μm) to lodge in the end arterioles and capillaries in the tumor (and normal tissues) which minimizes or prevents delivery of the injected radioactivity to other organs or tissues in the body.[142,143] Several types of particles and microspheres have been utilized (including ^{90}Y-labeled-Y_2O_3 particles, ^{90}Y-ceramic microspheres, ^{90}Y-resin microspheres, and ^{90}Y-glass microspheres).[142,143] ^{90}Y-glass microspheres are currently the only radiolabeled microsphere product (i.e., TheraSpher) that is approved for routine use in North America (Canada) for treatment of cancers in humans. Approval for routine patient use in the United States is actively being pursued for treatment of primary hepatocellular carcinomas. ^{90}Y-labeled microspheres, initially developed by Ehrhardt and Day,[144] is a product in which ^{90}Y is an integral component of the insoluble glass sphere. The sterile pyrogen-free 20−30 μm glass spheres are preformed by incorporating ^{89}Y−oxide into the glass matrix and subsequently activated by neutron bombardment in nuclear reactors to convert ^{89}Y in the glass to ^{90}Y, before using the spheres as radiotherapeutic vehicles. With the ^{90}Y imbedded in the spheres, it is not leached from the glass nor metabolized, which prevents in vivo mobilization of ^{90}Y to distant organs or tissues in the body.[144,145] This agent is currently being used primarily as a radiopharmaceutical for treatment of cancerous tumors in the liver.[146]

C. Radiotherapeutic Agents for Bone Cancer Treatment

Therapeutic radiopharmaceuticals administered intravenously have been used since 1942 to relieve pain from skeletal metastases.[4,147] Currently, three radiopharmaceuticals are approved by the U.S. FDA for routine use in humans: ^{32}P-sodium orthophosphate, ^{89}Sr-strontium chloride, and ^{153}Sm−EDTMP. For many years, ^{32}P-sodium orthophosphate was the most widely used agent for palliation of bone cancer pain.[2,7] The use of ^{32}P-phosphate in a limited number of patients continues to the present day; however, the successes with this drug have been tempered by mild to severe bone marrow depression that follows intravenous administration of this drug.[2,132,148] As a result, ^{89}Sr−chloride (Metastron) and more recently ^{153}Sm−EDTMP (Quadramet) are more widely used in the United States than ^{32}P-orthophosphate. The greatest challenge in designing effective radiopharmaceuticals for treatment of bone cancer pain is maximizing the radiation dose to the cancer cells in bone and minimizing radiation-induced bone marrow suppression.

The penetrating ability of the β particles emitted from these radiopharmaceuticals may have profound implications on the potential success of these as well as other types of radiopharmaceuticals.[13,98,99,127,140] The longer the range (i.e., the higher energy) of the β particle, the greater the irradiation of cells further removed from the site of radionuclide deposition. For tumors in bone originating from nonosseous cancers (e.g., prostate or breast cancers), the higher energy β-particle emitters will be able to reach and irradiate cancerous tissues that are located at distances further from the osseous tissue at the bone/tumor interface on which the radiotracer is deposited.[127,149−152] On the other hand, the longer range of the higher energy β particles will also deliver high radiation doses to normal tissues that are also located at greater distances from the radiotracers deposited on bone surfaces. This could increase the severity of side effects (e.g., bone marrow suppression).[95,127,153] The bone marrow suppression produced by these agents primarily results from radiation doses delivered to the red marrow by β particles that are emitted from the radionuclide deposited on or near the surface of the bone/marrow interface.[95,127,153] The β-particle dose delivered to the cancerous tumor relative to the bone marrow dose will directly effect the therapeutic index of the radiotracer.[151,154,155] Further studies in humans are required to determine the optimal β particle energy for specific therapeutic applications.

1. Skeletal Localization Mechanisms

Definitive mechanistic information about the processes responsible for radiopharmaceutical deposition

in bone cancer lesions remains elusive due to many complicating factors. A major reason for this lack of understanding stems from the fact that there is no unified overview of the mechanisms controlling normal bone mineralization.[156−158] The chemical and biological processes involved in skeletal metastases or during repair of bone are even less well understood. Experimental results, however, suggest that three general mechanisms can be proposed to explain the increased accumulation of the radiotracers in sites of growing or remodeling bone: local hypervascularity, interaction with the organic matrix, and incorporation into the mineral phase during the process of calcification.[153,156,157] A better understanding of the relative importance of these and other factors will improve our ability to design new and more sophisticated radiolabeled compounds for bone cancer therapy.

The chemical properties of the radionuclide used in the radiopharmaceutical is a major determinant on the deposition and retention of the radioactivity on bone cancer lesions. Some important insights about the fundamental chemical aspects involved in radiometal uptake in normal and abnormal osseous tissues can be gained by examining the inorganic chemistry of the disparate elements used in this area. For example, the periodic trends which govern whether a metal forms an acidic or basic oxide will determine the thermodynamically stable form of the radioisotope in mammals and play an important role in determining their biolocalization properties.

Basic oxides are those that yield alkaline solutions when added to water, whereas acidic oxides yield acidic solutions when added to water. Those elements which form basic oxides but carry a low formal charge (i.e., +1 or +2) tend to form soluble hydroxides.[153] These soluble cations (e.g., ^{47}Ca(II) and ^{89}Sr(II)) are relatively mobile in vivo.[156,157,159,160] In contrast, basic oxides that carry a higher formal charge (e.g., +3 or +4) tend to form insoluble hydroxides and form insoluble compounds (e.g., hydroxides) in vivo and are immobile (including ^{153}Sm(III), ^{166}Ho(III), and ^{117m}Sn(IV)).[153,161−164]

Acidic oxides can bear high formal charges that are neutralized in aqueous media by oxo ligands (O^{2-}). These elements tend to exist as anionic forms which are soluble and mobile in vivo (e.g., ^{32}P-phosphate and $^{186/188}$Re0). Whether or not these radioisotopes exist as acidic or basic oxides depends on their oxidation state. For example, ^{188}Re(IV) compounds (e.g., ReO_2) can be immobile in vivo as a basic oxide, however, upon oxidation to the ^{186}Re-perrhenate will become water soluble and mobile as an acidic oxide.[161,165,166]

The chemical principles provide a foundation for understanding four possible mechanisms for deposition of radiotracers on skeletal tissues which have been described in the literature. The first process involves the alkaline earth metals (e.g., ^{89}Sr(II)) which absorb to bone by exchange with the surface calcium ions on the hydroxy apatite matrixes (the integral part of the mineral component of osseous tissues).[7,157,167] ^{89}Sr(II), as with Ca(II), is soluble in vivo, and the major process of concern is cation exchange at the surface of hydroxy apatite.[156,168,169]

The second mechanism involves the reaction of +3 and +4 charged cations with hydroxyapatite to yield insoluble phosphates or hydroxide salts firmly adhered to the surface of the mineral matrix in bone. ^{153}Sm(III), ^{166}Ho(III), and ^{117m}Sn(IV) are expected to be absorbed by this mechanism and will generate a thermodynamically stable state with minimal or no significant resorption or in vivo mobilization.[153,161] The third process involves chemisorption of phosphates and phosphonates to the surface of hydroxyapatite. Examples of agents expected to act by this mechanism include ^{32}P-orthophosphate and other organophosphonate compounds labeled with therapeutic radionuclides such as ^{32}P, ^{131}I, or ^{211}At.[170,171] The fourth mechanism involves bridging of radiometals to hydroxyapatite by multidentate phosphonate and carboxylate chelate systems. This is the mechanism considered to be employed by $^{186/188}$Re−HEDP[153,161] for its deposition in bone. This presumably is also the mechanism by which ^{153}Sm−EDTMP, ^{166}Ho−DOTMP, and ^{117m}Sn−DTPA are initially adsorbed on the mineral matrix in bone.[153,161] It is likely that following delivery of these basic oxides to hydroxyapatite, the radiometals will dissociate from their respective ligands and adhere directly to the hydroxyapatite surface. The rate and extent of this type of transmetalation reaction will depend on the kinetic and thermodynamic stability of the complexes relative to that of the respective metal complexes on the bone surface. In addition to these four mechanisms, which only consider a deposition of radiotracers on the mineral matrix in bone, other mechanisms (including incorporation into the organic osseous matrixes) may also play significant roles in facilitating localization of radiometalated drugs on skeletal tissues.[156,157]

2. ^{89}Sr–Chloride

Ionic ^{89}Sr(III) has been used to treat painful skeletal metastases for over 50 years[2,7,8] and was approved by the U.S. FDA for routine use in humans in 1995. ^{89}Sr(II) is an alkaline earth and exhibits in vivo reactivity toward osseous tissues by virtue of its similarity to Ca(II).[2,156,157,168] While there are differences between Sr(II) and Ca(II), it is generally accepted that Sr(II) binds to the hydroxyapatite matrix by exchange with Ca(II) or interacting with sites normally involved in Ca(II) binding during initial stages of calcification.[157,167,172] ^{89}Sr is reabsorbed from normal bone into blood and other fluids, as is Ca(II).[156,157,167,172] This is an important property that differentiates it from other bone-seeking radiopharmaceuticals. This redistributive property of Sr(II) makes β-particle microdosimetry with ^{89}Sr in bone somewhat different than the other "surface seeking" radiotracers.[155,159,173−175]

Monitoring of the uptake and pharmacokinetics of ^{89}Sr (a pure β-particle emitter) in humans was performed using co-injected tracer levels of ^{85}Sr(II), a Sr γ-ray emitting radioisotope that can be externally detected and imaged.[159] ^{89}Sr was shown to concentrate approximately 5-fold higher in bone

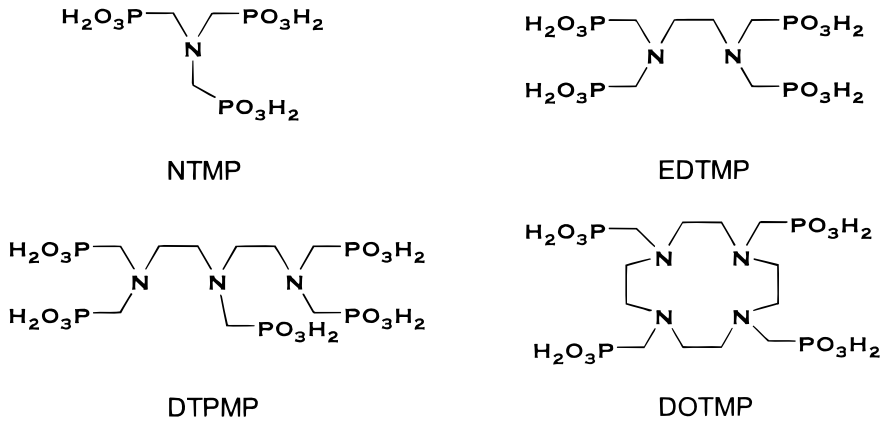

Figure 1. Nitrilotrimethylenephosphonic acid (NTMP), ethylenediaminetetramethylenephosphonic acid (EDTMP), diethylenetriaminepentamethylenephosphonic acid (DTPMP), 1,4,7,10-cyclododecyltetraaminetetramethylenephosphonic acid (DOTMP).

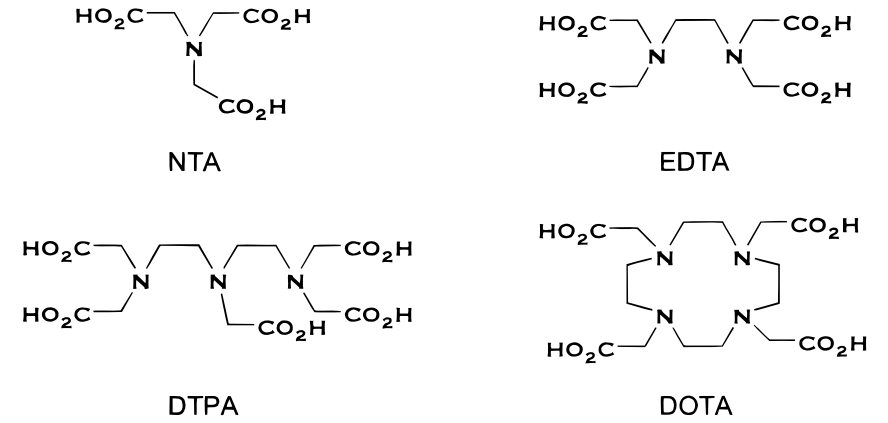

Figure 2. Nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), 1,4,7,10-cyclododecyltetraacetic acid (DOTA).

lesions than in normal bone.[176] Preferential uptake of $^{85/89}$Sr(II) occurs at osteoblastic sites in patients with nonosseous soft-tissue cancer (e.g., prostate cancer, breast cancer, etc.),[2,156,172] and the $^{85/89}$Sr concentration in the tumor site remained essentially unchanged for up to 100 days following injection.[177] The preferential uptake and long-term retention at metastatic cancer lesions in the skeleton provides for the high therapeutic index of ^{89}Sr, making it effective for treatment of painful bone cancers. Interestingly, ^{89}Sr redistributes (i.e., is not residualized) in primary bone cancers (e.g., osteosarcomas)[178] and, thus, has not been shown to be useful for treatment of cancers arising from osseous tissues. One might assume that redistribution of ^{89}Sr from these primary bone cancers occurs by mechanisms responsible for redistribution of ^{89}Sr from normal bone.

^{89}Sr−chloride (Metastron) has been shown to be efficacious for treatment of painful metastatic sites in the skeleton. Results from several human clinical trials demonstrate significant pain relief in approximately 70−80% of patients.[161,176,179,180] The standard intravenous administered dose in current clinical use is 4 mCi (148 MBq); alternatively, a dose of 55 μCi/kg (2.2 MBq/kg) is used.[179,180] Toxicity at this dose is limited to radiation-induced marrow suppression. This is usually manifested as thrombocytopenia, which reaches a nadir in 4−6 weeks, recovering slowly thereafter, reaching maximum recovery by 12 weeks.[179]

3. ^{153}Sm–EDTMP

^{153}Sm−ethylenediaminetetramethylenephosphonate (^{153}Sm−EDTMP) was first reported as a potential radiopharmaceutical for treatment of bone cancer lesions by Goeckeler and co-workers.[164,181] This particular radiolabeled chelate emerged as the most promising candidate based on results of a structure−activity-relationship (SAR) study with a series of multidentate phosphonic acid and multidentate carboxylic acid ligands.[153,182] An abbreviated series of some of the ligands studied are shown in Figures 1 and 2. The thermodynamic stability constant (K_s) of Sm(III) with all of the ligands in Figures 1 and 2 are high. The log K_s for the corresponding Sm(III) chelates with the phosphonate ligands shown in Figure 1 at neutral pH range from 16.9 to 25 (where K_s for Sm−NTMP < Sm−DTPMP < Sm−EDTMP < Sm−DOTMP).[153,182] The log K_s for the corresponding Sm(III) carboxylate chelates shown in Figure 2 at neutral pH range from 11.3 to 23.0 (where K_s for Sm−NTA < Sm−EDTA < Sm−DTPA < Sm−DOTA).[153,182] In the case of the ^{153}Sm−aminophosphonate complexes, there is no apparent relationship between K_s values and bone uptake (i.e., percent injected dose) of ^{153}Sm in the skeleton and all ^{153}Sm−aminophosphonates show high bone uptake in rats. These results suggest that ^{153}Sm−aminophosphonates are initially absorbed into the hydroxyapatite matrixes in bone via the interaction of the phospho-

nate group oxygen atoms on the ^{153}Sm chelate.[153,182] In contrast, there is a K_s relationship where aminocarboxylate chelates with higher K_s values (e.g., ^{153}Sm−DTPA and −DOTA) result in no significant bone uptake, while lower stability complexes exhibit high bone uptake.[153,182] Since the aminocarboxylate ligands themselves (i.e., nonmetalated) exhibit no significant long-term affinity for bone, it can be concluded that exchange of the radioactive metal from these chelates to hydroxyapatite is the primary localization mechanism responsible for deposition of ^{153}Sm into osseous tissues.[153,182]

The pharmacokinetics and in vivo clearance properties of ^{153}Sm−EDTMP in both animals and humans are excellent. In addition to the high and preferential localization of ^{153}Sm−EDTMP in bone cancer lesions, excretion of this radiolabeled chelate occurs almost exclusively and rapidly via the kidneys into the urine.[183−186] Within the first 15 min after intravenous administration of the complex, localization in the kidneys and the skeleton is high relative to all other organs and tissues.[184,187] Furthermore, within 30 min following administration, most of the nonbone associated radioactivity is in the urine.[153,183] There is no evidence of measurable in vivo metabolism of ^{153}Sm−EDTMP since it clears very efficiently from all nontarget tissues and fluids and is excreted unchanged into the urine, as analyzed by HPLC.[153,188] The rapid and efficient clearance of this radiopharmaceutical by the GFR route is directly related to the high negative charge of the complex.[153,183] Speciation computations for Sm−EDTMP indicate that the predominant species in solution at physiological pH is $[Sm-EDTMP]^{-5}$.[96,153] A more recent report estimates that in the hydroxyl form, $[Sm-EDTMP]^{-6}$ is the major secondary species at physiological pH.[96] $Sm[EDTMP]^{-4}$ is also present but comprises less than 10% of the total Sm−EDTMP species present at neutral pH.[96,153]

It is important to note that the ^{153}Sm−EDTMP radiopharmaceutical preparation (Quadramet) used for routine clinical applications has a ligand-to-metal ratio of approximately 250−300:1.[153,186,187] A large ligand excess is required, otherwise uptake of some ^{153}Sm is observed in the liver.[153,189] The presence of excess EDTMP in the blood is required to prevent dissociated ^{153}Sm(III) in the plasma from forming insoluble ^{153}Sm−hydroxyl species.[153,189] This is a reflection of the fact that despite the high K_s for Sm−EDTMP, the complex is not kinetically inert.[96,182] However, with excess EDTMP or Ca(II)−EDTMP in the plasma, formation of insoluble ^{153}Sm compounds does not occur. The uptake and residualization of ^{153}Sm−EDTMP in primary bone cancers (e.g., osteosarcoma, fibrosarcoma, and chondrosarcomas) was demonstrated in a study with tumor-bearing dogs by Lattimer and co-workers.[183,190,191] In this study, 40 dogs with an array of spontaneous primary bone cancers were treated with either 0.5 or 1.0 mCi/kg (18.5−37 MBq/kg) of ^{153}Sm−EDTMP. The results of individual treatments varied from spectacular to no effect. Four of the dogs were considered free of disease for 1 year and longer after administration of the radiopharmaceutical.[183,190] Twenty-five dogs had partial response to the ^{153}Sm−EDTMP treatment as evidenced by functional improvement, radiographic evaluation, and increased longevity.[183,190] These canine studies demonstrate that ^{153}Sm−EDTMP holds important potential for treatment of primary bone cancers. Results from ongoing clinical trials in humans will provide the necessary data to determine whether ^{153}Sm−EDTMP is also useful for treatment of primary bone cancer lesions in humans.

^{153}Sm−EDTMP (Quadramet) was approved by the U.S. FDA in 1997 for routine use in humans and has been shown to be efficacious for treatment of painful skeletal metastases. Several clinical trials demonstrated significant pain relief in approximately 70−80% of patients studied (i.e., a level similar to ^{89}Sr−chloride) at the standard intravenously administered dose of 1 mCi (37 MBq) per kg.[184−187,192] As with ^{89}Sr−chloride, toxicity is limited to bone marrow suppression manifested by both thrombocytopenia and decreased leukocyte counts, which reach a nadir at approximately 4 weeks, recovering to normal levels in approximately 6 weeks.

4. ^{186}Re–HEDP and ^{117m}Sn–DTPA

Both ^{186}Re−hydroxylethyenediphosphonate (^{186}Re−HEDP) and ^{117m}Sn−diethylenetriamine-pentaacetic acid (^{117m}Sn−DTPA) are being actively evaluated in human clinical trials as potential FDA-approved radiopharmaceuticals for treatment of painful skeletal metastases.[162,193−198] While several other agents have also been studied in humans,[170,171,199] significant efforts to conduct well-controlled clinical trials with ^{186}Re−HEDP and ^{117m}Sn−DTPA have been and continue to be made.

^{186}Re−HEDP. ^{186}Re−HEDP was first suggested as a possible therapeutic agent to palliate painful bone metastases in 1979.[200] ^{186}Re−HEDP is prepared by a method that is analogous to ^{99m}Tc-labeled diphosphonates which are widely used as skeletal imaging agents.[198,201] Perrhenate is reduced with stannous ion in the presence of excess diphosphonate ligand to form the drug product. This synthetic approach yields a complicated, time-dependent mixture of species which can be separated by various forms of chromatography.[202,203] While the synthetic chemistry and chemical composition of the ^{99m}Tc−diphosphonates and ^{186}Re−diphosphonates are similar, the biodistribution of these agents have some important differences. For example, the relative wash-off rate of ^{186}Re from femurs in normal rats is more rapid and extensive than the technetium analogue.[153,194] This is due to the fact that Re is more readily oxidized to perrhenate than is the technetium analogue.[165,166] Thus, the ^{186}Re(IV/V) bridged by HEDP is bonded to the bone surface; however, in vivo oxidation generates the soluble $^{188}ReO_4^-$ which undergoes wash-off and ultimately excretion, primarily through the renal system.[195,204] Understanding the fundamental chemistry underlying the wash-off of ^{186}Re−HEDP from osseous tissues allows flexibility in the design of new tracers. By varying the nature of the diphosphonate ligand, it may be possible to increase the wash-off of ^{186}Re from normal bone relative to the cancerous sites. This would, therefore, increase the abnormal/

Table 2. Radiopharmaceuticals for Bone Cancer Therapy

	radionuclide		
radiopharmaceutical	$t_{1/2}$ (days)	E_β (avg MeV)	max β^- range in tissue (mm)
^{89}Sr−chloride	50.5	0.58	6.7
^{32}P−orthophosphate	14.3	0.70	8.0
^{153}Sm−EDTMP	1.95	0.22	3.4
^{186}Re−HEDP	3.8	0.35	4.7
^{117m}Sn−DTPA	13.6	0.127, 0.129[a]	0.3[a]
^{166}Ho−DOTMP[b]	1.12	0.75	8.6

[a] Conversion electrons emitted by ^{117m}Sn are monoenergetic, and thus, their average energy is identical to their maximum energy [$E_{(max)}$]. Similarly, each monoenergetic conversion electron has the same range in tissue. [b] ^{166}Ho−DOTMP is the only radiopharmaceutical in this table not currently used for treatment of painful skeletal metastases. It is a bone-seeking radiopharmaceutical, but because of its high-energy β-particle, it is used to treat multiple myeloma and is being considered for use in bone marrow ablation.

normal bone ratio, which in turn could increase the therapeutic index.[193,204]

The standard dose of 30−35 mCi (1.11−1.3 GBq) is generally used for patient studies.[179,193,198,204] The ^{186}Re−HEDP radiopharmaceutical is prepared using excess ascorbic acid to provide a stabilized product.[198,204] The ascorbic acid is primarily used as an antioxidant to prevent oxidation of ^{186}Re from oxidizing to $^{186}Re^0$; however, it may also play a role as a free-radical scavenger to inhibit self-radiolysis. Recent studies with ^{188}Re−HEDP have been reported that compare it with ^{186}Re−HEDP for treatment of bone metastases.[205,206] ^{188}Re−HEDP is synthesized in a procedure similar to ^{186}Re−HEDP, producing an ascorbic acid stabilized radiopharmaceutical for human applications.[205−207]

^{117m}Sn−DTPA. This radiolabeled chelate was identified as a potential bone therapy agent by scientists at Brookhaven National Laboratories.[163,208] ^{117m}Sn−DTPA is a complex with high in vitro and in vivo stability which is consistent with the high thermodynamic stability of the complex and the presence of excess DTPA in the drug product and injectate.[209−211] This chelate is negatively charged and has a high polarity which is responsible for its clearance from the blood via the kidneys into the urine.[209,211] Since the DTPA ligand itself has minimal affinity for bone, it can be assumed that ^{117m}Sn(IV) transchelates from the complex to the bone surface where, as a basic oxide metal with a +4 charge, it is residualized.[153,161] Even though this complex has high thermodynamic stability, its kinetic stability is lower, permitting transchelation to a more kinetically inert association to the bone matrix. The 127 and 129 keV conversion electrons emitted from ^{117m}Sn (Table 2) are monoenergetic and have a definitive range in soft tissue (approximately 300 μm) and bone.[208,209,212] In contrast, the β-particle emitting radionuclides used in the other bone therapy agents emit particles with a spectrum of energies and their maximum β-particles have higher energies and longer ranges than ^{117m}Sn conversion electrons (Table 2). The short path length of these conversion electrons is postulated to reduce bone marrow toxicity.[162,209,211] Data from studies in humans show high bone lesion doses with relative sparing of the marrow.[162,197,213] Phase II and III clinical trials with this agent are underway.[213]

Preliminary results from these trials with both ^{188}Re−HEDP and ^{117m}Sn−DTPA indicate that response rates of patients to therapeutic treatment with these agents are similar to both ^{89}Sr−chloride and ^{153}Sm−EDTMP.[162,197,209,214] In addition, the toxicity of both of these agents is also limited to transient marrow suppression. In general, studies with all of the bone cancer therapeutic radiopharmaeuticals will provide important insights about the potential applications of unsealed source therapeutic agents in the treatment and management of patients with cancers. For example, various trials underway to further assess and quantify the effectiveness and applicability of one or more of these radiopharmaceuticals will be important. The value of employing these radiopharmaceuticals for multiple applications involving repeat doses, assessing dose fractionation schemes in patients with less advanced cancers, and adjunctive protocols with chemotherapeutic agents and/or external beam therapy regimens will be evaluated over the next decade. Results from these and other types of clinical trials with therapeutic radiopharmaceuticals for treatment of bone cancers will have a profound influence on the design of studies for assessment of the new site-directed radiolabeled drugs currently being developed for therapy.

5. ^{166}Ho−DOTMP

^{166}Ho−1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid (^{166}Ho−DOTMP) has been shown to hold potential for treatment of multiple myeloma patients and ablation of bone marrow.[215,216] ^{166}Ho complexed with DOTMP (see Figure 1) localizes in the skeleton and clears rapidly and exclusively via the kidney into the urine by virtue of its high overall negative charge.[215,217] Even though it is a bone-seeking complex, it is not considered useful for treatment of skeletal metastases since it emits a high-energy β particle (i.e., E_β(max) = 1.85 MeV). The long-range of these β particles (Table 2) produces excessive marrow suppression when compared to ^{153}Sm−EDTMP. Since these highly penetrating β particles destroy bone marrow cells remote from the surface of the bone where ^{166}Ho−DOTMP deposits, it can be used for eradication of multiple myeloma cells and the normal stem cells located in the marrow space.[154,155,215,216] In contrast, the β-particles emitted by ^{153}Sm−EDTMP located on bone surfaces following administration of extremely high doses of ^{153}Sm−EDTMP (i.e., $\gg$1.0 mCi/kg or 37 MBq/kg) were found to be too low for complete ablation of the normal bone marrow in dogs.[177,218]

The choice of DOTMP as the chelating agent (and not EDTMP) to deliver ^{166}Ho to the skeleton was made based on the mass of material to be administered. The use of sufficient quantities of ^{166}Ho (i.e., $\geq$2 Ci or 74 GBq) that are required for marrow ablation would require several milligrams of holmium target.[197,215] Since the ^{153}Sm−EDTMP preparation for human application uses nearly a 300-fold ligand excess, the amount of EDTMP required relative to the mass of holmium would be excessive.[153,189] In

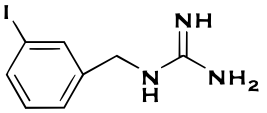

Figure 3. *m*-Iodobenzylguanidine (mIBG).

contrast, DOTMP has been shown to form a kinetically inert complex with ^{166}Ho.[153,215] This property has allowed the use of DOTMP complexed with ^{166}Ho for marrow ablation at ligand-to-metal molar ratios as low as 1.5:1.[215,216] Future studies with this agent in humans will provide critical data in determining the efficacy of this agent to ablate bone marrow in patients requiring bone marrow transplantation and for treatment of patients with multiple myeloma.

IV. Site-Directed Therapeutic Agents

The potential of using radiolabeled site-directed molecular carriers to treat cancerous tumors and micrometastases has long been recognized.[3,11−13,16,18,20] The basic premises underlying the approach of using "magic bullets" were first enunciated by Paul Ehrlich in the late 19th century.[219] The development of molecules labeled with particle-emitting radionuclides that exhibit high specificity in vivo targeting of cancer cells holds great promise for selective destruction of malignant cells. While considerable efforts have been devoted to the development of new magic bullets, none have received approval by the FDA (other than ^{131}I-sodium iodide) for routine use as therapeutic radiopharmaceuticals. Significant advances have been made over the past decade in the development of promising radiolabeled agents that have been shown to be effective for treatment of human cancers.[11,38,40,43,50,220] As a result of these efforts, several new therapeutic radiopharmaceuticals that selectively target cancer cells in vivo are being evaluated in FDA-approved phase II and III clinical trials.

A. ^{131}I-mIBG

Targeted radiotherapy is an attractive alternative to conventional therapies for treatment of neuroendocrine tumors, such as neuroblastoma and pheochromocytoma.[124,221,222] ^{131}I-*m*-iodobenzylguanidine (^{131}I-mIBG) developed by Wieland and co-workers[223−225] has been employed for imaging and adjunctive treatment of neuroendocrine tumors in the early-to-mid 1980s at the University of Michigan.[124,224,225] This compound (Figure 3) is a structural analogue of guanethidine that has structural similarities to norepinephrine. ^{131}I-mIBG is selectively taken up by adrenergic neurons, the adrenal medulla, and some neuroendocrine cancer cells by an active uptake mechanism at the cell membrane.[124,222] ^{131}I-mIBG is approved by the U.S. FDA for routine clinical use for diagnostic imaging but not for therapeutic applications. Despite the fact that numerous studies have demonstrated the effectiveness of this agent for treatment of neuroblastomas,[124,125] pheochromocytoma,[226] and other neuroendocrine tumors[124,227] in humans, considerable more work must be performed to establish the parameters essential in optimizing ^{131}I-mIBG therapy before approval for routine use can be considered.[125] Generally, doses between 100 and 200 mCi (3.7−7.4 GBq) are administered intravenously over a 4 h interval for therapeutic uses.[125,227] The major toxic side effect is radiation-induced bone marrow suppression.[121,126,222]

Over the past decade new formulations and analogues have been developed.[121,126,222] Vaidyanathan and Zalutsky[228] introduced in situ synthesis of ^{131}I-mIBG involving the iododesilylation of *m*-(trimethylsilyl)benzylguanidine to produce a no-carrier-added (NCA) ^{131}I-mIBG product, unlike the conventional preparation of ^{131}I-mIBG by halogen exchange methods.[223] Studies with this high specific activity preparation demonstrate increased tumor-to-nontarget ratios; however, increased uptake in normal tissues known to accumulate mIBG was also observed.[156,229] The reason for this observation is not understood. Thus, careful validation by human studies must be performed to assess possible advantages of using the NCA ^{131}I-mIBG preparation.[226]

The use of other halogen radionuclides (e.g., ^{125}I and ^{211}At) has also been considered.[121] Zalutsky and co-workers[222,230] have synthesized and studied ^{211}At-*m*-astatobenzylguanidine (^{211}At-mABG) as a potential alternative to ^{131}I-mIBG.[231] They employed the desilyation approach, used previously for ^{131}I-mIBG,[232] to synthesize ^{211}At-mABG.[230] ^{211}At is an α-particle emitting radionuclide. Since α particles are high-LET radiation, have a limited range in tissue, and are highly cytotoxic, they are attractive for radiotherapeutic applications.[89,221,233,234] Results of the studies by Zalutsky and co-workers showed that the carbon−astatine bond was stable and the in vivo deastatination rate was comparable to deiodination of ^{131}I-mIBG.[221] The cytotoxicity of ^{211}At-mABG on three human neuroblastoma cell lines, in vitro, was much higher than ^{131}I-mIBG,[221,233] and rates of uptake of the ^{211}At analogue in these cells was similar to ^{131}I-mIBG.[221,222,233] Results of these studies suggest that ^{211}At-mABG or other analogues are attractive alternatives as therapeutic agents for ^{131}I-mIBG; however, further in vivo studies are necessary to determine whether it is possible to exploit the higher radiotoxicity of ^{211}At-mABG for the therapy of neuroblastomas or other neuroendocrine tumors.

B. Immuno-Derived Molecules

Research with a multitude of site-specific targeting agents (including MAbs, antibody fragments, and receptor-avid biomolecules) labeled with a wide variety of radiometals has been and continues to be conducted at an accelerated rate.[12,57,86,97,235−240] The range of radiometals employed as the cytotoxic agent attached to cancer-specific biomolecules and used in multiple numbers of human clinical trials has been limited.[57,83,86,131,241,242] Yttrium-90 (^{90}Y) is the radioactive metal most frequently used in human studies, partly because of its routine availability as a high specific activity sterile pyrogen-free product.[131,235,239,240,243] Many of the bifunctional chelating agents (BFCAs) have been developed for use in conjugating this trivalent metal to molecular vectors.[243−245] Some BFCAs were developed for use

in radiolabeling biomolecules with other trivalent metals (e.g., lanthanide radioisotopes including, ^{177}Lu(III), ^{166}Ho(III), and ^{153}Sm(III) as well as ^{90}Y).[245−247] BFCAs with other trivalent metals (e.g., ^{105}Rh(III)) with therapeutic potential have been developed, but they have not been studied in humans.[50,248,249]

C. Radioimmunotherapeutic (RIT) Agents Labeled with β-Particle Emitters

Steady progress has been achieved over the past decade in the multidisciplinary field of radioimmunotherapy despite the many chemical, pharmacological, and biological obstacles that must be overcome.[141] Köhler and Milstein[142] revolutionized the field by developing a technique for the production of monoclonal antibodies (MAbs) of predefined specificity in large quantities.[142] This discovery formed the basis of worldwide research efforts to develop MAb conjugates for specific delivery of cytotoxic agents to cancer cells. Radioisotopes have two particular advantages over drugs or toxins conjugated to MAbs for eradication of cancerous tumors: (1) the α- or β-particles emitted by appended radionuclides can kill adjacent tumor cells regardless of whether they express the target antigen and (2) radioisotopes are not subject to multidrug resistance.[142,143] Interestingly, several studies of human tumor xenografts in mice suggest that radioimmunotherapy can, in certain instances, exert greater antitumor activity than dose-equivalent external beam radiation therapy.[142,143]

A large variety of MAbs and their fragments (including F_{AB}, F_{AB}', $(F_{AB}')_2$, and sFv) labeled with particle-emitting radionuclides have been studied in human clinical trials.[144] In fact, more than 100 radioimmunotherapy trials have been reported.[237,239] The majority of these trials involve MAbs labeled with ^{131}I.[250] Increasingly, more trials are being performed using MAbs labeled with radiometals. Studies comparing ^{131}I-labeled immunologically derived agents with those labeled with ^{90}Y and other radiometals demonstrate the important differences in their in vitro and in vivo performance characteristics.[26,219,251,252] The structure and physicochemical characteristics of the radiometal chelate and the conjugation chemistry has been shown to play a critical and pivotal role in determining the uptake and residualization of the radiometal−MAb conjugate in the tumor as well as influencing the uptake, retention, and clearance from nontarget tissues.[253,254] Additional research is required to identify and clarify the influence that chelation chemistry and conjugation chemistry has on determining the specificity of biolocalization in order to optimize radioimmunotherapy applications using radiometals.

The trivalent radiometals ^{90}Y, ^{111}In, and radiolanthanides cannot be attached to biomolecules by direct binding. In contrast, ^{131}I can be directly (covalently) attached to Tyr residues on proteins or peptides by well-established oxidative methods.[219,250] The trivalent metals must be linked by an intermediary molecule (chelate) that is, itself, covalently conjugated to the biomolecule.[65,243,255,256] It is of paramount importance that the radiometal chelate is exceptionally stable (ideally kinetically inert), even under extremely dilute conditions such that the in vivo behavior of the radiometal is fully controlled.[236,239,245] If the radiometal chelate moiety is not kinetically inert (or nearly so), even chelates with high thermodynamic stability will undergo in vivo dissociation or transchelation during the long periods (i.e., days) of circulating in the blood stream.

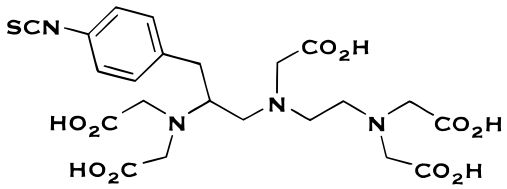

Figure 4. 2-(*p*-Isothiocyanatobenzyl)diethylenetriaminepentaacetic acid.

Functionalized polyaminocarboxylate chelates are the most prominent chelators used for complexation of trivalent radiometals (including ^{90}Y(III), ^{177}Lu(III), and ^{111}In(III)) for radioimmunotherapy applications. ^{111}In(III) is often used as a surrogate for ^{90}Y since this latter radionuclide does not emit γ-rays which are necessary for scintigraphic imaging (for determining biodistribution and radiation dosimetry studies) in humans.[239,241,257] While the chelation chemistry of ^{111}In with the polyaminocarboxylate ligands is often assumed to be similar to ^{90}Y, there can be significant differences making this an important issue for further study.[239,258−260]

DTPA, EDTA, and other common polyaminocarboxylic acid chelating agents have been used for many years to form ^{111}In-labeled radiopharmaceuticals.[255,258,261,262] DTPA conjugates have been shown to form ^{111}In-chelate structures with excellent in vivo stability and, for that reason, form the basis of several FDA-approved diagnostic ^{111}In-labeled radiopharmaceuticals. In contrast, the in vivo stability of DTPA conjugates with ^{90}Y and rare earth radiometals is usually unacceptably low. Even though the thermodynamic stability of ^{90}Y with DTPA derivatives is high, they are kinetically labile.[244,245,263] In vivo dissociated ^{90}Y, as well as the radiolanthanides, accumulate in bone to produce an undesirably high radiation dose to the radiosensitive bone marrow.[243,244,263]

Considerable efforts have been made to produce polyaminocarboxylate ligands that form complexes with ^{90}Y and the rare earth radionuclides with improved thermodynamic and kinetic stability.[245,246,261] As a result, several functionalized open-chain polyaminocarboxylate ligands and macrocyclic ligands have been developed and studied to produce chelates with ^{90}Y and the radioactive rare earths that have high in vivo stability. For example, Brechbiel, Gansow and co-workers[244,264] synthesized and evaluated a series of modified isothiocyanatobenzyl DTPA (Figure 4) ligands. Substitutions, particularly in the carbon atoms of the DTPA ligand backbone, sterically hinder the opening of the chelate ring that must occur during metal complex dissociation, increasing the kinetic inertness of the resulting chelates.[258,264,265] Several of these DTPA analogues have been studied as conjugates of MAbs and proteins.[264−266] These derivatives are constructed in a way that the a

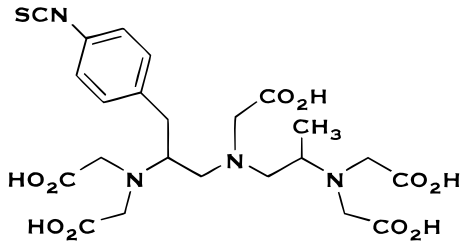

Figure 5. 2-(*p*-Isothiocyanatobenzyl)-6-methyldiethylenetriaminepentaacetic acid (1B4M-DTPA).

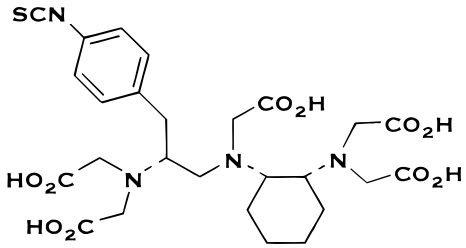

Figure 6. 2-(*p*-Isothiocyanatobenzyl)cyclohexyldiethylenetriaminepentaacetic acid (CHX-DTPA).

p-isothiocyanatobenzyl group is attached to one DTPA backbone ethylene group and one methyl group is appended to the second ethylene group in the DTPA backbone.[258,264,265] For example, 2-(*p*-isothiocyanotobenzyl)-6-methyl-diethylenetriamine-*N*,*N*′,*N*″,*N*″-pentaacetic acid (1B4M-DTPA) conjugates of MAbs continue to be used in several human clinical trials for treating patients with lymphomas.[225,235,259,267,268] Studies have shown that the 1B4M-DTPA BFCA (Figure 5) forms a ^{90}Y-complex with improved in vivo stability and is effective in reducing the deposition of ^{90}Y in the skeleton.[264,265]

Functionalized DTPA derivatives in which a cyclohexyl moiety replaces one of the ethylene groups produces complexes with trivalent metals that exhibit improved in vitro and in vivo stability.[269−272] The presence of the cyclohexyl group produces rigidity in the DTPA backbone, providing for increased kinetic inertness. The 2-(*p*-isothiocyanatobenzyl)-*trans*-cyclohexyl−DTPA (CHX−DTPA) bifunctional chelating agent (Figure 6) has received considerable attention for producing immunoconjugates labeled with trivalent radiometals. CHX−DTPA is a BFCA with two diastereomeric C-functionalized chelating moieties (CHX−A-DTPA and CHX−B-DTPA), both racemates.[269,270] There are distinct differences in the in vivo stability of the stereoisomers of these ligand complexes with $^{88/90}$Y and ^{212}Bi.[264,270] Further studies with the CHX-DTPA ligands are required to better understand the relationships of the stereochemistry of these chelates when attached to proteins or other biomolecules and their in vivo stability.

Most of the research over the past decade to radiolabel biomolecules with trivalent β-particle emitting radiometals has utilized functionalized macrocyclic polyaminocarboxylate BFCAs.[264,273,274] Complexes with macrocyclic ligands typically show enhanced thermodynamic and kinetic stability relative to the open-chain analogues.[257,264,273,275] The thermodynamic "macrocyclic effect" is attributed to the enthalpic and entropic contributions resulting from the "preorganization"[276] enjoyed by the macrocycle.[275,277] The 1,4,7,10-tetraazacyclododecane-*N*,*N*′,-*N*″,*N*‴-tetraacetic acid (DOTA) based BFCAs form the most studied series of ligands for labeling immunologically derived molecules with ^{90}Y, ^{177}Lu, and other trivalent radiometals.[257,264,275,277] DOTA (Figure 7) has been shown to form remarkably stable complexes with these metals and form radiometal complexes with high in vivo stability.[245,264,273,277]

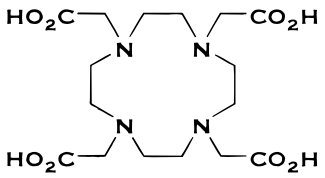

Figure 7. 1,4,7,10-Cyclododecane-1,4,7,10-tetraacetic acid (DOTA).

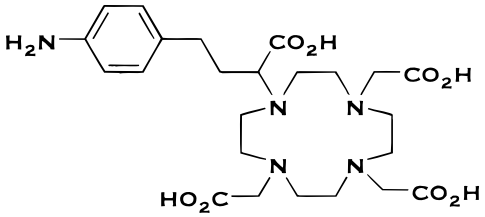

Figure 8. α-[2-(4-Aminophenyl)ethyl]-1,4,7,10-tetracyclododecane-1,4,7,10-tetraacetic acid (PA-DOTA).

Several approaches for conjugation of the DOTA ligand framework to proteins have been studied.[245,273,278−280] In some cases, one of the four carboxylic acid moieties on DOTA is activated (e.g., via anhydride formation) to facilitate their reaction with primary amines (i.e., primarily the ϵ-lysine-NH_2 group) on the protein to form stable amide bond linkages.[245,278,279] After conjugation by this route, only three carboxylate groups are available for complexation of the metal.

The in vivo stability of trivalent radiometal chelates with DOTA conjugates containing only three free carboxylate groups remains high. The availability of the fourth free carboxylic acid side could be useful for reducing the overall charge on these radiometal chelate moiety to −1. This offers a potential means for modifying in vivo pharmacokinetics of corresponding bioconjugates.

DOTA-based BFCAs have been synthesized that are linked to MAbs via one of the four side chains while maintaining four carboxylate groups for coordination of the metal.[245,281] These DOTA-BFCAs ensure the availability of all four carboxylate groups for coordination of the trivalent radiometals. An example of one of these derivatives α-[2-(4-aminophenyl)ethyl]-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (PA-DOTA) is shown in Figure 8. This chelator has been used to produce conjugates of $(F_{AB}')_2$ fragments and MAbs for labeling with ^{90}Y and rare earth radionuclides.[281,282] Following conversion of the aminophenyl group on PA-DOTA to the aryl isothiocyanate group, it is conjugated to MAbs and proteins via free NH_2 groups to form the thiourea linkage.[281,282] ^{177}Lu-labeled CC-49 MAbs, using the PA-DOTA BFCA, have been studied as a potential agent for treatment of patients with ovarian cancer following intraperitoneal injection.[40,281,282] The DOTA−

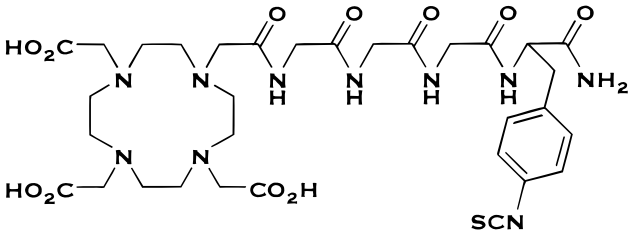

Figure 9. 1,4,7,10-Cyclododecane-1-glycylglycylglycyl-L-*p*-isothiocyanatophyenylalanine-4,7,10-triacetic acid.

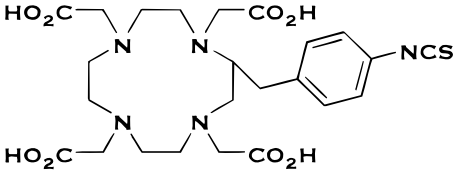

Figure 10. 2-(4-Isothiocyanatobenzyl)-1,4,7,10-tetraazacyclotetradecane-1,4,7,10-tetraacetic acid (p-NCS-Bz-DOTA).

Gly_3−L-(*p*-isothiocyanato)−Phe−amide BFCA (Figure 9) is a DOTA derivative also linked through one of the N-atoms on DOTA.[257,278,283,284] Conjugates with this BFCA have been used to prepare ^{90}Y-labeled chimeric L6 antibreast cancer MAbs and Lym-1-MAb.[278,285] DOTA-based BFCAs in which the side chain containing the activated group for conjugation to proteins is attached to one of the carbon atoms of the ligand backbone also preserves the integrity of all four $-CH_2COOH$ groups for metal coordination. Synthesis of these types of DOTA derivatives has been reported with the *p*-NCS−Bz−DOTA (Figure 10) BFCA (i.e., (2-(4-isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid) available for forming conjugates with trivalent radiometals.[277]

Results from in vivo studies with these metalated BFCAs demonstrate that the chemical and physiochemical characteristics of the trivalent radiometal chelates and the method used for conjugation play important roles in determining the therapeutic ratio of the radiolabeled immunoconjugates.[86,120,219,237−239,286] The goal in developing any therapeutic radiopharmaceutical is to maximize the uptake and retention of the agent in the tumor while minimizing the uptake and retention in normal tissues. In many cases, the metabolic products of many of the trivalent radiometal immunoconjugates studied are trapped inside of cells. If the radiometal chelate conjugates are deposited for extended periods inside of cells of normal tissues (e.g., the kidneys), the radiation dose to these tissues will limit their potential for radioimmunotherapy applications. For many of the radiometal conjugates, this has been problematic.[86,120,241,286,287] For example, metabolism of radiolabeled antibodies can ultimately produce the metabolically stable radiometal chelate conjugated via the ϵ-lysine amine group, which can have high and long-term uptake in the kidneys.[69,70,252,253,286,287] The inclusion of metabolizable spacer groups when linking the radiometal BFCA to the protein is one approach that can be used to accelerate clearance from the body (including the kidney) in order to reduce the radiation dose to normal tissues.[70−72,241,256,278,288]

Results from studies with ^{131}I-labeled anti B-1 antibodies that target the CD20 surface antigen for treatment of non-Hodgkin's lymphoma (NHL) offers an instructive example where therapeutic effectiveness was achieved by optimizing residualization and pharmacokinetic factors.[250,267,289] The CD20 surface antigen, expressed on 90% of B-cell lymphomas, is a target for therapy with radiolabeled antibodies.[267,290−292] This antigen, a more stable target than many other tumor-associated antigens, exhibits little or no shedding into the circulation and does not internalize when bound to the antibodies, thus providing for long-term retention of the radioimmunoconjugate on the tumors.[267,290−292] In contrast to many of the radiometal chelate conjugates, catabolic and metabolic products of ^{131}I-labeled antibodies are not residualized in cells (either cancer or normal cells) for extended periods.[65,86,94,219,250,289] The ^{131}I is easily removed from the antibodies primarily as ^{131}I-monoiodotyrosine by microsomal enzymes resulting in its rapid excretion from cells through the kidneys and into the urine.[250] Thus, elimination of the nonlocalized ^{131}I component from the body reduces the radiotoxicity of the radiopharmaceutical. Results of phase I/II trials indicate that radioimmunotherapy with the ^{131}I anti B1 MAb produces desirable clinical responses with minimal or modest toxicity in a high proportion of human patients.[239,250,289,293] Residualizing labels, such as ^{90}Y and other trivalent radiometals, may be more useful for targeting internalizing antigens as long as appropriate conjugation methods and radiometal chelate frameworks can be developed to facilitate efficient clearance from normal nontarget tissues.[94,239,248,294,295]

D. Tumor Pretargeting

Normal tissue toxicity, especially to the bone marrow, has become a major limiting factor in the application of radioimmunotherapy to solid tumors.[17,296] This is primarily related to the slow pharmacokinetics of radiolabeled MAbs.[17,296] Various attempts have been made to improve biodistribution of the radiolabeled MAbs, such as the use of a second antibody, local delivery, the use of metabolizable linkers, or MAb fragments that display faster blood clearance than do whole antibodies.[297] Further improvements are desirable. One of the approaches being developed in several laboratories to maintain high tumor uptake of therapeutic radionuclides while providing rapid clearance from the blood and nontarget tissues is "tumor pretargeting".[17,20,70,227,296−300] Several pretargeting strategies have been devised that utilize different macromolecule/radiolabeled-effector-molecule pairs.[296,301,302] The majority of pretargeting studies employ the avidin/streptavidin−biotin system.[20,298,300,303,304] This system is attractive since the K_a for avidin or streptavidin with biotin is approximately 10^{15}. This system has been used in a large number of human patients.[296,298,305]

The pretargeting design uses a three-step approach wherein three reagents are administered as sequential steps in a protocol designed to maximize the tumor radiation dose which minimizes radiation doses to bone marrow and other nontarget tis-

sues.[20,296,303] In the first pretargeting step, a long-lived circulating nonradiolabeled MAb (e.g., avidin/steptavidin-MAb or bivalent MAb) with a high affinity and specificity for antigens uniquely or overexpressed on cancer cells is administered. After sufficient time has passed for this MAb construct to localize in this tumor, a second injection of a chase molecule is given at peak tumor uptake, usually 1−3 days after administration of the MAb construct.[296] This second step provides a mechanism for rapid clearance of the MAb construct from the blood. One specific approach involves conjugating galactosyl residues to the chase macromolecule that steer the aggregates to the hepatic cells via the hepatocyte galactose receptor.[299]

The third step involves injection of the radiolabeled hapten or biotin conjugate. This is performed approximately 1 h after the chase molecule is administered.[17,296] Maximum tumor concentrations and tumor to nontarget ratios are typically achieved in approximately 1−3 h.[296] There is long-term retention of radioactivity associated with the streptavidin−MAb conjugate in the tumor (i.e., days), while the rapid elimination of unlocalized radioactivity attached to the small effector molecule, primarily via the kidneys, greatly reduces the radiation dose delivered to normal tissues.[17,296] Several effector molecules labeled with several radionuclides (including ^{90}Y, ^{131}I, ^{188}Re and ^{67}Cu) have received attention.[20,70,304] The majority of radiometalated effector molecules being studied, however, are ^{90}Y−DOTA conjugates (e.g., ^{90}Y−DOTA−biotin).[17,227,296,297]

Results from studies using the tumor-pretargeting approach are encouraging. Rapid clearance of the radiolabeled effector molecule from the blood stream makes it possible to consider use of short-lived radionuclides (e.g., including those in Table 1) and potentially the short-lived α-particle emitting radionuclides. The pretargeting principle offers an important opportunity for treatment of solid tumors, and refinement of the components and specific schemes should yield new radiolabeled agents that are capable of efficiently concentrating in the tumor while reducing radiotoxic side effects in human cancer patients.

E. α-Particle Emitting Radioimmunoconjugates

Because of their widespread application for chelating trivalent radiometals, functionalized polyaminocarboxylates have also been used for preparing $^{211/212}$Bi(III) immunoconjugates. The derivatives which have received the most attention are DTPA analogues and include DTPA anhydrides which yielded ^{212}Bi-labeled MAbs with poor in vivo stability.[306,307] Retention of radiobismuth on proteins was somewhat improved through the use of 2-(*p*-isothiocyanatobenzyl)−DTPA, 2-(*p*-isothiocyanatobenzyl)-5(6)-methyl−DTPA (MX−DTPA), and CHX−DTPA (see Figure 6).[101,270,306,307] The use of DOTA-based BFCAs resulted in higher in vivo stability; however, kinetics of complex formation with ^{212}Bi appear incompatible with its short half-life. Despite limitations on the in vivo stability of DTPA-based BFCAs with ^{212}Bi, studies with several ^{212}Bi-labeled MAbs demonstrate their potential for applications for site-specific therapy of human cancers.[30,101,234,306−308] Radioimmunoconjugates labeled with ^{212}Bi (or ^{213}Bi) may produce agents capable of treating leukemia, highly vascularized tumors, intraperitoneal malignancies, and micrometastases.[109,114,234,308,309]

Astatine-211 (^{211}At) labeled immunoconjugates have been synthesized and evaluated for their therapeutic potential. Proteins labeled with ^{211}At by direct electrophilic astatination were unstable by virtue of rapid loss of ^{211}At following in vivo administration.[106,310,311] As a result, Zalutsky and co-workers[106,312] synthesized *N*-succinimidyl-3-[^{211}At]astatobenzoate (SAB), an analogue of the radioiodinated intermediate that has been successfully employed for radioiodination of biomolecules. ^{211}At−SAB is prepared in good yields by electrophilic astato-destannylation of *N*-succinimidyl-3-(trimethylstannyl)benzoate.[106,310,313] The potential utility for labeling MAbs and MAb fragments with ^{211}At has been investigated using several MAb systems. Although studies involving intravenous administration of ^{211}At−MAbs demonstrate specific accumulation in tumors,[234,314] increased levels of ^{211}At in normal tissues indicates more rapid rates of ^{211}At catabolism. These studies demonstrate that the in vivo stability of radioimmunoconjugates labeled with ^{211}At−SAB may be acceptable, particularly for nonintravenous applications (e.g., intratumoral or intrathecal administration).[234,313−315]

F. Radiolabeled Receptor-Avid Agents

Interest in developing therapeutic receptor-avid peptides labeled with trivalent radiometals has been increasing as more effective radiolabeled peptides have been developed.[38,56,236,316,317] While numerous radiolabeled peptides are being studied, the majority of peptides labeled with trivalent metals that have advanced to clinical trials in humans, to assess their potential therapeutic applications, are those that target somatostatin (SSN) receptors.[41,55,294,318] Furthermore, most of these agents have been labeled with ^{90}Y or ^{111}In.[41,294,316,319] Since the ^{111}In−DTPA−D-Phe^{1}−octreotide (Figure 11) and its analogues are rapidly internalized by receptor-mediated endocytosis and intracellularly residualized following binding to cell surface SSN receptors, the administration of high doses of these agents has been considered to hold significant therapeutic potential.[41,69,79,319,320] The fact that ^{111}In−DTPA−D-Phe^{1}−octreotide and analogues are internalized makes it possible for the low-energy, poorly penetrating electrons emitted during decay of this radioisotope to deliver a high intracellular radiation dose.[321,322] Studies with high doses of ^{111}In-labeled peptides are required to determine the effectiveness of these low-energy electrons for treatment of tumors where heterogeneous deposition of the drug occurs.

The SSN receptor-avid peptides labeled with ^{111}In or ^{90}Y that have received the most attention are the DTPA and DOTA conjugates of octreotide analogues. Because of the higher in vivo stability of the ^{90}Y−DOTA chelate framework relative to ^{90}Y−DTPA, most of the research over the past few years has dealt with ^{90}Y−DOTA conjugates of SSN receptor-avid peptides.[47,239,319] The ^{90}Y−Bz−DTPA−octreotide analogue was shown to have improved stability relative

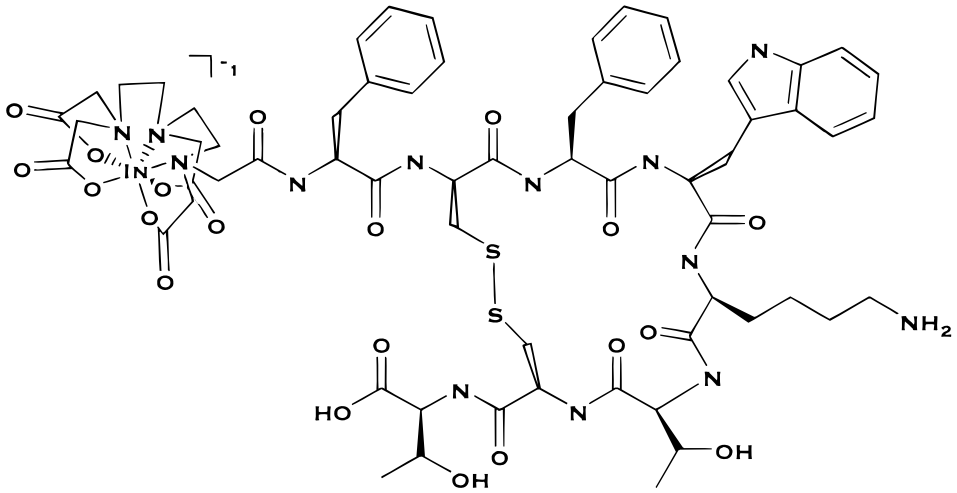

Figure 11. ^{111}In−DTPA−D-Phe^{1}−octreotide.

to ^{90}Y−DTPA−octreotide. However, comparisons of ^{90}Y−Bz−DTPA−octreotide with corresponding DOTA conjugates must be performed to determine their relative in vivo stabilities of these conjugates.[319,323] Recent studies with ^{111}In− and ^{90}Y−DOTA conjugates of ^{111}In− and ^{90}Y−[DOTA−D-Phe, Tyr^{3}]octreotide showed that these analogues had higher accumulation in SSN receptor-expressing tumors than the corresponding radiolabeled octreotide conjugates.[41,44] These results, as well as studies with other ^{90}Y-labeled octreotide derivatives, including studies in human patients, demonstrate the potential of these radiolabeled octreotide derivatives for treatment of cancers that overexpress SSN receptors.[16,41,324,325] While most current studies to develop therapeutically effective receptor-avid molecules relate to treatment of SSN-receptor expressing cancers, research is also being conducted to specifically target cancerous tumors that overexpress or uniquely express other types of receptors.[44,56,326] A limited number of studies involving therapeutic applications with radioactive compounds that target cancer cells that overexpress other types of receptors have been performed in humans.

It is important to re-emphasize that the physicochemical properties of the metal chelate and methods used to tether the chelate to receptor-avid peptides can have a more profound influence on the in vivo targeting capabilities of these smaller bioconjugates in comparison to the larger radiometalated immunoconjugates. Recent studies by Maecke and co-workers[44,327,328] offer an excellent example of the physicochemico properties which the radiometal chelate moiety can have on receptor binding characteristics and in vivo properties of site-specific targeting agents. Maecke and co-workers showed that Ga−DOTA−Tyr^{3}−octreotide has a >6-fold higher in vitro binding affinity for SSN receptors on AR42J tumor membranes than the corresponding Y−DOTA−Tyr^{3}−octreotide analogue.[327] More importantly, the in vivo targeting characteristics of ^{67}Ga−DOTA−Tyr^{3}−octreotide is far better than the corresponding ^{90}Y- and ^{111}In-labeled DOTA−Tyr^{3}−octreotide analogues.[327,328] For example, the specific accumulation of the ^{67}Ga analogue in SSN-expressing tumors is more than 2-fold higher than that in the ^{90}Y- and ^{111}In-analogues, with the efficiency of renal clearance of the ^{67}Ga-analogue also being far superior.[327,328] Structural analysis of Ga−DOTA−D-Phe−amide shows that this chelate moiety adopts a *cis*-pseudooctahedral geometry with a folded macrocyclic unit (2424 conformation).[328,329] Most importantly, one carboxylate group is free and deprotonated at physiological pH. This structural feature is known to contribute to efficient excretion of compounds through the kidneys.[70,330,331] Contrary to the Ga structure, the structure of Y−DOTA−D-Phe−amide exhibits a metal coordination number of eight, including the amido carboxy oxygen of the amido functional group used for coupling.[329,332,333] In this case, DOTA acts as an octadentate chelator forming a very compact, somewhat distorted square antiprismatic geometry with the 12-membered ring adopting the usual square [3333] conformation observed with several cyclam-derived complexes.[332,333] These structural and coordination differences between the three complexes can be traced back to the differences in ionic radii of the metal ions (e.g., ionic radii obtained from ref 334 are Ga^{3+}, 0.62 Å, In^{3+}, 0.80 Å, and Y^{3+}, 1.09 Å). Peptides are small (e.g., often 3−10 amino acid sequences) and the effect that the charge and structure of the chelate can have on the binding affinity of the conjugate to the cognate receptor can be enormous.[50,335,336] The inclusion of a spacer group between the radiometal chelate and the amino acid sequence that specifically binds to the cognate receptor can modify binding interactions substantially. Inclusion of a spacer group offers a mechanism to remove the metal chelate from the binding region so there is minimal steric or electronic interference with the specific receptor binding interaction.[50,335] The properties of the spacer group to which the radiolabeled complex is conjugated to the receptor binding moiety can also have a major effect on the level of renal retention of the radioisotope.[50,335,337] For example, a recent study with a ^{105}Rh(III)Cl_2−tetrathiamacrocycle complex, conjugated to bombesin (a peptide that binds with high affinity to gastrin releasing peptide (GRP) receptors) via a glutamine residue, showed minimal kidney retention in animals while retaining high residualization in GRP-expressing human cancer cells following receptor-mediated endocytosis.[50,338] These re-

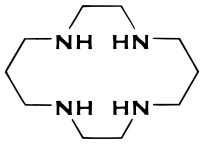

Figure 12. 1,4,8,11-Tetraazacyclotetradecane (CYCLAM).

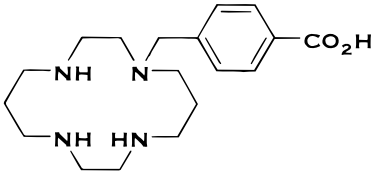

Figure 13. 4-[(1,4,8,11-Tetraazacyclotetradec-1-yl)methyl]-benzoic acid (CPTA).

sults, along with other studies, demonstrate the essential role that the chelation and conjugation chemistries play in determining the in vivo uptake and pharmacokinetic behavior of radiolabeled receptor-avid peptides being designed as potential therapeutic radiopharmaceuticals.

V. $^{64/67}$Cu Conjugates

^{64}Cu(II) and ^{67}Cu(II) are radionuclides that have been shown to hold potential for therapeutic applications.[212,239,339,340] Both of these copper radionuclides have been used as the cytotoxic component of bioconjugates (including MAbs and receptor-avid peptides) being evaluated in animals and humans to assess their potential for control or eradication of tumors.[61,341,342] Since the results of studies with $^{64/67}$Cu bioconjugates is presented in greater detail elsewhere in this issue (i.e., see C. J. Anderson and M. J. Wech), progress with radiocopper immunoconjugates will be only briefly reviewed.

In contrast to the use of functionalized DTPA and DOTA derivatives as the primary types of chelating frameworks used to form stable conjugates labeled with trivalent radiometals, most of the BFCAs used for $^{64/67}$Cu(II) are based on the 14-membered tetraazamacrocycle cyclam (1,4,8,11-tetraazacyclotetradecane) shown in Figure 12. This 14-membered ring has a cavity size and structure that provides an ideally suited "prearranged" coordination geometry for producing $^{64/67}$Cu(II)−cyclam chelates with excellent in vitro and in vivo stabilities.[339,343,344] Comparative studies with open-chain and macrocyclic structures demonstrate that the macrocyclic structure is essential to prevent in vitro and in vivo dissociation of $^{64/67}$Cu from the conjugates.[344] Several functionalized cyclam derivatives have been and are being used as the vehicle for labeling biomolecules with $^{64/67}$Cu(II). Two of the most commonly used BFCAs employed in formulation of $^{64/67}$Cu(II)-labeled conjugates for human clinical trials are 4-[1,4,8,11-tetraazacyclotetradec-1-yl)-methyl]benzoic acid (CPTA) and [6-(*p*-bromoacetamido)benzyl]-1,4,8,11-tetraazacyclotetradecane-*N*,*N*′,*N*′′′,*N*′′-tetracetic acid (BAT) shown in Figures 13 and 14, respectively.[68,239,341] The resulting $^{64/67}$Cu(II) chelate moiety in CPTA has an overall positive charge, while the $^{64/67}$Cu(II) chelates with the BAT moiety has a negative overall charge. The physicochemical properties of these chelate moieties

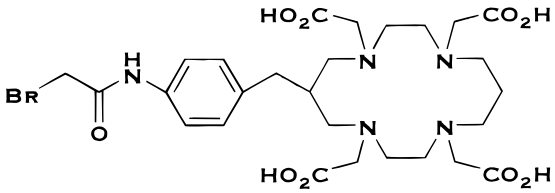

Figure 14. 6-[*p*-(Bromoacetamido)benzyl]-1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (BAT).

have been shown to have significant effects on the uptake and clearance of $^{64/67}$Cu from the tumor and normal tissues.[61,342,345,346] For example, the radiolabeled MAbs and peptides using $^{64/67}$Cu−CPTA generally have higher and more stable tumor accumulation than ^{131}I−MAbs but also exhibit a high level of accretion in the kidneys and liver that is related to intracellular trapping of the $^{64/67}$Cu−cyclam-containing metabolites.[61,75,239,342,343,347] Studies with $^{64/67}$Cu−BAT labeled biomolecules generally exhibit improved in vivo clearance properties relative to ^{67}Cu−CPTA conjugates, which is likely related to the overall negative charge on the appended ^{64}Cu−BAT chelate.[342,345,346] Clinical trials have been performed with ^{67}Cu-2IT-BAT-LYM-1 for treatment of non-Hodgkins lymphoma [341] and ^{67}Cu−CPTA-MAb35 and ^{67}Cu−CPTA-MAb C595 directed against colorectal tumors and bladder cancers, respectively.[339,348] Results from these and other studies with smaller radiocopper bioconjugates that specifically target cancers in vivo coupled with the development of new production approaches to make ^{64}Cu more readily available[349] demonstrate the feasibility of developing effective $^{64/67}$Cu-labeled site-specific therapeutic radiopharmaceuticals.

VI. $^{186/188}$Re-Labeled Biomolecules

^{186}Re and ^{188}Re ($^{186/188}$Re) have been identified as important radionuclides with therapeutic potential.[83,212,240,350] ^{188}Re is available in high specific activities as $^{188}ReO_4^-$ from ^{188}W/^{188}Re generators[84,212,351] and ^{186}Re in large quantities (i.e., ≥several curies) as the perrhenate acid or salts from the U.S. Department of Energy as well as in the form of aluminum perrhenate from the University of Missouri Research Reactor.[84,212] The availability of both ^{186}Re and ^{188}Re provide flexibility in designing radiolabeled agents that are compatible with their in vivo applications and pharmacokinetics properties. Since ^{188}Re (Table 1) has a higher β-particle energy and short half-life (17 h), it is generally more suitable for preparing radiopharmaceuticals that target larger tumors and have reasonably fast clearance from the blood and other nontarget tissues. In contrast, ^{186}Re is a medium-energy β-particle emitter (Table 1) and has a 3.7 day half-life which makes it generally amenable for use with those biomolecules that do not clear rapidly from the blood stream. The fact that ^{186}Re is not available as a high specific activity product limits its use to applications where high specific activity formulations are not mandatory. An important factor that adds value to $^{186/188}$Re-labeled drugs is the fact that ^{99m}Tc (the most widely used radionuclide for diagnostic imaging in nuclear medicine) is a chemical congener

of Re, making the chemistry of both ^{99m}Tc and $^{186/188}$Re the same or nearly identical in many cases.[240,245] Thus, when the chemistries of these radionuclides are similar, the ^{99m}Tc agents can be used as the "matched pair" for the corresponding $^{186/188}$Re agent, making it feasible to obtain excellent diagnostic imaging in patients allowing for pre- and post-assessment of patients treated with therapeutic $^{186/188}$Re analogues.

Despite the availability of $^{186/188}$Re reagents, these radionuclides have been used to a lesser extent than either ^{131}I and ^{90}Y to formulate perspective therapeutic radiopharmaceuticals. The reasons for the relatively low number of research and development studies with $^{186/188}$Re-labeled agents in humans relates partially to the more complex labeling chemistry required with $^{186/188}$Re. For example, more reducing agent (e.g., Sn(II)) is required to convert the rhenium from an oxidation state of VII in the $^{186/188}ReO_4^-$ reagents to a lower oxidation state(s) of Re to facilitate $^{186/188}$Re complexation to coordinating ligand frameworks since R^{VII} is more difficult to reduce than Tc^{VII}.[245,352] The presence of these strong reducing agents can alter the metalation reactions and/or the integrity of the biomolecular conjugate.[352,353] Overcoming these and other related issues (e.g., in general Re can be less stable at low oxidation states than Tc) to produce well-defined $^{186/188}$Re-labeled compounds in high yields that retain their ability for high specific in vivo targeting of cancers is not trivial. However, new approaches and methods to form $^{186/188}$Re-labeled biomolecules for use in humans are under active investigation.[45,240,353,354]

Strategies for synthesizing $^{186/188}$Re-labeled site-specific radiopharmaceuticals are based on, and parallel to, efforts being made to develop new ^{99m}Tc drugs.[245,338,355,356] Two general approaches are being employed: (1) direct labeling of biomolecules[245,353,357−359] and (2) metalation of bioconjugates via a covalently appended chelating framework.[92,360] This latter type of ^{99m}Tc- and $^{186/188}$Re-labeled agent can be produced by the "preformed" $^{186/188}$Re chelate method or by postconjugation labeling.[25,92,361] Direct labeling of proteins or peptides with $^{186/188}$Re that contain disulfide bonds have been performed via the reduction of disulfide bonds to sulfhydryl groups.[362] The sulfhydryl groups strongly coordinate Sn(II)-reduced $^{186/188}ReO_4^-$, and labeling with these radionuclides is performed in manners similar to direct labeling of biomolecules with ^{99m}Tc.[245,352,357] Since proteins (e.g., MAbs) have numerous coordination sites and thiol groups for complexation of ^{99m}Tc and $^{186/188}$Re, direct labeling is a nonspecific labeling method. For example, MAbs contain a number of inter- and intrachain disulfide bonds which are unequally susceptible to reduction, such that variation in radioactive agents and conditions can lead to an antibody with different numbers, or different arrays, of sulfhydryl groups. Methods for producing bioactive ^{188}Re-labeled MAbs, by the direct labeling method, in high yields that exhibit good in vitro and in vivo stabilities have been developed.[25,245,362,363] However, several of these in vivo studies have shown faster clearance of these ^{188}Re−MAbs from the blood relative to the corresponding ^{131}I- and ^{88}Y-labeled MAbs.[245,362,364,365] Comparisons of direct labeled and "preformed-chelate" labeled ^{188}Re−MAbs showed faster clearance of the former indicating that directly linked $^{186/188}$Re may dissociate from the MAbs more rapidly than other radiolabeled immunoconjugates prepared using the preformed chelate approach.[245,361,362]

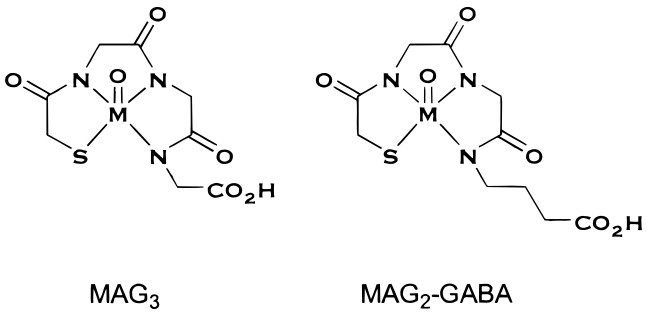

Figure 15. Tc/Re−mercaptoacetylglycylglycylglycine (MAG_3), mercaptoacetylgylcylglycyl-*g*-aminobutyric acid (MAG_2-GABA). M = Tc or Re.

Formation of $^{186/188}$Re-labeled bioconjugates using ligand frameworks that will form stable radiorhenium chelate moieties at a specific site on the biomolecule is required to produce well-defined products.[356] Several $^{186/188}$Re-labeled immunologically derived molecules and small biomolecular targeting agents, prepared using an appended metal chelation systems, have been evaluated in clinical trials.[25,350,366,367] The majority of BFCA systems used for $^{186/188}$Re are based on triaminothiol (N_3S) ligand framework.[25,251,368] Studies with diamidodithiol and diaminodithiol (N_2S_2) based BFCAs have also been reported with $^{186/188}$Re; however, most of the N_2S_2 systems form highly lipophilic Re/Tc complexes and are used in applications where hydrophobic targeting agents are most applicable.[31,369] Two of the N_3S chelators most frequently used as BFCAs for labeling MAbs, peptides, and other biomolecules are based on the mercaptoacetylglycylglycylglycine (MAG_3) framework. MAG_2−GABA (mercaptoacetylglycylglycyl-γ-aminobutyric acid) provides a spacer group between the Tc/Re−N_3S chelate and biomolecule. The GABA spacer group is 2-carbon units longer than the glycine spacer utilized in MAG_3, which can be advantageous for some applications. For example, the presence of the two carbon spacers may reduce steric hindrance and make labeling yields higher or may remove the ^{99m}Tc/^{188}Re chelate further from the binding region of the biomolecular targeting vector and improve binding affinities. MAG_3 and MAG_2−GABA form complexes with $^{99m}TcO^{3+}$ that have the identical structure as the corresponding $^{186/188}ReO^{3+}$ chelates (Figure 15), making it possible to use the ^{99m}Tc and $^{186/188}$Re as "matched pairs" when performing in vivo studies with N_3S bioconjugates.[366,370,371] The overall charge on the ^{99m}Tc− and $^{186/188}$Re−MAG_3 chelating framework is −1 since, along with the $-S^-$ from the coordinating thiol group, the three amido groups each lose a proton at neutral pH upon complexation with the ReO^{3+}/TcO^{3+} cores.[251,371] These polar chelate moieties promote more efficient renal clearance of ^{99m}Tc/$^{186/188}$Re−N_3S conjugates from the blood. Both the N_3S and N_2S_2 ligands are capable of forming high

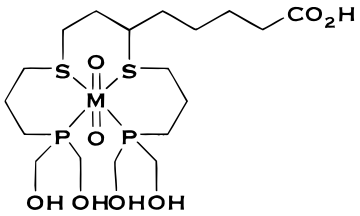

Figure 16. Dihydroxymethylenephosphinedithia BFCA (P_2S_2−COOH).

specific activity complexes and can be used for labeling biomolecules as preformed $^{186/188}$Re-BFCAs.[76,369] With some smaller biomolecules (e.g., steroids or low molecular peptides), these ligand frameworks can be used to bind $^{186/188}$Re to the conjugate via postconjugation labeling methods (i.e., either following direct ReO_4^- reduction or transmetalation).[360,370] Introduction of $^{186/188}$Re specifically into the conjugated chelate framework via the postconjugation labeling method becomes impossible when the biomolecule itself contains coordinating sites (e.g., thiol groups) that will nonspecifically complex the radiometal in direct competition with the appended chelator.

A variety of other approaches have been and are being used to develop site-specific $^{186/188}$Re-labeled bioconjugates with high in vivo stability.[354,372] It is important to keep in mind that any strategy used to prepare these conjugates should ideally produce high specific activity products in high yields in which the $^{186/188}$Re is bound to the conjugate in a specific and well-characterized manner. In many cases, it is desirable that the $^{186/188}$Re chelate moiety appended to the biomolecule be a polar entity (for targeting cell surface molecular entities) that will promote (potentially as metabolic products as well as the $^{186/188}$Re-labeled drug, itself) clearance from nontarget tissues and optimize clearance via the kidneys into the urine.[236,372] Clearance of therapeutic radiopharmaceuticals exclusively via this route is ideal to minimize the radiation dose to other normal tissues (e.g., GI tract and liver). Two examples of recent strategies to produce $^{186/188}$Re and ^{99m}Tc chelates that can be appended to biomolecules that are small, stable, polar, and can be formed in high specific activities employ (1) multidentate ligands based on inclusion hydroxymethylenephosphine (HMP) groups and (2) Re(I)−tricarbonyl synthons.

Katti and co-workers[373−377] developed synthetic methods to prepare a variety of ligands containing HMP groups (i.e., $-P(CH_2OH)_2$) that are capable of forming well-defined, stable complexes in high yields in aqueous solutions. Synthesis of the dihydroxymethylenephosphinedithia (P_2S_2) ligand system to which the $-(CH_2)_4$−COOH side chain is appended was reported.[326,378] This BFCA forms the Re(V)−P_2S_2−BFCA (Figure 16) which can be covalently linked, following activation of the uncomplexed −COOH group, to free amine groups on biomolecules.[326,378] ^{188}Re and ^{99m}Tc form +1 complexes with this P_2S_2 ligand framework (i.e., involving the Re(V) and Tc(V)−transdioxo cores) that have high polarity and are highly water soluble.[374,378] Results with a Re-/^{99m}Tc-metalated P_2S_2−bombesin (BBN) analogue demonstrate the ability of this bioconjugate to bind with high specificity and affinity to BBN-expressing cancer cells and showed efficient clearance of radioactivity from the blood, predominantly via the renal pathway.[379] These results, coupled with preliminary results with the other HMP-based ligand systems, hold important potential as a vehicle for formulating $^{186/188}$Re-labeled therapeutic agents.

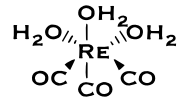

Figure 17. Tricarbonyl−Re(I) aqua ion. The Re(I)−CO bonds are substitution stable, whereas the Re(I)−OH_2 bonds (trans to each Re(I)−CO bond) are substitution labile.

Synthesis of $^{186/188}$Re (and ^{99m}Tc) using tricarbonyl synthons appears to be a promising approach to formulate high specific activity site-specific radiopharmaceuticals. New hydrophobic Re−tricarbonyl steroid conjugates are being produced and evaluated as agents capable of targeting progesterone or estradiol receptors.[33,380] Alberto and co-workers[381−384] have developed a facile method to label a variety of biomolecule conjugates with $^{186/188}$Re(I) using an organometallic aqua ion $[M(OH_2)_3(CO)_3]^+$ (M = Re,-Tc) synthon that is capable of producing hydrophilic Re/Tc chelate moieties. This synthon can be used to form the labeled biomolecules in aqueous media and can be prepared at NCA levels in water (also organic solvents, if desired) at atmospheric pressure in a short time from MO. The main characteristic of this carbonyl synthon is the high substitution stability of the three CO ligands and the substitution lability of the coordinated water molecules.[381,384] $[M(OH_2)_3(CO)_3]^+$ can be considered as a "semi-aqua ion".[381,384] The extraordinary kinetic inertness of different mononuclear tricarbonyl−Re(I) complexes form an important basis for the potential applications in the formulation of new $^{186/188}$Re-labeled therapeutic agents.[33,380,381,385] The exceptional stability of complexes formed with the "*fac*-$[M(CO)_3]^+$" moiety can be related to the d^6 electronic configuration of the M(I) metal ion in an octahedral field.

Results from studies involving the aqueous chemistry of the *fac*$[Re/Tc-(CO)_3]^+$ moiety show that the rate of substitution of the H_2O ligands by other donor groups is highly dependent upon the electronic characteristics of the incoming ligand.[381] The large differences in formation rates provides a high degree of selectivity for the reaction of the Re(I)− or Tc(I)−tricarbonyl aqua ion (Figure 17) with possible sites on biomolecules. This system has been reported to react to a minimal extent with most donor groups found on proteins or peptides (including aliphatic amines, amide group, and carboxylate groups).[381] Aromatic amines, including the imidazole moiety (a component of histidine) were shown to react rapidly with the $[Re(I)CO)_3(H_2O)_3]^+$ synthon.[381,386,387] Interestingly, preliminary results with an N-terminal-his analogue of neurotension[386] and a recombinant sFv fragment with a $his_{(5,6)}$ tail[388] indicate that only histidine molecules at the N-terminal end and not histidine residues incorporated into the protein se-

quence are capable of reacting efficiently at low concentrations (i.e., $<10^{-5}$M) with this synthon.[387] These results suggest, therefore, that $[^{186/188}Re(I)(CO)_3(H_2O)_3]^+$ can be used to selectively label biomolecules conjugated with histidine, or other moieties capable of rapidly reacting with this synthon, to produce well-defined, high specific activity $^{186/188}$Re agents with therapeutic potential.

Clearly, there is continued interest in designing and developing $^{186/188}$Re-labeled site-specific biomolecules for in vivo targeting and treatment of cancers in humans. The enormous potential that ^{186}Re and ^{188}Re hold as therapeutic radionuclides, based on their physical decay properties (Table 1), is widely recognized. Results from the limited number of in vivo studies with $^{186/188}$Re-labeled biomolecules provide strong evidence that radiorhenium conjugates can be effectively used for therapeutic applications.[25,251,350,366,389] It is essential that development of current ligand frameworks (e.g., N_3S-BFCAs) and expansion of efforts to develop new technologies for labeling molecular targeting agents with radiorhenium be continued in order to realize the potential of formulating $^{186/188}$Re-labeled pharmaceuticals for specific in vivo treatment of cancerous tumors.

VII. Summary

Site-specific radiopharmaceuticals that are labeled with particle-emitting radionuclides offer opportunities for selective in vivo cellular destruction. A limited number of radiolabeled drugs are being routinely used for treatment of cancers and other diseases in humans. The patient outcomes resulting from their utilization demonstrate the value of these types of agents for treatment of diffuse and otherwise untreatable conditions. One of the inherent problems associated with the design of novel radiolabled compounds relates to development of radiolabeled conjugates that have the capacity for achieving specific tissue targeting to meet therapeutic objectives with an acceptable degree of systemic toxicity.

Critical to the success of using unsealed therapeutic radioactive agent for cancer therapy is the need for the development of compounds labeled with particle-emitting radionuclides that possess the following attributes, either alone or in combination: (1) demonstrated ability to in vivo target cancer cells selectively relative to normal cells, (2) capability to achieve sufficiently high radioactivity concentration and spacial distributions in the cellular matrixes of tumors to irradiate all cells in the tumor, (3) ability to achieve long-term residualization in the tumor for delivery of cytotoxic radiation doses to the tumor, and (4) capability to clear the radiolabeled drug or its radioactive metabolites efficiently from nontarget tissues (particular radiosensitive normal cells) in order to minimize radiation-induced side effects. To successfully design, synthesize, and evaluate effective therapeutic radiopharmaceuticals, there is a great need for involvement of synthetic, organic, analytical, inorganic, and radiochemists working in concert with scientists with expertise in the biochemical, pharmacological, physiological, and oncological sciences. Interdisciplinary efforts of this level will provide reasonable possibilities for the efficient development of radiopharmaceuticals capable of delivering cytotoxic doses of radiation in high specificity to cancerous tumors.

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CR9804386