^{99m}Tc-Labeled Small Peptides as Diagnostic Radiopharmaceuticals

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I. Introduction

A. Radiopharmaceuticals

Radiopharmaceuticals are drugs containing a radionuclide and are used routinely in nuclear medicine departments for the diagnosis or therapy of various diseases. They are mostly small organic or inorganic compounds with definite composition. They can also be macromolecules such as monoclonal antibodies and antibody fragments that are not stoichiometrically labeled with a radionuclide. Radiopharmaceuticals can be divided into two primary classes: those whose biodistribution is determined exclusively by their chemical and physical properties and those whose ultimate distribution is determined by their receptor binding or other biological interactions. The latter class is often called target-specific radiopharmaceuticals.

The successful use of ^{111}In−DTPA−Octreotide (OctreoScan, Figure 1) in diagnosis of somatostatin receptor-positive tumors has intensified the search for new target-specific radiopharmaceuticals based on small biomolecules. Many small biomolecules have been synthesized, radiolabeled, and studied for their potential use as new diagnostic imaging agents for various diseases. These have been recently reviewed.[1−10] Table 1 shows some examples of radiolabeled receptor ligands (peptides and non-peptides) used for receptor imaging. These include ^{99m}Tc-labeled chemotactic peptides,[11−22] leukotriene B_4 (LTB_4) receptor antagonists,[23−26] and tuftsin receptor antagonists[27−29] for imaging focal sites of infection; somatostatin analogues,[30−39] bombesin analogues,[40−44]

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Shuang Liu was born in China. He received his B.S. degree in Chemistry at Shandong Normal University in 1982 and then completed his Master's degree in Pharmaceutical Sciences with Professor Huicai Wang at Shandong Medical University in 1985. He came to North America in 1987 to pursue his Ph.D. with Professor Robert C. Lucas at Memorial University of Newfoundland, St. John's, Canada, studying the coordination chemistry of macrocyclic thioether ligands with various transition metals. After completion of his Ph.D. in 1990, he spent the next 2.5 years as a NSERC (Natural Sciences and Enginerring Research Council of Canada) postdoctoral fellow with Professor Chris Orvig at the University of British Columbia, Vancouver, Canada, investigating the coordination chemistry of technetium, aluminum, gallium, indium, and lanthanide metal complexes with various ligand systems. In 1993, he joined DuPont Pharmaceuticals Company as a Senior Research Scientist and then Principal Research Scientist in the Medical Imaging Division (North Billerica, MA), conducting discovery research on receptor-based target-specific metalloradiopharmaceuticals, development of new techniques for the radiolabeling of small biomolecules, and formulation research. His research interests include new bifunctional chelators, radiolabeling of biomolecules, and inorganic chemistry and radiochemistry of metallopharmaceuticals.

Scott Edwards was born and raised in Georgia. He received his B.S. degree in Chemistry at the Georgia Institute of Technology in 1979 and his Ph.D. degree in Inorganic Chemistry from the Massachusetts Institute of Technology in 1983, studying organometallic transition-metal chemistry with Professor Richard Schrock. From 1983 to 1987, he worked in the Central Research Laboratories of Dow Chemical Company. In 1988, he joined what is currently the Medical Imaging Division of DuPont Pharmaceuticals Company as Senior Research Chemist. His research interests include new chelators and ligand systems for technetium and rhenium and their application to the design and synthesis of novel radiopharmaceuticals for imaging a variety of disease processes and for the treatment of cancer. He is presently Associate Director of the Discovery Chemistry group.

folate receptor antagonists,[45−47] and vasoactive intestinal peptide (VIP)[48−52] for imaging tumors; and platelet GPIIb/IIIa receptor antagonists for imaging thrombi.[53−64] Other radiolabeled receptor ligands such as neurotransmitter receptor antagonists, dopamine transporter antagonists, progestin receptor antagonists, and sigma receptor ligands have been recently reviewed.[1] Many excellent reviews have been published in the past decade discussing the technetium chemistry,[65−74] technetium radiopharmaceuticals,[75−83] radiolabeled antibodies for diagnosis and therapy,[84−90] potential radionuclides for diagnosis and therapy,[91−98] ^{99m}Tc-labeled target-specific small molecule radiopharmaceuticals,[1,9,99−105] radiolabeling of highly potent small peptides,[2,8] and pharmacokinetic characteristics of ^{99m}Tc-labeled small peptides.[106]

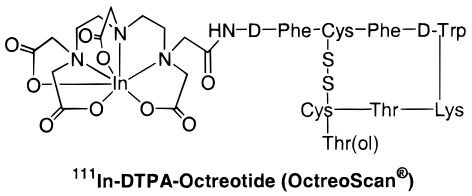


Figure 1. Structure of ^{111}In−DTPA−Octreotide (OctreoScan).

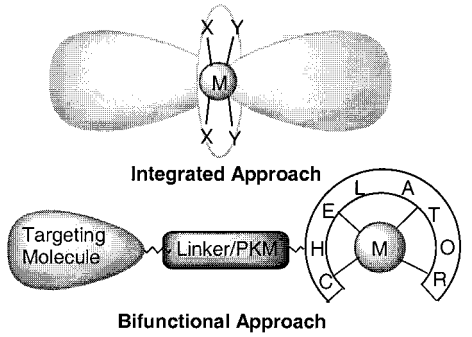


Figure 2. Schematic representation of two strategies of radiopharmaceutical design.

B. This Review

This review will focus on some fundamental aspects in the design and development of target-specific radiopharmaceuticals based on small peptide receptor ligands. It will also discuss the technetium chemistry associated with the ^{99m}Tc-labeling of highly active small peptides, technetium cores and bifunctional coupling agents (BFCAs), conjugation groups for peptide attachment, different radiolabeling approaches, three routes to prepare the ^{99m}Tc−BFCA−peptide complexes, quality control and characterization of ^{99m}Tc-labeled small peptides, and some examples of ^{99m}Tc-labeled receptor ligands as target-specific diagnostic radiopharmaceuticals.

II. Strategies for ^{99m}Tc Radiopharmaceutical Design

A. Integrated Approach

Figure 2 shows two strategies, the bifunctional approach and the integrated approach, for the design of receptor-based ^{99m}Tc radiopharmaceuticals. Both have been used in the design of ^{99m}Tc-labeled receptor ligands for imaging both extra- and intracellular receptors. The integrated approach involves the replacement of part of a known high-affinity receptor ligand with the requisite "unnatural" ^{99m}Tc chelate

Table 1. Examples of Radiolabeled Receptor Ligands as Diagnostic Radiopharmaceuticals

receptor ligand	receptor	radiolabeled compound	targeted disease	ref
		Peptides		
RGD-containing peptides	GPIIb/IIIa/platelet	RP419/DMP444	thrombosis	56−59, 189
CSVTCR	CD36/platelet	^{99m}Tc−GAGG−Aba−CSVTCR	thrombosis	391
RGD-peptidomimetic	GPIIb/IIIa/platelet	^{99m}Tc−P280/Thromboscan	thrombosis	61, 388, 394
TKPR-containing peptides	tuftsin	^{99m}Tc−RP128	infection/inflammation	28, 29, 414
chemotectic peptides	CTR/WBC[a]	f-MLFK−HYNIC−^{99m}Tc	infection/inflammation	12−22
P483	platelet factor 4	^{99m}Tc−P483H[b]	infection/inflammation	413
octreotide	somatostatin	^{111}In−DTPA−Octreotide	tumor (neuroendocrine)	420, 422−424
P-829	somatostatin	^{99m}Tc−P829	tumor (neuroendocrine)	33, 425, 426
vasoactive intestinal peptides VIP	^{99m}Tc−MAG_3−VIP/ ^{99m}Tc−1666		tumor	49, 237
vasoactive intestinal peptides vip		^{99m}Tc−GAGG−Aba−VIP	tumor	234
bombesin analogues	bombesin/GRP[c]	^{99m}Tc−Lys^3(DADT)−Bombesin	tumor	41, 42
P_2S_2−bombesin	bombesin/GRP[c]	^{99m}Tc−P_2S_2−Bombesin	tumor	43
HYNIC−bombesin	bombesin/GRP[c]	^{99m}Tc−HYNIC−Bombesin	tumor	44
RGD-containing peptides	vitronectin/integrin	^{125}I−D-Tyr−cyclo(RGDyV)	tumor	443
YIGSR	laminin/angiogenesis	^{131}I−YIGSR	tumor	445
α-MSH[d]	α-melanocyte	^{99m}Tc/^{188}Re−CCMSH	tumor (breast, prostate)	235, 236
		Non-Peptides		
leukotriene B_4 antagonists	LTB_4/WBC	RP517/RP532	infection/inflammation	23−26
folate antagonists	folate	^{111}In−DTPA−folate	tumor	45−47

[a] White blood cell. [b] ^{99m}Tc−P483 and heparin complex. [c] Gastrin release peptide. [d] Melanocyte stimulating hormone.

in such a way that there are minimal changes in size, conformation, and receptor binding affinity. The technetium chelate is a vital part of the receptor binding motif. Unfortunately, this approach often results in a more synthetically challenging target molecule or the loss of receptor binding affinity.[1] Apparently, the replacement of the C−C or C−heteroatom bonds with Tc−N or Tc−S coordination bonds usually has a significant impact on the size and conformation of the targeting molecule, which are critical for receptor binding. In addition, the introduction of the $[Tc{=}O]^{3+}$ core will lead to the change in the lipophilicity of the receptor ligand.

B. Bifunctional Approach

Unlike the integrated approach, the bifunctional approach uses a high binding affinity receptor ligand as the targeting molecule, a BFCA for the conjugation of the receptor ligand and the chelation of the radionuclide (^{99m}Tc), and a linker as the pharmacokinetic modifier (PKM). In this approach, the technetium chelate is often far apart from the receptor binding motif to minimize possible interference of the receptor binding by the technetium chelate. This is the more popular approach in the design of new receptor-based radiopharmaceuticals, in part, due to the likelihood of retaining the receptor binding affinity with a careful selection of the BFCA for the ^{99m}Tc-labeling and, in part, due to the fact that much of the coordination chemistry of small ^{99m}Tc chelates has already been well explored and understood. Therefore, it is not surprising that all the new receptor-based target-specific ^{99m}Tc radiopharmaceuticals approved or under clinical investigation use the bifunctional approach.

III. Fundamentals of Receptor Imaging

A. Target-Specific Radiopharmaceuticals

In general, a receptor-based target-specific radiopharmaceutical using the bifunctional approach (Figure 2) can be divided into four parts: a targeting molecule, a linker, a BFCA, and a radionuclide. The targeting molecule serves as the vehicle, which carries the radionuclide to the receptor site at the diseased tissue. The targeting molecules can be macromolecules, such as antibodies, or small biomolecules, including peptides, peptidomimetics, and nonpeptide receptor ligands. The choice of biomolecule depends on the disease target. The radionuclide is the radiation source. Between the targeting molecule and the radionuclide is the BFCA, which strongly coordinates to the metal ion and is covalently attached to the targeting molecule either directly or through a linker. Selection of a BFCA is largely determined by the nature and oxidation state of the metallic radionuclide. The linker can be a simple hydrocarbon chain or a long poly(ethylene glycol) (PEG), which is often used for modification of pharmacokinetics. Sometimes, a metabolizable linker is used to increase the blood clearance and to reduce the background activity, thereby improving the target-to-background (T/B) ratio.[107]

The use of metallic radionuclides offers many opportunities for designing new radiopharmaceuticals by modifying the coordination environment around the metal with a variety of chelators. The coordination chemistry of the metallic radionuclide will determine the geometry of the metal chelate and influence the solution stability of the radiopharmaceutical. Different metallic radionuclides have their different coordination chemistries and require BFCAs with different donor atoms and ligand frameworks. For "metal essential" radiopharmaceuticals, there is no targeting moiety and the biodistribution is exclusively determined by the physical properties of the metal chelate. For target-specific radiopharmaceuticals, the "metal tag" is not totally innocent because the target uptake and biodistribution will be affected by the metal chelate, the linker, and the targeting biomolecule. This is especially true for radiopharmaceuticals based on small molecules, such as peptides,

due to the fact that in many cases the metal chelate contributes greatly to the overall size and molecular weight. Therefore, the design and selection of the BFCA is very important for the development of a new radiopharmaceutical.

B. Receptors and Receptor Imaging

The term "receptor" was first introduced by Paul Ehrlich to describe the interaction of tetanus toxin with a specific protoplasmic site.[108] He is also credited with concept of the "magic bullet." Traditionally, biochemists define receptors as entities that can recognize a receptor ligand with high affinity and selectivity. The receptors are usually proteins embedded in a double layer of lipid molecules, the cell membrane, which separate the extracellular environment from the intracellular compartment. Since there are only a few structural determinations of receptors, a receptor is often characterized by its biological properties, including high ligand affinity, specificity, saturability, and distribution in relation to the physiological response.[100] The determination of these properties has to be performed experimentally and has been made possible by the development of high specific activity radiotracers such as ^{125}I-fibrinogen for the in vitro assay of the binding affinity of GPIIb/IIIa receptor antagonists. On the other hand, studies of various receptors and their connection with various diseases or disease states help the development of new imaging agents, which can be used to monitor changes in receptor and receptor function as the disease progresses.

Receptor imaging is usually reserved for localization of a radiolabeled compound that binds to receptors with high affinity and specificity. The high specificity of receptor binding results in selective uptake and distribution of the radiolabeled receptor ligand at the tissues, which are known to contain a substantial concentration of the target receptor. It is this high receptor binding affinity and specificity that makes receptor imaging advantageous over traditional scintigraphic imaging using simple technetium complex radiopharmaceuticals and over other diagnostic modalities such as magnetic resonance contrast imaging (MRI).

In general, the receptor protein is present in a limited concentration (10^{-6}–10^{-10} M or 10^{-9}–10^{-13} mol per gram of tissue).[109–111] MRI contrast imaging requires much higher concentrations of the imaging agent to achieve a reasonable contrast. It has been estimated that the minimal concentration required to achieve a 50% increase in image enhancement is about 10^{-4} M ($\sim 10^{-7}$ mol per gram of tissue) at 2 T using a standard T1 (spin lattice relaxation time) weighted spin–echo pulse sequence.[111] At this concentration, most receptors can be easily saturated. Therefore, it is virtually impossible to perform receptor imaging using monomeric gadolinium complex MRI contrast agents.

C. Agonists and Antagonists

Receptor binding is only the first step in both pharmacological and hormonal signaling. It is usually followed by a cascade of biological effects after receptor interaction. Some receptor ligands bind to the receptor site, compete with the "native ligand", and cause a cascade of biochemical effects. They are usually called "agonists". Some receptor ligands bind to the receptor site with comparable binding affinity and cause no physiological and biochemical reactions. They are considered as antagonists. For a receptor ligand to be useful as a targeting molecule, it should have very high receptor binding affinity with IC_{50} value in the nanomolar range. Low receptor binding affinity will result in low radioactivity uptake and short residence time at the receptor site. The receptor ligand should also have high specificity because the same peptide sequence or receptor binding motif might be able to bind to two or more receptors with a different affinity. For example, the RGD tripeptide sequence is known to bind the GPIIb/IIIa receptor on activated platelets[112,113] and vitronectin receptors (such as $\alpha v\beta 1$, $\alpha v\beta 3$, and $\alpha v\beta 5$) on endothelial cells.[114–116]

A radiopharmaceutical kit typically contains an excess of BFCA-derivatized receptor ligand relative to the amount of total technetium (^{99m}Tc and ^{99}Tc) in order to achieve a high radiolabeling yield, a reducing agent such as stannous chloride, if necessary, and other components such as a bulking agent or a weak transfer ligand. Kits can be purchased and stored for daily preparation. In many cases, the ^{99m}Tc-labeling can be accomplished simply by adding [^{99m}Tc]-pertechnetate to the kit. It is very important to note that the excess BFCA-derivatized receptor ligand does not cause receptor saturation and blockage of the binding of the radiolabeled receptor ligand. It also should not cause any side effects or unwanted pharmacological responses either. Thus, receptor antagonists are the candidates of choice for the development of receptor-based radiopharmaceuticals.

D. Intracellular and Extracellular Receptors

Receptors can be either intracellular or extracellular. The location of the receptor determines the radiopharmaceutical design, the selection of BFCA, and the degree of tolerance of the receptor ligand toward chemical modification such as attachment of the BFCA and the radionuclide. If the receptors are intracellular, the radiolabeled receptor ligand has to cross the cell membrane to reach the receptor site. In this case, the receptor ligand is usually small and has little tolerance for chemical modifications.[1] The metal chelate has to be neutral unless a specific intracellular transport mechanism is accessible. If the receptor is extracellular, the radiolabeled receptor ligand will not have to cross the cell membrane to interact with the receptor. In this situation, there is usually a higher degree of tolerance toward chemical modifications.[1] The metal chelate can be neutral or charged depending on the pharmacokinetic requirements. It is not surprising that most of the new radiopharmaceuticals approved or under clinical investigation are targeted to extracellular receptors. Examples include ^{111}In–DTPA–Octreotide and ^{99m}Tc–P829 for somatostatin receptors on the cell membranes of carcinoid tumors, as well as ^{99m}Tc–P280

and DMP444 for GPIIb/IIIa receptors on activated platelets.

The choice of a receptor system must be made on both chemical and clinical grounds. First, one has to identify a clinical need for a new radiopharmaceutical to diagnose a disease and the relationship between the disease and the expression of the receptors. There is no point to develop a radiopharmaceutical without clinical need. Second, the receptor concentration has to be sufficiently high. There is no value in developing a radiolabeled receptor ligand for a clinically important disease if the radiolabeled receptor ligand is not concentrated in the target tissue in such an amount that an external image can be obtained. Third, the receptor must be able to recognize the targeting molecule with high affinity and specificity. Otherwise, the new radiopharmaceutical will not be able to provide the necessary target uptake and target-to-background ratio and it will be difficult to differentiate various diseases or disease states with high sensitivity and specificity.

From a chemical equilibrium point of view, the number of radiolabeled molecules bound to the receptor is directly related to the affinity constant (K) and the receptor concentration (R_o). If one assumes that both the receptor and radiolabeled receptor ligands are homogeneous and there is no excess unlabeled receptor ligand to compete with the radiolabeled receptor ligand, the Scatchard form of the law of mass action is often used to describe the relationship between the target (bound, B) to background (free, F) ratio and the equilibrium constant (K) and the receptor concentration (R_o).[100,105,107]

$$\mathrm{B/F} = KR_o - KB \approx KR_o$$

If the receptor concentration (R_o) is much higher than that of the radiolabeled receptor ligand, the target-to-background ratio (B/F) is almost equal to KR_o. Obviously, both the receptor binding affinity and receptor population are important to the target-to-background ratio. It should be pointed out that this is an oversimplified equation useful only when the receptor concentration is in large excess over the receptor ligand (bound and free) and there is no excretion of radiolabeled receptor ligand. In practice, receptor imaging is much more complicated, particularly when receptors are intracellular.

E. Sensitivity of Receptor Imaging

The clinical application of a radiolabeled receptor ligand for receptor imaging will depend ultimately on its sensitivity, the counts recorded for a given amount of activity in a specific focus.[3] It is not surprising to observe that the greater the percentage of injected dose taken up by the tumor, the smaller the lesion detected. There are many factors that could influence the sensitivity of the radiopharmaceutical. These include the physical characteristics of the radionuclide, the properties of the radiopharmaceutical (receptor binding affinity and hydrophilicity), the size and location of the disease, receptor population, the rate and route of elimination of the radiolabeled receptor ligand, metabolism, nonspecific protein binding, competition from the unlabeled receptor ligand, internalization of receptors, and heterogeneity of the receptor population.

In general, higher receptor concentration gives better sensitivity. Receptor heterogeneity results in a lower number of binding sites for the radiolabeled receptor ligand and, consequently, leads to a decrease in the amount of radioactivity that is targeted to the disease and a loss of sensitivity for detection.[118] Small superficial lesions are more readily detectable than deep-seated ones. It is very important to remember that only a small portion of the injected radiopharmaceutical is delivered to the receptor site. The higher the amount of radiopharmaceutical delivered to the receptor site, the better the sensitivity of the radiopharmaceutical will be. The delivery of the radiopharmaceutical to the receptor site is dependent on the rate of blood clearance, the rate of diffusion across the cell membrane, and metabolic cleavage of the radiolabel from the receptor ligand.

F. Targeting Molecules

In the last two decades, monoclonal antibodies and their fragments have been studied for their potential applications in both diagnostic and therapeutic nuclear medicine. Radiolabeled monoclonal antibodies are often referred as "magic bullets". Although considerable progress has been made in this area,[84−90] clinical studies with radiolabeled antibodies have often demonstrated limited accumulation in the target and relatively slow blood clearance due to their high molecular weight, resulting in only modest target-to-background ratios. The lack of effectiveness of radiolabeled antibiodies is attributed, in part, to the inaccessibility of macromolecules to the tumor cells in solid masses and, in part, to the heterogeneous distribution of tumor-associated antigens on the tumor surface.[119] Antibodies have a high receptor binding affinity and high specificity. However, if the radiolabeled antibodies cannot be delivered to the target efficiently and clear from the nontarget organs fast enough, they are not good targeting molecules for receptor imaging. As Dr. Britton[120] stated "it is common to talk about the magic bullet. There are plenty of bullets but no magic, just a lot of hard work".

Peptides are compounds that contain amino acids (α-amino carboxylic acids) linked by amide (peptide) bonds. In mammals, 80% of the hormonal messengers are peptides, 15% are steroids, and 5% are tyrosine derivatives, the catecholamines and iodinated tyrosines.[119] Designed by nature for stimulating, inhibiting, or regulating numerous life functions, peptides have been considered ideal agents for therapeutic applications. Although the ^{111}In-labeled peptide was first explored for the use in nuclear medicine in 1981,[121] it was not until recently that ^{99m}Tc-labeled peptides have emerged as an important class of radiopharmaceuticals in diagnostic nuclear medicine.[2−10]

The difference between proteins and peptides is their sizes. The term "peptides" is usually used to refer to those containing less than 100 amino acids with a molecular weight of about 10 000 Da.[9] Small

peptides refer to peptides with less than 30 amino acids or a molecular weight less than 3500 Da. Compared to antibodies, small peptides offer several advantages. Peptides are necessary elements in more fundamental biological processes than any other class of molecule, and in many cases, the affinities of small peptides for their receptors are significantly greater than that of antibodies or their fragments. They can also tolerate harsher chemical conditions for modification or radiolabeling.[10] Small peptides are easy to synthesize and modify, less likely to be immunogenic, and can have rapid blood clearance. The faster blood clearance results in adequate T/B ratios earlier so that it is practical to use ^{99m}Tc, which is the preferred radionuclide for diagnostic nuclear medicine. In most cases, the primary sites of interactions of the peptides are specific receptors on the outer surface of the cell membrane (extracellular). All these factors make small peptides excellent candidates for the development of target-specific radiopharmaceuticals. In addition, other small molecule receptor antagonists such as peptidomimetics may also be used as targeting molecules.

Many biologically active peptides are known, and some of them are available commercially. These peptides are often developed as therapeutic drugs for the treatment of various diseases.[122,123] In some way, radiopharmaceutical research can take advantage of the new development in the therapeutic pharmaceutical industry, using the peptide lead compounds as a platform and modifying them with a BFCA for radiolabeling. For peptide-based therapeutic pharmaceuticals, bioavailability remains a major obstacle.[124] For radiopharmaceuticals, however, this is not an issue because they are administered via intravenous injection. At the same time, the results from radiopharmaceutical research will help the therapeutic pharmaceutical industry to have a better understanding of the possible biodistribution, excretion, and metabolism of the peptide lead compounds.

There are also disadvantages of using small peptides as targeting molecules. The first obstacle is their rapid proteolysis in plasma by endogenous peptidases and proteases. The peptides must be "molecularly engineered" to inhibit their enzymatic metabolism or destruction in order to increase their biological half-life in plasma. There are several ways to block the enzymatic destruction of peptides, including the use of D-amino acids (such as in Octreoscan),[121] substitution of the peptide bonds, replacement of the disulfide bond with a thioether linkage,[33] and insertion of unusual amino acids. Another obstacle to overcome in using small peptides as targeting molecules is the loss of binding affinity when conjugated to the BFCA and labeled with a radionuclide. In many cases, the peptide molecule contains only 4−6 amino acid residues. The attachment of a BFCA and the radionuclide has a significant contribution to the overall molecular weight and may cause conformational changes in the peptide receptor binding sequence. Therefore, whenever possible, the attachment of the BFCA and the radiolabel should be kept apart from the receptor binding sequence.

Table 2. Metallic Radionuclides Useful for Diagnosis

radionuclide	half-life	γ-energy (keV)	decay mode	source
^{64}Cu	12.8 h	511 (10%), 185 (24%)	EC	reactor
^{67}Ga	78.3 h	93 (10%), 185 (24%) 296 (22%)	EC	generator
^{99m}Tc	6.02 h	141 (89%)	IT	generator
^{111}In	2.83 d	171 (88%), 247 (94%)	EC	cyclotron
^{117m}Sn	14.0 d	159 (87%)	IT	cyclotron

G. Radionuclide

Table 2 shows several metallic radioisotopes useful for diagnostic nuclear medicine. The choice of the radionuclide depends largely on the physical and nuclear properties (half-life and γ-energy), availability, and cost. Nearly 80% of all radiopharmaceuticals used in the nuclear medicine department are ^{99m}Tc-labeled compounds. The 6 h half-life is long enough to allow a radiochemist to carry out radiopharmaceutical synthesis and for nuclear medicine practitioners to collect useful images. At the same time, it is short enough to permit the administration of millicurie amounts of ^{99m}Tc radioactivity without a significant radiation dose to the patient. The monochromatic 140 keV photons are readily collimated to give images of superior spatial resolution. Furthermore, ^{99m}Tc is readily available from commercial ^{99}Mo−^{99m}Tc generators at low cost.

^{99m}Tc is produced from a parent radionuclide, ^{99}Mo, a fission product with a half-life of 2.78 days. In a ^{99}Mo−^{99m}Tc generator, [^{99}Mo]molybdate is absorbed to an alumina column and ^{99m}Tc is formed by decay of ^{99}Mo. The ^{99m}Tc in the form of [^{99m}Tc]−pertechnetate is eluted from the column with saline. The ^{99m}Tc produced by the generator is never carrier-free because 15% of ^{99}Mo decays directly to the long-lived isotope ^{99}Tc ($t_{1/2} = 2.13 \times 10^5$ y), which is also the single decay product of ^{99m}Tc. The specific activity of eluted ^{99m}Tc is very high and is dependent upon the prior-elution time. In general, the total concentration of technetium (^{99m}Tc and ^{99m}Tc) in the ^{99}Mo−^{99m}Tc generator eluent is in the range of 10^{-7}−10^{-6} M.

H. Bifunctional Coupling Agents

A BFCA can be divided into three parts: a binding unit, a conjugation group, and a spacer (if necessary). An ideal BFCA is that which is able to form a stable ^{99m}Tc complex in high yield at very low concentration of the BFCA−peptide conjugate. There are several requirements for an ideal BFCA. The binding unit can selectively stabilize an intermediate or lower oxidation state of Tc so that the ^{99m}Tc complex is not subject to redox reactions; oxidation state changes are often accompanied by transchelation of ^{99m}Tc from a ^{99m}Tc−BFCA−peptide complex to the native chelating ligands in biological systems. The BFCA forms a ^{99m}Tc complex which has thermodynamic stability and kinetic inertness with respect to dissociation. The BFCA forms a ^{99m}Tc complex with a minimum number of isomers since different isomeric forms of the ^{99m}Tc chelate may result in significantly different biological and pharmacokinetic characteristics of the ^{99m}Tc−BFCA−peptide complex. Finally, the conjugation group can be easily attached to the peptide.

In simple technetium complex radiopharmaceuticals such as ^{99m}Tc−sestamibi, $[^{99m}Tc(MIBI)_6]^+$ (MIBI = 2-methoxy-2-methylpropylisonitrile), and ^{99m}Tc-bicisate [^{99m}TcO(ECD)] (ECD = *l*,*l*-ethylene dicycteine diethyl ester), the ligand (MIBI or ECD) is always present in large excess. The main factor influencing the ^{99m}Tc-labeling kinetics is the nature of the donor atoms and the radiolabeling conditions. For receptor-based peptide radiopharmaceuticals, however, the use of a large amount of BFCA−peptide may result in receptor site saturation, blocking the docking of the ^{99m}Tc-labeled BFCA−peptide conjugate, as well as unwanted side effects. To avoid these problems, the concentration of the BFCA−peptide conjugate in each kit has to be very low (10^{-6}−10^{-5} M). Otherwise, a postlabeling purification is often needed to remove excess unlabeled peptide, which is time-consuming and thus not amenable for clinical use. Compared to the total technetium concentration ($\sim 5 \times 10^{-7}$ M) in 100 mCi of [^{99m}Tc]pertechnetate (24 h prior-elution), the BFCA−peptide conjugate is not in overwhelming excess. In this situation, the ^{99m}Tc-labeling yield and the labeling kinetics may be affected by the amount of both BFCA−peptide conjugate and total technetium (^{99m}Tc and ^{99m}Tc) in the kit. Therefore, the BFCA attached to the peptide must have very high radiolabeling efficiency. It should be noted that the amount of BFCA−peptide conjugate is largely dependent on the receptor population, the type of receptor ligand, and the possible side effect caused by the use of a large amount of receptor ligand. A careful "peptide sensitivity" study is required to determine the optimum amount of BFCA−peptide conjugate in the kit formulation.

IV. General Considerations for ^{99m}Tc Radiopharmaceuticals

A. Requirements for ^{99m}Tc Radiopharmaceuticals

Discovery of a new class of drug is just the beginning of a long development process. In the development of a new ^{99m}Tc radiopharmaceutical, several factors need to be considered to satisfy the clinical requirements. The new radiopharmaceutical has to demonstrate its biological efficacy (high uptake in the target tissue, high target-to-background ratio, high specificity and sensitivity for the targeted disease or disease state). A kit formulation is required due to the 6 h half-life of ^{99m}Tc. The new radiopharmaceutical should have high radiochemical purity (RCP ≥ 90%) and high solution stability (shelf life preferably ≥ 6 h). The ^{99m}Tc-labeling should be accomplished in 10−30 min, preferably at room temperature.

B. Pharmacokinetic Considerations

For a new radiopharmaceutical, pharmacokinetics is one of the most important factors to consider. Pharmacokinetics is a term used conventionally to refer to the movement of a drug in the body. This includes absorption, distribution, metabolism, and elimination. When it is used in the context of radiopharmaceuticals, the term pharmacokinetics is often used to refer to the distribution and elimination of the radionuclide following administration of the radiopharmaceutical. Modification of pharmacokinetics of a new radiopharmaceutical involves weighing various factors such as blood retention time, receptor binding kinetics (fast binding and slow dissociation), and the excretion route.

The main pharmacokinetic consideration in the development of new imaging agents is that the radiolabeled compound is able to have its highest target uptake with a diagnostically useful signal-to-noise ratio in a short period of time.[106] To achieve this goal, the new radiopharmaceutical should have a relatively short blood residence time. The fast blood clearance is necessary to minimize nontarget radioactivity. On the other hand, the blood retention time should be long enough to allow the radiopharmaceutical to reach the receptor sites and achieve adequate accumulation of radioactivity at the targeted tissue or region of tissue. The time for the radiopharmaceutical to reach the target should also be short. Otherwise, it will take a long time to get diagnostically useful images. One of the problems associated with radiolabeled antibodies is their slow kinetics to reach the targeted tissue and to clear from circulating blood. The receptor binding rate of a radiopharmaceutical should be fast and the dissociation rate slow. In this way, the radioactivity accumulation at the target tissue can be maximized. Finally, the new radiopharmaceutical must have a rapid renal clearance to avoid accumulation of radioactivity in the gastrointestinal tract, which may obscure visualization of abdominal targets. It should be emphasized that the peptide and the ^{99m}Tc−BFCA−peptide complex should have high and specific receptor binding. However, if the ^{99m}Tc−BFCA−peptide complex does not show favorable pharmacokinetics, it would still be difficult to develop it into a commercial product for routine clinical applications.

C. Modification of Pharmacokinetics

There are several ways to modify the pharmacokinetics of a radiopharmaceutical. These include chemical modification of the targeting molecule or technetium chelate, the use of a linker as the pharmacokinetic modifier, and the choice of different technetium coligands if possible. The chemical modification of the targeting molecule can be achieved by introducing various hydrophilic or lipophilic groups onto the side chains of amino acids. Sometimes, an "innocent" peptide sequence such as polylysine, polyglycine, or polyaspartic acid can be used to improve the hydrophilicity of the targeting molecule. The chemical modification of the technetium chelate can be achieved using BFCAs with different charge and hydrophilicity. It has been demonstrated that BFCAs have a profound impact on the physical and biological properties of the ^{99m}Tc-labeled small peptides.[56,57] The choice of a linker depends on the pharmacokinetic requirement for the radiopharmaceutical. It can be a simple hydrocarbon chain to increase the lipophilicity, a poly(amino acid) sequence such as polyglycine to increase the hydrophilicity and renal clearance, or a long poly(ethyleneglycol) to slow extraction by

Table 3. Oxidation States and Stereochemistry of Technetium

oxidation state	example	coordination geometry	coordination number	magnetic moment (μB)
+7 (d^0)	$[TcH_9]^{2-}$	trigonal prism	9	diamagnetic
	TcO_4^-	tetrahedron	4	diamagnetic
+6 (d^1)	TcO_4^{2-}	tetrahedron	4	1.60
+5 (d^2)	$[Tc(NCS)_6]^-$	octahedron	6	diamagnetic
	$[Tc(Diars)_2Cl_4]^+$	dodecahedron	8	0.9 (?)
	$TcOCl_4^-$	square pyramid	5	diamagnetic
	[TcO(*l, l*-ECD)]	square pyramid	5	diamagnetic
	[TcO(*d,l*-HM-PAO)]	square pyramid	5	diamagnetic
	$[TcO_2$(tetrofosmin)$]^+$	octahedron	6	diamagnetic
+4 (d^3)	$[TcCl_6]^{2-}$	octahedron	6	4.05
+3 (d^4)	$[Tc(Diars)_2Cl_2]^+$	octahedron	6	diamagnetic
+2 (d^5)	$[TcCl_2(PhP(OEt)_2)_4]$	octahedron	6	1.4
+1 (d^6)	$[Tc(CNC(CH_3)_3)_6]^+$	octahedron	6	diamagnetic
	$[Tc(CO)_3([9]aneN_3)]^+$	octahedron	6	diamagnetic
0 (d^7)	$[Tc_2(CO)_{10}]$	octahedron	6	diamagnetic
−1 (d^8)	$[Tc(CO)_5]^-$	trigonal bipyramid	5	diamagnetic

the hepatocytes. It has been reported that the linker groups have a significant effect on the biodistribution of ^{111}In- or ^{99m}Tc-labeled antibodies.[125−128] Metabolizable linkers have been used to modify the pharmacokinetics of ^{111}In-labeled somatostatin analogues.[107] Depending upon the radionuclide and the BFCA, linker functional groups capable of rapid metabolism such as esters or disulfides can increase the clearance of radiolabeled receptor ligand from the blood via the renal system.

V. Technetium Chemistry

A. Diverse Redox Chemistry

Technetium chemistry has been reviewed extensively.[65−73] One of the characteristics of technetium is its rich and diverse redox chemistry. Table 3 summarizes various oxidation states and coordination geometries of technetium. Thus far, there is no effective chemistry that can be used to attach the pertechnetate anion to a small peptide. Therefore, the Tc(VII) in $^{99m}TcO_4^-$ has to be reduced to a lower oxidation state in order to produce a stable ^{99m}Tc−peptide complex or to a reactive intermediate complex from which ^{99m}Tc can be easily transferred to the BFCA−peptide conjugate. When $^{99m}TcO_4^-$ is reduced, the oxidation state of technetium depends on the nature of the reducing agent, the chelator, and reaction conditions. The following examples illustrate this quite dramatically. On the macro-scale (using $^{99}TcO_4^-$), the brown-black insoluble $TcO_2{\cdot}2H_2O$ is readily produced in an aqueous solution when $Na_2S_2O_4$ is used as the reducing agent.[129] If ethane-1,2-dithiol is present, an orange Tc(V) complex, $[TcO(SCH_2CH_2S)_2]^-$, is obtained.[130] If pentane-2,4-dione is used as a ligand, the red Tc(III) complex, $[Tc(acac)_3]$,[131] results while the N-substituted pyridinone ligands form very stable tris-ligand Tc(IV) complexes $[Tc(L)_3]^+$ (L = N-substituted pyridinonate).[132] If the ligand contains P donors, Tc(IV), Tc(III), and Tc(I) complexes are isolated depending on the number of P donors and coligands in the complex.[133−135] Isonitrile ligands form colorless Tc(I) complexes, $[(Tc(CNR)_6]^+$, almost quantitatively.[136] In the presence of carbon monoxide, $^{99}TcO_4^-$ can be reduced by sodium borohydride to form the Tc(I) complex cation $[(Tc(H_2O)_3(CO)_3]^+$.[137] The rich and diverse redox chemistry of technetium makes it difficult to control the oxidation state and the stability of the Tc complex. At the same time, it provides more opportunities to modify the structure and properties of Tc complexes by the choice of the chelating system, through the use of donor atoms having a high affinity for a specific oxidation state of the Tc, as well as the introduction of nondonating functional groups.

B. Isomerism

Another aspect of technetium chemistry is isomerism, including geometric isomers, epimers, enantiomers, and diastereomers. Epimers are often found in square pyramidal or octahedral oxotechnetium complexes containing chelating ligands with substituents on the ligand backbone or a tertiary amine-N donor atom.[138−165] The formation of these isomers is due to the relative orientation (*anti* and *syn*) of the substituents to the $[Tc{=}O]^{3+}$ core. Enantiomers are often found in oxotechnetium complexes such as $[TcO(MAG_3)]^-$ because of the asymmetrical bonding of MAG_3^{4-} to the $[Tc{=}O]^{3+}$ core even though the free ligand H_4MAG_3 does not have a chiral center. If a technetium complex contains two or more chiral centers, diastereomers may be formed. Diastereomers can often be separated using appropriate HPLC methods.

Isomers often have different lipophilicities and biodistributions in biological systems. This is particularly true for simple technetium complex radiopharmaceuticals as their biological properties are determined exclusively by the physical and chemical characteristics of the technetium complex. For example, the complex $[TcO(map)]^-$ (map = 2,3-bis-(mercaptoacetamido)propanoate) has two epimers (*anti* and *syn*) due to the disposition of the COOH group on the chelate ring relative to the Tc=O moiety. It was reported that in humans 58% of the *syn* isomer was excreted at 30 min as compared to only 19% of the *anti* isomer.[158,160] For receptor-based radiopharmaceuticals, the target uptake is largely dependent on the receptor binding affinity of the radiolabeled receptor ligand, receptor population and the blood clearance, which is determined by the physical properties of both the peptide and techne-

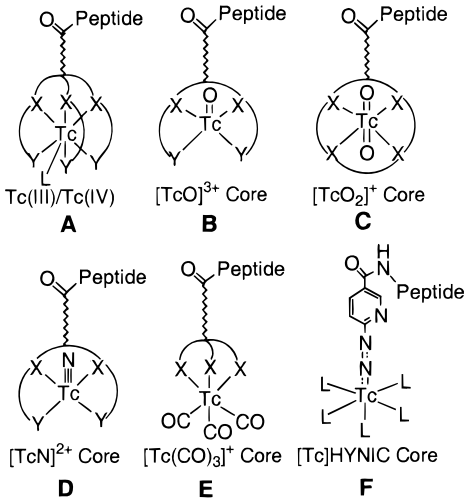


Figure 3. Technetium cores useful for the ^{99m}Tc-labeling of biomolecules.

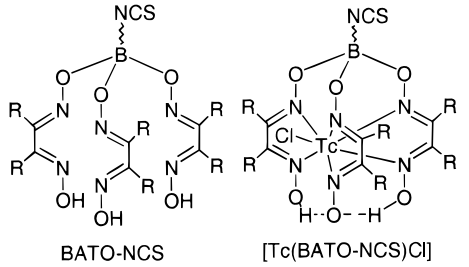


Figure 4. Bifunctional BATOs.

tium chelate. Therefore, the formation of isomers for the technetium chelate can have a significant impact on the biological properties of a radiopharmaceutical. The choice of BFCA should be those which form technetium complexes with minimal isomerism.

VI. Technetium Cores and Bifunctional Chelators

The technetium core determines the design of the BFCA framework and the choice of donor atoms. Figure 3 shows some technetium cores, which can be used for ^{99m}Tc-labeling of biomolecules. Table 4 lists examples of technetium cores and BFCAs that have been used for ^{99m}Tc-labeling of biomolecules such as antibodies, antibody fragments, peptides, peptidomimetics, and other non-peptide receptor ligands.

A. "Naked" Tc Atom

In Figure 3A, the technetium core is a "naked" Tc atom. The oxidation state for the Tc can be +3 or +4 depending upon the donor atoms. The donor atoms can be all imine nitrogens such as those in BATOs (boronic acid adducts of technetium dioximes)[166−168] or a combination of amine nitrogens and phenolate oxygens as in amine phenols.[169] In case of N_3O_3 amine phenols, the Tc(IV) is completely wrapped by three amine-N and three phenolate-O donor atoms with a coordination geometry between octahedron and a trigonal prism. For BATOs, the Tc(III) is seven-coordinate with six imine-N donors and a monodentate chloride coligand in a trigonal prismatic geometry.[166] BATOs (Figure 4) have been used as BFCAs for the ^{99m}Tc-labeling of antibodies,[170,171] as well as

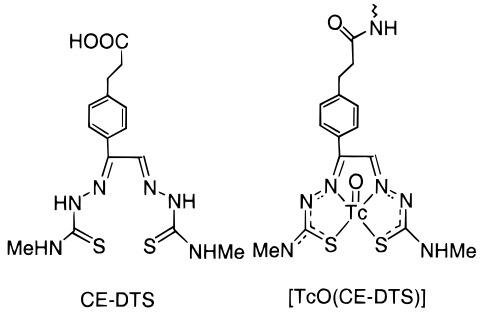


Figure 5. CE-DTS as a BFCA.

small molecules such as a nitroimidazole[172] and a muscarinic acetylcholine receptor ligand.[172]

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

The $[Tc{=}O]^{3+}$ core (Figure 3B) is very stable in the presence of a strong chelating group in aqueous media. It is the most frequently used technetium core for ^{99m}Tc-labeling of biomolecules (Table 4). The $[Tc{=}O]^{3+}$ core forms square pyramidal oxotechnetium complexes with tetradentate chelators, including N_4 propylene amine oxime (PnAO),[30] N_3S triamidethiols,[56,139,141−144] N_2S_2 diamidedithiols (DADS),[56,138] N_2S_2 monoamidemonoaminedithiols (MAMA),[56,152,162,164] and N_2S_2 diaminedithiols (DADT).[145−153]

p-Carboxyethylphenylglyoxal-di(*N*-methylthiosemicarbazone) (CE-DTS, Figure 5) contains an N_2S_2 donor set. It has been used for the ^{99m}Tc-labeling of antibodies.[174,175] Conjugation of CE-DTS with IgG at a 1:1 molar ratio was carried out by the phosphoryl azide method, but the yield was only 16.5% with retained immunoreactivity.[174] The purified CE-DTS−IgG conjugate was incubated with $^{99m}TcO_4^-$ in the presence of $SnCl_2$ and ascorbic acid to give the ^{99m}Tc-(CE-DTS)−IgG complex with 60% incorporation of ^{99m}Tc at 30 min, rising to 87−90% incorporation of ^{99m}Tc at 3 h.[175] Despite reported good in vivo stability of the ^{99m}Tc-labeled antibody, potential application of CE-DTS as a BFCA is severely limited by the high lipophilicity and low radiolabeling efficiency (slow radiolabeling and low labeling yield).

An N_2S_4 diaminetetrathiol (Figure 6) containing two amine-N and four thiolate-S donors has been used for the ^{99m}Tc- and ^{186}Re-labeling of antibodies.[176,177] It was designed not only to form a stable complex with ^{99m}Tc, but also to use one of the four thiols in coupling with an antibody via a disulfide linkage. The N_2S_4-modified antibody was labeled by transferring ^{99m}Tc from [^{99m}Tc]glucoheptonate with the labeling yield of 95%. Since the transfer of ^{99m}Tc to the N_2S_4 modified antibody was fast and nearly quantitative, no postlabeling purification was required. The radiolabeling could also be achieved by coupling the antibody with a $[^{99m}TcO(N_2S_4)]$ complex, which was preformed by reacting N_2S_4 with [^{99m}Tc]-pertechnetate in the presence (or absence) of sodium borohydride. The labeling yield in this way was only 50%. It has been suggested that an one-to-one coupling took place between the antibody and N_2S_4 BFCA through a disulfide linkage.[176] Although there is no structural evidence in the technetium literature

Table 4. BFCAs and Technetium Cores Useful for ^{99m}Tc-Labeling of Biomolecules[a]

BFCA	donor atoms	Tc core	biomolecule	conjugation group	ref
BATO	N_6Cl	Tc(III)	antibody	isothiocyanate	170−173
CE-DTS	N_2S_2	$[Tc{=}O]^{3+}$	antibody	activated carboxylate	174, 175
N_2S_4 aminethiol	N_2S_2(?)	$[Tc{=}O]^{3+}$	antibody	disulfide linkage	176, 177
DADS	N_2S_2	$[Tc{=}O]^{3+}$	peptide/antibody	activated ester	56, 178−181
MAG_3	N_3S	$[Tc{=}O]^{3+}$	peptide/antibody	activated ester	56, 182, 183
DADT	N_2S_2	$[Tc{=}O]^{3+}$	peptide/antibody	activated ester	194−212
MAMA	N_2S_2	$[Tc{=}O]^{3+}$	peptide/antibody	activated ester	56, 214−217
PnAO	N_4	$[Tc{=}O]^{3+}$	peptide	activated ester	30, 31, 225−228
Gly−Gly−Cys	N_3S	$[Tc{=}O]^{3+}$	peptide	amide/peptide	9, 61, 413
Me_2Gly−Ser−Cys	N_3S	$[Tc{=}O]^{3+}$	peptide	amide/peptide	232
Pic−Ser−Cys	N_3S	$[Tc{=}O]^{3+}$	peptide	amide/peptide	27
DAP−Gly−Cys	N_3S	$[Tc{=}O]^{3+}$	peptide	amide/peptide	33, 34
Lys−Gly−Cys	N_3S	$[Tc{=}O]^{3+}$	peptide	amide/peptide	9, 61
Cys−Gly−Cys	N_2S_2	$[Tc{=}O]^{3+}$	peptide	amide/peptide	9, 61
Gly−Ala−Gly−Gly	N_4	$[Tc{=}O]^{3+}$	peptide	amide/peptide	234, 391
cyclam/tetraamine	N_4	$[TcO_2]^+$	peptide/antibody	disulfide linkage	31, 274, 275
dithio−bisphosphine	S_2P_2	$[TcO_2]^+$	peptide	activated ester	42, 282, 283
PADA	N_2O	$[Tc(CO)_3]^+$	biomolecules	activated esyer	137
Cp	C_5	$[Tc(CO)_3]^+$	biomolecules	?	306
HYNIC	hydrazido/O_4(?)	[Tc]HYNIC	peptide/antibody	activated ester	12−21, 307−18
HYNIC	diazenido/NO_3P	[Tc]HYNIC	peptide/non-peptide	activated ester	55, 58
HYNIC	diazenido/ NNO_3	[Tc]HYNIC	peptide/non-peptide	activated ester	318, 321, 323

[a] BATO= boronic acid adducts of technetium dioximes; CE-DTS = *p*-Carboxyethylphenylglyoal-di(*N*-methylthiosemicarbazone); DADS = diamidedithiol; DADT = diaminedithiol; MAG_3 = mercaptoacetylglycylglycylglycine; MAMA = monoamine-monoamide-dithiol; PnAO = propylenediamine dioxime.

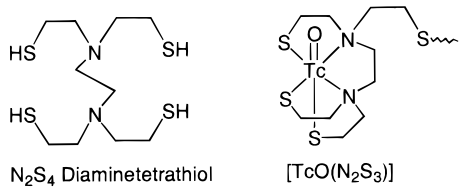


Figure 6. N_2S_4 diaminetetrathiol as BFCA.

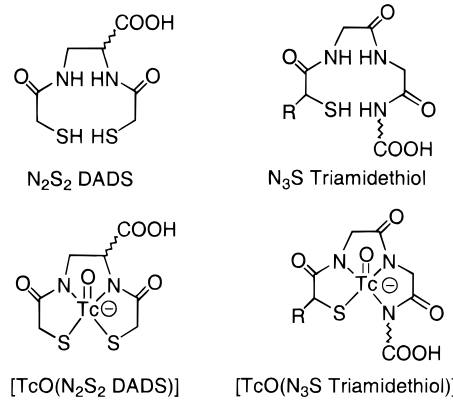


Figure 7. N_2S_2 diamidedithiol (DADS) and N_3S triamidethiol BFCAs.

where a sulfur atom coordinates to a $[Tc{=}O]^{3+}$ core in complexes containing the N_2S_2 diaminedithiol systems, it is still quite possible for the N_2S_3 donors to coordinate to the $[Tc{=}O]^{3+}$ core and form the six-coordinate technetium complex because of the neutrality requirement.

The N_2S_2 diamidedithiols (DADS, Figure 7) contain two amide-N and two thiolate-S donors and form stable anionic oxotechnetium complexes with the $[Tc{=}O]^{3+}$ core.[130,138] Like N_2S_2 diamidedithiols, N_3S triamidethiols also form very stable anionic oxotechnetium complexes.[139,141−144] Fritzberg and co-workers first reported the use of 4,5-bis(thioacetamido)pentanoate (mapt) as the BFCA in labeling antibodies

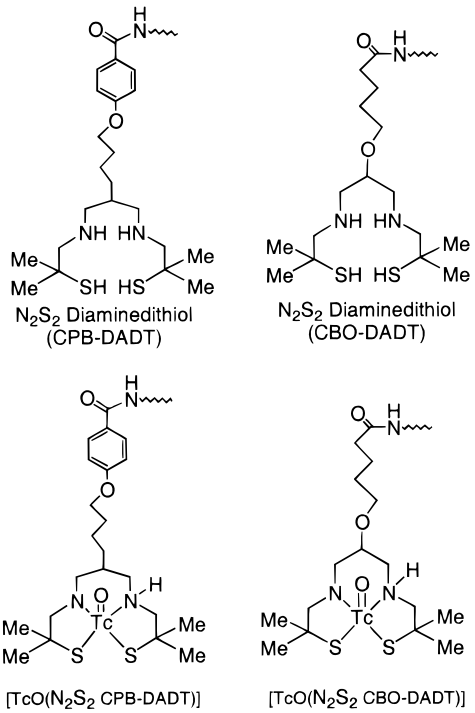


Figure 8. N_2S_2 diaminedithiol (DATS) BFCAs.

and their fragments with ^{99m}Tc by the preformed chelate approach.[178] It was found that the attachment of BFCA and incorporation of the radionuclide (^{99m}Tc or ^{99m}Re) does not significantly affect the physical and biological properties of the antibodies or antibody fragments since the radiolabeling occurs only on a small portion of these macromolecules. Other N_2S_2 diamidedithiols and N_3S triamidethiols have also been used as BFCAs for the ^{99m}Tc-labeling of biomolecules, including proteins,[179−186] biotin,[187] peptides,[56,188−192] and antisense oligodeoxynucleotides.[193]

N_2S_2 diaminedithiols (DADT, Figure 8) represent another class of BFCAs that bind the $[Tc{=}O]^{3+}$ core

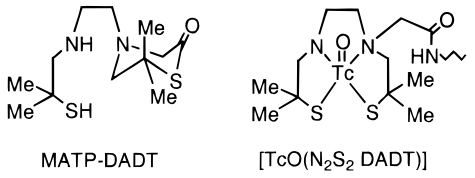


Figure 9. MATP−DADT as an N_2S_2 BFCA.

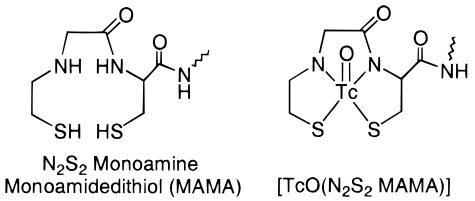


Figure 10. N_2S_2 monoamidemonoaminedithiol (MAMA) as a BFCA.

strongly to form stable technetium(V) complexes. They can be tribasic utilizing two thiolate sulfurs, one deprotonated amine nitrogen, and one neutral amine nitrogen to form neutral Tc(V) oxo complexes[145−150] or dibasic using two thiolate sulfurs and two tertiary amine nitrogens to form cationic Tc(V) oxo complexes.[153] Studies on antibody labeling with ^{99m}Tc using the *N*-hydroxysuccinimide ester of 6-[4′-(*p*-carboxyphenoxy)butyl]-2,10-dimercapto-2,10-dimethyl-4,8-diazaundecane(CPB-DADT) as the BFCA have been reported.[194−200] The ^{99m}Tc−DADT−IgG antibody conjugate was stable, and the presence of 1000-fold excess of DTPA did not cause transchelation of ^{99m}Tc.[197] Other DADT-based BFCAs were also *syn*thesized and used for the ^{99m}Tc-labeling of proteins and other biomolecules.[201−212]

The BFCA 4-[2[(2-mercapto-2-methylpropyl)methylamino]ethyl]-6,6-dimethyl-2-thiomorphilinone (MATP-DSDT, Figure 9) is of particular interest because it contains a thiomorpholinone ring, which can be used to react with primary amino groups of lysine residues. MAPT-DADT could be easily coupled to proteins under mild conditions, and the radiolabeling proceeded efficiently by transferring ^{99m}Tc from a weak ^{99m}Tc intermediate. The ^{99m}Tc-labeled proteins were highly stable both in vivo and in vitro with ^{99m}Tc binding to DADT.[210,212] MATP-DSDT has also been used for the ^{99m}Tc-labeling of muscarinic cholinergic receptor ligands[210,212] and small peptides.[11,40,41] One major drawback of using N_2S_2 diamidedithiol BFCAs is the high lipophilicity of their technetium chelate. The high lipophilicity often leads to high liver uptake and hepatobillary excretion of the radiolabeled compound. However, the lipophilicity of DADT chelators can be controlled by introducing various hydrophilic groups.

N_2S_2 monoamidemonoaminedithiols (MAMA, Figure 10) contain a secondary amine nitrogen, an amide nitrogen and two thiolate sulfur donors. They bind to the $[Tc{=}O]^{3+}$ core as tribasic ligands to form neutral complexes, [^{99m}TcO(MAMA)],[213] provided that the functional group is not charged. N_2S_2 MAMA BFCAs have been used for the ^{99m}Tc-labeling of biomolecules such as progesterone receptor ligands,[214] platelet GPIIb/IIIa receptor antagonists,[56,188] and dopamine transporters.[160,162,215−217] It has been reported that the ^{99m}Tc-labeled GPIIb/IIIa receptor antagonists remain stable for 24 h in saline solution after HPLC purification.[56]

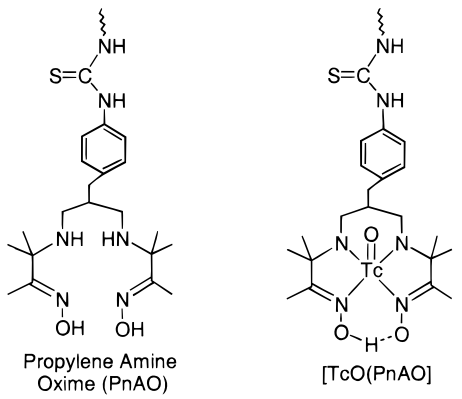


Figure 11. Propylene amine oxime (PnAO) as BFCA.

Tetradentate ligand PnAO (3,3,9,9-tetramethyl-4,8-diazaundecane-2,10-dione dioxime) was first shown by Troutner and co-workers to react with the $[Tc{=}O]^{3+}$ core and form a neutral technetium complex [TcO(PnAO)],[218] which demonstrates transient flow-related brain uptake in animal models and in humans.[219] As a result, a large number of PnAO derivatives were synthesized and studied as brain imaging agents.[220−224] It has been shown that PnAO technetium-oxo complexes all have a square pyramidal coordination geometry with the two amine N being deprotonated and the two hydroxy groups sharing the same proton via hydrogen bonds.[220,221] The PnAO chelating group, an example of which is shown in Figure 11, has been used for the ^{99m}Tc-labeling of a nitroimidazole,[225−227] biotin,[228] and a somatostatin analogue.[30,31] The advantage of using PnAO type of BFCAs is that the radiolabeling can be performed at ambient temperature.[228] The disadvantages include low specific activity for the radiolabeled biomolecules, solution instability, and extremely high lipophilicity of the technetium chelate.

For the last several years, there has been a growing interest in using small peptides, such as Gly−Ala−Gly−Gly and Gly−Ser−Cys, as BFCAs. There are several advantages of using small peptides as BFCAs. First, the attachment of the BFCA can be easily incorporated into solid-phase peptide synthesis. Second, these tripeptide chelating sequences usually form stable technetium complexes with the $[Tc{=}O]^{3+}$ core. Finally, the hydrophilicity of the technetium chelate can be tuned by changing side chains of the tripeptide or polypeptide chelating sequence.

Tetrapeptides contain a common N_4 aminetriamide chelating unit and have been investigated as a replacement of MAG_3 (by substituting the thiol with an amino group).[229,230] Unlike the N_3S triamidethiol, the tetrapeptide can be stored as the unprotected form. The ^{99m}Tc-labeling of the tetrapeptide can be achieved without a boiling step. Figure 12 shows the structures for the technetium complexes of several tripeptide sequences which have been used for the ^{99m}Tc-labeling of various biomolecules.[231−237] They all form stable square pyramidal technetium complexes with the $[Tc{=}O]^{3+}$ core. Recently, complexes [MO(RP294)] (M = Tc and Re; RP294 = Me_2N−Gly−Ser−Cys(Acm)−Gly−NH_2) were reported.[232] These com-

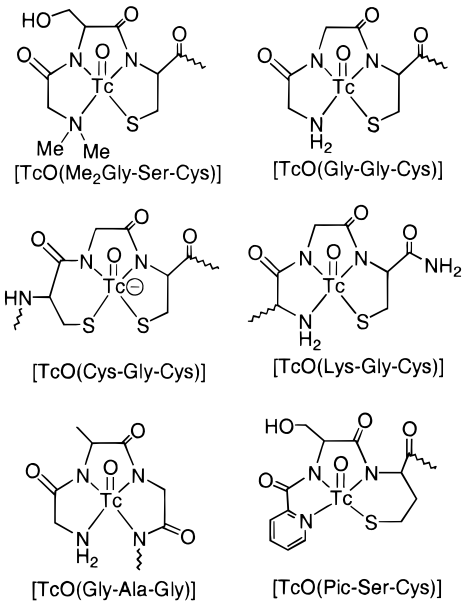


Figure 12. Tripeptide sequences as BFCAs.

plexes can serve as models for ^{99m}Tc-labeled peptides containing an N_3S monoaminediamidethiol BFCA. The Acm (acetamidomethyl) protecting group is removed upon coordination to the $[M{=}O]^{3+}$ core. It was found that RP294 forms neutral technetium- and rhenium-oxo complexes with two isomeric forms (*syn* and *anti*), which interconvert rapidly in aqueous solution at room temperature. The *syn* isomer of the complex, [ReO(RP294)], was isolated from the solution. A structural study of the *syn* isomer revealed a distorted square pyramidal coordination geometry with the N_3S donor set occupying the four basal sites and the oxo-O atom at the apical site.[232]

In 1989, Pietzsch[238] and co-workers found that technetium-oxo complexes, [TcO(L)(L′)] (L = tridentate thioetherdithiols or aminedithiols; L′ = monodentate thiolate), could be readily prepared by reacting [TcO(gluconate)$_2$] with a mixture of monothiolate and tridentate ligand. The combination of a dianionic tridentate SNS or SSS ligand with a monodentate thiolate produces a binary ligand system (3 + 1) that can bind to the $[M{=}O]^{3+}$ (M = Tc and Re) core and form neutral metal complexes with the distorted square pyramidal or trigonal bipyramidal coordination geometry.[154−157,239−249] The technetium complexes are useful as brain imaging agents.[154−157] The "3 + 1" concept could be expanded to "*n* + 1" systems (Figure 13). It has been used for ^{99m}Tc-labeling of dopamine transporters,[245,249,250] for the synthesis of 5-HT$_{2A}$ serotonin receptor ligands,[251,252] and for the synthesis of an estrogen receptor ligand.[252,253] In the case of serotonin receptor ligands, the integrated approach is used for the radiopharmaceutical design. The technetium chelate mimics the quinazoline−dione portion of ketanserin and is designed as a vital part of the whole molecule for receptor binding.

It was also found that at the tracer (^{99m}Tc) level the mixed ligand 3 + 1 complex has to be prepared in two steps.[254] First, the monodentate thiolate ligand reacts with [TcO(gluconate)$_2$] to give the intermediate complex $[TcO(L')_4]^-$. The tridentate dithiol ligand is

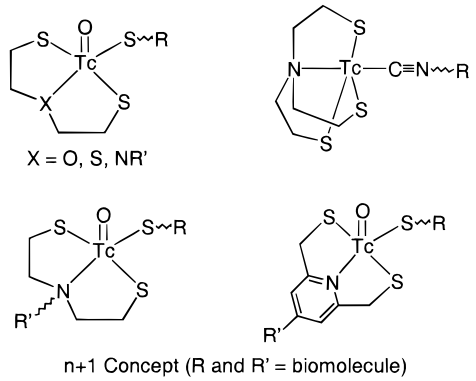


Figure 13. The *n* + 1 concept.

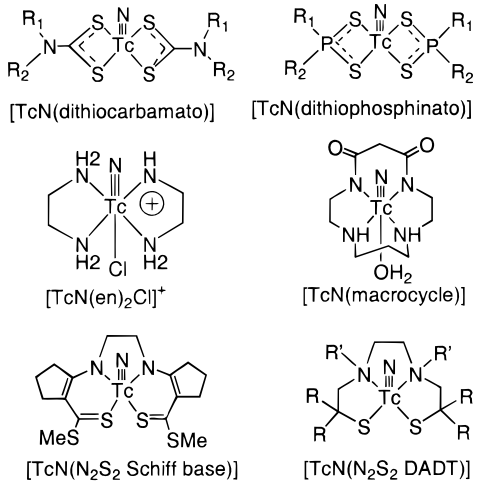


Figure 14. Technetium complexes containing the $[Tc{\equiv}N]^{2+}$ core.

then reacted with the intermediate complex to form the mixed binary ligand complex, [TcO(L)(L′)]. The best results (radiolabeling yield = 70−90%) were obtained with 0.2−0.5 mg of the monothiolate and 0.02−0.05 mg of the tridentate ligand. Using one-step synthesis, the mixed ligand 3 + 1 complexes were obtained in ∼40% yield.[251,254] The mixed-ligand 3 + 1 complexes, [TcO(L)(L′)], were found to be stable for up to 24 h.[254] However, the monodentate thiolate ligand could be substituted by "native" thiol-containing molecules such as cysteine.[255]

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

The $[Tc{\equiv}N]^{2+}$ core (Figure 14) is isoelectronic with the $[Tc{=}O]^{3+}$ core. The nitrido ligand is a powerful π-electron donor and shows a high capacity to stabilize the Tc(V) oxidation state. The $[Tc{\equiv}N]^{2+}$ core forms technetium(V) nitrido complexes with a variety of chelators.[256−273] The technetium(V) nitrido complex, [^{99m}TcN(noet)$_2$] (noet = *N*-ethyl-*N*-ethoxydithiocarbamato), has been developed as a new myocardial imaging agent by CIS−Bio International.[269] However, very little effort has been made to use the $[Tc{\equiv}N]^{2+}$ core for the ^{99m}Tc-labeling of biomolecules.

D. $[O{=}Tc{=}O]^{+}$ Core

The $[O{=}Tc{=}O]^{+}$ core (Figure 15) forms octahedral technetium complexes with linear or macrocyclic tetraamines such as cyclam. It has been used in the

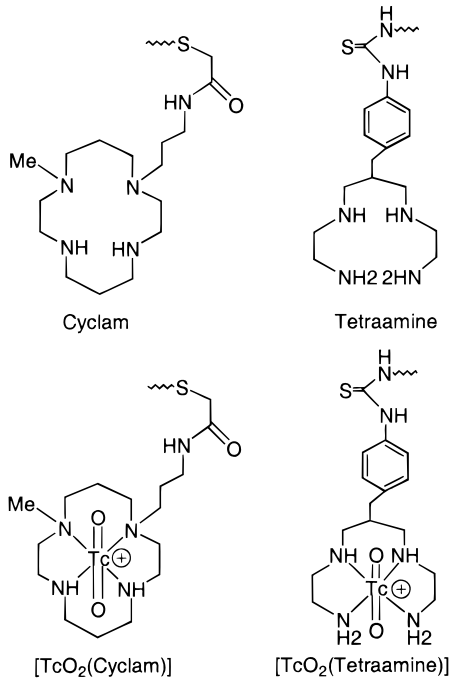


Figure 15. Linear and macrocyclic tetraamines as BFCAs.

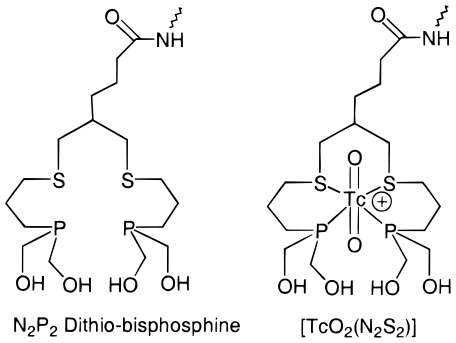


Figure 16. A water soluble phosphine BFCA.

^{99m}Tc-labeling of cyclam- or tetraamine-derivatized biomolecules such as antibodies[274] and a somatostatin analogue.[31,275] The radiolabeling efficiency of a cyclam-based BFCA is very low. Bidentate phosphines also form octahedral technetium complexes with the $[O{=}Tc{=}O]^+$ core.[276] An example of a technetium radiopharmaceutical using the $[O{=}Tc{=}O]^+$ core is $[^{99m}TcO_2(\text{tetrofosmin})_2]^+$ (tetrofosmin = 1,2-bis[bis(2-ethoxyethyl)phosphino]ethane), in which the two phosphine ligands bind to the Tc at the four equatorial positions with the oxo-O donors at the two apical positions.[276] This complex was developed as a heart imaging agent.[276−278]

Water-soluble phosphines (Figure 16) also form stable metal complexes with the $[O{=}M{=}O]^+$ core (M = Tc and Re).[279−283] A water-soluble dithiobisphosphine and its technetium and rhenium complexes were reported.[279−281] In the rhenium complex, $[ReO_2(S_2P_2)]^+$, the S_2P_2 ligand binds to the Re at four equatorial positions and the two oxo-O donors at the remaining two apical sites. The hydroxy-O atoms are not used as donor atoms. The presence of the hydroxymethyl groups is believed to be responsible for the fast clearance of the ^{99m}Tc complex from blood and nontarget tissues.[282] The combination of thioether-S with phosphine donors produces ^{99m}Tc complexes with high in vitro and in vivo stability. A functionalized dithiobisphosphine BFCA has been synthesized and used for the ^{99m}Tc-labeling of a bombesin antagonist.[42,282,283]

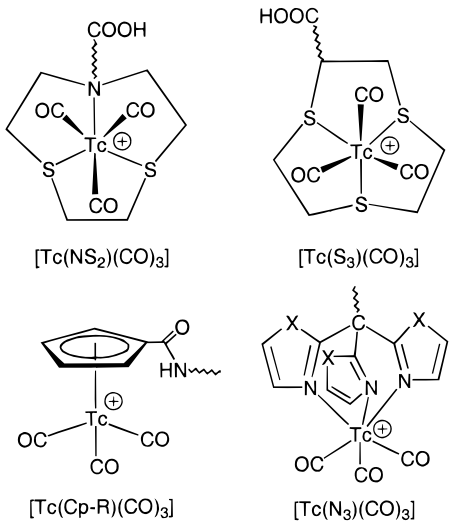


Figure 17. BFCAs bonding to the $[Tc(CO)_3]^+$ core.

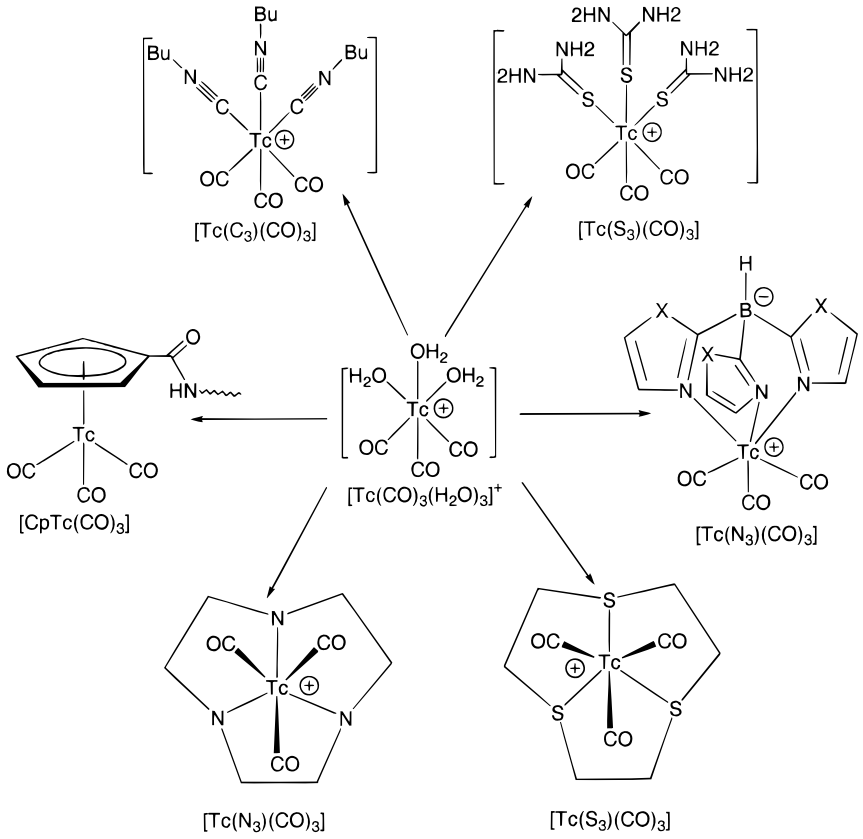
E. $[Tc(CO)_3]^+$ Core

The chemistry of technetium radiopharmaceuticals has been dominated by compounds containing the $[Tc{=}O]^{3+}$ core with technetium in its +5 oxidation state. Organometallic complexes of technetium in its low oxidation states received little attention until Davison and co-workers discovered that monodentate isonitrile ligands form very stable water-soluble organometallic Tc(I) complexes, $[Tc(CNR)_6]^+$, in almost quantitative yields.[136] Because of his pioneering efforts in this area, ^{99m}Tc−Sestamibi, $[^{99m}Tc(MIBI)_6]^+$, has been developed as the first successful organometallic radiopharmaceutical for myocardial imaging.

In an effort to develop new organometallic precursors for radiopharmaceutical applications, Alberto and co-workers recently published a new atmospheric pressure synthesis for complexes $[Et_4N]_2[MX_3(CO)_3]$ (M = Tc and Re; X = Cl and Br).[284] It was found that these complex anions are water soluble and remain stable in aqueous solution as the cationic form $[M(H_2O)_3(CO)_3]^+$, which readily undergoes ligand exchange reactions (Scheme 1) with a variety of chelators.[285−295] It was also found that the cationic complex $[Re([9]aneS_3)(CO)_3]^+$ was extremely stable under widely varying chemical conditions. Functionalization of macrocyclic or tripodal chelators is expected to result in a variety of BFCAs (Figure 17) useful for the ^{99m}Tc-labeling of biomolecules. A bidentate dithioether has been used to prepare Tc(I) and Re(I) complexes, [MX(*S,S*-estradiol)$(CO)_3$] (M = Tc, X = Cl; M = Re, X = Br).[296] However, neither tracer (^{99m}Tc) level chemistry nor the solution stability of these complexes was reported. Functionalized macrocyclic thioethers have been proposed as BFCAs for the radiolabeling of biomolecules. The advantage of using thioethers as BFCAs is that the radiolabeling of the BFCA−peptide conjugate is not pH dependent.

Recently, Alberto and co-workers[137] reported a one-step synthesis of the complexes $[M(H_2O)_3(CO)_3]^+$

Scheme 1. Reactions of $[Tc(H_2O)_3(CO)_3]^+$ with Various Ligands



(M = ^{99m}Tc and ^{188}Re) by direct reduction of [^{99m}Tc]-pertechnetate or [^{188}Re]perrhenate with sodium borohydride in aqueous solution in the presence of carbon monoxide. The yield of the ^{99m}Tc complex was >95%. The reaction of the complex $[^{99m}Tc(H_2O)_3(CO)_3]^+$ with PADA (picolinamine-*N*,*N*-diacetic acid) produces a neutral complex $[^{99m}Tc(PADA)(CO)_3]$ with very high yield and specific activity. The complex $[^{99m}Tc(PADA)(CO)_3]$ can also be prepared by an one-step synthesis from the direct reduction of [^{99m}Tc]pertechnetate with sodium borohydride in the presence of PADA. A structural study of the corresponding ^{99m}Tc analogue $[^{99}Tc(PADA)(CO)_3]$ showed a tridentate PADA with one carboxylic group remaining uncoordinated. The free carboxylic group can be used for attachment of biomolecules. This study clearly demonstrates that the complexes $[M(H_2O)_3(CO)_3]^+$ (M = ^{99m}Tc and $^{186/188}$Re) are versatile intermediates for synthesis of both diagnostic M = ^{99m}Tc and therapeutic $^{186/188}$Re organometallic radiopharmaceuticals. The $[Tc(CO)_3]^+$ core is an emerging new technetium core. Some important issues have not been fully addressed on the use of the $[Tc(CO)_3]^+$ core for the ^{99m}Tc-labeling of biomolecules. These include the possible toxicity of carbon monoxide, the CO-generating source if CO gas cannot be directly used in the radiolabeling process, and the possibility for a kit formulation.

There have been recent efforts to prepare η^5-cyclopentadienyl−tricarbonyl technetium(I) and rhenium(I) complexes, $CpM(CO)_3$, because of their small size and high stability.[300−305] The coordination chemistry of these organometallic complexes is very different from traditional coordination complexes of both metals. It is not surprising that preparation of these organometallic species has been cumbersome and often requires harsh chemical conditions. Recently, Katzenellenbogen and co-workers reported an "one-pot" three-component reaction for the synthesis of halo-, carbonyl-, and hydroxy-substituted $CpRe(CO)_3$ complexes using diazocyclopentadiene (CpN_2) as the Cp precursor.[306] It was found that the complex $[Et_4N]_2[ReBr_3(CO)_3]$ could readily react with CpN_2 in acetonitrile to form the complex (Br−Cp)$Re(CO)_3$. The bromide anion can be replaced by acetate or other protected amino acids. The amino group of amino acids is ideal for attachment of biomolecules. It should be noted that all these reactions were performed in acetonitrile. It is not practical to develop a kit formulation for the diagnostic ^{99m}Tc radiopharmaceutical. For therapeutic radiopharmaceuticals, however, the drug substance has to be made on the manufacturing site and is then delivered to hospitals. Therefore, this discovery will have very important applications for future development of therapeutic $^{186/188}$Re radiopharmaceuticals.

F. [Tc]HYNIC Core

Abrams and co-workers first reported the use of the [Tc]HYNIC core (Figure 3F) for the ^{99m}Tc-labeling of polyclonal IgG.[307−310] Since then, the [Tc]HYNIC core has been used for ^{99m}Tc-labeling of chemotactic peptides,[12−21,311] somatostatin analogues,[312−316] "Stealth" liposomes,[317] antisense oligonucleotides,[318] and a GPIIb/IIIa receptor antagonist.[55,58,318−323] Since the HYNIC can only occupy one or two coordination sites, a coligand such as tricine is required to complete the square pyramidal or octahedral coor-

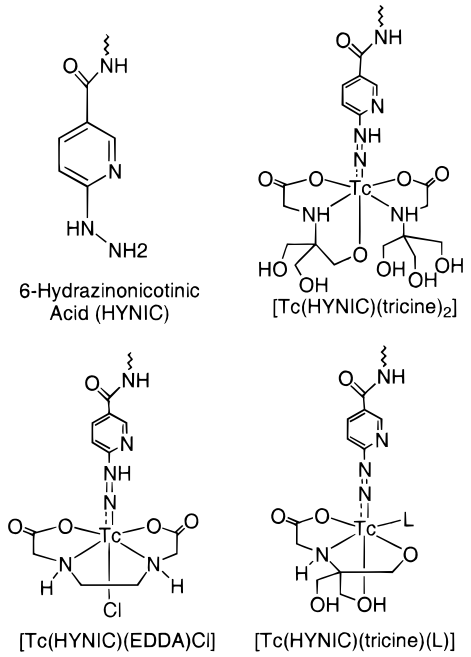


Figure 18. The [Tc]HYNIC core for the ^{99m}Tc-labeling of biomolecules.

dination sphere of the technetium (Figure 18). The advantage of using HYNIC as the BFCA is its high labeling efficiency (rapid and high yield radiolabeling) and the choice of various coligands such as tricine and glucoheptonate, which allows easy modification of the hydrophilicity and pharmacokinetics of the ^{99m}Tc-labeled small peptides. However, the use of tricine or glucoheptonate as coligands suffers two major drawbacks: the solution instability of technetium complexes [^{99m}Tc(HYNIC−BM)(L)$_2$] (BM = biomolecule; L = tricine, glucoheptonate, and EDDA) in the absence of excess coligand and the presence of multiple species of these complexes in solution due to different bonding modalities of HYNIC and the tricine or glucoheptonate coligands.

A ternary ligand system has been used in the ^{99m}Tc-labeling of HYNIC-derivatized biomolecules, including a GPIIb/IIIa receptor antagonist,[58] chemotactic peptides,[311] and LTB_4 receptor antagonists[23−26] using tricine and water-soluble phosphines as coligands (Figure 19). It is surprising that three different ligands (HYNIC−BM, tricine, and a phosphine) combine with technetium and form ternary ligand complexes [^{99m}Tc(HYNIC−BM)(tricine)(phosphine)] in high yield and high specific activity. It was found that these ternary ligand technetium complexes have extremely high solution stability and often show two isomeric forms if the BM contains one or more chiral centers. Like phosphines, imine-N containing heterocycles (Figure 19) have also been used as coligands for the ^{99m}Tc-labeling of HYNIC−BM.[318,321,322] It was found that the complexes, [^{99m}Tc(HYNIC−BM)(tricine)(heterocycle)] (heterocycle = ISONIC, ISONIC-L-Asp-(OMe)-OMe, ISONIC−HE, MTE, PA, PES, and PSA), are also formed as equal mixtures of two isomers. It has been demonstrated that the presence of two radiometric peaks is due to the resolution of two diastereomers resulting from the chiral centers on the peptide backbone and the chiral technetium

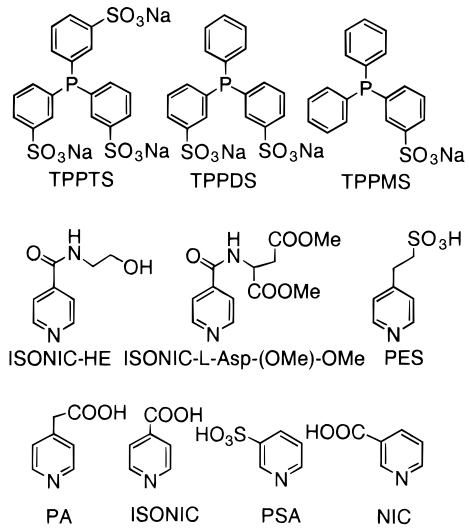


Figure 19. Coligands for the ternary ligand technetium complexes, [^{99m}Tc(HYNICtide)(tricine)(L)].

chelate.[318,320] The 1:1:1:1 composition for Tc:HYNIC:L:tricine (L = phosphines or imine-N heterocycle) was determined through a series of mixed-ligand experiments[58,318] and has been confirmed by the FAB-MS and LC-MS spectral data for technetium complexes at both ^{99m}Tc and ^{99}Tc levels.[323]

Obviously, the use of these ternary ligand systems offers several advantages. The biomolecule can be radiolabeled with extremely high specific activity. Three different ligands (HYNIC−BM, tricine, and phosphine/heterocycle) combine with Tc and form ternary ligand ^{99m}Tc complexes with only two detectable isomers if the HYNIC−BM or imine-N containing heterocyclic coligand contains one or more chiral centers. These ternary ligand technetium complexes have very high solution stability. The hydrophilicity of the ternary ligand ^{99m}Tc complexes can be tuned by changing functional groups on the phosphine coligand or imine-N containing heterocycle. The tricine coligand can also be substituted by other aminocarboxylates such as dicine (*N*-bis(hydroxymethyl)methylglycine) and bicine (*N*,*N*-bis(hydroxymethyl)glycine).

In principle, these ternary ligand systems can be used for the ^{99m}Tc-labeling of any biomolecules: peptides, peptidomimetics, and non-peptide receptor ligands. There are also limitations using these ternary ligand systems. Problems may arise when they are used for ^{99m}Tc-labeling of biomolecules containing one or more disulfide linkages, which are often vital to keep the rigid cyclic conformation of the biomolecule and to maintain the high receptor binding affinity. The use of reducing agents such as $SnCl_2$ and TPPTS in combination with high-temperature heating may reduce the S−S disulfide bonds and adversely affect the biological properties of the HYNIC−BM conjugate.

While the composition of the ternary ligand ^{99m}Tc complexes has been determined via a series of mixed-ligand experiments and by LC-MS at the tracer (^{99m}Tc) level, the technetium oxidation state remains unclear. It has been proposed that the technetium oxidation state in the [^{99m}Tc]tricine complex is +5.[308,309] The oxidation state might change when HYNIC and

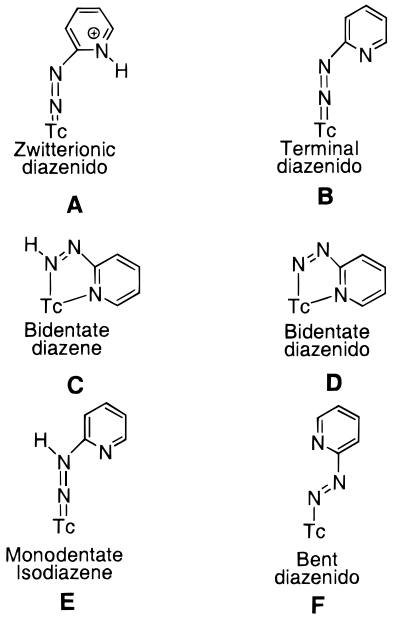


Figure 20. Possible bonding modalities for HYPY.

phosphine or an imine-N containing heterocyclic coligand are bonded to the Tc center. Since organohydrazides are good reducing agents, the reaction of HYNIC with [^{99m}Tc]pertechnetate or a intermediate complex such as [^{99m}TcO(tricine)$_2$] should be treated as a redox reaction instead of simple deprotonation of the hydrazine functionality. Therefore, the charge on the organohydrazide ligand cannot be assigned simply as a function of the number of hydrogens lost when it is bonded to the Tc center.

Complexes containing Tc−hydrazido and Tc−diazenido bonds have been previously reported and characterized by X-ray crystallography.[342−349] Davison, Zubieta, and co-workers recently reported a series of Tc(III)/Re(III) complexes of 2-hydrazinopyridine (HYPY) and hydrazinopyrimidine.[324−349] Although there are many possible bonding modalities for organohydrazides such as HYPY,[349] only four bonding modalities have been structurally characterized by X-ray crystallography, including neutral monodentate pyridiniumdiazenido (Figure 20 A), anionic monodentate pyridyldiazenido (Figure 20B), neutral bidentate pyridyldiazene (Figure 20 C), and anionic bidentate pyridyldiazenido (Figure 20 D). There is no structural information available for the monodentate pyridylisodiazene (Figure 20 E) and anionic monodentate pyridyldiazenido (Figure 20F). The pyridine-N atom is not "innocent". Unlike phenylhydrazine, HYPY can be a bidentate ligand in bonding to the Tc and form a five-membered chelate ring. In addition, the pyridine-N is an electron-withdrawing heteroatom and the aromatic pyridine ring is electron-deficient, which makes it easier for the β-nitrogen to be deprotonated.

It should be noted that the binding modality of HYPY and the number of HYPY ligands in the Tc complex are largely dependent on the nature and availability of the coligand. For example, the reaction of $NH_4[TcO_4]$ with HYPY·2HCl in the presence of other coligands produces a complex [$TcCl_3$(HN═NC_5H_4N)(N═NC_5H_4NH)][345,346] in which the Tc is

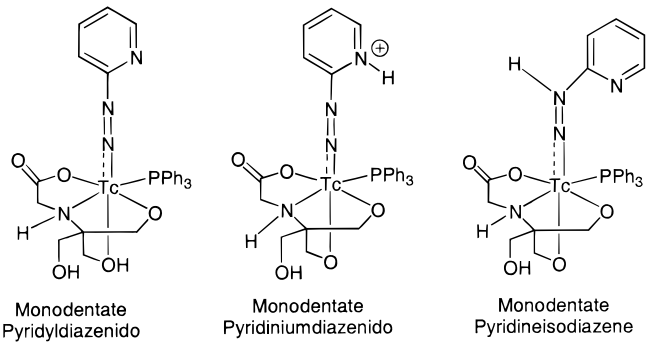


Figure 21. Three possible structures for the ternary ligand technetium complex [^{99m}Tc(HYPY)(tricine)(PPh_3)].

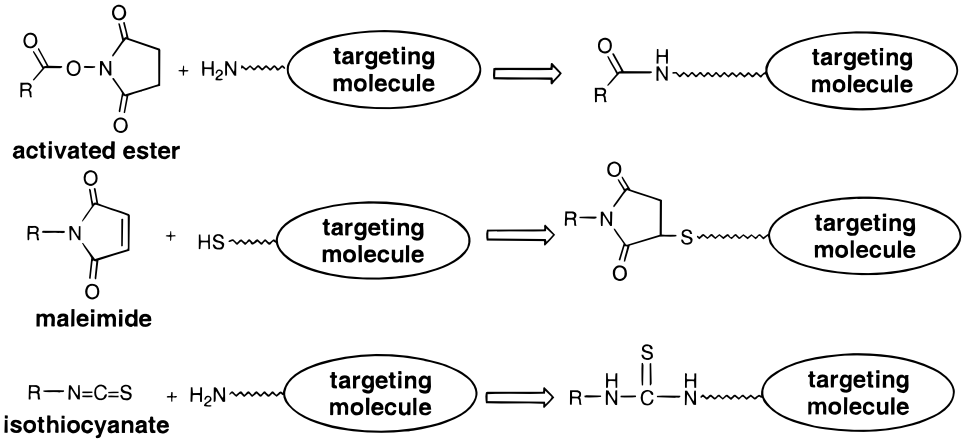
bonded by three chlorides, a neutral bidentate pyridyldiazene, and a neutral pyridiniumdiazenido. The reaction of HYPY·2HCl with [n-Bu_4N][$TcOCl_4$] or [NH_4][TcO_4] in the presence of excess tricine and PPh_3 produces Tc(III) complexes [Tc(HYPY)(PPh_3)$_2$-Cl_2] (major product) and [Tc(HYPY)(tricine)(PPh_3)] (minor product).[320] The HYPY ligand in [Re(HYPY)-Cl_2(PPh_3)$_2$] was found to be a neutral bidentate pyridyldiazene.[346] The complex [Tc(HYPY)(tricine)-(PPh_3)] serves as a model compound for ternary ligand ^{99m}Tc complexes [^{99m}Tc(HYNIC−BM)(tricine)-(L)] (L = TPPTS, TPPDS, and TPPMS). The NMR (^{1}H and ^{13}C) spectral data suggests that the complex [Tc(HYPY)(tricine)(PPh_3)] has a distorted octahedral coordination sphere with a monodentate HYPY, a tetradentate tricine, and a monodentate PPh_3 coligand.[320] However, the exact bonding modality of the HYPY in [Tc(HYPY)(tricine)(PPh_3)] remains unclear due to the difficulty in determining which atom is bonded to the exchangeable hydrogen (Figure 21A−C). In aqueous solution, the hydrogen is deprotonatable and there is no difference between these three modalities.

Like HYPY, the HYNICtide bonds to the Tc most likely via a Tc−diazenido linkage. The technetium chelate is chiral due to the asymmetrical bonding of the tricine coligand and should be formed in an equal mixture of D and L enantiomers. These enantiomers in combination with the chiral centers on the peptide backbone of HYNIC−BM result in the formation of two diastereomers. Therefore, it is not surprising that the HYNIC−BM with one or more chiral centers forms ternary ligand ^{99m}Tc complexes showing two radiometric peaks in their radio-HPLC chromatograms while the HYNIC−BM without a chiral center forms ternary ligand ^{99m}Tc complexes showing only one radiometric peak in their radio-HPLC chromatograms.

G. ^{99m}Tc-Labeling Efficiency

The ^{99m}Tc-labeling efficiency is a term used to describe the ability of a BFCA to achieve a high radiolabeling yield (>90%) of its ^{99m}Tc complex. There are several factors that influence the labeling efficiency of a BFCA. These include the identity of donor atoms, the chelator concentration, and reaction conditions such as temperature, time, and pH.[350] If the chelator concentration is fixed, the conditions used for the ^{99m}Tc-labeling are largely dependent

Scheme 2. Three Conjugation Groups



upon the nature of donor atoms. For example, high pH and heating at 100 °C for 30 min is required for the successful ^{99m}Tc-labeling of N_3S triamidethiol and N_2S_2 diamidedithol at low concentrations (10^{-5}−10^{-6} M) while the N_2S_2 monoamidemonoaminedithiol can be well labeled under milder conditions. For the N_2S_2 diaminedithiol, the ligand exchange with [^{99m}Tc]-glucoheptonate can be completed within 60 min at room temperature. In general, HYNIC and N_2S_2 diaminedithiols are better candidates as BFCAs due to their high ^{99m}Tc-labeling efficiency for labeling small peptides with very high potency. In some cases, N_3S triamidethiol, N_2S_2 diamidedithol, or N_2S_2 monoamidemonoamine−dithiol can be used as BFCAs if the use of larger amounts (>100 μg/mL) of BFCA−biomolecule conjugate does not cause unwanted side effects. For example, there is 100 μg of bibapcitide (P280) in each lyophilized AcuTect vial. This allows the use of less than 50 mCi of [^{99m}Tc]pertechnetate for the radiolabeling.

The ^{99m}Tc-labeling efficiency of a BFCA is also affected by the presence of protecting groups on the donor atom(s) and the identity of the exchange ligand or coligand. For example, it has been reported that tricine gives a much higher specific activity for the ^{99m}Tc-labeled HYNIC−IgG than glucoheptonate.[310] The use of protecting group(s) for thiol-containing chelators usually slows down the rate of radiolabeling.[351] The use of the appropriate combination of BFCA and exchange ligand (or coligand) is needed for the successful ^{99m}Tc-labeling of small biomolecules.

VII. Three Conjugation Groups for Peptide Attachment

A conjugation group is part of a BFCA and is used for attachment of the biomolecule. Peptides are polymers of amino acids containing a number of possible active side chains. In addition to these intrinsically reactive groups such as the ω-amino group from lysine, phenol moiety from tyrosine, thiol group from cysteine, and carboxylate group from aspartic or glutamic acid, specific reactive groups can be introduced into the peptide by chemical modification. These reactive groups, whether they are naturally part of the peptide or artificially introduced, can serve as "handles" for the attachment of a BFCA using the following three conjugation groups (Scheme 2).

A. *N*-Hydroxysuccinimide (NHS) Esters

The NHS-activated esters can be attached to a lysine primary amine group or an amino group from the linker through an amide bond. The NHS-activated esters have intermediate reactivity toward amines, with high selectivity for aliphatic amines. The optimum pH for reaction in aqueous systems is 8.0−9.0. The amide bond is very stable under physiological conditions. Virtually any molecule that contains a carboxylic group can be converted into its NHS ester, making the NHS-activated ester groups among the most powerful and the most commonly used conjugation groups for proteins and small biomolecules.

B. Isothiocyanates

Like NHS esters, isothiocyanates are amine-reactive groups with an intermediate reactivity and form thiourea bonds with primary amines of proteins or peptides. They are somewhat more stable in water than the NHS esters with respect to hydrolysis and react with amines in aqueous solution optimally at pH 9.0−9.5. Thus, isothiocyanates may not be suitable for modifying biomolecules that are sensitive to alkaline pH conditions.

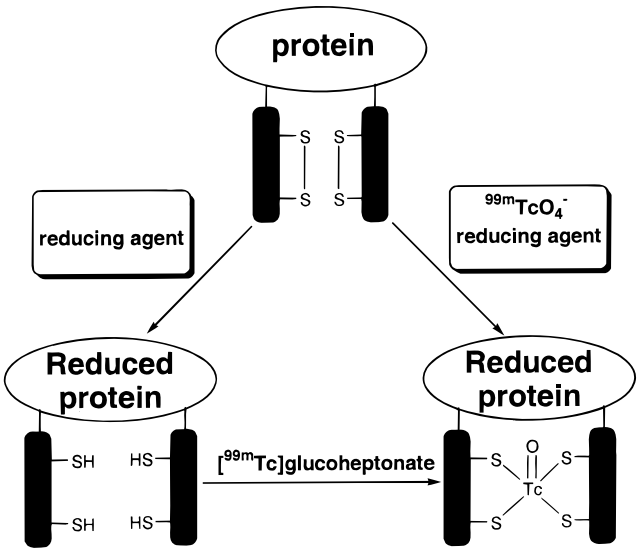
C. Maleimide

Maleimide is a thiol-reactive group and reacts selectively with a thiol from the antibody or peptide to form a thioether bond without any interference from histidine and other reactive groups. The optimum pH for the reaction is near 7. At pH higher than 8, maleimides may hydrolyze to form nonreactive maleamic acids.

VIII. Three Radiolabeling Approaches

There are several approaches for the ^{99m}Tc-labeling of biomolecules. The choice of radiolabeling approach depends on the type of biomolecules to be labeled and the purpose of the study (just for proof of principle or for product development). In the last two decades,

Scheme 3. Direct Labeling Approach



a large number of techniques have been developed for the ^{99m}Tc-labeling of biomolecules and extensively reviewed.[354−366] They are often classified into three main categories: the direct labeling approach, the prelabeling approach, and the postlabeling approach.

A. Direct Labeling Approach

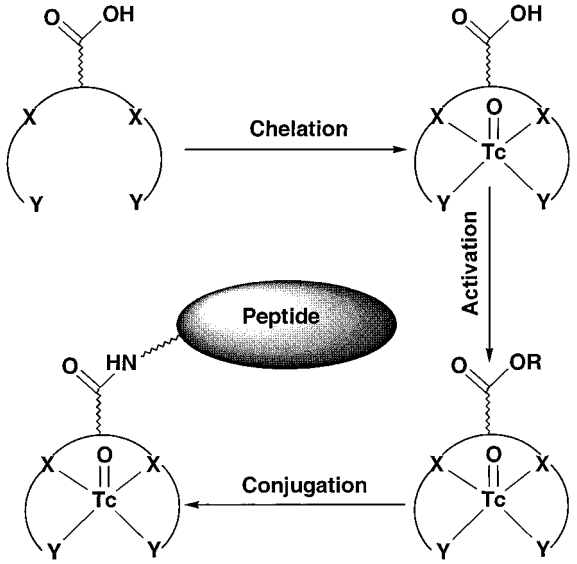
The direct labeling approach (Scheme 3) usually uses a reducing agent such as stannous chloride to convert a disulfide linkage into free thiols, which are able to bind the Tc. The advantage of this approach is that it is easy to carry out. However, very little is known about the number of donor atoms and the coordination geometry around the Tc center. There is little control over the stability of the ^{99m}Tc complex. In addition, this method applies only to antibodies or antibody fragments because many small peptides do not have any disulfide bonds or in some cases the disulfide bond is too critical for maintaining their biological properties to be reduced.

Somatostatin analogues (e.g., BIM-23014, RC-160, and Sandostatin), which contain a S−S disulfide bond in the cyclic peptide backbone, have also been radiolabeled by the direct labeling approach.[39] It is believed that when the S−S bond is reduced, the thiolate-S atoms bond to the Tc center and form stable ^{99m}Tc complexes. It was also reported that these ^{99m}Tc-labeled somatostatin analogues have not shown any apparent loss of biological activity nor have they shown any abnormal blood clearance in experimental animals compared to that with ^{111}In−DTPA−octreotide. However, several critical questions remain to be answered for this approach. What is the oxidation state of technetium? How many peptides are bonded to the Tc center? If only one peptide binds to the Tc, which tripeptide sequence (Cys−AA−AA or AA−AA−Cys) is involved in Tc bonding? How many ^{99m}Tc species are in the radiolabeled kit? What is the impact of ^{99m}Tc-labeling on the receptor binding affinity of the cyclic peptide?

B. Prelabeling Approach

The prelabeling approach (Scheme 4) involves formation of the ^{99m}Tc complex with a BFCA and

Scheme 4. Prelabeling Approach



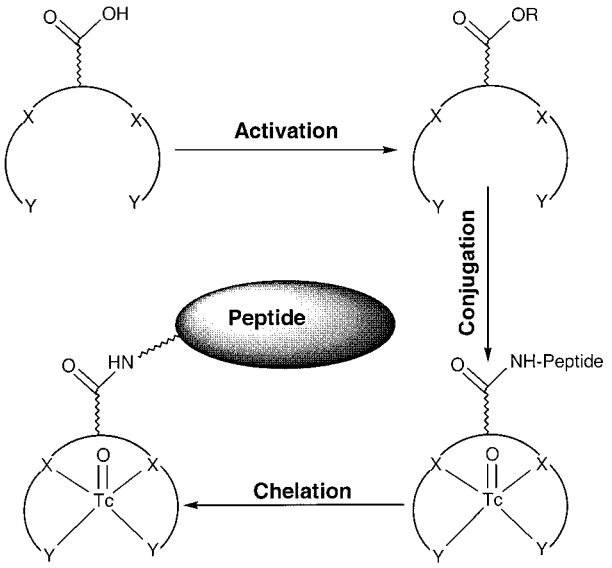
conjugation of the ^{99m}Tc−BFCA complex to a protein or peptide in a separate step on the tracer level. In this approach, the chemistry is better defined and the peptide or protein is not exposed to the sometimes forcing conditions used in the chelation step. For research purposes, this approach is very useful to demonstrate the proof of principle in a short period of time before making extensive efforts in preparing the peptide−BFCA conjugate. However, it is too complex and time-consuming for routine clinical use. The multiple-step tracer-level synthesis makes it very difficult to develop a kit formulation.

The prelabeling approach has been used for the ^{99m}Tc-labeling of antibodies and small peptides such as cyclic GPIIb/IIIa receptor antagonists using N_2S_2 diamidedithiols, N_3S triamidethiols, and an N_2S_2 monoaminemonoamidedithiol as BFCAs.[58,188] It was found that the ^{99m}Tc-labeled cyclic GPIIb/IIIa receptor antagonists remain stable in solution for at least 24 h. It was also found that BFCAs have significant effects on the physical and biological properties of the ^{99m}Tc−BFCA−peptide complex.[56,57] This is probably due to the fact that the ^{99m}Tc−BFCA chelates contribute nearly one-third of the total molecular weight of the ^{99m}Tc−BFCA−peptide conjugates.

C. Postlabeling Approach

In the postlabeling approach (Scheme 5), a BFCA is first attached to the peptide to form a BFCA−peptide conjugate. The BFCA can be attached to the C- or N-terminus of the peptide. It can also be attached to the side chain of the peptide or incorporated in the peptide backbone provided that incorporation of the BFCA does not significantly affect the receptor binding affinity. This approach involves multiple-step organic synthesis. However, the radiolabeling can be accomplished by direct reduction of $^{99m}TcO_4^-$ in the presence of a BFCA−peptide conjugate or by ligand exchange with an intermediate ^{99m}Tc complex such as [^{99m}Tc]glucoheptonate. This approach combines the ease of direct labeling with the well-defined chemistry of the preformed chelate approach. The indirect labeling is the most practical

Scheme 5. Postlabeling Approach



approach for the development of commercial peptide-based target-specific radiopharmaceuticals.

IX. Three Routes to Prepare ^{99m}Tc–BFCA–Peptide Complexes

A. Direct Reduction

Once it is decided to use the indirect labeling approach, the next question will be how to synthesize the ^{99m}Tc−BFCA−peptide complex. There are three routes for preparation of the ^{99m}Tc−BFCA−peptide complex. The first one is the reduction route, in which $^{99m}TcO_4^-$ is reduced in one step in the presence of the BFCA−peptide conjugate or a mixture of the BFCA−peptide conjugate and a weak chelating agent. Many of the synthetic conditions employed in these preparations are dictated by the 6 h half-life of ^{99m}Tc, the low concentration of $^{99m}TcO_4^-$, and the chemical stability of the BFCA−peptide conjugate. The reaction generally produces a mixture of reduced ^{99m}Tc species, and in many cases, the chemical form and the oxidation state of these species are not known.[65] However, the ^{99m}Tc−BFCA−peptide complex can be prepared as the predominant product by the choice of the appropriate reducing agent and an efficient BFCA under well-controlled conditions.

A variety of reducing agents can be used for the reduction of $^{99m}TcO_4^-$.[129] These include Sn(II), Ti(III), Cu(I), Fe(II), Sn(0), Zn(0), sodium borohydride, dithionate, dithionite, hypophosphoric acid, organic hydrazine, hydroxamine, formamidine sulfinic acid, dithiothreitol, and water-soluble phosphines. While most of these reductants can be used for the synthesis of small technetium complex radiopharmaceuticals, only a few of them have been used for commercial kits of the peptide-based target-specific radiopharmaceuticals. This is mainly due to limitations imposed by the stability of the BFCA−peptide conjugate. Special attention must be paid to make sure that the reducing agent matches the type of BFCA and the chemical stability of the peptide. For example, the use of borohydride and thiols may prove to be detrimental for cyclic peptides containing one or more S−S disulfide bonds because they are able to reduce the S−S disulfide bonds, particularly at elevated temperatures. This will lead to the destruction of the cyclic configuration, which is often vital for the receptor binding. Cu(I) is not suitable for the ^{99m}Tc-labeling of an N_2S_2 diaminedithiol conjugated peptide because it forms stable Cu(I) complexes with the N_2S_2 diaminedithiols. Organic hydrazines and hydroxamine may not be able to reduce $^{99m}TcO_4^-$ to the required oxidation state in a short period of time (<30 min). Sodium dithionite and sodium dithionate are often used at pH = 10 for effective reduction of $^{99m}TcO_4^-$. Harsh reaction conditions such as high pH should be avoided during the radiolabeling because the BFCA−peptide conjugate may decompose or undergo racimization under these conditions.

Sn(II) is the most commonly used reducing agent in commercial kits for the rapid preparation of ^{99m}Tc radiopharmaceuticals due to its fast reduction kinetics. However, the use of Sn(II) often leads to several problems.[367,368] For example, during the synthesis of a ^{99m}Tc radiopharmaceutical, initial reduction of $^{99m}TcO_4^-$ leads rapidly to the formation of the Tc(VI) intermediate $^{99m}TcO_4^{2-}$, which is unstable with respect to disproportionation to $^{99m}TcO_4^-$ and insoluble $^{99m}TcO_2$. The reduction of $^{99m}TcO_4^-$ can lead to the formation of Tc(IV), which undergoes rapid hydrolysis in aqueous solution to form $^{99m}TcO_2$. Sn(IV) also undergoes rapid hydrolysis to form insoluble SnO_2 colloid. The formation of colloids ($^{99m}TcO_2/SnO_2$) compromises the radiolabeling yield of the ^{99m}Tc−BFCA−peptide complex. Therefore, a weak chelating agent such as glucoheptonate (GH) or tricine is often used to stabilize both Sn(II) and Tc in its intermediate oxidation state.

B. Ligand Exchange

The second route for the ^{99m}Tc-labeling of the BFCA−peptide is the two-step ligand exchange synthesis. This route involves reduction of $^{99m}TcO_4^-$ by a reducing agent in the presence of a chelating agent such as glucoheptonate to form the intermediate complex $[^{99m}TcO(GH)_2]^{n-}$, which is then allowed to react with the BFCA−peptide conjugate under milder conditions to give the final product, the ^{99m}Tc−BFCA−peptide complex. This route is often used for the ^{99m}Tc-labeling of biomolecules such as antibodies and antibody fragments, which are sensitive to harsh reaction conditions (e.g., high pH and heating at elevated temperatures). $[^{99m}TcO(GH)_2]^-$ has also been used for the ^{99m}Tc-labeling of small peptides.[22,33,34] The intermediates $[^{99m}TcO(L)_2]^-$ (L = tricine, mannitol, and glucamine) have been used for ^{99m}Tc-labeling of HYNIC-conjugated biomolecules such as polyclonal IgG and small peptides.[11−21,307−310] Unlike tetradentate thiol-containing chelators, which replace the glucoheptonate in [^{99m}Tc]glucoheptonate complex, HYNIC reacts with the $[Tc{=}O]^{3+}$ core. The exchange ligand is the oxo-O atom, while the Tc is reduced to Tc(III) when HYNIC binds to the Tc center. Tricine or glucoheptonate serves two purposes: as a ligand to stabilize the reduced ^{99m}Tc in its Tc(V) oxidation

state and as a coligand to stabilize the [^{99m}Tc]HYNIC core.

C. Reduction–Substitution

The third route is the reduction−substitution route, which involves the use of a reducing ligand such as bidentate dmpe (1,2-bis(dimethylphosphino)-ethane)[369,370] and monodentate triphenylphosphine or sulfonated triphenylphosphine[256,257,371] which become incorporated into the coordination sphere of the technetium. First, the reducing ligand reduces $^{99m}TcO_4^-$ to a lower oxidation state, the exact nature of which is largely dependent upon other chelators/ligands bonded to the Tc center and serves as a ligand in bonding to the Tc. Examples include dmpe in the complex $[^{99m}Tc(dmpe)_2Cl_2]^+$ and TPPTS in ternary ligand complex [^{99m}Tc(HYNIC−BM)(tricine)-(TPPTS)].[372] If the phosphine coligand is replaced by an imine-N containing heterocycle, $SnCl_2$ has to be used for the reduction of $^{99m}TcO_4^-$. TPPTS has also been used as a reducing agent for the synthesis of nitrido technetium complexes [^{99m}TcN(dithiocarbamate)$_2$].[268]

X. Quality Control of the ^{99m}Tc-Labeled Peptides

A. Thin-Layer Chromatography (TLC)

TLC is the most frequently used procedure for the quality control of ^{99m}Tc-labeled small peptides. It is simple and quick, usually taking only 10−15 min to complete the whole procedure. In the radiolabeled kit, there are several ^{99m}Tc species: [^{99m}Tc]pertechnetate, [^{99m}Tc]colloid, the ^{99m}Tc−BFCA−peptide complex, and other radioimpurities. Ideally, one TLC paper strip or plate is needed for the separation of [^{99m}Tc]-pertechnetate, [^{99m}Tc]colloid, and the ^{99m}Tc−BFCA−peptide complex. The mobile phase is often comprised of an organic solvent (acetone, ethanol, acetonitrile, methyl ethyl ketone, or isopropyl alcohol) and saline or a buffer. The [^{99m}Tc]colloid remains at the origin, while the [^{99m}Tc]pertechnetate migrates to the solvent front. The ^{99m}Tc-labeled BFCA−peptide conjugate appears in the middle of the paper strip or plate. In some cases, two paper strips or TLC plates are needed for quality control. In this situation, saline (0.9% sodium chloride solution) is usually used for checking the presence of [^{99m}Tc]colloid. Organic solvents are usually used to check the presence of migrating [^{99m}Tc]pertechnetate at the solvent front and the water-soluble ^{99m}Tc-labeled BFCA−peptide conjugate at the origin. It should be noted that TLC methods give only the radiolabeling yield, which includes all ^{99m}Tc species containing the peptide. It does not tell the radiochemical purity (RCP) for the ^{99m}Tc−BFCA−peptide complex and is not suitable for separation of different isomeric forms.

B. High-Pressure Liquid Chromatography (HPLC)

For the past decade, HPLC has become a routine quality control technique for ^{99m}Tc radiopharmaceuticals. The advantage of radio-HPLC is its capability to determine the RCP for the ^{99m}Tc−BFCA−peptide complex and to separate different ^{99m}Tc species in the kit matrix. It is also a very powerful tool for separation of different isomers such as epimers and diastereomers. The separation of optical isomers requires the use of chiral chromatographic conditions (chiral column or chiral mobile phase). Radio-HPLC also has its limitations. For example, the radio-HPLC cannot be used for the assessment of the [^{99m}Tc]colloid formation. A TLC method is needed in combination with HPLC to assess both the radiolabeling yield and the RCP for the ^{99m}Tc-labeled peptide.

It should be emphasized that a single peak in the radio-HPLC chromatogram does not mean that there is only one ^{99m}Tc species. It is particularly true when the chromatographic conditions are not appropriately developed. For example, it has been reported that the complex [^{99m}Tc(fMLFK−HYNIC)(glucoheptonate)$_2$] showed a single peak in its radio-HPLC chromatogram using a sharp gradient HPLC method with acetonitrile from 0% to 100% over 10 min.[372,373] The same complex showed several radiometric peaks using a slower gradient reverse phase HPLC method.[311] Therefore, it is very important to develop a good radio-HPLC method for the accurate assessment of the RCP of the ^{99m}Tc−BFCA−peptide complex. It is also important that one should use different chromatographic conditions to confirm that one peak in the chromatogram is really one peak and there are no other radioimpurities or isomeric forms coeluting with the ^{99m}Tc−BFCA−peptide complex because these impurities often have different biological properties (biodistribution and excretion route) in biological systems.

XI. Characterization of the ^{99m}Tc-Labeled Peptides

One of the most important aspects of radiochemistry is to know the composition and structure of the radiopharmaceutical prepared in the radiolabeled kit. A quick and accurate method would help radiochemists understand the fundamental chemistry at the tracer (^{99m}Tc) level. As discussed previously, the total technetium (^{99m}Tc and ^{99m}Tc) concentration in the generator eluate is very low (10^{-8}−10^{-6} M). At this concentration, it is impossible to use classical spectroscopic (IR, UV/vis, NMR, and FAB-MS) methods for the characterization of ^{99m}Tc radiopharmaceuticals.

Traditionally, characterization of a new radiopharmaceutical involves synthesis of the corresponding ^{99}Tc complex at the macroscopic level. The composition and structure of the ^{99}Tc complex are determined by IR, NMR, FAB-MS, and X-ray crystallography. An HPLC concordance experiment is performed to demonstrate that the same technetium complex is prepared at both the macroscopic and the tracer levels. For peptide-based technetium radiopharmaceuticals, isolation of the ^{99}Tc−peptide complex usually involves multiple-step synthesis and tedious HPLC purification. It is also very difficult to grow single crystals for the highly water-soluble ^{99}Tc−peptide complex. In addition, the chelation chemistry for some chelating systems may differ at the macroscopic and tracer levels. Therefore, there is a growing need for a quick and accurate method to determine the composition

Table 5. LC-MS Data for Ternary Ligand ^{99m}Tc Complexes, [^{99m}Tc(HYNICtide)(tricine)(L)]

L	formula	formula weight	found $(M + H)^+$	found $(M + 2H)^{2+}$
TPPTS	$C_{62}H_{78}N_{14}O_{23}PS_3Tc$	1613.44	1614.6	807.2
TPPDS	$C_{62}H_{79}N_{14}O_{20}PS_2Tc$	1533.38	1534.5	768.2
TPPMS	$C_{62}H_{80}N_{14}O_{17}PSTc$	1453.31	1454.3	727.2
TPPTS	$C_{62}H_{78}N_{14}O_{23}PS_3Re$	1700.3	1701.1	850.2
TPPDS	$C_{62}H_{79}N_{14}O_{20}PS_2Re$	1620.4	1620.2	810.1
TPPMS	$C_{62}H_{80}N_{14}O_{17}PSRe$	1500.3	1501.3	770.2
ISONIC−HE	$C_{52}H_{73}N_{16}O_{16}Tc$	1276.4	1277.3	639.3
ISONIC−Asp−(OMe)−OMe	$C_{56}H_{77}N_{16}O_{19}Tc$	1377.2	1378.3	689.5
ISONIC−Sorb	$C_{56}H_{81}N_{16}O_{20}Tc$	1397.3	1398.3	699.2

and structure of the radiopharmaceutical in the radiolabeled kit at the tracer level.

A. Mixed-Ligand Experiment

The mixed-ligand experiment was used to determine the composition of ternary ligand ^{99m}Tc complexes, [^{99m}Tc(HYNICtide)(tricine)(L)] (L = water-soluble phosphines or imine-N containing heterocycles), at the tracer (^{99m}Tc) level.[58,318] In this experiment, the HYNICtide and a model compound, HYNIC−D-Phe−OMe, were used in the same reaction mixture. After the radiolabeling, the reaction mixture was analyzed by HPLC. If only one HYNICtide is bonded to the Tc, the chromatogram is expected to show two sets of peaks, one set from the complex [^{99m}Tc(HYNICtide)(tricine)(TPPTS)] and the other from the complex [^{99m}Tc(HYNIC−D-Phe−OMe)-(tricine)(TPPTS)]. If there were two HYNIC-containing ligands in each complex, a third set of peaks from the mixed-ligand complex, [^{99m}Tc(HYNICtide)-(HYNIC−D-Phe−OMe)(tricine)(TPPTS)], is expected. The presence of only two sets of peaks in the HPLC chromatogram demonstrates clearly that there is only one HYNICtide bonded to the Tc in [^{99m}Tc-(HYNICtide)(tricine)(L)]. The same experiment was used to determine the number of tricine and phosphine or imine-N containing heterocyclic coligand. This methodology also applies to the composition determination for ^{99m}Tc complexes with other binary or ternary ligand systems. The mixed-ligand experiment is simple and only takes several hours to know composition of the ^{99m}Tc complex.

B. LC-MS Method

Mass spectrometry has been a powerful tool for the study of drug metabolism and biodeposition for a number of years.[374−378] These types of studies have been traditionally carried out by GC-MS techniques. However, the use of LC-MS has been growing in importance, especially since the introduction of the thermospray interface, which works well for compounds of moderate polarity. More recently, with the advent of modern liquid-phase ionization techniques, such as electrospray, it has become possible to use LC-MS methods for the structural characterization of highly polar molecules at very low concentrations.

LC-MS has been used for the characterization of ternary ligand ^{99m}Tc complexes, [^{99m}Tc(HYNICtide)-(tricine)(L)] (L = TPPTS, TPPDS, and TPPMS). The LC-MS spectral data (Table 5) supports the proposed structure (Figure 18) and is consistent with the composition determined via the mixed-ligand experiments on the tracer (^{99m}Tc) level.[58,323] Using LC-MS, the amount of sample can be as low as 10^{-11} mol, particularly for complexes with a lower molecular weight and neutral charge. For example, the amount of sample for [^{99m}TcO(ED)] and [^{99m}Tc(MIBI)$_6$]$^+$ can be as low as 10^{-12} mol.[323] Compared to FAB-MS, LC-MS shows much lower fragmentation due to the use of the electrospray interface. While the MS is used for determination of the composition, the LC−UV and MSD profiles are commonly used for identification of the radiolabeled compound to be analyzed. For short-lived radionuclides such as ^{99m}Tc, the short half-life results in high specific radioactivity for the radiolabeled compound. In this situation, it is much better to use the radiodetector for identification of the radiopharmaceutical. In combination with a radiation detector, LC-MS can be used to determine the chemical composition of both the radiopharmaceutical and radioimpurities in the radiolabeled kit. LC-MS is a powerful technique for composition and structural studies at the tracer level and will become a routine technique for characterization of new radiopharmaceuticals.

XII. ^{99m}Tc-Labeled Peptides as Radiopharmaceuticals

For the last several years, a large number of radiolabeled receptor ligands have been synthesized and studied for potential use as target-specific radiopharmaceuticals. In general, they can be classified into three major categories according to their diagnostic applications: thrombus imaging, infection/inflammation imaging, and tumor imaging. These are the three major areas in which radiolabeled receptor ligands have been well-investigated in both preclinical and clinical studies. Some of them have been developed successfully into marketable products, while some of them are still in clinical trials.

A. Thrombus Imaging

Venous and arterial thrombus formation are common and potentially life-threatening events. A venous thrombus is an intravascular deposit predominantly comprising fibrin and aggregates of platelets. Intra-arterial platelet−fibrin thrombus formation is the final common pathway that leads to most complications of atherosclerosis, including acute myocardial infarction, unstable angina, stroke, and sudden death. In addition, intra-arterial thrombus is a major cause of the complications associated with intravascular prosthetic materials such as arterial bypass grafts

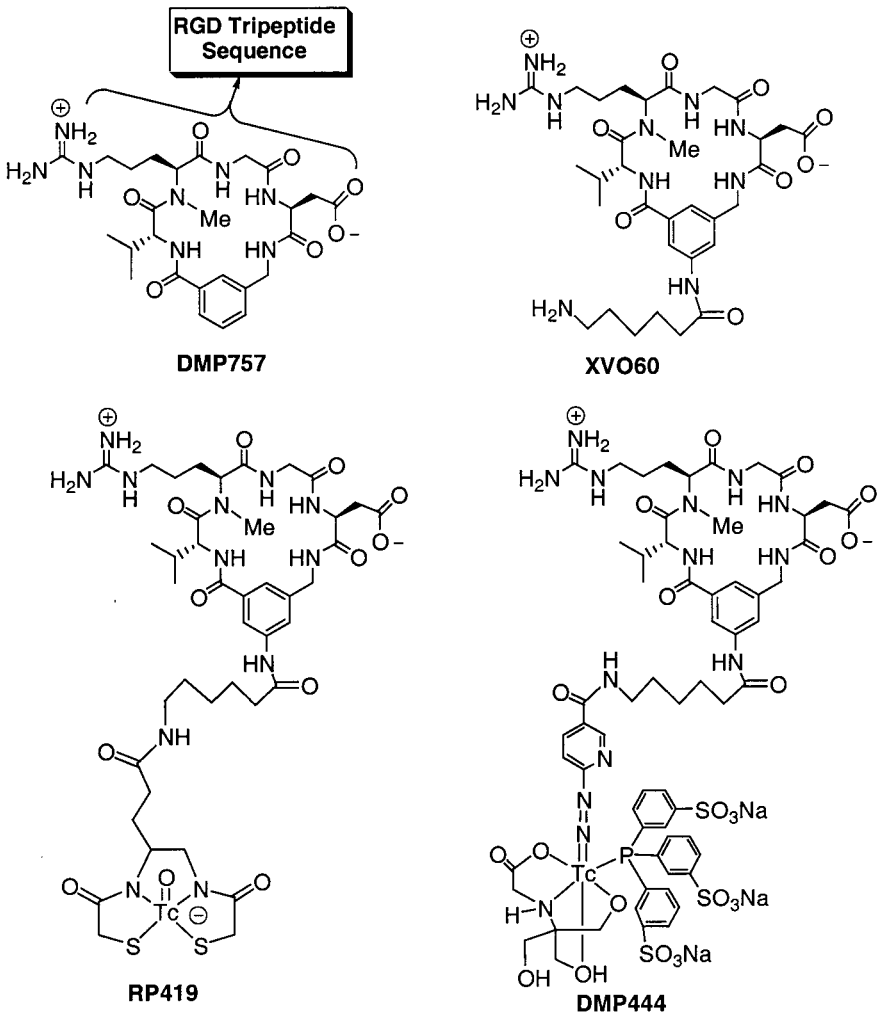


Figure 22. Structures of RP419 and RP444.

and heart valves. Thus, the development of a noninvasive imaging agent that is able to detect thrombus (both arterial and DVT) with high accuracy would be a significant advance in diagnostic nuclear medicine.

In the rapidly growing thrombus, platelets are activated. The activated platelets express the GPIIb/IIIa receptor which recognizes proteins and peptides bearing the RGD tripeptide sequence, while nonactivated circulating platelets express virtually none of the receptor in its active conformation. A number of RGD-containing GPIIb/IIIa receptor peptide antagonists have been synthesized and studied for their antithrombotic activities.[379−385] These small peptides represent a rapidly growing class of antithrombotics.

RP419 (Figure 22) is a cyclic platelet GPIIb/IIIa receptor antagonist. It can be prepared by either the prelabeling approach[56] or the postlabeling approach.[189,190] In a canine AV shunt model, RP419 was found to be incorporated into the growing thrombus under both arterial and venous conditions. In the canine DVT model, RP419 showed rapid uptake in thrombi with images clearly diagnostic within 15 min postinjection.[57]

DMP444 (Figure 22) is another GPIIb/IIIa receptor antagonist. HYNIC is used as the BFCA in DMP444 while tricine and TPPTS are used as coligands. In the canine AV shunt model,[59] DMP444 was adequately incorporated into the arterial and venous portions of the growing thrombus (7.8−9.9% and 0.2−3.7% ID/g, respectively). In the canine DVT model, the thrombus uptake (%ID/g = 2.86 ± 0.4) of DMP444 was much higher than that of the negative control, [^{99m}Tc]albumin. DMP444 showed a moderate blood clearance with a $t_{1/2}$ of approximately 90 min. Visualization of DVT can be as early as 15 min postinjection and improves over time with a thrombus/muscle ratio of 9.7 ± 1.9 at 120 min postinjection. Phase I clinical trials demonstrated an excellent safety profile and acceptable dosimetry.[386] Preliminary results from phase II trials showed that DMP444 could detect DVT within 60 min with 93% sensitivity, 75% specificity, and 82% accuracy.[387] [^{99}Tc]DMP444 and its two diastereomers were evaluated in an in vitro assay against fibrinogen binding to activated canine platelets. It was found that both isomers have the same binding affinity (IC_{50} = 12 ± 1 nM).[320] Compared to the unlabeled peptide (Figure 23, IC_{50} = 6 ± 2 and 8.1 ± 1.7 nM against fibrinogen binding to the activated human and canine platelets, respectively); [^{99}Tc]DMP444 remains a high-affinity GPIIb/IIIa receptor antagonist. The incorporation of the radionuclide did not significantly decrease the receptor binding affinity, even though the technetium chelate contributes more than 50% of the total molecular weight of DMP444.

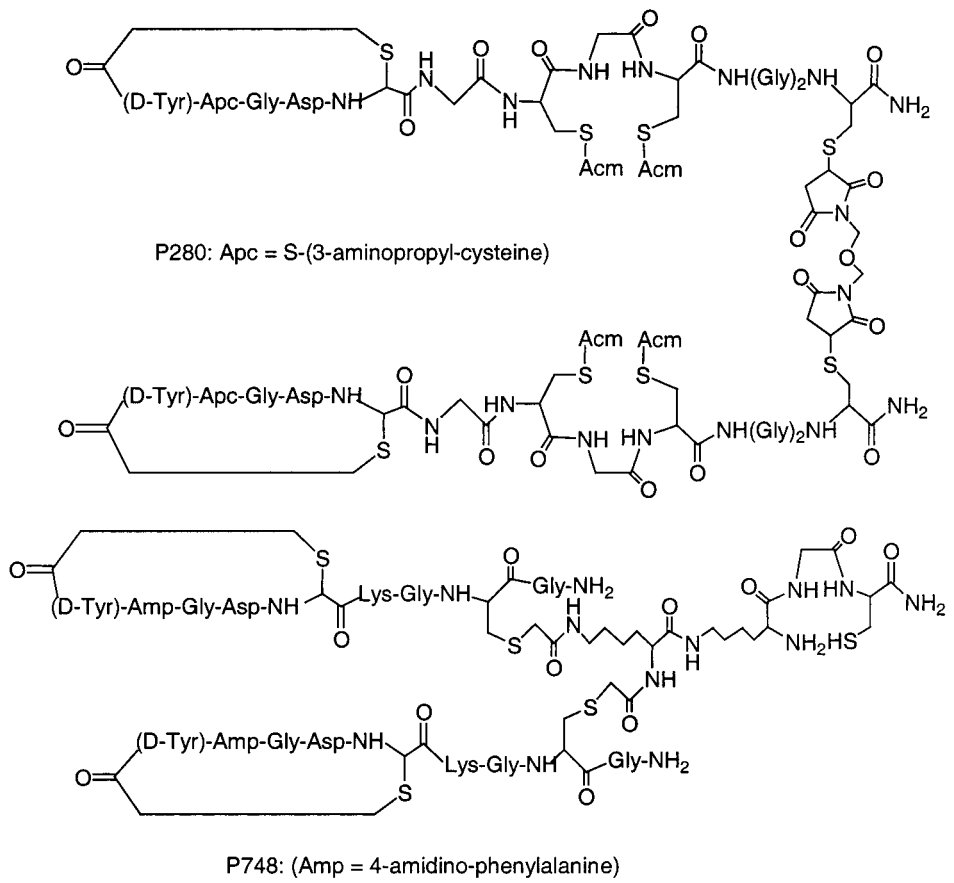


Figure 23. Structures of P280 and P748.

P280 (Figure 23) is a small oligopeptide consisting of two identical cyclic 13 amino acid monomers. Each monomer contains a −(*S*-aminopropylcysteine)−Gly−Asp tripeptide sequence, which is mimetic of the RGD sequence found in molecules such as fibrinogen and fibronectin. P280 has high affinity for the GPIIb/IIIa receptor with the IC_{50} of 87 nM for inhibition of human platelet aggregation.[61] Each monomer contains a Cys(Acm)−Gly−Cys(Acm) tripeptide sequence, which can be used for ^{99m}Tc-labeling. Because of the low labeling efficiency of N_2S_2 diamidedithiols and the presence of the Acm protecting groups on the two thiol groups, the specific activity for the ^{99m}Tc−P280 complex is low (60 mCi/μmol) compared to that of DMP444 (20 000 mCi/μmol). ^{99m}Tc−P280 was reported to give excellent images of DVT in a canine DVT model, while the thrombus uptake is only 0.0059 ± 0.0025%ID/g at 4 h postinjection.[61] The low thrombus uptake is most likely related to the lower binding affinity of P280 (IC_{50} = 87 nM) for the GPIIb/IIIa receptor and fast blood clearance. Clinical experience showed that ^{99m}Tc−P280 may enable imaging of venous thrombus within 60 min postinjection with 78% (7/9) accuracy.[388] ^{99m}Tc−P280 has recently been approved as a commercial product (AcuTec). However, there is no structural and compositional data available for both ^{99m}Tc−P280 and ^{99}Tc−P280. Recently, the solution coordination mode of a ^{99m}Tc-labeled endothelin derivative (Asp−Gly−Gly−*Cys*−Gly−*Cys*−Phe−(D-Trp)−Leu−Asp−Ile−Ile−Trp), which contains the identical Cys−Gly−Cys tripeptide sequence for the ^{99m}Tc-labeling, was determined by X-ray absorption spectroscopy (XAS). XAS analysis revealed that two peptide molecules bond to the $[TcO]^{3+}$ core to form the purely *S*-coordinate 2:1 complex.[389]

P748 (Figure 23) is another GPIIb/IIIa receptor antagonist. ^{99m}Tc−P748 is under investigation for imaging pulmonary embolism. P748 has a higher receptor binding affinity (IC_{50} = 28 nM against aggregation of human platelets) than P280. ^{99m}Tc−P748 shows slower blood clearance with $t_{1/2}\alpha$ = 9.6 min and $t_{1/2}\beta$ = 145 min, higher thrombus uptake (%ID/g = 0.018 ± 0.0068 at 4 h postinjection), and a higher target-to-background ratio than ^{99m}Tc−P280.[9,61] Unlike P280, P748 uses an N_3S monoamine diamidethiol chelating unit for the ^{99m}Tc-labeling. Because of the asymmetric character of the chelator, two epimers are seen, one of which is predominant. It was reported that P748 can be easily labeled by ligand exchange with [^{99m}Tc]glucoheptonate at room temperature. The specific activity for ^{99m}Tc−P748 is about 2000 mCi/μmol. Apparently, the presence of the amine-N donor and an unprotected thiol in the chelator improves the radiolabeling efficiency.

CYT-379 (acetyl-SYGRGDVRGDFKCTCCA-amide) is a low molecular weight small peptide. It was derived from a sequence present in the CDR region of the monoclonal antibody PAC 1.1 and contains two RGD sequences which bind to the GPIIb/IIIa receptor on the activated platelets. It also contains a technetium chelating group (KCTCCA). The animal studies showed that ^{99m}Tc−CYT-379 preferentially localizes in fresh thrombi in rabbits and dogs 1−2 h postinjection.[390] A phase I clinical study showed that ^{99m}Tc−CYT-379 has an initial component of rapid clearance

with $t_{1/2}\alpha$ of 12 min, followed by a slow component with $t_{1/2}\beta = 160$ min. It was also found that ^{99m}Tc−CYT-379 has a low sensitivity and accuracy (43%, 3/7) in detecting thrombi in patients, particularly those who were receiving heparin at the time of administration of ^{99m}Tc−CYT-379. The low sensitivity and accuracy of ^{99m}Tc−CYT-379 was also attributed to its slow clearance from blood plasma and the partial heptabilary excretion.[390]

Other ^{99m}Tc-labeled receptor ligands for thrombus imaging include TP-1300 (CSVTCR),[391] snake venom peptides,[392,393] and MP-2026 (Thromboscan),[394] which is an N_3S-conjugated RGD peptidomimetic analogue. TP-1300 was modified with an N_4 Gly−Ala−Gly−Gly binding group for ^{99m}Tc-labeling and an Aba (2,4-diaminobutanoic acid) linker. It has been shown that the ^{99m}Tc-labeled TP1300 was able to accumulate on thrombin-activated platelets. In humans, MP-2026 has been shown to monitor the localization and the functional activity of fresh thrombi. No structural information was disclosed for Thromboscan. Bitistatin, a 9000 Da peptide isolated from Bitis arietans venom, was labeled with ^{99m}Tc using HYNIC as BFCA and glucoheptonate as the coligand. In a canine PE model, the ^{99m}Tc−HYNIC−bitistatin complex showed rapid PE accumulation with the PE uptake of 2.5%ID/g and the PE/Blood ratio of 90 ± 13.[392]

B. Infection/Inflammation Imaging

For a number of years, imaging of inflammation and infection has been performed using either ^{67}Ga−citrate or ^{111}In-labeled white blood cells (WBCs).[395,396] ^{99m}Tc-labeled polyclonal IgG was also used for detection of infection foci.[397−403] Although these agents are well-accepted and efficacious, it usually takes 24 h to obtain diagnostically useful images. Imaging using ^{99m}Tc−hexamethylpropylene amine oxime (HMPAO)−WBCs[404,405] and ^{99m}Tc[albumin] colloid−WBCs[406] can be completed on the same day; but these procedures may impose significant risks to laboratory personnel and patients, particularly with the increasing prevalence of human immunodeficiency virus in the population.[407] Radiopharmaceuticals for imaging inflammation and infection have recently been reviewed extensively.[408−410]

White blood cells (WBCs), particularly polymorphonuclear leukocytes (PMNLs) and monocytes, accumulate in high concentrations at sites of infection. Therefore, research has been directed toward radiolabeling small molecules that bind to both circulating granulocytes and leukocytes. Fischman and co-workers have investigated a series of radiolabeled chemotactic peptides as infection/inflammation imaging agents.[12−18] These peptides were modified with HYNIC (for ^{99m}Tc-labeling) or DTPA (for ^{111}In-labeling) at the C-terminus. The ^{99m}Tc-labeling of HYNIC-modified chemotactic peptides can be achieved using various coligands such as glucoheptonate, mannitol, and glucamine.[13] It was reported that very high specific activity (20 000 mCi/μmol) could be achieved using glucoheptonate as the coligand. Different biodistributions were observed for various coligands.[13] Recently, van der Laken[19−21] and co-workers also reported the use of the ^{99m}Tc-labeled fMLFK−HYNIC for imaging acute infection and sterile inflammation. Animal studies have shown evidence of binding to leukocytes and localization at sites of infection. However, these studies have not addressed problems associated with the presence of multiple species of the binary ligand ^{99m}Tc complexes and their solution stability.

The conjugate fMLFK−HYNIC was recently radiolabeled using a ternary ligand system: fMLFK−HYNIC, tricine, and TPPTS.[311] The ternary ligand technetium complex [^{99m}Tc(fMLFK−HYNIC)(tricine)-(TPPTS)] (RP463) was evaluated in two animal (guinea pig and rabbit) models of focal infection. It was found that RP463 was incorporated rapidly into the infected tissues with a T/B of 5−7:1 at 4 h postinjection. In the rabbit infection model, a transient decrease in the white blood cell count of 35% was observed during the first 30 min after injection of the HPLC-purified RP463. Similar neutropenic response was also reported for the ^{99m}Tc-labeled thienylalanyl−leucyl−phenylalanyl chemotactic peptides,[411] ^{99m}Tc−RP050 and ^{99m}Tc−RP056.[22] Since RP463 was purified by HPLC, it should be free of unlabeled fMLFK−HYNIC. The neutropenic effect is probably due to the fact that RP463 is a high-affinity agonist for chemotactic peptide receptors on leukocytes.

The use of ^{99m}Tc-labeled potent agonists as radiopharmaceuticals suffers a major drawback, severe reduction of peripheral leukocyte count. There are several approaches to avoid this problem. The first approach is to separate the radiolabeled peptide from the excess unlabeled peptide using HPLC. This results in a product almost at its theoretical specific activity but is inconvenient for routine clinical use. This approach may not be enough because even the HPLC-purified ^{99m}Tc-labeled peptide can still cause the neutropenic effect if the ^{99m}Tc complex itself is a very potent agonist. The second approach involves using less active agonists with similar binding characteristics. However, lower binding affinity may result in less accumulation of the radioactivity at the receptor sites. The third approach is to use antagonist peptides as targeting molecules. Unfortunately, the receptor binding affinity of the antagonists tested to date appear to be much lower than that of the agonists.[412]

Peptide P483 (Figure 25) is an analogue of the C-terminal tridecapeptide of human PF-4, which is a 29-K Da homotetrameric protein. It contains a Cys−Gly−Cys tripeptide chelating unit for ^{99m}Tc-labeling. The pentalysine sequence on the N-terminus was used to promote renal clearance.[413] It was reported that complexation of ^{99m}Tc−P483 with heparin substantially enhanced the binding to WBCs and resulted in improved uptake in sites of infection in a rabbit infection model.[413] The in vitro distribution in human blood suggests that ^{99m}Tc−P483H associates with specific WBCs, particularly monocytes. In a rabbit infection model, ^{99m}Tc−P483H showed slightly higher infection uptake (0.062 ± 0.022%ID/g) than ^{111}In−oxine−WBCs (0.051 ±

0.008%ID/g) and a 6-fold higher target-to-background ratio, probably due to rapid blood clearance.[413]

Tuftsin is a tetrapeptide (TKPR) derived from the Fc portion of IgG. It promotes phagocytosis and chemotaxis of neutrophils and monocyte/macophages. Recently, a ^{99m}Tc-labeled tuftsin receptor antagonist (Pic−SC(Acm)G−TKPPR; Pic = picolinic acid) was used for imaging inflammation.[27] The Pic−SC(Acm)G sequence forms an N_3S chelating unit for ^{99m}Tc bonding. Radiolabeling was achieved by ligand exchange with [^{99m}Tc]glucoheptonate. Animal studies showed that the ^{99m}Tc-labeled tuftsin antagonist was able to give excellent images with target-to-background ratios of 3.6, 5.0, and 16.2 at 0.5, 3, and 17 h postinjection, respectively. Another ^{99m}Tc-labeled tuftsin receptor antagonist is ^{99m}Tc−RP128 (RP128 = dimethylGSC(Acm)G−TKPPR). In an inflammatory bowel disease (IBD) model, ^{99m}Tc−RP128 showed much better imaging quality than ^{111}In−oxine−WBCs.[29] Target (inflamed terminal colon) to background (proximal noninflamed colon) ratios of 2.14, 2.51, 2.90, and 1.90 were obtained at 0.5, 1, 3, and 18 h postinjection, respectively. Both agents were rapidly excreted via the renal system.[28] A pilot phase II clinical study demonstrated that ^{99m}Tc−RP128 could be used to visualize inflammatory lesions in patients with Crohn's disease within 4 h postinjection.[414]

Radiolabeled cytokines are a promising class of peptide radiopharmaceuticals that may have diagnostic potential in several pathological conditions.[415] Cytokines act via an interaction with specific surface receptors expressed on known cell populations. Cytokine receptors, usually of high affinity, are generally expressed at low levels on resting cells, but their expression can be upregulated during activation infections, autoimmune diseases, and other pathological conditions. Although radiolabeled cytokines have the potential for the study of pathophysiology of several diseases, very few ^{99m}Tc-labeled cytokines have been used for diagnosis of inflammation or infection.[415]

C. Tumor Imaging

The incidence of malignancies in the United States, Europe, and Japan may be estimated to be 3.3 MM in 1997. The most common malignancies in the United States and European countries are lung, colorectal, breast, and prostate. Despite a number of imaging modalities currently available in the clinic, there is still an unmet need for the development of new diagnostic agents, which can detect both the primary and metastastic tumors with more specificity and accuracy. Radiolabeled receptor-based biomolecules such as small peptides are of great interest because they have the potential to detect primary sites, identify occult metastatic lesions, guide surgical intervention, stage tumors, and predict the efficacy of therapeutic agents. When labeled with a suitable radionuclide, they can also be used as therapeutic radiopharmaceuticals.

The peptide that has attracted the greatest interest is somatostatin, a tetradecapeptide which exhibits an inhibitory effect on the secretion of numerous hormones, including growth hormone, thyrotrophin, insulin, glucogon, vasoactive intestinal peptide (VIP), and secretin. Somatostatin receptors are overexpressed on a number of human tumors and their metastases[416−419] thereby serving well as the target for tumor imaging. Although the first report of in vivo imaging with a somatostatin analogue appeared in 1976,[420] further development was hampered due to rapid degradation of the native peptide by plasma and tissue proteases.[421] For this reason, analogues of somatostatin have been synthesized using D-amino acids to prolong the in vivo half-life by inhibiting the action of amino and carboxypeptidases. Octreotide (Sandostatin, SMS 201-995) is a metabolically stable analogue of somatostatin and has been successfully used for the treatment of acromegaly and GEP tumors.[422−424] ^{111}In−DTPA−octreotide (OctreoScan) has become one of the most commonly used radiopharmaceuticals in the clinic for tumor imaging. However, it suffers several drawbacks such as the long half-life ($t_{1/2}$ = 67 h, g = 173, (89%) and 247 keV (94%)) and the high cost of ^{111}In. For diagnostic purposes, ^{99m}Tc is more desirable because of its low cost, easy availability, and ideal nuclear characteristics, which better match the rapid blood clearance and fast tumor accumulation of octreotide.

In the last several years, various somatostatin analogues have been labeled with ^{99m}Tc using different BFCAs. Of particular interest are ^{99m}Tc-labeled peptides P587 and P829 (Figure 24). Both peptides contain the Tyr−(D-Trp)−Lys−Val sequence, which is responsible for somatostatin receptor binding. The cyclic peptide backbone does not contain the S−S disulfide linkage and is not susceptible to reductive cleavage. In P587, the Gly−Gly−Cys tripeptide constitutes an N_3S triamidethiol chelator for ^{99m}Tc-bonding while P829 uses the (β-Dap)−Lys−Cys tripeptide sequence to form an N_3S monoaminediamide−thiol chelating unit. Studies in CA20948 tumor-bearing rats showed that the tumor uptake of ^{99m}Tc−P587 and ^{99m}Tc−P829 is comparable to or better than that of OctreoScan.[33] ^{99m}Tc−P829 is excreted predominantly through the renal system. Preliminary clinical results showed that ^{99m}Tc−P829 is useful not only for the detection of primary nonsmall cell lung cancer (NSCLC) with an accuracy of 100% (7/7), but also for metastatic sites of NSCLC with 82% accuracy (9/11).[425] In 23 non-Hodgkin's lymphoma and 8 Hodgkin's lymphoma patients, the sensitivity and specificity were 81% and 93%, respectively.[425] In 18 breast cancer patients, the sensitivity and specificity were 86% and 91%, respectively.[426] No adverse events were experienced in any of the patients. The diagnostic quality is such that when FDG is not available, ^{99m}Tc−P829 can be used as an alternative for detection of NSCLC.[425] ^{99m}Tc−P829 has been submitted for FDA approval as a new radiopharmaceutical for tumor imaging.

HYNIC was also used to label somatostatin analogues with ^{99m}Tc.[37,38,312−316] The use of tricine as a coligand produced a very high specific activity (~6000 mCi/μmol). In a tumor-bearing mouse model (AR4−2J), all three ^{99m}Tc-labeled HYNIC−octreotide conjugates showed comparable tumor uptake to ^{111}In−

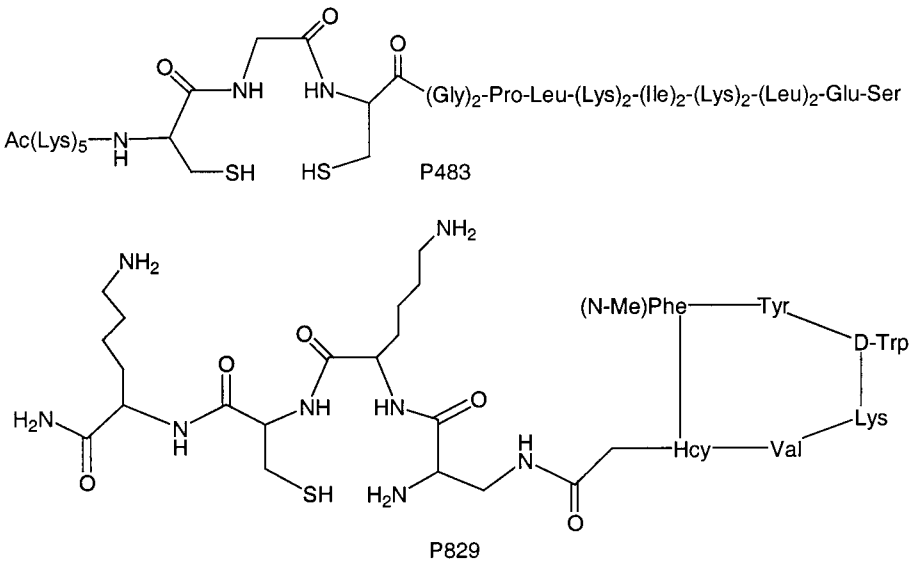


Figure 24. Structures of P483 and P829.

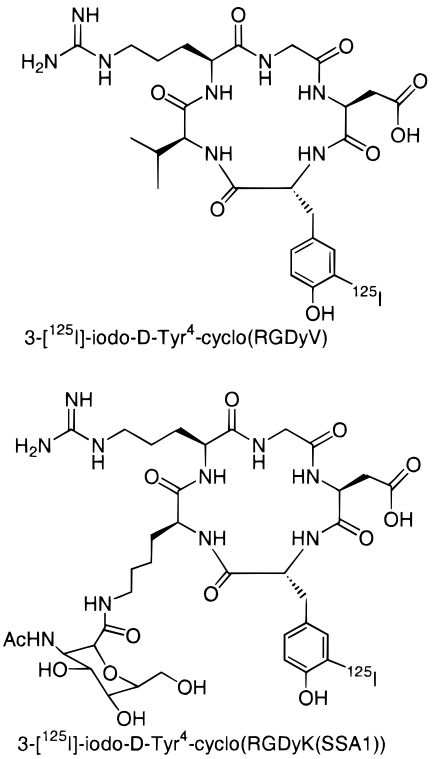


Figure 25. Structures of 3-^{125}I−D-Tyr^4-cyclo(RGDyV) and 3-^{125}I−D-Tyr^4-cyclo(RGDyK(SAA1)).

DTPA−octreotide.[37,38] The observed high blood activity is probably related to the instability of ^{99m}Tc−HYNIC−octreotide−tricine complexes and plasma protein binding. This assumption is supported by the fact that imine-N containing heterocycles can act as coligands and form stable ternary ligand complexes with the ^{99m}Tc-labeled HYNIC−peptide conjugates.[320]

A major drawback of somatostatin receptor imaging is the fact that several categories of tumors such as pancreatic carcinomas are not visualized because they do not express the somatostatin receptor.[99] For CNE (central nervous system) tumors, somatostatin receptor imaging shows different results depending on whether the tumor is within or outside the brain blood barrier (BBB).[427] When the tumor is outside the BBB, it can be easily visualized and the intensity of the scintigraphic signal correlates well with the tumor cell somatostatin receptor density. In tumors located inside the BBB, however, somatostatin receptor imaging provides only limited clinical information depending on the integrity or disruption of the BBB. Therefore, it has been of interest to search for alternative neuropeptide receptors expressed particularly in tumors that lack somatostatin receptor.[99,102]

Like somatostatin receptors, a large number of high-affinity receptors for VIP are expressed on various tumors including adenocarcinoma, endocrine-related tumors, melanoma, and lymphoma. Therefore, the ^{99m}Tc-labeled vasoactive intestinal peptide (VIP) and its derivatives (agonists and antagonists) may become new classes of tumor imaging agents. Studies using ^{123}I−VIP have clearly demonstrated its utility in localizing intestinal adenocarcinoma and endocrine tumors as well as metastatic tumor sites in humans.[48−52] Recently, Lister-James and co-workers reported a ^{99m}Tc-labeled VIP analogue (P1666 = [des-Met^{17}, *S*-(CH_2CO−Gly−Gly−Cys−Lys−amide)-Hcy^{17}]VIP).[237] It was found that the metal complex [ReO(P1666)] has a much higher receptor binding affinity than the parent peptide P1666. The ^{99m}Tc−P1666 complex showed low GI uptake and fast blood clearance and has the potential for imaging colorectal cancer. Thakur and co-workers also reported the ^{99m}Tc-labeling of VIP using cyclam, HYNIC, and MAG_3 as BFCAs. The gamma camera imaging shows the tumor/blood ratio to be less than 1, and the results from the cell binding assay were also discouraging.[49]

For the past decade, radiolabeled somatostatin analogues and VIP receptor ligands have demonstrated their utility for imaging neuroendocrine tumors positive with somatostatin receptors.[99,102] However, endocrine tumors are only a small part of the tumor population, and not all tumors express somatostatin and/or VIP receptors. This, in turn, results in only limited use of radiolabeled somatostatin and VIP receptor ligands in clinical applications.

Therefore, there is a growing interest in radiolabeled small peptides targeted at receptors with a larger tumor population.

Angiogenesis, the formation of new blood vessels, is a requirement for malignant tumor growth and metastasis.[428−433] Clinical studies have demonstrated that the degree of angiogenesis is correlated with the malignant potential of several cancers, including breast cancer, colorectal carcinoma, and malignant melanoma.[434−436] The angiogenic process depends on vascular endothelial cell migration and invasion, regulated by cell adhesion receptors.[434−436] The integrin $\alpha v\beta 3$ (vitronectin receptor) is such a cell adhesion receptor[440−442] and interacts with proteins and peptides containing the RGD tripeptide recognition sequence.[116,428−440] Many peptide vitronectin receptor antagonists have been shown to inhibit neovascularization, tumor-induced angiogenesis, and tumor growth.[116,440] These antagonists have a very high binding affinity for the vitronectin receptor. Therefore, they can serve as targeting molecules to carry the radionuclide for imaging angiogenesis and tumor vasculature.

Recently, a synthetic linear decapeptide αP2 (RGDSCRGDSY) was radiolabeled with ^{99m}Tc by ligand exchange with [^{99m}Tc]glucoheptonate.[443] The peptide contains two RGD sequences for receptor binding. It was believed that the cysteine residue inserted in the primary structure is responsible for the ^{99m}Tc binding. There is no structural information reported for the corresponding ^{99m}Tc complex. In humans, six out of eight lymph node metastases (75%) and all other neoplastic sites (11 sites) were successfully imaged using the ^{99m}Tc-labeled peptide αP2.[443] The ^{99m}Tc-labeled peptideαP2 shows rapid clearance from circulation via the renal system.

The RGD sequence is part of the proteins in the extracellular matrix such as vitronectin and thrombospondin. It also occurs in the blood protein fibrinogen, which decisively contributes to blood coagulation. Peptides containing the RGD sequence will bind to both the platelet GPIIb/IIIa receptor and the vitronectin receptor. A major problem in the design of vitronectin receptor antagonists is their selectivity.[116] It has been shown that incorporation of the RGD sequence in a cyclic pentapeptide (Figure 25) results in improved selectivity for the vitronectin receptor.[444−446] Further SAR studies showed that the amino acid substitution in position 5 has no influence on the activity.[447] Recently, Kessler[448] and co-workers reported two ^{125}I-labeled cyclic pentapeptides (Figure 25): 3-^{125}I−D-Tyr^4-cyclo(RGDyV) and 3-^{125}I−D-Tyr^4-cyclo(RGDyK(SAA1)) (SAA = sugar amino acid). It was found that 3-^{125}I−D-Tyr^4-cyclo(RGDyV) has fast hepatobiliary and renal excretion. The tumor/muscle and tumor/blood ratios for melanoma in nude mice were 5.5 and 9.5, respectively, at 60 min postinjection. Substitution of leucine with a SAA-functionalized lysine amino acid residue resulted in improved blood retention time, renal excretion, and better target-to-background ratio.[448] A blocking study using 3 mg/Kg of the $\alpha v\beta 3$ selective cyclo(RGDfV) demonstrated that the localization of radioactivity in the tumor is due to $\alpha v\beta 3$ receptor binding.

Laminin is a major cell-binding molecule of basement membranes.[449] Basement membrane binding capacity is strongly related to the metastatic potential of cancer cells. The interactions between the metastatic cell surface and laminin play a critical role during metastasis because of the specific laminin binding sites on the cancer cell surface.[450−452] Various laminin-derived synthetic peptides have been shown to inhibit experimental metastasis by competing with laminin.[453−455] A radioiodinated peptide YIGSR (^{131}I−YIGSR) was recently reported for lung carcinoma imaging in mice.[456] It was found that the radiolabeled peptide is excreted via the renal system, and the tumor uptake was 1.15% ID/g at 24 h postinjection. Autoangiography showed that the peptide did accumulate on the surface of certain tumor cells but not on the surface of any normal cell, demonstrating the specific binding of the radiolabeled peptide on cells that are rich in high-affinity laminin receptors. Similar results were obtained for the ^{99m}Tc-labeled YIGSR with better imaging quality due to the intrinsic nuclear characteristic of ^{99m}Tc.[457]

XIII. Conclusions

Development of a target-specific radiopharmaceutical is a long and complicated process. It involves a clear definition of the clinical need for a specific type of radiopharmaceutical, the selection of receptor system and peptide receptor ligand for the intended disease or disease state, identification of lead peptide based on a series in vitro and in vivo assays, selection of an efficient BFCA, modification of pharmacokinetics, proof of biological efficacy based on the biological performance in various animal models, and the development of a kit formulation. Once a new compound and a kit formulation is identified, it must undergo clinical evaluations (phases I−III) in humans to assess its safety and efficacy for the intended disease or disease state, as well as specificity, sensitivity, and accuracy as compared to other well-established diagnostic modalities. Once clinical studies are completed, the results will be submitted to the FDA for approval as a commercial radiopharmaceutical product.

Significant progress has been made in the development of peptide-based target-specific radiopharmaceuticals. In a very short period of time, ^{99m}Tc-labeled peptides have become an important class of imaging agents for the detection of various diseases or disease states such as tumors, thrombosis, and infection/inflammation. DMP444, ^{99m}Tc−P280, ^{99m}Tc−P483H, ^{99m}Tc−P748, ^{99m}Tc−P829, and ^{99m}Tc−P128 represent successful examples of ^{99m}Tc-labeled peptides as a new generation of radiopharmaceuticals. ^{99m}Tc−P280 has been approved by the FDA as a commercial product (AcuTect) for thrombus imaging; ^{99m}Tc−P829 has been submitted for FDA approval as a tumor imaging agent, while others are still under clinical evaluation. In general, results from preclinical and clinical studies have lived up to their expectations: high specificity, high uptake in the target organ, and high target-to-background ratios due to high receptor binding affinity and rapid blood clearance of the ^{99m}Tc-labeled small peptides.

The term "magic bullet" was originally used for antibodies (radiolabeled or unlabeled) for diagnosis and therapy of various diseases. This term also applies to radiolabeled small molecules including peptides, peptidomimetics, and non-peptide receptor ligands. The magic is not just the high receptor binding affinity and specificity. It is a combination of several factors: receptor binding affinity, receptor population, receptor specificity, and favorable pharmacokinetics. There will be no magic if the radiolabeled receptor ligand does not have a favorable pharmacokinetic profile (fast receptor binding, long receptor residence time, and fast blood clearance via the renal system).

Radiolabeled small peptides continue to attract interest because of their favorable physical and biological characteristics compared to antibodies. Small peptides, once the receptor binding sequence is identified, can be easily synthesized and modified according to their pharmacokinetic requirements. The isotope of choice for radiolabeling will continue to be ^{99m}Tc for diagnostic radiopharmaceuticals because of its ideal nuclear properties, low cost, and easy availability. The experience learned from ^{99m}Tc-labeling of small peptides can be applied to other highly potent receptor binding small molecules. In addition, these biologically active small peptides can also be used for the development of therapeutic radiopharmaceuticals when labeled with appropriate radionuclides such as ^{99m}Y and ^{186}Re.

^{99m}Tc-labeling of highly potent small peptides is different from that of simple organic chelators. For the development of receptor-based radiopharmaceuticals, it is very important to remember that the receptor population is often limited to$10^{-8}-10^{-6}$ M. The use of a large amount of the BFCA−peptide conjugate may saturate the receptor sites and cause unwanted side effects. Therefore, the BFCA−peptide conjugate has to be labeled with a high specific activity. One of the challenges for inorganic chemists is to design new ligand systems that have a very high labeling efficiency and form technetium complexes with high stability and minimal isomerism.

It should be emphasized that the discovery of a new class of biologically active peptides for the intended receptor is just the first step of a long process. The development of new ^{99m}Tc-labeling technologies is equally important. Pharmacokinetics is an important characteristic of a new ^{99m}Tc-labeled peptide radiopharmaceutical. If the ^{99m}Tc-labeled peptide does not have a good pharmacokinetic profile, it will be very difficult to develop into a product for routine clinical use even if the peptide has a high receptor binding affinity and specificity. Improvement of the pharmacokinetics of ^{99m}Tc-labeled peptides can be achieved by modification of both the peptide and the Tc chelate. The development of a new ^{99m}Tc radiopharmaceutical is a multidisciplinary effort and requires the collaboration from scientists in organic chemistry, inorganic chemistry, analytical chemistry, biochemistry, formulation chemistry, and nuclear medicine. As Jurrisson[68] stated, "Without their joint efforts nuclear medicine would not be where it is today, nor will it progress".

XIV. References

(1) Hom, R. K.; Katzenellenbogen, J. A. *Nucl. Med. Biol.* **1997**, *24*, 485.

(2) Liu, S.; Edwards, D. S.; Barrett, J. A. *Bioconjugate Chem.* **1997**, *8*, 621.

(3) Goldsmith, S. J. *Semin. Nucl. Med.* **1997**, *27*, 85.

(4) Fischman, A. J.; Babich, J. W.; Strauss, H. W. *J. Nucl. Med.* **1993**, *34*, 2253.

(5) Fischman, A. J.; Babich, J. W.; Rubin, R. H. *Semin. Nucl. Med.* **1994**, *24*, 154.

(6) Reubi, J. C. *Q. J. Nucl. Med.* **1997**, *41*, 63.

(7) Signore, A. *Q. J. Nucl. Med.* **1995**, *39*, 83.

(8) Thakur, M. L. *Nucl. Med. Commun.* **1995**, *16*, 724.

(9) Lister-James, J.; Moyer, B. R.; Dean, R. T. *Q. J. Nucl. Med.* **1996**, *40*, 221.

(10) McAfee, J. G.; Neumann, R. D. *Nucl. Med. Biol.* **1996**, *23*, 673.

(11) Baidoo, K. E.; Scheffiel, U.; Finley, P.; Lever, S. Z.; Wagner, H. N., Jr. *J. Nucl. Med.* **1994**, *35*, 19P (abstract 67).

(12) Babich, J. W.; Solomon, H.; Pike, M. C.; Kroon, D.; Graham, W.; Abrams, M. J.; Tompkins, R. G.; Rubin, R. H.; Fischman, A. J. *J. Nucl. Med.* **1993**, *34*, 1964.

(13) Babich, J. W.; Fischman, A. J. *Nucl. Med. Biol.* **1995**, *22*, 25.

(14) Fischman, A. J.; Rauh, D.; Solomon, H.; Babich, J. W.; Tompkins, R. G.; Kroon, D.; Strauss, H. W.; Rubin, H. R. *J. Nucl. Med.* **1993**, *34*, 2130.

(15) Babich, J. W.; Graham, W.; Barrow, S. A.; Fischman, A. J. *Nucl. Med. Biol.* **1995**, *22*, 643.

(16) Babich, J. W.; Solomon, H.; Pike, M. C.; Kroon, D.; Graham, W.; Abrams, M. J.; Tompkins, R. G.; Rubin, R. H.; Fischman, A. J. *J. Nucl. Med.* **1993**, *34*, 1964.

(17) Babich, J. W.; Graham, W.; Barrow, S. A.; Dragotakes, S. C.; Tompkins, R. H.; Rubin, R. H.; Fischman, A. J. *J. Nucl. Med.* **1993**, *34*, 2176.

(18) Babich, J. W.; Tompkins, R. G.; Graham, W.; Barrow, S. A.; Fischman, A. J. *J. Nucl. Med.* **1997**, *38*, 1316.

(19) van der Laken, C. J.; Boerman, O. C.; Oyen, W. J. G.; van de Ven, M, T. P.; Edwards, D. S.; Barrett, J. A.; van der Meer, J. W.; Corstens, F. H. *J. Nucl. Med.* **1997**, *38*, 1310.

(20) Claessens, R. A. M. J.; Boerman, O. C.; Koenders, E. B.; Oyen, W. J. G.; van der Meer, J. W. M.; Corstens, F. H. M. *Eur. J. Nucl. Med.* **1996**, *23*, 414.

(21) van der Laken, C. J.; Boerman, O. C.; Oyen, W. J. G.; van der Ven, M. P. P.; van der Meer, J. W. M.; Corstens, F. H. M. *Eur. J. Nucl. Med.* **1998**, *25*, 347.

(22) Pollak, A.; Goodbody, A. E.; Ballinger, J. R.; Duncan, G. S.; Tran, L. L.; Dunn-Dufault, R.; Meghji, K.; Lau, F.; Andrey, T. W.; Boxen, I.; Sumner-Smith, M. *Nucl. Med. Commun.* **1996,** *17*, 132.

(23) Rajopadhye, M.; Overoye, K. L.; Nguyen, H. M.; Barrett, J. A.; Heminway, S. J.; Liu, S.; Edwards, D. S. *Abstracts of Papers*, 216th ACS National Meeting, Boston, MA, 1998; American Chemical Society: Washington, DC, 1998; abstract 005 (medicinal chemistry).

(24) Harris, T. D.; Glovacka, D.; Kalogeropoulos, S. A.; Edwards, D. S.; Liu, S.; Barrett, J. A.; Heminway, S. J. *Abstracts of Papers*, 216th ACS National Meeting, Boston, MA, 1998; American Chemical Society: Washington, DC, 1998; abstract 035 (medicinal chemistry).

(25) Barrett, J. A.; Harris, T. D.; Heminway, S. J.; McKay, L.; Vining, M.; Crocker, A. C.; Rajopadhye, M.; Liu, S.; Edwards, D. S. *J. Nucl. Med.* **1998**, *39*, 215P (abstract 957).

(26) Barrett, J. A.; Harris, T. D.; Hemingway, S. J.; McKay, L.; Vining, M.; Crocker, A. C.; Lazewasky, J.; Rajopadhye, M.; Liu, S.; Edwards, D. S. *Eur. J. Nucl. Med.* **1998**, *25*, 887 (OS-202).

(27) Goodbody, A. E.; Ballinger, J.; Tran, L. L.; Sumner-Smith, M.; Lau, F.; Meghji, K.; Pollak, A. *Eur. J. Nucl. Med.* **1994**, *21*, 790 (abstract 262).

(28) Cavelier, V.; Goodbody, A. E.; Tran, L. L.; Bossuyt, A.; Thornback, J. *Eur. J. Nucl. Med.* **1996**, *23*, 1131 (abstract OMo398).

(29) Peers, S. H.; Tran, L. L.; Eriksson, S. J.; Ballinger, J.; Goodbody, A. E. *J. Nucl. Med.* **1995**, *36*, 114P (abstract 465).

(30) Maina, T.; Stolz, B.; Albert, R.; Bruns, C.; Koch, P.; Mäcke, H. *Eur. J. Nucl. Med.* **1994**, *21*, 437.

(31) Maina, T.; Stolz, B.; Albert, R.; Nock, B.; Bruns, C.; Mäcke, H. *Technetium and Rhenium in Chemistry and Nuclear Medicine;* Nicolini, M., Banoli, G., Mazzi, U., Eds.; SGEditorali: Padova, 1995; Vol. 4, pp 395−400.

(32) Kolan, H.; Li, J.-H.; Thakur, M. L. *Peptide Res.* **1996**, *9*, 144.

(33) Vallabhajosula, S.; Moyer, B. R.; Lister-James, J.; McBride, W. J.; Lipszyc, H.; Lee, H.; Bastidas, D.; Dean, R. T. *J. Nucl. Med.* **1996**, *37*, 1016.
(34) Pearson, D. A.; Lister-James, J.; McBride, W. J.; Wilson, D. M.; Martel, L. J.; Civitello, E. R.; Taylor, J. E.; Moyer, B. R.; Dean, R. T. *J. Med. Chem.* **1996**, *39*, 1361.
(35) Leimer, M.; Kurtaran, A.; Raderer, M.; Smith-Jones, P.; Bischof, C.; Petcov, W.; Brunner, C.; Schima, W.; Lister-James, J.; Virgolini, I. *J. Nucl. Med.* **1998**, *39*, 39P (abstract 145).
(36) Virgolini, I.; Leimer, M.; Handmaker, H.; Lasttoria, S.; Bischof, C.; Muto, P.; Pangerl, T.; Gludovacz, D.; Peck-Radosavljevic, M.; Lister-James, J.; Hamilton, G.; Kaserer, K.; Valent, P.; Dean, R. T. *Cancer Res.* **1998**, *58*, 1850.
(37) Mäcke, H. R.; Behe, M. *J. Nucl. Med.* **1996,** *37*, 29P (abstract 107).
(38) Behe, M.; Mäcke, H. R. *Eur. J. Nucl. Med.* **1995,** *22*, 791 (abstract 267).
(39) Thakur, M. L.; John, E.; Li, J.; Reddy, H. R.; Halmos, G.; Schally, A. V. *J. Nucl. Med.* **1995,** *36*, 92P (abstract 374).
(40) Thakur, M. L.; Halmos, G.; Jan, J. *J. Nucl. Med.* **1994,** *35*, 259P (abstract 1063).
(41) Baidoo, K. E.; Lin, K.-S.; Zhan, Y.-G.; Finley, P.; Scheffel, U.; Wagner, H. N., Jr. *Bioconjugate Chem.* **1998**, *9*, 218.
(42) Baidoo, K. E.; Zhan, Y.-G.; Lin, K.-S.; Scheffel, U.; Wagner, H. N., Jr. *J. Nucl. Med.* **1998**, *39*, 7P (abstract 16).
(43) Schibli, R.; Karra, S.; Katti, K. V.; Gali, H.; Higginbotham, C.; Sieckman, G.; Hoffman, T. J.; Volkert, W. A. *J. Nucl. Med.* **1998**, *39*, 225P (abstract 997).
(44) Zinn, K. R.; Smyth, C. A.; Wu, Q.; Kirkman, R. L.; Mountz, J. M.; Buchsbaum, D. J.; Rodgers, B. E. *J. Nucl. Med.* **1998**, *39*, 224P (abstract 994).
(45) Wang, S.; Luo, J.; Lantrip, D. A.; Waters, D. J.; Mathias, C. J.; Green, M. A.; Fuchs, P. L.; Low, P. S. *Bioconjugate Chem.* **1997**, *8*, 673.
(46) Mathias, C. J.; Wang, S.; Lee, R. J.; Waters, D. J.; Low, P. S.; Green, M. A. *J. Nucl. Med.* **1996**, *37*, 1003.
(47) Mathias, C. J.; Wang, S.; Waters, D. J.; Turek, J. J.; Low, P. S.; Green, M. A. *J. Nucl. Med.* **1998**, *39*, 1579.
(48) Kurtaran, A.; Raderer, M.; Müller, C.; Prokesch, R.; Kaserer, K.; Eibenberger, K.; Koperna, K.; Niederle, B.; Virgolini, I. *J. Nucl. Med.* **1997**, *38*, 880.
(49) Kolan, H. R.; Pallela, V.; Thakur, M. L. *J. Labelled Compd. Radiopharm.* **1997**, *40*, 455.
(50) Raderer, M.; Kurtaran, A.; Hejna, M.; Vorbeck, F.; Angelberger, P.; Scheithauer, W.; Virgolini, I. *Eur. J. Nucl. Med.* **1997**, *24*, 884 (abstract 19.1).
(51) Virgolini, I.; Kurtaran, A.; Leimer, M.; Kaserer, K.; Peck-Radosvljevic, M.; Angelberger, P.; Hübsch, P.; Dvorak, M.; Valent, P.; Niederle, B. *J. Nucl. Med.* **1998**, *39*, 1575.
(52) Raderer, M.; Kurtaran, A.; Yang, Q.; Meghdadi, S.; Vorbeck, F.; Hejna, M.; Angelberger, P.; Kornek, G.; Pidlich, J.; Scheithauer, W.; Virgolini, I. *J. Nucl. Med.* **1998**, *39*, 1570.
(53) Barrett, J. A.; Heminway, S. J.; Damphousse, D. J.; Thomas, J. R.; Looby, R. J.; Edwards, D. S.; Harris, T. D.; Rajopadhye, M.; Liu, S.; Carroll, T. R. *J. Nucl. Med.* **1994**, *35, 52P* (abstract 202).
(54) Barrett, J. A.; Bresnick, M. R.; Crocker, A. C.; Damphousse, D. J.; Hampson, J. R.; Heminway, S. J.; Mazaika, T. J.; Kagan, M.; Lazewatsky, J. L.; Edwards, D. S.; Liu, S.; Harris, T. D.; Rajopadhye, M.; Carroll, T. R. *J. Nucl. Med.* **1995**, *36*, 16P (abstract 55).
(55) Liu, S.; Edwards, D. S.; Looby, R. J.; Harris, A. R.; Poirier, M. J.; Barrett, J. A.; Heminway, S. J.; Carroll, T. R. *Bioconjugate Chem.* **1996**, *7*, 63.
(56) Liu, S.; Edwards, D. S.; Looby, R. J.; Poirier, M. J.; Rajopadhye, M.; Bourque, J. P.; Carroll, T. R. *Bioconjugate Chem.* **1996**, *7*, 196.
(57) Barrett, J. A.; Damphousse, D. J.; Heminway, S. J.; Liu, S.; Edwards, D. S.; Looby, R. J.; Carroll, T. R. *Bioconjugate Chem.* **1996**, *7*, 203.
(58) Edwards, D. S.; Liu, S.; Barrett, J. A.; Harris, A. R.; Looby, R. J.; Ziegler, M. C.; Heminway, S. J.; Carroll, T. R. *Bioconjugate Chem.* **1997**, *8*, 146.
(59) Barrett, J. A.; Crocker, A. C.; Damphousse, D. J.; Heminway, S. J.; Liu, S.; Edwards, D. S.; Lazewatsky, J. L.; Kagan, M.; Mazaika, T. J.; Carroll, T. R. *Bioconjugate Chem.* **1997**, *8*, 155.
(60) Knight, L. C.; Radcliffe, R.; Maurer, A. H.; Rodwell, J. D.; Alvarez, V. L. *J. Nucl. Med.* **1994**, *35*, 282.
(61) Lister-James, J.; Knight, L. C.; Mauer, A. H.; Bush, L. R.; Moyer, B. R.; Dean, R. T. *J. Nucl. Med.* **1996**, *37*, 775.
(62) Muto, P.; Lastoria, S.; Varrella, P.; Vergara, E.; Salvatore, M.; Morgano, G.; Lister-James, J.; Bernardy, J. D.; Dean, R. T.; Wencker, D.; Boer, J. S. *J. Nucl. Med.* **1995**, *36*, 1384.
(63) Pearson, D. A.; Lister-James, J.; McBride, W. J.; Wilson, D. M.; Martel, L. J.; Civitello, E. R.; Dean, R. T. *J. Med. Chem.* **1996**, *39*, 1372.
(64) Lister-James, J.; Vallabhajosula, S.; Moyer, B. R.; Pearson, D. A.; McBride, B. J.; De Rosch, M. A.; Bush, L. R.; Machac, J.; Dean, R. T. *J. Nucl. Med.* **1997**, *38*, 105.
(65) Deutsch, E.; Libson, K.; Jurrison, S.; Lindoy, L. F. In *Progress in Inorganic Chemistry*; Lippard, S. J., Ed.; John Wiley & Sons: New York, 1983; Vol. 30, p 75.
(66) Deutsch, E.; Vanderhedyden, J. L.; Gerundini, D.; Libson, K.; Hirth, W.; Colombo, F.; Savi, A.; Fazio, F. *J. Nucl. Med.* **1987**, *28*, 1870.
(67) Dewanjee, M. K. *Semin. Nucl. Med.* **1990**, *20*, 5.
(68) Jurisson, S.; Berning, D.; Jia, W.; Ma, D.-S. *Chem. Rev.* **1993**, *93*, 1137.
(69) Tisato, F.; Refosco, F.; Bandoli, G. *Coord. Chem. Rev.* **1994**, *135/136*, 325.
(70) Blauenstein, P. *New J. Chem.* **1990**, *14*, 405.
(71) Dilworth, J. R.; Parrott, S. J. In *Current Directions in Radiopharmaceutical Research and Development*; Mather, S. J., Ed.; Kluwer Academic Publishers: Netherlands, 1996; pp 1−29.
(72) Dilworth, J. R. *Coord. Chem. Rev.* **1996**, *96*, 163.
(73) Dilworth, J. R.; Parrott, S. J. *Chem. Soc. Rev.* **1998**, *27*, 43.
(74) Pinkerton, T. C.; Desilets, C. P.; Hoch, D. J.; Mikelsons, M. V.; Wilson, G. M. *J. Chem. Educ.* **1985**, *62*, 965.
(75) Eckelman, W. C.; Steigman, J.; Paik, C. In *Diagnostic Nuclear Medicine*, 3rd Ed.; Williams & Wilkins: Baltimore, 1996; pp 199−216.
(76) Archer, C. M.; Edwards, B.; Powell, N. A. In *Current Directions in Radiopharmaceutical Research and Development*; Mather, S. J., Ed.; Kluwer Academic Publishers: Netherlands, 1996; pp 81−88.
(77) Saha, G. B.; Go, R. T.; Macintyre, W. J. *Nucl. Med. Biol.* **1992**, *19*, 1.
(78) Walovitch, R. C.; Williams, S. J.; Lafrance, N. D. *Nucl. Med. Biol.* **1990**, *17*, 77.
(79) Nunn, A. D. *Semin. Nucl. Med.* **1990**, *20*, 111.
(80) Britton, K. E. *Eur. J. Nucl. Med.* **1990**, *16*, 373.
(81) Verbruggen, A. M. *Eur. J. Nucl. Med.* **1990**, *17*, 346.
(82) Schwochau, K. *Angew. Chem., Int. Ed. Eng.* **1994**, *33*, 2258.
(83) Antar, M. A. *Nucl. Med. Biol.* **1990**, *17*, 103.
(84) Keenan, A. M.; Hartert, J. C.; Larson, S. M. *J. Nucl. Med.* **1985**, *26*, 531.
(85) Khaw, B.-A.; Strauss, H. W.; Narula, J. *J. Nucl. Med.* **1993**, *34*, 2264.
(86) Harzra, D. K.; Britton, K. E.; Lahiri, V. L.; Gupta, A. R.; Khanna, P.; Saran, S. *Nucl. Med. Commun.* **1995**, *16*, 66.
(87) Serafini, A. N. *J. Nucl. Med.* **1993**, *34*, 533.
(88) Huston, J. S.; George, A. J. T.; Adams, G. P.; Stafford, W. F.; Jamar, F.; Tai, M.-S.; McCartney, J. E.; Oppermann, H.; Heelan, B. T.; Peters, A. M.; Houston, L. L.; Bookman, M. A.; Wolf, E. J.; Weiner, L. M. *Q. J. Nucl. Med.* **1996**, *40*, 320.
(89) Delaloye, A. B.; Delaloye, B. *Eur. J. Nucl. Med.* **1995**, *22*, 571.
(90) Behr, T. M.; Goldenberg, D. M. *J. Nucl. Med.* **1996**, *37*, 834.
(91) Ruth, T. J.; Pate, B. D.; Robertson, R.; Porter, J. K. *Nucl. Med. Biol.* **1989**, *16*, 323.
(92) Spencer, R. P. *Nucl. Med. Biol.* **1986**, *13*, 461.
(93) Fawwaz, R. A.; Wang, T. S. T.; Srivastava, S. C.; Hardy, M. A. *Nucl. Med. Biol.* **1986**, *13*, 429.
(94) Atkins, H. L. *Appl. Radiat. Isot.* **1998**, *49*, 277.
(95) Schubiger, P. A.; Alberto, R.; Smith, A. *Bioconjugate Chem.* **1996**, *7*, 165.
(96) Karelin, Y. E. A.; Toporov, Y. U. G. *Appl. Radiat. Isot.* **1998**, *49*, 299.
(97) Ehrhardt, G. J.; Ketring, A. R.; Ayers, L. M. *Appl. Radiat. Isot.* **1998**, *49*, 295.
(98) Knapp, F. F., Jr. (Russ); Mirzadeh, S.; Beets, A. L.; O'Doherty, M.; Blower, P. J.; Verdera, E. S.; Gaudiano, J. S. Kropp, J.; Guhlke, J.; Palmedo, H.; Biersack, H.-J. *Appl. Radiat. Isot.* **1998**, *49*, 309.
(99) Reubi, J. C. *J. Nucl. Med.* **1995**, *36*, 1825.
(100) Eckelman, W. C. *Nucl. Med. Biol.* **1994**, *21*, 759.
(101) Katzenellenbogen, J. A. *J. Nucl. Med.* **1995**, *36 (Suppl.),* 8S.
(102) Seregni, E.; Chiti, A.; Bombardieri, E. *Eur. J. Nucl. Med.* **1998**, *25*, 639.
(103) Schmidt, M.; Scheidhauer, K.; Luyken, C.; Voth, E.; Hildebrandt, G.; Klug, N.; Schicha, H. *Eur. J. Nucl. Med.* **1998**, *25*, 675.
(104) Pauwels, E. K.; McCready, V. R.; Stoot, J. H. M. B.; van Deurzen, D. F. P. *Eur. J. Nucl. Med.* **1998**, *25*, 277.
(105) Eckelman, W. C. *Eur. J. Nucl. Med.* **1995**, *22*, 249.
(106) Lister-James, J.; Moyer, B. R.; Dean, R. T. *Q. J. Nucl. Med.* **1997**, *41*, 111.
(107) Smith-Jones, P. M.; Stolz, B.; Albert, R.; Knecht, H.; Bruns, C. *Nucl. Med. Biol.* **1997**, *24*, 761.
(108) Ehrlich, P.; Herter, C. A. *Z. Physiol. Chem.* **1904**, *61*, 379.
(109) Eckelman, W. C. In *Radiopharmaceuticals. Chemistry and Pharmacology*; Nunn, A. D., Ed.; Dekker: New York, 1992; pp 167−219.
(110) Langstorm, B.; Hartvig, P. In *Radiopharmaceuticals. Chemistry and Pharmacology;* Nunn, A. D., Ed.; Dekker: New York, 1992; pp 21−66.
(111) Nunn, A. D.; Linder, K. E.; Tweedle, M. F. *Q. J. Nucl. Med.* **1997**, *41*, 155.
(112) Weller, T.; Alig, L.; Müller, M. H.; Kouns, W. C.; Steiner, B. *Drugs Future* **1994**, *19*, 461.

(113) Cook, N. S.; Kottirsch, G.; Zerwes, H.-G. *Drugs Future* **1994**, *19*, 135.
(114) Hynes, R. O. *Cell* **1992**, *69*, 11.
(115) Felding-Habermann, B.; Cheresh, D. A. *Curr. Opin. Cell Biol.* **1993**, *5*, 864.
(116) Giannis, A.; Rübsam, F. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 588.
(117) Eckelman, W. C. *Nucl. Med. Biol.* **1998**, *25*, 169.
(118) Reily, R. M.; Gariepy, J. *J. Nucl. Med.* **1998**, *39*, 1036.
(119) Roth, J. In *Cell Membrane Receptors for Viruses, Antigens and Antibodies, Polypeptide Hormones, and Small Molecules;* Beers, R. F.; Bassett, E. G., Eds.; Raven Press: New York, 1976; pp 7−16.
(120) Britton, K. E. *Nucl. Med. Commun.* **1997**, *18*, 992.
(121) Zoghbi, S. S.; Thakur, M. L.; Gottschalk, A. *J. Nucl. Med.* **1981**, *22*, P32.
(122) Prevost, G.; Mormont, C.; Gunning, M.; Thomas, F. *Acta Oncol.* **1993**, *32*, 209.
(123) Giannis, A.; Kolter, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244.
(124) Fix, J. A. *Pharm. Res.* **1996**, *13*, 1760.
(125) Deshapande, S. V.; DeNardo, S. L.; Meares, C. F.; McCall, M. J.; Adams, G. P.; Denardo, G. L. *Nucl. Med. Biol.* **1989**, *16*, 587.
(126) Weber, R. W.; Boutin, R. H.; Nedelman, M. A.; Lister-James, J.; Dean, R. T. *Bioconjugate Chem.* **1990**, *1*, 431.
(127) Ultee, M. E.; Bridger, G. J.; Abrams, M. J.; Longley, C. B.; Barton, C. A.; Larsen, S. K.; Henson, G. W.; Padmanabhan, S.; Gaul, F. E.; Schwartz, D. A. *J. Nucl. Med.* **1997**, *38*, 133.
(128) Bridger, G. J.; Abrams, M. J.; Padmanabhan, S.; Gaul, F. E.; Larsen, S. K.; Henson, G. W.; Schwartz, D. A.; Longley, C. B.; Burton, C. A.; Ultee, M. E. *Bioconjugate Chem.* **1996**, *7*, 255.
(129) Jones, A. G.; Orvig, C.; Trop, H. S.; Davison, A.; Davis, M. A. *J. Nucl. Med.* **1980**, *21*, 279.
(130) Davison, A.; Jones, A. G.; Orvig, C.; Sohn, M. *Inorg. Chem.* **1981**, *20*, 1629.
(131) Abrams, M. J.; Davison, A.; Jones, A. G.; Costello, C. E. *Inorg. Chim. Acta* **1983**, *77*, L235.
(132) Edwards, D. S.; Liu, S.; Poirier, M. J.; Zhang, Z.; Webb, G. A.; Orvig, C. *Inorg. Chem.* **1994**, *33*, 5607.
(133) Deutsch, E.; Vanderheyden, J.-L.; Gerundini, P.; Libson, K.; Hirth, W.; Colombo, F.; Savi, A.; Fazio, F. *J. Nucl. Med.* **1987**, *28*, 1870.
(134) Vanderheyden, J.-L.; Libson, K.; Nosco, D. L.; Ketring, A. R.; Deutsch, E. *Int. J. Appl. Radiat. Isot.* **1983**, *34*, 1611.
(135) Doyle, M. N.; Libson, K.; Woods, M.; Sullivan, J. C.; Deutsch, E. *Inorg. Chem.* **1986**, *25*, 3367.
(136) Abrams, M. J.; Davison, A.; Jones, A. G.; Costello, C. E.; Pang, H. *Inorg. Chem.* **1983**, *22*, 2798.
(137) Alberto, R.; Schibli, R.; Egli, A.; Schubiger, A. P. *J. Am. Chem. Soc.* **1998**, *120*, 7987.
(138) Rao, T. N.; Adhikesavalu, D.; Camerman, A.; Fritzberg, A. R. *J. Am. Chem. Soc.* **1990**, *112*, 5798.
(139) Eshima, D.; Taylor, A., Jr.; Fritzberg, A. R.; Kasina, S.; Hansen, L.; Sorenson, J. F. *J. Nucl. Med.* **1987**, *28*, 1180.
(140) Grummon, G.; Rajagopalan, R.; Palenik, G. J.; Koziol, A. E.; Nosco, D. L. *Inorg. Chem.* **1995**, *34*, 1764.
(141) Subhani, M.; Cleynhens, B.; Bormans, G.; Hoogmartens, M.; De Roo, M.; Verbruggen, A. M. In *Technetium and Rhenium in Chemistry and Nuclear Medicine;* Nicolini, M, Banoli, G., Mazzi, U., Eds.; Cortina International: Verona, 1989; Vol. 3, pp 453−461.
(142) Bormans, G.; Cleynhens, B.; Hoogmartens, M.; De Roo, M.; Verbruggen, A. M. In *Technetium and Rhenium in Chemistry and Nuclear Medicine;* Nicolini, M, Banoli, G., Mazzi, U., Eds.; Cortina International: Verona, 1989; Vol. 3, pp 661−666.
(143) Bormans, G.; Cleynhens, B.; Adriaens, P.; De Roo, M.; Verbruggen, A. M. *J. Labelled Compds. Radiopharm.* **1993**, *33*, 1065.
(144) Vanbilloen, H. P.; Bormans, G. M.; De Roo, M. J.; Verbruggen, A. M. *Nucl. Med. Biol.* **1996**, *22*, 325.
(145) Edwards, D. S.; Cheesman, E. H.; Watson, M. W.; Maheu, L. J.; Nguyen, S. A.; Dimitre, L.; Nason, T.; Watson, A. D.; Walovitch, R. In *Technetium and Rhenium in Chemistry and Nuclear Medicine;* Nicolini, M., Banoli, G., Mazzi, U., Eds.; Cortina International: Verona, 1989; Vol. 3, pp 431−444.
(146) Oya, S.; Kung, M.-P.; Frederick, D.; Kung, H. F. *Nucl. Med. Biol.* **1995**, *22*, 749.
(147) Kung, H. F.; Guo, Y. Z.; Yu, C. C.; Billings, J.; Subramanyam, V.; Calabrese, J. C. *J. Med. Chem.* **1989**, *32*, 433.
(148) Mach, R. H.; Kung, H. F.; Guo, Y. Z.; Yu, C. C.; Subramanyam, V.; Calabrese, J. C. *Nucl. Med. Biol.* **1989**, *16*, 829.
(149) Francesconi, L. C.; Graczyk, G.; Wehrli, S.; Shaikh, S. N.; McClinton, D.; Liu, S.; Zubieta, J.; Kung, H. F. *Inorg. Chem.* **1993**, *32*, 3114.
(150) Meegalla, S.; Plüssl, K.; Kung, M.-P.; Chumpradit, S.; Stevenson, D. A.; Frederick, D.; Kung, H. F. *Bioconjugate Chem.* **1996**, *7*, 421.
(151) O'Neil, J. P.; Wilson, S. R.; Katzenellenbogen, J. A. *Inorg. Chem.* **1994**, *33*, 319.
(152) Lever, S. Z.; Baidoo, K. E.; Mahmood, A. *Inorg. Chim. Acta* **1990**, *176*, 183.
(153) Marchi, A.; Marvelli, L.; Rossi, R.; Magon, L.; Bertolasi, V.; Ferretti, V.; Gilli, P. *J. Chem. Soc., Dalton Trans.* **1992**, 1485.
(154) Spyriounis, D. M.; Pelecanou, M.; Stassinopoulou, C. I.; Raptopoulou, C. P.; Terzis, A.; Chiotellis, E. *Inorg. Chem.* **1995**, *34*, 1077.
(155) Mastrostamatis, S. G.; Papadopoulos, M. S.; Pirmettis, I. C.; Paschali, E.; Varvarigou, A. D.; Stassinopoulou, C. I.; Raptopoulou, C. P.; Terzis, A.; Chiotellis, E. *J. Med. Chem.* **1994**, *37*, 3212.
(156) Pirmettis, I. C.; Papadopoulos, M. S.; Mastrostamatis, S. G.; Raptopoulou, C. P.; Terzis, A.; Chiotellis, E. *Inorg. Chem.* **1996**, *35*, 1685.
(157) Papadopoulos, M. S.; Pelecanou, M.; Pirmettis, I. C.; Spyriounis, D. M.; Raptopoulou, C. P.; Terzis, A.; Stassinopoulou, C. I.; Chiotellis, E. *Inorg. Chem.* **1996**, *35*, 4478.
(158) Klingensmith, W. C., III; Fritzberg, A. R.; Spitzer, V. M.; Johnson, D. L.; Kuni, C. C.; Williamson, M. R.; Washer, G.; Weil, R. III *J. Nucl. Med.* **1984**, *25*, 42.
(159) Bormans, G.; Cleynhens, B.; Jose, D.; Hoogmartens, M.; De Roo, M.; Verbruggen, A. *Nucl. Med. Biol.* **1990**, *17*, 499.
(160) Bubeck, B.; Brandau, W.; Reinbold, F.; Dreikorn, K.; Steinbächer, M.; Eisenhut, M.; Georgi, P. *Nucl. Med. Biol.* **1988**, *15*, 99.
(161) Walovitch, R. C.; Cheesman, E. H.; Maheu, L. J.; Hall, K. M. *J. Cereb. Blood Flow Metab.* **1994**, *14*, S4.
(162) Kung, H. F.; Bradshaw, J. E.; Chumpradit, S.; Zhang, Z. P.; Kung, M. P.; Mu, M.; Frederick, D. In *Technetium and Rhenium in Chemistry and Nuclear Medicine;* Nicolini, M., Banoli, G.; Mazzi, U., Eds.; SGEditorali: Padova 1995; Vol. 4, pp 293−298.
(163) Meegalla, S.; Plössl, K.; Kung, M.-P.; Stevenson, D. A.; Liable-Sands, L. M.; Rheingold, A. L.; Kung, H. F. *J. Am. Chem. Soc.* **1995**, *117*, 11037.
(164) Meegalla, S.; Plössl, K.; Kung, M.-P.; Chumpradit, S.; Stevenson, D. A.; Kushner, S. A.; McElgin, W. T.; Mozley, P. D.; Kung, H. F. *J. Med. Chem.* **1997**, *40*, 9.
(165) Alarabi, H.; Bell, R. A.; Faggiani, R.; Howard-Lock, H. E.; Lock, C. J. L.; Kramer, A. V. *Inorg. Chem.* **1995**, *34*, 1688.
(166) Treher, E. N.; Francesconi, L. C.; Gougoutas, J. Z.; Malley, M. F.; Nunn, A. D. *Inorg. Chem.* **1989**, *28*, 3411.
(167) Linder, K. E.; Malley, M. F.; Gougoutas, J. Z.; Unger, S. E.; Nunn, A. D. *Inorg. Chem.* **1990**, *29*, 2428.
(168) Linder, K. E.; Nowotnik, D. P.; Malley, M. F.; Gougoutas, J. Z.; Nunn, A. D. *Inorg. Chim. Acta* **1991**, *190*, 249.
(169) Marmion, M. E.; Woulfe, S. R.; Newmann, W. L.; Pilcher, G.; Nosco, D. L. *Nucl. Med. Biol.* **1996**, *23*, 567.
(170) Linder, K. E.; Wen, M. D.; Nowotnik, D. P.; Malley, M. F.; Gougoutas, J. Z.; Nunn, A. D.; Eckelman, W. C. *Bioconjugate Chem.* **1991**, *2*, 160.
(171) Linder, K. E.; Wen, M. D.; Nowotnik, D. P.; Ramalingam, K.; Sharkey, R. M.; Yost, F.; Narra, R. K.; Nunn, A. D.; Eckelman, W. C. *Bioconjugate Chem.* **1991**, *2*, 407.
(172) Linder, K. E.; Chan, Y. W.; Cyr, J. E.; Nowotnik, D. P.; Eckelman, W. C.; Nunn, A. D. *Bioconjugate Chem.* **1993**, *4*, 326.
(173) Jurisson, S. S.; Pirro, J.; DiRocco, R. J.; Rosenapire, K. C.; Jagoda, E.; Nanjappan, P.; Eckelman, W. C.; Nowotnik, D. P.; Nunn, A. D. *Nucl. Med. Biol.* **1995**, *22*, 269.
(174) Arano, Y.; Yokoyama, A.; Furukawa, T.; Horiuchi, K.; Yahata, T.; Saji, H.; Sakahara, H.; Nakashima, T.; Koizumi, M.; Endo, K.; Torizuka, K. *J. Nucl. Med.* **1987,** *28*, 1027.
(175) Arano, Y.; Yokoyama, A.; Magata, Y.; Saji, H.; Horiuchi, K.; Torizuka, K. *Int. J. Nucl. Med. Biol.* **1986,** *12*, 425.
(176) Najafi, A.; Alauddin, M. M.; Siegel, M. E.; Epstein, A. L. *Nucl. Med. Biol.* **1991,** *18*, 179.
(177) Najafi, A.; Alauddin, M. M.; Sosa, A.; Ma, G. Q.; Chen, D. C. P.; Epstein, A. L.; Siegel, M. E. *Nucl. Med. Biol.* **1992,** *19*, 205.
(178) Fritzberg, A. R.; Abrams, P. G.; Beaumier, P. L.; Kasina, S.; Morgan, A. C.; Rao, T. N.; Reno, J. M.; Sanderson, J. A.; Srinivasan, A.; Wilbur, D. S. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4025.
(179) Eary, J. F.; Schroff, R. W.; Abrams, P. G.; Fritzberg, A. R.; Morgan, A. C.; Kasina, S.; Reno, J. M.; Srinivasan, A.; Woodhouse, C. S.; Wilbur, D. S.; Natale, R. B.; Collins, C.; Stehlin, J. S.; Mitchell, M.; Nelp, W. B. *J. Nucl. Med.* **1989**, *30*, 25.
(180) Kasina, S.; Rao, T. N.; Srinivasan, A.; Sanderson, J. A.; Fitzner, J. N.; Reno, J. M.; Beaumier, P. L.; Fritzberg, A. R. *J. Nucl. Med.* **1991**, *32*, 1445.
(181) Majocha, R. E.; Reno, J. M.; Friedland, R. P.; VanHaight, C.; Lyle, L. R.; Marotta, C. A. *J. Nucl. Med.* **1992**, *33*, 2184.
(182) Kasina, S.; Sanderson, J. A.; Fitzner, J. N.; Srinivasan, A.; Rao, T. N.; Hobson, L. J.; Reno, J. M.; Axworthy, D. B.; Beaumier, P. L.; Fritzberg, A. R. *Bioconjugate Chem.* **1998**, *9*, 108.
(183) Gano, L.; Patricio, L.; Marques, E.; Cantinho, G.; Pena, H.; Martins, T.; Hnatowich, D. J. *Nucl. Med. Biol.* **1998**, *25*, 395.
(184) George, A. J. T.; Jamar, F.; Tai, M.-S.; Heelan, B. T.; Adams, G. P.; McCartney, J. E.; Houston, L. L.; Weiner, L. M.; Oppermann, H.; Peters, A. M.; Huston, J. S. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 8358.

(185) Ram, S.; Buchsbaum, D. J. *Int. J. Peptide Protein Res.* **1996**, *48*, 79.
(186) Rusckowski, M.; Qu, F.; Chang, F.; Hnatowich, D. J. *J. Peptide Res.* **1997**, *50*, 393.
(187) von Gog, F. B.; Visser, G. W. M.; Gowrising, R. W. A.; Snow, G. B.; van Dogen, G. A. M. S. *Nucl. Med. Biol.* **1998**, *25*, 619.
(188) Liu, S.; Edwards, D. S. In *Technetium and Rhenium in Chemistry and Nuclear Medicine;* Nicolini, M., Banoli, G., Mazzi, U., Eds.; SGEditorali: Padova, 1995; Vol. 4, pp 383−393.
(189) Rajopadhye, M.; Edwards, D. S.; Bourque, J. P.; Carroll, T. R. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1737.
(190) Rajopadhye, M.; Harris, T. D.; Yu, K.; Glowacka, D.; Damphousse, P. R.; Barrett, J. A.; Hemingway, S. J.; Edwards, D. S.; Carroll, T. R. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 955.
(191) Hnatowich, D. J.; Qu, T.; Chang, F.; Ley, A. C.; Ladner, R. C.; Rusckowski, M. *J. Nucl. Med.* **1998**, *39*, 56.
(192) Guhlke, S.; Schaffland, A.; Zamora, P. O.; Sartor, J.; Diekmann, D.; Bender, H.; Knapp, F. F.; Biesack, H.-J. *Nucl. Med. Biol.* **1998**, *25*, 621.
(193) Hjelstuen, O. K.; Tønnesen, H. J.; Bremer, P. O.; Verbruggen, A. M. *Nucl. Med. Biol.* **1998**, *25*, 651.
(194) Eisenhut, M.; Brandau, W.; Mi*β*feldt, M. *Nucl. Med. Biol.* **1989,** *16*, 805.
(195) Eisenhut, M.; Mi*β*feldt, M.; Matzku, S. *Eur. J. Nucl. Med.* **1990,** *16*, 423 (abstract 132).
(196) Eisenhut, M.; Missfeldt, M.; Matzku, S. *J. Labelled Compds. Radiopharm.* **1991,** *29*, 198.
(197) Eisenhut, M.; Missfeldt, M.; Lehmann, W. D.; Karas, M. *J. Labelled Compd. Radiopharm.* **1991,** ***30***, 1283.
(198) Eisenhut, M.; Lehmann, W. D.; Becker, W.; Behr, T.; Elser, H.; Strittmatter, W.; Steinstrasser, A.; Baum, R. P.; Valerius, T.; Repp, R.; Deo, Y. *J. Nucl. Med.* **1996**, *37*, 362.
(199) Eisenhut, M.; Missfeldt, M.; Lehmann, W. D.; Karas, M. *J. Labelled Compd. Radiopharm.* **1991**, *29*, 1283.
(200) Eisenhut, M.; Lehmann, W. D.; Becker, W.; Elser, H.; Strittmatter, W.; Baum, R. P.; Valerius, T.; Repp, R.; Deo, Y. *J. Nucl. Med.* **1996**, *37*, 362.
(201) O'Neil, J. P.; Anderson, C. J.; Carlson, K. E.; Welch, M. J.; Katzenellenbogen, J. A. *J. Nucl. Med.* **1993**, *33*, 18P (abstract 61).
(202) DiZio, J. P.; Fiaschi, R.; Davison, A.; Jones, A. G.; Katzenellenbogen, J. A. *Bioconjugate Chem.* **1991**, *2*, 352.
(203) DiZio, J. P.; Anderson, C. J.; Davison, A.; Ehrhardt, G. J.; Carlson, K. E.; Welch, M. J.; Katzenellenbogen, J. A. *J. Nucl. Med.* **1992**, *33*, 558.
(204) Misra, H. K.; Virzi, F.; Hnatowich, D. J.; Wright, G. *Tetrahedron Lett.* **1989,** *30*, 1885.
(205) Liang, F. H.; Virzi, F.; Hnatowich, D. J. *Nucl. Med. Biol.* **1987**, *14*, 555.
(206) Baidoo, K. E.; Lever, S. Z. *Tetrahedron Lett.* **1990**, *31*, 5701.
(207) Baidoo, K. E.; Lever, S. Z. *Bioconjugate Chem.* **1990,** *1*, 132.
(208) Baidoo, K. E.; Scheffel, U.; Lever, S. Z. *Cancer Res.* **1990**, *50* (*Suppl.*), 799s.
(209) Kao, C.-H. K.; Baidoo, K. E.; Lever, S. Z. *Nucl. Med, Biol.* **1997**, *24*, 499.
(210) Lever, S. Z.; Baidoo, K. E.; Mahmood, A.; Matsumura, K.; Scheffel, U.; Wagner, H. N., Jr. *Nucl. Med. Biol.* **1994**, *21*, 157.
(211) Lever, S. Z.; Sun, S.-Y.; Scheffel, U. A.; Kaltovich, F. A.; Baidoo, K. E.; Goldfarb, H.; Wagner, H. N., Jr. *J. Pharm. Sci.* **1994**, *83*, 802.
(212) Del Rosario, R. B.; Jung, Y.-W.; Baidoo, K. E.; Lever, S. Z.; Wieland, D. M. *Nucl. Med. Biol.* **1994**, *21*, 197.
(213) Mahmood, A.; Wolff, J. A.; Davison, A.; Jones, A. G. In *Technetium and Rhenium in Chemistry and Nuclear Medicine;* Nicolini, M., Banoli, G., Mazzi, U., Eds.; SGEditorali: Padova, 1995; Vol. 4, pp 211−215.
(214) O'Neil, J. P.; Carlson, K. E.; Anderson, C. J.; Welch, M. J.; Katzenellenbogen, J. A. *Bioconjugate Chem.* **1994**, *5*, 182.
(215) Meegalla, S.; Plössl, K.; Kung, M.-P.; Stevenson, D. A.; Mu, M.; Kushner, S. A.; Liable-Sands, L. M.; Rheingold, A. L.; Kung, H. F. *J. Med. Chem.* **1998**, *41*, 428.
(216) Kung, M.-P.; Stevenson, D. A.; Plössl, K.; Meegalla, S.; Beckwith, A.; Essman, W. D.; Mu, M.; Lucki, I.; Kung, H. F. *Eur. J. Nucl. Med.* **1997**, *24*, 372.
(217) Kung, H. F.; Kim, H.-J.; Kung, M.-P.; Meegalla, S.; Plössl, K.; Lee, H.-K. *Eur. J. Nucl. Med.* **1996**, *23*, 1527.
(218) Troutner, D. E.; Volkert, W. A.; Hoffman, T. J.; Holmes, R. A. *Int. J. Radiat. Isot.* **1984**, *35*, 467.
(219) Chaplin, S. B.; Oberle, P. A.; McKenzie, E. H.; Hoffman, T. J.; Volkert, W. A.; Holmes, R. A.; Canning, L. R.; Pickett, R. D.; Nowotnik, D. P. Neirinckx, R. D. *Nucl. Med. Biol.* **1986**, *13*, 261.
(220) Jurisson, S.; Schlemper, E. O.; Troutner, D. E.; Canning, L. R.; Nowotnik, D. P.; Neirinckx, R. D. *Inorg. Chem.* **1986**, *25*, 543.
(221) Jurisson, S.; Aston, K.; Fair, K. F.; Schlemper, E. O.; Sharp, P. R.; Troutner, D. E. *Inorg. Chem.* **1987**, *26*, 3576.
(222) Neirinckx, R. D.; Canning, L. R.; Piper, I. M.; Nowotnik, D. P.; Pickett, R. D.; Holmes, R. A.; Volkert, W. A.; Forster, A. F.; Weisner, P. S.; Marriott, J. A.; Chaplin, S. B. *J. Nucl. Med.* **1987**, *28*, 191.
(223) Roth, C. A.; Hoffman, T. J.; Corlija, M.; Volkert, W. A.; Holmes, R. A. *Nucl. Med. Biol.* **1992**, *19*, 783.
(224) Paganelli, G.; Magnani, P.; Zito, F.; Lucignani, G.; Sudati, F.; Truci, G.; Motti, E.; Terreni, M.; Pollo, B.; Giovanelli, M.; Canal, N.; Scotti, G.; Comi, G.; Koch, P.; Mäcke, H. R.; Fazio, F. *Eur. J. Nucl. Med.* **1994**, *21*, 314.
(225) Linder, K. E.; Chan, Y.-W.; Cyr, J. E.; Malley, M. F.; Nowotnik, D. P.; Nunn, A. D. *J. Med. Chem.* **1994**, *37*, 9.
(226) Ballinger, J. R.; Kee, J. W. M.; Rauth, A. M. *J. Nucl. Med.* **1996**, *37*, 1023.
(227) Di Roco, R. J.; Bauer, A. A.; Pirro, J. P.; Kuczynski, B. L.; Belnavis, L.; Chan, Y. W.; Linder, K. E.; Narra, R. K.; Nowotnik, D. P.; Nunn, A. D. *Nucl. Med. Biol.* **1997**, *24*, 201.
(228) Ramalingam, K.; Raju, N.; Nanjappan, P.; Nowotnik, D. P. *Tetrahedron Lett.* **1995**, *51*, 2875.
(229) Vanbilloen, H. P.; Bormans, G. M.; De Roo, M. J.; Verbruggen, A. M. *Nucl. Med. Biol.* **1995**, *22*, 325.
(230) Vanbilloen, H. P.; De Roo, M. J.; Verbruggen, A. M. *Eur. J. Nucl. Med.* **1996**, *23*, 40.
(231) Rajagopalan, R.; Grummon, G. D.; Bugaj, J.; Hallemann, L. S.; Webb, E. G.; Marmion, M. E.; Vanderheyden, J.-L.; Srinivasan, A. *Bioconjugate Chem.* **1997**, *8*, 407.
(232) Wong, E.; Fauconnier, T.; Bennett, S.; Valliant, J.; Nguyen, T.; Lau, F.; Lu, L. F. L.; Pollak, A.; Bell, R. A.; Thornback, J. R. *Inorg. Chem.* **1997,** *36*, 5799.
(233) Wong, E.; Bennett, S.; Nguyen, T.; Lu, L. F. L.; Fauconnier, T.; Bell, R. A.; Eshima, D.; Thornback, J. R. *J. Nucl. Med.* **1998,** *39*, 7P (abstract 19).
(234) Pallela, V.; Chakdar, S.; Rattan, S.; Thakur, M. L. *J. Nucl. Med.* **1998,** *39*, 64P (abstract 245).
(235) Reilly, R. M.; Kiarash, R.; Sandhu, J.; Gairepy, J. *J. Nucl. Med.* **1998,** *39*, 77P (abstract 296).
(236) Chen, J.-Q.; Quinn, T. P. *J. Nucl. Med.* **1998,** *39*, 222P (abstract 984).
(237) Lister-James, J.; Virgolini, I.; Nelson, C. A.; Pearson, D. A.; Leimer, M.; Moyer, B. R.; Wilson, D. M.; Dean, R. T. *J. Nucl. Med.* **1998,** *39*, 225P (abstract 998).
(238) Pietzsch, H.-J.; Spies, H.; Hoffmann, S. *Inorg. Chim. Acta* **1989**, *165*, 163.
(239) Spies, H.; Johannsen, B. *Analyst* **1995**, *120*, 775.
(240) Spies, H.; Fietz, T.; Glaser, M.; Pietzsch, H.-J.; Johannsen, B. In *Technetium and Rhenium in Chemistry and Nuclear Medicine;* Nicolini, M., Banoli, G., Mazzi, U., Eds.; SGEditorali: Padova, 1995; Vol. 4, pp 243−246.
(241) Spies, H.; Fietz, T.; Pietzsch, H.-J.; Johannsen, B.; Leibnitz, P.; Reck, G.; Scheller, D.; Kloostermann, K. *J. Chem. Soc., Dalton Trans.* **1995**, 227.
(242) Papadopoulos, M.; Pirmettis, I.; Raptopoulou, C. P.; Terzis, A.; Chiotellis, E. In *Technetium and Rhenium in Chemistry and Nuclear Medicine;* Nicolini, M., Banoli, G., Mazzi, U., Eds.; SGEditorali: Padova, 1995; Vol. 4, pp 223−226.
(243) Mastrostamatis, S.; Pirmettis, I.; Papadopoulos, M.; Paschali, E.; Raptopoulou, C. P.; Terzis, A.; Chiotellis, E. In *Technetium and Rhenium in Chemistry and Nuclear Medicine;*. Nicolini, M., Banoli, G., Mazzi, U., Eds.; SGEditorali: Padova, 1995; Vol. 4, pp 409−412.
(244) Glaser, M.; Spies, H.; Berger, R.; Hahn, F. E.; Lügger, T.; Johannsen, B. *Inorg. Chim. Acta* **1997**, *257*, 143.
(245) Johannsen, B.; Berger, R.; Brust, P.; Pietzsch, H.-J.; Scheunemann, M.; Seifert, S.; Spies, H.; Syhre, R. *Eur. J. Nucl. Med.* **1997**, *24*, 316.
(246) Mastrostamatis, S.; Pirmettis, I.; Papadopoulos, M.; Chiotellis, E.; Friebe, M.; Berger, R.; Spies, H.; Johannsen, B. *Appl. Radiat. Isot.* **1998**, *49*, 961.
(247) Noll, B.; Noll, St.; Jankowsky, R.; Spies, H.; Johannsen, B. *Forschungszent.* Ros*sendorf [Ber.] FZR* **1997**, *200*, 85.
(248) Reich, T.; Bernard, G.; Nitsche, H.; Spies, H.; Johannsen, B. *Forschungszent.* Ros*sendorf [Ber.] FZR* **1996**, *123*, 94.
(249) Meegalla, S.; Plössl, K.; Kung; M−P.; Chumpradit, S.; Stevenson, D. A.; Frederick, D.; Kung, H. F. *Bioconjugate Chem.* **1996,** *7*, 421.
(250) Hoepping, A.; Spies, H.; Johannsen, B. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2871.
(251) Johannsen, B.; Scheunemann, M.; Spies, H.; Brust, P.; Wober, J.; Syhre, R.; Pietzsch, H.-J. *Nucl. Med. Biol.* **1996**, *23*, 429.
(252) Wüst, F.; Skaddan, M. B.; Katzenellenbogen, J. A.; Spies, H. *Q. J. Nucl. Med.* **1998**, *42 (Suppl.),* 32.
(253) Seifert, S.; Pietzsch, H.-J.; Scheunemann, M.; Spies, H.; Syhre, R.; Johannsen, B. *Appl. Radiat. Isot.* **1998**, *49*, 5.
(254) Syhre, R.; Seifert, S.; Spies, H.; Gupta, A.; Johannsen, B. *Eur. J. Nucl. Med.* **1998**, *25*, 793.
(255) Pelecanou, M.; Pirmettis, I. C.; Nock, B. A.; Papadopoulos, M.; Chiotellis, E.; Stassinopoulou, C. I. *Inorg. Chim. Acta* **1998**, *281*, 148.
(256) Marchi, A.; Marvelli, L.; Rossi, R.; Roncara, P.; Uccelli, L.; Giganti, M. In *Technetium and Rhenium in Chemistry and Nuclear Medicine;* Nicolini, M., Banoli, G., Mazzi, U., Eds.; SGEditorali: Padova, 1995; Vol. 4, pp 113−116.

(257) Duatti, A.; Marchi, A.; Pasqualini, R. *J. Chem. Soc., Dalton Trans.* **1990**, 3729.
(258) Pasqualini, R.; Comazzi, V.; Bellande, E.; Dautti, A.; Marchi, A. *Appl. Radiat. Isot.* **1992**, *43*, 1329.
(259) Coulais, Y.; Cros, G.; Darbieu, M. H.; Tafani, J. A. M.; Belgadj-Tahar, H.; Bellande, E.; Pasqualini, R.; Guiraud, R. *Nucl. Med. Biol.* **1994**, *21*, 263.
(260) Belgadj-Tahar, H.; Coulais, Y.; Cros, G.; Darbieu, M. H.; Tafani, J. A. M.; Fabre, J.; Esquerre, J. P.; Guiraud, R. *Nucl. Med. Biol.* **1996**, *23*, 353.
(261) Cros, G.; Belhadj-Tahar, H.; deMontauzon, D.; Gleizes, A.; Coulais, Y.; Guiraud, R.; Bellande, E.; Pasqualini, R. *Inorg. Chim. Acta* **1994**, *227*, 25.
(262) Tisato, F.; Mazzi, U.; Bandoli, G.; Cros, G.; Darbieu, M.-H.; Coulais, Y.; Guiraud, R. *J. Chem. Soc., Dalton Trans.* **1991**, 1301.
(263) Marchi, A.; Garutti, P.; Duatti, A.; Magon, L.; Rossi, R.; Ferretti, V.; Bertolasi, V. *Inorg. Chem.* **1990**, *29*, 2091.
(264) Marchi, A.; Rossi, R.; Marvelli, L.; Bertolasi, V. *Inorg. Chem.* **1993**, *32*, 4673.
(265) Marchi, A.; Rossi, R.; Magon, L.; Duatti, A.; Casellato, U.; Graziani, R.; Vidal, M.; Riche, F. *J. Chem. Soc., Dalton Trans.* **1990**, 1935.
(266) Archer, C. M.; Dilworth, J. R.; Griffiths, D. V.; McPartlin, M.; Kelly, J. D. *J. Chem. Soc., Dalton Trans.* **1992**, 183.
(267) Abram, U.; Lang, E. S.; Abram, S.; Wegmann, J.; Dilworth, J. R.; Kirmse, R.; Woollins, J. D. *J. Chem. Soc., Dalton Trans.* **1997**, 623.
(268) Pasqualini, R.; Duatti, A.; Bellande, E.; Comazzi, V.; Brucato, V.; Hoffschir, D.; Fagret, D.; Comet, M. *J. Nucl. Med.* **1994**, *35*, 334.
(269) Pasqualini, R.; Duatti, A. *J. Chem. Soc., Chem. Commun.* **1992**, 1354.
(270) Fang, P.; Wang, B.-C.; Zhou, X.; Zhou, X.-Q.; Chen, Z.-P. *Appl. Radiat. Isot.* **1998**, *49*, 1591.
(271) Demaimacy, F.; Dazord, L.; Roucoux, A.; Noiret, N.; Patin, H.; Moisan, A. *Nucl. Med. Biol.* **1997**, *24*, 701.
(272) Bellande, E.; Comazzi, V.; Laine, J.; Lecayon, M.; Pasqualini, R.; Duatti, A.; Hoffschir, D. *Nucl. Med. Biol.* **1995**, *22*, 315.
(273) Marchi, A.; Marvelli, L.; Rossi, R.; Magon, L.; Uccelli, L.; Bertolasi, V.; Ferretti, V.; Zanobini, F. *J. Chem. Soc., Dalton Trans.* **1993**, 1281.
(274) Franz, J.; Volkert, W. A.; Barefield, E. K.; Holmes, R. A. *Nucl. Med. Biol.* **1987,** *14*, 569.
(275) Thakur, M. L.; Kolan, H.; Li, J.; Wiaderkiewicz, R.; Pallela, V. R.; Duggaraju, R.; Schally, A. V. *Nucl. Med. Biol.* **1997**, *24*, 105.
(276) Kelly, J. D.; Forster, A. M.; Higley, B.; Archer, C. M.; Booker, F. S.; Canning, L. R.; Chiu, K. W.; Edwards, B.; Gill, H. K.; McPartlin, M.; Nagle, K. R.; Latham, I. A.; Pickett, R. D.; Storey, A. E.; Webbon, P. M. *J. Nucl. Med.* **1993**, *34*, 222.
(277) Higley, B.; Smith, F. W.; Smith, T.; Gemmell, H. G.; Gupta, P. D.; Gvozdanovic, D. V.; Graham, D.; Hinge, D.; Davidson, J.; Lahiri, A. *J. Nucl. Med.* **1993**, *34*, 30.
(278) Jones, S.; Hendel, R. C. *J. Nucl. Med. Technol.* **1993**, *21*, 191.
(279) Smith, C. J.; Katti, K. V.; Volkert, W. A.; Barbour, L. J. *Inorg. Chem.* **1997**, *36*, 3928.
(280) Reddy, V. S.; Berning, D. E.; Katti, K. V.; Barnes, C. L.; Volkert, W. A.; Ketring, A. R. *Inorg. Chem.* **1996**, *35*, 1753.
(281) Katti, K. V.; Gali, H.; Smith, C. J.; Berning, D. E. *Acc. Chem. Res.* **1999**, *32*, 9.
(282) Berning, D. E.; Katti, K. V.; Singh, P. R.; Higginbotham, C.; Reddy, S.; Volkert, W. A. *Nucl. Med. Biol.* **1996**, *23*, 617.
(283) Smith, C. J.; Li, N.; Katti, K. V.; Higginbotham, C.; Volkert, W. A. *Nucl. Med. Biol.* **1997**, *24*, 685.
(284) Alberto, R.; Schibli, R.; Egli, A.; Schubiger, P. A.; Herrmann, W. A.; Artus, G.; Abram, U.; Kaden, T. A. *J. Organomet. Chem.* **1995**, *493*, 119.
(285) Alberto, R.; Schibli, R.; Schubiger, P. A.; Abram, U.; Kaden, T. A. *Polyhedron* **1996**, *15*, 1079.
(286) Abram, U.; Abram, S.; Alberto, R.; Schibli, R. *Inorg. Chim. Acta* **1996**, *248*, 193.
(287) Findeisen, B. L. M.; Schmidt, K. *Isotopenpraxis* **1991**, *27*, 5.
(288) Alberto, R.; Herrmann, W. A.; Kiprof, P.; Baumgärtner, F. *Inorg. Chem.* **1992**, *31*, 895.
(289) Castro, H. H. K.; Hissink, C. E.; Teuben, H. J. H.; Vaaburg, W.; Panek, K. *Recl. Trav. Chim. Pays-Bas* **1992**, *111*, 105.
(290) Alberto, R.; Schibli, R.; Schubiger, P. A.; Abram, U.; Berke, H.; Kaden, T. A. *J. Chem Soc., Chem. Commun.* **1996**, 1291.
(291) Egli, A.; Alberto, R.; Schibli, R.; Schaffland, A. O.; Abram, U.; Abram, S.; Kaden, T. A.; Schubiger, P. A. *J. Labelled Compds. Radiopharm.* **1997**, *39*, 443.
(292) Alberto, R.; Schibli, R.; Egli, A.; Abram, U.; Hübener, R.; Kaden, T. A.; Pietzsch, H.-J.; Schubiger, P. A. *J. Labelled Compd. Radiopharm.* **1997**, *39*, 442.
(293) Alberto, R.; Schibli, R.; Abram, U.; Egli, A.; Knapp, F. F.; Schubiger, P. A. *Radiochim. Acta* **1997**, *79*, 99.
(294) Schibli, R.; Alberto, R.; Abram, U.; Abrams, S.; Egli, A.; Schubiger, P. A.; Kaden, T. A. *Inorg. Chem.* **1998**, *37*, 3509.
(295) Alberto, R.; Egli, A.; Schibli, R.; Abram, U.; Kaden, T. A.; Schaffland, A. O.; Schwarzbath, R.; Schubiger, P. A. *Q. J. Nucl. Med.* **1998**, *42 (Suppl.)*, 9.
(296) Egli, A.; Alberto, R.; Schibli, R.; Abram, U.; Schaffland, A. O.; Allemann-Tannahill, L.; Tourwe, D.; Schubiger, P. A. *Q. J. Nucl. Med.* **1998**, *42 (Suppl.)*, 32.
(297) Waibel, R.; Alberto, R.; Willuda, J.; Egli, A.; Schbiger, P. A. *Q. J. Nucl. Med.* **1998**, *42 (Suppl.)*, 32.
(298) Reisgys, M.; Wüst, F.; Alberto, R.; Schibli, R.; Schubiger, P. A.; Pietzsch, H.-J.; Spies, H.; Johannsen, B. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2243.
(299) Alberto, R.; Schibli, R.; Egli, A. Europian Patent Application No. 97201232, 1997.
(300) Top, S.; El Hafa, H.; Vessieres, A.; Quivy, J.; Vaissermann, J.; Hughes, D. W.; McGlinchey, M. J. J.; Mornon, J.-P.; Thoreau, E.; Jaouen, G. *J. Am. Chem. Soc.* **1995**, *117*, 8372.
(301) Salmain, M.; Gunn, M.; Gorfti, A.; Top, S.; Jaouen, G. *Bioconjugate Chem.* **1993**, *4*, 425.
(302) Hom, R. K.; Katzenellenbogen, J. A. *J. Org. Chem.* **1997**, *62*, 6290.
(303) Chi, D. Y.; O'Neil, J. P.; Anderson, C. J.; Welch, M. J.; Katzenellenbogen, J. A. *J. Med. Chem.* **1994**, *37*, 928.
(304) Hom, R. K.; Chi, D. Y.; Katzenellenbogen, J. A. *J. Org. Chem.* **1996**, *61*, 2624.
(305) Spradau, T. W.; Katzenellenbogen, J. A. *Organometallics* **1998**, *17*, 2009.
(306) Minutolo, F.; Katzenellenbogen, J. A. *J. Am. Chem. Soc.* **1998**, *120*, 4514.
(307) Abrams, M. J.; Juweid, M.; tenKate, C. I.; Schwartz, D. A.; Hauser, M. M.; Gaul, F. E.; Fuccello, A. J.; Rubin, R. H.; Strauss, H. W.; Fischman, A. J. *J. Nucl. Med.* **1990**,*31*, 2022.
(308) Schwartz, D. A.; Abrams, M. J.; Hauser, M. M.; Gaul, F. E.; Larsen, S. K.; Rauh, D.; Zubieta, J. A. *Bioconjugate Chem.* **1991**, *2*, 333.
(309) Larson, S. K.; Abrams, M. J.; Higgins, J. D., III; Solomon, H. F.; Babich, J. W.; Fischman, A. J. *J. Nucl. Med.* **1994**, *35*, 105P (abstract 418).
(310) Larson, S. K.; Solomon, H. F.; Caldwell, G.; Abrams, M. J. *Bioconjugate Chem.* **1995**, *6*, 635.
(311) Edwards, D. S.; Liu, S.; Ziegler, M. C.; Harris, A. R.; Crocker, A. C.; Hemingway, S. J.; Barrett, J. A.; Bridger, G. J.; Abrams, M. J.; Higgins, J. D., III *Bioconjugate Chem.*, in press.
(312) Bangard, M.; Behe, M.; Bender, H.; Guhlke, S.; Risse, J.; Grünwald, F.; Mäcke, H.; Biersack, H.-J. *Eur. J. Nucl. Med.* **1998**, *25*, 837 (abstract OS-2).
(313) Decristoforo, C.; Mather, S. J. *Eur. J. Nucl. Med.* **1998**, *25*, 866 (abstract OS-118).
(314) Stalteri, M. A.; Zhang, Y. M.; Mather, S. J. *Q. J. Nucl. Med.* **1998**, *42 (Suppl.)*, 48.
(315) Decristoforo, C.; Mather, S. J. *Q. J. Nucl. Med.* **1998**, *42 (Suppl.)*, 46.
(316) Krois, D.; Riedel, C.; Angelberger, P.; Kalchberger, P.; Virgolini, I.; Lehner, H. *Liebigs Ann. Chem.* **1996**, 1463.
(317) Laverman, P.; Dams, E. T. M.; Oyen, W. J. G.; Storm, G.; Koenders, E. B.; Prevost, R.; van der Meer, J. W. M.; Corstens, F. H. M.; Boerman, O. C. *Eur. J. Nucl. Med.* **1998**, *25*, 865 (abstract OS-116).
(318) Liu, S.; Edwards, D. S.; Harris, A. R. *Bioconjugate Chem.* **1998**, *9*. 583.
(319) Edwards, D. S.; Cheesman, E. H.; Harris, T. D.; Liu, S.; Rajopadhye, M.; Sworin, M. *J. Nucl. Med.*, **1998**, *39*, 217P (abstract 964).
(320) Liu, S.; Edwards, D. S.; Harris, A. R.; Hemingway, S. J.; Barrett, J. A. *Inorg. Chem. Inorg. Chem.* **1999**, *38*, 1326.
(321) Liu, S.; Edwards, D. S.; Harris, A. R. *Q. J. Nucl. Med.* **1998**, *42 (Suppl.)*, 35.
(322) Liu, S.; Edwards, D. S.; Harris, A. R. *J. Nucl. Med.*, **1998**, *39*, 215P.
(323) Liu, S.; Ziegler, M. C.; Edwards, D. S.; Glajch, J. L. *Q. J. Nucl. Med.* **1998**, *42 (Suppl.)*, 17.
(324) Abrams, M. J.; Larsen, S. K.; Shaikh, S. N.; Zubieta, J. *Inorg. Chim. Acta* **1991**, *185*, 7.
(325) Nicholson, T.; Zubieta, J. *J. Chem. Soc., Chem. Commun.* **1985**, 167.
(326) Nicholson, T.; Zubieta, J. *Inorg. Chim. Acta* **1987,** *134*, 191.
(327) Nicholson, T.; Lombardi, P.; Zubieta, J. *Polyhedron* **1987,** *6*, 1577.
(328) Nicholson, T.; Zubieta, J. *Polyhedron* **1988,** *7*, 171.
(329) Dilworth, J. R.; Thompson, R. M.; Archer, C. M.; Kelly, J. D.; Povey, D. C. In *Technetium and Rhenium in Chemistry and Nuclear Medicine;* Nicolini, M., Bandoli, G., Mazzi, U., Eds.; Cortina International: Verona, 1989; Vol. 3, pp 109−111.
(330) Archer, C. M.; Dilworth, J. R.; Jobanputra, P.; Parrot, S. J.; Thompson, R. M.; Kelly, J. D. In *Technetium and Rhenium in Chemistry and Nuclear Medicine;* Nicolini, M., Bandoli, G., Mazzi, U., Eds.; Cortina International: Verona, 1989; Vol. 3, pp 51−54.

(331) Archer, C. M.; Kelly, J. D.; Dilworth, J. R.; Povey, D. C.; Smith, G. W. In *Technetium and Rhenium in Chemistry and Nuclear Medicine;* Nicolini, M., Bandoli, G., Mazzi, U., Eds.; Cortina International: Verona, 1989; Vol. 3, pp 159−163.
(332) Coutinho, B.; Dawson, D.; Dilworth, J. R.; Miller, J. R.; Rosser, M.; Archer, C. M.; Kelly, J. D. In *Technetium and Rhenium in Chemistry and Nuclear Medicine;* Nicolini, M., Bandoli, G., Mazzi, U., Eds.; Cortina International: Veronapp, 1989; 109−111.
(333) Dilworth, J. R.; Harrison, S. A.; Walton, D. R. M.; Schweda, E. *Inorg. Chem.* **1985,** *24*, 2594.
(334) Archer, C. M.; Dilworth, J. R.; Jobanputra, P.; Thompson, R. M.; McPartlin, M.; Povey, D. C.; Smith, G. W.; Kelly, J. D. *Polyhedron* **1990,** *9*, 1497.
(335) Dilworth, J. R.; Jobanputra, P.; Thompson, R. M.; Archer, C. M.; Kelly, J. D.; Hiller, W. *Z. Naturforsch.* **1991,** *46b*, 449.
(336) Archer, C. M.; Dilworth, J. R.; Jobanputra, P.; Thompson, R. M.; McPartin, M.; Hiller, W. *J. Chem. Soc., Dalton Trans.* **1993,** 897.
(337) Dilworth, J. R.; Jobanputra, P.; Thompson, R. M.; Povey, D. C.; Archer, C. M.; Kelly, J. D. *J. Chem. Soc., Dalton Trans.* **1994,** 1251.
(338) Dilworth, J. R.; Jobanputra, P.; Miller, J. R.; Parrott, S. J.; Chen, Q.; Zubieta, J. *Polyhedron* **1993**, *12*, 513.
(339) Coutinho, B.; Dilworth, J. R.; Jobanputra, P.; Thompson, R. M.; Schmid, S.; Strähle, J.; Archer, C. M. *J. Chem. Soc., Dalton Trans.* **1995,** 1663.
(340) Nicholson, T.; de Vries, N.; Davison, A.; Jones, A. G. In *Technetium and Rhenium in Chemistry and Nuclear Medicine;* Nicolini, M., Bandoli, G., Mazzi, U., Eds.; Cortina International: Verona 1990; Vol. 3, pp 95−108.
(341) Cook, J.; Davision, A.; Jones, A. G.; Davis, W. M. In *Technetium and Rhenium in Chemistry and Nuclear Medicine* Nicolini, M., Bandoli, G., Mazzi, U., Eds.; Cortina International: Verona, 1990; Vol. 3, pp 65−68.
(342) Nicholson, T.; Hirsch-Kuchma, M.; Freiberg, E.; Davison, A.; Jones, A. G. *Inorg. Chim. Acta* **1998**, *279*, 206.
(343) Rose, D. J.; Maresca, K. P.; Nicholson, T.; Davison, A.; Jones, A. G.; Babich, J.; Fischman, A.; Graham, W.; DeBord, J. R. D.; Zunieta, J. A. *Inorg. Chem.* **1998**, *37*, 2701.
(344) Nicholson, T.; Cook, J.; Davison, A.; Rose, D. J.; Maresca, K. P.; Zubieta, J. A.; Jones, A. G. *Inorg. Chim. Acta* **1996,** *252*, 421.
(345) Nicholson, T.; Cook, J.; Davison, A.; Rose, D. J.; Maresca, K. P.; Zubieta, J. A.; Jones, A. G. *Inorg. Chim. Acta* **1996,** *252*, 427.
(346) Hirsch-Kuchma, M.; Nicholson, T.; Davison, A.; Davis, W. M.; Jones, A. G. *Inorg. Chem.* **1997**, *36*, 3237.
(347) Nicholson, T.; Hirsch-Kuchma, M.; Davison, A.; Davis, W. M.; Jones, A. G. *Inorg. Chim. Acta* **1998**, *271*, 191.
(348) Hirsch-Kuchma, M.; Nicholson, T.; Davison, A.; Jones, A. G. *J. Chem. Soc., Dalton Trans.* **1997**, 3189.
(349) Hirsch-Kuchma, M.; Nicholson, T.; Davison, A.; Davis, W. M.; Jones, A. G. *J. Chem. Soc., Dalton Trans.* **1997**, 3185.
(350) Liu, S.; Edwards, D. S.; Harris, A. R.; Singh, P. R. *Appl. Radiat. Isot.* **1997**, *48*, 1103.
(351) Rao, T. N.; Gustavson, L. M.; Srinivasan, A.; Kasina, S.; Fritzberg, A. R. *Nucl. Med. Biol.* **1992**, *19*, 889.
(352) Eckelman, W. C.; Paik, C. H.; Steigman, J. *Nucl. Med. Biol.* **1989**, *16*, 171.
(353) Fritzberg, A. R.; Berninger, R. W.; Hadley, S. W.; Wester, D. W. *Pharma. Res.* **1988**, *5*, 325.
(354) Otsuka, F. L.; Welch, M. J. *Nucl. Med. Biol.* **1987**,*14*, 243.
(355) Hnatowich, D. J. *Nucl. Med. Biol.* **1990**,*17*, 49.
(356) Hnatowich, D. J. *Semin. Nucl. Med.* **1990**, *20*, 80.
(357) Srivastava, S. C.; Mease, R. C. *Nucl. Med. Biol.* **1991**, *18*, 589.
(358) Delmon-Moingeon, L. I.; Mahmood, A.; Davison, A.; Jones, A. G. *J. Nucl. Biol. Med.* **1991**, *35*, 47.
(359) Rhodes, B. A. *Nucl. Med. Biol.* **1991**, *18*, 667.
(360) Eckelman, W. C.; Steigman, J. *Nucl. Med. Biol.* **1991**, *18*, 3.
(361) Liu, Y.-F.; Wu, C.-C. *Pure Appl. Chem.* **1991**, *63*, 427.
(362) Griffiths, G. L.; Goldenberg, D. M.; Jones, A. L.; Hansen, H. J. *Bioconjugate Chem.* **1992**, *3*, 91.
(363) Bhargava, K. K.; Acharya, S. A. *Semin. Nucl. Med.* **1989**, *19*, 187.
(364) Wolf, W.; Shani, J. *Nucl. Med. Biol.* **1986**, *13*, 319.
(365) Gansow, O. A. *Nucl. Med. Biol.* **1991**, *18*, 369.
(366) Parker, D. *Chem. Soc. Rev.* **1990**, *19*, 271.
(367) Francis, M. D.; Tofe, A. J.; Hiles, R. A.; Birch, C. G.; Bevan, J. A.; Grabensteller, R. J. *Int. J. Nucl. Med. Biol.* **1981**, *8*, 145.
(368) Srivastava, S. C.; Neiken, G.; Smith, T. D.; Richards, P. *Int. Appl. Radiat. Isot.* **1977**, *28*, 83.
(369) Gerson, M. C.; Deutsch, E.; Nishiyama, H.; Libson, K. F.; Adolph, R. J.; Grossman, L. W.; Sodd, V. J.; Fortman, D, L.; Vanderheyden, J.-L. E.; Williams, C. C.; Saenger, E. L. *Eur. J. Nucl. Med.* **1983**, *8*, 371.
(370) Deutsch, E.; Bushong, W.; Glavan, K. A.; Elder, R. C.; Sodd, V. J.; Scholz, K. L.; Fortman, D. L.; Lukes, S. J. *Science* **1981**, *214*, 85.
(371) Edwards, D. S.; Liu, S. U.S. Patent No. 5,744,120, 1998.
(372) Babich, J. W.; Graham, W.; Fischman, A. J. *J. Nucl. Med.* **1995**, *36*, 148P (abstract 675).
(373) Babich, J. W.; Fischman, A. J. *J. Nucl. Med.* **1994**, *35*, 19P (abstract 68).
(374) Lee, M. S.; Kerns, E. H.; Hail, M. E.; Liu, J.-P.; Volk, K. J. *LC-GC* **1997**,
(375) *15*, 542.
(376) Niessen, W. M. A.; Tinke, A. P. *J. Chromatogr.* **1995**, *703*, 37.
(377) Gelpé, E. *J. Chromatogr.* **1995**, *703*, 59.
(378) Regnier, F.; Huang, H. *J. Chromatogr.* **1996**, *750*, 3.
(379) Bruins, A. P. *J. Chromatogr.* **1991**, *554*, 39.
(380) Ali, F. E.; Bennett, D. B.; Calvo, R. R.; Elliott, J. D.; Hwang, S. M.; Ku, T. W.; Lago, M. A.; Nichols, A. J.; Romoff, T. T.; Shah, D. H.; Vasko, J. A.; Wong, A. S.; Yellin, T. O.; Yuan, C.-K.; Samanen, J. M. *J. Med. Chem.* **1994**, *37*, 769.
(381) Cheng, S.; Craig, W. S.; Mullen, D.; Tschopp, J. F.; Dixon, D.; Pierschbacher, M. D. *J. Med. Chem.* **1994**, *37*, 1.
(382) Zablocki, J. A.; Miyano, M.; Garland, R. B.; Pireh, D.; Schretzman, L.; Rao, S. N.; Lindmark, R. J.; Panzer-Knodle, S. G.; Nicholson, N. S.; Taite, B. B.; Salyers, A. K.; King, L. W.; Campion, J. G.; Feigen, L. P. *J. Med. Chem.* **1993**, *36*, 1811.
(383) Bach, A. C., II; Eyermann, C. J.; Gross, J. D.; Bower, M. J.; Harlow, R. L.; Weber, P. C.; DeGrado, W. F. *J. Am. Chem. Soc.* **1994**, *116*, 3207.
(384) Jackson, S.; DeGrado, W. F.; Dwivedi, A.; Parthasarathy, A.; Higley, A.; Krywko, J.; Rockwell, A.; Markwalder, J.; Wells, G.; Wexler, R.; Mousa, S.; Harlow, R. L. *J. Am. Chem. Soc.* **1994**, *116*, 3220.
(385) Xue, C.-B.; DeGrado, W. F. *J. Org. Chem.* **1995**, *60*, 946.
(386) Wityak, J.; Fevig, J. M.; Jackson, S. A.; Johnson, A. L.; Mousa, S. A.; Parthasarathy, A.; Wells, G. J.; DeGrado, W. F.; Wexler, R. R. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2097.
(387) Line, B. R.; Crane, P.; Lazewatsky, J.; Barrett, J. A.; Cloutier, D.; Kagan, M.; Lukasiewicz, R.; Holmes, R. A. *J. Nucl. Med.* **1996**, *37*, 117P (abstract 458).
(388) Line, B. R.; Seabold, J. E.; Edell, S. L.; Weiland, F. L.; Heit, J. A.; Crane, P. D.; Widner, P. L.; Haber, S. J. *J. Nucl. Med.* **1998**, *39*, 218P (abstract #969).
(389) VandeStreek, P. R.; Weiland, F. L.; Bosco, P.; Obranovich, G. T.; Carretta, R. F. *Eur. J. Nucl. Med.* **1998**, *25*, 879 (abstract OS-172).
(390) Johannsen, B.; Jankowsky, R.; Noll, B.; Spies, H.; Reich, T.; Nitsche, H.; Dinkelborg, L. M.; Hilger, C. S.; Semmler, W. *Appl. Radiat. Isot.* **1997**, *48*, 1045.
(391) Ben-Haim, S.; Kahn, D.; Weiner, G. J.; Madsen, M. T.; Waxman, A. D.; Williams, C. M.; Maguire, R. T. *Nucl. Med. Biol.* **1994**, *21*, 131.
(392) Pallela, V.; Consigny, P. M.; Shi, R.; Thakur, M. L. *J. Nucl. Med.* **1998**, *39*, 64P (abstract 244).
(393) Knight, L. C.; Baidoo, K. E.; Romano, J. E.; Brown, K.; Gabriel, J.; Maurer, A. H. *J. Labelled Compd. Radiopharm.* **1997**, *40*, 461.
(394) Knight, L. C.; Maurer, A. H.; Romano, J.; Buczala, S. *J. Nucl. Med.* **1993**, *34*, 66P (abstract 258).
(395) Vanoli, E.; Rossetti, C.; Carpinelli, A.; Savi, A.; D'Angelo, A.; Crippa, R.; Schmidt, M. A.; Srinivasan, A.; Fazi, F. *J. Nucl. Med.* **1998**, *39*, 30P (abstract 111).
(396) McAfee, J. G. *J. Nucl. Med.* **1990,** *31*, 413 (Editorial).
(397) Claessens, R. A. M. J.; Koenders, E. B.; Oyen, W. J. G.; Corstens, F. H. M. *Eur. J. Nucl. Med.* **1996,** *23*, 1536.
(398) Buscombe, J. R.; Lui, D.; Ensing, G.; de Jong, R.; Ell, P. J. *Eur. J. Nucl. Med.* **1990,** *16*, 649.
(399) Lei, K.; Rusckowski, M.; Chang, F.; Qu, T.; Mardirossion, G.; Hnatowich, D. J. *Nucl. Med. Biol.* **1996,** *23*, 1536.
(400) Barrow, S. A.; Graham, W.; Jyawook, S.; Dragotakes, S. C.; Solomon, H. F.; Babich, J. W.; Rubin, R. H.; Fischman, A. J. *J. Nucl. Med.* **1993,** *34*, 1975.
(401) Callahan, R. J.; Barrow, S. A.; Abrams, M. J.; Rubin, R. H.; Fischman, A. J. *J. Nucl. Med.* **1996,** *37*, 843.
(402) Fischman, A. J.; Solomon, H. F.; Babich, J. W.; Abrams, M. J.; Callahan, R. J.; Strauss, H. W. *Nucl. Med. Biol.* **1994,** *21*, 111.
(403) Peters, A. M.; Danpure, H. J.; Osman, S.; Hawker, R. J.; Henderson, B. L.; Hodgson, H. J.; Kelly, J. D.; Neirinckx, R. D.; Lavender, J. P. *Lancet* **1986,** *2*, 946.
(404) Vorne, M.; Soini, I.; Lantto, T.; Paakkinen, S. *J. Nucl. Med.* **1989,** *30*, 1332.
(405) Charron, M.; Orenstein, S. R.; Bhargava, S. *J. Nucl. Med.* **1994,** *35*, 451.
(406) Peters, A. M. *Nucl. Med. Commun.* **1996,** *17*, 455.
(407) Lange, J. M. A.; Boucher, C. A. B.; Hollak, C. E. M.; Wiltink, E. H. H.; Reiss, P.; van Royen, E. A.; Roos, M.; Danner, S. D.; Goudsmit, J. *N. Eng. J. Med.* **1990,** *322*, 1375.
(408) Rojas-Burke, J. *J. Nucl. Med.* **1992,** *33*, 13N.
(409) Oyen, W. J. G.; Boerman, O. C.; van der Laken, C. J.; Claessens, R. A. M. J.; van der Meer, J. W. M.; Corsten, F. H. M. *Eur. J. Nucl. Med.* **1996,** *23*, 459.
(410) Chianelli, M.; Mather, S. J.; Martin-Comin, J.; Signore, A. *Nucl. Med. Commun.* **1997**, *18*, 437.

(411) van der Laken, C. J.; Boerman, O. C.; Oyen, W. J.; van der Ven, M. T. P.; van der Meer, J. W. M. *Eur. J. Nucl. Med.* **1998,** *25*, 535.
(412) Rajopadhye, M.; Overoye, K. L.; Barrett, J. A.; Bresnick, M.; Heminway, S. J.; McKay, L.; Vining, M.; Edwards, D. S.; Liu, M.; Ziegler, M. C.; Harris, T. D.; Carroll, T. R. J. *J. Labelled Compd. Radiopharm.* **1997**, *40*, 449.
(413) Solomon, H. F.; Derian, C. K.; Beblavy, M.; Jester, D.; Santulll, R.; Pike, M.; Kroon, D.; Hoey, K.; Fischman, A. J. *J. Nucl. Med.* **1994,** *35*, 45P (abstract 172).
(414) Moyer, B. R.; Vallabhajosula, S.; Lister-James, J.; Bush, L. R.; Cyr, J. E.; Snow, D. A.; Bastidas, D.; Lipszyc, H.; Dean, R. T. *J. Nucl. Med.* **1996**, *37*, 673.
(415) Iles, D. L.; Goodbody, A. E.; Cockeram, A.; Beaudin, D.; Beyea, D.; Reid, J.; Fitzpatrick, J.; Peers, S. H.; Eshima, L.; Thornback, J. R. *J. Nucl. Med.* **1998**, *39*, 271P (abstract 1189).
(416) Mather, S. J.; Chianelli, M. *Q. J. Nucl. Med.* **1996**, *40*, 290.
(417) Reubi, J. C. *Endocrinol. Metab. Clin. North Am.* **1993,** *22*, 917.
(418) Virgolini, I.; Pangerl, T.; Bischof, C.; Leimer, M.; Kurtaran, A.; Yang, Q.; Peck-Radosavjlevic, M.; Kaserer, K.; Niederle, B.; Angelberger, P.; Gangl, A.; Valent, P. *Eur. J. Nucl. Med.* **1996,** *23*, 1101 (abstract Omo274).
(419) Maini, C. L.; Tofani, A.; Scuito, R.; Carapella, C.; Cioffi, R.; Crecco, M. *Nucl. Med. Commun.* **1993,** *14*, 550.
(420) Reubi, J. C. *J. Nucl. Med.* **1995,** *36*, 1825.
(421) Bardfeld, P. A.; Chervu, L. R.; Myrty, D. R. K. *Br. J. Radiol.* **1976,** *49*, 381.
(422) Wangberg, B.; Nilsson, O.; Theodørsson, E.; Dahlstrøm, A.; Ahlman, H. *Br. J. Cancer* **1991,** *64*, 23−28.
(423) Lamberts, S. W. J. *Endocr. Rev.* **1988,** *9*, 417.
(424) Bauer, W.; Briner, U.; Doepfner, W.; Haller, R.; Huguenin, R.; Marbach, P.; Petcher, T. J.; Pless, J. *Life Sci.* **1982,** *31*, 1133.
(425) Weckbecker, G.; Liu, R.; Tolcsvai, L.; Bruns, C. *Cancer Res.* **1992**, *52*, 4973.
(426) Lister-James, J.; Coel, M. N.; Ko, J.; Baran, Y. M.; Aten, E. M. *Eur. J. Nucl. Med.* **1997,** *25*, 885 (abstract 19.5).
(427) Lister-James, J.; Baran, Y.; Handmaker, H.; Virgolini, I.; Dean, R. T. *Q. J. Nucl. Med.* **1998**, *42 (Suppl. 1−3)*, 47.
(428) Haldemann, A. R.; Rösler, H.; Barth, A.; Waser, B.; Geiger, L.; Godoy, N.; Markwalder, R. V.; Seiler, R. W.; Sulzer, M.; Reubi, J. C. *J. Nucl. Med.* **1995,** *36*, 403.
(429) Folkman, J. *Semin. Cancer Biol.* **1992**, *3*, 65.
(430) Weinstat-Saslow, D.; Steeg, P. S. *FASEB J.* **1994**, *8*, 401.
(431) Folkman, J. *Nature Med.* **1995**, *1*, 27.
(432) Mousa, S. A. *Drugs Future* **1998**, *23*, 51.
(433) Brown, J. M.; Giaccia, A. J. *Cancer Res.* **1998**, *58*, 1408.
(434) Jones, J. L.; Walker, R. A. *Int. J. Oncol.* **1997**, *11*, 609.
(435) Horak, E. R.; Leek, R.; Klenk, N.; LeJeune, S.; Smith, K.; Stuart, N.; Greenall, M.; Stepniewska, K.; Harris, A. L. *Lancet* **1992**, *340*, 1120.
(436) Meitar, D.; Crawford, S. E.; Rademaker, A. W.; Cohn, S. L. *J. Clin. Oncol.* **1996**, *14*, 405.
(437) Takebayashi, Y.; Aklyama, S.; Yamada, K.; Akiba, S.; Aikou, T. *Cancer* **1996**, *78*, 226.
(438) Blood, C. H.; Zetter, B. R. *Biochim. Biophys. Acta* **1990**, *1032*, 410.
(439) D'Amore, P. A. *Semin. Cancer Biol.* **1992**, *3*, 49.
(440) Strömblad, S.; Cheresh, D. A. *Trends Cell Biol.* **1996**, *6*, 462.
(441) Brooks, P. C.; Montgomery, A. M. P.; Rosenfeld, M.; Reisenfeld, R.; Hu, T.; Klier, G.; Cheresh, D. A. *Cell* **1994**, *79*, 1157.
(442) Drake, C. J.; Cheresh, D. A.; Little, C. D. *J. Cell Sci.* **1995**, *108*, 2655.
(443) Varner, J. A.; Cheresh, D. A. *Curr. Opin. Cell Biol.* **1996**, *8*, 724.
(444) Sivolapenko, G. B.; Skarlos, D.; Pectasides, D.; Stathopoulou, E.; Milonakis, A.; Sirmalis, G.; Stuttle, A.; Courtenay-Luck, N. S.; Kostantinides, K.; Epenetos, A. A. *Eur. J. Nucl. Med.* **1998**, *25*, 1383.
(445) Gurrath, M.; Müller, G.; Kessler, H.; Aumailley, M.; Timpl. R. *Eur. J. Biol.* **1992**, *210*, 911.
(446) Aumailley, M.; Gurrath, M.; Müller, G.; Calvete, J.; Timpl., R.; Kessler, H. *FEBS Lett.* **1991**, *291*, 50.
(447) Pfaff, M.; Tangemann, B.; Müller, B.; Gurrath, M.; Müller, G.; Kessler, H.; Timpl R.; Engel, J. *J. Biol. Chem.* **1994**, *269*, 20233.
(448) Haubner, R.; Gratias, R.; Diefenbach, B.; Goodman, S. L.; Jonczyk, A.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 7461.
(449) Huabner, R.; Wester, H. J.; Senekowitsch-Schmidtke, R.; Diefenbach, B.; Kessler, H.; Stöcklin, G.; Schwaiger, M. *J. Labelled Compd. Radiopharm.* **1997**, *40*, 383.
(450) Timpl, R.; Dziadek, M. *Int. Rev. Exp. Pathol.* **1986**, *29*, 1.
(451) Liotta, L. A.; Rao, C. N.; Wiener, V. M. *Annu. Rev. Biochem.* **1986**, *55*, 1037.
(452) Koliakos, G. G.; Tsilibary, E. C.; Charonis, A. S. *Connect. Tissue Res.* **1991**, 26, 167.
(453) Mercurio, A. M. *Curr. Opin. Cell Biol.* **1990**, *2*, 845.
(454) Fields, G. B. *Peptide Res.* **1993**, *6*, 115.
(455) Graf, J.; Ogle, R. C.; Robey, F. A.; Sasaki, M.; Martin, G. R.; Yamada, Y.; Kleinman, H. K. *Biochemistry* **1987**, *26*, 6896.
(456) Yamamura, K.; Kibbey, M. C.; Kleinman, H. K. *Cancer Res.* **1993**, *53*, 423.
(457) Koliakos, G.; Trontzos, C.; Kouzi-Koliakos, K.; Kanellaki, M.; Grammaticos, P. *J. Nucl. Med.* **1997**, *38*, 1940.
(458) Zamora, P. O.; Eshima, D.; Graham, D.; Shuttuck, L.; Rhodes, B. A. *Biochim. Biophys. Acta* **1993**, *1182*, 197.

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