

REVIEWS

Synthesis of Target-Specific Radiolabeled Peptides for Diagnostic Imaging

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INTRODUCTION

Radiopharmaceuticals are drugs containing atoms of some radioactive elements. They are designed for diagnostic or therapeutic purposes, to deliver small doses of ionizing radiation to the disease sites in the body. Radiopharmaceuticals, unlike classical chemotherapeutics, act against malignant cells with high specificity. Radiopharmaceuticals are mostly small organic molecules, such as peptides or peptidomimetics, but they can also be macromolecules, for example antibodies.

Biodistribution of radiopharmaceuticals can be determined either by their chemical and physical properties or by their biological interactions. Radiopharmaceuticals, which act through receptor binding, are called target-specific. Ideally, these radiopharmaceuticals are designed to locate with high specificity at cancerous tumors, even if their location in the body is unknown, while producing minimal radiation damage to normal tissues (1–6). In the past decade significant progress has been made in the development of peptide-based target-specific radiopharmaceuticals, which have become an important class of imaging agents for the detection of various diseases, such as tumors, thrombosis, and inflammation.

Many excellent reviews have been published discussing different aspects of radionuclide chemistry and therapy (7–9) and the use of radiopharmaceuticals for diagnosis and treatment of different pathological conditions (10–19). Most popular technetium radiopharmaceuticals (20–28), as well as those incorporating other radionuclides (29, 30), have been reviewed. Antibodies (24, 25), peptides (23, 30–35), and steroids (36) as targeting molecules have been described.

In this review, which is limited to the use of small peptides as targeting molecules, we attempt to summarize, from the chemical point of view, the development of labeling methods, in particular the application of different bifunctional chelating agents. We also give a short description of radionuclides used with these agents. Radiopharmaceuticals based on small peptides, which are in clinical use or under investigation in preclinical and clinical trials, are also mentioned.

LABELING METHODS

Direct Labeling. Direct labeling methods (Table 1) are mostly based on the binding of a radionuclide to thiol groups in the targeting molecule, that seems relatively

easy to perform (37, 38). However, such a labeling process is difficult to control, for its detailed chemistry is unknown and may lead to unplanned changes in the structure, stability, and pharmacokinetic properties of the labeled molecule. Furthermore, very little is known about the number of donor atoms in the labeled molecule and the geometry of radionuclide coordination. The stability in vivo of a synthesized complex also remains uncertain.

The direct labeling approach is rather unsuitable for small peptides, which either do not possess disulfide linkage or are unable to maintain their activity after reduction. For example Thakur (39) has reported the alteration of the receptor binding properties of the radiolabeled somatostatin analogues, when the disulfide bridge was reduced to free thiol groups and subsequently radiolabeled with ^{99m}Tc . However, direct labeling has been successfully applied for labeling of the platelet receptor-binding peptide with ^{99m}Tc (40, 41) and for high molecular weight proteins such as antibodies and their fragments (42, 43).

Chelate Methods. In chelate methods (Table 1) a radionuclide is bound to the targeting molecule indirectly, through a bifunctional chelating agent (BFCA) (Figure 1).

In general, a radiopharmaceutical containing a BFCA consists of the following parts: a targeting molecule, BFCA, radionuclide, and a linker (22). The targeting molecule is a carrier of a radionuclide to the receptor site in vivo. A radionuclide serves as a radiation source. A BFCA, covalently attached to the targeting molecule, functions as the coordinator of the radionuclide. A linker, not always necessary, is a spacer residue, which separates a targeting molecule from a chelating agent.

Functional groups, naturally present in the peptide or introduced synthetically, are responsible for covalent attachment of BFCA. Naturally occurring functional groups include terminal, as well as side-chain amino and carboxy groups, thiol groups from cysteine, and *p*-hydroxyphenyl from tyrosine. BFCA must contain a conjugation group, which is used for attachment of the peptide.

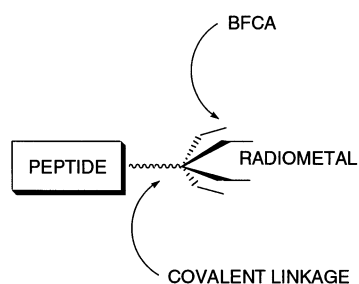
Several types of conjugation groups, active esters, isothiocyanates, maleimides, hydrazides, and α -haloamides are used to form BFCA–peptide linkages (Figure 2).

Active esters can be used to form an amide bond between a carboxy group of a BFCA and an amino group in a peptide ligand. Since they are at a low level of activation, side-reactions during coupling are generally less of a problem than with most amide bond forming procedures. Commonly used are *p*-nitrophenyl, pen-

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Table 1. General Overview of Applied Labeling Methods

labeling method	main principle	targeting molecule	advantages	disadvantages	ref
direct labeling	radionuclide binds directly to active groups present in the targeting molecule	high molecular weight molecules	easy to perform	unknown chemistry	(32, 35, 37, 38)
chelate methods				unknown geometry of a radionuclide-targeting molecule complex possible damage to targeting molecule during labeling process	
prelabeling	labeling of BFCA followed by conjugation with the targeting molecule	small peptides	relatively easy to control, well-defined chemistry	time-consuming	(26, 32)
postlabeling	conjugation of BFCA to targeting molecule, followed by labeling of conjugate	small peptides	most popular method	complicated purification of obtained radiopharmaceutical possible damage to targeting molecule during labeling process	(35, 44)
			targeting molecule functional groups remain unlabeled		
			well-defined chemistry possible use of classical solid-phase or solution methods of the peptide synthesis		

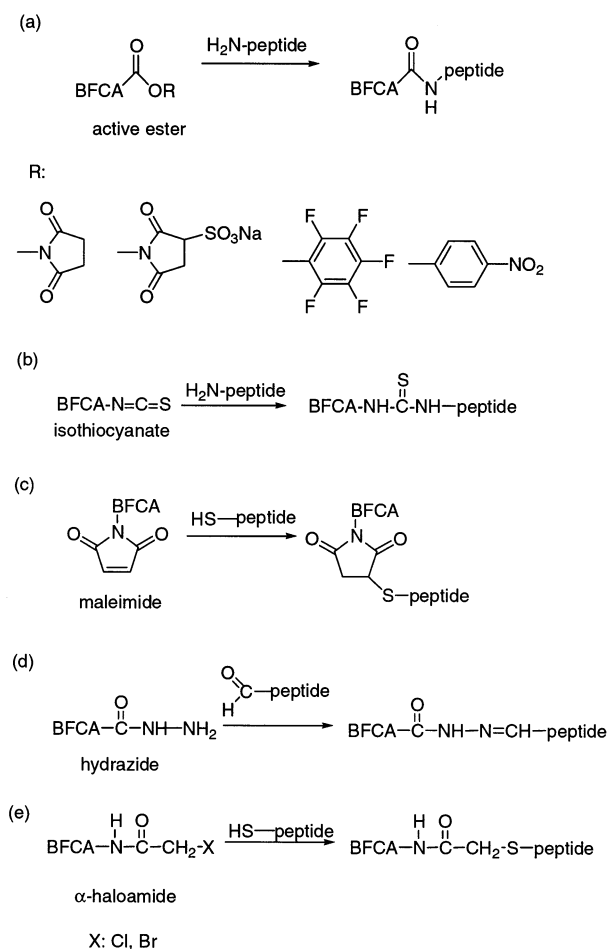
**Figure 1.** Schematic structure of a radiopharmaceutical. BFCA can complex a metal and also contains a functional group which forms a covalent linkage to a biological molecule, such as peptide.

tafluorophenyl, *N*-hydroxysuccinimide, and sulfo-*N*-hydroxysuccinimide active esters. The *N*-hydroxysuccinimide esters are very reactive with high selectivity toward aliphatic amines (22, 45). The choice of an active ester to be used for BFCA–peptide bond formation is partly dictated by sheer reactivity, but the ease of coproduct removal is also an important consideration. Thus, for water-insoluble BFCA–peptide conjugates, a succinimide ester coupling is especially convenient because both *N*-hydroxysuccinimide and sulfo-*N*-hydroxysuccinimide are very water-soluble (46, 47) and easy to remove. But for water-soluble BFCA–peptide conjugates *p*-nitrophenyl or pentafluorophenyl esters, which are ether-soluble, may be a better choice (48).

Isothiocyanates, like active esters, react with amino groups of a peptide forming thiourea bonds. Since they react best in the higher pH, they cannot be used with peptides susceptible to alkaline conditions.

The third class are maleimides, which react with thiols and form thioether bonds. The optimum pH for the reaction is near 7. At higher pH maleimides may hydrolyze to form nonreactive maleic acids (49, 50).

Hydrazides are conjugation groups which react with the aldehyde group of a peptide to form a hydrazone–

**Figure 2.** Reaction schemes for BFCA–peptide conjugation.

peptide conjugate. They are suitable for peptides containing 2-amino alcohol structure like in serine, threonine, and hydroxylysine, which can be very rapidly oxidized

by periodate at pH = 7 to generate an aldehyde. The use of a low molar ratio of periodate to peptide minimizes the potential for side-reactions during the oxidation. The formed hydrazones are stable at pH 6–8 for at least 12 h at 22 °C (51).

α -Haloamides are suitable for conjugation with peptides containing a free SH group. Chloroacetyl- or bromoacetyl amides are used (52, 53).

The bifunctional approach is often associated with the term 'pharmacokinetic modifier' (PKM). Liu and Edwards (22) define PKM as a linker between a targeting molecule and a BFCA. PKM is a spacer residue, separating the radionuclide from the binding site of the molecule to minimize the risk of its undesired modification. The use of a linker helps to choose a BFCA and a radionuclide more independently. The most popular linkers are long poly(ethylene glycol) (PEG) or hydrocarbon chains to increase the lipophilicity and polyamino acid sequences, such as polyglycine, to increase the hydrophilicity, as well as esters and disulfides capable of rapid metabolism.

The distinction between chelate methods can be made depending on the sequence of the steps used for the synthesis of a radiolabeled peptide.

Prelabeling Method. The pre-labeling method (pre-formed chelate approach) is based on the labeling of a BFCA with its subsequent activation and conjugation, through covalent bonds, to a peptide. In this approach the chemistry of a process is well defined and easily controlled. As the labeling and conjugation steps are separated, it can be ensured that the radionuclide is attached directly to a chelate moiety and the peptide amino groups remain unlabeled (22). However, the pre-labeling of a BFCA may impair the conjugation process and complicate purification of a radiopharmaceutical. The method is time-consuming, so not appropriate for use with short-lived isotopes, generally associated with imaging. As such the prelabeling method is rarely used for synthesis of radiolabeled peptides.

Postlabeling Method. The postlabeling method (indirect labeling approach), the most popular approach in the synthesis of radiopharmaceuticals, requires the synthesis of a BFCA–peptide conjugate and is followed by its labeling. In this method BFCA may be attached to N- or C-terminus, as well as to a side chain of a peptide or it can be even incorporated into a peptide backbone. The postlabeling method is characterized by a well-defined chemistry and relative simplicity. For convenient, high yield synthesis of radiopharmaceuticals, applied BFCA should be compatible with the solid-phase or solution methods of peptide synthesis. However, harsh conditions required for effective labeling of conjugates may sometimes cause changes in the amino acid sequence or peptide backbone conformation and even begin the decomposition of the whole radiopharmaceutical (35).

BIFUNCTIONAL CHELATING AGENTS

BFCAs are used to connect a radionuclide and a targeting molecule to form a radiopharmaceutical. An ideal BFCA should coordinate the radionuclide with a high yield, to form a relatively stable complex. The agent must comply with the nature and oxidation state of a radionuclide and should prevent any accidental changes in its redox potential.

It is important to carefully choose a proper BFCA, as the conjugation with targeting molecule requires specific conditions: pH, temperature, reaction time. The stereochemistry of a BFCA is important when synthesizing radiopharmaceuticals targeting specific receptors.

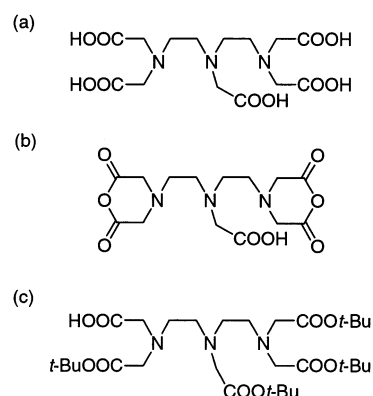


Figure 3. Structure of DTPA and its analogues: (a) DTPA; (b) cDTPA; (c) mDTPA.

DTPA. DTPA (N^{α} -diethylenetriaminopentaacetic acid) belongs to the group of polyaminocarboxy chelates (Figure 3a). It is a strong chelating group, mostly linked with ^{111}In , a trivalent radionuclide. It can be attached to larger proteins, e.g., albumins and antibodies (49, 50, 54), as well as to small peptides, like somatostatin analogues (55, 56). The conjugation of DTPA with macromolecules has been successfully performed by the use of isobutyl chloroformate as a coupling reagent (57). For small peptides, however, DTPA derivatives such as DTPA bicyclic anhydride (cDTPA) and monoreactive DTPA derivative, 3,6-bis(carboxymethyl)-9-(((2-maleimidoethyl)-carbamoyl)methyl)-3,6,9-triazaundecanedioic acid (mDTPA), have been applied (Figure 3b and 3c).

Hnatowich et al. (54) have developed a simple method of covalent coupling of cDTPA to peptides at their amino groups. The efficiency of this method is relatively high and it has several advantages. The coupling reaction runs in an aqueous solution and is a simple, one-step process. The side product of the reaction, a double substituted DTPA derivative, and unreacted material can be both easily separated from the main product by gel chromatography. The conjugated peptide maintains its affinity toward specific receptors. The sample, purified before the addition of a radionuclide, can be stored and labeled only when required. For peptides containing lysine the conjugation occurs especially at its ϵ -amino group, as more basic than N-terminal amino group. The method is therefore inappropriate for somatostatin analogues, as the lysine residue is situated within the active site of the molecule and the conjugation may result in the loss of receptor binding activity. For that reason a modified method, proposed by Bakker et al. (55) for DTPA-octreotide, is used to conjugate the somatostatin analogue with cDTPA. In this approach lysine residue within the active site of the peptide is protected with *tert*-butoxycarbonyl group (Boc), before the reaction with cDTPA and deprotected after the conjugation (Figure 4). This method enables a selective reaction of the N-terminal amino group with BFCA, whereas the lysine residue within the bioactive site remains unsubstituted.

A monoreactive DTPA derivative, mDTPA, with four carboxy groups protected as *tert*-butyl esters, was introduced by Arano et al. (57) (Figure 5). Since mDTPA possesses only one free carboxy group, the formation of undesired intermolecular linkages with peptides is prevented. High solubility of mDTPA in various solvents makes this BFCA appropriate for both liquid- and solid-phase peptide synthesis.

A great obstacle in the efficient radiolabeling of DTPA conjugates is the presence of trace metals in the prepara-

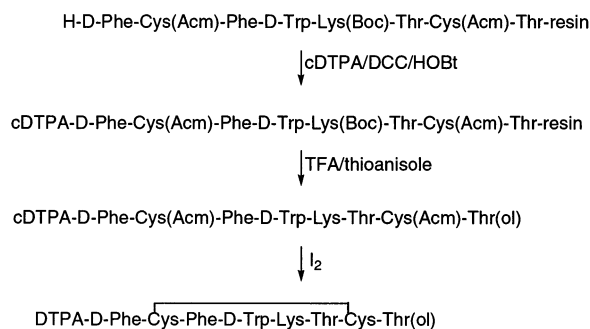


Figure 4. Synthesis of DTPA–octreotide, starting with cDTPA.

tion, as they compete with radionuclides in the process of labeling. For that reason a significant, 40- to 70-fold molar excess of peptide conjugate and ultrapure radionuclide derivative of the highest possible specific activity are required (55).

DTPA conjugates have been shown to form ^{111}In -chelate structures (Figure 6), which are eight coordinate, using all three amino and four carboxy groups, while the eighth position around the radionuclide is occupied by the amide carbonyl oxygen (58). ^{111}In –DTPA conjugates possess excellent *in vivo* stability (59–61).

Many research groups put much effort in the synthesis of kinetically stable DTPA–peptide conjugates that form complexes with ^{90}Y (62). Substitutions, particularly in the carbon atoms of the DTPA backbone, sterically hinder the opening of the chelate ring that must occur during radionuclide complex dissociation and increase the *in vivo* stability of the radiopharmaceutical. The first class of modified DTPA conjugates was constructed by attaching *p*-isothiocyanatobenzyl moiety to one DTPA backbone ethylene group and appending methyl to another ethylene group in the same backbone (Figure 7a,b). The second class of modified DTPA conjugates was developed by replacing one of the ethylene groups by a cyclohexyl moiety (Figure 7c). Such modifications increase the rigidity in the DTPA backbone and the *in vivo* stability of obtained radiopharmaceuticals (62–64). Synthesis of new derivatives constructed on DTPA core has been recently reported (65, 66).

$^{99\text{m}}\text{Tc}$ is less suitable for the labeling of DTPA–peptide conjugates, as this radionuclide, even at high concentrations, has low affinity and poor selectivity to the binding sites of this BFCA (67).

DOTA. DOTA (1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid) (Figure 8a) and its derivatives proved to be a good alternative for DTPA. They play an important role in clinical applications, as they form

very stable complexes with a variety of trivalent radionuclides, such as $^{66,67,68}\text{Ga}$, $^{86,90}\text{Y}$, ^{111}In , ^{149}Pm , ^{177}Lu (68–73) and divalent radionuclides, ^{27}Mg , ^{47}Ca , ^{64}Cu (74).

Two different approaches for DOTA conjugation with peptides have been developed. In the first approach one of the four carboxy groups in DOTA is activated to facilitate the reaction with primary amines in the peptide and form a stable amide bond linkage. In the second approach DOTA derivatives with additional side chains are used. A peptide ligand is attached to the side chain of DOTA derivative. Several DOTA derivatives have been synthesized so far, like PA–DOTA (α -[2-(4-aminophenyl)-ethyl]-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) and *p*-NCS-Bz–DOTA ((2-(4-isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid) (Figure 8b and 8c) (69). Recently Eisenwiener et al. (44) have introduced two new DOTA derivatives, DOTASA(*t*-Bu)₄, (1-(1-carboxy-2-carbo-*tert*-butoxyethyl)-4,7,10-(carbo-*tert*-butoxymethyl)-1,4,7,10-tetraazacyclododecane) and DOTAGA(*t*-Bu)₄, (1-(1-carboxy-3-carbo-*tert*-butoxypropyl)-4,7,10-(carbo-*tert*-butoxymethyl)-1,4,7,10-tetraazacyclododecane) (Figure 8d), which convenient synthesis is outlined in Figure 9. The conjugation of all DOTA derivatives to a peptide is performed through an amino group of a peptide.

Fully eight coordinate structure has been reported for all DOTA complexes (60) using four amino and four carboxy groups. In the case when one carboxy group is used for conjugation, the amide carbonyl oxygen occupies the eighth position around the radionuclide.

DOTA and derivatives were successfully conjugated to a number of somatostatin analogues, and obtained radiopharmaceuticals had good pharmacological parameters (72–78). DOTA conjugates are especially suitable for radionuclide therapy, as they can be radiolabeled with ^{67}Ga (75), ^{90}Y (71, 76), and ^{111}In (73). De Jong et al. (68) have demonstrated that ^{90}Y –DOTA conjugates have very good pharmacokinetic properties *in vivo*. However, in these conjugates the chelate is situated closer to the peptide, the labeled conjugate is more rigid and less flexible, which makes binding with the receptor more difficult. Reubi et al. (79) reported the best pharmacological properties for ^{67}Ga –DOTA complexes. The radionuclide coordination geometry, including the number of uncomplexed carboxy and amino groups, increases the flexibility of a ligand and allows its better adjustment to the receptor binding site.

TETA. TETA (1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid) (Figure 10) is one of the most studied chelating agents for copper in peptide targeted radio-

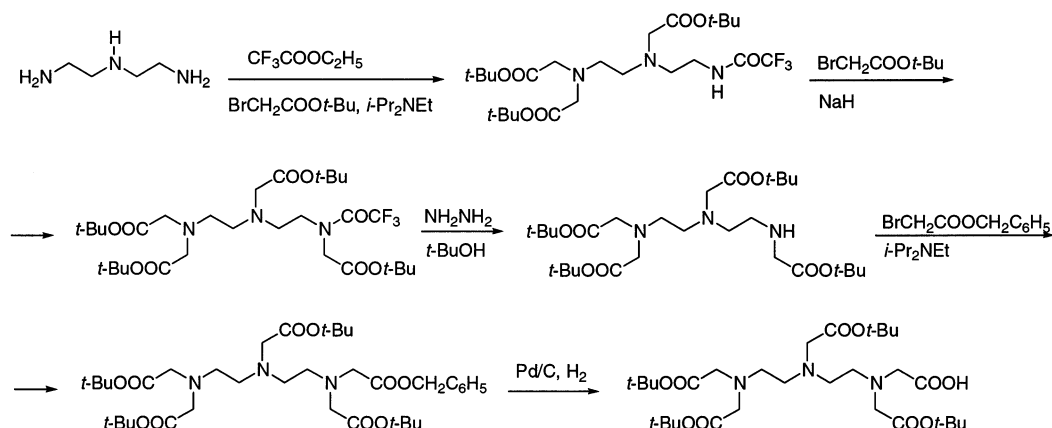


Figure 5. Reaction scheme for synthesis of mDTPA.

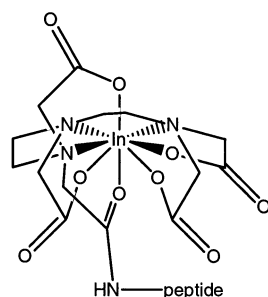


Figure 6. Possible structure of In^{3+} -DTPA-peptide.

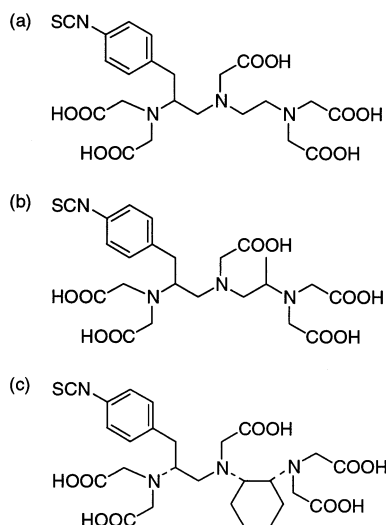


Figure 7. Structures of DTPA derivatives: (a) 2-(*p*-isothiocyanatobenzyl) diethylenetriaminopentaacetic acid; (b) 2-(*p*-isothiocyanatobenzyl)-6-methyldiethylene-triaminopentaacetic acid (1B4H-DTPA); (c) 2-(*p*-isothiocyanatobenzyl) cyclohexyldiethylene-triaminopentaacetic acid (CHX-DTPA).

therapy. TETA has been successfully used as a BFCA with somatostatin analogues (30).

NOTA. NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid) (Figure 11a), its phosphonate analogue NOTP (1,4,7-triazacyclononane-*N,N',N''*-tris(methylenephosphonic) acid) (Figure 11b) and the monoethyl ester of NOTP, NOTPME (1,4,7-triazacyclononane-*N,N',N''*-tris(methylenephosphonate-monoethyl ester)) (Figure 11c) were studied for possible use in radiopharmaceuticals. Complexes with ^{67}Ga (80) and ^{111}In (81) were reported.

A monoreactive NOTA derivative, NODAGA(*t*Bu)₃ (1-(1-carboxy-3-carbo-*tert*-butoxypropyl)-4,7-(carbo-*tert*-butoxymethyl)-1,4,7-triazacyclononane) (Figure 11d) was synthesized by Eisenwiener et al. (82). The synthesis is outlined in Figure 12. This BFCA is useful for the coupling to the N-terminus of peptides on solid-phase and in solution. The NODAGA-peptide conjugates were labeled with ^{67}Ga and ^{111}In in high yields and good specific activities. NODAGA-based derivatives carry a spacer function between the BFCA and the peptide which improves the receptor binding affinity.

HYNIC. HYNIC (2-hydrazinonicotinic acid) (Figure 13), first described by Abrams et al. (83) has been used as a BFCA for radiolabeling of different groups of molecules, such as γ -globulins (83, 84), chemotactic peptides (85, 86), and somatostatin analogues (87–90). Structural organization of HYNIC determines its application, as it can only occupy one or two coordination sites of the radionuclide. That is why a coligand such as tricine or EDDA (ethylenediaminediacetic acid) should be also coordinated to complete the coordination sphere

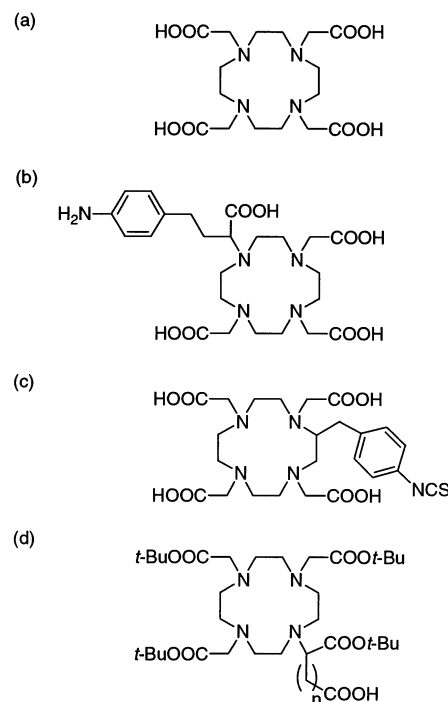


Figure 8. Structures of DOTA and its derivatives: (a) DOTA; (b) PA-DOTA; (c) *p*-NCS-Bz-DOTA; (d) DOTASA(*t*-Bu)₄, *n* = 1 and DOTAGA(*t*-Bu)₄, *n* = 2.

of a radionuclide (91, 92) (Figure 14). The conjugation of coligands helps in modifying the properties of obtained radiopharmaceutical, such as hydrophilicity or pharmacokinetics. However, the requirement for the use of coligands makes the chemistry of the synthesis more complicated, and multiple possible products and side-products can be obtained.

HYNIC-coligand conjugates were reported to have low stability (22). The search for stable HYNIC-coligand complexes is now carried on and phosphines seem to be the most promising coligands so far (92, 93). HYNIC derivatives, together with phosphines and tricine, form ternary complexes [$^{99\text{m}}\text{Tc}(\text{HYNIC-TM})(\text{tricine})(\text{phosphine})$] (TM-targeting molecule). Such complexes are stable in solution, and their hydrophilicity can be modified by changing functional groups attached to phosphine backbone or by substitution of tricine by other glycine derivatives.

HYNIC is often used as a BFCA for somatostatin analogues. The desired amide bond formation should occur between the carboxy group of HYNIC and the N-terminal amino group of a peptide. However, in somatostatin analogues the presence of lysine makes it difficult to obtain a monosubstituted product. Krois et al. (87) have compared available methods of HYNIC-octreotide conjugation, and none of them seemed efficient enough. The method of protecting the lysine amino group with (Boc)₂O reported by Bakker et al. (55) proved to be unsatisfactory, as the final product was contaminated with Boc-disubstituted derivative, difficult to separate. The activation of HYNIC to *N*-hydroxysuccinimide ester to facilitate the conjugation step, a method suggested by Abrams et al. (83), also produced poor results. An improved method of HYNIC-octreotide conjugation, reported by Krois et al. (87), is based on the incorporation of the chelator at an early stage of *ab ovo* peptide synthesis performed by solution method. That approach produced high yield of conjugate, practically free of any contamination.

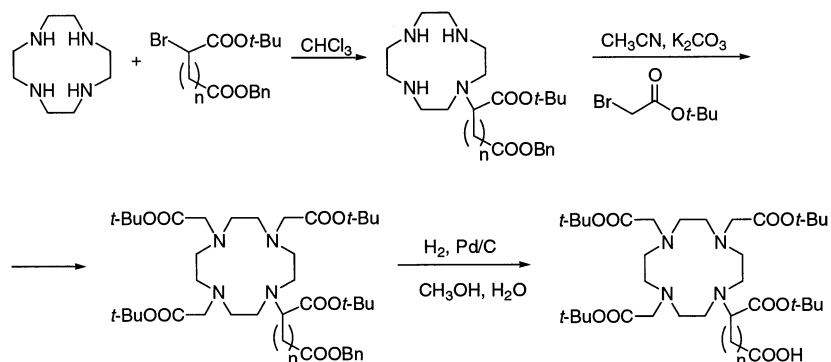


Figure 9. Synthesis of DOTASA(*t*-Bu)₄ and DOTAGA(*t*-Bu)₄.

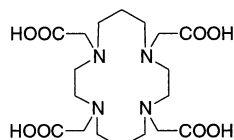


Figure 10. Structure of TETA.

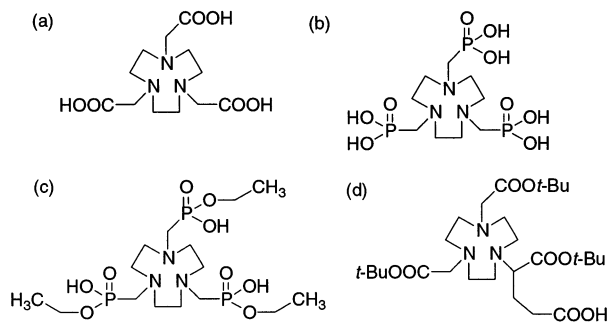


Figure 11. Structures of NOTA and its derivatives: (a) NOTA, (b) NOTP, (c) NOTPME, (d) NODAGA(*t*-Bu)₃.

Multidentate Chelators. The class of tetradentate chelators includes N₃S triamidothiol, N₂S₂ diamidodithiols (94), and N₂S₄ diaminotetrathiols (26) (Figure 15) and a number of their derivatives, containing sulfur and nitrogen atoms, incorporated into chelating backbones.

N₂S₄ chelators contain two amino and four thiol donors and they have been used for ^{99m}Tc- and ¹⁸⁶Re-labeling of antibodies (95, 96). N₂S₂ containing two amido and two thiol donors have been used for ^{99m}Tc- and ¹⁸⁶Re-labeling of proteins, peptides, and oligonucleotides. Since most of the N₂S₂ derivatives form highly lipophilic rhenium and technetium complexes, they are used whenever hydrophobic targeting systems are applied (97). Both N₃S- and

N₂S₂-derived BFCAs form high specific activity complexes, and for that reason they can be used in the preformed chelate labeling method. In case of small molecules (steroids, low molecular weight peptides) these BFCAs complex ^{186/188}Re in the postconjugation labeling process (28). BFCAs based on N₃S backbone are used for the synthesis of ^{186/188}Re radiopharmaceuticals (98).

The most frequently applied BFCAs from that group are MAG₃ (mercaptoacetyl-glycylglycylglycine) and MAG₂-GABA (mercaptoacetyl-glycylglycyl-γ-butyric acid), containing the spacer groups glycine and GABA, respectively (Figure 16). GABA spacer group is two carbon units longer than glycine; therefore, it may exhibit more useful properties, such as higher yield in radionuclide labeling or better receptor binding due to the changes in intramolecular organization (99–101).

BFCAs listed above have been widely used as metal chelating agents in both diagnostic and therapeutic procedures. Attempts to design new derivatives with improved properties are still under way (66, 102).

RADIONUCLIDES

Each radionuclide is characterized by a specific coordination chemistry and therefore must be conjugated with a specific BFCA with specific donor atoms and ligand frameworks to retain its own radioactivity.

The selection of an appropriate radionuclide is an inherent determinant in developing any therapeutic radiopharmaceutical. Important factors to consider include half-life of the radioactive nuclide, its mode of decay, and its cost and availability (Table 2). The half-life is a critical factor. For diagnostic imaging the half-life of a radionuclide must be long enough to enable the synthesis of the labeled compound and to facilitate the accumulation in the target tissue, while allowing clear-

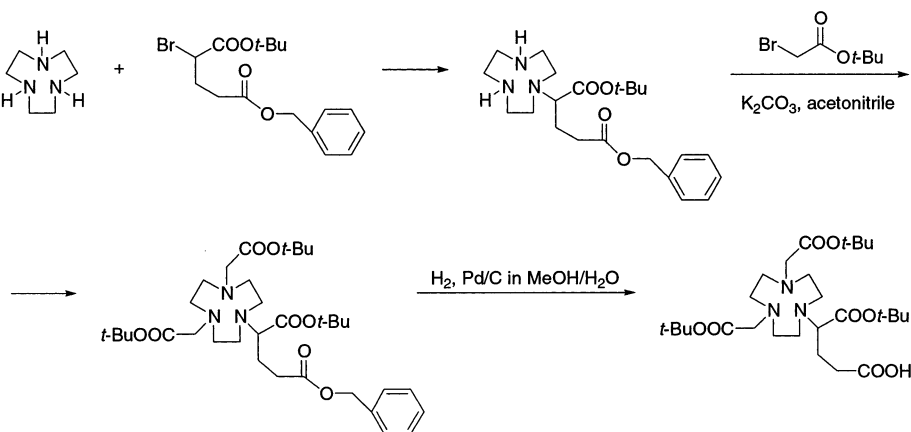
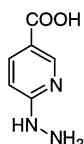
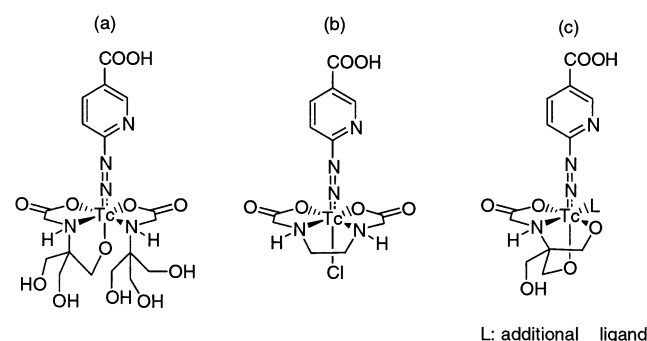
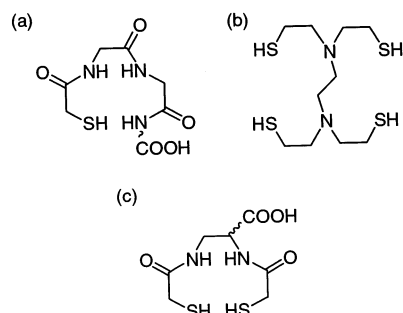
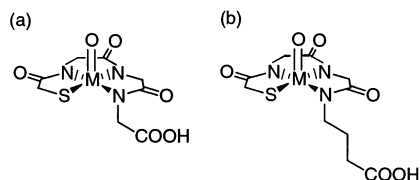


Figure 12. Synthesis of NODAGA(*t*-Bu)₃.

Table 2. γ -, β^- -, and β^+ -Emitting Radionuclides^a Used for Radiolabeling of Radiopharmaceuticals (103)

radionuclide	$t_{1/2}$ (h)	γ -energy (keV)	β^- -energy (keV)	β^+ -energy (keV)	decay mode
⁶⁴ Cu	12.7	-	579	653	β^+ (17.4%) β^- (39%) EC
⁶⁶ Ga	9.5	-	-	4150, 935	β^+ (56%) EC (44%)
⁶⁷ Ga	78.3	91, 93, 185, 296, 388	-	-	EC (100%)
⁶⁸ Ga	1.1	-	-	1880, 770	β^+ (90%) EC (10%)
⁸⁶ Y	14.7	-	-	2335, 2019, 1603, 1248, 1043	β^+ (33%) EC (66%)
⁹⁰ Y	64.1	-	2288	-	β^- (72%)
^{99m} Tc	6.0	141	-	-	IT (100%)
¹¹¹ In	67.9	245, 172	-	-	EC (100%)
¹⁴⁹ Pm	53.0	286	1070	-	β^- (95.9%)
¹⁷⁷ Lu	160.8	-	236	-	β^- (100%)

**Figure 13.** Structure of HYNIC.**Figure 14.** Structures of HYNIC–technetium complexes: (a) [Tc(HYNIC)(tricine)₂]; (b) [Tc(HYNIC)(EDDA)Cl]; (c) [Tc(HYNIC)-(tricine)(L)].**Figure 15.** Structures of multidentate chelators: (a) N₃S triamidethiol; (b) N₂S₄ diaminotetrathiol; (c) N₂S₂ diamidodithiol.**Figure 16.** Structures of: (a) MAG₃; (b) MAG₂-GABA.

ance through the nontarget organs. Ideally, the half-life should be as short as possible to reach these two goals. Radionuclides most commonly used in radiopharmaceuticals range in half-lives from minutes (⁶²Cu) to days (¹⁷⁷Lu).

Copper (⁶⁴Cu). There are two radionuclides of copper used in the radiopharmaceutical labeling. ⁶⁴Cu ($t_{0.5}$ = 12.7 h) is a β^+ -, 0.653 MeV (17.4% abundance), and β^- -emitter, 0.579 MeV (39% abundance), and it decays by electron capture. A good review on the biological chemistry of copper is a book by Linder (104).

Gallium (⁶⁶Ga, ⁶⁷Ga, ⁶⁸Ga). Gallium radionuclides can be either used for γ -scintigraphy or positron emission tomography (PET) imaging. ⁶⁶Ga ($t_{0.5}$ = 9.5 h) is a medium half-life β^+ -emitting radionuclide obtained from ⁶⁶Zn(p,n)⁶⁶Ga cyclotron, applied in the limited number of cases. ⁶⁷Ga ($t_{0.5}$ = 78.3 h) is produced in ⁶⁸Zn(p,2n)⁶⁷Ga cyclotron. ⁶⁸Ga ($t_{0.5}$ = 1.1 h) is obtained from the ⁶⁸Ge/⁶⁸Ga generator and it has been used in a limited number of clinical studies (105, 106).

Yttrium (⁸⁶Y, ⁹⁰Y). There are two radionuclides of yttrium used in the radiopharmaceutical labeling. ⁸⁶Y ($t_{0.5}$ = 14.7 h) is a β^+ -emitting radionuclide, often used as an equivalent for ⁹⁰Y in the PET imaging. ⁹⁰Y ($t_{0.5}$ = 64 h) is a β^- -emitter, which is probably the most frequently used radionuclide for targeted radiotherapy in human studies. It has a relatively high energy transfer and therefore can be used for imaging of bulky disease sites of solid tumors. ⁹⁰Y can be relatively easily obtained in a high-specific activity from ⁹⁰Sr (107–109). ⁹⁰Y is also available as a GMP commercial product.

Technetium (^{99m}Tc). ^{99m}Tc is used in about 85% of all diagnostic applications. It has ideal properties for diagnostic imaging. The half-life of 6 h is long enough to synthesize the ^{99m}Tc-labeled radiopharmaceuticals and perform imaging studies, yet short enough to minimize the radiation dose to a patient. ^{99m}Tc emits a 140 keV γ -ray with 89% abundance which is close to optimum for imaging with the present γ cameras. This energy is sufficient for emerging from inside the body and for imaging internal organs. ^{99m}Tc is readily available at low costs from its parent nuclide ⁹⁹Mo ($t_{0.5}$ = 66 h) from a ⁹⁹Mo/^{99m}Tc generator. At the concentration levels used for imaging ($<10^{-6}$ M) neither its γ -radiation nor the soft β -decay is hazardous (110).

Indium (¹¹¹In). ¹¹¹In has the half-life of 67 h which makes this isotope ideal for labeling immunoglobulins, where imaging is performed over intervals of several days. This nuclide decays by electron capture with emission of γ -photons of 173 and 247 keV (89% and 95% abundance, respectively), which allows its use in γ -scintigraphy.

The first peptide radiopharmaceutical approved for clinical use was the ¹¹¹In labeled somatostatin analogue OctreoScan. Nevertheless, because of its less favorable half-life, ¹¹¹In is inferior to ^{99m}Tc for diagnosis. ¹¹¹In is often used as an equivalent for ⁹⁰Y in scintigraphic

Table 3. Regulatory Peptides

regulatory peptide	no. of amino acid residues	receptor type (subtypes)	in vivo activity
SST (somatostatin)	14	SST receptors (sst1/sst2/sst3/sst4/sst5)	inhibition of hormone and exocrine secretion
BN/GRP (bombesin/gastrin releasing peptide)	14	BN/GRP receptors (GRP, NMB, BRS-3)	gut hormone release, regulation of exocrine secretion
VIP (vasoactive intestinal peptide)	28	VIP receptors (–)	vasodilation, water, and electrolyte secretion in the gut
RGD-containing peptides/RGD-peptidomimetics	–	GPIIb/IIIa/platelet and vitronectin/integrin receptors	inhibition of adhesive and aggregatory functions of platelets
α -MSH (α -melanocyte stimulating hormone)	13	α -MSH receptors (–)	melanogenesis
NT (neurotensin)	13	NT receptors (NT1, NT2, NT3)	vasoconstriction, regulation of cardiac activity raise in vascular permeability
SP (substance P)	11	SP receptors (NK1)	hypotension, salivary gland secretion, transmission of pain

Table 4. Radiopharmaceuticals Based on Small Peptides^a

group	peptide	BFCA	targeted disease	ref
SST analogues	octreotide	DTPA	tumor (neuroendocrine)	(3, 113)
		TETA		(152, 153)
		HYNIC		(61, 118)
	Tyr ³ -octreotide (Y3-TETA)	TETA	tumor (neuroendocrine)	(152, 153)
BN/GRP analogues	BN	HYNIC	tumor (neuroendocrine)	(114, 115)
		S–S (direct)		(116)
		HYNIC		(117)
	vapreotide (RC-160)	N ₂ S ₂	tumor	(94)
VIP analogues	VIP	HYNIC	tumor	(119)
		MAG ₃	tumor	(120–122)
		HYNIC	thrombosis	(40, 41, 123, 124)
	CCMSH	S–S (direct)	tumor (breast, prostate)	(125, 126)
RGD-containing peptides/ RGD-peptidomimetics	SP	DTPA	tumor	(127, 128)
	fMLF	HYNIC	infection/inflammation	(129, 130)

^a ST, somatostatin; BN, bombesin; GRP, gastrin releasing peptide; VIP, vasoactive intestinal peptide; RGD sequence, Arg-Gly-Asp sequence; α -MSH, α -melanocyte stimulating hormone; SP, substance P.

imaging in humans by using dosimetry cameras, since ⁹⁰Y radionuclide does not emit γ -rays (32).

Promethium (¹⁴⁹Pm). ¹⁴⁹Pm is a moderate energy β^- -emitter (1.07 MeV, 95.9% abundance) with a half-life of 53 h. ¹⁴⁹Pm also emits a low abundance of an imageable γ -ray (286 keV, 3%) that may allow in vivo tracking of the therapeutic dose (111).

Lutetium (¹⁷⁷Lu). The most frequently used radionuclide of lutetium is ¹⁷⁷Lu ($t_{0.5}$ = 160.8 h) which is a β^- - and also a γ -emitter (103).

RADIOPHARMACEUTICALS BASED ON SMALL PEPTIDES

Small peptides play a very important role in vivo, where they regulate many physiological processes (Table 3). Low molecular weight peptides have attracted much attention recently as radiopharmaceuticals due to their relatively low immunogenicity, good pharmacokinetic properties, and binding affinities. They are relatively easier to synthesize and modify than larger molecules (112). Many radiopharmaceuticals based on small peptides have been used as diagnostics for clinical purposes (Table 4). They have been radiolabeled with various radionuclides (Table 5).

Because of the lack of tertiary structure, small peptides are less susceptible to a loss of integrity through labeling conditions. However, when minor modifications occur in their structure, they can result in important changes in the lipophilicity and charge with consequences for bio-distribution and kinetics.

The physicochemical properties of the radiopharmaceuticals based on small peptides, including binding affinity in vivo, are determined not only by the nature of

Table 5. Radionuclides Used with Different Peptide Ligands^a

radionuclide	labeled peptide	ref
⁶⁴ Cu	SST analogues	(152, 153)
	chemotactic peptides	(18, 30)
^{66/67/68} Ga	SST analogues	(131)
	chemotactic peptides	(131)
^{86/90} Y	SST analogues	(18, 30, 132–136)
	SST analogues	(114, 115, 117, 118, 137)
^{99m} Tc	BN/GRP analogues	(119)
	VIP analogues	(120–122)
	RGD-containing peptides/ RGD-peptidomimetics	(40, 41, 123, 124)
	α -MSH analogues	(125, 126)
	chemotactic peptides	(129, 130, 138)
	SST analogues	(113)
¹¹¹ In	α -MSH analogues	(139, 140)
	NT analogues	(141, 142)
	SP analogues	(127, 128, 143)
	chemotactic peptides	(144)

^a ST, somatostatin; BN, bombesin; GRP, gastrin releasing peptide; VIP, vasoactive intestinal peptide; RGD sequence, Arg-Gly-Asp sequence; α -MSH, α -melanocyte stimulating hormone; NT, neurotensin; SP, substance P.

the targeting molecule. They are also influenced by the radionuclide–BFCA complex properties, as a BFCA contributes greatly to the overall size and molecular weight of a conjugate. Furthermore, the incorporation of a spacer-linker between the radionuclide–BFCA complex and a peptide can also modify the binding interactions (22, 142, 143).

Somatostatin Analogues. The peptide that has attracted the greatest interest as an imaging agent is somatostatin (SST). Somatostatin is a tetradecapeptide that regulates the secretion of numerous hormones. In addition, receptors for somatostatin are expressed on a variety of human tumors and that fact has become a basic

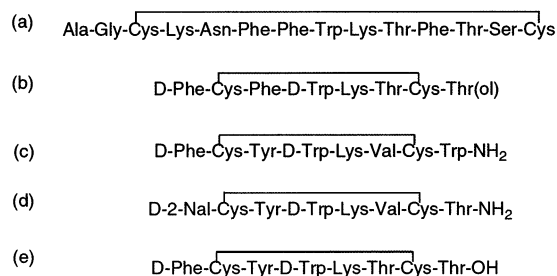


Figure 17. Structure of somatostatin and its analogues: (a) somatostatin; (b) octreotide; (c) vapreotide (RC-160); (d) lanreotide; (e) Tyr³-octreotide (Y3-TATE).

Table 6. Radiopharmaceuticals Based on ST Analogs

ST analog	BFCA	radionuclide
octreotide (OC)	DTPA	¹¹¹ In
	TETA	⁶⁴ Cu
	HYNIC	^{99m} Tc
Tyr ³ -octreotide (Y3-TATE)	DTPA	¹¹¹ In
	DOTA	^{66,67,68} Ga, ^{86/90} Y, ¹¹¹ In
	TETA	⁶⁴ Cu
vapreotide (RC-160)	DTPA	¹¹¹ In
	DOTA	^{66,67,68} Ga, ^{86/90} Y, ¹¹¹ In
	HYNIC	^{99m} Tc
lanreotide (LAN)	DOTA	¹¹¹ In

principle for the use of somatostatin analogues in radiochemistry and tumor imaging (147–150).

Although the first report of *in vivo* imaging with a somatostatin analogue appeared in 1976 (151), further development was delayed because the native peptide is rapidly degraded by plasma and tissue proteases. In years that followed somatostatin analogues that are more resistant to biological degradation have been developed.

The cyclic octapeptide, octreotide (152), is a good replacement for somatostatin in the clinical application, since it shows similar bioactivity, it has a relatively high metabolic stability and its pharmacokinetic properties are better. Octreotide is less susceptible to enzymatic degradation *in vivo* due to the incorporation of the N-terminal D-Phe and the C-terminal amino alcohol, Thr(ol), into its molecule (153, 154).

The pharmacophoric group in octreotide is a sequence of four amino acids: -Phe³-D-Trp⁴-Lys⁵-Thr⁶-, organized into a β -turn conformation by a disulfide bond, formed between cysteine residues at the N- and C-terminus of the peptide backbone (112).

Many octreotide analogues have been synthesized and some of them proved to be useful as targeting molecules (113). Their structures are shown in Figure 17.

Somatostatin radiopharmaceuticals which have received the most attention were octreotide conjugates with DTPA, DOTA (132–136), and HYNIC (115–118, 137) (Table 6). Since the *in vivo* stability of DOTA octreotide conjugates is higher than DTPA derivatives, the former draw more attention of research groups.

⁶⁴Cu-TETA-octreotide and ⁶⁴Cu-TETA-Tyr³-octreotide can be used to detect somatostatin receptor-positive tumors in humans. Their favorable dosimetry and pharmacokinetics indicate that they are promising radiopharmaceuticals for PET imaging of patients with endocrine tumors. The radiotherapy results, along with absorbed dose estimates to target and clearance organs, confirm that ⁶⁴Cu-labeled somatostatin analogues warrant continued consideration as agents for targeted radiotherapy (152, 153).

Bombesin Analogues. Bombesin (BN), a 14-amino acid peptide originally isolated from frog skin and its mammalian equivalent, a 27-amino acid gastrin-releas-

ing peptide (GRP) (157, 158) belong to the bombesin family of peptides. Receptors for bombesin/GRP are expressed in the central nervous system and peripheral tissues such as intestines or pancreas. They are also present on tumors, which include lung, prostate, breast, gastric, colon, and pancreatic cancer. Since bombesin/GRP peptides function in most cases as autocrine growth factors, the disruption of bombesin/GRP triggered biochemical pathways would result in the inhibition of cancer growth. This is the main principle of bombesin/GRP analogues application in tumor diagnosis (159–161).

Several attempts have been made to synthesize and evaluate the properties of bombesin/GRP analogues as future radiopharmaceuticals. Baidoo et al. (119) have synthesized N₂S₂-bombesin derivative, subsequently labeled with ^{99m}Tc, which proved to be a promising diagnostic agent. Bombesin-DTPA conjugates labeled with ^{99m}Tc have also been reported (162, 163).

Vasoactive Intestinal Peptide Analogues. Vasoactive intestinal peptide (VIP) is a 28-amino acid neuroendocrine mediator, one of the inhibitory neurotransmitters in the gut (62). VIP receptors are expressed on central nervous system and pancreatic tissues, as well as on adenocarcinomas.

The synthesis of VIP analogue conjugates seems complicated, since both N- and C-terminus play an important role and their substitution effects intrinsic activity and receptor binding, respectively. However, the ^{99m}Tc-radio-labeled derivatives of VIP have been reported (124, 164).

RGD-Containing Peptides and RGD Peptidomimetics. Peptides containing RGD (Arg-Gly-Asp) sequence interact *in vivo* with integrins, adhesion molecules present on cell membranes of most tissues, responsible for platelet adhesion and activation and leukocyte mobility, as well as cancer cell dissemination. As a result, RGD-containing peptides and RGD peptidomimetics bind to cancer cells and may be used as tumor diagnostic agents (123). Peptides with RGD sequence bind also to glycoprotein IIb/IIIa receptors expressed on activated platelets and are used as thrombus imaging agents or antithrombotics (165–169).

A group of potent RGD-containing analogues, candidates for thrombus imaging agents, was reported (170–172). To minimize the effect of ^{99m}Tc-labeling on RGD sequence integrity, Jackson et al. (170) have incorporated spacers between a BFCA and a targeting molecule. New thrombus imaging agents contain modified RGD sequences (52, 165, 173), so that the amino acid residue in the peptidomimetic triplet forms a N₂S₂ group and functions also as a BFCA.

α -Melanocyte Stimulating Hormone. Labeled analogues of a tridecapeptide α -melanocyte stimulating hormone (α -MSH) are used for melanoma tumor imaging, as its receptors are overexpressed on the membranes of malignant cells (174–176). The most promising derivatives so far are ¹¹¹In-labeled conjugates with DTPA, as well as analogues containing N₃S chelating moiety, labeled with ^{99m}Tc at the N-terminus (139, 140, 177).

Neurotensin Analogues. Neurotensin (NT) is a 13-amino acid neurotransmitter in the central nervous system and a local hormone stimulating growth functions. Neurotensin analogues are used for detection of tumors, such as meningiomas or pancreas, prostate, lung, and colon carcinomas. So far, DTPA conjugates with NT and analogues were synthesized (141, 142).

Substance P Analogues. The receptors for substance P (SP), an 11-amino acid neuromodulator and neurotransmitter, are overexpressed on breast, thyroid, and

glial tumors (143). DTPA conjugates of SP analogues, complexed with ^{111}In , were reported (127, 128).

Chemotactic Peptides. *N*-Formylmethionyleucylphenylalanine (fMLF) is a microbial product, which binds to receptors present on inflammatory cells, granulocytes, and leukocytes. $^{99\text{m}}\text{Tc}$ -HYNIC and ^{111}In -DTPA conjugates were synthesized by means of SPPS method and evaluated as potential infection and inflammation imaging agents (144).

CONCLUSIONS

Nature has designed peptides to regulate many body functions. The concept of designing radiopharmaceuticals based on small peptides is very promising. In the past decade radiolabeled peptide-based radiopharmaceuticals have become an important class of imaging agents for the detection of a wide variety of tumors, as well as thrombosis, infection, and inflammation. The major advantage of developing peptide-based radiopharmaceuticals is that, once the receptor-binding sequence of the peptide molecule is identified, it may be readily synthesized and modified according to pharmacokinetic requirements.

The application of radiolabeled peptides for diagnostic imaging and therapy is rapidly gaining importance in nuclear medicine. Biologically active molecules that selectively interact with specific cell types are attractive vehicles for the delivery of radioactivity to target tissues. The typical approach for imaging a cellular receptor is to synthesize appropriate radiolabeled peptide analogues that possess high binding affinity for the receptor of interest. Various receptors are overexpressed on particular tumor types, and peptides binding to these receptors can be used to visualize tumor lesions scintigraphically.

Small radiolabeled synthetic peptides are preferred over macromolecules such as proteins and antibodies for imaging applications, since they present several advantages. Small peptides can be easily synthesized. They can withstand harsher chemical conditions for radiolabeling. They penetrate tumors more easily than bigger molecules, and they are less likely to induce an immunogenic response.

However, the negative aspects of use of radiolabeled peptides should also be pointed out. Among them one should take into consideration a low tumor uptake relative to a total dose and a normal tissue uptake, very rapid targeting and clearance which creates the necessity to use shorter-lived isotopes. Other pharmacokinetic and toxicity issues still need to be addressed.

The synthesis of radiolabeled peptides is not an easy process. Binding of a radionuclide to a peptide can be achieved either directly or through a bifunctional chelating agent (BFCA). In the latter approach two methods can be distinguished: prelabeling method, based on the labeling of BFCA with its subsequent conjugation to a peptide and postlabeling method which requires the synthesis of BFCA-peptide conjugate, followed by its labeling. The postlabeling chelate method is the most popular approach in the synthesis of radiopharmaceuticals. Several BFCAs have been designed so far. The most popular are DTPA, DOTA, NOTA, and HYNIC. Conjugation of a BFCA to a peptide is not easy to perform considering multiple functional groups in a BFCA, as well as in a peptide molecule. Not many radiopharmaceuticals based on bioactive peptides have been in clinical use so far. The majority of them are somatostatin analogues. Further studies must be undertaken to explain the chemistry of radiolabeling and to develop better methods

of BFCA-peptide conjugate synthesis. The development of new radiopharmaceuticals is a multidisciplinary effort and requires the collaboration from scientists in organic, inorganic, and analytical chemistry, as well as biochemistry and nuclear medicine.

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LITERATURE CITED

- (1) Hoefnagel, C. A. (1991) Anticancer radiopharmaceuticals. *Anticancer Drugs* 2, 107–132.
- (2) Schubiger, P. A., Alberto, R., and Smith, A. (1996) Vehicles, chelators, and radionuclides: choosing the “building blocks” of an effective therapeutic radioimmunoconjugate. *Bioconjugate Chem.* 7, 165–179.
- (3) Reubi, J. C. (1995) Neuropeptide receptors in health and disease: the molecular basis for in vivo imaging. *J. Nucl. Med.* 36, 1825–1835.
- (4) Verbruggen, A. M. (1990) Radiopharmaceuticals: state of the art. *Eur. J. Nucl. Med.* 17, 346–364.
- (5) Antar, M. A. (1990) Radiopharmaceuticals: state of the art. *Nucl. Med. Biol.* 17, 103–128.
- (6) Pauwels, E. K., McCready, V. R., Stoot, J. H. M. B., and van Deurzen, D. F. P. (1998) The mechanism of accumulation of tumour-localising radiopharmaceuticals. *Eur. J. Nucl. Med.* 25, 277–305.
- (7) Wessels, B. W., and Meares, C. F. (2000) Physical and chemical properties of radionuclide therapy. *Semin. Radiat. Oncol.* 10, 115–122.
- (8) Liu, S., and Edwards, D. S. (1995) *Technetium and Rhenium in Chemistry and Nuclear Medicine* (Nicolini, M., Banoli, G., Mazzi, U., Eds.) pp 383–393, SG Editoriale, Padova.
- (9) Park, C. H. (1997) The role of radioisotopes in radiation oncology. *Semin. Oncol.* 24, 639–654.
- (10) Saha, G. B., Go, R. T., and Macintyre W. J. (1992) Radiopharmaceuticals for cardiovascular imaging. *Nucl. Med. Biol.* 19, 1–20.
- (11) Walovitch, R. C., Williams, S. J., and Lafrance, N. D. (1990) Radiolabeled agents for SPECT imaging of brain perfusion. *Nucl. Med. Biol.* 17, 77–83.
- (12) Nunn, A. D. (1990) Radiopharmaceuticals for imaging myocardial perfusion. *Semin. Nucl. Med.* 20, 111–118.
- (13) Britton, K. E. (1990) The development of new radiopharmaceuticals. *Eur. J. Nucl. Med.* 16, 373–385.
- (14) Eckelman, W. C. (1994) The application of receptor theory to receptor-binding and enzyme-binding oncologic radiopharmaceuticals. *Nucl. Med. Biol.* 21, 759–769.
- (15) Seregni, E., Chiti, A., and Bombardieri, E. (1998) Radionuclide imaging of neuroendocrine tumours: biological basis and diagnostic results. *Eur. J. Nucl. Med.* 25, 639–658.
- (16) Schmidt, M., Scheidhauer, K., Luyken, C., Voth, E., Hildebrandt, G., Klug, N., and Schicha, H. (1998) Somatostatin receptor imaging in intracranial tumours. *Eur. J. Nucl. Med.* 25, 675–686.
- (17) Spencer, R. P. (1991) Receptor-mediated radiopharmaceutical imaging of the liver. *Targeted Diagn. Ther.* 4, 321–338.
- (18) Volkert, W. A., and Hoffmann, T. J. (1999) Therapeutic radiopharmaceuticals. *Chem. Rev.* 99, 2269–2292.
- (19) Weiner, R. E., and Thakur, M. L. (2001) Radiolabeled peptides in diagnosis and therapy. *Semin. Nucl. Med.* 31, 296–311.
- (20) Lister-Jones, J., Moyer, B. R., and Dean, R. T. (1990) Small peptides radiolabeled with $^{99\text{m}}\text{Tc}$. *Q. J. Nucl. Med.* 40, 221–233.
- (21) Eckelman, W. C. (1995) Radiolabeling with technetium- $^{99\text{m}}$ to study high-capacity and low-capacity biochemical systems. *Eur. J. Nucl. Med.* 22, 249–263.
- (22) Liu, S., and Edwards, D. S. (1999) $^{99\text{m}}\text{Tc}$ -labeled small peptides as diagnostic radiopharmaceuticals. *Chem. Rev.* 99, 2235–2268.

- (23) Jurisson, S. S., and Lydon, J. D. (1999) Potential technetium small molecule radiopharmaceuticals. *Chem. Rev.* 99, 2205–2218.
- (24) Hnatovich, D. J. (1990) Recent developments in the radio-labeling of antibodies with iodine, indium, and technetium. *Semin. Nucl. Med.* 20, 80–91.
- (25) Schwachau, K. (1994) Technetium radiopharmaceuticals – fundamentals, synthesis, structure and development. *Angew. Chem., Int. Ed. Engl.* 33, 2258–2267.
- (26) Liu, S., Edwards, D. S., and Barrett, J. A. (1997) ^{99m}Tc labeling of highly potent small peptides. *Bioconjugate Chem.* 8, 621–636.
- (27) Okarvi, S. M. (1999) Recent developments in ^{99m}Tc -labeled peptide-based radiopharmaceuticals: An overview. *Nucl. Med. Commun.* 20, 1092–1112.
- (28) Hom, R. K., and Katzenellenbogen, J. A. (1997) Technetium- ^{99m} -labeled receptor-specific small-molecule radiopharmaceuticals: recent developments and encouraging results. *Nucl. Med. Biol.* 24, 485–498.
- (29) Okarvi, S. M. (2001) Recent progress in fluorine-18 labeled peptide radiopharmaceuticals. *Eur. J. Nucl. Med.* 28, 929–938.
- (30) Anderson, C. J., and Welch, M. J. (1999) Radiometal-labeled agents (non-technetium) for diagnostic imaging. *Chem. Rev.* 99, 2219–2234.
- (31) Srivastava, S. C., and Mease, R. C. (1991) Progress in research on ligands, nuclides and techniques for labeling monoclonal antibodies. *Int. J. Rad. Appl. Instrum. B.* 18, 589–603.
- (32) Fischman, A. J., Babich, J. W., and Strauss, W. (1993) A ticket to ride: Peptide radiopharmaceuticals. *J. Nucl. Med.* 34, 2253–2263.
- (33) Ehrhardt, G. J., Ketrang, A. R., and Ayers, L. M. (1998) Reactor-produced radionuclides at the University of Missouri Research Reactor. *Appl. Radiat. Isot.* 49, 295–297.
- (34) Langer, M., and Beck-Sickinger, A. G. (2001) Peptides as carrier for tumor diagnosis and treatment. *Curr. Med. Chem.-Anti-Cancer Agents* 1, 71–93.
- (35) Thakur, M. L. (1995) Radiolabeled peptides: Now and the future. *Nucl. Med. Commun.* 16, 724–732.
- (36) Katzenellenbogen, J. A. (1995) Designing steroid receptor-based radiotracers to image breast and prostate tumors. *J. Nucl. Med.* 36(Suppl), 8S–13S.
- (37) Baidoo, K. E., Scheffel, U., and Stathis, M. (1998) High-affinity no-carrier-added ^{99m}Tc -labeled chemotactic peptides for studies of inflammation in vivo. *Bioconjugate Chem.* 9, 208–217.
- (38) Zamora, P. O., Gohlke, S., Bender, H., Diekmann, D., Rhodes, B. A., Biersack, H., and Knapp, F. F., Jr. (1996) Experimental radiotherapy of receptor-positive human prostate adenocarcinoma with ^{188}Re -RC-160, a directly radiolabeled somatostatin analogue. *Int. J. Cancer* 65, 214–220.
- (39) Thakur, M. L. (1993) Tc- ^{99m} labeled sandostatin: preparation and preliminary evaluation. *J. Labeled Compd. Radiopharm.* 32, 365–367.
- (40) Lister-James, J., Vallabhajosula, S., Moyer, B. R., Pearson, D. A., McBride, B. J., De Rosch, M. A., Bush, L. R., Machac, J., and Dean, R. T. (1997) Pre-clinical evaluation of technetium- ^{99m} platelet receptor binding peptide. *J. Nucl. Med.* 38, 105–111.
- (41) Lister-James, J., Knight, L. C., Maurer, A. H., Bush, L. R., Moyer, B. R., and Dean, R. T. (1996) Thrombus imaging with a technetium- ^{99m} -labeled, activated platelet receptor-binding peptide. *J. Nucl. Med.* 37, 775–781.
- (42) Eckelman, W. C. (1990) Development of radiochemically pure antibodies. *Cancer Res.* 3, 686–692.
- (43) Delmon-Moingeon, L. I., Mahmood, A., Davison, A., and Jones, A. G. (1991) Strategies for labeling monoclonal antibodies and antibody-like molecules with technetium- ^{99m} . *J. Nucl. Biol. Med.* 35, 47–59.
- (44) Eisenwiener, K. P., Powell, P., and Maecke, H. R. (2000) A convenient synthesis of novel bifunctional prochelators for coupling to bioactive peptides for radionuclide labeling. *Bioorg. Med. Chem. Lett.* 10, 2133–2135.
- (45) Anderson, G. W., Zimmerman, J. E., and Callahan, F. M. (1967) A reinvestigation of the mixed carbonic anhydride method of peptide synthesis. *J. Am. Chem. Soc.* 89, 5012–5017.
- (46) Anderson, G. W., Zimmerman, J. E., and Callahan, F. M. (1964) The use of esters of *N*-hydroxysuccinimide in peptide synthesis. *J. Am. Chem. Soc.* 86, 1839–1842.
- (47) Grumbach, I. M., and Veh, R. W. (1991) Sulpho-*N*-hydroxysuccinimide activated long chain biotin. A new microtitre plate assay for the determination of its stability at different pH values and its reaction rate with protein bound amino groups. *J. Immunol. Methods* 140, 205–210.
- (48) Gohlke, S., Diekmann, D., Zamora, P. O., Knapp, F. F., and Biersack, H. J. (1995) MAG3 *p*-nitrophenyl ester for ^{99m}Tc and ^{188}Re labeling of amines and peptides. *Technetium and Rhenium in Chemistry and Nuclear Medicine* 4. (M. Nicolini, G. Bandoli, U. Mazzi, Eds.) pp 363–366, SGEEditoriali, Padua.
- (49) Meares, C. F. (1986) Chelating agents for the binding of metals to antibodies. *Nucl. Med. Biol.* 13, 311–318.
- (50) McMurry, T. J., Pippin, C. G., Wu, C., Deal, K. A., Brechbiel, M. W., Mirzadeh, S., and Gansow, O. A. (1998) Physical parameters and biological stability of yttrium(III) diethylene-triaminepentaacetic acid derivative conjugates. *J. Med. Chem.* 41, 3546–3549.
- (51) Geoghegan, K. F., Stroh, J. G. (1992) Site-directed conjugation of nonpeptide groups to peptides and proteins via periodate oxidation of a 2-amino alcohol. Application to modofoculation at N-terminal serine. *Bioconjugate Chem.* 3, 138–146.
- (52) Pearson, D. A., Lister-James, J., McBride, W. J., Wilson, D. M., Martel, L. J., Civitello, E. R., and Dean, R. T. (1996) Thrombus imaging using technetium- ^{99m} -labeled high-potency GPIIb/IIIa receptor antagonists. Chemistry and initial biological studies. *J. Med. Chem.* 39, 1372–1382.
- (53) Pearson, D. A., Lister-James, J., McBride, W. J., Wilson, D. M., Martel, L. J., Civitello, E. R., Taylor, J. E., Moyer, B. R., and Dean, R. T. (1996) Somatostatin receptor-binding peptides labeled with technetium- ^{99m} : chemistry and initial biological studies. *J. Med. Chem.* 39, 1361–1371.
- (54) Hnatowich, D. J., Layne, W. W., Childs, R. L., Lantaigne, D., Davis, M. A., Griffin, T. V., and Doherty, P. W. (1983) Radioactive labeling of antibody: A simple and efficient method. *Science* 220, 613–615.
- (55) Bakker, W. H., Albert, R., Bruns, C., Breeman, W. A. P., Hofland, L. J., Marbach, P., Pless, J., Pralet, D., Stolz, B., Koper, J. W., Lamberts, S. W. J., Visser, T. J., and Krenning, E. P. (1991) [^{111}In -DTPA-D-Phe]-octreotide, a potential radiopharmaceutical for imaging of somatostatin receptor-positive tumors: synthesis, radiolabeling and in vitro validation. *Life Sci.* 49, 1583–1591.
- (56) Krejcarek, G. E., and Tucker, K. L. (1977) Covalent attachment of chelating groups to macromolecules. *Biochem. Biophys. Res. Commun.* 2, 581–585.
- (57) Arano, Y., Akizawa, H., Uezono, T., Akaji, K., Ono, M., Funakoshi, S., Koizumi, M., Yokoyama, A., Kiso, Y., and Saji, H. (1997) Conventional and high-yield synthesis of DTPA-conjugated peptides: application of a monoreactive DTPA to DTPA-D-Phe¹-octreotide synthesis. *Bioconjugate Chem.* 8, 442–446.
- (58) Maecke, H. R., Riesen, A., and Ritter, W. (1989) The molecular structure of Indium-DTPA. *J. Nucl. Med.* 30, 1235–1239.
- (59) Wang, S., Luo, J., Lantrip, D. A., Waters, D. J., Mathias, C. J., Green, M. A., Fuchs, P. L., and Low, P. S. (1997) Design and synthesis of [^{111}In]DTPA-folate for use as a tumor-targeted radiopharmaceutical. *Bioconjugate Chem.* 8, 673.
- (60) Breeman, W. A. P., van Hagen, P. M., Kwekkeboom, D. J., Visser, T. J., and Krenning, E. P. (1998) Somatostatin receptor scintigraphy using [^{111}In -DTPA⁰]RC-160 in humans: a comparison with [^{111}In -DTPA⁰]octreotide. *Eur. J. Nucl. Med.* 25, 182–186.
- (61) Kolan, H., Li, J., and Thakur, M. L. (1996) Sandostatin labeled with ^{99m}Tc : In vitro stability, in vivo validity and comparison with ^{111}In -DTPA-Octreotide. *Pept. Res.* 9, 144–150.

- (62) Brechbiel, M. W., and Gansow, O. A. (1991) Backbone-substituted DTPA ligands for 90Y radioimmunotherapy. *Bioconjugate Chem.* 2, 187–194.
- (63) Kobayashi, H., Wu, C., Yoo, T. M., Sun, B. F., Drumm, D., Pastan, I., Paik, C. H., Gansow, C. A., Carrasquillo, J. A., and Brechbiel, M. N. (1998) Evaluation of the in vivo biodistribution of yttrium-labeled isomers of CHX-DTPA-conjugated monoclonal antibodies. *J. Nucl. Med.* 39, 829–836.
- (64) Wu, C., Kobayashi, H., Sun, B., Yoo, T. M., Paik, C. H., Gansow, O. A., Carrasquillo, J. A., Pastan, I., and Brechbiel, M. W. (1997) Stereochemical influence on the stability of radio-metal complexes in vivo. Synthesis and evaluation of the four stereoisomers of 2-(p-nitrobenzyl)-trans-CyDTPA. *Bioorg. Med. Chem.* 5, 1925–1934.
- (65) Safavy, A., Smith, D. C., Bazooband, A., and Buchsbaum, D. J. (2002) De novo synthesis of a new diethylenetriamine-pentaacetic acid (DTPA) bifunctional chelating agent. *Bioconjugate Chem.* 13, 317–326.
- (66) Safavy, A., Smith, D. C., Jr., Bazooband, A., and Buchsbaum, D. J. (2002) Synthesis of the first diethylenetriamine-pentahydroxamic acid (DTPH) bifunctional chelating agent. *Bioconjugate Chem.* 13, 327–332.
- (67) Blok, D., Feitsma, R. I. J., Vermeij, P., and Pauwels, E. J. K. (1999) Peptide radiopharmaceuticals in nuclear medicine. *Eur. J. Nucl. Med.* 26, 1511–1519.
- (68) de Jong, M., Bakker, W. H., Krenning, E. P., Breeman, W. A. P., van der Pluijm, M. E., Bernard, B. F., Visser, T. J., Jermann, E., Behe, M., Powell, P., and Maecke, H. R. (1997) Yttrium-90 and indium-111 labeling, receptor binding and biodistribution of [DOTA⁰, D-Phe¹, Tyr³]octreotide, a promising somatostatin analogue for radionuclide therapy. *Eur. J. Nucl. Med.* 24, 368–371.
- (69) McMurry, T. J., Brechbiel, M., Kumar, K., and Gansow, O. A. (1992) Convenient synthesis of bifunctional tetraaza macrocycles. *Bioconjugate Chem.* 3, 108–117.
- (70) DeNardo, S. J., Kukis, D. L., Miers, L., Winthrop, M. D., Kroger, L. A., Salako, Q., Shen, S., Lamborn, K. R., Gumerlock, P. H., Meares, C. F., and DeNardo, G. L. (1998) Yttrium-90-DOTA-peptide-chimeric L6 radioimmunoconjugate: efficacy and toxicity in mice bearing p53 mutant human breast cancer xenografts. *J. Nucl. Med.* 39, 842–849.
- (71) DeNardo, S. J., Zhong, G. R., Salako, Q., Li, M., DeNardom G. L., and Meares, C. F. Pharmacokinetics of chimeric L6 conjugated to indium-111- and yttrium-90-DOTA-peptide in tumor-bearing mice. *J. Nucl. Med.* 36, 829–836.
- (72) Otte, A., Jermann, E., Behe, M., Goetze, M., Bucher, H. C., Roser, H. W., Heppeler, A., Mueller-Brand, J., and Maecke, H. R. (1997) DOTATOC: a powerful new tool for receptor mediated radionuclide therapy. *Eur. J. Nucl. Med.* 24, 792–795.
- (73) Virgolini, I., Szilvasi, I., Kurtaran, A., Angelberger, P., Raderer, M., Havlik, E., Vorbeck, F., Bischof, C., Leimer, M., Dorner, G., Kletter, K., Niederle, B., Scheithauer, W., and Smith-Jones, P. (1998) Indium-111-Dota-¹lanreotide: biodistribution, safety and radiation absorbed dose in tumour patients. *J. Nucl. Med.* 39, 1928–1936.
- (74) Keire, D. A., Jang, Y. H., Li, L., Dasgupta, S., Goddard, W. A., III, and Shively, J. E. (2001) Chelators for radioimmunotherapy: I. NMR and ab initio calculation studies on 1,4,7,10-tetra(carboxyethyl)-1,4,7,10-tetraazacyclododecane (DO4Pr) and 1,4,7-tris(carboxymethyl)-10-(carboxyethyl)-1,4,10-tetraazacyclododecane (DO3A1Pr). *Inorg. Chem.* 40, 4310–4318.
- (75) Otte, A., Mueller-Brand, J., Dellas, S., Nitzsche, E. U., Herrmann, R., and Maecke, H. R. (1998) Yttrium-90-labeled somatostatin-analogue for cancer treatment. *Lancet* 351, 417–418.
- (76) Smith-Jones, P., Bischof, C., Leimer, D., Gludovacz, D., Angelberger, P., Pangerl, T., Peck-Radosavljevic, M., Hamilton, G., Kaserer, K., Steiner, G., Schlagbauer-Wadl, H., Maecke, H., and Virgolini, I. (1998) “Mauritius”, a novel somatostatin analogue for tumour diagnosis and therapy. *J. Nucl. Med.* 39 Suppl: 223P.
- (77) Heppeler, A., Behe, M., Froidevaux, S., Hennig, M., Jermann, E., and Maecke, H. R. (1998) Metal coordination chemical aspects and tumour targeting of a promising somatostatin analogue. *J. Nucl. Med.* 39 Suppl, 63P.
- (78) Stolz, B., Weckbecker, G., Smith-Jones, P., Albert, R., Raulf, F., and Bruns, C. (1998) The somatostatin receptor targeted radiotherapeutic [⁹⁰Y-DOTA-D-Phe¹, Tyr³]octreotide (⁹⁰Y-SMT 487) eradicates experimental rat pancreatic CA20948 tumours. *Eur. J. Nucl. Med.* 7, 668–674.
- (79) Reubi, J. C., Waser, B., Schaer, J. C., Laederach, U., Erion, J., Srinivasan, A., Schmidt, M. A., and Bugaj, J. E. (1998) Unsulfated DTPA- and DOTA-CCK analogues as specific high affinity ligands for CCK-B receptor- expressing human and rat tissues in vitro and in vivo. *Eur. J. Nucl. Med.* 25, 481–490.
- (80) Broan, C. J., Cox, J. P. L., Craig, A. S., Katakya, R., Parker, D., Harrison, A., Randall, A. M., and Ferguson, G. (1991) Structure and solution stability of indium and gallium complexes of 1,4,7-triazacyclononanetriacetate and of Yttrium complexes of 1,4,7,10-tetraazacyclododecanetetracetate and related ligands: Kinetically stable complexes for use in imaging and radioimmunotherapy. X-ray molecular structure of the indium and gallium complexes of 1,4,7-triazacyclononan-1,4,7-triacetic acid. *J. Chem. Soc., Perkin Trans.* 2, 87–98.
- (81) Clarke, E. T., and Martell, A. (1991) Stabilities of the Fe(III), Ga(III) and In(III) chelates of N,N',N''-triazacyclononanetriacetic acid. *Inorg. Chem. Acta* 181, 273–280.
- (82) Eisenwiener, K. P., Prata, M. I., Buschmann, I., Zhang, H. W., Santos, A. C., Wenger, S., Reubi, J. C., and Macke, H. R. (2002) NODAGATOC, a new chelator-coupled somatostatin analogue labeled with [67/68Ga] and [111In] for SPECT, PET and targeted therapeutic applications of somatostatin receptor (hsst2) expressing tumors. *Bioconjugate Chem.* 13, 530–541.
- (83) 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., and Fischman, A. J. (1990) Technetium-99m-human polyclonal IgG radiolabeled via the hydrazino nicotinamide derivative for imaging focal sites of infection in rats. *J. Nucl. Med.* 31, 2022–2028.
- (84) Schwartz, D. A., Abrams, M. J., Hauser, M. M., Gaul, F. E., Larsen, S. K., Rauh, D., and Zubieta, J. A. (1991) Preparation of hydrazino-modified proteins and their use for the synthesis of 99mTc-protein conjugates. *Bioconjugate Chem.* 2, 333–336.
- (85) Babich, J. W., Solomon, H., Pike, M. C., Kroon, D., Graham, W., Abrams, M. J., Tompkins, R. G., Rubin, R. H., and Fischman, A. J. (1993) Technetium-99m-labeled hydrazino nicotinamide derivatized chemotactic peptide analogues for imaging focal sites of bacterial infection. *J. Nucl. Med.* 34, 1964–1974.
- (86) Babich, J. W., and Fischman, A. J. (1995) Effect of “co-ligand” on the biodistribution of 99mTc-labeled hydrazino nicotinamide acid derivatized chemotactic peptides. *Nucl. Med. Biol.* 22, 25–30.
- (87) Krois, D., Riedel, C., Angelberger, P., Kalchauer, H., Virgolini, I., and Lehner, H. (1996) Synthesis of N-α-(6-hydrazinonicotinoyl)-octreotide: a precursor of a [^{99m}Tc] complex. *Liebigs. Ann.* 1463–1469.
- (88) Bangard, M., Behe, M., Gohlke, S., Otte, R., Bender, H., Maecke, H., and Biersack, H.-J. (1998) Detection of somatostatin receptor -positive tumours using the new 99mTc-tricine-HYNIC-d-Phe1-Tyr3-octreotide: first results in patients and comparison with 111In-DTPA-d-Phe1-octreotide. *Eur. J. Nucl. Med.* 27, 628–637.
- (89) Decristoforo, C., and Mather, S. J. (1999) Technetium-99m somatostatin analogues: effect of labeling methods and peptide sequence. *Eur. J. Nucl. Med.* 26, 869–876.
- (90) Decristoforo, C., Melendez-Alafort, L., Sosabowski, J. K., and Mather, S. J. (2000) 99mTc-HYNIC-[Tyr3]-octreotide for imaging somatostatin-receptor-positive tumors: preclinical evaluation and comparison with 111In-octreotide. *J. Nucl. Med.* 41, 1114–1119.
- (91) Edwards, D. S., Liu, S., Barrett, J. A., Harris, A. R., Looby, R. J., Ziegler, M. C., Heminway, S. J., and Carroll, T. R. (1997) New and versatile ternary ligand system for technetium radiopharmaceuticals: water soluble phosphines and tricine as coligands in labeling a hydrazinonicotinamide-modified

- cyclic glycoprotein IIb/IIIa receptor antagonist with ^{99m}Tc . *Bioconjugate Chem.* 8, 146–154.
- (92) Liu, S., Edwards, D. S., and Harris, A. R. (1998) A novel ternary ligand system for ^{99m}Tc -labeling of hydrazino nicotinamide-modified biologically active molecules using imine-N-containing heterocycles as coligands. *Bioconjugate Chem.* 9, 583–595.
- (93) Barrett, J. A., Crocker, A. C., Damphousse, D. J., Hem-inway, S. J., Liu, S., Edwards, D. S., Lazewatsky, J. L., Kagan, M., Mazaika, T. J., and Carroll, T. L. (1997) Biological evaluation of thrombus imaging agents utilizing water soluble phosphines and tricine as coligands when used to label a hydrazinonicotinamide-modified cyclic glycoprotein IIb/IIIa receptor antagonist with ^{99m}Tc . *Bioconjugate Chem.* 8, 155–160.
- (94) Liu, S., and Edwards, D. S. (1995) New N_2S_2 diamidedithiol and N_3S triamidedithiols as bifunctional chelating agents for labeling small peptides with technetium-99m. In *Technetium and Rhenium in Chemistry and Nuclear Medicine 4* (Nicolini M., Banoli G., Mazzi U., Eds.) pp 383–393, SG Editoriali, Padova.
- (95) Najafi, A., Alauddin, M. M., Siegel, M. E., and Epstein, A. L. (1991) Synthesis and preliminary evaluation of a new chelate N2S4 for use in labeling proteins with metallic radionuclides. *Int. J. Rad. Appl. Instrum. B.* 18, 179–185.
- (96) Najafi, A., Alauddin, M. M., Sosa, A., Ma, G. Q., Chen, D. C., Epstein, A. L., and Siegel, M. E. (1992) The evaluation of ^{186}Re -labeled antibodies using N2S4 chelate in vitro and in vivo using tumor-bearing nude mice. *Int. J. Rad. Appl. Instrum. B.* 19, 205–212.
- (97) Kasina, S., Rao, T. N., Srinivasan, A., Sanderson, J. A., Fitzner, J. N., Reno, J. M., Beaumier, P. L., and Fritzberg, A. R. (1991) Development and biologic evaluation of a kit for preformed chelate technetium-99m radiolabeling of an antibody Fab fragment using a diamide dimercaptide chelating agent. *J. Nucl. Med.* 32, 1445–1451.
- (98) Wong, E., Fauconnier, T., Bennett, S., Valliant, J., Nguyen, T., Lau, F., Lu, L. F. L., Pollak, A., Bell, R. A., and Thornback, J. R. (1997) Rhenium(V) and Technetium(V) Oxo Complexes of an $\text{N}(2)\text{N}'\text{S}$ Peptidic Chelator: Evidence of Interconversion between the Syn and Anti Conformations. *Inorg. Chem.* 36, 5799–5808.
- (99) Mathias, C. J., Sun, Y., Welch, M. J., Green, M. A., Thomas, J. A., Wade, K. R., and Martell, A. E. (1988) Targeting radiopharmaceuticals: comparative biodistribution studies of gallium and indium complexes of multidentate ligands. *Int. J. Rad. Appl. Instrum. B.* 15, 69–81.
- (100) Guhlke, S., Schaffland, A., Zamora, P. O., Sartor, J., Diekmann, D., Bender, H., Knapp, F. F., and Biesack, H. J. (1998) ^{188}Re - and ^{99m}Tc -MAG3 as prosthetic groups for labeling amines and peptides: approaches with pre- and postconjugate labeling. *Nucl. Med. Biol.* 25, 621–631.
- (101) Gano, L., Patricio, L., Marques, E., Cantino, G., Pena, H., Martins, T., and Hnatowich, D. J. (1998) Human polyclonal immunoglobulin labeled with technetium-99m via NHS-MAG3: a comparison of radiochemical behavior and biological efficacy with other labeling methods. *Nucl. Med. Biol.* 25, 395–403.
- (102) Benshafut, R., Haran, A., Shvarts, D., and Schneider, B. (2002) Synthesis and characterization of a new tetradentate ligand for Cu(II) metal ions. *J. Org. Chem.* 67, 4040–4044.
- (103) Firestone, R. B. (1996) *Table of isotopes* (Shirley, W. S., Ed.), Horizon Pubs & Distributors Inc.,
- (104) Linder, M. C. (1991) *Biochemistry of copper*, Plenum Press, New York.
- (105) Goethals, P., Coene, M., Slegers, G., Agon, P., Deman, J., and Schelstraete, K. (1988) Cyclotron production of carrier-free ^{66}Ga as a positron emitting label of albumin colloids for clinical use. *Eur. J. Nucl. Med.* 14, 152–154.
- (106) Loc'h, C., Maziere, B., and Comar, D. (1980) A new generator for ionic gallium-68. *J. Nucl. Med.* 21, 171–173.
- (107) Herzog, H., Rosch, F., Stocklin, G., Lueders, C., Quaim, S. M., and Feinendegen, L. E. (1993) Measurement of pharmacokinetics of yttrium-86 radiopharmaceuticals with PET and radiation dose calculation of analogous yttrium-90 radiotherapeutics. *J. Nucl. Med.* 34, 2222–2226.
- (108) Wester, H. J., Brockmann, J., Rosch, F., Wutz, W., Herzog, H., Smith Jones, P., Stolz, B., Bruns, C., and Stocklin, G. (1997) PET-pharmacokinetics of ^{18}F -octreotide: a comparison with ^{67}Ga -DFO- and ^{86}Y -DTPA-octreotide. *Nucl. Med. Biol.* 24, 275–286.
- (109) Rosch, F., Herzog, H., Stolz, B., Brockmann, J., Kohle, M., Muhlensiepen, H., Marbach, P., and Muller-Gartner, H. W. (1999) Uptake kinetics of the somatostatin receptor ligand [^{86}Y]DOTA-DPhe1-Tyr3-octreotide ([^{86}Y]SMT487) using positron emission tomography in nonhuman primates and calculation of radiation doses of the ^{90}Y -labeled analogue. *Eur. J. Nucl. Med.* 26, 358–366.
- (110) Sattelberger, A. P., and Atcher, R. W. (1999) Nuclear medicine finds the right chemistry. *Nat. Biotechnol.* 17, 849–850.
- (111) Hu, F., Cutler, C. S., Hoffman, T., Sieckman, G., Volkert, W. A., and Jurisson, S. S. (2002) Pm-149 DOTA bombesin analogues for potential radiotherapy, in vivo comparison with Sm-153 and Lu-177 labeled DO3A-amide-betaAla-BBN(7–14)NH(2). *Nucl. Med. Biol.* 29, 423–430.
- (112) Signore, A. (1995) Receptor ligands. *Q. J. Nucl. Med.* 39, 83–85.
- (113) Bakker, W. H., Krenning, E. P., and Breeman, W. A. (1990) Receptor scintigraphy with a radiolabeled somatostatin analog: radiolabeling, purification, biological activity and in vivo application in animals. *J. Nucl. Med.* 31, 1501–1509.
- (114) Bangard, M., Behe, M., and Bender, H. (1998) Technetium-99m-octreotide for the detection of somatostatin receptor positive (SSTR+) tumors: preliminary results. *Eur. J. Nucl. Med.* 25, 837.
- (115) Maecke, H. R., Heppeler, A., and Nock, B. (1999) Somatostatin Analogues Labeled with Different Radionuclides. *Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine 5* (M. Nicolini, U. Mazzi, Eds.) pp 77–91, SGEditoriali, Padova.
- (116) Thakur, M. L., Kolan, H. R., and Rifat, S. (1996) Vapreotide labeled with ^{99m}Tc for imaging tumors: preparation and preliminary evaluation. *Int. J. Oncol.* 9, 445–451.
- (117) Decristoforo, C., and Mather, S. J. (1999) In vitro and in vivo Comparison of HYNIC Versus N_3S Derivatized Somatostatin Analogues Labeled with ^{99m}Tc . *Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine 5* (M. Nicolini, U. Mazzi, Eds.) pp 77–91, SGEditoriali, Padova.
- (118) Thakur, M. L., Eshbach, J., Wilder, S., John, E., and McDevitt, M. R. (1993) Tc-99m labeled Sandostatin: Preparation and preliminary evaluation. *J. Label. Compds. Radiopharm.* 32, 365–367.
- (119) Baidoo, K. E., Lin, K. S., Zhan, Y. G., Finley, P., Scheffel, U., and Wagner, H. N., Jr. (1998) Design, synthesis and initial evaluation of high-affinity technetium bombesin analogues. *Bioconjugate Chem.* 9, 218–225.
- (120) Rao, P. S., Thakur, M. L., Pallela, V. R., Patti, R., Reddy, K., Li, H., Sharma, S., Pham, H. L., Diggles, L., Minami, C., and Marcus, C. S. (2001) ^{99m}Tc labeled VIP analog: evaluation for imaging colorectal cancer. *Nucl. Med. Biol.* 28, 445–450.
- (121) Pallela, V. R., Thakur, M. L., Chakder, S., and Rattan, S. (1999) ^{99m}Tc -labeled vasoactive intestinal peptide receptor agonist: functional studies. *J. Nucl. Med.* 40, 352–360.
- (122) Virgolini, I., Kurtaran, A., and Raderer, M. (1995) Vasoactive intestinal peptide receptor scintigraphy. *J. Nucl. Med.* 36, 1732–1739.
- (123) Sivolapenko, G. B., Skarlos, D., and Pectasides, D. (1998) Imaging of metastatic melanoma utilizing a technetium-99m labeled RGD-containing synthetic peptide. *Eur. J. Nucl. Med.* 25, 1383–1389.
- (124) Varner, J. A., and Cheresh, D. A. (1996) Integrins and cancer. *Curr. Opin. Cell Biol.* 8, 724–730.
- (125) Chen, J., Cheng, Z., Hoffman, T. J., Jurisson, S. S., and Quinn, T. P. (2000) Melanoma-targeting properties of (^{99m}Tc)-technetium-labeled cyclic alpha-melanocyte-stimulating hormone peptide analogues. *Cancer Res.* 60, 5649–5658.

- (126) Chen, J., Giblin, M. F., Wang, N., Jurisson, S. S., and Quinn, T. P. (1999) In vivo evaluation of $^{99m}\text{Tc}/^{188}\text{Re}$ -labeled linear alpha-melanocyte stimulating hormone analogues for specific melanoma targeting. *Nucl. Med. Biol.* 26, 687–693.
- (127) van Hagen, P. M., Breeman, W. A., Reubi, J. C., Postema, P. T., van der Anker-Lugtenburg, P. J., Kwekkeboom, D. J., Laissue, J., Waser, B., Lamberts, S. W., Visser, T. J., and Krenning, E. P. Visualization of the thymus by substance P receptor scintigraphy in man. *Eur. J. Nucl. Med.* 23, 1508–1513.
- (128) Breeman, W. A., van Hagen, P. M., Visser-Wisselaar, H. A., van der Pluijm, M. E., Koper, J. W., Setyono-Han, B., Bakker, W. H., Kwekkeboom, D. J., Hazenberg, M. P., Lamberts, S. W., Visser, T. J., and Krenning, E. P. (1996) In vitro and in vivo studies of substance P receptor expression in rats with the new analogue [Indium-111-DTPA-Arg1]-substance P. *Eur. J. Nucl. Med.* 37, 108–117.
- (129) Babich, J., Graham, W., Barrow, S. A., Fischman, A. J. (1995) Comparison of the infection imaging properties of a ^{99m}Tc labeled chemotactic peptide with ^{111}In IgG. *Nucl. Med. Biol.* 22, 643–648.
- (130) Babich, J. W., Graham, W., Barrow, S. A., Dragotakes, S. C., Tompkins, R. G., Rubin, R. H., and Fischman, A. J. (1993) Technetium- ^{99m} -labeled chemotactic peptides: comparison with indium-111-labeled white blood cells for localizing acute bacterial infection in the rabbit. *J. Nucl. Med.* 34, 2176–2181.
- (131) Moerlein, S. M., Welch, M. J., and Hotchkiss, R. M. (1993) A gallium-68-labeled chemotactic peptide analogue for imaging focal sites of bacterial infection. *J. Labeled. Compds. Radiopharm.* 32, 426–427.
- (132) Otte, A., Jermann, E., Behe, M., Bucher, H. C., Roser, H. W., Heppeler, A., Mueller-Brand, J., and Maecke, H. R. (1997) DOTATOC: a powerful new tool for receptor-mediated radionuclide therapy. *Eur. J. Nucl. Med.* 24, 792–795.
- (133) de Jong, M., Bakker, W. H., Breeman, W. A. P., Hofland, L. J., Visser, T. J., Srinivasan, A., Schmidt, M., Behe, M., Maecke, H., and Krenning, E. P. (1998) Pre-clinical comparison of [DTPA0] octreotide, [DTPA0, Tyr3] octreotide and [DOTA0, Tyr3] octreotide as carriers for somatostatin receptor-targeted scintigraphy and radionuclide therapy. *Int. J. Cancer* 5, 406–411.
- (134) Krenning, E. P., Kwekkeboom, D. J., Bakker, W. H., Breeman, W. A., Kooij, P. P., Oei, H. Y., van Hagen, M., Postema, P. T., de Jong, M., and Reubi, J. C. (1993) Somatostatin receptor scintigraphy with [^{111}In -DTPA-D-Phe1]- and [^{123}I -Tyr3]-octreotide: the Rotterdam experience with more than 1000 patients. *Eur. J. Nucl. Med.* 20, 716–731.
- (135) Heppeler, A., Froidevaux, S., Eberle, A. N., and Maecke, H. R. (2000) Receptor targeting for tumor localisation and therapy with radiopeptides. *Curr. Med. Chem.* 7, 971–994.
- (136) de Jong, M., Bernard, B. F., de Bruin, E., van Gameren, A., Bakker, W. H., Visser, T. J., Maecke, H. R., and Krenning, E. P. (1988) Internalization of radiolabeled [DTPA0]octreotide and [DOTA0, Tyr3]octreotide: peptides for somatostatin receptor-targeted scintigraphy and radionuclide therapy. *Nucl. Med. Commun.* 19, 283–288.
- (137) Maina, T., Stolz, B., Albert, R., Bruns, C., Koch, P., and Maecke, H. (1994) Synthesis, radiochemistry and biological evaluation of a new somatostatin analogue (SDZ 219–387) labeled with technetium- ^{99m} . *Eur. J. Nucl. Med.* 21, 437–444.
- (138) Fischman, A. J., Babich, J. W., and Rubin, R. H. (1994) Infection imaging with technetium- ^{99m} -labeled chemotactic peptide analogues. *Semin. Nucl. Med.* 24, 154–168.
- (139) Bagutti, C., Stolz, B., Albert, R., Bruns, C., Pless, J., and Eberle, A. N. (1993) [^{111}In]DTPA-labeled analogues of alpha-MSH for the detection of MSH receptors in vitro and in vivo. *Ann. N.Y. Acad. Sci.* 680, 445–447.
- (140) Bard, D. R. (1995) An improved imaging agent for malignant melanoma, based on [Nle4, D-Phe7]alpha-melanocyte stimulating hormone. *Nucl. Med. Commun.* 16, 860–866.
- (141) Reubi, J. C., Waser, B., Friess, H., Buchler, M., and Laissue, J. (1998) Neurotensin receptors: a new marker for human ductal pancreatic adenocarcinoma. *Gut* 42, 546–550.
- (142) Reubi, J. C., Waser, B., Schaer, J. C., and Laissue, J. (1999) Neurotensin receptors in human neoplasms: high incidence in Ewing's sarcomas. *Int. J. Cancer* 82, 213–218.
- (143) Hennig, I. M., Laissue, J. A., Horisberger, U., and Reubi, J. C. (1995) Substance-P receptors in human primary neoplasms: tumoral and vascular localization. *Int. J. Cancer* 61, 786–792.
- (144) Fischman, A. J., Pike, M. C., and Kroon, D. (1991) Imaging focal sites of bacterial infection in rats with indium-111-labeled chemotactic peptide analogues. *J. Nucl. Med.* 32, 483–491.
- (145) Liu, S., Edwards, D. S., Looby, R. J., Poirier, M. J., Rajopadhye, M., Bourque, J. P., and Carroll, T. R. (1996) Labeling cyclic glycoprotein IIb/IIIa receptor antagonists with ^{99m}Tc by the preformed chelate approach: effects of chelators on properties of [^{99m}Tc]chelator-peptide conjugates. *Bioconjugate Chem.* 7, 196–202.
- (146) Barrett, J. A., Damphousse, D. J., Heminway, S. J., Liu, S., Edwards, D. S., Looby, R. J., and Carroll, T. R. (1996) Biological evaluation of ^{99m}Tc -labeled cyclic glycoprotein IIb/IIIa receptor antagonists in the canine arteriovenous shunt and deep vein thrombosis models: effects of chelators on biological properties of [^{99m}Tc]chelator-peptide conjugates. *Bioconjugate Chem.* 7, 203–208.
- (147) Lamberts, S. W. J. (1988) The role of somatostatin in the regulation of anterior pituitary hormone secretion and the use of its analogues in the treatment of human pituitary tumors. *Endocr. Rev.* 9, 417–436.
- (148) Lamberts, S. W. J., Krenning, E. P., and Reubi, J. C. (1991) The role of somatostatin and its analogues in the diagnosis and treatment of tumours. *Endocrine Rev.* 19, 450–482.
- (149) Thakur, M. L., Kolan, H., Li, J., Wiaderkiewicz, R., Pallela, V. R., Duggaraju, R., and Schally, A. V. (1997) Radiolabeled somatostatin analogues in prostate cancer. *Nucl. Med. Biol.* 24, 105–113.
- (150) de Jong, M., Breeman, W. A. P., and Bernard, H. F. (1999) Therapy of neuroendocrine tumours with radiolabeled somatostatin analogues. *Q. J. Nucl. Med.* 43, 356–366.
- (151) Bardfeld, P. A., Chervu, L. R., and Myrty, D. R. K. (1976) The organ distribution of ^{131}I -tryosyl somatostatin. *Br. J. Radiol.* 49, 381–382.
- (152) Anderson, C. J., Dehdashti, F., Cutler, P. D., Schwarz, S. W., Laforest, R., Bass, L. A., Lewis, J. S., and McCarthy, D. W. (2001) ^{64}Cu -TETA-octreotide as a PET imaging agent for patients with neuroendocrine tumors. *J. Nucl. Med.* 42, 213–221.
- (153) Lewis, J. S., Lewis, M. R., Cutler, P. D., Srinivasan, A., Schmidt, M. A., Schwarz, S. W., Morris, M. M., Miller, J. P., and Anderson, C. J. (1999) Radiotherapy and dosimetry of ^{64}Cu -TETA-Tyr3-octreotide in a somatostatin receptor-positive, tumor-bearing rat model. *Clin. Cancer Res.* 5, 3608–3616.
- (154) Bauer, W., Briner, U., Doepfner, W., Haller, R., Huguenin, R., Marbach, P., Petcher, T. J., and Pless, J. (1982) SMS 201–995: a very potent and selective octapeptide analogue of somatostatin with prolonged action. *Life Sci.* 31, 1133–1140.
- (155) Pless, J., Bauer, W., Briner, U., Doepfner, W., Marbach, P., Maurer, R., Pletcher, T. J., Reubi, J. C., and Vonderscher, J. (1986) Chemistry and pharmacology of SMS 201–995, a long acting octapeptide analogue of somatostatin. *Scand. J. Gastroenterol. Suppl.* 119, 54–64.
- (156) Edwards, W. B., Fields, C. G., Anderson, C. J., Pajean, T. S., Welch, M. J., and Fields, G. B. (1994) Generally applicable, convenient solid-phase synthesis and receptor affinities of octreotide analogues. *J. Med. Chem.* 37, 3749–3757.
- (157) McDonald, T. J., Jornvall, J., Nilsson, G., Vagne, M., Ghatei, M., Bloom, S. R., and Mutt, V. (1979) Characterization of a gastrin releasing peptide from porcine nonatral gastric tissue. *Biochem. Biophys. Res. Commun.* 90, 227–233.
- (158) Moody, T. W., Carney, D. N., Cuttita, F., Quattrocchi, K., and Minna, J. D. (1985) High affinity receptors for bombesin/GRP-like peptides on human small cell lung cancer. *Life Sci.* 37, 105–113.

- (159) Moody, T. W., Mahmoud, S., Staley, J. S., Naldini, L., Cirillo, D., South, V., Felder, S., and Kris, R. (1989) Human glioblastoma cell lines have neuropeptide receptors for bombesin/GRP. *J. Mol. Neurosci.* 1, 235–242.
- (160) Yano, T., Pinski, J., Szepeshazi, K., Groot, K., and Schally, A. V. (1992) Stimulation by bombesin/gastrin releasing peptide antagonist RC-3095 of growth of human breast cancer lines. *Cancer Res.* 52, 4545–4547.
- (161) Breeman, W. A., Hofland, L. J., de Jong, M., Bernard, B. F., Srinivasan, A., Kwekkeboom, D. J., Visser, T., and Krenning, E. P. (1999) Evaluation of radiolabeled bombesin analogues for receptor-targeted scintigraphy and radiotherapy. *Int. J. Cancer* 81, 658–665.
- (162) Breeman, W. A., de Jong, M., Bernard, B. F., Kwekkeboom, D. J., Srinivasan, A., van der Pluijm, M. E., Hofland, L. J., Visser, T. J., and Krenning, E. P. (1999) Pre-clinical evaluation of [(111)In-DTPA-Pro(1), Tyr(4)]bombesin, a new radioligand for bombesin-receptor scintigraphy. *Int. J. Cancer* 83, 657–663.
- (163) Moody, T. W., and Perry, D. C. (1989) Peptide Receptors. *Receptor Pharmacology and Function* (M. Williams, R. A. Glennon, P. B. M. W. M. Timmermans, Eds.) pp 527–570, Dekker, New York.
- (164) Pallela, V. R., Reddy, M. V. R., Senadhi, V. K., and Thakur, M. L. (1998) Synthesis and evaluation of Tc-99m-labeled VIP analogues as potential tumour imaging agents. *J. Nucl. Med.* 39, 226P.
- (165) Ali, F. E., Bennett, D. B., Calvo, R. R., and Elliott, J. D. (1994) Conformationally constrained peptides and semipeptides derived from RGD as potent inhibitors of the platelet fibrinogen receptor and platelet aggregation. *J. Med. Chem.* 37, 769–780.
- (166) Cheng, S., Craig, W. S., Mullen, D., Tschopp, J. F., Dixon, D., and Pierschbacher, M. D. (1994) Design and synthesis of novel cyclic RGD-containing peptides as highly potent and selective integrin $\alpha_{IIb}\beta_3$ antagonists. *J. Med. Chem.* 37, 1–8.
- (167) Teng, W., Rose, J. W., Philips, D. R., Nannizzi, L., Arsen, A., Campbell, A. M., and Charo, I. F. (1993) Design of potent and specific integrin antagonists. *J. Biol. Chem.* 268, 1066–1073.
- (168) Zablocki, J. A., Miyano, M., and Garland, R. B. (1993) Potent in vitro and in vivo inhibitors of platelet aggregation based upon the Arg-Gly-Asp-Phe sequence of fibrinogen. A proposal on the nature of the binding interaction between the Arg-guanidine of RGD mimetics and the platelet GP IIb/IIIa receptor. *J. Med. Chem.* 36, 1811–1919.
- (169) Bach, A. C., Eyermann, C. J., and Gross, J. D. (1994) Structural studies of a family of a high affinity ligands for GPIIb/IIIa. *J. Am. Chem. Soc.* 116, 3207–3219.
- (170) Jackson, S., DeGrado, W. F., and Dwivedi, A. (1994) Template-constrained cyclic peptides: design of high affinity ligands for GPIIb/IIIa. *J. Am. Chem. Soc.* 116, 3220–3230.
- (171) Xue, C. B., and DeGrado, W. F. (1995) An Efficient synthesis of GPIIb/IIIa inhibitor DMP 728. A novel synthesis of N $^{\alpha}$ -methylarginine-containing peptide. *J. Org. Chem.* 60, 946–952.
- (172) Muto, P., Lastoria, S., and Varrella, P. (1995) Detecting deep venous thrombosis with technetium-99m-labeled synthetic peptide P280. *J. Nucl. Med.* 36, 1384–1391.
- (173) Lister-Jones, J., Knight, L. C., Mauer, A. H., Bush, L. R., Moyer, B. R., and Dean, R. T. (1996) Thrombus imaging with technetium-99m-labeled, activated platelet receptor binding peptide. *J. Nucl. Med.* 37, 775–781.
- (174) Siegrist, W., Oestreicher, M., Stutz, S., Girard, J., and Eberle, A. N. (1988) Radioreceptor assay for alpha-MSH using mouse B16 melanoma cells+. *J. Recept. Res.* 8, 323–343.
- (175) Siegrist, W., Solca, F., Stutz, S., Giuffre, L., Carrel, S., Girard, J., and Eberle, A. N. (1989) Characterization of receptors for alpha-melanocyte-stimulating hormone on human melanoma cells. *Cancer Res.* 49, 6352–6358.
- (176) Tatro, J. B., Atkins, M., Mier, J. W., Hardarson, S., Wolfe, H., Smith, T., Entwistle, M. L., and Reichlin, S. (1990) Melanotropin receptors demonstrated in situ in human melanoma. *J. Clin. Invest.* 85, 1825–1832.
- (177) Chen, J., Giblin, M. F., Wang, N., Jurisson, S. S., and Quinn, T. P. (1999) In vivo evaluation of 99mTc/188Re-labeled linear alpha-melanocyte stimulating hormone analogues for specific melanoma targeting. *Nucl. Med. Biol.* 26, 687–693.

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