Design of Radiolabeled Peptide Radiopharmaceuticals

20

It is one of the more striking generalizations of biochemistry - which surprisingly is hardly ever mentioned in the biochemical textbooks - that the twenty amino acids and the four bases, are, with minor reservations, the same throughout Nature. (Francis Crick)

20.1 Introduction

The first amino acid (AA or aa) was discovered in 1806 by two French chemists, Louis-Nicolas Vauquelin and Pierre Jean Robiquet. They isolated a compound from asparagus that was subsequently named asparagine. However, the first use of the term "amino acid" (AA) in the English language dates from 1898, while the German term, *Aminosäure*, was used earlier. In 1902, Emil Fischer and Franz Hofmeister independently proposed that proteins are formed from many AAs, whereby bonds are formed between the amino group of one AA with the carboxyl group of another, resulting in a linear structure that Fischer termed *peptide* (Fig. 20.1). A peptide bond is an amide type of covalent chemical bond linking two consecutive α-AAs. The amino acids that have been incorporated into peptides are termed "residues," since a water molecule is released when a peptide (amide) bond is formed. All peptides except cyclic peptides have an N-terminal (NH_2, amine group) and C-terminal (COOH, carboxyl group) residue at the end of the peptide.

Peptides are short chains of AAs containing less than 50 AAs. A peptide chain containing 10–15 AAs is called oligopeptide. A polypeptide is a longer, continuous, unbranched peptide chain, and a polypeptide that contains >50 AAs is known as a protein. A peptide is defined, however, by the FDA as a polymer composed of less than 40 amino acids (500–5000 Da) [1].

20.1.1 Proteinogenic and Non-proteinogenic AAs

Technically, any organic compound with an amine ($-NH_2$) and a carboxylic acid (-COOH) functional group is an AA. As of 2020, >500 naturally occurring AAs are known, although 20 appear in the genetic code and are considered as "standard" alpha AAs, (or proteinogenic AAs) because their generic structure (except for proline) includes a primary amino group (NH_2) and a carboxylic acid group (COOH) bonded to the same carbon, known as the α-carbon (C_α), as shown in Fig. 20.1. The standard α-AAs with their letter symbols are shown in Table 20.1. The chemical structures of the standard α-AAs are

S. Vallabhajosula, *Molecular Imaging and Targeted Therapy*,
https://doi.org/10.1007/978-3-031-23205-3_20

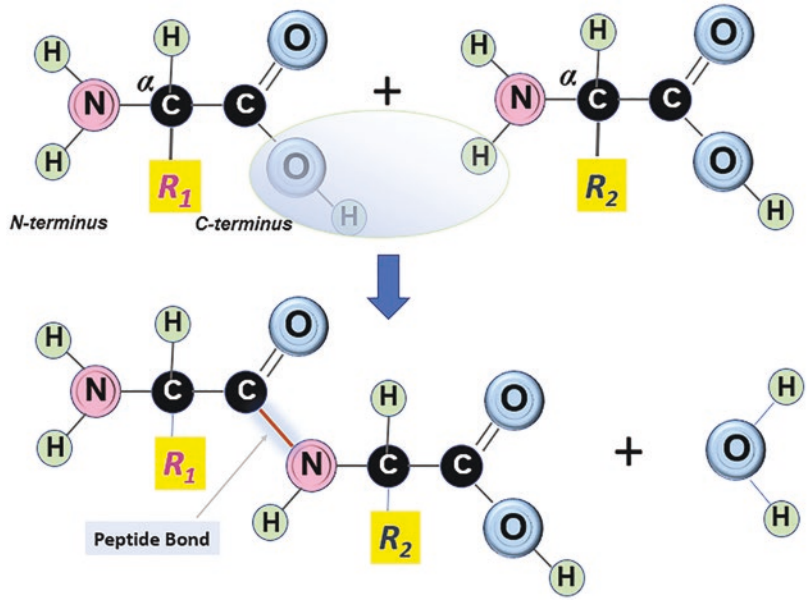

Fig. 20.1 The peptide bond is an amide type of covalent chemical bond formed between the amino group of one amino acid with the carboxyl group of another, linking two consecutive α-amino acids, resulting in a linear structure

Table 20.1 The standard proteinogenic alpha amino acids

No.	Amino acid	Letter code			Formula	Mol. wt.	Description
1	Alanine	ALA	Ala	A	$C_3H_7N_1O_2$	89.09	Aliphatic AAs with hydrophobic side chain
2	Isoleucine	ILE	Ile	I	$C_6H_{13}N_1O_2$	131.17	
3	Leucine	LEU	Leu	L	$C_6H_{13}N_1O_2$	131.17	
4	Valine	VAL	Val	V	$C_5H_{11}N_1O_2$	117.15	
5	Phenylalanine	PHE	Phe	F	$C_9H_{11}N_1O_2$	165.19	Aromatic AAs with hydrophobic side chain
6	Tryptophan	TRP	Trp	W	$C_{11}H_{12}N_2O_2$	204.23	
7	Tyrosine	TYR	Tyr	Y	$C_9H_{11}N_1O_3$	181.19	
8	Asparagine	ASN	Asn	N	$C_4H_8N_2O_3$	132.12	AAs with neutral side chain
9	Cysteine	CYS	Cys	C	$C_3H_7N_1O_2S_1$	121.16	
10	Glutamine	GLN	Gln	Q	$C_5H_{10}N_2O_3$	146.15	
11	Methionine	MET	Met	M	$C_5H_{11}N_1O_2S_1$	149.21	
12	Serine	SER	Ser	S	$C_3H_7N_1O_3$	105.09	
13	Threonine	THR	Thr	T	$C_4H_9N_1O_3$	119.12	
14	Arginine	ARG	Arg	R	$C_6H_{14}N_4O_2$	174.2	AAs with positive charged side chain
15	Histidine	HIS	His	H	$C_6H_9N_3O_2$	155.16	
16	Lysine	LYS	Lys	K	$C_6H_{14}N_2O_2$	146.19	
17	Aspartic acid	ASP	Asp	D	$C_4H_7N_1O_4$	133.1	AAs with negative charged side chain
18	Glutamic acid	GLU	Glu	E	$C_5H_9N_1O_4$	147.13	
19	Glycine	GLY	Gly	G	$C_2H_5N_1O_2$	75.07	Unique AAs
20	Proline	PRO	Pro	P	$C_5H_9N_1O_2$	115.18	

shown in the Fig. 20.2. There are two extra proteinogenic AAs: selenocysteine and pyrrolysine.

In AAs, the α-carbon atom is attached to four different groups; it is asymmetric and, therefore, exhibits optical isomerism. D-Amino acids involve the mirror image of the naturally occurring L-isomers. They are used for a range of applications, mostly to increase resistance against a range of degradation enzymes. Peptides containing D-amino acids are, therefore, significantly

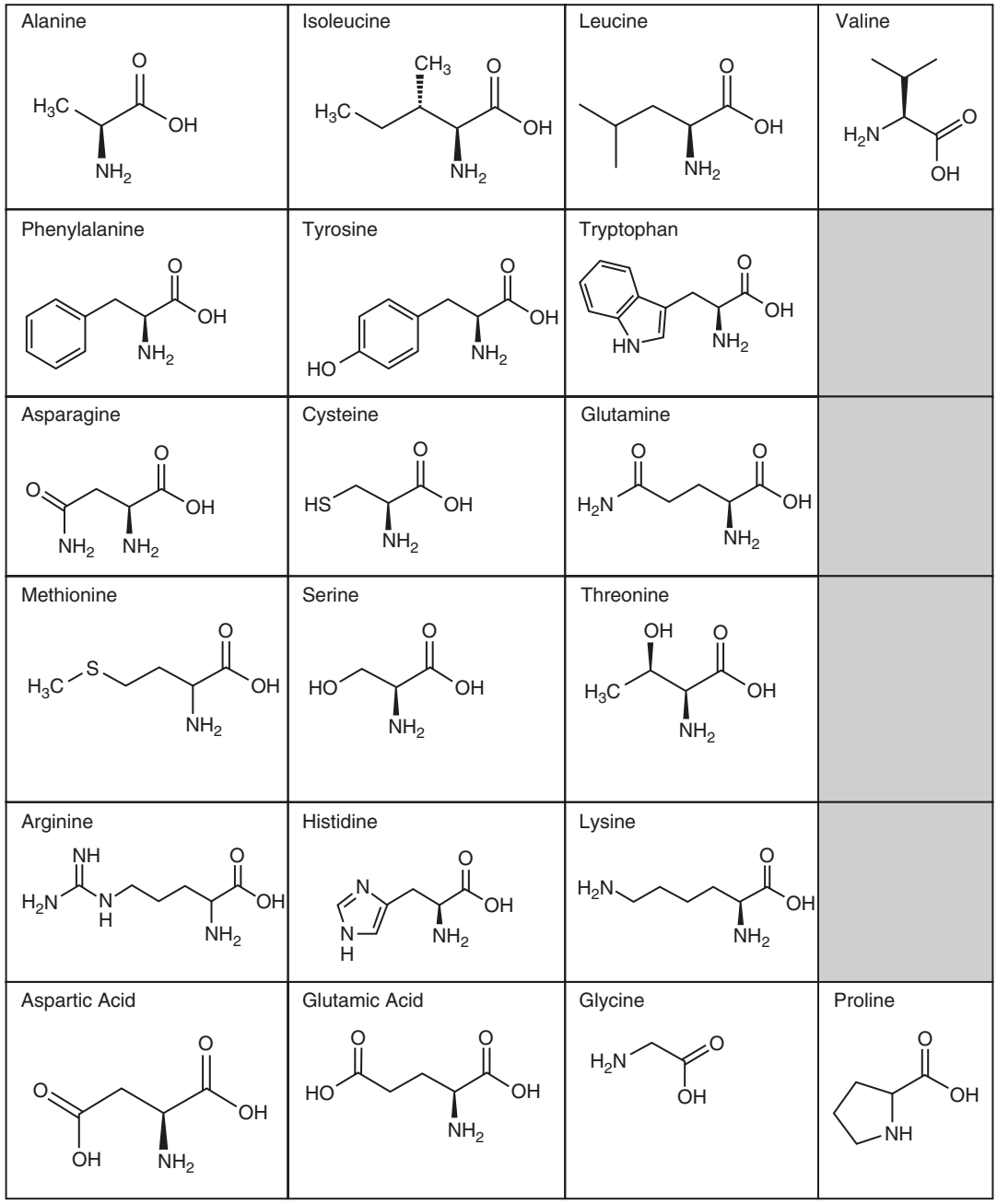

Fig. 20.2 The chemical structures of alpha amino acids

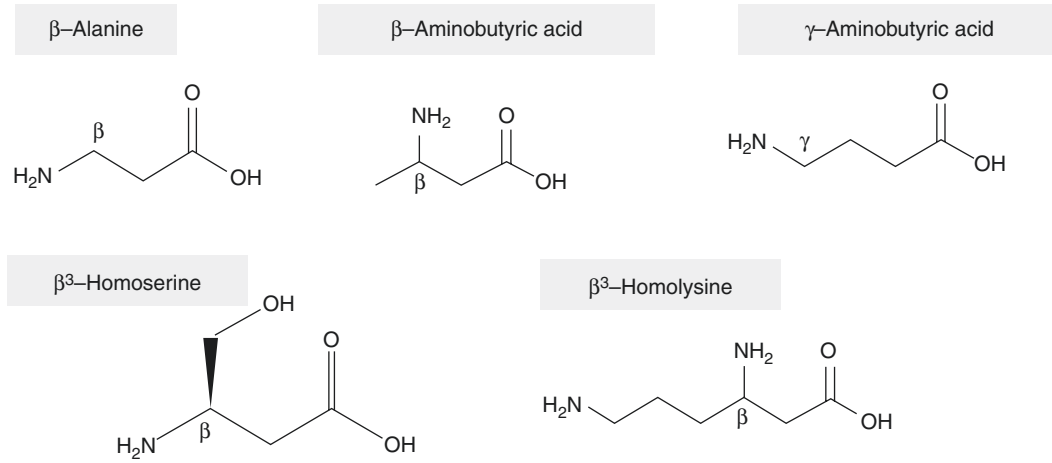

Fig. 20.3 Examples of non-natural amino acids

more stable than peptides containing only L-amino acids.

Most natural AAs are α-AAs in the *L* conformation but, some non-natural AAs (NNAAs) also exist in nature. In these AAs, the amine group and the carboxy groups are not attached to the same α-carbon atom. The most common examples are β-alanine and γ-aminobutyric acid (GABA), an inhibitory neurotransmitter in brain (Fig. 20.3).

20.1.2 Peptide Therapeutics

In 1902, Bayliss and Starling discovered the blood borne chemical messenger "secretin" and later coined the term "hormone" for the blood borne chemical messengers. Peptide hormones may contain three to several hundred AAs. For example, Somatostatin (SST), also known as growth hormone-inhibiting hormone (GHIH), is a peptide hormone made up of either 14 or 28 AAs, whereas human growth hormone (hGH) is a protein of 191 AAs. Peptide therapeutics have played a notable role in medical practice since the advent of "*insulin therapy*" in the 1920s. As of 2020, >70 peptide products have been approved and >100 commercial products are available in the global market. In addition, >160 therapeutic peptide products were reported in active clinical trials and >200 were reported in the preclinical development [2].

Table 20.2 Approved radiolabeled peptide drug conjugates in clinical use

Radiotracer	Target	Year
^{123}I-(Tyr^3-octreotide)	SSTR-2	1989
^{111}In-DTPA-octreotide (OctreoScan(R*)	SSTR-2	1994
^{99m}Tc-Apcitide (^{99m}Tc-P280, AcuTect)	GP II_b/III_a	1997
^{68}Ga-DOTA-TOC (^{68}Ga-endotreotide, Somakit)	SSTR-2	2016, 2020
^{99m}Tc-Hynic-octreotide (Tektreotyd)	SSTR-2	2018
^{68}Ga-DOTA-TATE (NETSPOT®)	SSTR-2	2018
^{177}Lu-DOTA-TATE (LUTATHERA®)	SSTR-2	2018
^{64}Cu-DOTA-TATE (DetectNet®)	SSTR-2	2020
^{68}Ga-PSMA-11	PSMA	2020
^{177}Lu-PSMA-617	PSMA	2022

Peptide drug conjugates (PDC) can offer a multifunctional approach for advancing targeted cancer therapeutics. In 1989, ^{123}I-204-090 (Tyr^3-octreotide) was the first radiolabeled peptide used in humans for imaging studies but, the first radiolabeled PDC (RPDC) approved by US-FDA was ^{111}In-DTPA-octreotide (OctreoScan®) in 1994. Several approved radiolabeled PDCs (RPDCs) for imaging and therapy are summarized in Table 20.2.

In the design and development of RPDCs for theranostic applications, the pharmacokinetic (PK) properties, such as absorption, distribution, metabolism, and excretion (ADME) as well as pharmacodynamic (PD) properties need to be optimized to improve safety, reduce normal organ toxicities, and to enhance target specificity.

20.1.3 Advantages and Disadvantages of Peptides

The biological actions of the peptides are mediated upon binding with high affinity to specific receptors. Many of these regulatory peptide receptors are massively overexpressed in numerous cancers, compared to their relatively low density in physiological organs. Table 20.2 summarizes the expression of peptide receptors on different tumors and lists the peptides currently being studied for the development of receptor targeted PDCs.

Peptides have several advantages over small molecule, organic molecules, or large proteins and antibodies [3–6]:

- Peptides are naturally occurring biologics and, hence, safer than synthetic drugs and have a greater efficacy, selectivity, and specificity for the target receptor.
- In contrast to synthetic drugs, peptides are degraded into their component proteinogenic AAs without leading to toxic metabolites.
- Because of the short half-lives, peptide drugs are associated with less accumulation in the body, thereby reducing the risks that may arise from their degradation products.
- Compared with larger proteins and antibodies, peptides can penetrate and diffuse further into the tumor tissue because of their low molecular mass, and relatively small size.
- Due to high concentration in the tumor, rapid clearance from the blood and non-target tissues and high receptor affinity on tumor cells lead to optimal target-to-non-target ratios.
- Peptides are less immunogenic than recombinant proteins and antibodies. In addition, they are associated with lower manufacturing costs, higher activity, and greater stability (they can be stored at room temperature).
- For diagnostic imaging, usually, tiny quantities of peptides (<10 μg) are sufficient. Even for therapeutic purposes, <500 μg is needed to deliver the therapeutic doses of radionuclides.
- Peptide molecules provide the flexibility for chemical modification and/or insertion of synthetic (non-natural) AAs in the sequence to optimize biodistribution, and enhance target binding.
- Peptide molecules provide the possibility of attaching a bifunctional chelator (BFC) at either the C- or N-terminus to facilitate easier labeling with radiometals. Peptides can tolerate the severe conditions (pH, temperature, organic solvent) associated with chemical modifications.
- Peptides can also be readily synthesized using conventional peptide synthesizers and, any desired modifications to the structure, can be easily engineered by making the appropriate changes to the peptide sequence during synthesis and/or by adding other structural modifications after synthesis.

The major disadvantages include short plasma-half-life, poor in vivo stability, and renal clearance.

- Natural peptides have a short half-life, due to their rapid degradation caused by many peptidases and proteases found in plasma.
- Short half-lives of peptides are also experienced due to rapid renal clearance. The glomeruli pores within the kidney have a size of ~8 nm. The circulating hydrophilic peptides that are <25 kDa are filtered through the glomeruli but, partly retained in the kidney due to reuptake by renal proximal tubules mediated by megalin receptors.
- Combination with a chelator and/or incorporation of a radionuclide may result in reduction of receptor-binding affinity compared to

the original peptide or chelate-conjugated peptide.
- The relatively high uptake and prolonged retention of radiolabeled peptides within the kidneys may lead to renal damage.

20.2 Design of Peptide Radiopharmaceuticals (PRP)

Except for radiohalogens (^{18}F, $^{123/124/131}$I), most PRPs for imaging and therapy are based on metallic radionuclides, such as ^{111}In, ^{64}Cu, ^{68}Ga, ^{90}Y, ^{177}Lu and ^{225}Ac. The chemical components of a target specific PRP may have four or five different individual components, such as targeting vehicle (peptide), spacer and/or linker, and finally, a bifunctional chelator (BFC) to complex the radiometal (Fig. 20.4). The significance of each of these components will be discussed in greater detail under different sections.

The development of a PRP for successful receptor targeting can be summarized in the following major steps [3]:

1. Identification of the molecular target (the peptide receptor) using receptor autoradiography or immunohistochemistry.
2. Synthesis of a peptide and/or its analogs based on the original natural peptide sequence. Modifications in the sequence may be needed to produce a metabolically stabilized peptide analog which preserves most of the biological activity of the original peptide molecule.
3. The peptide is covalently conjugated to a BFC via a spacer/linker for radiometal labeling.
4. The peptide may be chemically modified to have a prosthetic group for labeling to radiohalogens.
5. Optimization of a radiolabeling protocol.
6. Study the in vitro characteristics of PRP, such as labeling efficiency, radiochemical purity, specific activity, etc.
7. Determine the in vivo stability, specificity, and the diagnostic/or therapeutic potential based on preclinical studies.
8. Based on in vitro and in vivo studies in animal models, further optimizations of the PRP may be needed to improve the biological performance of the PRP.

The design and development of PRPs involve various strategies to improve the stability and PK (such as ADME) and PD characteristics of the final drug product. The PK of natural amino acid

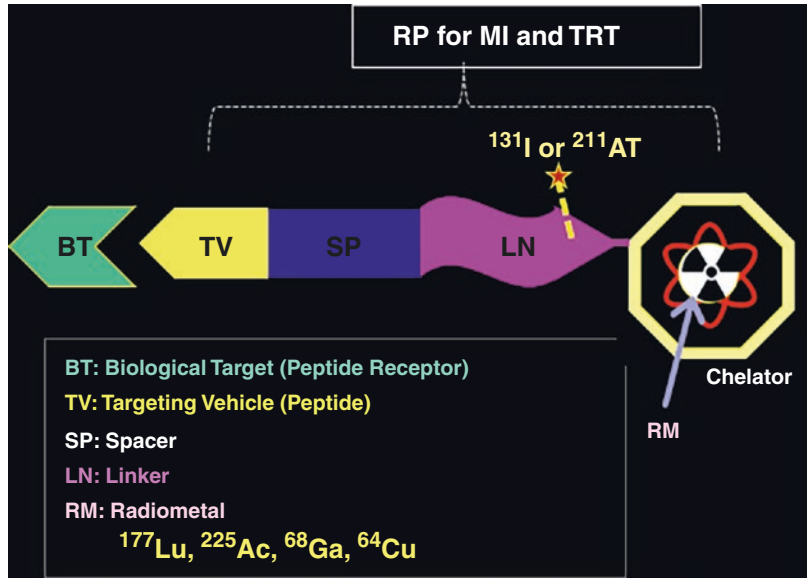

Fig. 20.4 Schematic of a target specific peptide radiopharmaceutical showing different components, such as targeting vehicle (vector), spacer, linker, and chelating agent

sequences can be optimized through the introduction of (a) conformational constraints (i.e., induced by cyclization, or insertion of non-natural amino acids in the peptide sequences) providing unfavorable changes in the binding entropy; and (b) conjugation with glycosylated moieties or polyether compounds at the N-terminus end of synthetic peptides. The stability of peptides can also be increased by the formation of dimers, tetramers, or heterodimers, which improve the stability and the affinity of synthetic peptide chains to their receptors [4, 6].

20.2.1 Peptide Modification and Insertion of Non-natural AAs

Cyclic peptide (CP) structures are mainly due to the formation of disulfide bonds between the thiol groups of two unprotected cysteines within the linear peptide. However, CPs can also be generated from lactam bridges between amino and carboxyl groups. Several cyclized peptides are currently used in nuclear medicine, such as somatostatin, RGD tripeptide, cholecystokinin, and minigastrin, as well as bombesin, and vasoactive intestinal peptide (VIP). Cyclization of peptides provides conformational and functional characteristics that are critical for their application as target-specific vectors to carry radionuclides. Cyclization also brings about conformational constraints that lead to stability against proteolytic degradation and improved receptor binding affinity.

20.2.1.1 Somatostatin (SST) Analogs

Various modifications in the AA sequence of somatostatin produced successful SST analogs for imaging and therapy (Fig. 20.5) [7]. Some of the important modifications are summarized here.

- SST-14 is a cyclic peptide with a disulfide bridge between Cys^3 and Cys^{14} and has a short half-life (<3 min) in circulation. Octreotide, the 8 AA analog was synthesized by inserting the D-AAs such as D-Phe at N-terminus and the amino-alcohol Thr-ol at C-terminus, and preserving a disulfide bridge, between Cys^2 and Cys^7. Octreotide showed enhanced biological activity, reduced metabolic degradation with a half-life of about 2 h [3].
- Two octreotide analogs were developed by replacing Phe^3 at position-3 with Tyr^3. While Tyr^3-octreotide (TOC) has the amino-alcohol Thr-ol at C-terminus, octreotate (TATE) has a free carboxyl group at C-terminus. Octreotate showed higher affinity to SSTR-2 than octreotide and Tyr^3-Octreotide (Table 20.3).
- Replacing Phe^3 in octreotide with 1-naphthyl alanine $(1\text{-Nal})^3$ produced an SST analog NOTATOC with higher affinity not only for SSTR-2 but, also for SSTR-3 and 5 (Table 20.3).
- Incorporation of stable AAs, such as β-DAP (β-(L-1,2-diamino propionic acid) and homocysteine (Hcy) in depreotide and (2-naphthyl)-D-alanine in lanreotide as well as the amidation of their C-terminus improved the tumor uptake in comparison to octreotide.
- The design of peptide antagonists required several chemical modifications, such as deletions or the introduction of NNAAs with different chirality. The fundamental differences in chirality between L- and D-AAs mean that peptides built on D-AAs are not recognized by many proteins, including proteases. The result of this lack of recognition is that while most L-peptides are vulnerable to enzymatic degradation in vivo, the analogous D-peptides are resistant to degradation and have low immunogenicity. In the octreotide scaffold, the inversion of chirality at positions 2 and 3 was shown to cause relevant structural modifications converting an SST agonist into an antagonist [8]. Several new SST antagonists (LM3, JR10, JR11) were developed (Fig. 20.6) by the introduction of D-4-aminocarbamoyl-Phenylalanine (D-Aph(Cbm)) in place of D-Trp, and 4-amino-L-hydroorotyl-phenylalanine (Aph(Hor)) in position Tyr to improve the receptor affinity [10].

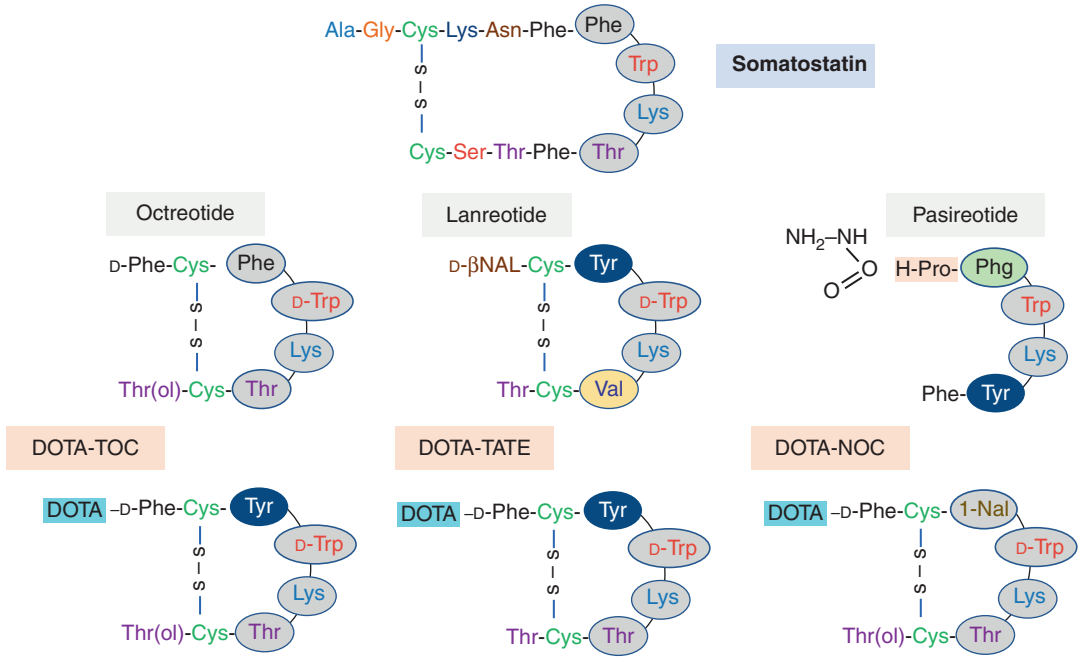

Fig. 20.5 Somatostatin analogs octreotide, lanreotide, and pasireotide are used as chemotherapeutic drugs to treat patients with neuroendocrine tumors. DOTA-conjugated octreotide analogs were developed to synthesize radiopharmaceuticals for molecular imaging and TRT

20.2.1.2 Cholecystokinin and Minigastrin (MG) Peptide

Cholecystokinin and minigastrin (MG) peptide hormones bind to the gastrin/CCK-2 receptor. Replacement of Met residues at positions 3 and 6 in CCK8 sequence with norleucine (NLe) residue produced CCK8(NLe) analog with higher affinity to the receptor. Similarly, replacement of Met residue at position 11 in MG sequence with NLe and also replacement of Phe residue at position 13 with naphthylalanine (NaI), generated two very important analogs (PP-F11N and MGS5) (Fig. 20.7) with higher affinity for the CCK-2 receptor and in vivo stability.

Bombesin (BN) is an amphibian 14-AA analog of the 27-AA human gastrin-releasing peptide (GRP). It has high binding affinity to the G protein-coupled gastrin-releasing peptide receptor (GRPR/BB2). The C-terminal 7–14 AA sequence (Fig. 20.7) is known to be critical for receptor binding and more stable in vivo than the full-length peptide molecule. Several chemical modifications have been introduced in the synthetic bombesin to stabilize the structure, to increase circulation time, to increase the binding affinity, and to potentiate agonist or antagonist properties. These analogs are usually modified by replacing Arg^3 by Lys, and at the C-terminus, Leu^{13} and Met^{14} by NNAAs such as statin (Sta) and NLe. Three important Bombesin analogs, AMBA, RM1, and RM2 (Fig. 20.7) were developed for theranostic studies.

20.2.2 Peptide Cyclization

Cyclic peptides (CPs) are generally generated from linear peptides by introducing disulfide bridges between sulfhydryl (thiol) groups of two peptidyl-cysteine residues. Cyclization of a linear peptide is usually achieved by linking the C-terminus to the N-terminus of the peptide backbone or by linking the C- or N-terminus to a side chain, or linking one side chain to another side chain. Peptide cyclization increases the pep-

Table 20.3 Affinity of SSTR peptides. Somatostatin analogs: affinity profiles IC_{50}[a] for the somatostatin receptor (SSTR) subtypes

Peptide	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
Somatostatin	5.2	2.7	7.7	5.6	4.0
Octreotide	>1000	0.4	4.4	>1000	5.6
Lanreotide	2129	0.75	98	1826	5.2
Pasireotide	9.3	1	1.5	>100	0.16
BFC-peptide conjugates					
DTPA-octreotide	>10,000	12	376	>1000	299
DOTA-TOC	>10,000	14	880	>1000	393
DOTA-TATE	>10,000	1.5	>1000	433	>1000
DOTA-NOC	>10,000	1.9	40	260	7.2
Metal-labeled chelate-peptide conjugates					
In-DTPA-octreotide	>10,000	22	182	>1000	237
In-DOTA-TOC	>10,000	4.6	120	230	130
Y-DOTA-TOC	>10,000	11	389	>1000	114
Ga-DOTA-TOC	>10,000	2.5	613	>1000	60
Ga-DOTA-TATE	>10,000	0.2	<1000	300	377
Lu-DOTA-TATE	>1000	2.0	162	>1000	1000
Ga-DOTA-NOC	>1000	1.9	40	260	7.2
Lu-DOTA-NOC	>1000	3.4	12.0	747	14.0
In-DOTA-BASS	>1000	9.4	>1000	380	>1000
In-DOTA-JR11	>1000	3.8	>1000	>1000	>1000
Ga-DOTA-JR11 (Ga-OPS201)	>1000	29	>1000	>1000	>1000
Lu-DOTA-JR11 (Lu-OPS201)	>1000	0.73	>1000	>1000	>1000
Ga-NODAGA-JR11 (Ga-OPS202)	>1000	1.2	>1000	>1000	>1000

[a]IC_{50} values expressed in nanomoles [9, 10]

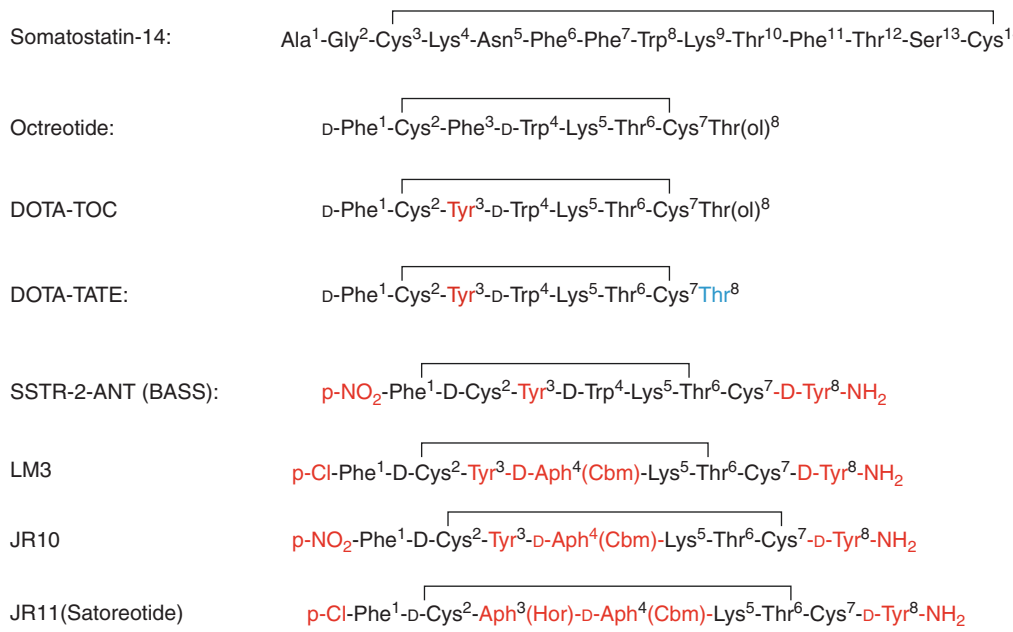

Fig. 20.6 Amino acid sequences of somatostatin (or octreotide) analogs with specific binding to SSTR-2 receptors. Octreotide, Dotatoc, and Dotatate are agonists, while BASS, LM3, JR10, and JR11 are antagonists

CCK8: D-Asp^{1}-Tyr^{2}-Met^{3}-Gly^{4}-Trp^{5}-Met^{6}-Asp^{7}-Phe^{8}-NH_2

sCCK8: D-Asp^{1}-Tyr^{2}(OSO_3H)-Met^{3}-Gly^{4}-Trp^{5}-Met^{6}-Asp^{7}-Phe^{8}-NH_2

CCK8(Nle): D-Asp^{1}-Tyr^{2}-Nle^{3}-Gly^{4}-Trp^{5}-Nle^{6}-Asp^{7}-Phe^{8}-NH_2

MG Leu^{1}-$(Glu)_5^{2\text{-}6}$-Ala^{7}-Gly^{9}-Tyr^{10}-Met^{11}-Asp^{12}-Phe^{13}-NH_2

PP-F11N $(DGlu)_6$-Ala^{7}-Tyr^{8}-Gly^{9}-Nle^{11}-Asp^{12}-Phe^{13}-NH_2

MGS5: DGlu-Ala-Tyr-Gly-Trp-(N-Me)Nle-Asp-1-Nal-NH_2

Bombesin: Pyr^{1}-Gln^{2}-Arg^{3}-Leu^{4}-Gly^{5}-Asn^{6}-Gln^{7}-Trp^{8}-Ala^{9}-Val^{10}-Gly^{11}-His^{12}-Leu^{13}-Met^{14}-NH_2

AMBA: DO3A-CH_2-CO-Gly-4-aminobenzoyl-[Gln^{7}-Trp^{8}-Ala^{9}-Val^{10}-Gly^{11}-His^{12}-Leu^{13}-Met^{14}-NH_2]

RM1: DOTA-Gly-aminobenzoyl-[D-Phe^{6}-Gln^{7}-Trp^{8}-Ala^{9}-Val^{10}-Gly^{11}-His^{12}-Sta^{13}-Leu^{14}-NH_2

RM2: DOTA-4-amino-1-carboxymethyl-piperidine-[D-Phe^{6}-Gln^{7}-Trp^{8}-Ala^{9}-Val^{10}-Gly^{11}-His^{12}-Sta^{13}-Leu^{14}-NH_2]

Fig. 20.7 Cholecystokinin (CCK), Minigastrin (MG), and Bombesin analogs specific for Gastrin-Releasing Peptide Receptor (GRPR)

tides' structural rigidity and metabolic stability by locking the peptide into a conformation that is less susceptible toward proteolytic enzymes. It can also be used to increase biological activity by locking the peptide into a more biologically active conformation [10, 11].

The integrin family comprises numerous transmembrane receptors (such as $\alpha_v\beta_3$ and $\alpha_v\beta_5$) regulating cell adhesion and interaction with the extracellular matrix [12]. These receptors are recognized by several proteins and peptides containing the tripeptide L-arginine-glycine-L-aspartic acid (RGD) sequence. However, linear RGD peptides showed in general low binding affinity (IC_{50} > 100 nmol/L), lack of specificity to $\alpha_v\beta_3$, and instability in the bloodstream due to the high degradation rate caused by the high susceptibility to proteases of the aspartic acid residue. Cyclization and incorporation of D-AA residues provided antagonists with increased stability and affinity by reducing structural flexibility as in the case of a cyclic pentapeptide, c(RGDfV). The methylated analog, c(RGDf(NMe)V) named cilengitide, with a half-life of ~2 h showed very high affinity for $\alpha_v\beta_3$ (0.61 nM) and $\alpha_v\beta_5$ (8.4 nM) integrins [6, 13]. Replacement of the Val^{5} in c(RGDfV^{5}) by Lys or Glu generated c(RGDfK) and c(RGDfE), respectively (Fig. 20.8), without altering the integrin $\alpha_v\beta_3$ binding affinity, and at the same time creating analogs which are useful for conjugating BFCs.

20.2.3 Insertion of β-Amino Acids

The incorporation of single or multiple β-AAs into peptides can decrease recognition by proteases and enhance in vivo metabolic stability and potency due to their different electronic environments, and backbone/side chain configurations compared to their α-amino acid analogs [11]. Incorporation of βAla–βAla linker, into bombesin peptide, however, did not improve in vivo stability. Bombesin analog modified with β^3-homoglutamic acid in the linker, with one single negative charge showed a significant increase in tumor uptake and tumor-to-tissue ratio but, did not necessarily increase the metabolic stability [11]. Also, the introduction of the *N*-methylated β-alanine linker in a statine-based GRPR-antagonist did not disrupt the binding affinity and presented a similar in vivo stability compared to the unmodified compound [14].

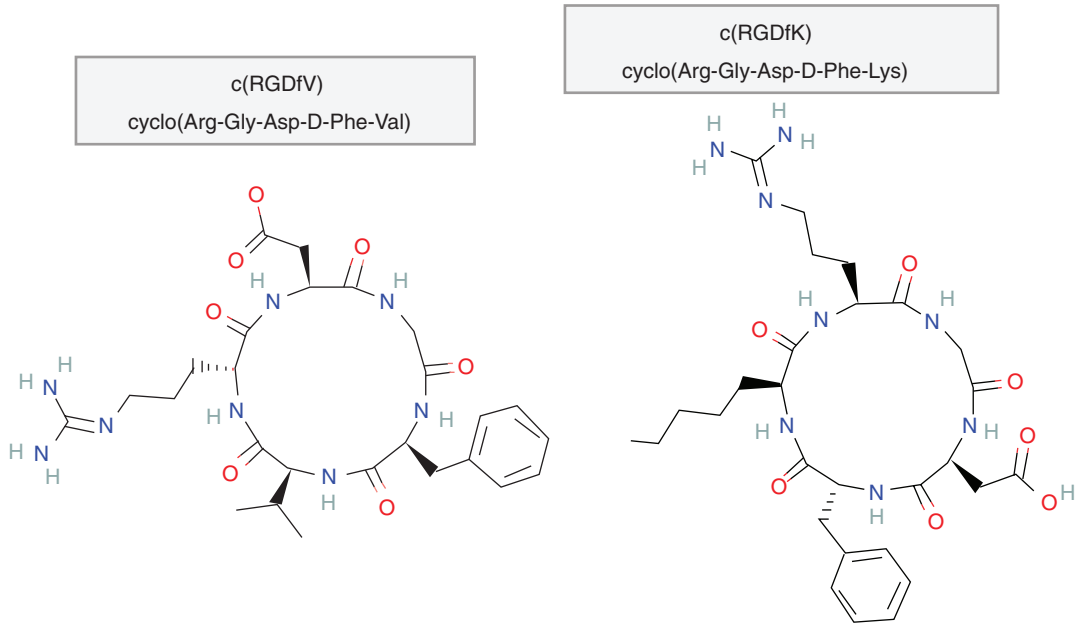

Fig. 20.8 Cyclic pentapeptide RGD analogs, c(RGDfV) and c(RGDfK) with high affinity for $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins useful for developing radiopharmaceuticals for imaging

20.2.4 Substitution of Amides with Sulfonamides

Sulfonamides are amide analogs in which the carbonyl moiety is replaced with an isosteric SO_2 group. Sulfonamide groups contain a tetrahedral achiral sulfur atom bound to two electronegative oxygen atoms. To increase the metabolic stability of peptides-based radiopharmaceuticals, one or more amide groups in the backbone of a peptide can be substituted with relatively stable sulfonamide groups [11]. The most common method is to substitute the amide with the sulfonamide group at the cleavage site. However, it has also been found that the substitution of amides close to cleavage sites can also increase metabolic stability [11]. The potential benefits of substituting amide for sulfonamide bonds have not been fully explored in the design of radiopharmaceuticals.

20.2.5 N-Methylation (N-Alkylation)

Peptide modification through *N*-methylation constitutes substituting one or more NH groups in a peptide backbone with *N*-methyl (NCH_3) substituents. N-methylation has the potential to enhance resistance to proteases, increase membrane permeability, and biological activity. The *N*-methyl group will influence the conformational flexibility of both the peptide backbone and the side chains of the residues close to the *N*-CH_3 AAs [11].

N-methylation of the amide bond between the Ile^{19} and Ile^{20} residues in the endothelin peptide antagonist derivatives increased the half-life and receptor binding affinity compared to unmodified compound [15]. The minigastrin analog MGS5 (Fig. 20.7) shows several modifications in the C-terminal sequence of the peptide, namely, replacement of Met with Nle and of Phe with 1-naphtyl-alanine (1-Nal), as well as N-methylation of the peptide bond between Trp and Nle. Preclinical studies with radiolabeled DOTA-MGS5 demonstrated high and persistent tumor uptake and favorable tumor to-background activity ratios, including kidneys, suggesting that new radiopharmaceuticals based on DOTA-MGS5 will be powerful peptide probes in the localization and treatment of patients with CCK2R-expressing tumors [16].

20.2.6 PEGylation

The technique of chemical modification of the peptide using polyethylene glycol (PEG) polymer (Fig. 20.9) by covalent or non-covalent attachment is known as PEGylation. PEG possesses useful properties, including high solubility in water and many organic solvents, non-toxicity, and non-immunogenicity, and has been approved by the FDA for human use. PEGylation can improve both the physiochemical and PK performance of the peptides. The effects of PEGylation on peptide pharmacokinetics include avoidance of reticuloendothelial (RES) clearance, mitigation of immunogenicity, and reduction of enzymatic proteolysis and of losses by renal filtration, with potentially beneficial changes in biodistribution [11, 17]. These effects can dramatically increase the half-life of a peptide in circulation but, without adversely affecting binding and activity of the peptide ligand.

PEG's most common form is a linear or branched polyether with terminal hydroxyl groups. Monofunctional methoxy-PEG (mPEG) is the most common reagent used for PEGylation. PEG can also be derivatized with several linkage moieties yielding methoxyPEG-amines, -maleimides, or carboxylic acids (Fig. 20.9). The length and shape of PEGs (linear, branched, or dendritic) have been shown to influence the pharmacological properties of the PEGylated peptides and proteins, with branched PEG structures often most effective [11]. Many PEGylated peptide-based radiopharmaceuticals have been developed and shown to possess improved pharmacokinetic properties compared to their unmodified analogs, including increased receptor binding affinity, increased tumor uptake, and decreased kidney uptake [6, 11, 17]. Based on preclinical studies, radiolabeled DOTA-bombesin analogs with PEG chain as a linker between DOTA and the peptide showed superior PK properties and increase in half-life [18]. Incorporation of a mini-PEG (three ethylene oxide units) spacer into an RGD cyclic peptide was used to produce [^{18}F]-FB-mini-PEG-E[c(RGDyK)]^2. In preclinical studies, this agent showed greater radiolabeling yield, reduced renal clearance, and similar tumor uptake compared to the non-PEGylated analog [19].

20.2.7 Glycosylation

Glycosylation is a chemical reaction used to attach a carbohydrate (or glycan) to a hydroxyl or other functional group of another molecule. In N-linked glycosylation reactions, the glycans are attached to a nitrogen of asparagine or arginine side chain. In case of O-linked reactions, the glycans are attached to the hydroxyl oxygen of AA residues such as serine, threonine, and tyrosine. The introduction of carbohydrate moieties into a peptide changes the physiological properties of peptides, and may protect AA's side chain from oxidation, increase meta-

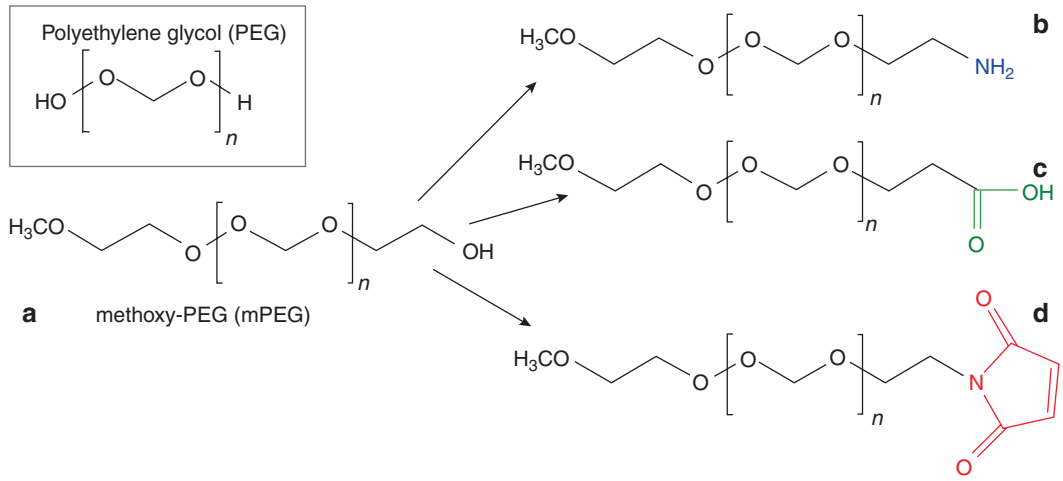

Fig. 20.9 Pegylation is a technique of chemical modification of the peptide using polyethylene glycol (PEG) polymer. Methoxy-PEG (mPEG) (**a**) is the most common reagent used for PEGylation. PEG can also be derivatized with several linkage moieties yielding methoxyPEG-amines (**b**), -carboxylic acids (**c**), or maleimides (**d**)

bolic stability, improve penetration through biological membranes, and even may facilitate active transport of the modified peptide by targeting glucose transporters on the cell membrane [20]. Conjugation of radiolabeled bombesin analogs with glucose moiety (through a triazole group) reduced abdominal accumulation and increased the uptake by tumors without affecting the cell internalization of the modified peptides [21]. Glycosylation of CCK analog, obtained by glucose binding to the Lys side chain at N-terminal region of the synthetic peptide, contributed to decrease its lipophilicity and to improve sensitivity, specificity, and pharmacokinetics in CCKR-expressing tumors [22]. Glycosylation and pegylation of modified cyclic RGD peptide have also shown to improve the pharmacokinetics. In particular, the F-Galacto-c(RGDfK) and c(RGDfK)-Peg-MPA) (MPA, mercapto propionic acid) showed IC_{50} of 100 nM and 8–15 nM, respectively [6]. Several examples (Fig. 20.10) for the application of ^{18}F-fluoroglycosylation as a strategy for the suc-

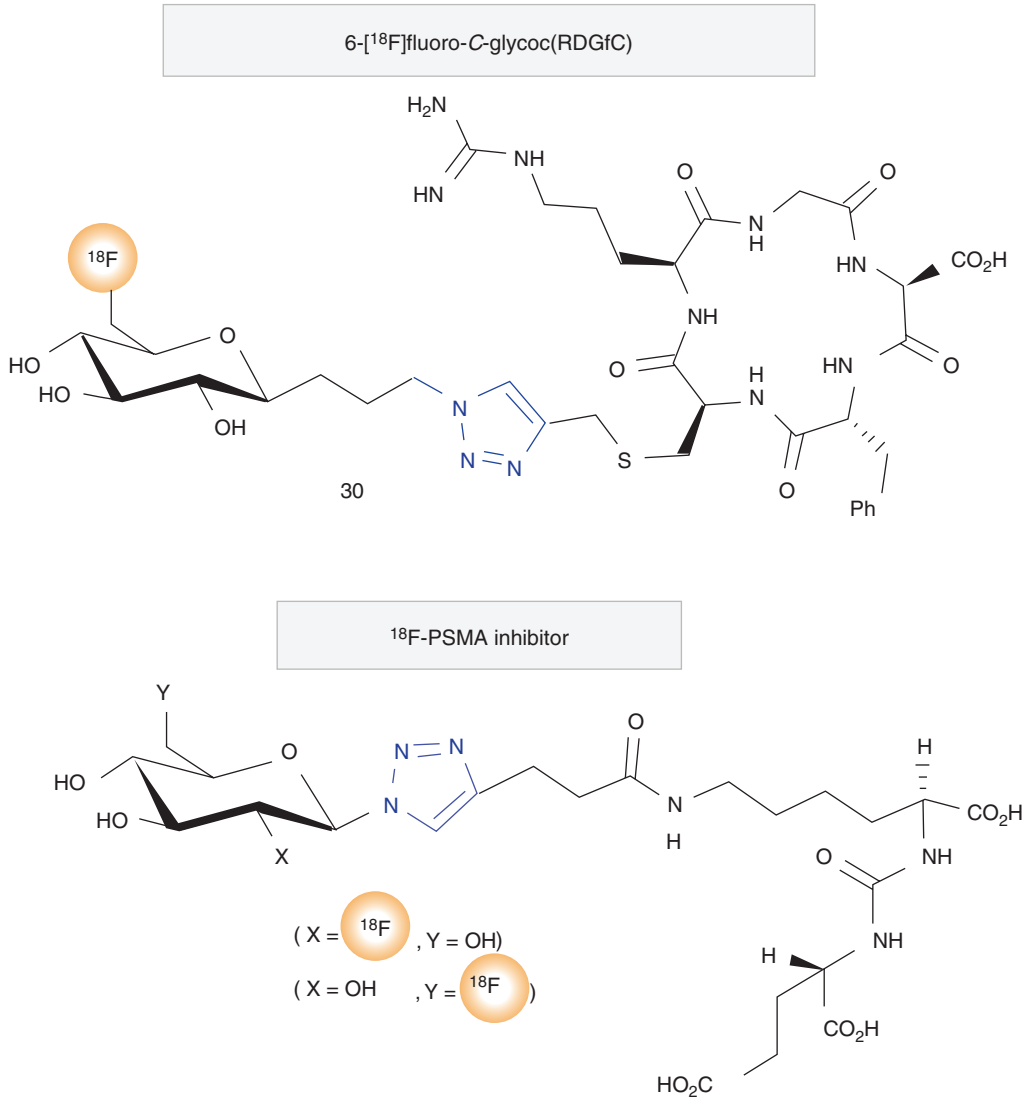

Fig. 20.10 Examples of ^{18}F labeled-RGD peptide and PSMA inhibitor synthesized based on click chemistry via Cu(I)-catalyzed Huisgen 1,3-cycloaddition reaction of an azide and an acyclic alkyne (CuAAC) using the ^{18}F-labeled prosthetic group 6-deoxy-6-[1^{8}F]fluoro-β-glucosyl azide. (From [23])

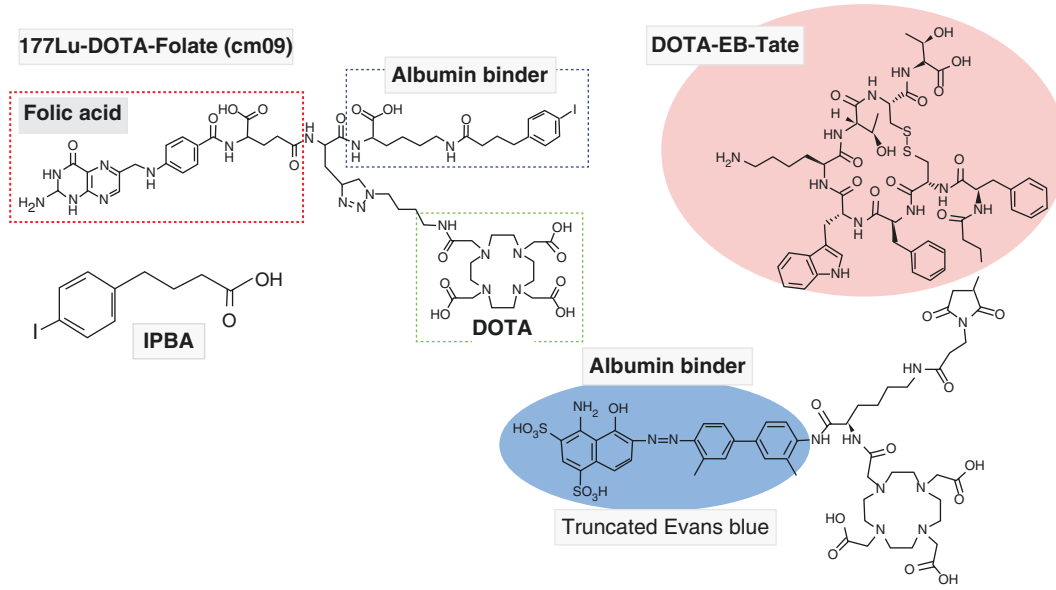

Fig. 20.11 Examples of ^{177}Lu labeled radiotracers with albumin binding molecule attached to the target specific vector. Folate analog was developed using 4-(p-iodophenyl)butyric acid (IPBA) as the albumin binder, while truncated Evans blue was used as the albumin binder to develop Dotatate analog. (Figures modified from Muller et al. [26] and Tian et al. [27])

cessful development of PET tracers has been recently reviewed [23].

20.2.8 Albumin Binding

The plasma protein, human serum albumin (HAS) has a long half-life of about 19 days, and because of its high molecular weight (67 kDa), it has low renal clearance making the protein a valuable candidate as a drug delivery system and a means to extend the half-life of peptides [4, 24, 25]. HSA is a widely recognized carrier for the passive targeting to solid tumors and has been frequently used to develop drug conjugates for longer plasma half-life. The covalent or non-covalent attachment of peptides to albumin can reduce the glomerular filtration rate and extend the half-life of peptides by increasing the size of peptide-based drugs. Albumin is also found to specifically target tumor regions because of its enhanced permeability and retention (EPR) effect as well as albumin receptor binding, which is a unique advantage as the carrier for tumor-targeted drug delivery [25].

In addition to fatty acids, serum albumin can intrinsically bind a large diversity of small endogenous and exogenous organic molecules, shielding their hydrophobic character and strongly increasing their solubility in plasma [25]. Albumin binding ligands based on the lead structure 4-(p-iodophenyl)butyric acid (IPBA) (Fig. 20.11) have been identified by screening DNA-encoded chemical libraries [28]. The best derivative of IPBA known as Albutag was used to develop radiolabeled folate conjugates for imaging and therapy [26]. Albutag was also used to develop a novel class of trifunctional ligands, consisting of the high-affinity PSMA-binding domain, the Albutag, and the DOTA chelator, to facilitate the modification of the three moieties independently and ultimately enable the generation of spatial optimized conjugates PSMA conjugates for prostate cancer theranostics [29]. Preclinical studies demonstrated that the trifunctional ligands had high and persistent tumor

uptake with absorbed doses that were four times greater than those observed for a similar compound lacking the albumin-binding moiety. Albutag was also used to develop albumin-binding PSMA-targeting PET radioligands based on NODAGA chelator [30].

Amino acid analogs of IPBA (Lys-Glu-IPBA and Lys-Asp-IPBA) were used to investigate whether prolonging blood residence time of [^{177}Lu]Lu-DOTATATE with albumin binders could increase tumor accumulation and tumor-to-kidney ratios for improved therapeutic efficacy. Preclinical studies demonstrated that addition of an albumin binder to DOTATATE increased blood residence time and tumor uptake of [^{177}Lu]Lu-AspAB-DOTATATE; however, the increase in kidney uptake was proportionally higher, thus reducing the therapeutic index and clinical usefulness [31].

Evans blue (EB) is an azo dye that binds to serum albumin with low micromolar affinity, and each albumin molecule can bind up to 14 molecules of EB [32]. Several EB derivatives were developed to facilitate the development of radiolabeled peptides [32]. EB was used to increase the diagnostic and therapeutic efficacy of radiolabeled DOTATATE in patients with SSTR-2 positive neuroendocrine tumors. Preclinical studies in various animal tumor models demonstrated that the circulation half-life of radiolabeled EB-TATE was twice than that of DOTATATE and the tumor accumulation was much higher. Clinical studies with ^{177}Lu-DOTA-EB-TATE in patients with metastatic NETs demonstrated higher blood retention, and achieved a 7.9-fold enhancement of tumor activity. However, the effective renal dose of ^{177}Lu-DOTA-EB-TATE was significantly higher than that of ^{177}Lu-DOTA-TATE [33].

20.2.9 Spacers/Linkers

Spacers are inert molecules used to increase the distance of peptides from chelators to prevent steric influence and loss of activity on the cell receptors upon functionalization [34]. In fact, the molecular size, lipophilicity, and the flexibility of the functional moiety can influence the binding of the bioactive peptide to its target [6]. Many spacers of different kinds, such as hydrocarbon chain, amino acid sequence, and PEG can be introduced to modify the pharmacokinetic properties of these biomolecules. Spacers with different charge and hydrophilicity affect the characteristics of the peptide conjugate. It has been shown that the complex with uncharged and hydrophobic spacers leads to increased liver uptake, while the composition with positively charged spacers results in high kidney retention. Therefore, the pharmacokinetics of radio complexes correlates to the structure and total charge of the conjugates [35]. In fact, the molecular size, lipophilicity, and the flexibility of the functional moiety can influence the binding of the bioactive radiolabeled peptide to its receptor [18, 36].

The influence of different spacers (PEG2, PEG4, N-acetyl glucosamine (GlcNAc), triglycine, β-alanine, aspartic acid, and lysine) between the chelator DOTA and the SST analog NOC were investigated. It was observed that in general, the spacers marginally influenced the binding affinities to the SSTR2 and SSTR5 receptor subtypes but, resulted an almost complete loss of SSTR3 affinity of the [^{111}In-DOTA]-X-NOC peptides [3]. In preclinical studies, the influence of PEG spacers of different lengths on the biological profile of bombesin-based radiolabeled peptide antagonists were investigated. Among all the analogs studied, the PEG_4 and PEG_6 showed significantly better properties; very high tumor-to-non-target organ ratios, in particular, tumor-to-kidney ratios [18, 37].

20.2.10 Dimerization and Multimerization

Over the last years, many radiolabeled peptides were developed for diagnostic imaging and therapy, including peptides consisting of more than one copy of the targeting peptide. The radiolabeled peptides in routine clinical use (such as SST analogs), however, are primarily monovalent and composed of monomeric domain specific for one receptor. In contrast, multimeric

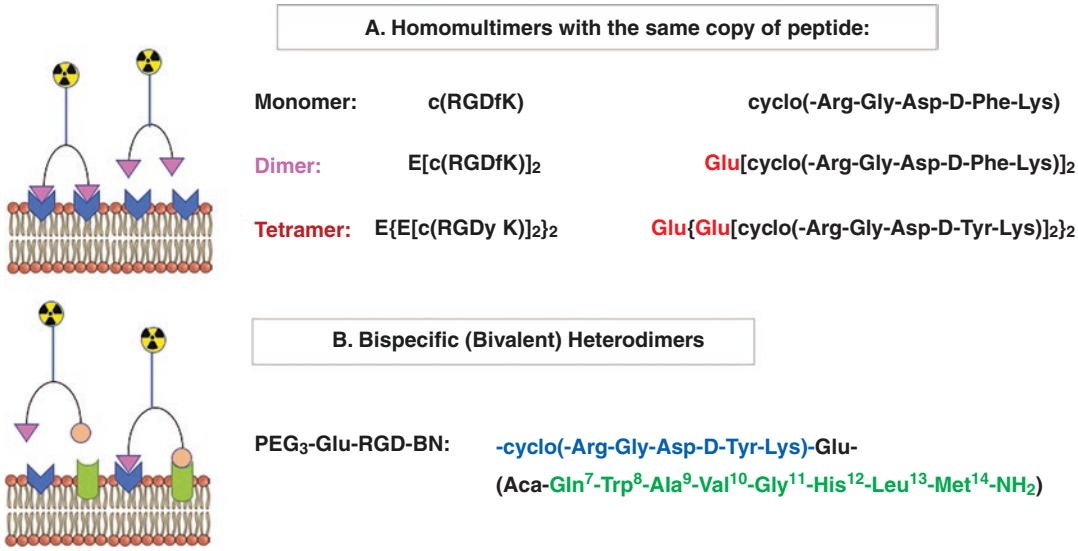

Fig. 20.12 Dimerization and multimerization of targeted peptide radiopharmaceuticals

ligands are composed of more than one monomeric domain attached to a backbone molecule so that the compound has the capability to bind to multiple binding sites on the same receptor or different receptors simultaneously (Fig. 20.12). Radiotracers being composed of two or more different target affine ligands designed to bind to several different receptors are known as heterobivalent ligands (or heteromultimers). If they bind to only one receptor by at least one allosteric interaction, such tracers are called bitopic ligands (or homomultimers).

In preclinical studies, ^{99m}Tc-HYNIC labeled dimeric RGD-peptide was compared with those of the monomeric analog [38]. Binding assays demonstrated a tenfold higher binding affinity to $\alpha_v\beta_3$ integrin for the dimeric analog compared with the monomeric (IC_{50} values: 0.1 and 1.0 nM). The tumor uptake of both analogs was good but, was considerably greater for the dimeric RGD-peptide. In addition, the dimeric RGD-peptide showed greater kidney uptake and enhanced retention due to the difference in the molecular masses of the peptides. Similar studies with ^{64}Cu labeled tetrameric and octameric RGD peptides showed that ^{64}Cu-DOTA-octameric-RGD peptide had the greatest binding affinity, greater tumor uptake, and enhanced tumor retention compared with ^{64}Cu-DOTA-RGD tetramer [39].

Since several cancer cells express both integrin $\alpha_v\beta_3$ and GRP receptors, both RGD and BN radiopeptides have been widely used. ^{18}F-labeled PEGylated-RGD-Bombesin heterodimer was developed and compared to the non-PEGylated analog, and the corresponding monomers. The receptor binding affinities of heterodimer were comparable to the corresponding monomers. Biodistribution and μPET imaging studies in nude mice with PC-3 cells confirmed that ^{18}F-FB-PEG_3-Glu-RGD-BN displayed rapid and high tumor uptake with high tumor contrast and favorable PK with reduced liver accumulation [4, 40].

Radiolabeled heterobivalent peptidic ligands (HBPLs), being able to address different receptors, are highly interesting tumor imaging agents as they can offer multiple advantages over monovalent peptide receptor ligands. Furthermore, the application of a radiolabeled heterobivalent agent can solve the ubiquitous problem of limited tumor visualization sensitivity caused by differential receptor expression on different tumor lesions [4]. In the last 10 years, heterodimeric peptides for imaging and therapy were developed with several combinations of receptors, such as $\alpha_v\beta_3$ and GRPR, $\alpha_v\beta_3$ and PSMA and $\alpha_v\beta_3$ and STTR. In a pilot study, the safety, radiation dosimetry, and diagnostic performance of ^{68}Ga-BBN-RGD heterodimer peptide radiophar-

maceutical was studied in patients with breast cancer [32, 33, 41]. Both the primary cancer and metastases showed positive ^{68}Ga-B1/2BN-RGD accumulation.

20.3 Radiolabeling of Peptides

As described earlier, the first radiolabeled peptide used in 1989 for imaging studies was ^{123}I-Tyr^3-octreotide to detect SSTR-2 receptor positive NETs. In the last three decades, hundreds of radiolabeled peptides were developed and evaluated in preclinical studies to assess the potential for molecular imaging and TRT studies. Some of the most common radionuclides used in the development of radiolabeled peptides are shown in Table 20.4. Except for the radioisotopes of halogens (F, I, and At), all other radionuclides are metals.

The choice of the labeling approach is driven by the nature and the chemical properties of the radionuclide. The choice of radionuclide for peptide labeling, and different methods of labeling of radionuclides to the peptides will be discussed briefly here. More detailed discussions of this topic are also presented in Chaps. 8 and 18.

20.3.1 Radionuclides

Radionuclides are useful for molecular imaging studies based on PET or SPECT decay either by positron (β^+) emission, electron capture (*EC*) or isomeric transition (IT). The radionuclides that are used for therapy decay by emitting either β^- particles or α particles.

The decay characteristics of some of the important radionuclides routinely used for imaging and therapy are listed in Table 20.4. Among the radionuclides listed in this table, radioisotopes of F, I, and At are non-metals and belong to the halogen (Group-7) family. All other radionuclides are metals differing in valency, oxidation state, and co-ordination chemistry. The γ-emissions of radionuclides decaying by IT or

Table 20.4 Radionuclides for imaging and therapy

Radionuclide	$T_{1/2}$	Decay Mode	%	γ Energy keV	%	Particle, E_{mean} keV	%	
^{99m}Tc	6 hours	IT	98	140				SPECT
^{123}I	13.22 days	EC	97	159	83.6			SPECT
^{111}In	2.8 days	EC	100	171.3 245.4	90.7 94.1			SPECT
^{18}F	109.8 min	β^+	96.73			250	96.73	PET
^{68}Ga	67.7 min	β^+	87	1077	3.22	830	99.9	PET
^{64}Cu	12.7 hours	β^+ β^- EC	17.6 38.5 44			278	17.6	PET
^{89}Zr	3.3 days	β^+ EC	23 77	909	99	396	22.7	PET
^{124}I	4.18 days	β^+ EC	23 77	603 723 1691	62.9 10.4 11.2	822	22.7	PET
^{131}I	8.025 days	β^-	100	364.5	81.5	181.9	100	Therapy
^{90}Y	2.66 days	β^-	100			932.3	100	Therapy
^{177}Lu	6.647 days	β^-	100	113 208	6.23 10.4	133.6	100	Therapy
^{213}Bi	45.6 min	α	100	440.5	25.9	8440	100	Therapy
^{211}At	7.2 hours	α	41.8			5869	41.8	Therapy
^{225}Ac	9.92 days	α	100	218[a] 440.5[a]	11.5 25.9	5830	100	Therapy

[a]Gamma photons from the daughters Fr-221 and Bi-213

EC are useful for planar and SPECT imaging studies, while PET is based on the annihilation radiation (511 keV photons) from radionuclides decaying by positron (β^+) emission.

The radionuclides that are used for therapy decay by emitting either β^- particles or α particles. The cytotoxic effects of therapy radionuclides are primarily due to irreversible DNA damage and cell death. Alpha particles with high LET deposit their high kinetic energy (5–8 MeV) over short distances (<100 μm) compared to low LET β^- particles (<15 mm). The decay mode and the kinetic energy of non-penetrating particle radiation will determine the particle range in the tissue the amount of energy absorbed (expressed in Grays) in the tissue.

It is also important to recognize that several radionuclides are also available as theranostic-pair, ideal for both imaging and therapy. Isotopes of the same element (such as ^{123}I, ^{124}I, and ^{131}I) have similar chemistry and the in vivo behavior of radiotracers labeled with isotopes of the same element will be identical. This contrasts with nonchemically identical matched pairs of isotopes (such as ^{111}In/^{90}Y and ^{68}Ga/^{177}Lu), which may have different biodistribution and PK. Therefore, ^{111}In or ^{68}Ga labeled diagnostic radiopharmaceuticals can only be regarded as chemical/biological surrogates for ^{90}Y, ^{177}Lu, ^{225}Ac, and other trivalent metal-labeled radiopharmaceuticals.

Radionuclidic purity and specific activity (SA) of radionuclides are very important quality control indicators, and provide quantitative information regarding the purity of the radionuclide, and the mass of radionuclide for a given amount of activity (MBq or mCi per mg or μmole). The radionuclide purity and SA, however, are very much dependent on the methods and nuclear reactions used to produce the radionuclides by cyclotrons and reactors. (please refer to Chap. 8, regarding the production of radionuclides).

20.3.2 Radiolabeling Methods

The labeling of peptides with radionuclides can be performed by direct labeling, with the addition of a prosthetic group. Direct labeling is the method used to label peptides without using intermediates, such as BFCs. Direct labeling technique are generally used mostly for radioiodination and in some cases labeling with Tc-99m. Prosthetic groups are small molecules able to bind with radionuclides in one site of the structure and, simultaneously, with a peptide at a second site. Prosthetic groups are bifunctional agents that consist of a suitable site for radioiodination or fluorination and functional groups to allow covalent attachment of the peptide. Radiometals specifically require bifunctional chelating agents (BFC or BFCA) to obtain the best conjugation of radiometal with peptides. The bifunctional nature of the chelators means that they have functional groups that can coordinate (form a complex) a metal ion and can also be covalently attached to the peptide.

20.3.3 Peptide Labeling with Radioiodine

Radioiodination of peptides can be performed either by electrophilic substitution (direct method) or via conjugation (indirect method) [3, 42, 43]. The reaction consists of the electrophilic substitution of an aromatic proton by electrophilic radioiodine and it takes place in an amino acid residue of the peptide which affords strong (but also weak) electron donating groups (e.g., -OH, -NH_2, -OR, -SR, -NHCOR) [3]. The tyrosine (Tyr) or histidine (His) side chains in peptides offer the possibility of electrophilic aromatic substitution by electrophilic radioiodine with high efficiency under mild conditions. Since radioiodide is stable and chemically non-reactive, several oxidizing agents (such as chloramine T, Iodogen®, lactoperoxidase/H_2O_2) can be used for the generation of electrophilic iodine species ((HO^*I, H_2O^*I)). With Tyr residue, substitution of a hydrogen ion with the reactive iodonium ion occurs *ortho-* to the phenolic hydroxyl group, while with histidine, substitution occurs at the second position of the imidazole ring. Electrophilic substitutions can often be per-

formed fast on a nonderivatized substrate under mild reaction conditions.

In an indirect method, the incorporation of radioiodine can be performed by the utilization of radioiodinated prosthetic groups, which can be used for conjugation with specific functionalities introduced previously into the peptide precursors, such as amine, aminooxy or thiol groups [43]. Active esters, such as N-succinimidyl-4-iodobenzoate (PIB), N-succinimidyl-3-iodobenzoate (SIB), and N-succinimidyl-5-[*I] iodo-3-pyridine carboxylate (SIPC), have been developed. Aldehydes, such as 4-iodobenzaldehyde, have been used for the coupling of peptides to form stable radiolabeled oximes. This methodology has been proposed for radioiodination of multimeric cyclic RGD peptides [42]. Recently, the first bimodal fluorinated and iodinated prosthetic group, tetrafluorophenyl 4-fluoro-3-iodobenzoate (TFIB), was introduced as a suitable acylating agent for the labelling of a wide variety of primary amine-containing compounds. [^{125}I]/[^{18}F]TFIB was successfully used to tag tumor-targeting peptides, such as PEG3[c(RGDyK)]$_2$ and NDP-MSH, targeting αvβ3 integrin and MC1R receptors, respectively [43]. ^{125}I ($T_{1/2}$ = 59.4 days) labeled peptides are especially useful for all the preclinical development work to optimize the design of peptides for imaging and therapy (Fig. 20.13).

20.3.4 Peptide Labeling with Fluorine-18

^{18}F has the most ideal half-life and has a unique, and diverse chemistry for labeling of peptides. The most common method of cyclotron production is based on ^{18}O(p, n)^{18}F nuclear reaction, and ^{18}F-fluoride is produced in high amount of radioactivity (74–370 GBq), and SA (100 GBq/μmol) for use as a nucleophile. Fluorination of peptides may be conducted by direct methods or indirect methods. Direct methods are those in which the ^{18}F-fluoride is reacted directly with the peptide, which may have been previously modified to facilitate radiolabeling, and only subsequent purification is required to obtain the final product. Indirect methods require the prior radiosynthesis of a prosthetic group and subsequent bioconjugation to a peptide that has been modified for site specific reaction [3, 44–47].

Several successful attempts to introduce ^{18}F directly into small peptides have been reported in the literature. Direct substitution methods, how-

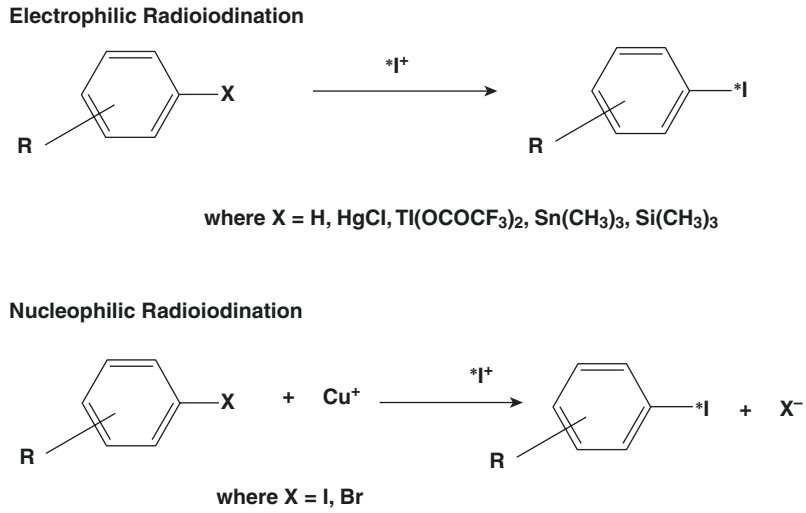

Fig. 20.13 Electrophilic and nucleophilic radioiodination

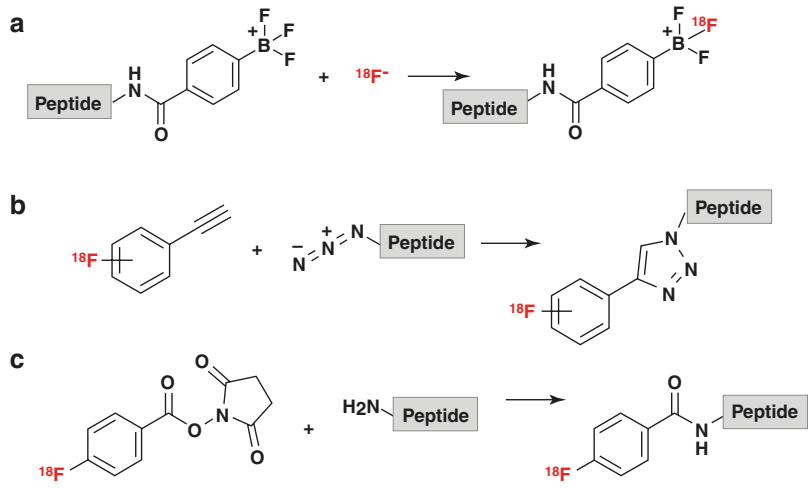

Fig. 20.14 Examples of radiofluorination techniques based on substitution or addition reactions commonly used in the synthesis ^{18}F labeled peptides. Isotope exchange (**a**), click chemistry (**b**), and Fluorinate prosthetic group (**c**)

ever, usually require some non-physiological conditions of pH or temperature and most peptides do not tolerate such conditions, and may undergo hydrolysis. The use of prosthetic groups for the direct ^{18}F-labeling of peptides was first reported based on trimethylammonium-substituted modified peptides. This methodology has demonstrated usefulness toward direct ^{18}F-fuorination of peptides containing histidine, tryptophan, lysine, and arginine residues without the need of protecting groups [44]. Several new strategies for direct ^{18}F labeling have also been developed recently. An elegant site-selective C–H ^{18}F-fuorination of leucine residue within complex peptide using [^{18}F]-N-fluorobenzenesulfonimide ([^{18}F]NFSI) showed that a combination of photoactivated sodium decatungstate and ([^{18}F]NFSI effects site-selective ^{18}F-fluorination at the branched position in leucine residues in the unprotected and unaltered native peptides [48]. A method to introduce a 4-[^{18}F]fluorophenylalanine residue into peptide sequences by chemo-selective radiodeoxyfluorination of a tyrosine residue can label small peptides by formally replacing a single hydrogen, the para-hydrogen atom in Phenylalanine residue with [^{18}F]fluoride [49].

In the indirect labeling methods, the peptides are attached to the prosthetic groups mostly through amine- or thiol-reactive groups via acylation, alkylation, amidation, imidation, oxime, hydrazone formation, or using click chemistry [45]. Selected examples of prosthetic groups include [^{18}F]SFB, [^{18}F]FBA, [^{18}F]FBAM, and [^{18}F]FBEM. The choice of prosthetic group, however, is critical for radiotracer development, as they may adversely alter the physical and physiological characteristics of the labeled molecule. Several examples of radiofluorination techniques based on substitution or addition reactions are shown in Fig. 20.14.

Since fluorine was known to bind and form stable complexes with many metals, a successful method was developed to form a stable NOTA Al-^{18}F complex of a peptide conjugated with macrocyclic chelators (such as NOTA) [50]. This labeling methodology has been used by several groups for the labeling of small molecules, peptides, and proteins using several acyclic and macrocyclic chelators (Fig. 20.15) [45, 52, 53]. The Al-[^{18}F]fluoride ([^{18}F]AlF) radiolabeling method combines the favorable decay characteristics of fluorine-18 with the convenience and familiarity of metal-based radiochemistry and has been used to parallel ^{68}Ga radiopharmaceutical developments. As such, the [^{18}F]AlF method is popular and widely implemented in the development of radiopharmaceuticals for the clinic [51].

20.3.5 Peptide Labeling with Trivalent Radiometals

Among the metals shown in Table 20.5, Tc-99 m is a unique transition metal exhibiting oxidation

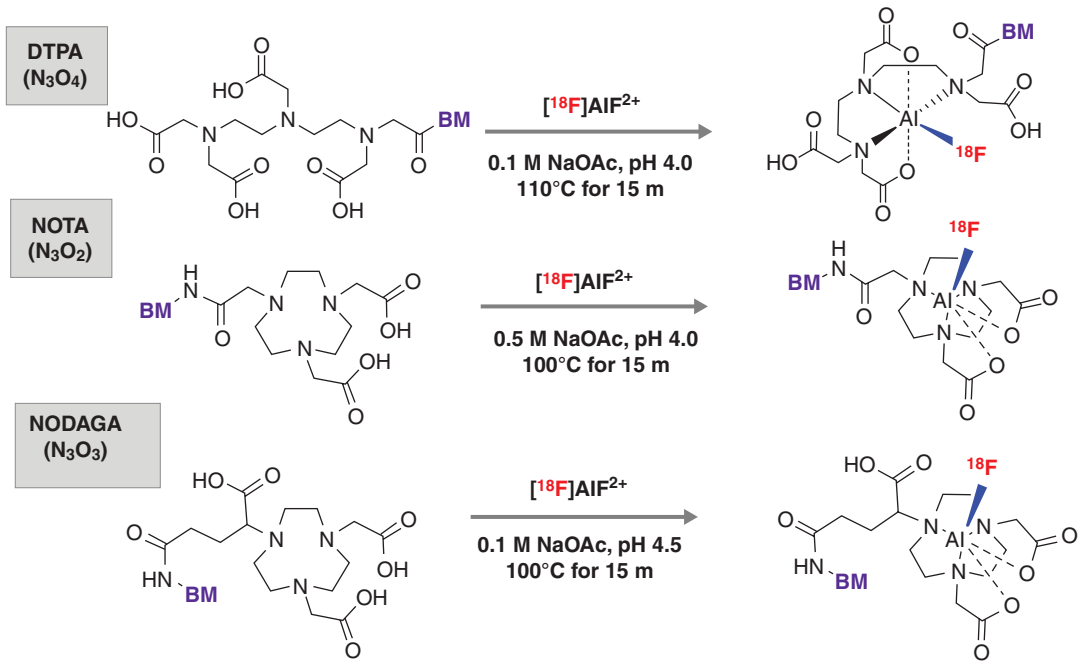

Fig. 20.15 ^{18}F labeling of peptides or biomolecules (BM) using aluminum-fluoride method [^{18}F]AlF. (Figure modified from Archibald and Allott [51])

Table 20.5 Physical and chemical characteristics of radiometals

	Transition metals			Post-transition metals				Lanthanides	Actinides	
Property	Cu	Y	Tc-99 m	Ga	In	Pb	Bi	Lu	Ac	Th
Atomic number	29	39	43	31	49	82	83	71	89	90
Group	11	3	7	13	13	14	15	3	n/a	n/a
Atomic radius (pm)	128	181	136	122	163	175	156	174		108–135
Ionic radius (pm)	80	90–108	51–78.5	47–62	62–92	79–143	96–117	86–103	112	105
Electron structure	[Ar] $3d^{10}$ $4s^1$	[Kr] $4d^1$ $5s^2$	[Kr] $4d^5$ $5s^2$	[Ar] $3d^{10}$ $4s^2$ $4p^1$	[Kr] $4d^{10}$ $5s^2$ $5p^1$	[Xe] $4f^{14}$ $5d^{10}$ $6s^2$ $6p^2$	[Xe] $4f^{14}$ $5d^{10}$ $6s^2$ $6p^3$	[Xe] $4f^{14}$ $5d^1$ $6s^2$	[Rn] $6d^1$ $7s^2$	[Rn] $6d^2$ $7s^{2X}$
Electronegativity	1.90	1.22	1.9	1.81	1.78	1.87 2.33	2.02	1.27	1.1	1.3
Oxidation state	+1, +2	+3	−3 to +7	+3	+3	+2, +3	+3	0 to +4	+3	+1 to +4
Coordination number	4	6–9	5–7	4–6	4–8	4–8	5–8	6–9	6–8	8

states from +7 to −1 and it can be stabilized using various ligands and coordination environments. Therefore, the chemistry of Tc-99 m will be discussed separately. All other metals (except copper) predominantly are hard Lewis acids and exist as trivalent cations (3^+) and share similar chelation chemistry. Copper is an intermediate Lewis acid and prefers 2^+ oxidation state. Hard metal ions prefer hard donating groups (e.g., carboxylic acids), which possess dense anionic character. Conversely,

soft metals have low charge density and polarizable electron shells, and form covalent bonds with softer, more electron-disperse donor groups.

20.3.5.1 Bifunctional Chelators (BFCs)

Chelation is a type of bonding of ions and molecules to metal ions. It involves the formation of two or more separate coordinate bonds between a polydentate ligand (known as chelators or chelating agents) and a single central metal atom. DTPA and DOTA are the two most frequently used chelators in nuclear medicine. The chelating agents have been modified to function as BFCs since they have a metal binding moiety function for the sequestration of the metallic radionuclide and also possess a chemically reactive functional group that can provide the requisite chemistry for covalent attachment to a targeting vector of interest, such as a small molecule peptide. The primary goal of radiometal complexation using BFCs is the formation of robust coordination complexes to prevent the release of free metals in vivo. Based on the metal chemistry, it is important to identify the most appropriate BFC that provides both the thermodynamic stability in vitro, and kinetic stability in vivo (Table 20.6).

Polyaminocarboxylate ligands (containing nitrogen and oxygen donor atoms) have both acyclic, and macrocyclic options to encapsulate the metal ion. Initially, acyclic BFCs, such as EDTA, DTPA, and their derivatives were used to prepare ^{111}In and ^{90}Y labeled antibodies. To improve the in vivo stability of ^{90}Y labeled antibodies, full octadentate macrocyclic bifunctional DOTA derivatives have been developed for complexing trivalent radiometals, such as ^{111}In, ^{86}Y/^{90}Y, and ^{177}Lu [3, 6, 54, 55]. The most common chelators and BFCs used in the development of radiolabeled peptides are shown in the Figs. 20.16 and 20.17. Each of these chelating agents (Table 20.6) differ in size and offer different donor groups like carboxylic acids, alcohols, amines, thiols, and phosphonic acids.

Macrocyclic BFCs are generally more kinetically inert and require minimal physical manipu-

Table 20.6 Acyclic and macrocyclic ligands (or chelating agents) used for the labeling of peptides with radiometals

Chelator	Chemical name	Donor atoms	Coordination number (CN)	Metals
DTPA	Diethylenetriaminepentaacetic acid	N_3O_5	8	Ga, In
EDTA	Ethylenediaminetetraacetic acid	N_2O_4	6	Ga, In
HBED-CC	3-[3-[4-[5-(2-carboxyethyl)-2-hydroxyphenyl]-1,4-bis(carboxymethylamino)butyl]-4-hydroxyphenyl]propanoic acid	N_2O_6	8	Ga
NOTA	1,4,7-triazacyclononane-1,4,7-tri-acetic acid	N_4O_3	6	Ga
DOTA	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid	N_4O_4	8	In, Ga, Y, Lu, Bi, Ac, Th, Cu
TCMC	1,4,7,10-tetrakis(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane	N_4O^4	8	Pb
TETA	1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid	N_4O_4	8	Cu
CB-TE2A	4,11-bis-(carboxymethyl)-1,4,8,11-tetraazabicyclo[6.6.2]-hexadecane	N_4O_2	6	Cu
SarAr	1-*N*-(4-aminobenzyl)-3,6,10,13,16,19-hexaazabicyclo[6.6.6]eicosane-1,8-diamine (SarAr)	N_6	6	Cu
PCTA	3,6,9,15-Tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene-3,6,9-triacetic acid	N_4O_3	7	Ga, Cu
PEPA	1,4,7,10,13-pentaazacyclopentadecane-N,N′,N″,N‴,N⁗-pentaacetic acid	N_5O_5	10	Ac
HEHA	1,4,7,10,13,16-hexaazacyclohexadecane-N,N′,N″,N‴,N⁗,N⁗′-hexaacetic acid	N_6O_6	12	Ac

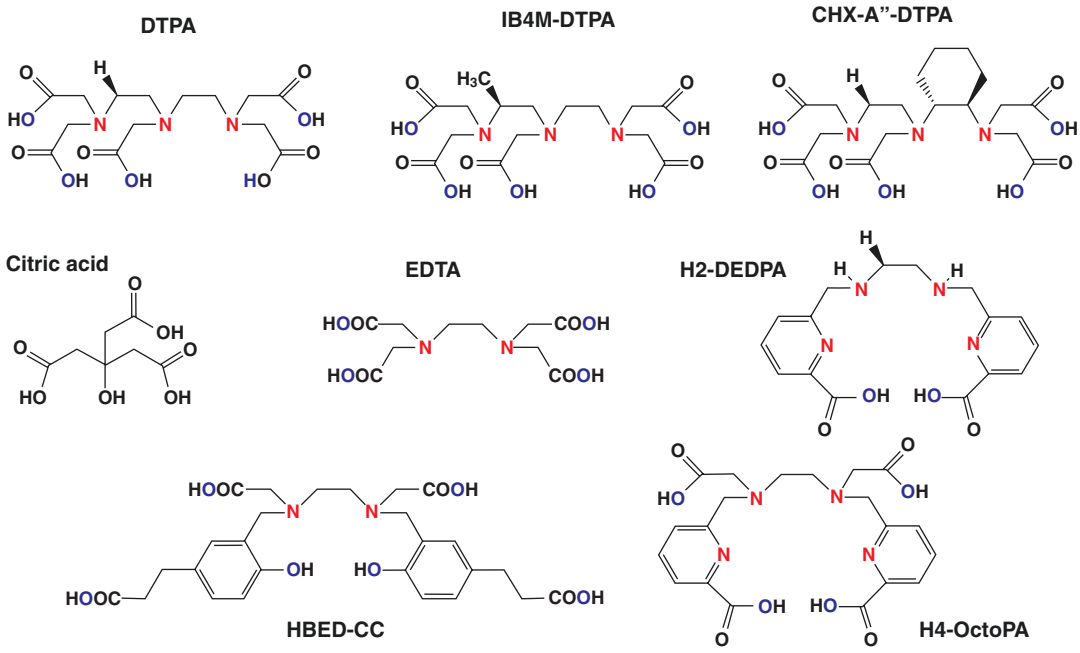

Fig. 20.16 Acyclic chelators used for the synthesis of radiometal-labeled peptide radiopharmaceuticals

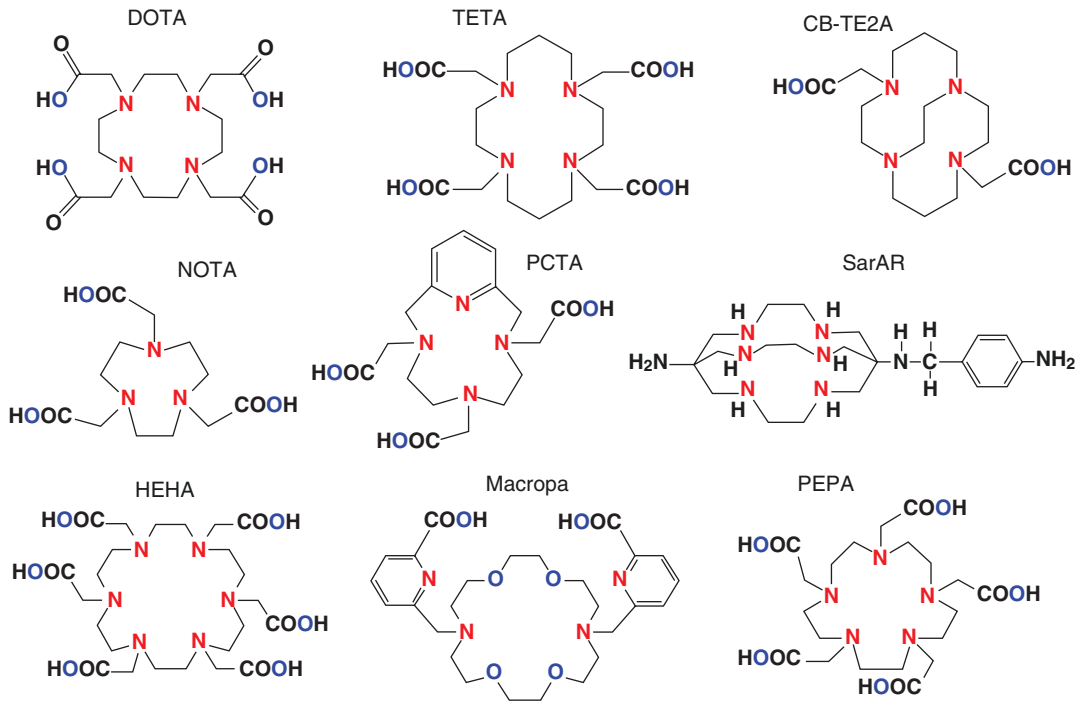

Fig. 20.17 Macrocyclic chelators used for the synthesis of radiometal-labeled peptide radiopharmaceuticals

lation during metal ion coordination, as they possess inherently constrained geometries and partially pre-organized metal binding sites. In contrast, acyclic chelators must undergo more drastic change in physical orientation and geometry in solution so as to enable the donor atoms to coordinate with metal ion. However, radiolabeling with acyclic chelators can happen at RT in a short time, whereas macrocycles may require higher temperatures and longer incubation times [55]. The stability of the metal–ligand complex is defined by the stability constant (K_S or K_{ML}) when the system reaches an equilibrium between interacting chemical species. The higher the value of K_{ML}, the greater the thermodynamic stability of the metal–ligand complex. The values of K_{ML} (such as 10^4 or 10^{30}) are normally represented as log K_{ML} values (such as 4 and 30). A more useful thermodynamic parameter is the *pM value* ($-\log[M]_{Free}$), and the pM values are linearly correlated with K_{ML} values, and express the extent to which a metal ion complex is formed in solution under physiologically relevant conditions [57]. The K_{ML} and pM values for different DOTA and DTPA with several metals is shown in Table 20.7.

20.3.5.2 Covalent Attachment of BFC to Peptide

BFCs are chelators (Fig. 20.18) with reactive functional groups that can be covalently coupled (or conjugated) to a primary amine (NH_2) or thiol (SH) group of the peptides. The bioconjugation techniques may utilize functional groups, such as carboxylic acids groups of the BFC, or BFC derivatives can be prepared with reactive groups that can facilitate the conjugation with peptides. Some common BFC derivatives are activated esters (*such as N*-hydroxysuccinimide NHS-ester) for amide couplings, isothiocyanates (NCS) for thiourea couplings, and maleimides for thiol couplings.

BFCs are often placed at the N- or C-terminus of the peptide. Often, additional spacers are used, which increases the molecular weight of whole structure, an adverse fact especially for small peptides. The labeling can influence charge or lipophilicity of the compound which, in turn, may change its biodistribution or excretion, particularly for small ligands. The right selection of labeling method is therefore crucial to obtain appropriate resolution of images, concentration in tumors cells, and excretion methods with efficient clearance.

20.3.5.3 Matching BFC to Radiometal

To identify the most appropriate chelator for a specific radiometal, the following factors are important:

- Fast radiolabeling kinetics at ambient temperature preferably, especially for heat sensitive peptides.
- The reaction time should be short (<10 min), especially for radionuclides with short half-life.
- The labeling yield and RCP should be high and preferably does not require HPLC purification of the drug product.
- The in vitro and in vivo stability (kinetic inertness) should be high to prevent the biodistri-

Table 20.7 Metal-Chelate complexes: coordination numbers (CN) and stability constants

	DOTA			DTPA		
	CN	Log K_{ML}	p*M*	CN	Log K_{ML}	p*M*
Ga^{3+}	N_4O_2	21.3–26.1	15.2, 18.5	N_3O_4	24.3, 25.5	20.2
Y^{3+}	N_4O_4	24.3–24.9	19.3, 19.8	N_3O_5	21.9, 22.5	17.6, 18.3
In^{3+}	N_4O_4	23.9	17.8, 18.8	N_3O_5	29.0, 29.5	24.4, 25.7
Lu^{3+}	N_4O_4	21.6, 23.5	17.1	N_4O_5	22.4, 22.6	19.1
Bi^{3+}	N_4O_4	30.3	27.0	N_4O_5	33.9, 35.2	
AC^{3+}						
Cu	N_4O_2	22.7, 22.7	17.6	N_3O_3	21.4	

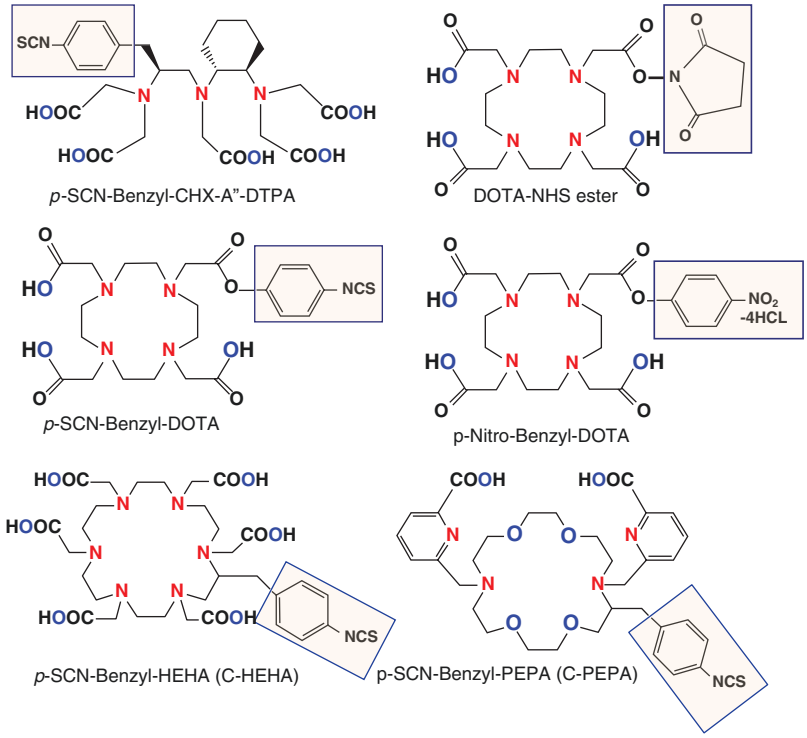

Fig. 20.18 Bifunctional chelating (BFCs) BFCs have a metal binding moiety function for the sequestration of the metallic radionuclide, and also possess a chemically reactive functional group that can provide the requisite chemistry for covalent attachment to a targeting vector of interest, such as a peptide

bution of free radiometal species to non-target organs. Kinetic inertness is the most important consideration for selecting the best chelator, especially for therapeutic radiometals.

The preference of various chelators for different radiometals is summarized in Table 20.6. Among the acyclic chelators, DTPA is acceptable for the chelation of ^{111}In, ^{90}Y, and ^{177}Lu but, CHX-A"-DTPA is the best acyclic chelator for these three metals. DOTA is one of the primary workhorse chelators for the development of radiolabeled peptides and one of the current "gold standards" for several radiometals including ^{111}In, ^{90}Y, ^{177}Lu, and ^{225}Ac. DOTA has also been used to complex metals, such as ^{64}Cu, ^{213}Bi, and ^{212}Pb, and clinical studies with radiolabeled peptides documented the potential safety of these peptides. DOTA has been extensively used with $^{67/68}$Ga but, it is widely accepted to be less stable than NOTA. ^{68}Ga-DOTATOC has been shown to exhibit superior in vivo properties to ^{111}In-DOTATOC (Octreoscan) despite non-optimal stability [55]. ^{68}Ga-DOTATATE, ^{177}Lu-DOTATATE, and ^{68}Ga-DOTATOC (Fig. 20.20) have received FDA approval for routine clinical studies (Fig. 20.19).

Although DOTA has been used, successfully with ^{212}Pb, but, its slow radiolabeling kinetics and stability properties were not ideal. The DOTA derivative TCMC is considered a good chelator for both ^{212}Pb and $^{212/213}$Bi [55].

The majority of Cu^{2+} chelator development in the last two decades has focused on tri- and tetraaza macrocycle-based polyamino carboxylates, such as NOTA and TETA. The best chelators to date are CB-TE2A, NOTA, and DiamSar. The clinical studies with ^{64}Cu-DOTATATE (DetectNet), however, showed adequate safety and efficacy to receive the FDA approval for the detection of SSTR-2 positive NETs.

A fundamental critical component of a radiometal-based radiopharmaceutical is the BFC, the ligand system that binds the radiometal ion in a tight stable coordination complex so that it can be properly directed to a desirable molecular target in vivo. The experimental methods by which chelators are assessed for their suitability with a variety of radiometal ions

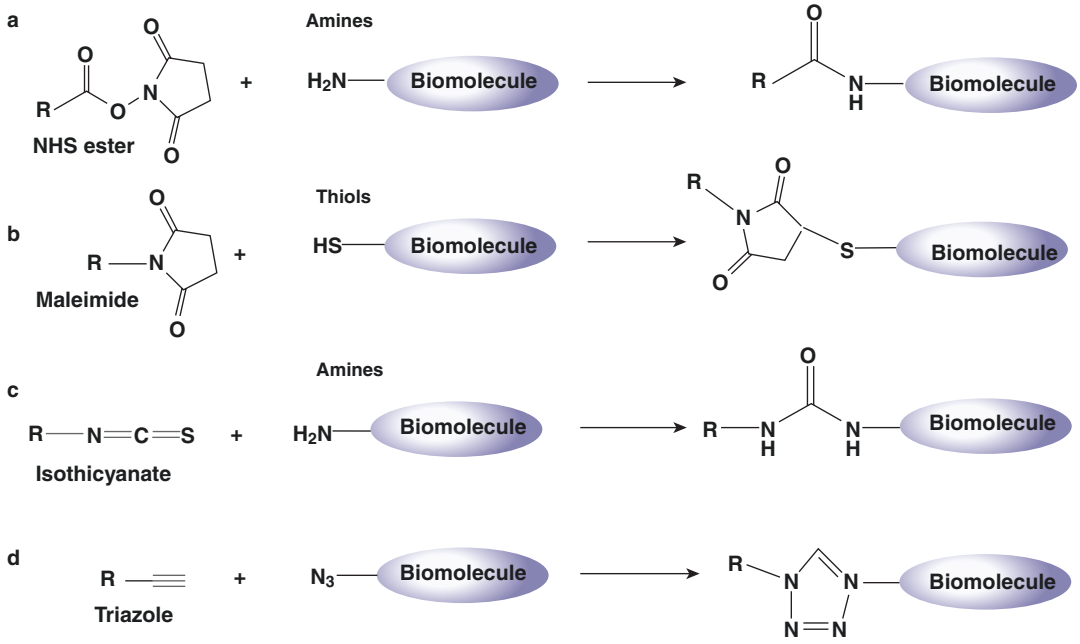

Fig. 20.19 Conjugation reactions commonly used for the covalent attachment of BFCA (R) to a targeting biomolecule with the formation of an amide (**a**), thioether (**b**), thiourea (**c**), and triazole bond (**d**)

was reviewed in several publications [3, 6, 54, 55] and also discussed in Chaps. 12 and 18.

20.3.6 Peptide Labeling with ^{99m}Tc

^{99m}Tc can be readily obtained from the ^{99}Mo → ^{99m}Tc generator as pertechnetate ($^{99m}TcO_4^-$) by elution of the generator with 0.9% saline solution. One of the characteristics of Tc is its diverse redox chemistry. The chemically stable pertechnetate ion (VII) must be reduced to lower oxidation states (I-V) to prepare ^{99m}Tc-labeled radiopharmaceuticals. During reduction by the stannous ion (Sn^{2+}), in an appropriate buffer and pH, the presence of a ligand stabilizes Tc in its lower oxidation state. In a specific Tc-complex, the oxidation state of Tc, however, depends on the chelate and pH. As a transition metal, Tc can adopt many coordination geometries, depending on the donor atoms and the type of the chelating agent. Several donor atoms, such as N, S, O and P, geometrically arranged in a chelating molecule, can form coordination complexes with technetium.

The radiopharmaceutical chemistry of Tc(V) is dominated by the $[TcO]^{3+}$ core, which is stabilized by a wide range of donor atoms (N, S, O) but, prefers thiolate, amido, and alkoxide ligands. Several tetra-ligand chelates designed to bind to Tc(V), typically form complexes (such as N_2S_2, N_3S, N_3O, and N_4) having square pyramidal geometries. The ^{99m}Tc complex of mercaptoacetyltriglycine (MAG3) forms a square pyramidal complex with Tc(V) with the basal plane consisting of three nitrogen atoms and one sulfur donor atom. A variety of BFCs, such as N_2S_2 diamidedithios, N_3S triamidethiols, N_4 tetraamines, or hydrazinonicotinic acid (HYNIC) have been evaluated upon conjugation to peptides to achieve labeling with ^{99m}Tc.

The direct method of ^{99m}Tc labeling to peptides uses a reducing agent to break a disulfide bridge of a peptide for binding of ^{99m}Tc to thiol groups in the peptide molecules. This method often suffers from lack of specificity and poor in vivo stability. In the indirect method, ^{99m}Tc is

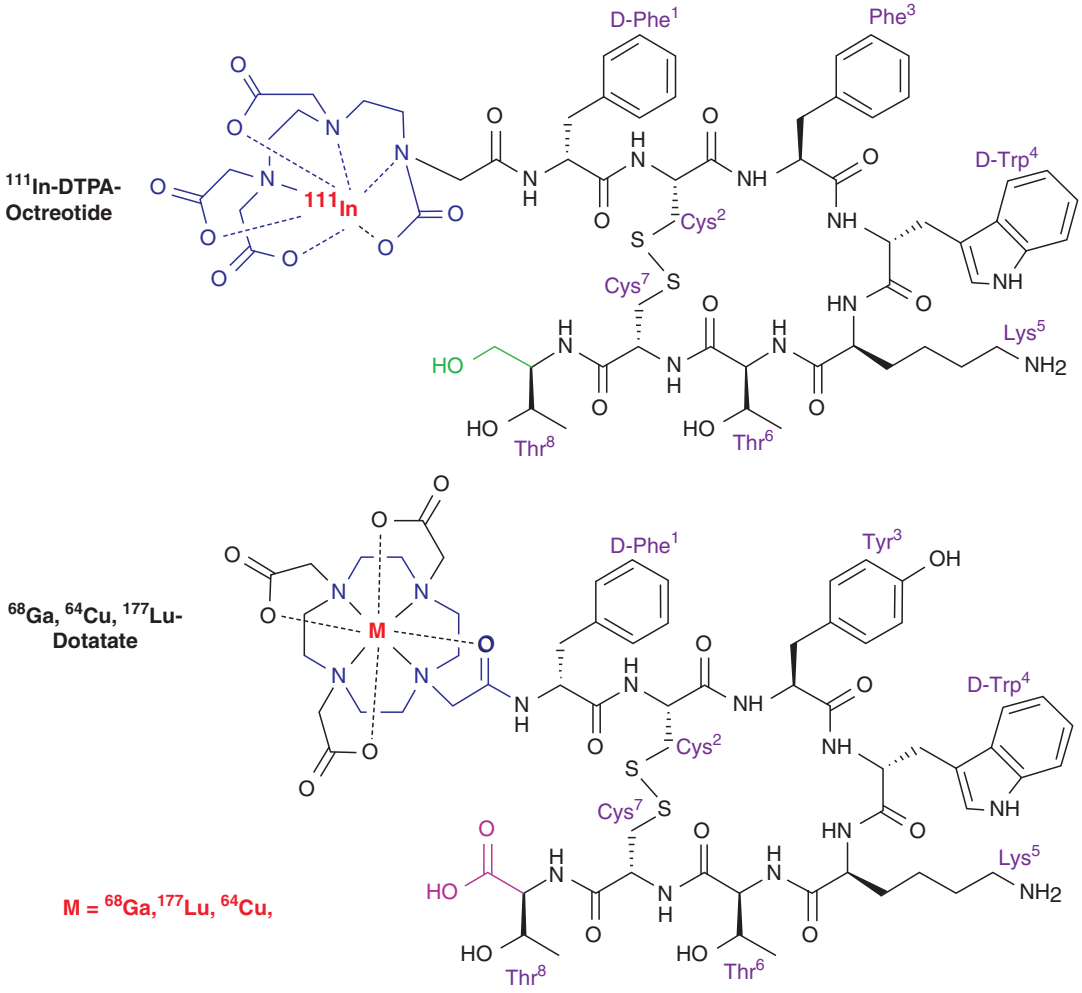

Fig. 20.20 Chemical structures of ^{111}In-DTPA-Octreotide (Octreoscan) and ^{68}Ga, ^{64}Cu, or ^{177}Lu-Dotatate (DOTA-Tyr^3-octreotate)

bound to the peptide through a BFC, which can be conjugated to the peptide either before (post-labeling approach) or after labeling with ^{99m}Tc. In the 1990s, several approaches were developed to label peptides and proteins with ^{99m}Tc. Three important labeling methods [57, 58] have been developed based on three commonly used Tc-coordination environments (cores), as shown in Fig. 20.21.

- The MAG_3-based bifunctional chelates (Tc(V) oxo core).
- The *N*-oxysuccinimidylhydrazino-nicotinamide system and (Tc(V)HYNIC core).
- The recently described single amino acid chelates for the Tc(I)-fac-tricabonyl core.

Mixed aminothiol-based chelators, such as N_2S_2 ligand bisaminoethanethiol (BAT) and N3S ligand mercaptoacetyltriglycine (MAG_3) were developed to label biomolecules based on Tc(V) O core. ^{99m}Tc-MAG3 (Mertiatide) was developed in 1986 as an anionic kidney functional imaging agent. The parent ligand is readily derivatized as the *S*-acetyl MAG_3-ethyl ester, containing a *p*-isothiocyanatobenzyl substituent, or as the *S*-acetyl MAG3-hydroxysuccinimidyl ester for conjugation to biomolecules [59]. In the 1990s, MAG3 ligand was used to develop ^{99m}Tc-P829 peptide (Depreotide) for somatostatin receptor imaging. The original octreotide peptide was modified to eliminate the disulfide bridge to prevent reduction during the synthesis of ^{99m}Tc-P829

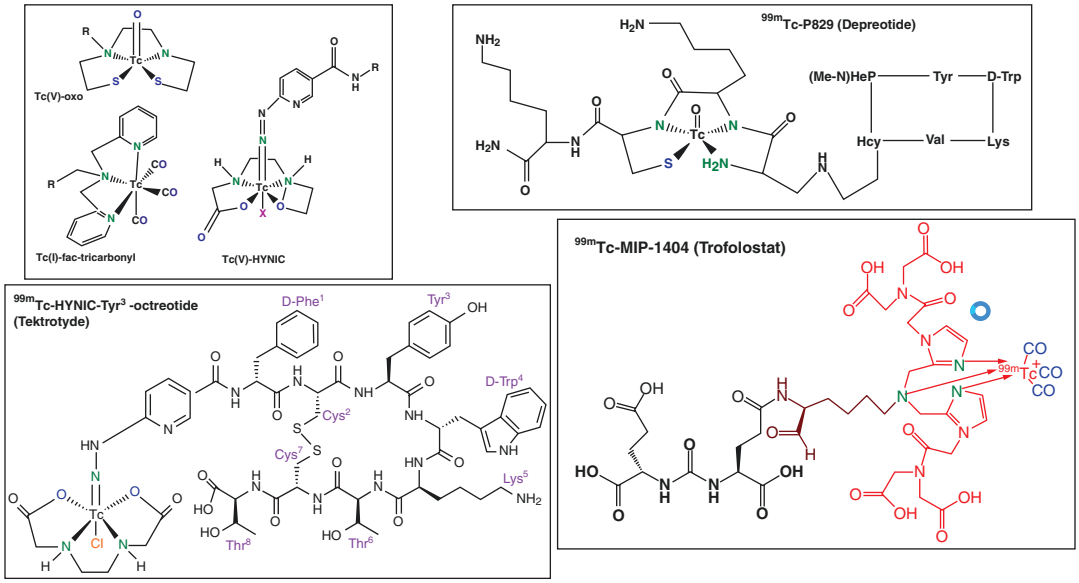

Fig. 20.21 Tc-coordination cores commonly used to develop radiometal labeled peptides

complex (Fig. 20.21). Tc-MAG3 core is robust and provides chemical versatility for the development of bifunctional tracers. There are, however, drawbacks, such as the use of stannous chloride as a reducing agent and the need for elevated pH condition, that may lead to aggregation of proteins, as well as nonquantitative radiolabeling yields [54].

An alternative pendant approach to ^{99m}Tc radiolabeling of biomolecules was provided by the introduction of hydrazinenicotinic acid (HYNIC) as a bifunctional chelator [60]. HYNIC with co-ligands like tricine and ethylenediamine diacetic acid (EDDA), in the presence of $SnCl_2$ performs fast and efficient labeling. Based on this Tc(V)HYNIC core, many small molecules, peptides, and proteins were labeled as imaging agents [5]. For example, Tyr^3-octreotide (TOC) was successfully labeled based on Tc-HYNIC core (Fig. 20.21). Since the HYNIC occupies only one or two coordination positions for ^{99m}Tc, co-ligands (such as tricine and EDDA) are needed to complete Tc's coordination positions. Changes in co-ligand number and type will have an effect on the in vivo behavior of the radiopharmaceutical. It has been understood that [HYNIC-^{99m}Tc(tricine)$_2$] complexes, usually, are not very stable in solutions and could appear in different isomeric forms based on pH, temperature, and time [61].

20.3.6.1 Tc-Tricarbonyl Core $[Tc(CO)_3]^+$

A major advancement in Tc chemistry has been the discovery that a highly adaptable tricarbonyl Tc core makes it possible to prepare organometallic complexes in aqueous solution [61]. In an effort to develop new organometallic precursors, for the preparation of ^{99m}Tc-complexes, investigators have shown that, by treating pertechnetate $\left(TcO_4^-\right)$ with sodium borohydride ($NaBH_4$) in the presence of carbon monoxide (CO) gas, one can produce the reactive Tc(I) species, $[Tc(CO)_3(OH_2)_3]^+$ [62, 63]. In this complex, the three facially oriented water molecules are sufficiently labile so that they can be readily displaced by a variety of mono-, bi- and tridentate ligands. Since it is difficult to work with CO gas, the technology is based on the use of a solid reagent, potassium boranocarbonate ($K_2H_3BCO_2$), which acts as both a reducing agent and a source of CO gas [64]. The kit is available from Mallinckrodt (Tyco) Medical under the trade name Isolink. Further, it has been shown that both, bidentate and tridentate chelates bind rapidly to the

$[Tc(CO)_3]^+$ core on a macroscopic scale and at the tracer level. ^{99m}Tc-tricarbonyl core is the favorite strategy for labeling of peptides because: (a) a high labeling yield is achieved; (b) purification is not needed after labeling protocol; and (c) attachment of ^{99m}Tc-tricarbonyl to peptide is easy and convenient [61]. Preclinical studies of several biomolecules labeled with Tc-tricarbonyl core revealed that these labeled compounds are biologically, kinetically, and thermodynamically stable for imaging studies.

Based on the chemistry of the organometallic fragment $[^{99m}Tc][Tc(CO)_3(H_2O)_3]+$, two radiopharmaceuticals, ^{99m}Tc-MIP-1404 and ^{99m}Tc-MIP-1405, were developed by Molecular Insight Pharmaceuticals (MIP). The preparation of these complexes was accomplished using a standard methodology and commercially available IsoLink kits (Covidien, Dublin, Ireland) and the imidazole chelator, which contains three nitrogen atoms suitable for binding to the ^{99m}Tc(I)-tricarbonyl-core (Fig. 20.21). The lead compound ^{99m}Tc-MIP-1404 (Trofolastat) completed phase III clinical trials as an imaging agent for the detection of prostate-specific membrane antigen (PSMA) positive prostate cancer [65, 66].

References

1. Cooper BM, Legre J, O'Donovan DH, et al. Peptides as a platform for targeted therapeutics for cancer: peptide–drug conjugates (PDCs). Chem Soc Rev. 2021;50:1480–94.
2. Zhang Y, Zhang H, Ghosh D, Williams RO. Just how prevalent are peptide therapeutic products? A critical review. Int J Pharma. 2020;587:119491.
3. Fani M, Maeske HR, Okavi SM. Radiolabeled peptides: valuable tools for the detection and treatment of cancer. Theranostics. 2012;2(5):481–501.
4. Gharibkandi NA, Conlon JM, Hosseinimehr SJ. Strategies for improving stability and pharmacokinetic characteristics of radiolabeled peptides for imaging and therapy. Peptides. 2020;133(12):170385.
5. Mikulová MB, Mikuš P. Advances in development of radiometal labeled amino acid-based compounds for cancer imaging and diagnostics. Pharmaceuticals. 2021;14:167.
6. Tornesello AL, Buonaguro L, Tornesello ML, Buonaguro FM. New insights in the design of bioactive peptides and chelating agents for imaging and therapy in oncology. Molecules. 2017;22(8):1282.
7. Maleki F, Farahani AM, Rezazedeh F, et al. Structural modifications of amino acid sequences of radiolabeled peptides for targeted tumor imaging. Bioorg Chem. 2020;99:103802.
8. Bass RT, Buckwalter BL, Patel BP, et al. (1996) Identification and characterization of novel somatostatin antagonists. Mol Pharmacol 50:709–715.
9. Günther T, Tulipano G, Dournaud P, et al. International Union of Basic and Clinical Pharmacology. CV. Somatostatin receptors: structure, function, ligands, and new nomenclature. Pharmacol Rev. 2018;70:763–835.
10. Fani M, Nicolas GP, Wild D. Somatostatin receptor antagonists for imaging and therapy. J Nucl Med. 2017;58:61S–6S.
11. Evans BJ, King AT, Katsifis A, et al. Methods to enhance the metabolic stability of peptide-based PET radiopharmaceuticals. Molecules. 2020;25:2314. https://doi.org/10.3390/molecules25102314.
12. Gai Y, Jiang Y, Long Y, et al. Evaluation of an integrin α(v)β(3) and aminopeptidase N dual-receptor targeting tracer for breast cancer imaging. Mol Pharm. 2020;17:349–58.
13. Kapp TG, Rechenmacher F, Neubauer S, et al. Comprehensive evaluation of the activity and selectivity profile of ligands for RGD-binding integrins. Sci Rep. 2017;7:39805.
14. Popp I, Pozzo D, Waser B, et al. Approaches to improve metabolic stability of a statine-based GRP receptor antagonist. Nucl Med Biol. 2017;45:22–9.
15. Cod WL, He JX, Reily MD, et al. Design of a potent combined pseudopeptide endothelin-A/endothelin-B receptor antagonist, Ac-d Bhg^{16}-Leu-Asp-Ile-[NMe] $Ile\text{-}Trp^{21}$ (PD 156252): examination of its pharmacokinetic and spectral properties. J Med Chem. 1997;40:2228–40.
16. Klingler M, Summer D, Rangger C, et al. DOTA-MGS5, a new cholecystokinin-2 receptor-targeting peptide analog with an optimized targeting profile for theranostic use. J Nucl Med. 2019;60:1010–6.
17. Wu H, Huang J. PEGylated peptide-based imaging agents for targeted molecular imaging. Curr Protein Pept Sci. 2016;7(6):582–95.
18. Jamous M, Tamma ML, Gourni E, et al. PEG spacers of different length influence the biological profile of bombesin-based radiolabeled antagonists. Nucl Med Biol. 2014;41:464–70.
19. Wu Z, Li Z-B, Cai W, et al. ^{18}F-labeled mini-PEG spacered RGD dimer (^{18}F-FPRGD2): synthesis and microPET imaging of αvβ3 integrin expression. Eur J Nucl Med Mol Imaging. 2007;34:1823.
20. Moradi SV, Hussein WM, Varamini P, et al. Glycosylation, an effective synthetic strategy to improve the bioavailability of therapeutic peptides. Chem Sci. 2016;7:2492–500.
21. Schweinsberg C, Maes V, Brans L, et al. Novel glycated $[^{99m}Tc(CO)_3]$-labeled Bombesin analogues for improved targeting of gastrin-releasing pep-

tide receptor-positive tumors. Bioconjug Chem. 2008;19:2432–9.
22. Cho EH, Lim JC, Lee SY, Jung SH. An assessment tumor targeting ability of ^{177}Lu labeled cyclic CCK analogue peptide by binding with cholecystokinin receptor. J Pharmacol Sci. 2016;131:209–14.
23. Shinde SS, Maschauer S, Prante O. Sweetening pharmaceutical radiochemistry by ^{18}F-Fluoroglycosylation: recent progress and future prospects. Pharmaceuticals. 2021;14:1175. https://doi.org/10.3390/ph14111175.
24. Liu Z, Chen X. Simple bioconjugate chemistry serves great clinical advances: albumin as a versatile platform for diagnosis and precision therapy. Chem Soc Rev. 2016;45(5):1432–56.
25. Zorzi A, Linciano S, Angelini A. Non-covalent albumin-binding ligands for extending the circulating half-life of small biotherapeutics. Med Chem Commun. 2019;10:1068.
26. Muller C, Struthers H, Winiger C, et al. DOTA conjugate with an albumin-binding entity enables the first folic acid-targeted ^{177}Lu-radionuclide tumor therapy in mice. J Nucl Med. 2013;54(1):124–31.
27. Tian R, Jacobson O, Niu G. Evans blue attachment enhances somatostatin receptor Subtype-2 imaging and radiotherapy. Theranostics. 2018;8(3):735–45.
28. Dumelin CE, Trussel S, Buller F, et al. A portable albumin binder from a DNA encoded chemical library. Angew Chem Int Ed Engl. 2008;47:3196–201.
29. Kelly JM, Amor-Coarasa A, Ponnala S, et al. Albumin-binding PSMA ligands: implications for expanding the therapeutic window. J Nucl Med. 2019;60(5):656–63.
30. Umbricht CA, Benesova M, Hasler R, et al. Design and preclinical evaluation of an albumin-binding PSMA ligand for (64)Cu-based PET imaging. Mol Pharm. 2018;15(12):5556–64.
31. Rousseau E, Lau J, Zhang Z, et al. Effects of adding an albumin binder chain on [(177)Lu] LuDOTATATE. Nucl Med Biol. 2018;66:10–7.
32. Jacobson O, Kiesewetter DO, Chen X. Albumin-Binding Evans Blue Derivatives for Diagnostic Imaging and Production of Long-Acting Therapeutics Bioconjugate Chem. 2016;27(10):2239–47.
33. Zhang J, Wang H, Jacobson O, et al. Safety, pharmacokinetics, and dosimetry of a long-acting radiolabeled somatostatin analog (177)Lu-DOTA-EB-TATE in patients with advanced metastatic neuroendocrine tumors. J Nucl Med. 2018;59(11):1699–705.
34. Arezou FM, Fariba M, Nourollah S. The influence of different spacers on biological profile of peptide radiopharmaceuticals for diagnosis and therapy of human cancers. Anti Cancer Agents Med Chem. 2020;20(4):402–16.
35. Farahani AM, Maleki F, Sadeghzadeh N. The influence of different spacers on biological profile of peptide radiopharmaceuticals for diagnosis and therapy of human cancers. Anti Cancer Agents Med Chem. 2020;20(4):402–6.
36. Antunes P, Ginj M, Walter MA, et al. Influence of different spacers on the biological profile of a DOTA-somatostatin analogue. Bioconjug Chem. 2007;18:84–92.
37. Jia Y, Shi W, Zhou Z, et al. Evaluation of DOTA-chelated neurotensin analogs with spacer-enhanced biological performance for neurotensin-receptor-1-positive tumor targeting. Nucl Med Biol. 2015;42:816–23.
38. Janssen M, Oyen WJ, Massuger LF, et al. Comparison of a monomeric and dimeric radiolabeled RGD-peptide for tumor targeting. Cancer Biother Radiopharm. 2002;17(6):641–6.
39. Li ZB, Cai W, Cao Q, et al. (64)Cu-labeled tetrameric and octameric RGD peptides for small-animal PET of tumor alpha(v)beta(3) integrin expression. J Nucl Med. 2007;48(7):1162–71.
40. Liu Z, Yan Y, Chin FT, et al. Dual integrin and gastrin-releasing peptide receptor targeted tumor imaging using ^{18}F-labeled PEGylated RGD-bombesin heterodimer ^{18}F-FB-PEG3-Glu-RGD-BBN. J Med Chem. 2009;52(2):425–32.
41. Li H, Liu Z, Yuan L, et al. Radionuclide-based imaging of breast cancer: state of the art. Cancers (Basel). 2021;13(21):5459.
42. Jamous M, Haberkorn U, Mier W. Synthesis of peptide radiopharmaceuticals for the therapy and diagnosis of tumor diseases. Molecules. 2013;18:3379–409.
43. Oliveira MC, Correia JDG. Biomedical applications of radioiodinated peptides. Eur J Med Chem. 2019;179:56–77.
44. Ajenjo J, Destro G, Cornelissen B, Gouverneur V, et al. Closing the gap between ^{19}F and ^{18}F chemistry. EJNMMI Radiopharm Chem. 2021;6:33–70.
45. Jacobson O, Kiesewetter DO, Chen X. Fluorine-18 radiochemistry, labeling strategies and synthetic routes. Bioconjug Chem. 2015;26:1–18.
46. Richter S, Wuest F. ^{18}F-labeled peptides: the future is bright. Molecules. 2014;19:20536–56.
47. Halder R, Ritter T. ^{18}F-fluorination: challenge and opportunity for organic chemists. J Org Chem. 2021;86(20):13873–84.
48. Yuan Z, Nodwell MB, Yang H, et al. Site-selective, late-stage C−H ^{18}F-fuorination on unprotected peptides for positron emission tomography imaging. Angew Chemie Int Ed. 2018;57:12733–6.
49. Rickmeier J, Ritter T. Site-specific deoxyfluorination of small peptides with [^{18}F]fluoride. Angew Chem Int Ed. 2018;57:14207–11.
50. McBride WJ, Sharkey RM, Karacay H, et al. A novel method of ^{18}F radiolabeling for PET. J Nucl Med. 2009;50:991–8.
51. Archibald SJ, Allott L. The aluminum-[^{18}F]fluoride revolution: simple radiochemistry with a big impact for radiolabelled biomolecules EJNMMI. Radiopharm Chem. 2021;6:30.
52. Kumar K. ^{18}F-AlF-labeled biomolecule conjugates as imaging pharmaceuticals. J Nucl Med. 2018;59(8):1218–9.

53. Fersing C, Bouhlel A, Cantelli C, et al. A comprehensive review of non-covalent radiofluorination approaches using aluminum [^{18}F]fluoride: will [^{18}F]AlF replace ^{68}Ga for metal chelate labeling? Molecules. 2019;24(16):2866.
54. Boros E, Packard A. Radioactive transition metals for imaging and therapy. Chem Rev. 2019;119:870–90.
55. Price EW, Orvig C. Matching chelators to radiometals for radiopharmaceuticals. Chem Soc Rev. 2014;43:260.
56. Kostelnik TI, Orvig C. Radioactive main group and rare earth metals for imaging and therapy. Chem Rev. 2019;119(2):902–56.
57. Boschi A, Uccelli L, Martini P. A picture of modern Tc-99m radiopharmaceuticals: production, chemistry, and applications in molecular imaging. Appl Sci. 2019;9:2526.
58. Rezazadeh F, Sadeghzade N. Tumor targeting with ^{99m}Tc radiolabeled peptides: clinical application and recent development. Chem Biol Drug Des. 2019;93:205–21.
59. Ram S, Buchsbaum DJ. A peptide-based bifunctional chelating agent for ^{99m}Tc and ^{186}Re labeling of monoclonal antibodies. Cancer. 1994;73(s3):769–73.
60. Babich JW, Fischman AJ. Effect of co-ligand on the biodistribution of ^{99m}Tc labeled hydrazine nicotinic acid derivatized chemotactic peptides. Nucl Med Biol. 1995;22:25–30.
61. Piramoon M, Hosseinimehr SJ. The past, current studies and future of organometallic 99mTc(CO)3 labeled peptides and proteins. Curr Pharm Des. 2016;22:4854–67.
62. Alberto R, Schlibi R, Schubiger AP. First application of fac-[^{99m}Tc$(OH_2)_3(CO)_3]^+$ in bioorganometallic chemistry: design, structure, and in vitro affinity of a 5-HT1A receptor ligand labeled with ^{99m}Tc. J Am Chem Soc. 1999;121:6076–7.
63. Waibei R, Alberto R, Willude J, et al. Stable one-step technetium-99m labeling of His-tagged recombinant proteins with a novel Tc(I)-carbonyl complex. Nat Biotechnol. 1999;17:897–901.
64. Alberto R, Ortner K, Wheatley N, et al. Synthesis and properties of boranocarbonate: A convenient in situ CO source for the aqueous preparation of [^{99m}Tc$(OH^2)^3(CO)^3$]+. J Am Chem Soc. 2001;123:3135–136.
65. Schmidkonz C, Götz TI, Atzinger A, et al. ^{99m}Tc-MIP-1404 SPECT/CT for assessment of whole-body tumor burden and treatment response in patients with biochemical recurrence of prostate cancer. Clin Nucl Med. 2020;45(8):e349–57.
66. Vallabhajosula S, Nikolopoulou A, Babich JW (2014) ^{99m}Tc-labeled small-molecule inhibitors of prostate-specific membrane antigen: pharmacokinetics and biodistribution studies in healthy subjects and patients with metastatic prostate cancer. J Nucl Med 55(11):1791–1798.