Radiopharmaceuticals for Molecular Imaging

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An experiment is a question which science poses to Nature, and a measurement is the recording of Nature's answer. (Max Planck)

9.1 Radiotracer Vs. Radiopharmaceutical

In the 1920s, George de Hevesy, coined the term *radioindicator* or *radiotracer* and introduced the *tracer principle* in biomedical sciences. Initially, β^--emitting radioisotopes were used as therapeutic agents. For example, ^{32}P was used for the treatment of *polycythemia vera* and *leukemia*, and ^{131}I was used for the treatment of thyroid disease (toxic and nontoxic goiter) and thyroid cancer.

Following the discovery of the scintillation scanner by Benedict Casen, ^{131}I was introduced as a radiotracer for diagnostic imaging purpose. In 1956, Merrill Bender introduced ^{131}I-labeled serum albumin as a radiotracer to image brain tumors. The introduction of the Anger camera and the ^{99m}Tc generator, in the 1960s, stimulated the development of a number of radiolabeled compounds as radiotracers for diagnostic studies in nuclear medicine.

A radiotracer can be defined as a specific radiolabeled molecule (or probe) that resembles or traces the in vivo behavior of a natural molecule and can be used to provide information about a specific biological process. The degree of similarity between the radiotracer and the natural substance, however, may vary depending on the particular radiotracer. For example, [^{11}C] glucose and [^{14}C]glucose are true tracers of glucose because they are chemically identical to natural glucose, while [^{18}F]fluorodeoxyglucose (FDG), an analog of glucose, also traces glucose, but does not behave identically to glucose since it is chemically different.

One of the most important characteristics of a true radiotracer is the ability to study the components of a homeostatic system without disturbing their function. Occasionally, the term *radioligand* is also used in the context of imaging studies. A radioligand can be defined as any radiolabeled molecule that can bind with another molecule or substance (binder) in a predictable way under controlled conditions. For example, ^{68}Ga-Dotatate (Netspot) is a radioligand that binds, specifically, to somatostatin type 2 receptors (SSTR-2) in patients with neuroendocrine tumors (NETs), while [^{18}F]Fluorodeoxyglucose (FDG) is a radiotracer used to image glucose metabolism in tumor tissue, heart, and brain.

9.1.1 Radiopharmaceutical Vs. Radiochemical

All radiolabeled compounds or substances used for the purpose of diagnosis or therapy have been defined as *radioactive drugs* or *radiopharmaceuticals* by the U.S. Food and Drug Administration

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(FDA). Diagnostic radiopharmaceuticals are administered in trace amounts (<100 μg) and, typically, do not induce any physiological response or pharmacological effect in patients. The term radioindicator, however, may be more appropriate to describe radiolabeled compounds or substances used for the purpose of diagnosis.

In 1954, the U.S. Atomic Energy Commission (AEC) and, subsequently, the U.S. Nuclear Regulatory Commission (NRC) were given the responsibility for directing the medical use of reactor-produced byproducts. Since the 1960s, the stated mission of the FDA has been to "assure safety and efficacy in marketed medicinal agents and medicinal devices." In 1972, the FDA took over the responsibility to regulate the medical usage of reactor-produced radiolabeled compounds from the NCR. Subsequently, in 1975, the FDA decided to also assume the responsibility for the cyclotron-produced radiolabeled compounds. In addition, the FDA adopted the same regulations for radiolabeled compounds (or nuclear medicine imaging probes) as those in existence for traditional drugs (pharmaceuticals). As a result, the term "radiopharmaceutical" has become the official FDA categorization for all radioindicators and radiotracers used for diagnosis and therapy. Although the field of nuclear medicine evolved into a more sophisticated molecular imaging technology, the FDA continues to extend the usage of the term "radiopharmaceuticals" to include the novel radiolabeled molecular imaging probes or radiotracers.

The term *radiochemical* is sometimes used for any radiolabeled compound or radiotracer. From the standpoint of chemistry and radiochemical purity, there is no difference between the terms radiochemical and radiopharmaceutical. From a regulatory point of view, however, a radiopharmaceutical must also be sterile, pyrogen free, safe for human use, and efficacious for a specific indication. In contrast, a radiochemical may not be sterile and pyrogen-free and is not an FDA-approved agent for routine human use.

9.2 Radiopharmaceuticals for Molecular Imaging (RP-MI)

The terms, radiotracer, radioligand, and radiolabeled molecular imaging probe (RMIP), have specific meaning depending on their specific use and application. However, from a regulatory point of view, the term radiopharmaceutical represents any radiolabeled molecule intended for human use.

The members of the molecular imaging center of excellence (MICoE) standard definitions task force recently developed the following standard definitions and terms [1]:

- Molecular imaging is the visualization, characterization, and measurement of biological processes at the molecular and cellular levels in humans and other living systems.
- Molecular imaging agents are "probes used to visualize, characterize, and measure biological processes in living systems". Both, endogenous molecules and exogenous probes can be molecular imaging agents.

Because the emphasis of this textbook is on molecular imaging and targeted radionuclide therapy, it is important to understand the subtle difference between the conventional nuclear imaging technology and the molecular imaging technology. Some of the radiopharmaceuticals used in nuclear medicine are *nonspecific*, while all the RP-MI, by definition, are highly *specific* for the measurement of a specific biological process and, therefore, can be regarded as targeted radiopharmaceuticals for imaging.

The current FDA-approved radiopharmaceuticals for SPECT and PET can be divided into "specific" and nonspecific agents (Table 9.1). Several examples would clearly illustrate the difference between specific and nonspecific agents. Radioiodide (^{123}I, ^{124}I, or ^{131}I) is a very good example of a molecular imaging probe; just like

Table 9.1 FDA-approved radiopharmaceuticals for planar imaging, SPECT, and PET

Radiopharmaceuticals	Trade Name	Indications
Non-specific radiopharmaceuticals		
^{99m}Tc pertechnetate		Salivary and lacrimal gland imaging, vesicoureteral imaging
^{99m}Tc-macroaggregated albumin (MAA)		Evaluation of pulmonary perfusion Evaluation of peritoneo-venous shunt patency
^{99m}Tc-sulfur colloid		Image area of functioning RES in liver, spleen and bone marrow, localization of lymph nodes draining a primary tumor
^{99m}Tc-tilmanocept	Lymphoseek®	Lymphatic mapping and to locate lymph nodes draining a primary tumor site
^{99m}Tc-pyrophosphate		Bone imaging and blood pool imaging to detect GI bleeds
^{99m}Tc-mebrofenin	Choletec®	Hepatobiliary imaging agent
^{99m}Tc-bicisate	Neurolite®	To assess rCBF and to localize stroke
^{99m}Tc-exametazine	Ceretec™	To assess rCBF and to localize stroke
^{99m}Tc-medronate (MDP)		Bone imaging to delineate areas of altered osteogenesis
^{99m}Tc-oxidronate (HDP)		Bone imaging to delineate areas of altered osteogenesis
^{99m}Tc-Pentetate (DTPA)		Renal imaging and function
^{99m}Tc-mertiatide	MAG3™	Renal imaging and function
^{99m}Tc-succimer (DMSA)		Evaluation of renal parenchymal disorders
^{99m}Tc-sestamibi	Cardiolite®	Myocardial perfusion and evaluation of breast lesions
^{99m}Tc-tetrofosmin	Myoview™	Myocardial perfusion
^{201}Tl chloride		Myocardial perfusion
^{67}Ga-citrate		Detection of certain tumors and acute infection
^{111}In pentetate		Radionuclide cisternography
^{111}In oxyquinoline		Label leukocytes and to localize areas of acute infection
Specific (targeted radiopharmaceuticals or radiolabeled molecular imaging probes (RMIP))		
^{123}I sodium iodide (Liquid or capsules)		Thyroid uptake by active transport via NaI transporter evaluation of thyroid function and morphology
^{131} sodium iodide (Liquid or capsules)		Evaluation of thyroid function and detection of metastases associated with thyroid malignancies
^{111}In pentetreotide	Octreoscan™	Localization of primary and metastatic NETs bearing SSTRs
[^{123}I]Iobenguane	Adreview™	Detection of primary or metastatic pheochromocytoma or neuroblastoma via norepinephrine transporter
[^{123}I]Ioflupane	DaTscan™	Imaging striatal dopamine transporters in patients with parkinsonian syndrome
^{82}Rb chloride	Cardiogen-82®, Rubi-fill®	As a K^+ analog, to evaluate regional myocardial perfusion
[^{13}N]Ammonia		To evaluate regional myocardial perfusion
[^{18}F]Fludeoxyglucose (FDG)		To assess abnormal glucose metabolism in oncology To assess myocardial hibernation To identify foci of epileptic seizures
[^{18}F]Sodium Fluoride		To delineate areas of altered osteogenesis
[^{18}F]Florbetapir	Amvid™	To estimate β-amyloid neuritic plaque density in patients with cognitive impairment
[^{18}F]Florbetaben	Neuraceq™	To estimate β-amyloid neuritic plaque density in patients with cognitive impairment
[^{18}F]Flutemetamol	Vizamyl™	To estimate β-amyloid neuritic plaque density in patients with cognitive impairment
[^{18}F]Flortaucipir	Tauvid™	To estimate the density and distribution of aggregated tau neurofibrillary tangles (NFTs)
[^{18}F]Piflufolastat	Pylarify®	To detect PSMA-positive lesions in prostate cancer

(continued)

Table 9.1 (continued)

Radiopharmaceuticals	Trade Name	Indications
[^{11}C]Choline		To help identify potential sites of prostate cancer recurrence
[^{18}F]Fluoroestradiol	Cerianna™	For the detection of estrogen receptor-positive lesions in patients with breast cancer
[^{18}F]Fluciclovine	Auxumin™	Prostate cancer recurrence
[^{18}F]Fluorodopa		To visualize dopaminergic nerve terminals in the striatum in patients with suspected Parkinsonian syndromes (PS)
^{68}Ga-DOTATATE	NETspot	For localization of SSTR-positive NETs
^{64}Cu-DOTATATE	Detectnet	For localization of SSTR-positive NETs
^{68}Ga-DOTATOC		For localization of SSTR-positive NETs
^{68}Ga-PSMA-HBED-CC		PSMA-positive lesions in prostate cancer

the natural iodide (I^-), it is actively transported into the thyroid gland via sodium iodide symporter (NIS) and incorporated into the thyroid hormones. In contrast, ^{99m}Tc pertechnetate (TcO_4^-), while useful for thyroid imaging, is not a specific radiotracer to assess thyroid function. ^{67}Ga citrate is a nonspecific diagnostic imaging agent to identify both tumor tissue and abscess (or infection), while ^{111}In-labeled leukocytes are highly specific to detect the sites of acute infection but, not tumor tissue.

The metabolic and molecular information provided by [^{18}F]FDG-PET can be considered as the first validated "clinically useful" molecular imaging technique. FDG is a substrate for the enzyme *hexokinase* and highly specific in assessing the glucose metabolism of any tissue. FDG, however, is a non-specific molecular imaging probe to image malignant tissue, since it is also taken up by inflammatory tissue, macrophages, and many other normal cells that have augmented glucose utilization. In contrast, ^{111}In-DTPA-octreotide (OctreoScan) or ^{68}Ga-Dotatate (NetSpot) are highly specific agents to image neuroendocrine tumors (NETs) expressing somatostatin type 2 (SSTR 2) receptors. Therefore, ^{111}In-DTPA-octreotide and ^{68}Ga-Dotatate are truly targeted radiopharmaceuticals (or RMIPs) designed for molecular imaging studies based on SPECT and PET, respectively [2].

9.2.1 Molecular Medicine and Theranostics

As previously discussed, the term *homeostasis* is used by physiologists to describe maintenance of static, or constant, conditions in the internal environment by means of positive and negative feedback of information. Diseases can be defined as abnormal processes as well as abnormalities in molecular concentrations of different biological markers, signaling molecules and receptors. In the last two decades, there has been a revolution in our basic understanding of the biology and biochemistry of disease. With the elucidation of the human genome and the description of the genetic abnormalities, responsible for numerous diseases, we now have a better understanding of the basic molecular pathways, proteins, and signal transduction processes that are present in the normal cell.

The basis of molecular medicine is that chemical disturbances will precede anatomical abnormalities in disease. The new generation of molecular therapeutics is based on the rational drug design to reverse or control chemical imbalances by targeting key specific receptors, enzymes, membrane transporters, and antigens. The ultimate goal of molecular medicine is to treat the disease in its early stages with an appropriate patient-specific "targeted molecular

therapy." In order to achieve this goal, it is essential to develop highly specific RP-MI. In the design and development of an ideal radiotracer, it is important to identify first a specific biological target, and then design a radiopharmaceutical, which may be a biochemical or a synthetic molecule (targeting vehicle or vector), specific for the biological target (receptor, enzyme, protein, antigen) or a biological process (such as metabolism, angiogenesis, and apoptosis) in an organ, or tissue of interest. Subsequently, an appropriate radionuclide (such as ^{18}F, ^{123}I, ^{68}Ga, ^{64}Cu) can be used to synthesize a radiopharmaceutical suitable for imaging based on either PET or SPECT techniques. A schematic of a targeted radiopharmaceutical for molecular imaging studies is shown in Fig. 9.1.

The development of RP-MI will greatly benefit from the science of molecular medicine and from the chemical insights of molecular therapeutics. The human brain uses glucose as the primary source of energy for its physiological functions. Alteration of blood glucose levels leads to hypo/hyperglycemic conditions. Consequently, the brain undergoes dysregulation of glucose metabolism which is further associated with various pathological disorders causing cognitive impairment and loss of critical brain functions. Glucose metabolism is connected with multiple other metabolic pathways in order to generate adequate energy for neuronal cells to carry out their functions. Therefore, glucose homeostasis plays a prominent role in the maintenance of healthy brain physiology. The Warburg effect, first proposed in 1920s, describes a phenomenon in which cancer cells take up glucose more and preferentially metabolize glucose by glycolysis, producing lactate as an end product, even in the presence of oxygen. 2-deoxy-2-[^{18}F]fluoro-D-glucose (FDG), a glucose analog was developed in 1970s, by substituting ^{18}F for the normal hydroxyl group at the C-2 position in the glucose molecule (Fig. 9.2). FDG-PET was used for 20 years to image glucose metabolism of brain and heart before it was discovered that FDG is useful as a diagnostic radiopharmaceutical for the detection of various tumors

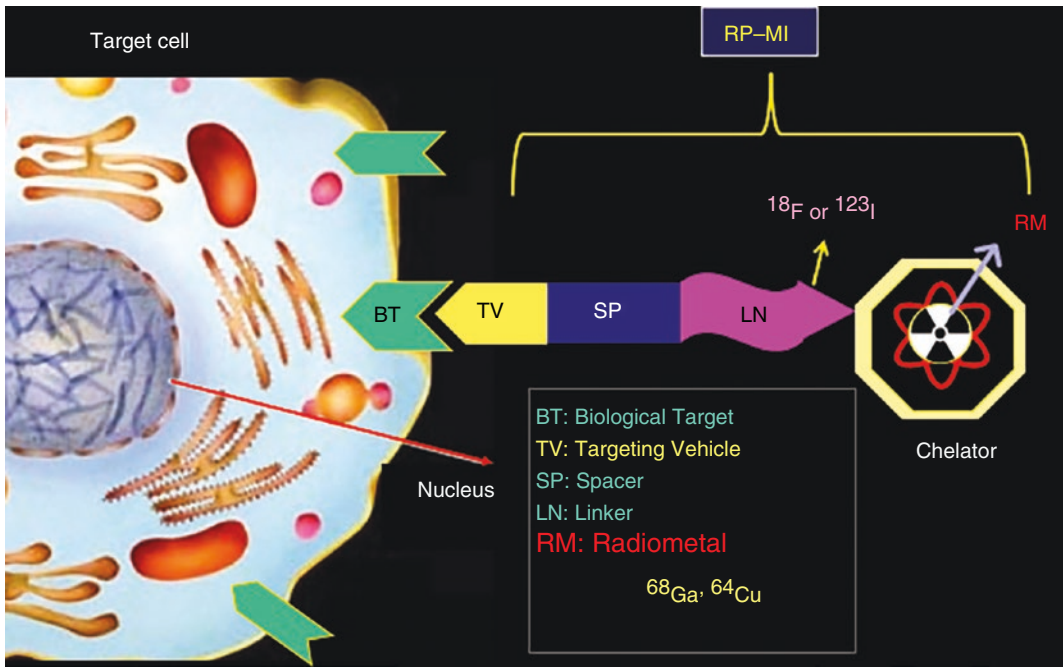

Fig. 9.1 Schematic of a targeted radiopharmaceutical for molecular imaging based on PET or SPECT. The targeting vehicle (vector) specific for a biological target can be labeled with ^{18}F or ^{123}I. However, labeling (or complexation) with radiometals such as ^{68}Ga or ^{64}Cu requires conjugation of a chelator to the targeting vehicle via spacer/linker

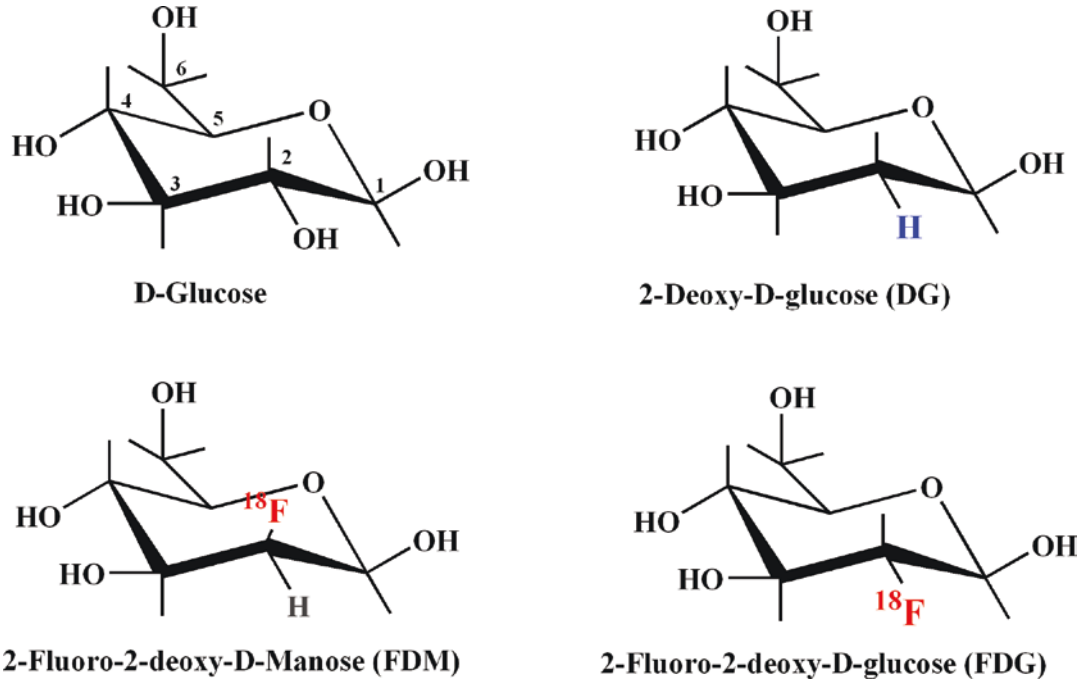

Fig. 9.2 Analogs of D-glucose: Substitution of hydrogen or fluorine atom for the hydroxyl group on C-2 position of glucose would produce DG and FDG, which can be transported into the cell by glucose transporters similar to that of D-glucose

and also to assess tumor response to chemotherapy. FDG revolutionized nuclear medicine and accelerated the development of molecular imaging technology. The glucose molecule, however, is not suitable for radiolabeling with beta emitters such as ^{131}I, ^{90}Y, and ^{177}Lu.

Molecular imaging agents and drugs (therapeutics) share common concepts of structural design and mechanisms of localization and/or action because they target the same enzyme, receptor, or antigen. Drugs block or inhibit their targets and restore the chemical imbalance associated with a disease, while RPMIs, at tracer levels, can provide noninvasive quantitative assessment of the functional status of the molecular target in a specific disease. For example, the discovery of SSTRs in patients with NETs led to the development of chemotherapy based on octreotide (Sandostatin®), which binds specifically to SST type 2 receptors. Radiolabeled analogs of octreotide such as ^{111}In-DOTA-octreotide, ^{68}Ga-Dotatate, and ^{68}Ga-dotatoc provided imaging agents to detect SSTR-2 positive lesions in patients with NETs. The same molecule used for imaging is labeled with β^- emitting radionuclide (^{177}Lu) to synthesize a therapeutic agent (^{177}Lu-dotatate or Lutathera™) for TRT of patients with NETs. Receptor specific chemotherapeutic drug lead to the development of MIPs, and MIPs in turn lead to the development of drugs for TRT. Thus, a diagnostic imaging agent and a therapeutic drug may have similar structural requirements, being the same molecule or structural analogs of each other [3].

The concept of theranostics integrates two distinct approaches that both encompass all steps of patients' management. Theranostics in nuclear medicine is a personalized approach to treating cancer, using similar (or same) molecules for both imaging (diagnosis) and therapy. A target specific biomolecule is designed in such a manner that it can be labeled with a γ or β^+ emitting radionuclide for SPECT or PET imaging, and it can also be labeled with a therapeutic radionu-

Table 9.2 Theranostic radiopharmaceuticals

Disease/Indication	Target	Radiopharmaceutical for	
		Molecular imaging	Therapy
Therapy of neuroendocrine tumors (NETs)	SSTR-2 Agonist	^{68}Ga-Dotatate[a]	^{177}Lu-Dotatate[a]
		^{64}Cu-Dotatate[a]	^{177}Lu-EB-Tate
			^{177}Lu-Dotatoc
			^{90}Y-Dotatoc
			^{177}Lu-Satoreotide tetraxetan
	SSTR-2 antagonist	^{68}Ga-NODAGA-JR11 (OPS202)	^{177}Lu-DOTA-JR11 (OPS201)
	Glucagon-like peptide-1 (GLP-1R)	^{68}Ga-DOTA-Exendin-4	^{177}Lu-DOTA-Exendin-4
Melanoma, multiple myeloma, small-cell lung cancer, NETs	CXCR4	^{68}Ga-Pentixafor	^{177}Lu-Pentixafor
Metastatic castration resistant prostate cancer (mCRP)	Bone mineral (hydroxyapatite)	[^{18}F]sodium fluoride[a]	^{223}Ra dichloride[a] ^{153}Sm-EDTMP
	Prostate specific membrane a antigen (PSMA)	^{68}Ga-PSMA-11[a]	^{177}Lu-PSMA-617 ^{225}Ac-PSMA-617
		[^{18}F]PSMA-1007	^{177}Lu-PSMA-I&T ^{225}Ac-PSMA-617
		[^{18}F]rhPSMA-7.3	
	GRPR	^{68}Ga-RM2	^{177}Lu-RM2
		^{68}Ga-NeoBOMB-1	
Pancreatic adenocarcinomas	Neurotensin receptor (NTSR1)	^{111}In-3B-227	^{177}Lu-3B-227
Bone marrow ablation in leukemias	CD-45	^{131}I-IOMAB, Apamistamab	^{131}I-IOMAB, Apamistamab

[a]Radiopharmaceuticals approved by FDA for routine clinical studies

clide decaying by β^-, α, or *EC* (emitting Auger electrons) [4–8]. Several theranostic radiopharmaceuticals of clinical importance are listed in Table 9.2.

The success of theranostics in the clinic has already been well established with the introduction of somatostatin analogues for PET/SPECT imaging and TRT in patients with SSTR-positive NETs. The future of theranostics is very promising and several theranostic radiopharmaceuticals in phase II/III clinical studies for both imaging and therapy are summarized in Table 9.2.

9.2.2 RPMI: Categories and Types

A number of biological processes and biochemical targets (Table 9.3) have been identified in order to develop target specific molecular imaging radiopharmaceuticals for PET and SPECT. On the basis of these molecular targets, imaging probes can be divided into nine different categories:

1. Probes for the determination of perfusion and membrane transport
2. Probes based on specific substrates for metabolism
3. Probes based on enzyme-mediated transformation
4. Probes based on receptor-mediated interactions
5. Probes based on antigen-antibody interactions
6. Probes based on abnormal protein expression or protein deposits
7. Probes based on nucleic acids (DNA and RNA)
8. Probes based on nanoparticles
9. Probes based on cellular migration or trafficking

Table 9.3 Radiopharmaceuticals for molecular imaging: Biological target and biochemical process

Biochemical process	Specific target	Radiopharmaceutical
Thyroid function	Sodium iodide symporter (NIS)	$^{123, 124, 131}$I sodium iodide
Glucose metabolism	*Glucose transporters and hexokinase*	[^{18}F]FDG
Fatty acid metabolism	*Thiokinase*	[^{18}F]FTHA
Membrane synthesis	*Choline kinase*	[^{18}F]Fluorocholine
DNA synthesis	*Thymidine kinase*	[^{11}C]thymidine, [^{18}F]FLT
Amino acid transport	Sodium dependent and sodium independent carrier mediated process	[^{18}F] FACBC or fluciclovine [^{18}F]fluoro-m-tyrosine
Dopamine synthesis and metabolism	*Aromatic amino acid decarboxylase (AAADC*	[^{18}F]FDOPA
Nigrostriatal neuronal degeneration	Dopamine transporter	[^{123}I]Ioflupane (DatScan)
Dopamine receptor	Dopamine D2 receptor	[^{11}C]Raclopride, ^{123}I-IBZM
Dopamine reuptake	Dopamine presynaptic transporter	[^{18}F]FP-CIT, ^{123}I-β-CIT
Altered osteogenesis	Bone mineral (hydroxyapatite)	[^{18}F]Sodium Fluoride
Neuroendocrine tumor	Norepinephrine transporter	[^{123}I]iobenguane (MIBG) (Adreview™)
Receptor binding	Estrogen receptor	[^{18}F]Fluoroestradiol
	Androgen receptor	[^{18}F]Fluorodihydroxytestosterone (FDHT)
	Somatostatin receptor (SSTR-2)	^{111}In-DTPA-Octreotide ^{68} Ga-Dotatate, ^{64}Cu-Dotatate
Macrophage (microglia)	Peripheral benzodiazepine receptor	[^{11}C]PK11195
Tumor antigen	Prostate-Specific Membrane Antigen (PSMA)	^{68}Ga-PSMA-11, [^{18}F]Piflufolastat, [^{18}F] PSMA-1007, [^{18}F]-rhPSMA-7.3 ^{111}In-DOTA-huJ591 mAb ^{89}Zr-IABM2 minibody
Dementia, amyloid burden	*β-Amyloid*	[^{11}C]PIB, [^{18}F]Florbetapir, [^{18}F]Florbetaben, [^{18}F]Flutemetamol
Dementia, tau burden	Aggregated tau neurofibrillary tangles (NFTs)	[^{18}F]Flortaucipir
Apoptosis	Phosphatidylserine	^{124}I-Annexin V
Angiogenesis	Integrin receptors, $\alpha_V \beta_3$	^{18}F-FB– E[c(RGDyK)]2
Hypoxia	Acidic pH in cells	[^{18}F]FMISO

Imaging radiopharmaceuticals may also be classified based on their clinical utility and the nature of application for which they are designed as tools in the drug development program [9]. Four classes of RMIPs have been identified:

1. A *radiolabeled drug substance* in which the cold stable atom is replaced by a radioisotope of the same element, which can be used for assessing the pharmacokinetics and biodistribution of the parent drug.
2. A *radioligand* with good binding affinity for a biologic target, which can be used to evaluate the effect of other unlabeled compounds at that target.
3. A *pathway marker* interacting with one component of a set of related biologic molecules, which may be used to probe the overall status of that system.
4. A *biomarker*, or surrogate marker, which provides a more general readout at the level of cell or organ for a specific biological process.

9.2.3 Choice of Radionuclide for SPECT and PET

A number of radionuclides that emit either γ photons or β+ are available (Table 9.4) for developing radiopharmaceuticals for imaging studies. These radionuclides, basically, belong to three groups of chemical elements: elements of organic radionuclides (C, N, and O), halogens (F and I), and metals (Cu, Ga, Y, Sc, In, Zr, and Tc). Certain

Table 9.4 Radionuclides useful for developing radiopharmaceuticals for PET and SPECT

	For PET			For SPECT		
Elements	Radionuclide	$T_{½}$	β^+ (MeV)	Radionuclide	$T_{½}$	γ (MeV)
Organic elements	^{11}C	20.4 min	0.959			
	^{13}N	9.96 min	1.197			
	^{15}O	2.03 min	1.738			
Halogens	^{18}F	109.8 min	0.635			
	^{75}Br	98 min	1.74			
	^{76}Br	16.1 hours	3.98			
	^{124}I	4.2 days	2.13	^{123}I	13.2 h	0.159
				^{131}I	8.04 d	0.364
Metals	^{66}Ga	9.45 hours	4.153	^{67}Ga	78.2 h	0.093, 0.184, 0.296
	^{68}Ga	68.3 min	1.898			
				^{111}In	67.2 h	0.173, 0.247
	^{44}Sc	3.92 hours	1.470			
	^{61}Cu	3.32 hours	1.220	^{67}Cu	2.6 d	0.185, 0.92
	^{62}Cu	9.76 min	2.910			
	^{64}Cu	12.8 hours	0.656			
	^{86}Y	14.74 hours	3.150			
	^{89}Zr	78.4 hours	0.900			
	^{94m}Tc	52 min	2.440	^{99m}Tc	6.0 h	0.140
	^{52}Mn	5.6 days				

elements (I, Cu, Ga, and Tc) are useful for the development of radiotracers for both PET and SPECT using a radioisotope of the same chemical element.

The organic radionuclides that decay by positron emission are ^{11}C, ^{13}N, and ^{15}O. These three radionuclides are isotopes of natural elements that are part of the majority of biochemicals and drugs. Interestingly, for these three elements there are no corresponding radioisotopes that decay by γ emission. Radiopharmaceuticals developed with the organic positron emitters are true molecular imaging radiotracers since these probes, biochemically, are indistinguishable from their natural counterparts. For example, [^{11}C]thymidine, [^{11}C]choline, [^{13}N]NH_3, or [^{15}O] water is chemically and biochemically the same as the corresponding unlabeled molecule.

The group of elements known as halogens (F, Cl, Br, and I) are very unique in nature. While chloride and iodide ions are quite common in the human body, fluorine, and bromine atoms, generally, are not part of the natural molecules. Among all the halogens, however, the fluorine atom is the only one that closely mimics the hydrogen atom in size (Table 9.5) [10]. The van der Wall's radii of fluorine and hydrogen are very similar, 1.35 and 1.2 Å, respectively. As a result, one can expect that, in any given organic molecule, the C-F bond closely mimics the biological behavior of the C-H bond. In addition, the fluorine atom is also the most electronegative of all halogens. As a result, the fluorine atom introduces a polarity more akin to a hydroxyl substituent in a molecule. The other halogens are bigger in size and are less electronegative compared to fluorine. Consequently, labeling a biochemical with bromine or iodine radioisotopes would alter the biological behavior of the molecule. However, halogen atoms in drug molecules are quite common and, sometimes, the halogen-containing drug molecules may have even greater affinity for a receptor or an enzyme, in vivo, than the nonhalogenated molecules [10]. In the last two decades, several radiopharmaceuticals were developed based on ^{123}I for SPECT imaging studies. Compared to the positron emitter, ^{124}I, the theoretical specific activity (Table 9.6) of ^{123}I is even higher (because of shorter half-life). Organic molecules containing aromatic rings can be eas-

Table 9.5 Comparison of physico-chemical parameters of halogens with hydrogen and hydroxyl group

	Hydrogen	Fluorine	Oxygen (OH)	Iodine
Electronegativity	2.2	3.98	3.44	2.66
[a]Bond length, Å	1.09	1.39	1.43	2.14
Van Der Wal's radius, Å	1.20	1.35	1.40	2.15
Bond energy, KCal/mol^{-1}	99	116	85	51

[a]Bond length for $-CH_2X$ reported here where X = H, F, or OH. Table modified from Park et al. [10]

Table 9.6 Specific activity (SA) of radionuclides

	Maximum SA		Practical SA
Nuclide	mCi/μg	Ci/μmole	Ci/μmole
^{11}C	838,000	9220	<100
^{18}F	95,000	1170	10–20 as F^- < 0.03 as F_2
^{68}Ga	40,600	2766	
^{67}Ga	597	47	<3.35
^{111}In	423	40	<5.55
^{123}I	1926	237	
^{124}I	250	31	

ily labeled with radionuclides. Therefore, ^{123}I and ^{124}I play a major role in the development of radiotracers for molecular imaging studies.

Among the metals (Table 9.4), Ga, In, and Y are trivalent and have similar chemistries. The transition metals such as copper, scandium, and zirconium have complex coordination chemistries. The radionuclides of all these metals can be used to label peptides and proteins using bifunctional chelating agents. ^{111}In, ^{64}Cu, and ^{68}Ga-labeled octreotide analogs (^{111}In-DTPA-octreotide, ^{64}Cu-dotatate, and ^{68}Ga-dotatate) have already been approved by FDA as molecular imaging probes for SPECT and PET imaging studies to detect SSTR-2 positive NETs. Among the transition metals, radioisotopes of copper have useful physical characteristics to develop RMIPs. Several other positron-emitting metals, such as ^{44}Sc, ^{89}Zr, and ^{94m}Tc (Table 9.4), may also be useful for developing imaging agents.

9.2.4 General Criteria for the Design of RP-MI

For any radiopharmaceutical to be successful as an imaging agent for PET or SPECT, the radiopharmaceutical must have the following ideal characteristics:

- Rapid plasma clearance to reduce blood pool background in the target tissue
- Rapid washout or clearance from nonspecific areas
- Low nonspecific binding
- Preferably must be stable in vivo, and no metabolism
- High membrane permeability and intracellular trapping
- Target specificity and high affinity for molecular targets
- Specific activity must be high to prevent saturation of specific binding sites
- Tissue distribution, localization, and target binding should be favorable for developing simple kinetic modeling to estimate quantitative data
- Radiation dosimetry must be favorable for multiple diagnostic imaging studies (if necessary)
- Synthesis of radiopharmaceutical under GMP conditions must be rapid and suitable for automation using automated synthesis modules

Developing a specific molecular imaging radiotracer involves careful design of the structural requirements in a molecule in order to optimize target specificity and at the same time optimize the pharmacokinetic and pharmacodynamic behavior of the probe to meet the demand of the imaging technique. The physicochemical properties of the radiopharmaceutical, such as size, charge, solubility, lipophilicity, and SA are very important criteria, and must be addressed in designing the structural features of the molecule. Factors, such as rapid metabolism and plasma

protein binding (PPB), and nonspecific binding in nontarget tissues are not desirable for optimal in vivo behavior. It is important to identify the most appropriate structural analog that meets most of the criteria for an ideal imaging agent. Some of these criteria will be discussed in greater detail with specific examples, later, in order to emphasize the importance of a careful design of structural features in order to develop a clinically useful MIP.

9.2.4.1 The Size of MIP

The molecular size or molecular mass of the MIP is one of the major properties of a molecule that determines the rate of clearance from circulation. Small organic molecules, natural or synthetic, clear from circulation rapidly and provide much higher target/background ratios. With small organic molecules, however, it may not be possible to put more than one radiolabel per molecule. Also, with small molecules, it may be difficult to optimize high SA and high target affinity, and to achieve appropriate pharmacokinetic and metabolic behavior. Therefore, with large proteins, such as antibody molecules (150,000 Da), it may be possible to label with more than one radiolabel per molecule. Liposomes and nanoparticles, because of their size, shape, structural flexibility, multivalency/multifunctionality characteristics, may offer greater potential for developing high specific activity imaging agents. Among the radiopharmaceuticals approved for routine clinical studies (Table 9.1), all of them are small molecules (<1500 Da) as shown in Fig. 9.3.

Since small molecules clear from circulation rapidly, radiolabeling with radionuclides with

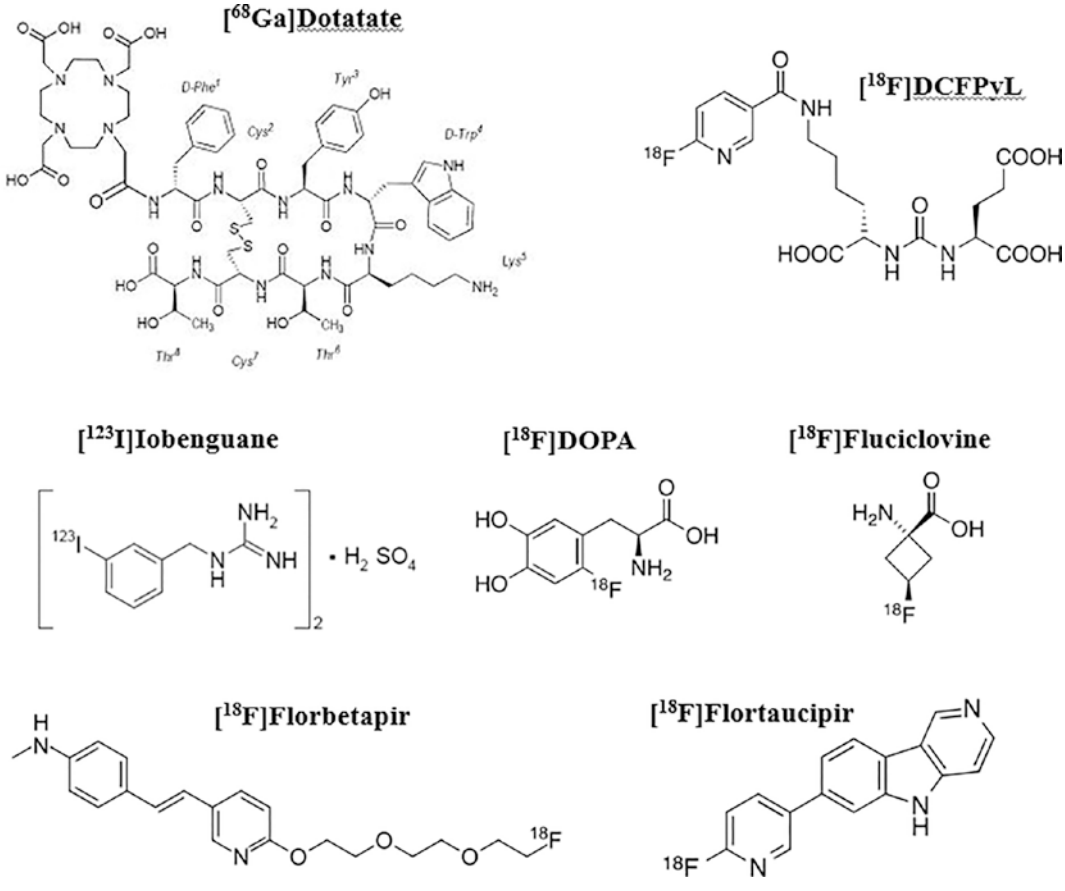

Fig. 9.3 Examples of small molecule radiopharmaceuticals for PET. Among the agents shown in the table, ^{68}Ga-Dotatate is relatively large (1500 Da) compared to Fluciclovine (132 Da)

short-half life (such as ^{18}F, ^{68}Ga, and ^{44}Sc) also facilitates developing radiopharmaceuticals that deliver less radiation dose to the critical organs. Therefore, radioimmunoimaging based on monoclonal antibodies labeled with longer-lived nuclides (such as ^{124}I and ^{89}Zr) are becoming relatively less popular for routine diagnostic imaging studies. However, radioimmunoimaging with mAbs is very important in the research and development of radioimmunoconjugates for RIT.

9.2.4.2 The Position of Radiolabel in the Radiotracer

The position of the radiolabel in the molecular imaging probe must be carefully selected so that the presence of a radionuclide at that position preserves the overall chemical and pharmacological properties of the parent unlabeled molecule. In addition, the position of the radiolabel must also preserve the metabolic stability of the radiopharmaceutical during the time course of the imaging study. The importance of the position of the radiolabel in the imaging probe is illustrated using FDG and FLT molecules.

Design of FDG Molecule

2-[^{18}F]Fluoro-2-deoxy-d-glucose (FDG) is regarded as the most important, clinically useful, and successful radiopharmaceutical in nuclear medicine. The design of FDG [11, 12] is a perfect example to illustrate the significance of the position of the ^{18}F atom in the FDG molecule (Fig. 9.2).

In the 1940s, 6-deoxy-6-[^{19}F]fluoro-6-deoxy-D-glucose ([6-^{19}F]FDG) was first reported [12]. In 1954, it was suggested that the hydroxyl group on carbon 2 was not essential for (1) the carrier-mtediated membrane transport into the cell, and (2) the substrate specificity of D-glucose for *hexokinase*-mediated phosphorylation reaction [13]. It also became apparent that the hydroxyl group on C-2 was essential for further metabolism of the glucose-6-phosphate. Subsequently, 2-fluoro-[^{19}F]2-deoxy glucose ([2-^{19}F] FDG) was developed as an anticancer agent [12]. However, due to the very high toxicity of FDG, further clinical studies with FDG were abandoned. Later, 2-[^{14}C]-2-deoxy glucose (CDG) was developed as a tracer to measure cerebral glucose metabolism in animals based on autoradiography [14].

On the basis of Sokoloff's work, [^{11}C]CDG would be an appropriate tracer for PET imaging studies [15]. However, the attachment of the ^{18}F atom to the carbon 2 atom in the D-glucose molecule is justified since FDG is a good substrate for *hexokinase*. The kinetic constant (K_m) of the reaction with hexokinase for FDG (0.19 ± 0.03) is very similar to that of D-glucose (0.17). Attaching the ^{18}F atom to carbon 3 or 4 alters the substrate specificity significantly ($K_m > 70$) [3]. Also, the ^{18}F atom in the C-2 position prevents further metabolism of the molecule. As a result, FDG-6-phosphate accumulates in the cell.

Another interesting difference between FDG and CDG concerns the renal excretion of the molecule. The hydroxyl group on C-2 is essential for the active transport across the renal tubules for reabsorption of D-glucose. In contrast to CDG, FDG is not reabsorbed, but excreted. As a result, the body background of the ^{18}F activity is less and the radiation dosimetry with FDG is much more favorable.

Design of FLT Molecule

The DNA synthesis is a measure of cell proliferation. The four nucleotides required for DNA synthesis are cytosine, guanine, adenine, and thymidine. Thymidine is the only one incorporated exclusively into the DNA and not into the RNA [16]. Intracellularly, thymidine is first phosphorylated in the cytoplasm by the enzyme *thymidine kinase-1* (*TK-1*) to thymidine monophosphate (TMP), prior to incorporation into the DNA. [^{3}H] Thymidine was introduced to measure thymidine incorporation into DNA (thymidine labeling index) in tumor tissue [17, 18]. The nonradioactive 3-[^{19}F]fluoro-3-deoxythymidine (FLT) (Fig. 9.4) was first developed in 1969 as an anticancer agent [19]. Subsequently, [^{11}C]thymidine was introduced as a PET tracer [20]. Almost two decades later, 3-[^{18}F]fluoro-3-deoxythymidine (FLT), the metabolically stable thymidine analog, was introduced for PET imaging studies [21].

FLT is transported into the cell similar to thymidine and then phosphorylated to [^{18}F]FLT-5′-monophosphate by the enzyme, *TK-1*. FLT-MP is

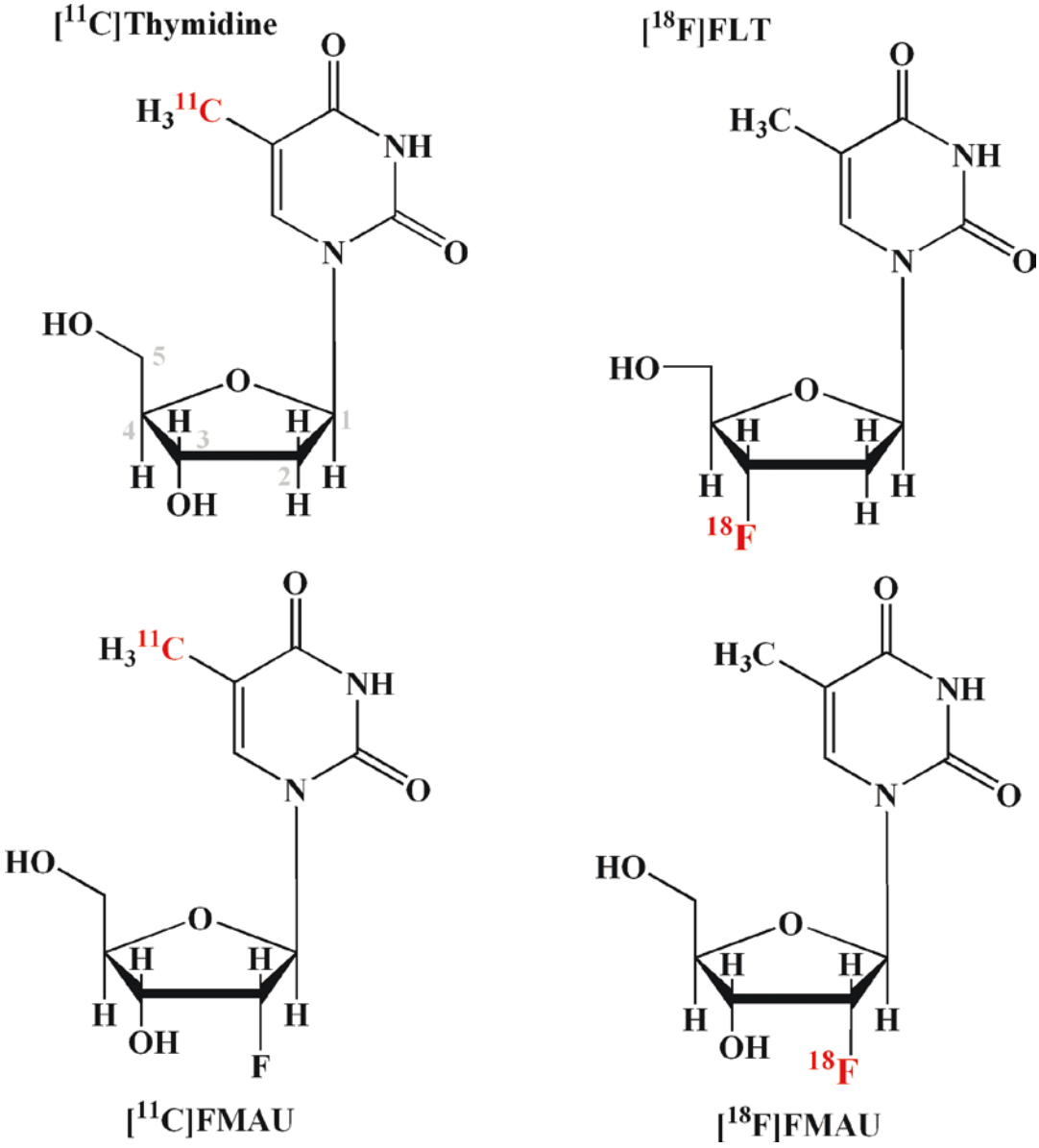

Fig. 9.4 Radiolabeled analogs of thymidine for imaging DNA synthesis: The hydroxyl group on C-3 in the sugar is essential for incorporation into DNA synthesis

further phosphorylated to FLT-TP by the enzyme *thymidylate kinase* [22]. FLT phosphates, however, are (1) impermeable to the cell membrane, (2) resistant to degradation, and (3) metabolically trapped inside the cells. The incorporation of FLT into the DNA, however, is relatively insignificant (<1%).

The pyrimidine analogs, 2′-fluoro-5-[^{11}C] methyl-1-β-D-arabinofuranosyluracil (FMAU) and 2′-[^{18}F]fluoro-5-methyl-1-β-D-arabinofuranosyluracil (FMAU) have been shown to be useful for imaging tumor cell proliferation [23, 24]. FMAU can be taken up by cells and phosphorylated by TK-1 and TK-2 and sub-

sequently incorporated into DNA, by *DNA polymerase*. FMAU employs the same DNA synthetic pathway as thymidine and, therefore, has the potential to image DNA synthesis. It is important to recognize that FMAU is incorporated into DNA synthesis, but not into FLT [22, 25]. This observation suggests that the hydroxyl group on the C-3 atom (Fig. 9.4) is essential for the incorporation of thymidine analogs into DNA, but not for substrate specificity of the enzyme TK-1. Despite the fact that FLT lacks the hydroxyl group on C-3, necessary for its incorporation into DNA, it appears to outperform both FMAU and FIAU in terms of uptake and retention based on in vitro studies [26].

Design of MIBG

In the 1980s, Dr. Wieland and his colleagues at the University of Michigan developed an analog of norepinephrine (NE), known as ^{131}I-*meta*-iodobenzylguanidine (MIBG), a diagnostic tracer to allow imaging of the adrenal medulla [27, 28]. MIBG was developed by linking the benzyl portion of bretylium with the guanidine group of guanethidine (Fig. 9.5). Among the three isomers of iodobenzylguanidines, the meta isomer (MIBG) has less in vivo deiodination and liver uptake than the other two isomers. In 1994, [^{131}I]MIBG, also known as iobenguane sulfate I-131 intravenous (*low SA (LSA) formulation*), received FDA approval as an imaging agent for the localization of specific sites of pheochromocytomas and neuroblastomas. In 2008, [^{123}I] MIBG or iobenguane I-123 injection was also approved by FDA as a tumor imaging agent (AdreviewTM; GE Healthcare). To develop derivatives of MIBG for PET studies, iodine atom was replaced with fluorine directly to obtain ^{18}F-meta-fuorobenzylguanidine, ([^{18}F]MFBG ([^{18}F]PFBG) [29].

9.2.4.3 Stereospecificity

As discussed in Chap. 6, *stereoisomers* have the same bonds, but exhibit different spatial arrangements of their atoms. One type, known as optical isomerism, is exhibited by molecules with asymmetric carbon atoms (chiral centers) that have non-superimposable mirror images. The optical isomers, called *enantiomers,* rotate plane-polarized

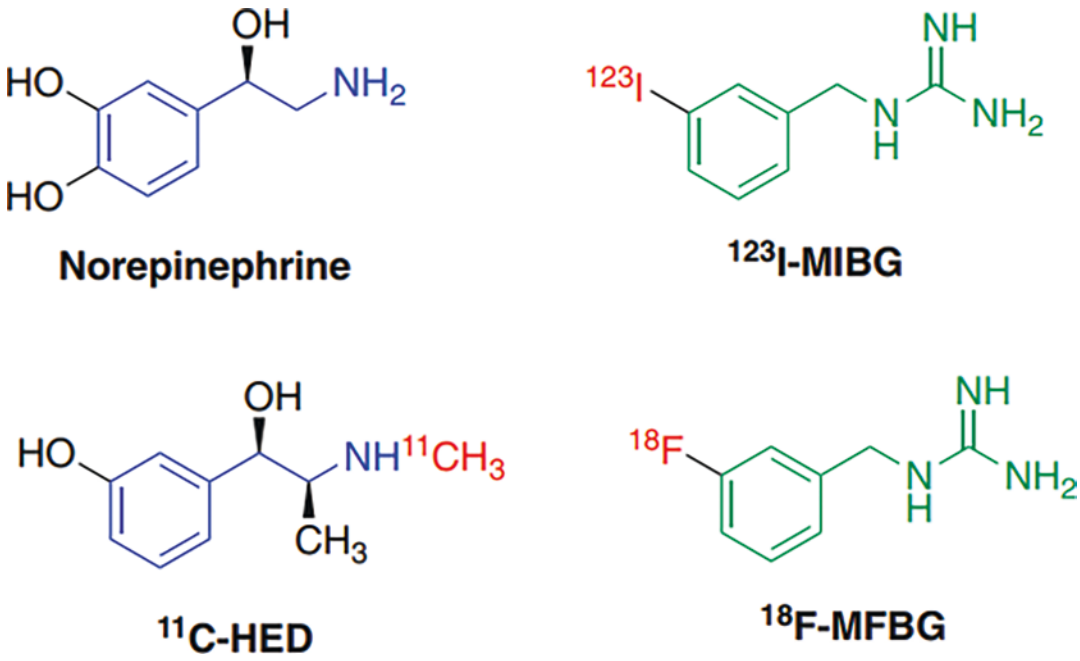

Fig. 9.5 Norepinephrine analogs specific for norepinephrine transporter. It is the meta position of iodine or fluorine atom in the aromatic ring that has better tumor uptake and retention

light in opposite *directions: dextrorotatory* (D or (+)) and *levorotatory* (L or (−)). These designations only refer to the experimental values of rotation, while the letters D and L relate asymmetric centers to glyceraldehydes (as in sugars), for which an absolute stereochemistry has been defined. On the basis of this designation, in the case of amino acids, L-amino acids are often dextrorotatory. Only L-amino acids are found in nature.

A molecule is said to be *chiral* if it exists as an *enantiomer*. Molecular targets such as receptors and enzymes often exhibit stereoselectivity or binding selectivity for one enantiomer over the other. The stereochemical requirements of biological activity have consequence for the radiolabeled amino acids and sugars [11]. For example, only the radiolabeled L-amino acid and D-glucose analogs are biologically active. Further, it is important to recognize that radiolabeled drug molecules also exhibit stereoselective binding to neuroreceptors. For example, only the [^{11}C]-(+) McN-5652 binds to the serotonin transporter [30] and [^{11}C]dexetimide binds to the muscarinic cholinergic receptor [31].

Development of [^{18}F]-rhPSMA-7.3

The importance of stereospecificity on the in vivo behavior and target specificity of a radiotracer is best illustrated based on the design and development of PSMA agent. To develop an ideal theranostic small molecule PSMA inhibitor, [^{18}F]rhPSMA-7 was developed as a radio hybrid (rh) PSMA inhibitor since the molecule can be labeled with ^{18}F as well as a radiometal (^{68}Ga, ^{177}Lu, or ^{225}Ac) [32, 33]. Subsequently, based on preclinical evaluation [^{18}F]rhPSMA-7.3 was selected as the lead compound since it is a single diastereoisomer form of ^{18}F-rhPSMA-7, which has been shown to have good diagnostic efficacy in patients with primary and recurrent prostate cancer [33].

[^{18}F,^{nat}Ga]rhPSMA-7 represents a mixture of four stereoisomers (Fig. 9.6), differing in the stereoconfiguration of the diaminopropionic acid branching unit (*D*-Dap or *L*-Dap) and the glutamic acid pendant arm at the DOTAGA-chelator (*R*-DOTA-GA or *S*-DOTA-GA). Four rhPSMA-7 isomers are as follows:

[^{18}F,^{nat}Ga]rhPSMA-7.1 (D-Dap–R-DOTA-GA)
[^{18}F,^{nat}Ga]rhPSMA-7.2 (L-Dap–R-DOTA-GA)
[^{18}F,^{nat}Ga]rhPSMA-7.3 (D-Dap–S-DOTA-GA)
[^{18}F,^{nat}Ga]rhPSMA-7.4 (L-Dap–S-DOTA-GA)

Based on HPLC analysis, the investigators discovered that [^{18}F,^{nat}Ga]rhPSMA-7.3 was the predominant species. Biodistribution studies in tumor bearing mice indicated that [^{18}F,^{nat}Ga]rhPSMA-7.3 has high tumor accumulation and low uptake in blood, liver, and kidneys. Therefore, it was selected as the preferred isomer and transferred into clinical studies [34].

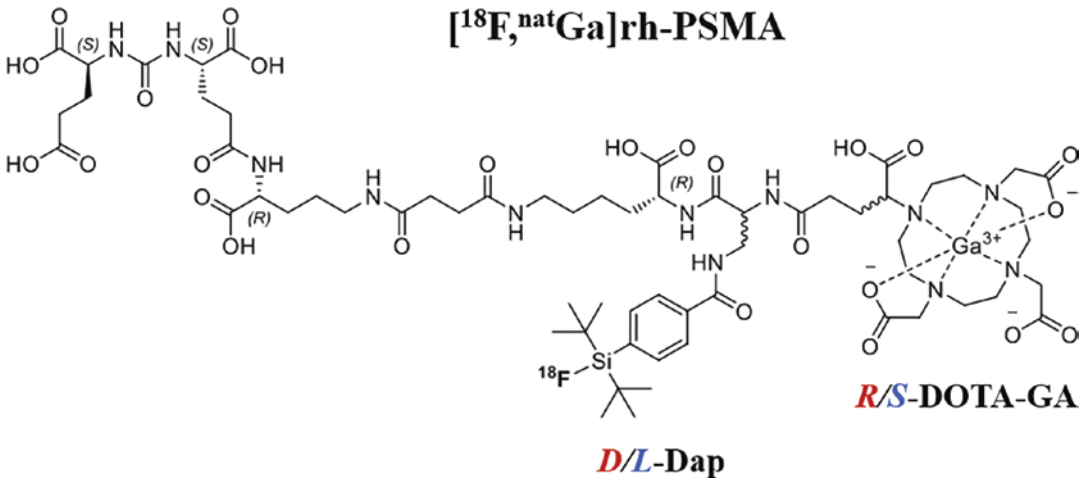

Fig. 9.6 [^{18}F,^{nat}Ga]rhPSMA-7 small molecule PSMA inhibitor: It is a mixture of four stereoisomers

9.2.4.4 Lipophilicity

Lipophilicity is the affinity of a molecule or moiety for a lipophilic environment. Molecular size, mass (weight), and hydrogen-bonding capacity also contribute to the overall lipophilicity of the molecule [35]. Lipophilicity is a fundamental physicochemical property of a compound and plays a pivotal role in the absorption, distribution, metabolism, and elimination of drug molecules. The most common experimental lipophilicity measurement involves partitioning of a compound between an octanol and a buffer, where the log of the ratios of the compound concentration in the octanol layer is divided by that in the buffer layer, often called the Log P. Lipophilicity is also expressed in several different ways, including terms such as Log P, clog P, delta Log P, and Log D, depending on the method of estimation [36].

Very polar compounds, normally, exhibit high water solubility, fast clearance through the kidneys, and often contain ionizable functional groups that limit blood-brain barrier (BBB) penetration. In general, only the neutral, lipophilic molecules can pass through the BBB and enter into the brain tissue. The brain penetration, and specific to nonspecific binding ratios, exhibited in vivo by radiotracers, involves a complex interplay between many critical factors, including lipophilicity, receptor affinity, metabolism, molecular size, and shape, etc. Based on an extensive literature search, Waterhouse recently noted that for most neutral radiotracers, the relationship between lipophilicity and molecular weight shows the expected parabolic relationship [36] (Fig. 9.7). The general criteria for adequate brain penetration and optimum target to nontarget ratios of high SA radiotracers are as follows:

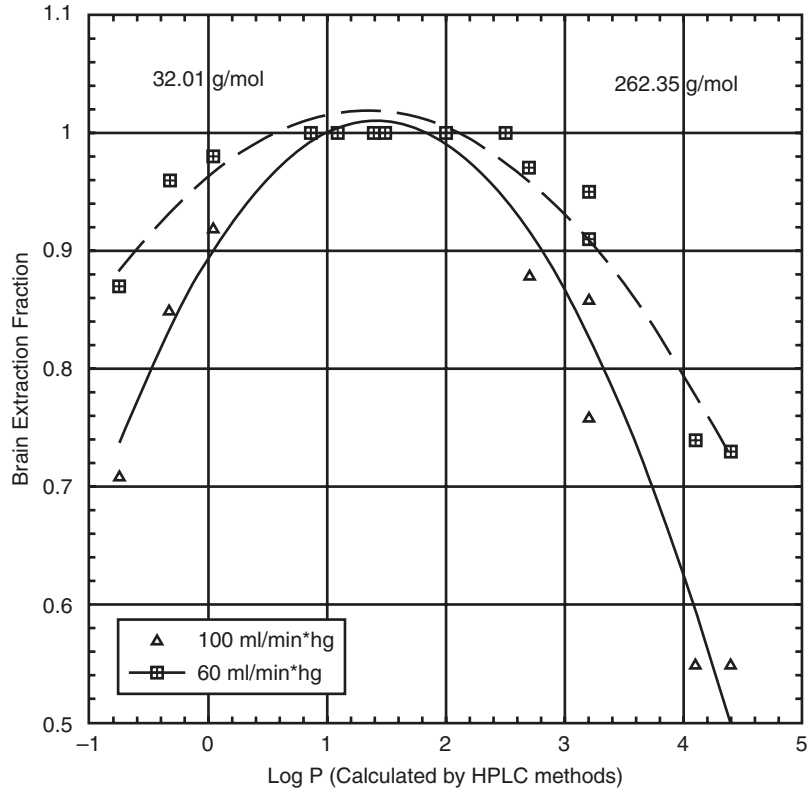

Fig. 9.7 Lipophilicity (log P) vs. brain uptake for simple low- mass radiolabeled compounds, such as [^{11}C]ethanol and [^{11}C] butanol. The results indicate that the brain uptake of these radiotracers does indeed correlate with lipophilicity and that the relationship is parabolic in nature. Also, the uptake is dependent on the blood flow [36]

- Log P or Log D < 3.5
- Molecular weight <450 g mol^{-1}
- An absence of functional groups that will strongly ionize at physiological pH
- No appreciable affinity for efflux pumps (such as PGP)
- No appreciable affinity for specific binding sites for high-capacity peripheral sites, including albumin or other plasma proteins
- Not a substrate for enzymes at the BBB

9.2.4.5 Plasma Protein Binding

It is well documented that both, specific and nonspecific binding (NSB) of drugs can occur with plasma proteins, cell membranes, and other components present in the blood [37]. NSB may be saturable when drugs are administered at high-enough mass and bioavailability may not be an issue. The NSB of radiotracers, however, may significantly reduce the bioavailability [37]. NSB to albumin and other plasma proteins is known to correlate positively and linearly with increasing lipophilicity [38]. Some radiotracers exhibit high NSB (>90%) and may enter the brain nearly as well as those that exhibit much lower plasma protein binding [35]. In addition, increasing lipophilicity has been correlated with increased lung deposition, liver and spleen uptake, and higher affinity to many metabolic enzymes such as cytochrome P450. Such processes serve to decrease the percentage of the administered dose that may enter the brain and other target organs, and tissues.

9.2.4.6 Metabolism

In the design of a radiotracer for molecular imaging, it is especially important that the label (radionuclide) is ideally in a metabolically stable position. When a radiotracer is injected into a human subject, the signal from the radionuclide is detected, regardless of whether it is coming from the intact radiotracer or from a radiolabeled metabolic fragment. The fate of the radionuclide, as a result of metabolism, must be considered in selecting the position of the label in a molecule. Peripheral metabolism, especially in the blood, may dramatically decrease the delivery of the probe to the target site. Also, metabolism of the radiopharmaceutical at or near the target site may decrease the specific binding to the molecular target. In addition, the quantitative estimation of molecular target concentration based on kinetic modeling may get complicated if the metabolic products are also trapped at the target site. Whatever the case may be, it is essential that all the metabolic products and their relative concentrations are known so that a meaningful interpretation of the imaging data can be made. Several examples will be discussed to emphasize the importance of metabolism in molecular imaging studies.

[^{18}F]FDOPA Metabolism

In the presynaptic dopaminergic neuron, the amino acid tyrosine is converted to dihydroxyphenylalanine (L-DOPA). Subsequently, *DOPA decarboxylase* or *aromatic amino acid decarboxylase* (*AAAD*) converts L-DOPA to dopamine. The position of the ^{11}C or ^{18}F label in the L-DOPA molecule clearly illustrates the importance of metabolism and its role in drug design (Fig. 9.8). A ^{11}C label can be introduced in the carboxyl group (COOH) as in the case of [^{11}C-carboxyl]L-DOPA. Since AAAD eliminates the carboxyl group, the ^{11}C label is quickly lost. However, if the β-carbon atom is labeled with ^{11}C, as in the case of [β-^{11}C]l-DOPA, the ^{11}C activity is still retained with the dopamine molecule [39].

[^{18}F]6-Fluoro-l-DOPA (FDOPA) was also developed as a tracer to examine the transport of dopamine precursor from plasma and its decarboxylation by AAAD to fluorodopamine [40]. FDOPA, however, undergoes extensive metabolism in vivo [41] as shown in Fig. 9.9. FDOPA is decarboxylated by *aromatic L-amino acid decarboxylase* (AAAD) to produce [^{18}F] Fluorodopamine (FDA). FDOPA can be *O*-methylated by catechol-*O*- methyltransferase (COMT) to 3-*O*-methyl-6-[^{18}F] fluoro-L-dopa (3-OMFDOPA), which is uniformly distributed throughout the brain. Fluorodopamine (FDA) can be oxidized by *monoamine oxidase (MAO)* to L-3,4-dihydroxy-6-[^{18}F]fluorophenylacetic acid ([^{18}F]FDOPAC), which is subsequently *O*-methylated by COMT to 6-[^{18}F] fluorohomovanillic acid ([^{18}F]FHVA). AAAD and *COMT* are also present in peripheral tissues, such as liver, kid-

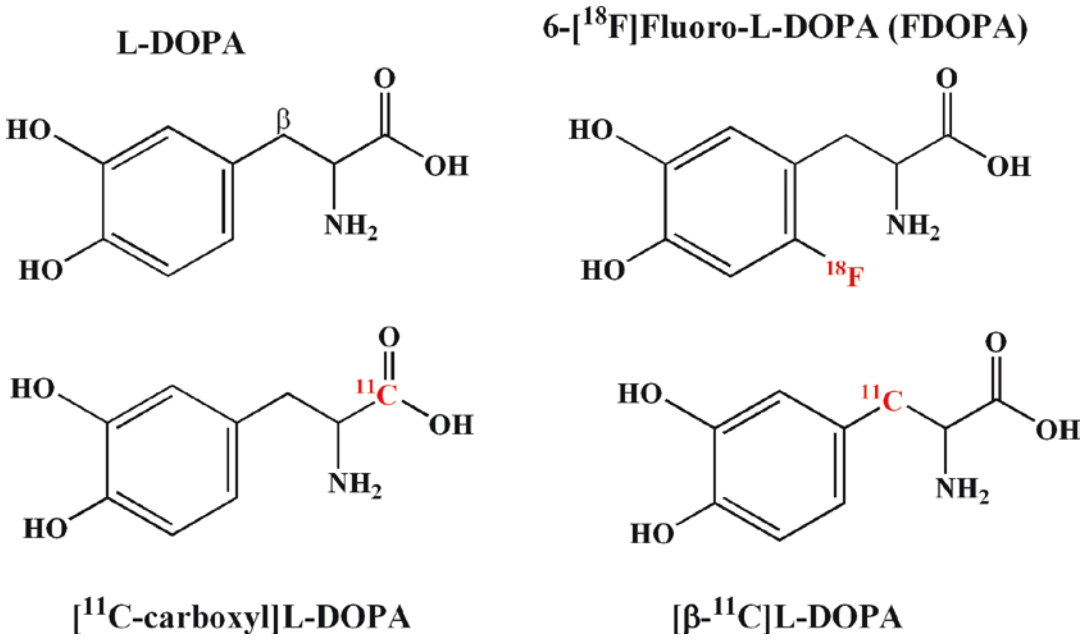

Fig. 9.8 Radiolabeled analogs of L-DOPA: The position of ^{11}C or ^{18}F label is very important to prevent metabolic degradation and elimination in vivo

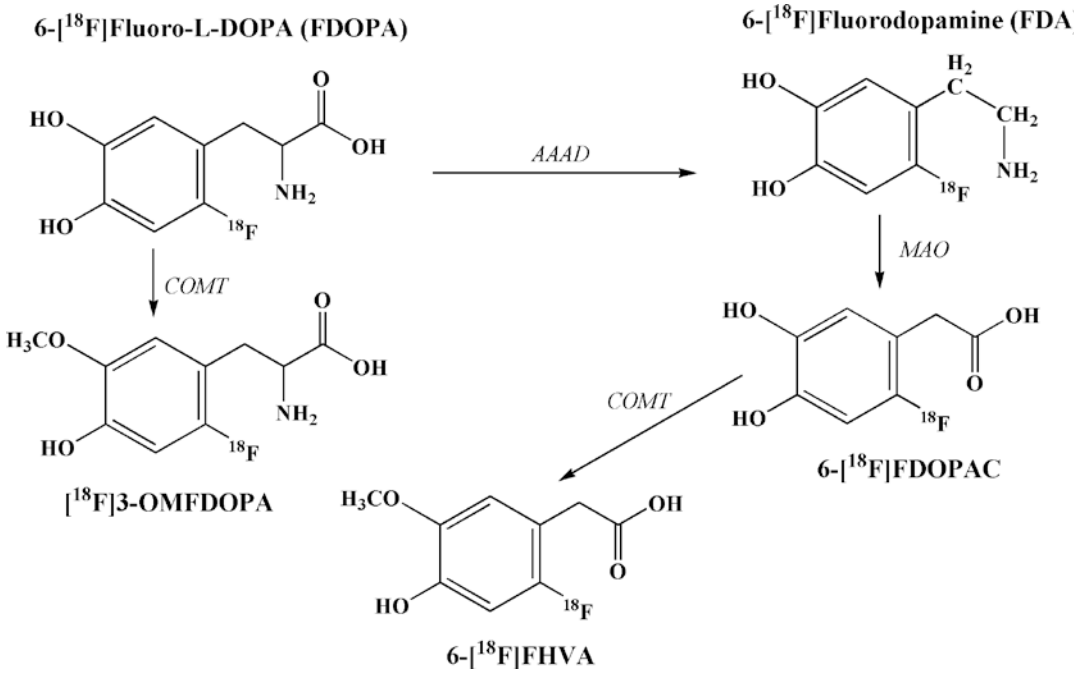

Fig. 9.9 In vivo metabolism of FDOPA

neys, and lung. Both, the decarboxylation of the compound and the release of radiometabolites into the blood, however, can be reduced with carbidopa, a decarboxylase inhibitor.

While FDOPA has already demonstrated its value as a diagnostic probe in Parkinson's disease and in neuroendocrine tumors, the metabolic profile of this probe, however, complicates the estimation of quantitative parameters by kinetic modeling.

Thymidine Metabolism

[^{11}C]Thymidine was developed as a PET tracer to image tumors and measure proliferation rate. Analysis of thymidine PET images, however, is complicated by the fact that the tracer is rapidly metabolized (Fig. 9.10). As a result, much of the ^{11}C activity imaged in thymidine studies is in the form of labeled metabolites, which are no longer available to be incorporated into the DNA.

The large pools of *thymidine phosphorylase* (*TP*) present in the blood, liver and spleen rapidly degrade thymidine in vivo. Thymidine can be degraded by *TP* to thymine and then be reduced to dihydrothymine (DHT), β-ureidoisobutyric acid (BUIB), and β-amino isobutyric acid (BAIB). For thymidine, labeled at the C-2 position, degradation products beyond BUIB do not contain the label, which leaves the pathway as [^{11}C]CO_2. Degradation takes place rapidly in the liver and blood, and labeled metabolites appear in significant quantities in the blood within minutes after injection [42].

Thymidine can be labeled with ^{11}C in the methyl ($^{11}CH_3$) or in the C-2 (^{11}CO) position of

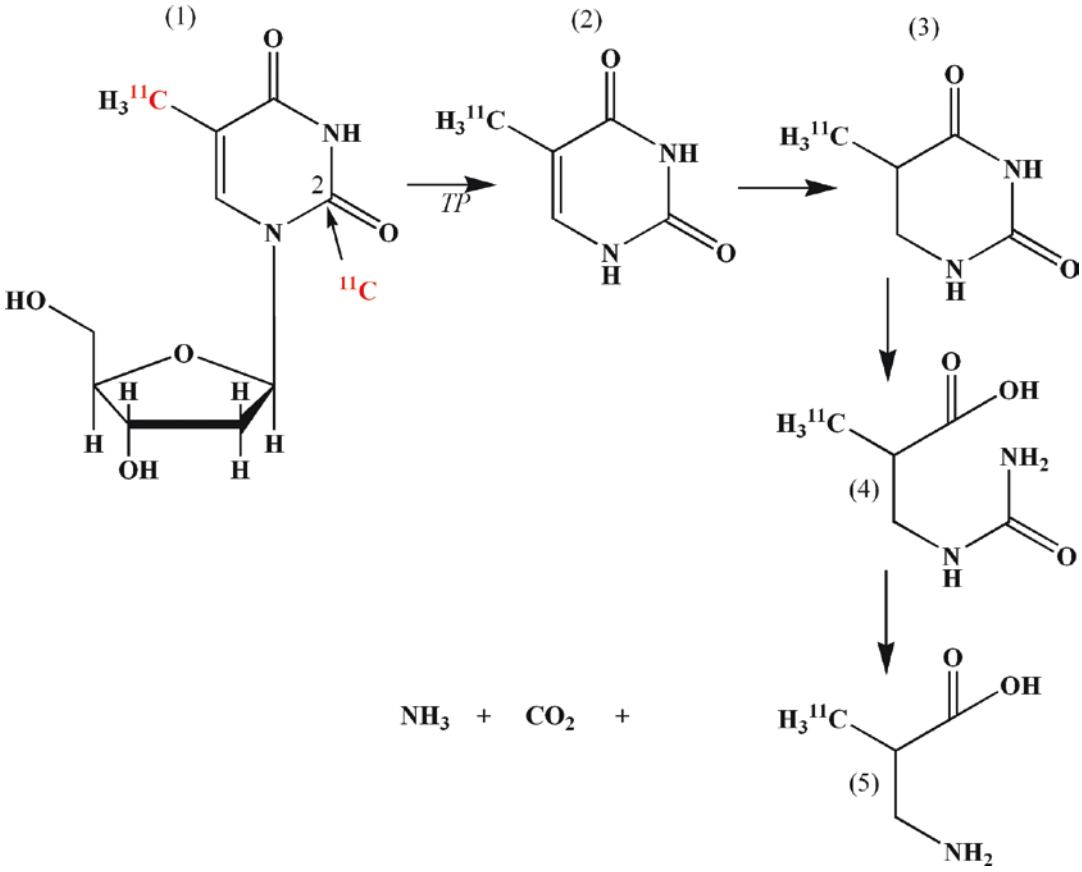

Fig. 9.10 In vivo metabolism of [^{11}C]thymidine: Labeling C-2 in the carbonyl group will result in the elimination of ^{11}C activity as CO_2. Labeling the methyl group with ^{11}C provides relatively more stable tracer for imaging DNA synthesis

the pyrimidine ring. The former gives rise to acidic metabolites, which can accumulate in cells in a number of pathways and limit the ability to develop a comprehensive, validated kinetic model to measure the DNA synthesis using methyl-labeled thymidine. On other hand, the C-2 label is lost as $[^{11}C]CO_2$, which rapidly enters the large bicarbonate pool in the body.

To overcome the practical limitations of $[^{11}C]$ thymidine imaging, metabolically stable ^{18}F-labeled thymidine analogs, FLT and FMAU (Fig. 9.4) have been developed [21, 43]. FLT does not generate significant metabolites during the imaging procedure as does $[^{11}C]$thymidine. FLT is not a substrate for *TP*, the enzyme that breaks the bond between the pyrimidine and deoxyribose. However, FLT is glucuronidated in the liver and then delivered to the blood. FLT-glucuronide is restricted to the vascular space and is cleared by the kidneys.

Metabolism of WAY-100,635

WAY-100,635 is a $5HT_{1A}$ receptor antagonist (Fig. 9.11). Initially, the ^{11}C label was introduced in the methoxy group (OCH_3). Subsequently, ^{11}C was introduced in the carbonyl (CO) position to reduce the contribution of labeled metabolites [44]. The molecule was finally modified resulting in a ^{18}F-labeled analog with improved metabolic stability and greater potential for commercial distribution.

A key step in the development of a new molecular imaging agent is to characterize its binding specificity in vivo by imaging studies. It is important to compare the biodistribution and pharmacokinetics of radiotracer with and without a pharmacological dose of the radiolabeled compound to assess the specificity and saturation ability. In order to address the significance of the metabolism on a specific binding at the target site, radiotracer should be labeled at different positions in the molecule and then the in vivo distribution and kinetics of these analogs should be compared.

9.2.4.7 Specific Activity

As previously discussed, SA (Ci/μmole^{-1}) of the RMIP is very important for molecular imaging studies based on PET and SPECT since it is a measure of the number of probe molecules that can give a radioactive signal in a given mass of RMIP. The SA needed for a given radiotracer depends on the concentration of the target molecules (receptors, enzymes of antigens) present in a given cell or tissue (Table 9.7). Typically, the concentration of high-affinity molecular targets is between 10^{-12}–10^{-9} moles L^{-1}. For neuroreceptor and gene imaging studies very high SA (2–10 Ci μmole^{-1}) is absolutely necessary. For enzyme-mediated molecular imaging studies, 100–1000 times lower SA may be adequate. For animal (rodent) studies, based on MicroPET and MicroSPECT, it is important to recognize that the SA requirements may be even higher than what is needed for clinical studies [45]. The relative size of the brain in different species (humans, rats, and mice) and dopamine receptor levels in striatum are compared in Table 9.7. With the typical dose of $[^{11}C]$raclopride (SA = 1.0 Ci μmol^{-1}) injected for imaging studies, the receptor occupancy in mice is almost 70% compared to relatively insignificant levels of occupancy in humans (Table 9.8). These results suggest that for MicroPET imaging studies, the SA of radiotracers must be at least ten times more than what is needed for human studies.

9.2.4.8 Radiopharmaceutical: Mechanism of Localization

Radiotracers can be generally classified based on their ability to image a specific biochemical process or based on their unique mechanism of localization in a specific organ/tissue of interest (Table 9.2). The localization of RMIP in a specific target organ or tissue of interest depends on three important phenomena:

- Rapid blood clearance and transport of radiotracer to the target organ or tissue of interest.
- Transport of radiotracer from capillaries into the extracellular fluid and subsequent transport into the cells through the cell membrane. Transport processes such as simple diffusion, facilitated diffusion, active transport, and receptor-mediated endocytosis (Chap. 7, Sect.

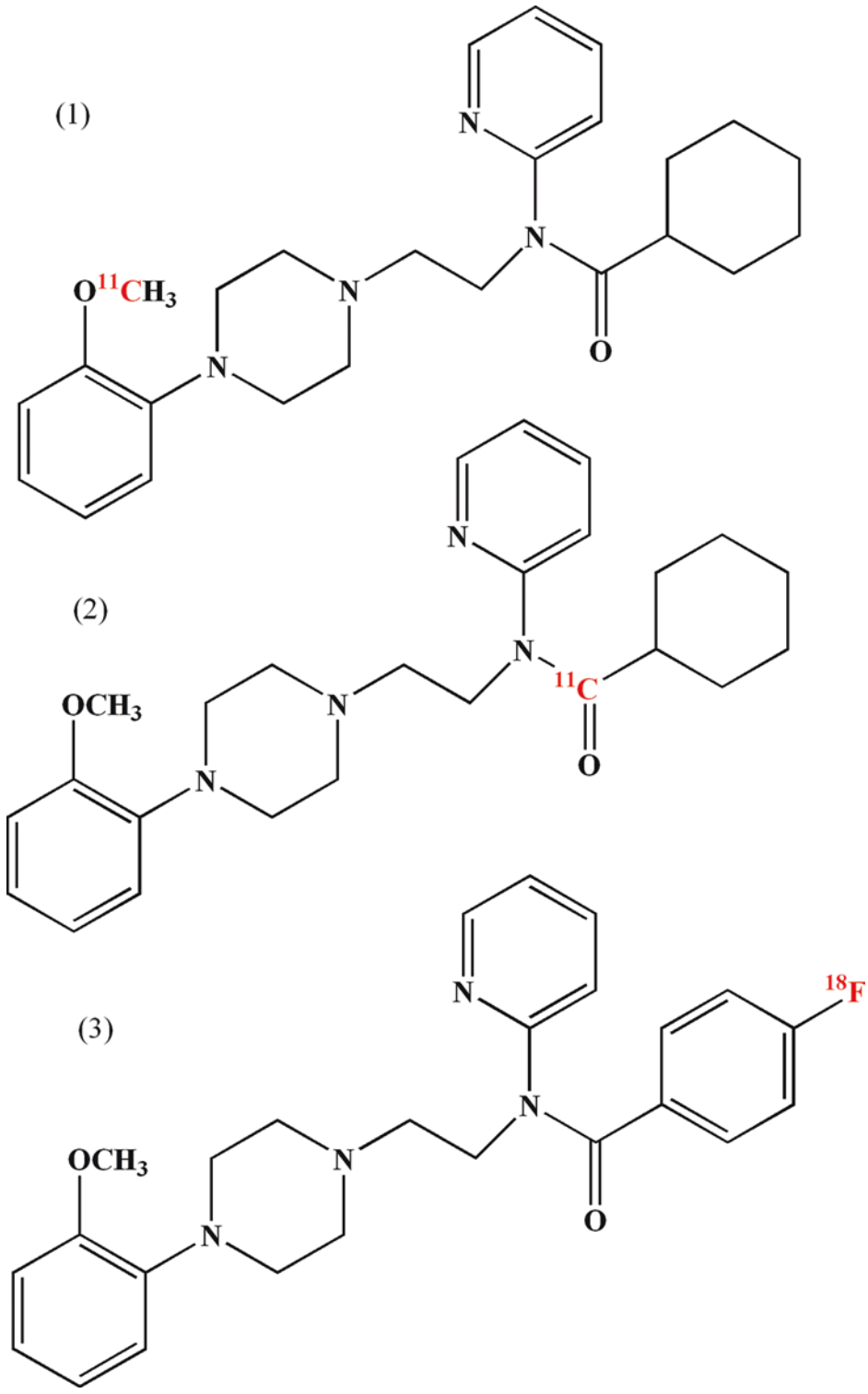

Fig. 9.11 Radiolabeled WAY-100,635, a $5HT_{1A}$ receptor antagonist: ^{11}C in the carbonyl position (2) provides a more stable radiotracer when compared to ^{11}C labeling in the methoxy group (1). The ^{18}F-labeled analog provides even greater in vivo stability and is the preferred radiotracer (3)

7.7) play a very important role in the localization of the radiotracer at the target site.

- Localization at the target site and intracellular trapping may be due to any one of these following biochemical processes:
  - Specific binding to receptors on the cell membrane or within the cell
  - Internalization of radiotracer-receptor complex
  - Specific binding to the extracellular or intracellular antigens
  - Specific enzyme-mediated intracellular metabolism, followed by trapping of metabolites
  - Incorporation into the biochemical synthesis of intracellular proteins or DNA

9.2.5 General Methods of Radiolabeling

Depending on the type of radioisotope chosen and the method used to radiolabel, three different strategies can be employed to synthesize radiopharmaceuticals as shown below:

Table 9.7 Specific activity requirements for molecular imaging probes

Imaging study based on	SA (Ci μmole^{-1}) of radiotracer for	
	PET	SPECT
Enzyme-mediated cellular trapping	0.01–0.1	0.1–0.5
Antigen-antibody binding	0.1–1.0	1–5
Neuroreceptor binding	2–10	2–10
Gene expression	2–10	>10

1. *Isotope exchange*: Radiopharmaceutical can be prepared by direct exchange (isotopic substitution) of one or more stable atoms of an element in a molecule with one or more nuclides of a radioisotope of the same element. The radiolabeled molecule and the unlabeled molecule are chemically identical and behave in vivo in a similar manner. This method of radiolabeling is generally used to prepare radioiodinated radiopharmaceuticals in which the stable ^{127}I atom is replaced with a ^{123}I or ^{124}I atom (Chap. 10). Also, in the preparation of ^{11}C compounds, the stable ^{12}C atom is replaced with a ^{11}C radionuclide but, the preparation of ^{11}C compounds may involve multistep alkylation reactions (Chap. 11).
2. *Introduction of a foreign element*: Radiotracers can be prepared by the introduction of a foreign element or radionuclide in a parent MIP. Most of the radiopharmaceuticals are prepared based on this method. The radiolabeled compound and the unlabeled molecule are not chemically identical and may have different in vivo behavior. For example, preparation of [^{18}F]FDG (Fig. 9.1) involves the introduction of an ^{18}F atom in the deoxyglucose (DG) molecule. Also, in the preparation of radioiodinated peptides and hormones, ^{123}I or ^{124}I is added to the parent molecule that does not contain a natural stable iodine atom.
3. *Metal chelation*: This method also introduces a foreign element, (radiometal such as ^{99m}Tc, ^{64}Cu, ^{68}Ga, and ^{111}In) into an organic compound, known as bifunctional chelating agent (BFC). One or more atoms (such as O, N, and S) in the BFC donate a pair of electrons to the foreign metal atom to form coordinate cova-

Table 9.8 Brain dopamine D_2 receptor occupancy of [^{11}C]Raclopride in different species[a]

Species	Body wt. (g)	Brain wt. (g)	Stratum wt. (g)	D_2/D_3 receptor mol kg^{-1}	Dose(mCi)	[^{11}C]Raclopride (mCi μmol^{-1})	Occupancy(%)
Humans	70,000	1500	40	13.6	10–15	1000	<1.0
Rats	250	1.5	0.05	19.8	1.0	1000	19.0
Mice	25	0.4	0.03	20.0	1.0	1000	70.0

[a]The above table was modified from reference [45]

lent bonds (Chap. 11). As a result, the chemical and biological properties of the radiometalchelate complex are different than that of the chelating agent. Two important radiopharmaceuticals prepared based on metal chelation are ^{68}Ga-PSMA-11 and ^{68}Ga-PSMA-617 using either acyclic chelator DTPA-HBED-CC or cyclic chelator DOTA. The chemical structures of ^{68}Ga-PSMA-HBED-CC (also known as PSMA-11), and ^{68}Ga-PSMA-617 are shown in the Fig. 9.12. Both the labeled and unlabeled molecules have affinity to prostate specific membrane antigen (PSMA).

4. Certain peptides and macromolecules such as monoclonal antibodies can be labeled with radiometals based on the metal chelation method, as described above. This technique, however, requires conjugation of a bifunctional chelate (BFC) to the peptide or protein first and then subsequent chelation of the radiometal by the BFC molecule. The radiometal is not directly incorporated into the peptide or protein molecule.

9.2.5.1 Important Factors of Radiolabeling

In developing an appropriate radiolabeling technique, suitable for a specific RMIP, several important factors need to be considered in order to optimize the synthesis procedure.

Efficiency of Radiolabeling During the synthesis of RMIP, the percent of radioactivity incorporated in the desired chemical form of RMIP compared to the total radioactivity in the reaction mixture is given by the labeling yield or labeling efficiency (LE), which may be expressed as a percent at the end of the synthesis (%LE @EOS) or at the end of bombardment (% LE @EOB), as in the case of cyclotron-produced PET radiopharmaceuticals. It is always desirable to have very

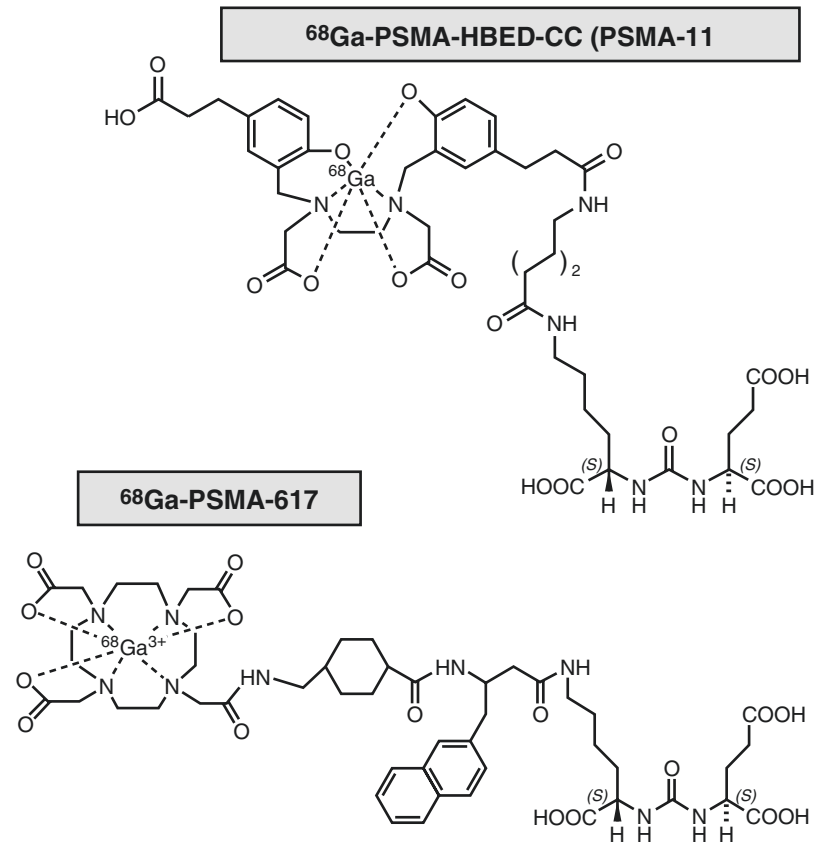

Fig. 9.12 Synthesis ^{68}Ga-PSMA-11 is based on acyclic chelator HBED-CC, while synthesis of ^{68}Ga-PSMA-617 is based on cyclic chelator DOTA

high labeling LE, but sometimes 2–5% LE is acceptable, provided the radiolabeled product is pure and acceptable for human studies.

Purification of the Final Product Most radiopharmaceutical preparations require a purification step to separate or isolate the desired RMIP from the unlabeled radiochemical contaminants and/or undesirable chemical species present in the reaction mixture. High-pressure liquid chromatography (HPLC) is one of the most common techniques used to obtain the purified RMIP. A number of solid-phase purification techniques, using mini cartridges based on alumina and ion exchange resins, are becoming increasingly popular. The *radiochemical purity* (RCP) of the final RMIP must be very high (>95%) for human studies, while the contamination of the final RMIP formulation with the undesirable radiochemical impurities must be very low (<5–10%). For every radiopharmaceutical, an acceptable quality control criterion, especially regarding the purity of the final radiolabeled drug product, must be carefully established.

Radiolysis Radiolabeled compounds may be degraded or decomposed by the high-energy radiations involving both γ photons and electrons (both β^- and β^+). When radiations from a radionuclide in a radiotracer molecule break the chemical bonds, within the same molecule, then such a process is known as *autoradiolysis*. The high-energy radiation may generate free radicals in water, such as hydrogen peroxide and perhydroxyl free radicals, which in turn may damage the radiotracer molecules. Radiolysis is very much dependent on the concentration of the final radiolabeled drug product formulation (activity/mL); the higher the concentration, the greater the radiolysis effect. Peptides and protein molecules may be more susceptible for radiation damage than small organic molecules.

Stability of the Radiopharmaceutical The in vitro chemical stability of radiotracer is very important for optimal shelf life. In addition, the in vivo stability is critical for optimal targeting of the imaging probe. Radiopharmaceuticals prepared, based on metal chelation, may be unstable in vivo because of the presence of competing metallic ions and binding agents.

9.2.6 Automated Synthesis Modules

Computer-controlled automation of the synthesis of PET radiopharmaceuticals is desirable for routine commercial production and to reduce the radiation exposure to the personnel involved in the production of these PET drugs. A number of automated synthesis modules (ASM) for routine production of radiolabeled precursors (such as $^{11}CH_3I$) or FDA-approved PET radiopharmaceuticals ready for clinical studies are commercially available (Fig. 9.13). The main purpose of the ASMs is to:

- Reduce exposure to high levels of harmful radiation
- Reduce the need for manual steps to accelerate the overall radiolabeling synthesis procedure
- Use the time of personnel efficiently and minimize operator errors due to the operator
- Obtain better reproducibility of RMIP synthesis from batch-to-batch
- Facilitate compliance with FDA regulations regarding GMP

ASMs are based on the principle of unit operations, in which a complex synthetic procedure is reduced to a series of simple operations (or reactions), such as evaporation, fluorination, chromatography, hydrolysis, purification, and sterilization. These operations are controlled by PCs with software programs that are user friendly and flexible enough to change various reactions conditions. For the development of ^{11}C PET radiopharmaceuticals, ASMs capable of generating [^{11}C]Methyl iodide or triflate are critical for the reliable synthesis of radiotracers.

Some of these ASMs, such as the TracerLab FX are based on the use of sterile disposable kits

Fig. 9.13 Automated synthesis modules for the production of PET radiopharmaceuticals. FASTlab HPLC+ for efficient HPLC purification and reformulation in one system with increased flexibility and efficiency of R&D tracer production for ^{18}F and ^{68}Ga radiopharmaceuticals, Neptis® perform an innovative synthesizer for the automated production of the largest range of [^{18}F] fluoride-based radiotracers via nucleophilic synthesis. Neptis® xSeed™ is a multi-run synthesizer designed specifically for research and development. Synthera®+, the most compact radiosynthesis module. Trasis-All-in-One PET tracer synthesizer, the cutting-edge solution for tracer production and development for ^{18}F, ^{11}C, and radio-metals. ITM-iQS-Theranostics Synthesizer for various radiometals for PET and therapy

with ready-to-use reagent vials for each batch production. At this time, several commercial ASMs are used routinely for the synthesis of ^{18}F-labeled radio-chemically pure, sterile, and pyrogen-free FDA-approved radiopharmaceuticals used in clinical studies. Several ASMs for the manufacture of radiolabeled metal complexes are also in routine use in many commercial manufacturing facilities.

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