11 Organic Radionuclides for Molecular Imaging (C, N, and O)

Science is built up of facts, as a house is built of stones; but an accumulation of facts is no more a science than a heap of stones is a house. (Henri Poincaré)

11.1 Advantages of Organic Radionuclides

All natural organic molecules or biochemicals in the human body and many drug molecules are made up of carbon, hydrogen, nitrogen, and oxygen. The organic radionuclides useful for developing radiotracers for PET are ^{11}C, ^{13}N, and ^{15}O (Table 11.1). These three elements, however, do not have any radionuclides suitable for developing radiotracers for SPECT.

Among the three organic radionuclides, ^{11}C offers the greatest potential to develop radiotracers for routine clinical applications because ^{11}C, as a label, can be easily substituted for a stable carbon in an organic compound without changing the biochemical and pharmacological properties of the molecule. Furthermore, the short half-life of ^{11}C provides favorable radiation dosimetry to perform multiple studies in the same subject under different conditions. The short half-life of ^{11}C may be disadvantageous for commercial production of radiotracers but, has significant potential for developing:

Table 11.1 Organic chemical elements: stable and radioactive isotopes

Element	Radionuclide	Decay mode	%	Half-life	β^+ Energy (MeV) β^+ E_{mean} (MeV)		Range (mm)	
					Max	Mean	Max	Mean
Carbon	^{10}C	β^+ and γ		19.3 s	3.65			
	^{11}C	β^+, EC	99.8	20.3 months	0.960	0.382	4.2	1.2
	^{12}C	Stable	98.9					
	^{13}C	Stable	1.1					
	^{14}C	β^-	100	5715 years	0.156 (β^-)			
Nitrogen	^{13}N	β^+ decay	99.8	9.97 months	1.1999	0.492	5.5	1.8
	^{14}N	Stable	99.634					
	^{15}N	Stable	0.366					
Oxygen	^{14}O	β^+ and γ		70.6 s	5.143			
	^{15}O	β^+ decay	99.9	122.2 s	1.732	0.735	8.4	3.0
	^{16}O	Stable	99.762					
	^{17}O	Stable	0.038					
	^{18}O	Stable	0.20					

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- Radiotracers with high specific activity (SA) to study drug interactions associated with exceedingly small concentrations of neuroreceptors.
- Radiolabeled drugs for monitoring the response to treatment.
- The relatively short physical half-life of ^{11}C (20 min) allows for multiple imaging studies to be obtained in the same subject within a brief period of time (3–4 h) with the same tracer (at base line followed by experimental intervention) or with multiple tracers to assess the specificity of the receptor interaction.

Compared to ^{11}C, the potential clinical utility of ^{13}N and ^{15}O radiotracers is extremely limited. In the last four decades, [^{13}N]ammonia and [^{15}O] water are the only tracers that have shown clinical utility in the assessment of regional blood flow, and perfusion. The radiochemistry of ^{13}N and ^{15}O is described briefly at the end of this chapter.

11.2 ^{11}C-Labeled Radiopharmaceuticals

^{11}C was first produced in 1934 [1] and the first biological application was based on the use of [^{11}C]CO_2 to investigate the photosynthesis in plants [2]. [^{11}C]CO was the first radiotracer used in human subjects to investigate the fixation of CO by red blood cells [3]. Several reviews have extensively discussed the chemistry and potential application of ^{11}C-labeled radiotracers [4–9]. In the last three decades, a spectrum of carbon-11 PET radiotracers has been developed to image many of the upregulated and emerging targets for the diagnosis, prognosis, prediction, and therapy in the fields of oncology, cardiology, and neurology.

11.2.1 Production of ^{11}C

The most commonly used method of ^{11}C production is based on the nuclear reaction, $^{14}N(p,\alpha)^{11}C$, in which the natural nitrogen gas is used as the target. One of the competing nuclear reactions is $^{14}N(p,pn)^{13}N$ or via $^{16}O(p,\alpha)^{13}N$ but, the relative amount of ^{13}N activity produced is dose-dependent and short irradiation times may lead to relatively enormous amounts of ^{13}N [5, 10]. With trace amounts of oxygen in the target (<1%), [^{11}C]CO_2 and [^{11}C]CO are formed [11]. With relatively higher proton energies (>13 MeV), longer irradiation times (>30 min) and higher beam currents (>30 μA), the most predominant ^{11}C precursor generated is [^{11}C]CO_2 gas. In the presence of hydrogen (5%) in the target, [^{11}C]methane (CH_4) and [^{11}C]hydrogen cyanide (HCN) can be produced by a recoil synthesis; however, due to radiolysis, [^{11}C]CH_4 is the main precursor available for processing [12, 13].

^{11}C radioactivity from the cyclotron target can be recovered in the form of two major precursors: [^{11}C]carbon dioxide or [^{11}C]methane. Subsequently, these gases can be converted into several secondary precursors, such as methyl iodide, methyl triflate, HCN, nitromethane, and phosgene.

11.2.1.1 Specific Activity (SA) of ^{11}C

The theoretical SA of ^{11}C is 9220 Ci μmol^{-1} or 9.22 Ci nmol^{-1} (Table 11.2). Since the contamination of the target and the gas lines with stable ^{12}C is unavoidable, ^{11}C is always contaminated with ^{12}C atoms. Also, both CO_2 and CH_4 gases are present in the atmosphere and provide a ubiquitous source of carrier as a contaminant that decreases the SA. As a consequence, the practical SA of ^{11}C precursors achieved from the typical production in a cyclotron target varies from 0.01 to 0.1 Ci nmol^{-1} depending on a number of

Table 11.2 Specific activity (SA) of ^{11}C and ^{18}F

	Half-life	Theoretical SA		Practical SA	
Radionuclide	min	Ci nmol^{-1}	nmol Ci^{-1}	Ci nmol^{-1}	nmol Ci^{-1}
^{11}C	20.4	9.22	0.108	0.01–0.1	10–100
^{18}F	110	1.71	0.585	0.001–0.02	50–1000

factors. In other words, every ^{11}C atom is contaminated with 100 or 1000 atoms of stable carbon atoms, which implies that the majority of the mass is mostly due to stable ^{12}C and extraordinarily little of the carbon is from the ^{11}C activity. There is a significant potential and also a need to improve the SA of ^{11}C production with the current cyclotron targets, and generate ^{11}C precursors with ultrahigh SA.

11.2.2 ^{11}C Precursors

The carbon-11 precursors (Fig. 11.1) are classified into primary and secondary based on their utility, and wide range of applications in the radiolabeling of various compounds like aliphatic, aromatic, and heterocyclic compounds after the production of carbon-11 radionuclide. The carbon-11 precursors in the chemical form of [^{11}C]CO_2 and [^{11}C]CH_4 are considered as the primary precursors, which can be produced during in-target production. These are converted into secondary precursors by rapid and efficient online or one-pot synthetic procedures to produce building blockers for generating carbon-11 radiotracers. Most of the ^{11}C tracers are synthesized using the secondary precursors. Several useful transformations for the generation of secondary precursors are summarized in Fig. 11.1. From the secondary precursors such as [^{11}C]methyl iodide, other secondary precursors can be synthesized, as shown in Fig. 11.2.

[^{11}C]Methyl iodide is the precursor of choice for introducing ^{11}C into organic molecules. However, a number of other precursors (Fig. 11.1) have been developed in the last few decades in order to meet the demands of synthetic strategies used for the development of ^{11}C-labeled radiotracers. [^{11}C]Methyl iodide can also be used to prepare a number of secondary ^{11}C precursors, such as methyl triflate ($CH_3OSO_2CF_3$), methyl lithium, nitromethane, and methyl magnesium iodide or bromide. Starting with [^{11}C]methane, precursors, such as hydrogen cyanide, cupric cyanide, carbon tetrachloride, and phosgene can be prepared [14, 15].

Methyl triflate, introduced in 1991 as an alkylating agent, is more advantageous than methyl iodide for alkylation reactions under mild conditions [16]. More specifically, it can be easily prepared by passing [^{11}C]methyl iodide through a small soda-glass column containing sliver triflate-impregnated graphitized carbon and the conversion to [^{11}C]methyl triflate is very efficient, and fast. The other precursor for methylations under mild conditions is methyl lithium [^{11}C] CH_3Li, which can be prepared by an equilibrium reaction between *n*-butyl lithium (*n*-BuLi) and [^{11}C] methyl iodide [17].

The Grignard reagent, methyl magnesium iodide, [^{11}C]CH_3MgI, is useful to add a methyl

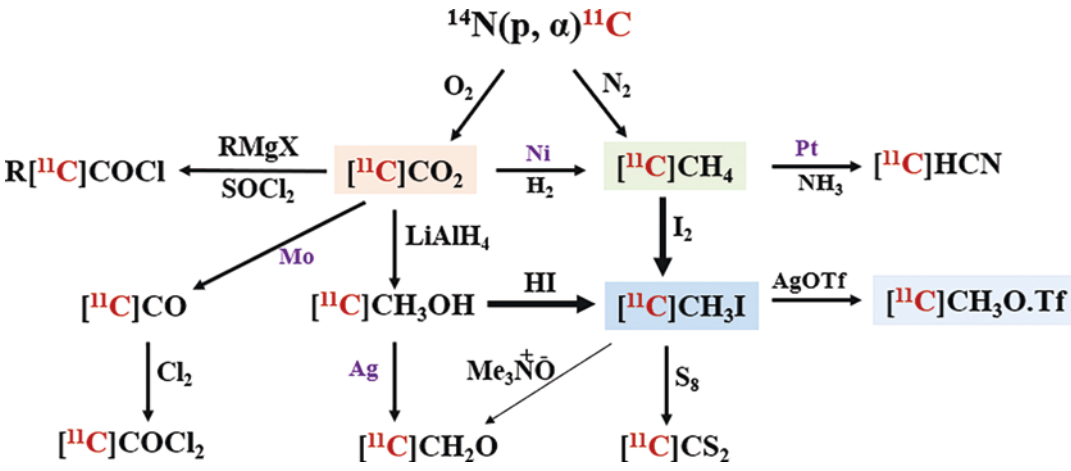

Fig. 11.1 ^{11}C-labeled precursors prepared from [^{11}C]carbon dioxide and [^{11}C] methane

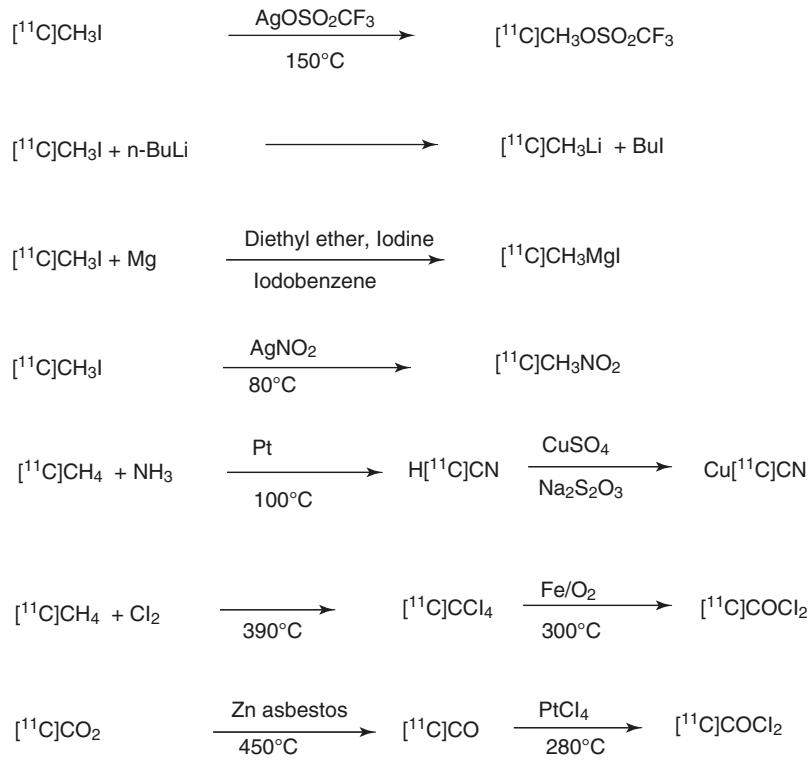

Fig. 11.2 Methods for the synthesis of ^{11}C-labeled precursors

group to a carbonyl (CO) group in a molecule. This precursor can be prepared by the interaction of [^{11}C]methyl iodide with magnesium turnings mixed with iodobenzene in ether [18].

Nitroalkanes, such as nitromethane [^{11}C]CH_3NO_2, can easily be converted into carbon nucleophile in the presence of a base. Also, an aldehyde group in a molecule can easily be substituted with a nitromethane. Subsequently, the nitrogroup can be reduced to an amine [19]. [^{11}C]CH_3NO_2 can also be easily prepared by the reaction of methyl iodide with silver nitrate at 80 °C [20]. Other nitroalkanes, such as nitroethane and nitropropane, can also be prepared similarly.

Cyanide (HCN) can be an extremely useful precursor for replacing halogen atoms, through nucleophilic substitution, with the cyano group. [^{11}C]HCN can be used to label amines, amino acids, aldehydes, and acids, and can be easily prepared by the reaction of [^{11}C]methane with ammonia over a platinum catalyst, at a very high temperature [12]. Since copper salts mediate certain aromatic nucleophilic substitutions, [^{11}C]HCN can easily be converted to Cu[^{11}C]CN [21].

Phosgene, [^{11}C]$COCl_2$) is a useful precursor that can be prepared easily by the catalytic chlorination of [^{11}C] CO, which typically is produced through the reduction of [^{11}C]CO_2 over hot zinc [22]. Phosgene can also be prepared by converting [^{11}C]methane to carbon tetrachloride, [^{11}C]CCl_4 through reaction with hot Cl_2 gas. Carbon tetrachloride is then mixed in a stream of oxygen gas and passed through a second furnace at 300 °C containing iron granules [23]. [^{11}C] Phosgene has been used to prepare [^{11}C] urea, a precursor for the synthesis of 2-[^{11}C]thymidine.

11.2.2.1 [^{11}C]Methylation Reaction

The most common method in ^{11}C chemistry is heteroatom methylation using [^{11}C]methyl iodide or iodomethane (CH_3I) and [^{11}C]methyl triflate (CH_3Tf), the most common precursors used to make ^{11}C radiotracers. CH_3I was first prepared in 1976 to synthesize [^{11}C]methionine [24]. Two methods are used for the synthesis of [^{11}C] CH_3I (Fig. 11.3). In a "liquid-phase" synthesis, [^{11}C]CO_2 is first reduced to methanol [^{11}C]CH_3OH, using lithium aluminum hydroxide ($LiAlH_4$)

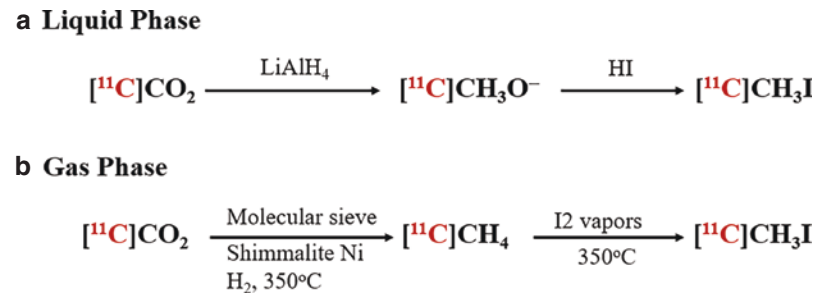

Fig. 11.3 Synthesis of [^{11}C]methyl iodide: Liquid phase method (**a**) and gas phase method (**b**)

GE TRACERlab™ FX2 MeI **TRACElab™ FX2 C**

Fig. 11.4 TRACERlab FX2 MeI provides a patented gas phase [^{11}C] methyl iodide production method via direct reaction of Iodine with [^{11}C] methane. [^{11}C] methane can either be supplied from a [11C] methane target, or in case a [^{11}C]CO_2 target is used, the integrated conversion step of [^{11}C]CO_2 to [^{11}C] methane can be utilized. TRACERlab FX2 C provides [^{11}C]methyl iodide or [^{11}C]methyl triflate production and methylation of PET tracers in one module. For labeling processes starting directly with [^{11}C]CO_2 the methylation step can be bypassed

which then reacts with hydroiodic acid (HI) to generate methyl iodide. In a "gas-phase" synthesis, [$^{11}CH_4$] gas (either from the target directly or produced from [$^{11}CO_2$]) reacts with iodine vapors generating methyl iodide [25, 26]. Commercial automated synthesis modules (Fig. 11.4) are available to synthesize [^{11}C]CH_3I and [^{11}C]CH_3. Tf. The [^{11}C]methylation reactions for making [^{11}C]-radiotracers are described according to the final bond between the carbon-11 and the other atom; the most common alkylations can be divided into N-alkylation, O-alkylation, and S-alkylation. The N-alkylation reaction uses [^{11}C]CH_3I or [^{11}C]CH_3OTf as an electrophile to react with a either a primary, secondary, or even tertiary amine group to give the corresponding [^{11}C]N-methyl radiotracer.

Generally, the radiochemical purity and SA of [^{11}C] CH_3I depend on the synthesis procedure and the automated module employed [27, 28]. The specific activity of [^{11}C]CH_3I (collected in DMF or acetone) can be determined by analytical HPLC using a Novapak C_{18} column (Waters, 4.6 × 150 mm) and a mobile phase consisting of acetonitrile/water (40/60) containing 0.1 M ammonium formate at a flow rate of 2 mL min^{-1}. The retention time of [^{11}C]CH_3I is 2.7 min (Fig. 11.5). The gas-phase method generates higher SA of [^{11}C]CH_3I and may be appropriate for receptor binding radiotracers [29].

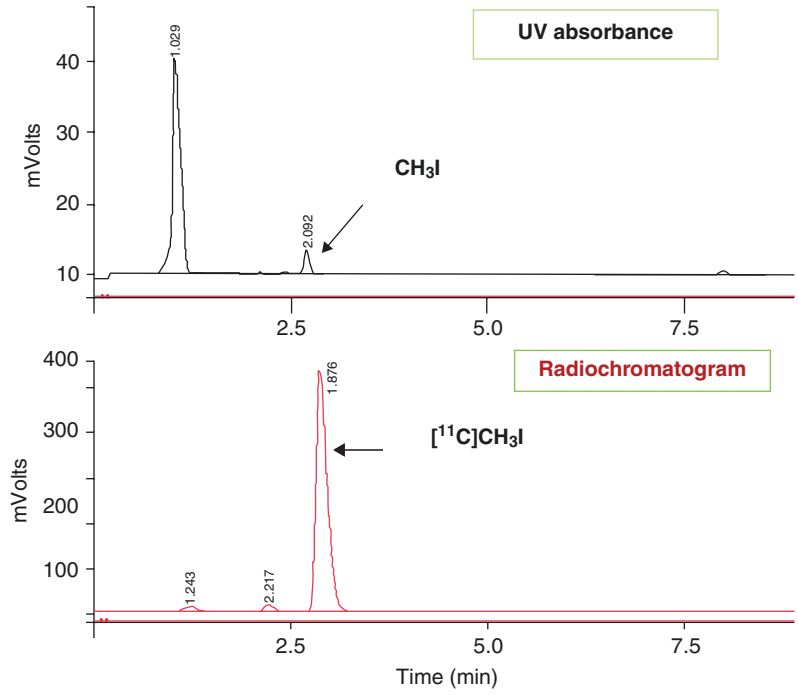

Fig. 11.5 Radiochemical purity of [^{11}C]methyl iodide (produced based on gas phase method) based on analytical HPLC

11.2.3 Synthesis of ^{11}C Labeled MIPs

A number of ^{11}C-labeled molecular imaging probes of significant clinical interest have been developed in the last few decades (Table 11.3). Historically, several different approaches have been used for the production of ^{11}C-labeled radiotracers, but the most practical approaches have been based on either (a) organic synthetic methods or (b) enzyme catalysis [4, 14].

The methods based on organic synthesis typically involve alkylations of C, N, O, and S nucleophiles with [^{11}C]methyl iodide or [^{11}C] methyl triflate. The alkylation reactions require an organic precursor, also known as *nor* compound (a molecule of interest without a methyl group on a specific C, N, O, or S atom). If a molecule of interest has several reactive groups, the organic precursors must have protective groups that can be easily deprotected by hydrolysis following methylation to generate the final drug product. This is the most common synthetic approach used in the routine production of ^{11}C-labeled radiopharmaceuticals and several examples are discussed below. ^{11}C labeling based on methyl-iodide or triflate method may not be possible in certain compounds and ^{11}C labeling through C-C bond formation may be the correct approach. Therefore, several different methods have been developed in recent years to synthesize ^{11}C-labeled radiotracers [5, 7]. The first novel method is the direct formation of ^{11}C-labeled carbonyl groups, where organic bases are utilized as "[^{11}C]CO_2 fixation agents." The second method is a low pressure [^{11}C]carbonylation technique that utilizes solvable xenon gas. These new methods have been reviewed recently in greater detail [7]. In summary, the following methods are utilized for the synthesis of ^{11}C-labeled tracers through C-C bond formation [7]:

Table 11.3 Examples of C-11-labeled PET radiotracers developed for applications in oncology, cardiology, neurology, and psychiatry

Biological process	Radiotracer	Target mechanism of uptake	Clinical application
Phosphatidylcholine (PC) Membrane synthesis	[^{11}C]Choline (CHO)	*Choline kinase*	Prostate cancer, brain tumors
DNA synthesis	[^{11}C]Thymidine, [^{11}C]4-DST	*Thymidine kinase (TK)*	Tumor aggressiveness (Ki67)
Amino acid transport and protein synthesis	[^{11}C]L-methionine [^{11}C]phenylalanine [^{11}C]ACBC [^{11}C]5–5-HTP) [^{11}C]AMT	Amino acid transporters (LAT 1–4)	Many tumors, including brain tumors, prostate cancer, and neuroendocrine tumors (NETs)
Cardiac oxygen metabolism	[^{11}C]Acetate	Utilized in TCA cycle and oxidative phosphorylation	Myocardial blood flow and oxygen consumption
Fatty acid metabolism	[^{11}C]Palmitate	β-Oxidation in mitochondria	Myocardial metabolism and infarct quantitation
Cardiac neuroreceptors	[^{11}C]CGP-12388	β-Receptor antagonists	Heart failure
Myocardial neuronal imaging	[^{11}C] Hydroxyephedrine (HED)	Norepinephrine reuptake transporter (NET)	Heart failure and NETs
Dopamine transporters (DATs)	[^{11}C]-L-DOPA	Analog of L-DOPA, substrate for AAAD	Parkinson's disease (PD)
	[^{11}C]Cocaine	Bind selectively to DAT	Cocaine addiction
	[^{11}C]β-CIT	DAT	Parkinson's disease (PD)
	[^{11}C]PE2i	DAT	Parkinson's disease (PD)
Vesicular monoamine transporter type 2 (VMAT2)	[^{11}C]DTBZ	DAT	Parkinson's disease (PD)
Dopamine D_1 receptors	[^{11}C]NNC-112 [^{11}C]SCH-23390	D_1 receptor antagonist	Schizophrenia, PD, cognitive disorders
Dopamine $D_{2/3}$ receptors	[^{11}C]Raclopride [^{11}C]FLB-457	Non-selective antagonists for $D_{2/3}$ receptors	Schizophrenia, PD, drug addiction, anxiety, Huntington's disease (HD)
	(+)-[^{11}C]PHNO	Specific for D_3 receptors	PD
Monoamine oxidase (MAO) expression	[^{11}C]clorgyline [^{11}C]-*L*-deprenyl	Suicide MAO inhibitors	PD
Adenosine receptors	[^{11}C]KF18446 [^{11}C]MPDX	A_{2A} receptor	HD, chronic diffuse axonal injuries
Glutamatergic receptors (GluR)	[^{11}C]ABP-688	Selective metabotropic GluR antagonist	HD, Alzheimer disease (AD)
	[^{11}C]MeNBI	GluN1/GluN2 containing NMDA receptors	Various neurological disorders
Opiate receptors	[^{11}C]Carfentanil	High-affinity μ opiate receptor agonist	Cocaine addiction
	[^{11}C]Diprenorphine	Nonselective partial opiate receptor (μ, δ, κ) agonist	HD
GABA receptors	[^{11}C]Flumazenil (FMZ)	$GABA_A$ receptor antagonist	Amyotrophic lateral sclerosis (ALS), epilepsy
	[^{11}C]PK11195	Peripheral benzodiazepine (PBR) receptor	Activate microglia in neuroinflammation
	[^{11}C]DPA-713		
Serotonergic system	[^{11}C]HTP	Serotonin synthesis	Serotonin metabolism NETs
	[^{11}C]AMT		

(continued)

Table 11.3 (continued)

Biological process	Radiotracer	Target mechanism of uptake	Clinical application
Serotonin receptors	[^{11}C]WAY100635	$5HT_{1A}$ receptor antagonist	ALS, AD-dementia, temporal lobe epilepsy
	[^{11}C]Desmethyl WAY100635		
	[^{11}C]MDL-100907	$5HT_{2A}$ receptor antagonist	
	[^{11}C]NMSP		
Serotonin transporter (SERT)	(+)-[^{11}C]McN-5652	Binds to SERT	Depression, AD-dementia
	[^{11}C]DSAB		
	[^{11}C]DASB		
	[^{11}C]Citalopram		
Cholinergic system	[^{11}C]Nicotine	nAchR subtype α4β2	Leaning, memory, and AD
	[^{11}C]epibatidine		
	[^{11}C]MP4P [^{11}C]MP4A	Acetylcholinesterase enzyme inhibitor	AD
Amyloid plaques	[^{11}C]6-OH-BTA-1(PiB)	Binds β-amyloid plaque	AD, mild cognitive impairment (MCI)
	[^{11}C]AZD2184		
	[^{11}C]SB-13		

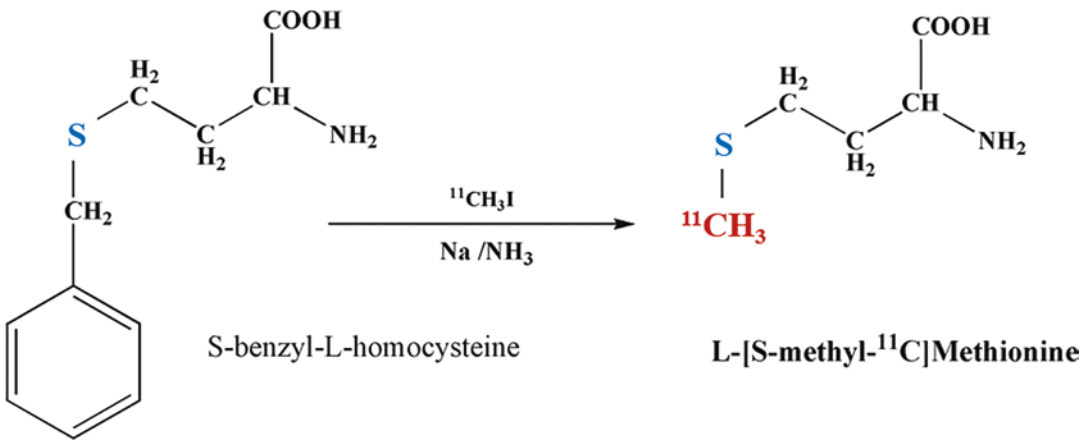

Fig. 11.6 Synthesis of L-[S-methyl-^{11}C]methionine

- The alkylation of carbanions (nucleophiles) with ^{11}C-labeled alkyl halides, nitroalkanes, and cyanide.
- The carboxylation of organometallic reagents, copper, and other metal-mediated catalysts.
- Transition-metal-mediated (Pd, Rh) chemical reactions with [^{11}C]CH_3I and [^{11}C]CO.
- Well-known reactions mechanisms like Stille reaction, Negishi coupling reaction, Suzuki cross-coupling reaction, and Heck reaction.

11.2.3.1 L-[S-Methyl-^{11}C]Methionine

The amino acid L-Methionine, labeled with ^{11}C in the methyl position, has been used for imaging of brain tumors. The routine production involves an alkylation on a sulfur nucleophile by the reaction of [^{11}C] methyl iodide with *S*-benzyl-L-homocysteine in the presence of liquid ammonia and sodium, as shown in Fig. 11.6 [24, 30]. Following reverse phase HPLC of the reaction mixture, the final drug

product, L-[S-methyl-[11]C]methionine, is eluted using a phosphate buffer.

11.2.3.2 Synthesis of [O-Methyl-[11]C] Raclopride

Raclopride is a dopamine D_2 receptor antagonist and is one of the most extensively used neuroreceptor imaging probes. Raclopride is labeled with [11]C by *O*-methylation using [[11]C]methyl iodide, as shown in Fig. 11.7. The enantiomerically pure *S*-precursor (*O*-desmethylraclopride) in DMSO is reacted with [[11]C] CH_3I in the presence of sodium hydroxide. The purified drug product, [[11]C]raclopride, is obtained following reverse phase HPLC of the reaction mixture using a C-18 column, a 10 mM phosphoric acid, and acetonitrile (70:30 v/v) as an eluent. The fraction containing [[11]C]raclopride is, subsequently, evaporated to remove acetonitrile, reformulated in physiological saline, and sterilized by membrane filtration.

11.2.3.3 Synthesis of R-[N-Methyl-[11]C] PK11195

PK11195, a peripheral benzodiazepine receptor ligand, labeled with [11]C, was originally developed as a tracer to image activated microglia in the brain [31, 32]. PK11195 can be labeled with [11]C by *N*-methylation using [[11]C]methyl iodide, as shown in Fig. 11.8. The precursor, *R*-desmethyl PK11195 (1.0 mg), is mixed with KOH (20 mg) in DMSO (0.4 mL) for 5 min. Subsequently, the

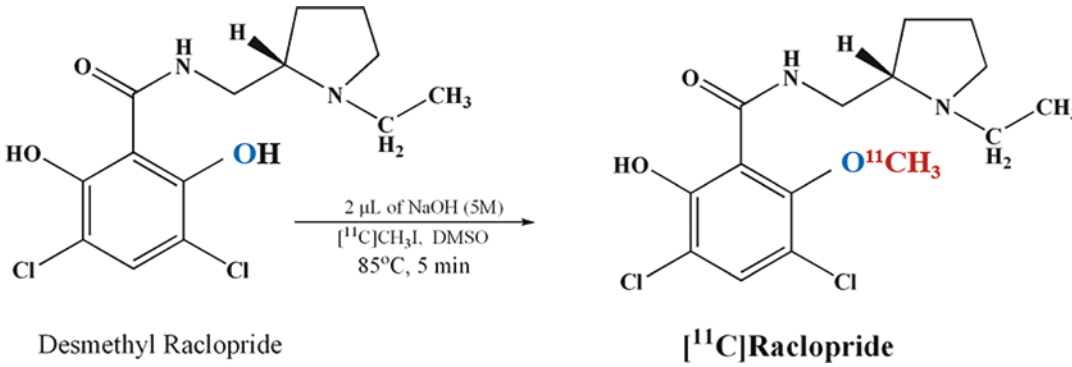

Fig. 11.7 Synthesis of [[11]C]Raclopride

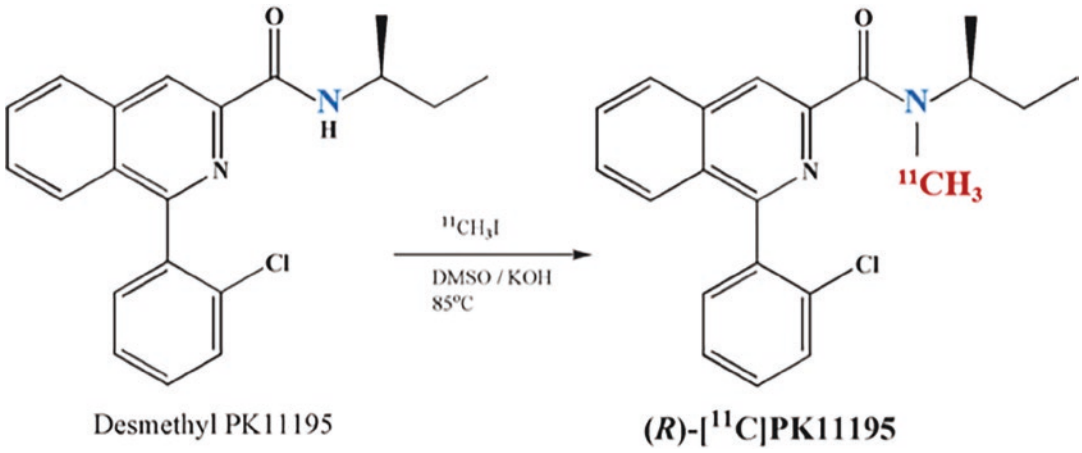

Fig. 11.8 Synthesis of [[11]C]PK11195

mixture is reacted with [^{11}C]CH_3I for 3 min at 80 °C. The mixture is diluted with 2–3 mL of mobile phase (70% methanol and 30% water) and purified using reverse phase HPLC column. The eluent fraction containing the drug product is passed through a C18 sep-pack cartridge to remove methanol. The final drug product is reformulated in 10% ethanol and physiological saline, and sterilized by membrane filtration.

11.2.3.4 [^{11}C]PIB

Based on an amyloid dye thioflavin-T, a ^{11}C tracer, *N*-Methyl-^{11}C-2-(4′-methylaminophenyl)-6-hydroxy-benzothiazole (^{11}C-6-OH-BTA-1, also known as "Pittsburgh Compound-B" or [^{11}C]-PIB), was developed to image brain amyloid plaques in patients with Alzheimer's disease [33, 34]. PIB can be labeled with ^{11}C by *N*-methylation using [^{11}C] methyl iodide, as shown in Fig. 11.9. The precursor, desmethyl PIB with a protective group known as MOMO-BTA-0 (1.5 mg) is mixed with KOH (10 mg) in DMSO (0.4 mL) for 5 min. Subsequently, the mixture is reacted with [^{11}C]CH_3I for 5 min at 125 °C. At the end, the protective group is removed by hydrolysis at 80 °C for 5 min using 0.5 mL of

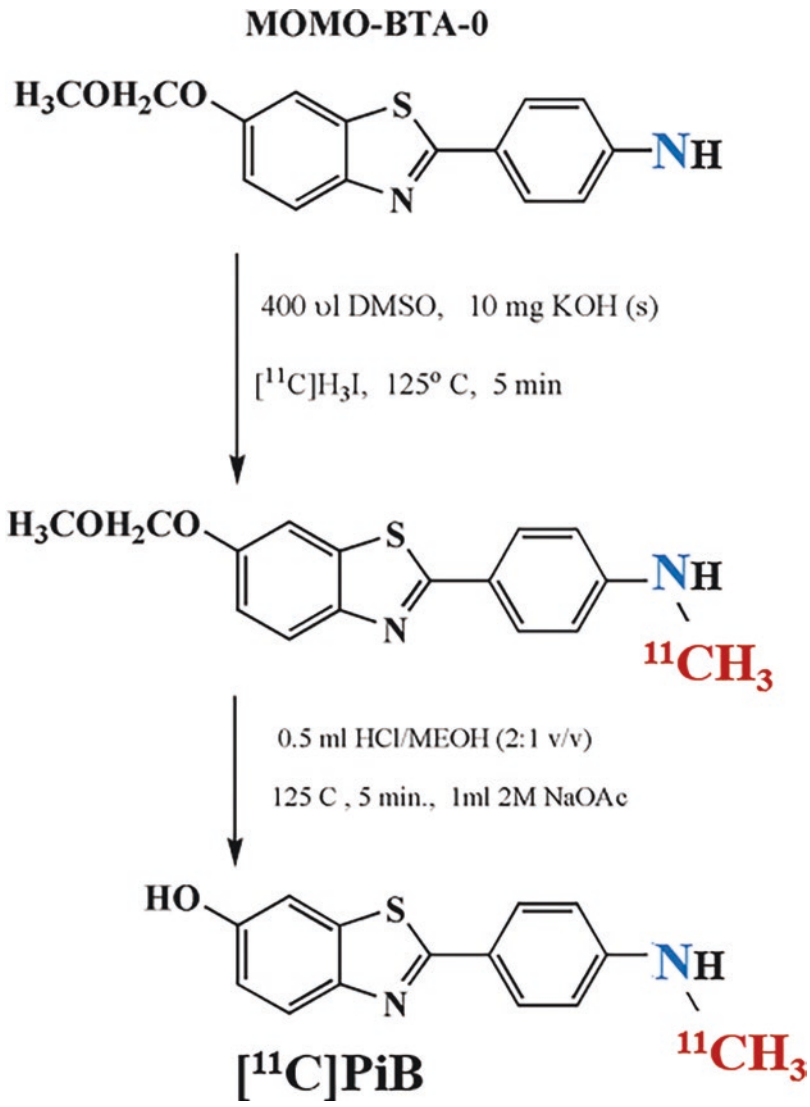

Fig. 11.9 Synthesis of [^{11}C]PIB

methanolic HCl (2:1). The mixture is diluted with mobile phase (35%) acetonitrile and 65% triethyl ammonium phosphate, pH 7.2), and purified using reverse phase HPLC. The eluent fraction containing [^{11}C]PIB is passed through the C18 sep-pak cartridge to remove the organic solvent. The final drug product can be reformulated in physiological saline with 10% ethanol and sterilized by membrane filtration.

The preparation of PiB based on the secondary precursors [^{11}C]CH_3I or [^{11}C]CH_3OTf is always time and activity consuming. As discussed before, any approach capable of eliminating the steps of synthesis of the ^{11}C-methylating agents would be advantageous for a better overall performance in terms of the RCY and the available activity for PET scans. [^{11}C]CO_2 is an attractive starting material for synthesizing ^{11}C-labeled tracers. The use of [^{11}C]CO_2 via the so-called fixation to synthesize ^{11}C-ureas, ^{11}C-carbamates, ^{11}C-oxazolidinones, ^{11}C-carboxilic acids, and ^{11}C-amides is well-documented in the literature [5, 7]. Therefore, several investigators developed methods to synthesize [^{11}C]PiB using [^{11}C]CO_2 directly from the cyclotron [35].

11.2.3.5 Synthesis of [^{11}C]5-Hydroxy-L-Tryptophan (HTP)

Preparation of certain ^{11}C radiopharmaceuticals can be very complicated and may involve many steps in the synthesis followed by purification procedures. ^{11}C-labeled amino acids can be prepared using enzyme catalyzed reactions (specially to prepare the desired enantiomer with biological activity rather than a racemic mixture). For example, in the synthesis of [^{11}C]5-HTP [36], [^{11}C]-L-alanine is synthesized first, by reacting [^{11}C]CH_3I with *N*-(Diphenyl methylene)glycine tertiary butyl ester. Subsequently, [^{11}C]-L-alanine is converted to pyruvic acid using enzymes GPT, DAO and GPT. The interaction of labeled alanine with 5-hydroxyindole, in the presence of *tryptophanase*, will finally produce [^{11}C]5-HTP (Fig. 11.10). The final drug product is purified by HPLC and sterilized by membrane filtration.

11.2.3.6 Synthesis of [^{11}C]Choline (CHO)

The biological basis of [^{11}C]choline as a cancer imaging PET tracer is its role as a precursor in the biosynthesis of phosphatidylcholine and the biosynthesis of phospholipid-rich mem-

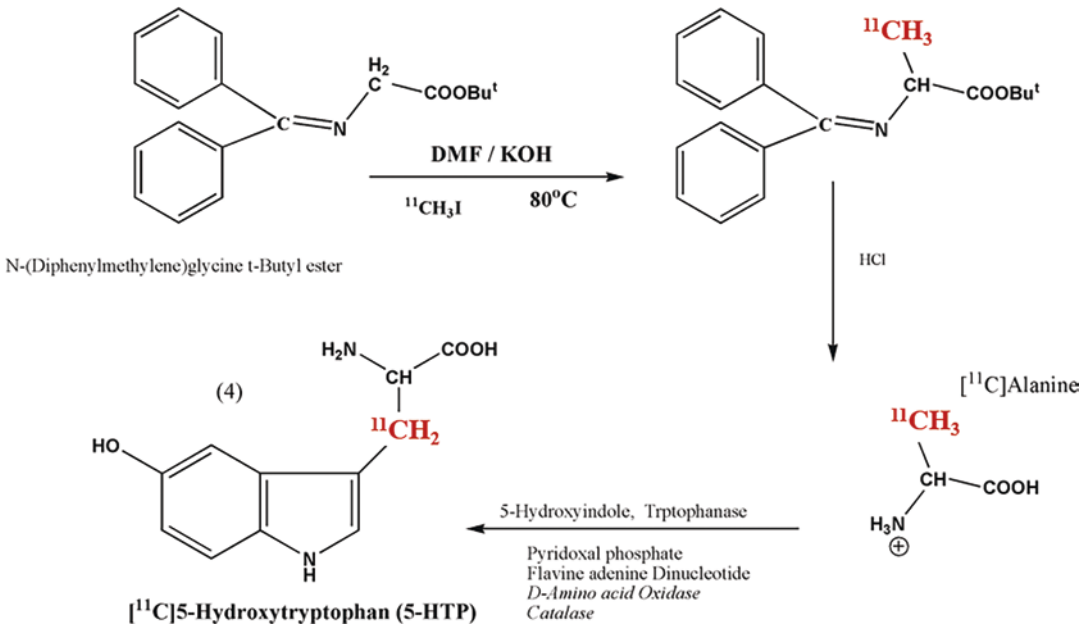

Fig. 11.10 Synthesis of [^{11}C]5-Hydroxytryptophan (5HTP)

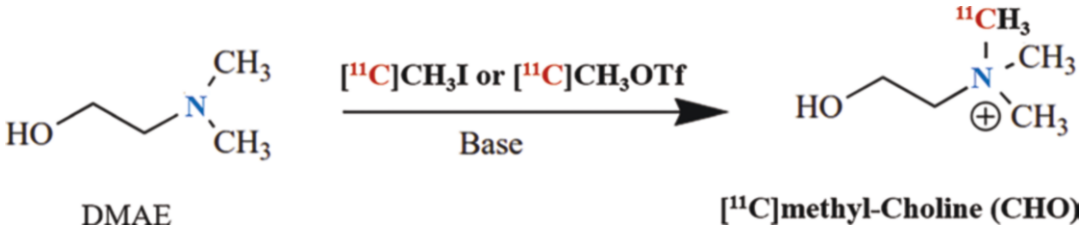

Fig. 11.11 Synthesis of [^{11}C]Choline based on [^{11}C]CH_3I (methyl iodide) and 2-amino-(N,N-dimethyl)-ethanol (DMAE) precursor

branes. CHO-PET is used to image tumors, prostate cancer, and breast cancer in patients. The original synthesis of [^{11}C] choline trapped the [^{11}C]CH_3I in a reaction flask, then reacted with 2-amino-(N,N-dimethyl)-ethanol (DMAE) in acetone with $KHCO_3$ as base (Fig. 11.11). After stirring the reaction mixture at room temperature for 20 min, the final HPLC product has high specific activity (37 GBq/μmol), high radiochemical purity as well as chemical purity (98%), in about 30% radiolabeling yield [37, 38]. Subsequently, investigators have been optimizing the procedure and modifying automated systems to make the production of CHO more efficient with greater activities per single batch run [9]. [^{11}C]choline has been produced via N-[^{11}C]methylation of the precursor DMAE with either [^{11}C]methyl iodide/bromide or [^{11}C]methyl triflate, followed by purification using solid phase extraction (SPE) method [39].

[^{11}C]Choline is the first and the only PET radiopharmaceutical approved by FDA for routine clinical studies. In spite of the extraordinary clinical investigations with hundreds of ^{11}C-labeled PET radiotracers in neurology, oncology, and cardiology, the clinical utility of these tracers is still not well established.

11.3 ^{13}N-Labeled Radiopharmaceuticals

^{13}N was first produced by the bombardment of boron with α particles using the $^{10}B(\alpha,n)^{13}N$ reaction [40]. The first ^{13}N radiotracer of biological interest was [^{13}N]ammonia (NH_3). A number of nuclear reactions were used over the years to produce ^{13}N [41]. However, the most popular method of producing ^{13}N is based on the proton (8–15 MeV) bombardment of natural oxygen gas, using the $^{16}O(p,\alpha)^{13}N$ reaction [42]. ^{13}N can also be produced by the proton (4–9 MeV) bombardment of isotopically enriched ^{13}C using the $^{13}C(p,n)^{13}N$ reaction [43].

11.3.1 [^{13}N]Ammonia (NH_3)

The most predominant chemical species of ^{13}N produced in the water target is [^{13}N]nitrate $\left(NO_3^-\right)$, while the other two species, [^{13}N]nitrite (NO_2) and [^{13}N] ammonia, may represent only a small fraction of the total ^{13}N radioactivity (Table 11.4). [^{13}N]ammonia is formed as the primary product by the abstraction of hydrogen atoms from the water, as shown in the Fig. 11.12. As the irradiation dose to target is increased, radiolytic oxidation occurs producing oxoanions of nitrogen, consisting of mainly nitrates and nitrites [44]. The most common method of increasing the radiochemical yield of [^{13}N] ammonia is by the addition of free radical scavengers, such as ethanol and acetic acid, to the target water [45]. Subsequently, the $\left[^{13}N\right]NH_4^+$ ion can be trapped on a small cation exchange cartridge from which it can be eluted using physiological saline.

[^{13}N]Ammonia can be used to prepare a number of ^{13}N-labeled amino acids for the determination of protein synthesis rates in tumors. [^{13}N]

Table 11.4 Methods for the production of ^{13}N and ^{15}O radiotracers

Target material	Nuclear reaction	In-target product(s)	Post irradiation treatment	Final product
H_2O	$^{16}O(p,\alpha)^{13}N$	NO_3^-, BO_2^-, NH_2^+	Reduction of anions using DeVarda's alloy and NaOH	$[^{13}N]NH_3$
H_2O/Ethanol (1 mM)	$^{16}O(p,\alpha)^{13}N$	NH_4^+	Radiochemical purification using cation exchange cartridge	$[^{13}N]NH_3$
N_2/O_2 (0.1–4.0%)	$^{14}N(d,n)^{15}O$	$[^{15}O]O_2$	Remove traces of NO_2 and O_3	$[^{15}O]O_2$
	$^{15}N(d,n)\ ^{15}O$			
N_2/CO_2 (0.1–2.0%)	$^{14}N(d,n)^{15}O$	$[^{15}O]CO_2$	Remove traces of $[^{15}O]O_2$	$[^{15}O]CO_2$ with trace ^{13}N levels
N_2/H_2 (5.0%)	$^{14}N(d,n)^{15}O$	$[^{15}O]CO_2$	Remove traces of $[^{15}O]O_2$	$[^{15}O]CO_2$ with trace ^{13}N levels

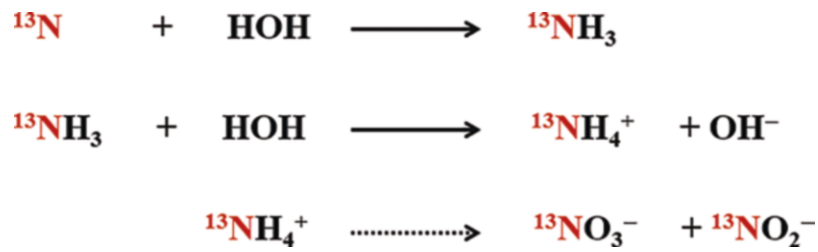

Fig. 11.12 Reactions of ^{13}N with water in the cyclotron target

Ammonia has also been used to prepare ^{13}N-labeled anticancer drugs, such as CCNU, BCNU, and cisplatin [44].

11.3.2 Synthesis of [^{13}N]Gemcitabine

Gemcitabine (Gemzar®, Eli Lilly) is a chemotherapy drug most commonly used to treat non-small cell lung, pancreatic, bladder, and breast cancer. [^{13}N]Gemcitabine (GT) can be prepared using [^{13}N]ammonia, as shown in Fig. 11.13. Following production of [^{13}N]ammonia based on $^{16}O(p,\alpha)^{13}N$ reaction, the target water is passed through an ion exchange CM cartridge to trap [^{13}N] ammonia. Subsequently, it is eluted into a vial containing a DeTet, (a gemcitabine precursor containing a tetrazol group) using 0.6 mL of sodium acetate buffer (1 M, pH 8.5). The mixture is then heated at 150–160 °C for 5 min. Finally, at the end of the reaction, the mixture is filtered and the filtrate is purified by HPLC to isolate the pure [^{13}N]GT, which is then sterilized using membrane filtration [46].

11.4 ^{15}O-Labeled Radiotracers

The potential utility of [^{15}O]oxygen to study regional tracer biology was first demonstrated in murine experimental neoplasms at the Washington University in St Louis [47]. Since that time, different chemical forms of ^{15}O, such as carbon dioxide (CO_2), carbon monoxide (CO), water (H_2O), and *n*-butanol ($CH_3(CH_2)_3OH$) have been used to study oxygen metabolism, blood volume, and blood flow in humans with PET.

^{15}O can be produced in a cyclotron using a variety of nuclear reactions [44] but, the most commonly used reactions are $^{14}N(d,n)^{15}O$ and $^{15}N(p,n)^{15}O$. The chemical forms of ^{15}O generated in the target vessel depend on the nuclear reaction, energy of the bombarding particle, and the mixture of target gases (such as N_2/O_2, N_2/CO_2, and N_2/H_2) (Table 11.4).

11.4.1 ^{15}O-Labeled Gases

When N_2 gas is bombarded with deuterons, the presence of oxygen (0.1–4.0%) leads to the gen-

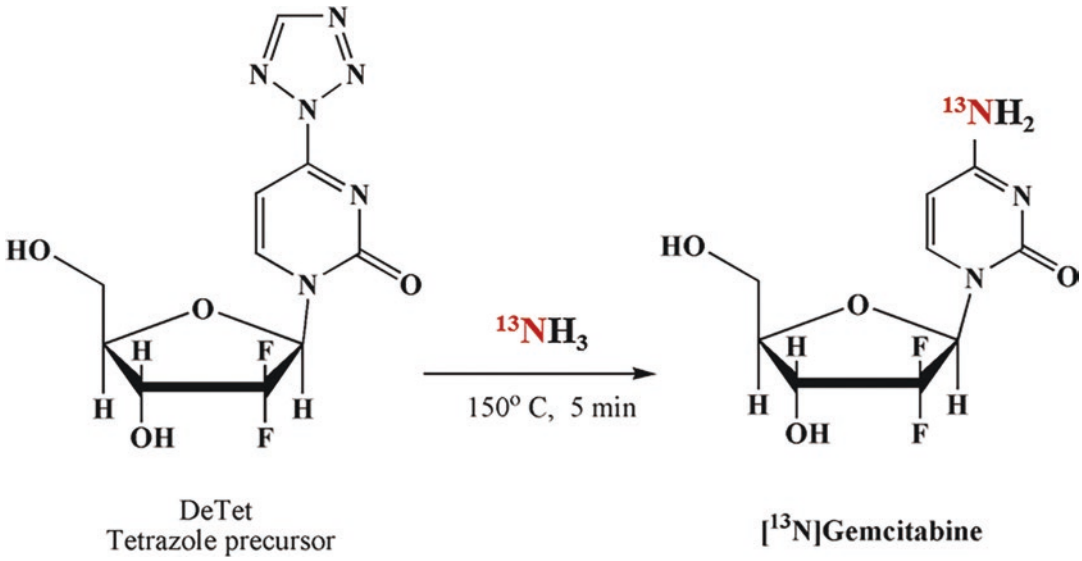

Fig. 11.13 Synthesis of [^{13}N]Gemcitabine

eration of [^{15}O]O_2 with higher radiochemical purity. Subsequently, [^{15}]O_2 can be used to synthesize labeled CO and CO_2 gases.

[^{15}O]CO can be synthesized, when [^{15}]O_2 is reacted with carbon (activated charcoal) at 900–950 °C. However, when [^{15}]O_2 reacts with carbon at 400–450 °C, the predominant species formed is [^{15}O]CO_2. Methods for the in-target production of ^{15}O-labeled CO and CO_2 gases have also been developed. When the target N_2 gas is mixed with minimal O_2 levels (0.25%), [^{15}O]CO is produced. Also, the presence of a source of hot carbon within the target volume has been shown to be optimal for the in-target production of [^{15}O]CO [48, 49]. When N_2/CO_2 gas mixture is used as the target gas, the product $^{15}O_2$ is converted to [^{15}O]CO_2 in the target. Due to secondary nuclear reaction, $^{12}C(d,n)^{13}N$, the major radionuclidic impurity in [^{15}O]CO_2 preparations is [^{13}N]N_2 gas.

11.4.2 Synthesis of [^{15}O]Water

When a N_2/H_2 mixture is bombarded with deuterons, the predominant ^{15}O-labeled product in the target vessel is [^{15}O]H_2O [50]. Also, [^{15}O]H_2O is readily synthesized outside the target by the palladium-catalyzed reaction of [^{15}O]O_2 with H_2 gas [51]. A flow of purified [^{15}O]O_2 in nitrogen is mixed with hydrogen and passed over a few pellets of palladium-alumina catalyst, and the resulting [^{15}O]H_2O vapor is trapped by bubbling the nitrogen carrier through a sterile saline solution. Based on this principle, an advanced automated system for the administration of [^{15}O]water at the bedside was developed by the investigators at the Hammersmith Hospital in London.

References

1. Crane HR, Lauritsen CC. Further experiments with artificially produced radioactive substances. Phys Rev. 1934;45:497.
2. Ruben S, Hassid WZ, Kamen MD. Reactive carbon in the study of photosynthesis. J Am Chem Soc. 1939;61:661.
3. Tobias CA, Lawrence JH, Roughton FJW. The elimination of carbon monoxide from human body with reference to possible conversion of CO to CO_2. Am J Phys. 1945;145:253.
4. Antoni G, Kihlberg T, Langstrom B. Aspects on the synthesis of ^{11}C-labeled compounds. In: Welch MJ, Redvanley CS, editors. Handbook of radiopharmaceuticals. West Sussex: Wiley; 2003.
5. Dahl K, Halldin C, Schou M. New methodologies for the preparation of carbon-11 labeled radiopharmaceuticals. Clin Transl Imaging. 2017;5:275–89.
6. Fowler JS, Ding Y-S. Chemistry. In: Wahl RL, Buchanan JW, editors. Principles and practice of positron emission tomography. Philadelphia, PA: Lippincott Williams and Wilkins; 2002.

7. Goud NS, Bhattacharya A, Joshi RK, et al. Carbon-11: radiochemistry and target-based PET molecular imaging applications in oncology, cardiology, and neurology. J Med Chem. 2021;64:1223–59.
8. Långström B, Kihlberg T, et al. Compounds labeled with short-lived β+ emitting radionuclides and some applications in life sciences. The importance of time as a parameter. Acta Chem Scand. 1999;53:651–69.
9. Tu Z, Mach RH. C-11 radiochemistry in cancer imaging applications. Curr Top Med Chem. 2010;10:1060–95.
10. Qaim SM, Clark JC, Crouzel C, et al. PET radionuclide production. In: Stöcklin P, editor. Radiopharmaceuticals for positron emission tomography. Dordrecht: Kluwer Academic; 1993.
11. Bida GT, Ruth TJ, Wolf AP. Experimentally determined thick target yields for the 14N (p,α) reaction. Radiochim Acta. 1978;27:181–5.
12. Christman DR, Finn RD, Kalstrom KI, et al. The production of ultra high specific activity ^{11}C labeled hydrogen cyanide, carbon dioxide, carbon monoxide, and methane via the 14N(p, α)11C reaction. Int J Appl Radiat Isot. 1975;26:435–41.
13. Lamb JF, James RW, Winchell HS. Recoil synthesis of high specific activity ^{11}C-cyanide. Int J Appl Radiat Isot. 1971;22:475–9.
14. Antoni G, Långström B. Progress in ^{11}C radiochemistry. In: Bailey DL, Townsend DW, Valk PE, Maisey MN, editors. Positron emission tomography- basic sciences. London: Springer; 2005. p. 223–36.
15. Ferrieri RA. Production and application of synthetic precursors labeled with carbon-11 and fluorine-18. In: Welch MJ, Redvanley CS, editors. Handbook of radiopharmaceuticals. West Sussex: Wiley; 2003.
16. Jewett DM. A simple synthesis of [^{11}C]methyl triflate. Appl Radiat Isot. 1991;43:1383–5.
17. Reiffers S, Vallburg W, Wiegman T, et al. Carbon-11 labeled methyl lithium as a methyl donating agent: the addition to 17-keto steroids. Int J Appl Radiat Isot. 1980;31:535–9.
18. Elsinga PH, Keller E, De Groot TJ, et al. Synthesis of [^{11}C]methyl magnesium iodide and its application to the introduction of [^{11}C]-*N*-ter-butyl groups and [^{11}C]-sec-alcohols. Appl Radiat Isot. 1995;46:227–31.
19. Schoeps K-O, Halldin C, Nagren K, et al. Preparation of [1-^{11}C]dopamine, [1-^{11}C]*p*-tyramine, and [1–^{11}C]*m*-tyramine. Nucl Med Biol. 1993;20:669–78.
20. Schoeps K-O, Stone-Elander S, Halldin C. On-line synthesis of [^{11}C]nitroalkanes. Appl Radiat Isot. 1989;40:261–2.
21. Ponchant M, Hinnen F, Demphel S, et al. [^{11}C] copper(I) cyanide: a new radioactive precursor for ^{11}C-cyanation and functionalization of haloarenes. Appl Radiat Isot. 1997;48:755–62.
22. Roeda D, Crouzel C, Zanten V. Synthesis of ^{11}C-urea for medical use. Radiochem Radioanal. 1978;33:175–6.
23. Steel CJ, Brady F, Luthra, et al. An automated radiosynthesis of 2-[^{11}C]thymidine using anhydrous [^{11}C] urea derived from [^{11}]phosgene. Appl Radiat Isot. 1999;51:377–88.
24. Långström B, Lundqvist H. The preparation of [^{11}C] methyl iodide and its use in the synthesis of [methyl-^{11}C]-L-methionine. Int J Appl Radiat Isot. 1976;27:357–63.
25. Larsen P, Ulin J, Dahlstrom K, et al. Synthesis of [^{11}C] iodomethane by iodination of [^{11}C]methane. Appl Radiat Isot. 1997;48:153–7.
26. Link JM, Krohn KA, Clark JC. Production of [^{11}C] CH_3I by single pass reaction of [^{11}C]CH_4 with I_2. Nucl Med Biol. 1997;24:93–7.
27. Kothari PJ, Suehiro S, Vallabhajosula S. Evaluation of commercial automated [^{11}C]methyl iodide (MeI-plus) synthesis module. J Label Compd Radiopharm. 2005;48:S226.
28. Makiko S, Kothari PJ, Vallabhajosula S. Quality control studies of [^{11}C]methyl iodide produced in liquid and gas phase synthesis modules. J Label Compd Radiopharm. 2005;48:S213.
29. Kothari PJ, Vallabhajosula S, Lampiri E, Goldsmith SJ. An improved gas phase module (GE TracerLab FXc) for the production of [^{11}C]methyl iodide. J Nucl Med. 2007;48:181p.
30. Schmitz F, Plenevaux A, Del-Fiore G, et al. Fast routine production of L-[^{11}C-methyl]-methionine with Al_2O_3/KF. Appl Radiat Isot. 1995;46:893–7.
31. Banati RB, Goerres GW, Myers R, et al. [^{11}C]*(R)*-PK11195 positron emission tomography imaging of activated microglia in vivo in Rasmussen's encephalitis. Neurology. 1999;53:2199–203.
32. Hashimoto KI, Inoue O, Suzuki K, et al. Synthesis and evaluation of ^{11}C-PK11195 for in vivo study of peripheral-type benzodiazepine receptors using positron emission tomography. Ann Nucl Med. 1989;3:63–71.
33. Mathis CA, Bacskai BJ, Kajdasz ST, et al. A lipophilic thioflavin-T derivative for positron emission tomography (PET) imaging of amyloid in brain. Bioorg Med Chem Lett. 2002;12:295–8.
34. Mathis CA, Wang Y, Holt DP, et al. Synthesis and evaluation of ^{11}C-labeled 6-substituted 2-aryl benzothiazoles as amyloid imaging agents. J Med Chem. 2003;46:2740–54.
35. Buccino P, Savio E, Porcal W. Fully automated radiosynthesis of the amyloid tracer [^{11}C]PiB via direct [^{11}C]CO_2 fixation-reduction EJNMMI. Radiopharm Chem. 2019;4:14.
36. Bjurling P, Watanabe Y, Tokushige M, et al. Synthesis of β-^{11}C-labeled L-tryptophan and 5-hydroxy-L-tryptophan using a multi-enzymatic reaction route. J Chem Soc Perkin Trans. 1989;1:1331–4.
37. Hara T, Kosaka N, Shinoura N, Kondo T. PET imaging of brain tumor with [methyl-^{11}C]choline. J Nucl Med. 1997;38(6):842–7.
38. Hara T, Kosaka N, Kishi H. PET imaging of prostate cancer using carbon-11-choline. J Nucl Med. 1998;39(6):990–5.
39. Pascali C, Bogni A, Iwata R, et al. [^{11}C]Methylation on a C18 Sep-Pak cartridge: a convenient way to pro-

duce [N-methyl-^{11}C] choline. J Labelelled Compd Rad. 2000;43(2):195–203.
40. Joliot F, Curie I. Artificial production of a new kind of radioelement. Nature. 1934;133:201–2.
41. Schlyer DJ. Production of radionuclides in accelerators. In: Welch MJ, REdvanley CS, editors. Handbook of radiopharmaceuticals, radiochemistry and applications. New York: Wiley; 2003.
42. Tilbury RS, Dahl JR, Marano SJ. N-13 species formed by proton irradiation of water. J Label Compd Radiopharm. 1977;13:208.
43. Ferrieri RA, Schlyer DJ, Wieland BW, et al. On-line production of [N-13]-nitrogen from solid enriched [C-13] targets and its application to [N-13]ammonia using microwave radiation. Int J Appl Radiat Isot. 1983;34:897–900.
44. Clark JC, Aigbirhio FI. Chemistry of nitrogen-13 and oxygen-15. In: Welch MJ, Redvanley CS, editors. Handbook of radiopharmaceuticals. West Sussex: Wiley; 2003.
45. Wieland BW, Bida G, Padgett H, et al. In-target production of ^{13}N-ammonia via proton irradiation of dilute aqueous ethanol and acetic acid mixtures. Appl Radiat Isot. 1991;42:1095–8.
46. Vallabhajosula S, Zatorski A, Kothari PJ, et al. [^{13}N] gemcitabine: a new PET tracer to assess gemcitabine (GT) tumor uptake. J Nucl Med. 2008;49(s1):98.
47. Ter-Pogossian MM, Powers WE. The use of radioactive oxygen-15 in the determination of oxygen content in malignant neoplasms. In: Exterman RC, editor. Radioisotopes in scientific research, III. Oxford: Pergamon Press; 1958.
48. Berridge MS, Terris AH, Cassidy EH. Low carrier production of water and carbon monoxide. Appl Radiat Isot. 1990;41:1173–5.
49. Votaw JR, Satter MR, Sunderland JJ, et al. The Edison lamp: [^{15}O]carbon monoxide production in the target. J Label Compd Radiopharm. 1986;23:1211–3.
50. Vera Ruiz H, Wolf AP. Direct synthesis of ^{15}O-labeled water at high specific activities. J Label Compd Radiopharm. 1978;15:185–9.
51. Clark JC, Crouzel V, Meyer CJ. Current methodology for oxygen-15 production for clinical use. Appl Radiat Isot. 1987;38:597–600.