Radiohalogens for imaging and therapy

Michael J. Adam[a] and D. Scott Wilbur[b]

Received 14th July 2004
First published as an Advance Article on the web 21st December 2004
DOI: 10.1039/b313872k

Radiohalogens play a very important role in radiopharmaceuticals used for medical imaging (now referred to as molecular imaging) and therapy applications. Development of new radiopharmaceuticals that have radiohalogens incorporated requires an understanding of parameters that are unique to chemistry involving these radionuclides. Those parameters include requirement for production and purification of the halogen radionuclides, as well as development of reaction conditions for use with high specific activity short-lived radionuclides. In this *tutorial review*, several radiohalogens, their radiolabeling chemistry and their application to medical imaging and therapy are discussed.

1 Introduction

The combination of technologically advanced imaging instrumentation and radionuclide labeled molecules, referred to as radiopharmaceuticals, provides a method for non-invasively visualizing human anatomy and physiology to aid in the diagnosis of human disease and to monitor therapy. Measurements of physiological functions and biochemical parameters known to be involved in human diseases, such as enzymatic reaction rates or cell surface receptor densities, provide valuable information that can be used in treatment of patients. Further, appropriately designed radiopharmaceuticals also have the potential for seeking out and killing aberrant cells in the body, such as cancer cells. This *in vivo* use of radionuclides, whether by themselves or chelated/bonded to a targeting molecule, prompted the development of the field of nuclear medicine. Radiohalogens have played, and continue to play, a major role in this developing field.

Radiohalogens used in nuclear medicine today fall into two main categories, those useful in imaging applications and those useful in therapy applications. Importantly, the chemical and nuclear decay properties of some radiohalogens are ideally suited for use in these divergent nuclear medicine applications. Although early medical imaging was conducted on planar gamma cameras, today nuclear medicine imaging is carried out on highly sophisticated Single Photon Emission Computed Tomography (SPECT) instruments available in most hospitals worldwide. More recently another imaging modality,

Michael J. Adam

Michael J. Adam was born in New Westminster, British Columbia, Canada in 1952. He obtained an Associate of Arts degree from Douglas College in 1973 and his B.Sc. from the University of British Columbia in 1975. He obtained a Ph.D. from the University of British Columbia in 1980 under the guidance of Laurance D. Hall on the synthesis of sugar conjugates. He began his career as a Research Scientist at TRIUMF in 1980 and is now a Senior Scientist at TRIUMF and an adjunct professor of Pharmaceutical Sciences and at the Department of Chemistry at UBC. Dr Adam's main research interest is in the area of Positron Emission Tomography (PET) and radiohalogen chemistry but also includes medicinal inorganic chemistry and the application of metals in medicine.

D. Scott Wilbur

D. Scott Wilbur was born in Bath, New York in 1950. He studied Chemistry at Eastern Oregon State College, La Gande, Oregon from 1969–1971, then moved to Portland State University, Portland, Oregon from 1971–1973, where he graduated with a B.S. in Chemistry. After a year of working at the Oregon Graduate Center in Beaverton, Oregon and taking post-graduate studies at Portland State University, he went to the University of California, Irvine for graduate studies in Chemistry. His graduate studies were with Professor Harold W. Moore, with whom he studied synthetic chemistry and cycloadditions relating to cyanoketenes. He graduated with a Ph.D. in Chemistry in 1978. After that he became a staff chemist in the Medical Radioisotope Research Group of the Isotope and Nuclear Chemistry division at the Los Alamos National Laboratory in Los Alamos, New Mexico. In that position he worked primarily with radiohalogens for labeling of small molecules for nuclear medicine applications. In 1984 he joined a start-up biotech company, NeoRx Corporation, in Seattle, Washington where he ultimately became Director of Radiopharmaceutical Chemistry. During his time at NeoRx Corporation he studied the use of pendant groups for labeling monoclonal antibodies with radiohalogens. In 1990, he joined the Department of Radiation Oncology at the University of Washington in Seattle, where he has risen to full Professor. At UW, he has continued his studies with radiohalogens and has had a focus on targeting of therapy radionuclides to cancer.

Positron Emission Tomography (PET), is being used clinically, but is not as widely available. Although the first clinical use of a radioiodine nuclide, conducted in 1940,[1] was a study of thyroid function, the free radionuclide can also be used in therapy of thyroid carcinoma. Indeed, radioiodine may be considered a corner stone in nuclear medicine, as it was the first radionuclide to be used for both imaging and therapy. Importantly, because of their availability and well-defined chemistry, radioiodine nuclides have also been used extensively in the development of many radiopharmaceuticals, even ones that ultimately do not employ radiohalogen nuclides.

Aside from radioiodine, which localizes specifically to thyroid and thyroid-originating cancers, for most practical purposes radiohalogens must be coupled with biomolecules to obtain radiopharmaceuticals that can be useful for diagnosis or therapy of human disease. Fortunately, the chemistry required to incorporate radiohalogens into organic molecules is founded in a vast body of literature on halogenation of organic compounds. For example, many commonly used reaction types such as nucleophilic, electrophilic, demetallation, and exchange reactions have been used to incorporate radiohalogens into organic compounds. However, halogenations with halogen radionuclides present some unusual challenges not of concern when using stable halogen nuclides. Some of the major challenges include: (1) working with radioactive materials, sometimes at very high levels of radioactivity; (2) working with short half-lived radionuclides, making reaction times, concentrations of reactants, and presence of impurities important factors in the reactions; (3) the requirement to obtain high specific activity compounds for certain applications, and (4) dealing with issues associated with production and purification of radiohalogens, including high cost.

When considering the application of radiohalogens, the handling and chemical requirements for developing imaging and therapeutic agents can be quite different. Thus, sections of this review are separated into two broad categories, Imaging and Therapy. Examples of the chemistry employed to obtain radiopharmaceuticals currently used in nuclear medicine, such as fluorodeoxyglucose (FDG), are included to show the breadth of the field. There are a large number of publications relating to radiopharmaceuticals containing radiohalogens. This tutorial review is purposefully brief. More in-depth information can be obtained from the many review articles describing various aspects of radiohalogen chemistry and their applications. Some review articles recommended for reading describe; (1) radiohalogenated carbohydrates for use in PET and SPECT,[2] (2) applications of positron-emitting radiohalogens in PET and oncology,[3] (3) incorporation of radiohalogens into organic molecules,[4] incorporation of radiohalogens into proteins,[5] and (4) use of radiohalogens in radioimmunotherapy.[6] While brief, this review is intended to be broad in scope and should provide the non-specialist with a good overview of the chemical factors that are important in radiohalogen chemistry used in both imaging and therapy. It should also provide insight into the many potential applications of radiohalogenated molecules and their significance in nuclear medicine.

2 Radiohalogen nuclides

Although there are only five halogens in the periodic chart, there are many more radiohalogens, those isotopes being distinguished from the stable nuclides by having differing numbers of neutrons in the nucleus. From an inspection of the chart of the nuclides, one finds listed 13 radiofluorine nuclides in addition to the stable nuclide (^{19}F); 14 radiochlorine nuclides in addition to two stable nuclides (^{35}Cl, ^{37}Cl); 23 radionuclides of bromine in addition to two stable nuclides (^{79}Br, ^{81}Br), 34 radioiodine nuclides in addition to the stable nuclide (^{127}I), and 31 radionuclides of astatine with no stable nuclides. Even though there are a large number of radiohalogen nuclides, for the purpose of this review, the ten radiohalogen nuclides that are currently of highest interest for application to the development of new radiopharmaceuticals are discussed. Those radiohalogen nuclides are listed in Table 1. The interest in these radionuclides is based on the fact that they have properties that make them useful for imaging or therapy applications, and methods are available for obtaining them. Even though other radiohalogen emission properties might make them useful, many are not practical because they have half-lives that are too short and/or they can not be readily produced. More information on the factors that make these radiohalogens useful in imaging and therapy is provided in the following sections.

2.1 Production of radiohalogen nuclides

Radiohalogens of interest for medical purposes are not available from natural sources and must be prepared. Some of the radiohalogens are produced by commercial sources and can be purchased (*e.g.* ^{123}I, ^{125}I, ^{131}I), but others must be prepared before each use. An important aspect of the application of radiohalogens is the method of production. A full description of methods for producing radiohalogen nuclides is beyond the scope of this tutorial, but information on the subject is available.[7] However, in general those radiohalogen nuclides that have atomic masses less than the mass of the stable nuclide are primarily produced by bombardment of protons (p,n reactions), deuterons (d,2n reactions) or alpha particles (α,2n reactions) on a target material that is one or two elements to the left of them on the periodic chart (groups 6a and 5a respectively). These nuclides are neutron deficient and may undergo a number of decay pathways, including emissions of photons (from internal conversion), low energy electrons, and/or positrons.

Table 1 Radiohalogens used in imaging and therapy

Radiohalogen	Half life	Type of emission	Application
^{18}F	110 m	β^+	PET Imaging
^{122}I	3.6 m	β^+	PET Imaging
^{123}I	13.2 h	γ	SPECT Imaging
^{124}I	4.18 d	β^+	PET Imaging
^{125}I	59.4 d	Auger e^-	Therapy
^{131}I	8.02 d	β^-	Therapy
^{75}Br	97 m	β^+	PET Imaging
^{76}Br	16.2 h	β^+	PET Imaging
^{77}Br	57 h	Auger e^-	Therapy
^{211}At	7.21 h	α	Therapy

Radiohalogens that have higher masses than the stable nuclides are generally prepared by irradiation of the stable halogens with neutrons in a nuclear reactor (n,xp reactions). Importantly, the method of production can have a dramatic effect on the chemistry of radiohalogenation, and can dictate which biological application the radiohalogenated molecules can be used in. Preparation of radiohalogens by irradiation of a target material can introduce significant challenges for the radiochemist. Some of the chemical problems involve introduction of radionuclidic impurities, chemical impurities, solvent removal and handling of high quantities of radioactivity. This latter problem often requires the use of specialty equipment such as remote handling in highly shielded hot cells.

2.2 Specific activity of radiohalogens

One of the very important considerations in the usefulness of a radiohalogen nuclide is its specific activity. Specific activity is an important concept for this area of chemistry since it can have a large effect on radiolabeling chemistry, and the ability to conduct molecular imaging or therapy. Briefly, this term is defined as the ratio of the amount of radioactivity per unit mass or molar quantity (*e.g.* millicuries (mCi)/μg or mCi/mmol). High specific activity radionuclides have relatively little carrier or non-radioactive nuclide contaminant. For example, high specific activity ^{18}F may be several thousand Curies (Ci)/mmol for [^{18}F]fluoride from a water target in comparison to only 1 Ci/mmol for carrier added [^{18}F]F_2 from a gas target. The specific activity of an isotope and consequently a labeled molecule is significant from a chemistry and biological perspective. From the chemistry perspective high specific activity radiofluorine may only be in nanomolar amounts making stoichiometry of the other reagents extreme in comparison to the radionuclide. From a biological and imaging perspective, high specific activity is often a requirement to achieve true tracer conditions where receptor sites are not being significantly populated with non-radioactive compound. This is also important for therapy where low specific activity radiohalogenated molecules can saturate targeted receptors without having sufficient radioactivity to kill a cell.

There are several factors that affect the specific activity of a radiolabeled compound. A major factor is the specific activity of the radionuclide itself, which is directly related to its half-life. The relatively short half-life radionuclides, such as the ones discussed in this review, have high theoretical specific activities. Radiohalogenations employing high specific activity radionuclides can result in low specific activity labeled compounds due to intentional or unintentional introduction of stable halogens into reaction mixtures. Intentional introduction of stable or carrier halogen can occur when preparing a specific halogenation reactant (as with the [^{18}F]F_2 described previously) or when employing an isotopic exchange reaction. Generally, introduction of stable halogens is avoided, but unintentional introduction of stable halogens can not be avoided. The reason for this is that halogens are ubiquitous in nature and the glassware, solvents, reactants, and oxidants used can introduce significant amounts of the stable halogen carrier when high specific activity reactions are conducted. Because of this unintentional introduction of stable nuclides, high specific activity reactions are referred to as no-carrier-added as opposed to carrier-free. Another factor that relates to specific activity, which may not be obvious, is the chemical form of the radiohalogenation agent. For example, when high specific activity radioiodide is oxidized *in-situ* it generates an electropositive iodine, but it is unlikely to form I_2. This is true because there is so little radioiodine present that statistically it is not possible for two iodine atoms to join together at the concentrations involved. Instead it is more likely to have a mixed halogen such as ICl formed, particularly if an oxidant such as *N*-chlorosuccinimide (NCS) or chloramine-T (ChT) is used. In oxidation reactions using *N*-chloro oxidizing agents, anionic iodide reacts with electropositive chlorine to form ICl. Importantly, when a mixed halogen species is formed, complete reaction of the electropositive radiohalogen is possible, rather than having a theoretical yield of only 50% when a dihalogen species (*e.g.* I_2) is used. An example where this is particularly important is destannylation reactions, where radioiodide oxidized *in situ* provides nearly quantitative radiochemical yields. If [*I]I_2 was the reactive species in the reaction, half the radioiodine would be lost to the trialkyltin moiety.

2.3 Radiohalogen nuclides useful in imaging

The radioactive decay property that allows the use of radionuclides for imaging is emission of photons with sufficient energy to detect in a device external to the body. Radionuclides that are useful for SPECT imaging emit photons in high abundance and have high enough energy (*e.g.* > 100 keV) to readily escape the body and be detected. In PET imaging, positrons emitted by the radionuclide interact with a negatron (electron) in an annihilation process to produce two coincident 511 keV photons, which are detected simultaneously in a detector ring. An important characteristic is the energy of the emitted positron. The higher its energy the further a positron will travel before the annihilation occurs. A longer distance of positron travel in tissue results in an overall loss of spatial resolution. Another important characteristic is the abundance of the positron emissions since a low abundance requires more radioactivity to be administered. Yet another consideration is the presence of other high-energy photon emissions that might complicate the detection of coincidental 511 photons.

From a purely physical point of view, ^{18}F has the most favourable nuclear properties for imaging with PET. The half-life (110 min), high percentage of β^+ emission (97%), and relatively low positron energy (0.635 MeV) make this the ideal PET halogen for high-resolution images. Other favourable features of ^{18}F are that the fluorine atom is small, it can accept a hydrogen bond, and the carbon–fluorine bond is very strong. However, there are no naturally occurring fluorine-containing compounds in the body, and fluorine-containing biomolecules are often not suitable substrates in normal metabolic pathways as they can be enzyme inhibitors. Despite these potential problems there are many ^{18}F labeled compounds used in research and clinical PET today.

^{75}Br is a potential PET imaging isotope with a reasonable half-life (97 min). However, compared to ^{18}F, it suffers from a

higher positron energy (1.74 MeV), a 71% positron emission and second high energy gamma, all of which contribute to its overall poorer resolution for imaging. ^{76}Br has gained more interest recently, partly due to applications that can be more effective using its longer half-life (16.2 h). However, its positron energy of 3.98,3.44 MeV and 54% positron emission make it less favourable when compared to ^{18}F for imaging resolution.[8] ^{77}Br is a single photon and positron emitter with a 57 h half-life. Due to its limited availability there has been little published on its use in imaging since the 1990's. Also, its two higher energy gamma rays, as well as low percent positron emission (1%), make it less desirable as a SPECT or PET isotope.

^{122}I is another positron emitting halogen that has favourable imaging properties. However, its very short half-life (3.6 min) makes it only useful for imaging a biological function that does not involve a long physiological process, such as blood flow. ^{123}I is a commercially available SPECT isotope that has very favourable SPECT imaging properties and a reasonable half-life (13.2 h). This isotope is widely used in nuclear medicine and can be shipped around the world. ^{124}I is a positron-emitting radionuclide that has gained considerable interest recently. The interest comes from the fact that it has a relatively long half-life (4.18 d), can be produced on an accelerator and has both imaging (PET) and therapeutic properties. However, the emission properties of this radionuclide are far from ideal.

From the forgoing discussion, it should be apparent that the choice of radiohalogen for imaging can not be solely based on optimum physical properties. Rather, other factors such as half-life, availability and halogen labeling chemistry play a significant role. Still other factors such as the amount of radiation damage (doses) delivered to target and non-target tissues in the body when different radiohalogens are used must be considered.

2.4 Radiohalogen nuclides useful in therapy

To understand the application of radionuclides to therapy, one must have a knowledge of some basic radiobiological effects of radiation.[9] Radiation interacts with biological tissues through absorption of energy to either excite or ionize electrons in atoms or molecules in its path. If the absorbed energy of the radiation is sufficient to eject one or more orbital electrons, ionization occurs. Importantly, the amount of energy dissipated in each ionization event is more than enough to break chemical bonds. Since the largest component of cells (*e.g.* 80%) is water, it may be considered the principal molecule for ionization. Ionized water molecules rapidly decay to hydroxyl free radicals, and these very reactive species can diffuse short distances in the cell to interact with, and cause damage to, critical cell components. Such interaction of radiation with biomolecules is considered an indirect action, as the damaging effect comes from the reactive hydroxyl radical not the radiation itself. Although cellular repair mechanisms can offset the damage caused by ionization in many examples, large amounts of damage to DNA can be lethal to the cell. In addition to the indirect action, particulate radiation (*e.g.* electrons, protons, neutrons, and α-particles) can collide with critical components of the cell such as DNA, and transfer energy to cause damage. This interaction of the radiation with the critical biomolecules of the cells is considered a direct action, and dominates the damage caused by heavy particles such as proton, neutrons and α-particles. Because these interactions transfer a large amount of energy in the distance traveled, these radiation types are considered to have high linear energy transfer (LET). This is important as the relative biological effectiveness (at cell killing) increases as the LET of the radiation increases.

Based on the principles outlined above, radiohalogens used for therapy applications have particle emissions such that they provide more or higher quality interaction (ionization) in biological tissues than the radionuclides used in imaging that emit photons. Indeed, in principle, any radiohalogen that emits an electron (β^-, β^+, or Auger electron) or α-particle (^{4}He nucleus) might be used for therapy. Emission of electrons occurs over a continuum of energies, and a maximum energy and average energy can be measured. This is important as the maximum energy defines the greatest distance that an electron (β^- or β^+) will travel through tissue (*e.g.* mm to cm) and the average energy defines where the most radiation dose will be deposited. α-Particle emissions are different in that they are of discrete energy, and thus, travel a specific distance in tissue. Irrespective of the type of radiation used, the desired effect is to selectively deliver a cell-killing dose to the target (*e.g.* cancer cells) while causing minimal toxicity to non-target tissues (*e.g.* bone marrow, kidney, liver). Although studies have been conducted for many years to develop therapy radiopharmaceuticals with radiohalogens, suitable molecules for targeting diseased cells such as cancer have not been available until more recently.

Even though there are several radiohalogen nuclides that could be used for therapy, few have been used. The most commonly used radiohalogen for therapy is ^{131}I. Because of the natural propensity of iodine to localize to the thyroid, ^{131}I has been used to treat thyroid cancer for many years.[10] Other factors that have made this radionuclide widely used are its availability and reasonably long half-life (8.02 d). This has made it relatively inexpensive and readily transported to clinical sites. Unfortunately, ^{131}I does not have optimal properties as its decay produces high energy photons (*e.g.* 364 keV, 637 keV). These photons are problematic as they make it difficult to conduct labeling at the required high levels and can cause the patient to be isolated to minimize the radiation exposure to health care professionals and family members. The photons do allow imaging of the distribution of radioiodine within a patient, albeit a high-energy collimator must be used and the quality of image is low. It is important to be able to evaluate the distribution of radionuclide in a patient so that an estimate of the radiation dose to the target tissue (*e.g.* tumor) and non-target tissues can be obtained. This estimate provides information that is important regarding the biological effect on normal tissues (*e.g.* maximum tolerated dose) and the potential for an effective therapy to the tumor.

The β-particle emissions of ^{131}I travel a few mm in tissue. This distance is important in tumors as the β-particle travels well beyond the cell it is attached to or internalized in, providing a radiation field effect (cross-fire effect). The field

effect alleviates the requirement that every cell have the radionuclide associated with it. Thus it is useful for solid tumors where penetration of the carrier molecule is difficult, or targeted receptors are not available on all cells. Another radiohalogen of high interest for therapy is ^{211}At. Although the half-life of ^{211}At is relatively short (7.2 h), it is the only α-emitting radiohalogen nuclide that is considered acceptable for use in humans. In contrast to β-particle emission, the α-particles emitted by ^{211}At decay have a range of 50–70 μm due to their much higher interaction with tissue. This high LET makes ^{211}At particularly attractive for therapy of metastatic cancer or in applications where single cells are targeted. While it is estimated to take several hundred (*e.g.* > 400) β-particle emitting radionuclides on a single cell to kill that cell due to the fact that most of the particles' energy is deposited outside of the cell, it is estimated that only a few (*e.g.* 1–14) associated α-particle emitting radionuclides can kill a cell. The closer the ^{211}At atom decays to the nucleus, the fewer atoms are required to kill the cell, making internalizing targeting agents particularly attractive.

Another radiohalogen nuclide of interest for therapy is ^{125}I. The particle emitted in radioactive decay of ^{125}I, that makes it of particular interest, is an Auger electron. The Auger electron deposits its energy in a very short distance (*e.g.* a few Å), which makes it high LET. While this short distance requires that the Auger electron emitting radiohalogen be internalized and localized in a specific location (*i.e.* associated with double strand DNA) to provide an effective therapy (cell killing), it causes little damage to non-target tissues. Although ^{125}I is readily available, it is not an ideal radionuclide for therapy as it has a relatively long half-life (59.4 d), so other Auger electron-emitting radiohalogens are of interest. The SPECT imaging radionuclide ^{123}I has Auger electron emissions and a much shorter half-life, however, few studies have been performed with this radionuclide to determine its effectiveness in therapy.

3 Radiohalogen labeling chemistry

Radiohalogen labeling generally follows the same chemistry useful for non-radioactive halogenations. Thus, nucleophilic substitution occurs with halogen anions and electrophilic substitution reactions occur with electropositive halogens. However, as indicated previously, the reactions are heavily influenced by the method of preparation of the radionuclide and by other factors such as half-life of the radionuclide and specific activity sought. Information is provided in the following sections on the most commonly conducted radiolabeling reactions, radiofluorination and radioiodination, followed by the less common radiobromination and astatination reactions.

3.1 Fluorine-18

Fluorine-18 is probably the single most important radioisotope used in PET.[11] As mentioned above, the ideal physical properties of this isotope of fluorine coupled with its favourable chemical properties make it highly desirable for radiopharmaceutical development. The production of ^{18}F is now commonly carried out on a cyclotron *via* the ^{18}O(p,n) process using either ^{18}O enriched water, for fluoride production, or ^{18}O-oxygen gas, for elemental fluorine production. The availability of ^{18}O-water and gas is good at present. For a more comprehensive discussion of the targetry, nuclear reactions and other production routes, the reader can go to previously published review articles on this topic.[12] The [^{18}F]fluoride obtained from a water target is in the no-carrier-added (*nca*) form. In contrast, the [^{18}F]F_2 obtained from the oxygen gas target is obtained in the carrier-added (*ca*) form due to the fact that stable [^{19}F]F_2 is added to the target to extract the highly reactive fluorine from its walls. The labeling chemistry for ^{18}F falls under one of two main classes of reactions, electrophilic or nucleophilic, depending on which of these two target systems is used.

3.1.1 Electrophilic labeling. Although fluoride reactions dominate most of the ^{18}F radiochemistry in PET today, electrophilic fluorinating agents such as [^{18}F]F_2 have played an important role in the development of ^{18}F labeled radiopharmaceuticals for PET. [^{18}F]F_2 was the first labeling reagent used in PET chemistry in the original synthesis of [^{18}F]fluorodeoxyglucose ([^{18}F]FDG).[2] In this synthesis, dilute F_2 (typically 1–5% in Ar, He or nitrogen) was added across the double bond of triacetylglucal to form a mixture of products. Superseding this reaction was the preparation of [^{18}F]acetyl hypofluorite, from [^{18}F]F_2, which also undergoes electrophilic addition across the glucal double bond to form FDG. This reaction had the advantage of adding more selectively to one face of the double bond yielding the glucose configuration in higher radiochemical yield than for F_2. Although there have been several electrophilic ^{18}F reagents prepared from F_2 over the years the commonly used reagents today are still [^{18}F]F_2 and [^{18}F]acetyl hypofluorite. The two main electrophilic reactions are the direct electrophilic substitution and demetallation reactions, conducted with organometallic intermediates such as organo-mercury acetate or organo-trialkyltin derivatives. Both of these reactions make use of either elemental fluorine or acetyl hypofluorite. Some of the routinely synthesized radiopharmaceuticals utilize the electrophilic substitution method. Examples of radiopharmaceuticals produced using this reaction; [^{18}F]fluorodeoxy-D-glucose ([^{18}F]FDG), 6-L-[^{18}F]fluorodopa ([^{18}F]FDOPA), 6-L-[^{18}F]-fluoro-*m*-tyrosine ([^{18}F]FMT) and 2-L-[^{18}F]fluorotyrosine ([^{18}F]FT), are shown in Fig. 1.

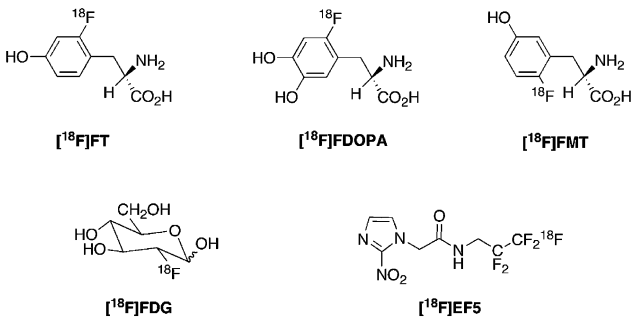
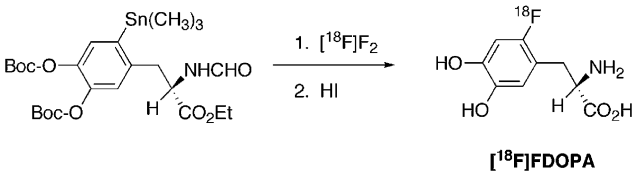


Fig. 1 Examples of radiofluorinated compounds prepared and investigated as PET imaging agents.

Although [^{18}F]FDG is now mainly synthesized by the nucleophilic route, electrophilic fluorination chemistry was used for many years. FDG is used to obtain glucose metabolic rates and as such is useful for imaging the heart, brain and tumors. The aromatic amino acids, such as fluorodopa and the fluorotyrosines, are more easily prepared with [^{18}F]F_2 (Scheme 1),[13] since the aromatic rings are deactivated toward

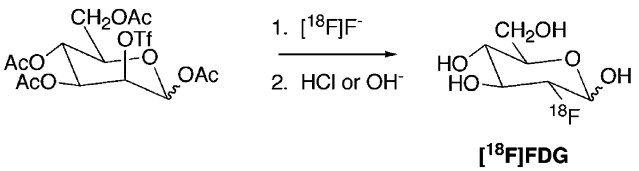


Scheme 1 Radiofluorination of a dopa derivative by electrophilic destannylation reaction.

nucleophilic attack and the product application of these particular compounds does not require high specific activity. The tyrosine tracers are used for brain and protein metabolism studies. Fluorodopa is decarboxylated upon entry into the brain, becoming fluorodopamine which mimics the neurotransmitter dopamine. This agent is useful in studying movement disorders such as Parkinson's disease. Other compounds that contain nitroimidazoles, such as [^{18}F]EF5 (Fig. 1), may be applicable to imaging hypoxia in tumors. This compound has also been labelled with [^{18}F]F_2 simply because attempts to label it with [^{18}F]fluoride have failed. Therefore, despite the emphasis on fluoride chemistry for labeling there remains a role for electrophilic fluorinations in PET radiochemistry for compounds that are difficult to label with fluoride and that do not require high specific activity in their biological application.

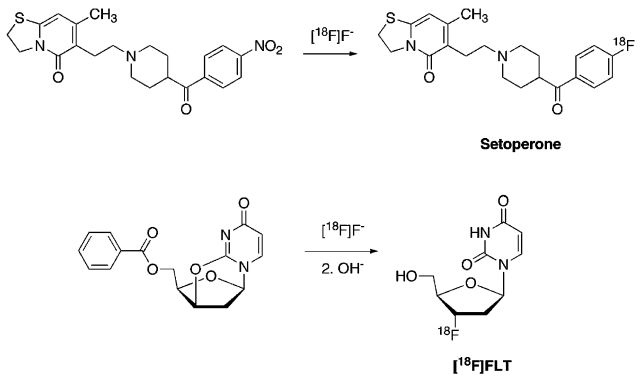
3.1.2 Nucleophilic labeling. Radiofluorination with [^{18}F]fluoride is now the preferred method for the incorporation of ^{18}F into organic compounds for molecular imaging with PET. This chemistry is generally used more than F_2 reactions for several reasons. First, [^{18}F]fluoride is prepared from an enriched ^{18}O-water target that can produce more ^{18}F than most ^{18}O gas targets can produce [^{18}F]F_2. The water target is less complicated to operate than the gas target, because gas targets require a double irradiation and cryogenic trapping of the enriched gas. Second, [^{18}F]fluoride generally gives higher radiochemical yields, since all the ^{18}F is available to the reaction, whereas only half of the ^{18}F from [^{18}F]F_2 is incorporated at the desired site on the molecule. The third reason is that [^{18}F]fluoride is obtained in much higher specific activity than [^{18}F]F_2, because carrier F_2 is required to extract this gas from the target. Unfortunately, it is not possible to oxidize fluoride to elemental fluorine *in-situ* as is the case for bromine and iodine. Many compounds such as [^{18}F]FDG have been labeled using [^{18}F]fluoride (Scheme 2).[14]

The standard method to prepare reactive [^{18}F]fluoride uses a phase-transfer Kryptofix 222/potassium carbonate system. Briefly, the fluoride from the water target is trapped onto a small anion exchange column and the expensive ^{18}O water is recovered. The trapped fluoride is then eluted with a mixture of water, acetonitrile, Kryptofix 222 (K222) and K_2CO_3. The



Scheme 2 Radiofluorination of a glucose derivative by nucleophilic displacement reaction.

eluted fluoride mixture is evaporated to dryness with heat and vacuum, then acetonitrile is added and evaporated two or three more times to drive off any residual water. After these steps, the reactive [^{18}F]fluoride is ready for use. The cryptand K222 is a polyether amine, cyclic molecule that sequesters the potassium ion to provide a "naked", reactive form of fluoride. The most commonly used nucleophilic reactions in labeling chemistry involve the displacement of a good leaving group, such as a tosylate or triflate, or the aromatic substitution of either a nitro group or trialkyl amine on a suitably activated ring. Examples of aromatic nucleophilic substitution reactions are shown in Scheme 3 for the synthesis of the serotonin



Scheme 3 Additional examples of radiofluorination by nucleophilic displacement reactions.

receptor antagonist [^{18}F]Setoperone[15] and the cell proliferation imaging agent [^{18}F]fluorothymidine ([^{18}F]FLT).[16]

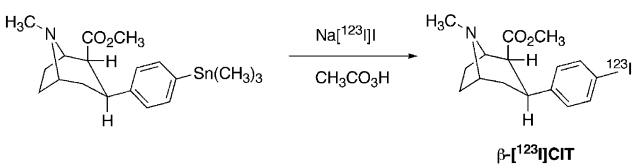
Setoperone and other serotonin receptor antagonists are used in the study of psychiatric disorders such as schizophrenia, and FLT is used as a PET tracer for oncology.

3.2 Iodine-122, -123, -124

As with fluorine labeling chemistry, radioiodination reactions are primarily conducted using electrophilic and nucleophilic substitution reactions.[17] The advantage that iodination reactions have over fluorine chemistry is that iodide is easily oxidized to an electrophilic form of iodine. This affords investigators the ability to use high specific activity iodide in a wide spectrum of electrophilic chemistry without adding carrier iodine. A disadvantage of iodine is that it has a lower stability towards the *in vivo* cleavage of the carbon–iodine bond. For this reason, aromatic C–I bonds, which are more stable than aliphatic C–I bonds, are most often formed to confer as high stability as possible. Release of radioiodine *in*

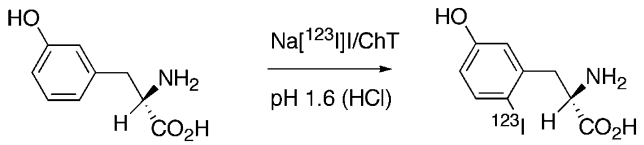
vivo is readily apparent by detection of significant quantities of iodine in the thyroid and stomach. As mentioned previously, the application of radiohalogen nuclides is highly dependent on their half-life and particle emissions. ^{122}I is a short-lived positron-emitting radioiodine, which has limited use due to its short half-life (3.6 min) and availability. In addition to the difficulties in preparing the radionuclide for use, the very short half-life makes it difficult to carry out complex organic chemistry. In contrast, another radioiodine nuclide, ^{123}I, is widely used throughout the world and is produced commercially on a cyclotron. This iodine isotope has excellent physical properties for imaging with SPECT and a long enough half-life (13.2 h) to make shipment feasible. A third radioiodine isotope, ^{124}I, has recently gained significant interest for both imaging and therapy applications. The reason for this is that ^{124}I has a longer half-life (4.18 d) than most other radionuclides used for quantitative imaging by PET, and has emissions that permit its use as a therapeutic isotope in oncology. This combination is particularly attractive as it allows physicians to follow the distribution and pharmacokinetics of therapeutic agents with longer biological half-lives.

3.2.1 Electrophilic labeling. Electrophilic labeling with radioiodine is the preferred overall chemistry route because of the ease with which iodide can be oxidized *in-situ* to an electropositive form of iodine. This is convenient since radioiodine nuclides are normally obtained in the form of sodium iodide. Electrophilic reactions can be performed simply by adding an *in-situ* oxidant such as peracetic acid, chloramine-T (ChT), *N*-chlorosuccinimide (NCS) or one of several other mild oxidants into the reaction with the appropriate precursor. These reactions usually proceed in high radiochemical yield. Although a number of organometallic intermediates can be used, the destannylation reaction is the favoured electrophilic route to radioiodinated compounds such as β-[^{123}I]CIT (Scheme 4).[18]



Scheme 4 An example of an electrophilic destannylation reaction for radioiodination of aryl groups.

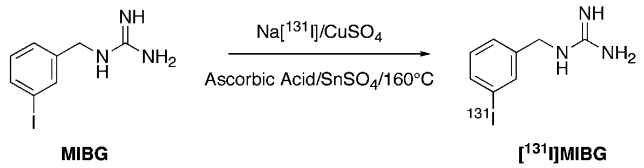
The destannylation reactions proceed well even when very small quantities of trialkyltin-precursors (< 50 μg) are used with high specific activity Na[*I]I. Importantly, this reaction works with deactivated aromatic compounds, providing a high *in vivo* stability. β-CIT and its analogs bind to dopamine reuptake receptor sites in the brain and as such are useful in research to study the dopaminergic system of the brain and in studying movement disorders such as Parkinson's disease. Direct electrophilic iodination of suitably activated aromatic compound, such as *m*-tyrosine[19] (Scheme 5) is also a feasible radiolabeling route. Iodination of proteins is readily achieved by the direct labeling of tyrosine residues with *in-situ* oxidized



Scheme 5 Direct radioiodination of *meta*-tyrosine.

iodine. However, one must be aware that the *in vivo* stability of the radioiodinated compound may be low when using activated aromatic rings, particularly phenols, for incorporating the radioiodine.

3.2.2 Nucleophilic labeling. Halogen exchange reactions are the most common nucleophilic method for the introduction of radioiodine nuclides into organic molecules. Frequently, either ammonium sulfate or copper(II) salts[20] are used to catalyze the iodide for iodine exchange, as is the case for the synthesis of *meta*-[^{131}I]iodobenzylguanidine ([^{131}I]MIBG) shown in Scheme 6.[21]



Scheme 6 Example of a radioiodination *via* an isotopic exchange reaction.

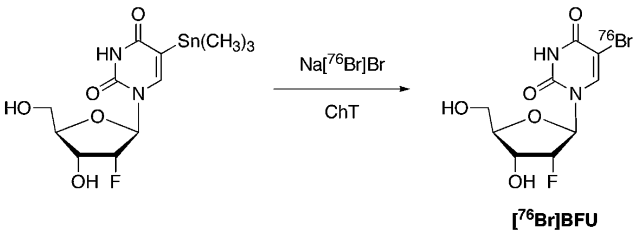
MIBG is used for the diagnosis and therapy of certain brain tumors depending on the iodine isotope used. It has been demonstrated that the exchange reactions work with all of the radioiodine isotopes, albeit varying yields are obtained based on the radionuclide used and its method of preparation. In the halogen exchange reactions both brominated and iodinated precursors can be used, but the use of iodinated precursors will contribute carrier iodine since the precursor (non-radioactive iodinated compound) cannot be separated from the radiolabelled product. The [^{131}I]MIBG has also been prepared by a destannylation, but high specific activity is not required so the exchange reaction has been chosen as this reaction does not require preparation of the requisite *meta*-trialkylstannylbenzylguanidine intermediate.

3.3 Bromine-75, -76, -77

Radiobromine labeled compounds are attractive since the chemistry is more readily conducted than fluorine chemistry, the carbon–bromine bond is stronger than the corresponding carbon–iodine bond and a bromine atom is smaller than an iodine atom. Bromide, like iodide, can be oxidized *in-situ* to conduct electrophilic reactions with high specific activity radionuclides or alternatively can be used directly in nucleophilic substitution reactions. However, bromine radionuclides are less available than iodine radionuclides. They can be produced on a cyclotron, but their targets and isotope extraction procedures (*e.g.* dry distillation) are complicated. Thus, they are only produced in a few centres around the

world. It is interesting to note that there are very few papers in the recent literature on the use of ^{75}Br and ^{77}Br, but several on the use of ^{76}Br.[22,23] This is probably due to the fact that ^{76}Br is a positron emitter with a longer half-life (16 h) than ^{75}Br, and has a higher abundance of positron emission than ^{77}Br.

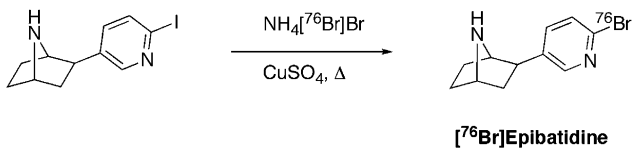
3.3.1 Electrophilic labeling. Electrophilic bromination reactions are also predominantly conducted using the destannylation reaction. An example of this type of reaction is the synthesis of [^{76}Br]bromofluorouracil ([^{76}Br]BFU) (Scheme 7).[24]



Scheme 7 An example of a radiobromination *via* an electrophilic destannylation reaction.

BFU is incorporated into DNA and is used in oncology for tumor imaging. With the use of *in-situ* oxidants such as chloramine-T (ChT), *N*-chlorosuccinimide (NCS), and hydrogen peroxide/acetic acid, the reactions are fast, high yielding and all of the radiobromide is available (as electropositive bromine) for the substitution reaction. As with the iodinations, direct electrophilic reactions on suitably activated aromatic systems can also be achieved using the *in-situ* oxidant reaction conditions. There is less of a concern about the *in vivo* stability of bromine radionuclides on activated aromatic rings.

3.3.2 Nucleophilic labeling. Radiobromination *via* aromatic nucleophilic substitution reactions can be achieved using similar reaction conditions to those used in radioiodine labeling *via* Cu^{2+} assisted exchange reaction. An example of this reaction is the radiobromination to form [^{76}Br]Epibatidine (Scheme 8).[23] Epibatidine binds to nicotinic acetylcholine



Scheme 8 An example of a radiobromination *via* non-isotopic nucleophilic aromatic substitution reaction.

receptors and can be used to image neurodegenerative disorders such as Alzheimer's disease. It should be noted that high specific activity radiobrominated compounds can be obtained from non-isotopic halogen exchange reactions if separations can be attained for the bromo and iodo derivatives.

The preponderance of radiobromination reactions are on aromatic rings, however, radiobromination can be achieved on aliphatic carbons using the same conditions as used for nucleophilic displacement reactions. Similar to the previously described radioiodination and radiofluorination reactions, a variety of leaving groups, including iodine, tosylate, mesylate, triflate *etc.*, can be used in the reactions. The *in vivo* stability of a radiobrominated compound in which the radiobromine is bonded to an aliphatic carbon is expected to be higher than for the corresponding radioiodinated compound.

3.4 Astatine labeling

Labeling of organic molecules with At generally follows the same procedures as employed for labeling with iodine radionuclides.[25] However, the stability of the astatine–carbon bond is much lower than that of an iodine–carbon bond,[26] causing difficulties with using the same chemistry for labeling biomolecules as used with radioiodine. For example, unlike radioiodine, an instability is noted when proteins are directly labeled with *in situ* oxidized At (using ChT). Data have been published that indicate electrophilic labeling of At occurs on sulfhydryls rather than on the expected tyrosine phenolic moiety. To circumvent this instability, methods for producing At-labeled aromatic compounds that could be coupled (conjugated) with proteins were developed.[5] Although a large number of aryl astatination reagents have been developed, the most commonly used reagents for At-labeling of proteins are the *para*-astatobenzoate *N*-hydroxysuccinimidyl ester, [^{211}At]PAB[27] and the *meta*-astatobenzoate *N*-hydroxysuccinimidyl ester, [^{211}At]ATE,[28] shown in Fig. 2.

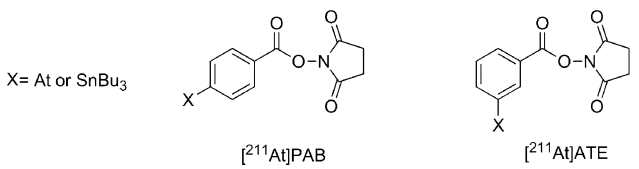


Fig. 2 Benzoate *N*-hydroxysuccinimide esters that contain the precursor trialkylstannane (X = $SnBu_3$), or ^{211}At label for conjugation with proteins.

Electrophilic destannylation of the trialkyltin functional group provides very rapid and efficient incorporation of the astatine into the reagents, but the conjugation of these reagents with proteins usually occurs in 40–60% yield.

These reagents, and others, have proven to be stable to deastatination on slowly metabolized proteins, such as intact monoclonal antibodies (MW $\sim$ 150 kDa), but are not stable to deastatination when smaller Fab′ fragments (MW $\sim$ 50 kDa) are used. This instability to metabolism is being addressed by evaluating other aromatic ring compounds and boron cage molecules.[29] Astatinated boron cage molecules appear to be more stable than the aromatic ring compounds, but their physical nature (lipophilicity and charge) causes non-target tissue retention *in vivo*. The issue of *in vivo* instability limits the application of ^{211}At, therefore, it is an area of active research.

4 Imaging applications

Clinical PET is dominated by the use of FDG because of its utility in oncology and cardiology. Since FDG gives

information about glucose metabolic rate, it is a very sensitive tracer for the detection of tumors, which generally metabolize more glucose, and for cardiac tissue viability, since only viable cardiac muscle tissue can metabolize glucose.[30,31] Thus, PET imaging with FDG has become the gold standard for diagnosing many forms of cancer and for predicting the success of cardiac bypass operations. On the other hand clinical research in PET and SPECT, using radiohalogenated ligands, has seen the synthesis of hundreds of labeled compounds that have been applied to a broad range of biological systems.[32] Research imaging applications have been dominated by studies of the functioning of the brain and its many receptor systems.[7] Compounds such as [^{123}I]Altropane or β-[^{123}I]CIT, tropane analogs, are used in the study and diagnosis of Parkinson's disease. These tracers bind to the post-synaptic reuptake sites of the neurotransmitter dopamine.[33] Other structurally similar agents labeled with ^{18}F have also been used for this application. Some dopaminergic applications include the use of [^{18}F]fluorodopa, and other ^{18}F- and ^{76}Br-labeled receptor drugs. More recent brain imaging research has involved agents labeled with radiohalogens, including β-amyloid markers for the study of Alzheimer's disease, seratonin receptor antagonists, such as [^{18}F]Setoperone, for psychiatric disorders including schizophrenia, and radiohalogenated opioid receptor-binding agents for the study of pain and reward, just to name a few. The brain research involving radiohalogenated radiopharmaceuticals has been accelerated in recent years with the development of very high-resolution tomographs. These sophisticated instruments, developed for imaging both humans and animals, provide an ability to observe the functioning of very small regions of the brain. The use of radiohalogenated radiopharmaceuticals to study human disease will continue to play a crucial role in nuclear medicine imaging.

5 Radiotherapy applications

The systemic administration (intravenous injection) of radiopharmaceuticals can provide selective targeting of cell-killing radioactivity to diseased cells, such as cancer cells. It is important to note that the major difference between conventional external beam irradiation and an administered therapeutic radiopharmaceutical is that the former provides a localized therapy whereas targeted radionuclide therapy (TRT) can seek out target cells throughout the body. Thus, TRT may provide a treatment of micrometastatic disease that can not be attained by conventional radiotherapy. Treatment of metastatic disease is a major hurdle to obtaining cures for cancer patients. As in imaging, radiohalogens play a key role in the development of TRT agents.

The use of radiohalogen nuclides for therapy of cancer is very actively under investigation. This is a large area of research encompassing most cancer types. The radionuclide-based cancer therapy that has been used longest and most widely is Na[^{131}I]I in thyroid carcinoma. However, broad application of radiohalogens to cancer requires that they be attached to specific targeting agents. The area that has been most studied is the use of radiohalogenated cancer-targeting monoclonal antibodies (mAbs), particularly radioiodinated mAbs. In addition to their being relatively inexpensive and readily available, the reason radioiodine nuclides (^{131}I and ^{125}I) have been used extensively in this application is that radiolabeling can be done easily and efficiently without prior modification of the protein. Radiohalogens other than radioiodine require attachment to a pendant group[5,6] (*e.g.* benzoate active esters) to attain radiolabeling that is stable and does not significantly affect the mAb binding to target cancer cells. Although mAbs are readily radioiodinated, other issues such as scale-up of the labeling reactions, radiolysis of the ^{131}I-labeled mAbs, and radiation safety concerns must be dealt with before clinical trials can begin. High levels of radiohalogens in isolated products can produce radiolysis of the carrier molecule, but this can be circumvented by the addition of anti-oxidants such as citric or ascorbic acid. Additionally, experience has shown that the radiation safety concerns can be successfully dealt with.[34] There are a number of other issues with using mAbs as cancer-targeting agents, which have slowed the development of this area, particularly for therapy of solid tumors.[35] However, within the past year the FDA has approved a ^{131}I-labeled mAb ([^{131}I]Bexxar) for treatment of non-Hodgkin's lymphoma. Many clinical studies are being conducted with radiolabeled antibodies. A large number of preclinical studies are being conducted with alternative forms of mAbs and alternative mAb-based methods of targeting (*e.g.* pretargeting) are being investigated to circumvent the problems with using radiolabeled antibodies. Radiohalogens will continue to play a major role in this developing field.

In addition to the use of mAbs as cancer targeting agents, more recently there has been considerable effort investigating a growing number of receptor-binding peptides (*e.g.* somatostatin, bombesin, annexin, VIP, *etc.*) as targeting agents.[36] While many of the early studies have included directly-labeled radioiodinated peptides, due to the rapid metabolism, which releases the radioiodine, the use of radiohalogens has not been encouraging. Chelated radiometals are most often used with internalizing receptors as they are retained (residualized) within the target cell. Studies are being conducted with charged functional groups (*e.g.* amidines, boron cage molecules) on pendant molecules that are radiohalogenated to residualize them. If this is successful, it is very likely that radiohalogens will be used in therapy where peptides are the targeting agent. Another approach that is being investigated to decrease the rate of metabolism is to increase the molecular weight of the peptides by attaching them to larger molecules or by combining several of them on one molecule (multi-dentate). This latter approach has provided promising results as an increase in binding affinity (avidity) is attained which can provide higher selectivity and better targeting.

Radiohalogenated small molecules are also of interest for therapy. A small molecule that has been under study for a number of years is *meta*-[^{131}I]iodobenzylguanidine ([^{131}I]MIBG; Scheme 6). MIBG is an analog of noradrenaline and is of interest for therapy of neuroblastoma.[37] Another broad area that has employed radiohalogens in its development is radiolabeled steroid hormones. Radiohalogens are particularly useful in this area as they do not greatly perturb the general shape and size of the steroid nucleus, allowing high affinity binding with the requisite protein carrier molecules. As

some of the radiohalogenated steroids have been shown to access the cell nucleus, radiohalogens that emit Auger electrons are particularly attractive for therapy of hormone-dependent tumors (*e.g.* breast and prostate). Another therapy agent that incorporates an Auger electron-emitting radiohalogen (^{125}I) under development is 5-[^{125}I]iodo-2′-deoxyuridine ([^{125}I]IUDR).[38] When administered, rapidly growing cancer cells incorporate [^{125}I]IUDR into the DNA and the Auger electron emission later breaks that DNA, killing the cells. Yet another potential therapy application involving a radiohalogenated small molecule is treatment of cancer metastases in bone. While cancer cures are the major goal, in this therapy the primary goal is to decrease (palliate) the pain associated with metastases growing in bone. Studies have shown that bone-seeking bis-phosphonate agents can be prepared in which radiohalogens are stabilized toward dehalogenation.[39] This is particularly important as it appears that β-emitting radionuclides, such as ^{131}I, do not alleviate all of the pain. In studies with radiometals, α-emitters and Auger electron-emitters have been shown to provide good pain palliation, so ^{211}At and ^{125}I might be investigated as a substitute for ^{131}I. A further area of research that appears to have great promise for radiohalogens in therapy is the use of anti-sense oligomers (*e.g.* DNA, RNA, PNA, morpholino-oligos, *etc.*) that bind selectively with segments of mRNA and/or DNA in the cell. These molecules labeled with α- or Auger electron-emitting radiohalogens have the potential to kill or alter the biochemical pathways in cancer cells.

In this section, a brief overview of some of the areas of active research into radiohalogenated molecules for therapy has been provided. It is important to note that very few radionuclide based therapy agents are approved and available for routine clinical use. However, the promise of this area is great and recently the knowledge about cellular function and message systems has increased greatly, hopefully allowing the researcher to choose the best target for therapy. Without question, radiohalogens will play a major role in the development of the new therapeutic agents.

6 Conclusions

The use of radiohalogenated radiopharmaceuticals continues to be an important and growing area of nuclear medicine for both imaging and radiotherapy. With the success of clinical PET using [^{18}F]FDG and the accompanying infrastructure that has been established, there is now incentive for the development of newer agents labeled with ^{18}F and positron emitting iodine and bromine nuclides. Along with the rapid growth of molecular imaging has come a renewed interest in radiotherapy agents, based on radiohalogens, since both the targeting and the efficacy of treatment can be monitored by imaging. In addition to those radiohalogen nuclides discussed in this review, a number of others have been studied (*e.g.* ^{34m}Cl, ^{80m}Br). While other halogen radionuclides may ultimately have application in nuclear medicine imaging or therapy, it seems likely that new radiopharmaceuticals developed in the future will contain one of the radiohalogens discussed in this review. Importantly, the future growth in this area of medicine will continue to be dependent on the implementation of radiohalogen chemistry and the development of new ways to incorporate radiohalogen nuclides into biologically important molecules.

Acknowledgements

The authors wish to thank the Canadian Institutes for Health Research, the Natural Sciences and Engineering Council of Canada, U.S. Department of Energy, U.S. National Institutes of Health and TRIUMF for funding our studies with radiohalogens. We also wish to thank the PET group at TRIUMF and UBC.

Michael J. Adam[a] **and D. Scott Wilbur**[b]

[a]*TRIUMF, University of British Columbia, 4004 Wesbrook Mall, Vancouver, BC, Canada, V6T 2A3. E-mail: adam@triumf.ca*

[b]*University of Washington, Department of Radiation Oncology, Seattle, WA 98104-2499, USA. E-mail: dswilbur@u.washington.edu*

References

1 G. Subramanian, *Radiopharmaceuticals*, The Society of Nuclear Medicine, New York, 1975.
2 M. J. Adam, *J. Labelled Compd. Radiopharm.*, 2002, **45**, 167.
3 M. Glaser, S. K. Luthra and F. Brady, *Int. J. Oncol.*, 2003, **22**, 253.
4 R. Bolton, *J. Labelled Compd. Radiopharm.*, 2002, **45**, 485.
5 D. S. Wilbur, *Bioconjugate Chem.*, 1992, **3**, 433.
6 M. R. Zalutsky, *Radioimmunotherapy of Cancer*, P. G. Abrams and A. R. Fritzberg, Eds., Chapter 4, pp. 81–106, Marcel Dekker, Inc., New York, 2000.
7 M. Pagani, S. Stone-Elander and S. A. Larson, *Eur. J. Nucl. Med.*, 1997, **24**, 1301.
8 M. J. Ribeiro, P. Almeida, D. Strul, N. Ferreira, C. Loc'h, V. Brulon, R. Trebossen, B. Maziere and B. Bendriem, *Eur. J. Nucl. Med.*, 1999, **26**, 758; R. Laforest, *IEEE Trans. Nucl. Sci.*, 2002, **49**, 2119.
9 E. J. Hall, *Radiobiology for the Radiologist*, 3rd Edn., J.B. Lippincott Company, Philadelphia, 1988.
10 S. Vallabhajosula, *Nuclear Oncology: Diagnosis and Therapy*, I. Khalkhali, J. C. Maublant and S. J. Goldsmith, Eds., Chapter 3, pp. 31–62, Lippincott, Williams & Wilkins, Philadelphia, 2001.
11 J. S. Fowler and A. P. Wolf, *Acc. Chem. Res.*, 1997, **30**, 181.
12 G. Stocklin and V. W. Pike, in: *Methodological Aspects. Developments in Nuclear Medicine*, P. Cox, Ed., Kluwer Academic Publishers, Dordrecht, 1993, p. 24.
13 M. Namavari, A. Bishop, N. Satyamurthy, G. Bida and J. R. Barrio, *Appl. Radiat. Isot.*, 1992, **43**, 989.
14 K. Hamacher, H. H. Coenen and G. Stocklin, *J. Nucl. Med.*, 1986, **27**, 235.
15 C. Crouzel, M. Venet, T. Irie, G. Sanz and C. Boullais, *J. Labelled Compd. Radiopharm.*, 1988, **25**, 403.
16 H.-J. Machulla, A. Blocher, M. Kuntzsch, M. Piert, R. Wei and J. R. Grierson, *J. Radioanal. Nucl. Chem.*, 2000, **243**, 843.
17 R. J. Seevers and R. E. Counsell, *Chem. Rev.*, 1982, **82**, 575.
18 J. L. Neumeyer, S. Wang, R. A. Milius, R. M. Baldwin, Y. Zea-Ponce, P. B. Hoffer, E. Sybirska, M. Al-Tikriti, D. S. Charney, R. T. Malison, M. Laruelle and R. B. Innis, *J. Med. Chem.*, 1991, **34**, 3144.
19 M. J. Adam, Y. Zea-Ponce and J. M. Berry, *J. Labelled Compd. Radiopharm.*, 1990, **28**, 1065.
20 A. S. El-Wetery, A. A. El-Mohty, S. Ayyoub and M. Raieh, *J. Labelled Compd. Radiopharm.*, 1997, **39**, 631.
21 A. R. Wafelman, M. C. P. Konings, C. A. Hoefnagel, R. A. A. Maes and J. H. Beijnen, *Appl. Radiat. Isot.*, 1994, **45**, 997; S. Chattopadhyay, M. K. Das, B. R. Sarkar, G. Prabhakar, K. S. Mehra and N. Ramamoorthy, *Appl. Radiat. Isot.*, 2001, **54**, 241.
22 L. Lu, L. Samuelsson, M. Bergstrom, K. Sato, K.-J. Fasth and B. Langstrom, *J. Nucl. Med.*, 2002, **43**, 1688.

23 K. Kassiou, C. Loc'h, F. Dolle, J. L. Musachio, L. Dolci, C. Crouzel, R. F. Dannals and B. Maziere, *Appl. Radiat. Isot.*, 2002, **57**, 713.
24 L. Lu, M. Bergstrom, K.-J. Fasth and B. Langstrom, *J. Nucl. Med.*, 2000, **41**, 1746.
25 K. Berei and L. Vasaros, *The chemistry of halides, pseudo-halides and azides*, (S. Patai and Z. Rappoport, Eds.), Chapter 14, pp. 787–819, John Wiley & Sons, Ltd, New York, 1995.
26 K. Berei, S. H. Eberle, H. W. Kirby, H. Munzel, K. Rossler, A. Seidel and L. Vasaros, *Astatine; Gmelin Handbook of Inorganic Chemistry*, 8th Edition, Springer-Verlag, New York, 1985.
27 D. S. Wilbur, S. W. Hadley, M. D. Hylarides, P. G. Abrams, P. A. Beaumier, A. C. Morgan, J. M. Reno and A. R. Fritzberg, *J. Nucl. Med.*, 1989, **30**, 216.
28 M. R. Zalutsky and A. S. Narula, *Appl. Radiat. Isot.*, 1988, **39**, 227.
29 M. F. Hawthorne and A. Maderna, *Chem. Rev.*, 1999, **99**, 3421.
30 H. N. Wagner, Jr., *J. Nucl. Med.*, 1991, **32**, 561.
31 S. S. Gambhir, J. Czernin, J. Schwimmer, D. H. S. Silverman, R. E. Coleman and M. E. Phelps, *J. Nucl. Med.*, 2001, **42**, 1–93.
32 M. J. Welch and C. S. Redvanly, *Handbook of Radiopharmaceuticals*, J. Wiley, England, 2003.
33 R. M. Baldwin, Y. Z-Ponce, S. S. Zoghbi, M. Laurelle, M. S. Al-Tikriti, E. H. Sybirska, R. T. Malison, J. L. Neumeyer, R. A. Milius, S. Wang, M. Stabin, E. O. Smith, D. S. Charney, P. B. Hoffer and R. B. Innis, *Nucl. Med. Biol.*, 1993, **20**, 597.
34 G. L. DeNardo, S. L. Mills, D. L. Kukis, V. Anderson and S. J. DeNardo, *Antibody, Immunoconjugates, Radiopharm.*, 1995, **8**, 1.
35 D. M. Goldenberg, *J. Nucl. Med.*, 2002, **43**, 693.
36 D. Kwekkeboom, E. P. Krenning and M. de Jong, *J. Nucl. Med.*, 2000, **41**, 1704.
37 T. Klingebiel, P. Bader, R. Bares, J. Beck, B. Hero, H. Jurgens, P. Lang, D. Niethammer, B. Rath and R. Handgretinger, *Eur. J. Cancer*, 1998, **34**, 1398.
38 A. I. Kassis, P. Y. Wen, A. D. Van den Abbeele, J. Baranowska-Kortylewicz, G. M. Makrigiorgos, K. R. Metz, K. Z. Matalka, C. U. Cook, S. K. Sahu, P. M. Black and S. J. Adelstein, *J. Nucl. Med.*, 1998, **39**, 1148.
39 R. H. Larsen, K. M. Murund, G. Akabani, P. Hoff, O. S. Bruland and M. R. Zalutsky, *J. Nucl. Med.*, 1999, **40**, 1197.